MECHANISMS OF KIDNEY DAMAGE—HUMAN AND ANIMAL STUDIES

1-OR

Tubular Markers, KIM1 and NAG, and Risk of Early Renal Function Loss in Type 1 Diabetes

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The aim of this study was to evaluate whether urinary markers of tubular injury are predictors of early glomerular filtration rate (GFR) loss in diabetes. Our study group consisted of 255 Joslin subjects with type 1 diabetes, no proteinuria and normal GFR at baseline. This cohort was followed for 5-7 years and multiple determinations of serum cystatin C were performed to identify patients with significant early GFR loss. As the outcome of the study rapid GFR loss during 5-7 years of follow-up was defined as an annual GFR decrease >4%. Baseline urinary concentrations of tubular markers, N-acetyl-b-D-glucosaminidase (NAG) and Kidney Injury Molecule-1 (KIM1) were measured by nephelometry and on the Luminex platform, respectively. Rapid GFR loss occurred in 49 subjects (20%) and was more frequent in those who had higher concentrations of KIM1 in urine, p value for trend: 0.0001. Proportion of cases stratified by KIM1 increasing quartiles is presented in the table below.

	KIM1-Q1	KIM1-Q2	KIM1-Q3	KIM1-Q4
GFR loss [%]	**8**	**11**	**27**	**31**
number at risk	64	63	64	64

Multivariate logistic analysis included baseline age, albumin excretion rate (AER), hemoglobin A1c and body mass index. Odds ratio of rapid GFR loss for subjects with KIM1 concentrations above median were OR (CI): 3.9 (1.9-7.9), p value 0.0002 in the univariate, and 2.7 (1.3-5.8), p value 0.009, in the multivariate model, respectively. Association of NAG with rapid GFR loss was borderline and did not remain significant in the multivariate analysis.

Urinary concentrations of KIM1, but not of NAG, predict early rapid GFR loss in subjects with type 1 diabetes. These findings also provide strong evidence that *tubular injury* is involved in the disease process, which underlies the development of early progressive renal function loss. **ADA-Funded Research**

2-OR

TIMP3 Deficiency Accelerates Diabetic Nephropathy in Mice

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Some experimental models of nephropathy have recently shown that activation of TACE/ADAM17 is involved in nephropathy, even though the exact role of ADAM17 and its inhibitor TIMP3 in a hyperglycemic environment is unclear. To test this hypothesis we treated WT mice with streptozocin to induce hyperglycemia, and then measured TACE activity in kidney; we found a significant activation of the enzyme in hyperglycemic (STZ, streptozocin-treated) mice compared to untreated controls ($p<0.001$). Next, we analyzed STZ-WT and STZ-*Timp-3*$^{-/-}$ kidneys. STZ-*Timp-3*$^{-/-}$ mice showed significantly incresead mean glomerular area and fractional mesangial area ($p<0.05$ and $p<0.001$, respectively), a thicker glomerular basement membrane, due to an increased amount of type IV collagen deposition ($p<0.01$). STZ-*Timp-3*$^{-/-}$ showed increased macrophage infiltration, measured by F4/80 staining ($p<0.01$). Oxidative stress markers staining revealed that STZ-*Timp-3*$^{-/-}$ mice are characterized by an increased expression of NOX4, RAGE and N-Carboxymethyl-lysine, a major product of oxidative modification of glycated proteins ($p<0.01$ for all). To analyze the role of TIMP3 in diabetic nephropathy at a molecular level we performed real time PCR on kidney RNA from STZ-*Timp-3*$^{-/-}$ and WT mice. MCP-1, a marker of inflammation, was significantly increased while nephrin and WT1, two podocyte markers were reduced in STZ-*Timp-3*$^{-/-}$ mice compared to the WT littermates ($p<0.01$ for all). Microarray profiling revealed up-regulation of different classes of genes involved in inflammation (*Cxcl9, Ccr5, Mcp-1, Mcp-5, Aif-1, Cd36, Mgl-1, Mgl-2*), renal cells proliferation and fibrosis (*Pdgf-d, Tgfb3, Fgf, Ghr*), lipid metabolism (*Fabp5, Fasn, Ldlr, Acaca, Acms3*) and transport (*Slc13a1, Slc7a13, Slc7a6, Slco4c1, Slc12a3, Glut8*) in STZ-*Timp-3*$^{-/-}$ mice compared to WT ones. We also found a significant reduction in the expression of genes connected to control of oxidative stress such as *Foxo1 and Foxo3a* in the kidney of STZ-*Timp-3*$^{-/-}$ mice. In conclusion, our results show that the deficiency of Timp3 is correlated to inflammation and oxidative stress accumulation in diabetic renal nephropathy.

Supported by: JDRF

3-OR

Down Regulation of Thiamine Transporter Expression in Human Tubular Epithelial Cells *In Vitro* and Increased Thiamine Clearance in Diabetes

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Increased renal clearance of thiamine (vitamin B_1) is common in diabetic patients linked to decreased renal reuptake of thiamine. Two recent clinical studies evaluating thiamine supplements to correct thiamine loss in patients with type 2 diabetes and microalbuminuria showed decreased urinary albumin excretion and reversal of microalbuminuria. The aim of our investigation is to elucidate the mechanism of decreased renal reuptake of thiamine in diabetes. Thiamine is actively scavenged from glomerular filtrate by two thiamine transporter proteins, THTR-1 and THTR-2, encoded by genes *SLC19A2* and *SLC19A3*. The location of these transporters in human kidneys and the effect of high glucose concentration on transporter expression in human tubular epithelial cells in primary culture were investigated.

Renal location of THTR-1 and THTR-2 was investigated by immunohistochemical staining of paraffin-embedded human kidneys. Freshly extracted human proximal tubular epithelial cells were grown in primary culture in medium containing low and high glucose concentrations (5 or 26 mmol/L, respectively) with 4 nmol/L thiamine and the expression of *SLC19A2* and *SLC19A3* investigated.

Immunohistochemical staining of human kidneys showed particularly intense staining of THTR-1 and THTR-2 in the proximal tubule. When human proximal tubular epithelial cells were incubated in high glucose concentration, *SLC19A2* and *SLC19A3* mRNA was decreased (-76% and -56% respectively; $p<0.001$). Thiamine transporter protein levels were also decreased in high glucose concentration (THTR-1 -77%; THTR-2 -83%; both $p<0.05$). Concomitantly, forward:reverse apparent permeability of monolayers of proximal tubule epithelial cells to [^{3}H]thiamine was decreased 23% in high glucose concentration ($p<0.001$).

We conclude that the proximal tubule is the likely major site in the kidney of reuptake of thiamine from glomerular filtrate. The decreased renal reuptake of thiamine in diabetes is likely due to hyperglycemia-induced decreased expression of thiamine transporters in the renal tubular epithelium.

Supported by: Diabetes UK

4-OR

TM5275 and TM5441, Novel Inhibitors of Plasminogen Activator Inhibitor-1, Prevent Renal Injury in Streptozotocin-Induced Diabetic Mice

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Diabetic nephropathy is the leading cause of end-stage renal disease worldwide. Plasminogen activator inhibitor-1 (PAI-1) is increasingly recognized as an important factor favoring extracellular matrix (ECM) accumulation in chronic kidney disease including diabetic nephropathy. We previously reported that diabetic nephropathy in PAI-1 null mice was attenuated compared to wild-type mice. The present study examined the protective effect of PAI-1 inhibitors on renal injury in streptozotocin (STZ)-induced diabetic mice. TM5275 and TM5441 have been developed as effective PAI-1 inhibitors through virtual screening and structure relationship activity study to improve oral pharmacokinetic properties (Izuhara et al. J Cereb Blood Flow Metab 30:904, 2010). TM5275 (10 and 50 mg/kg/day) or TM5441 (2 and 10 mg/kg/day) were administered orally for 16 weeks to STZ-induced diabetic mice and age-matched control mice. At 16 weeks after the injection of STZ, diabetic mice showed significantly increased plasma glucose and creatinine, kidney to body weight, glomerular volume, fractional mesangial area, and urinary albumin excretion compared to age-matched control. Renal fibronectin, collagen I, α-SMA, and PAI-1 expression were also upregulated in diabetic mice kidneys. Treatment with TM5275 or TM5441 effectively inhibited albuminuria, renal and glomerular hypertrophy, and ECM accumulation in diabetic kidneys. These data confirm that PAI-1 plays an important role in the development and progression of diabetic nephropathy and suggest that the administration of TM5275 and TM5441, novel inhibitors of PAI-1, would become an effective therapeutic modality for the prevention of diabetic renal injury.

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For author disclosure information, see page 785.

5-OR

BMP4/Smad1 Signaling: A Novel Therapeutic Target for the Progression of Diabetic Nephropathy

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Deposition of type IV collagen (Col4) in mesangial matrix is the hallmark of diabetic nephropathy. We have shown that Smad1 transcriptionally regulates Col4 and α smooth muscle actin (αSMA) *in vitro*. Here, we further addressed the role of Smad1 signaling in diabetic nephropathy *in vivo*. First, we established Smad1 overexpressing (Smad1-Tg) mice. Although Smad1-Tg mice did not show any glomerular lesions, streptozotocin (STZ)-induced diabetic Smad1-Tg mice showed more prominent mesangial expansion and more prominent phosphorylation of Smad1 than diabetic WT mice. To delineate the mechanisms further, we examined the role of bone morphogenetic protein 4 (BMP4) for the progression of diabetic nephropathy *in vivo*. By RT-qPCR in RNA from isolated glomeruli, expressions of BMP4 and its receptor were increased along with phospho-Smad1 in STZ-induced diabetic mice at 36 weeks. Immunohistochemistry confirmed that increased BMP4 was in podocytes, whereas its receptor, ALK3 mainly in the mesangial area. To prove the direct involvement of BMP4, we also made tamoxifen-inducible BMP4-transgenic mice. Tamoxifen dramatically increased the BMP4 expression in glomeruli, resulting in marked mesangial matrix expansion even in the absence of diabetes. Next, STZ mice were treated with either a neutralizing antibody against BMP4 (αB) or control IgG from 20 to 36 weeks. Diabetic mice with αB showed a significant decrease in Smad1 phosphorylation, resulting in less mesangial expansion and Col4 accumulation than those with control IgG. In mesangial cells, αB treatment inhibited the transcription of Col4 and αSMA in a dose dependent manner. BMP4 increased the expression of αSMA, and this increase was blocked by Dorsomorphin, an inhibitor of Smad1 phosphorylation. Furthermore, overexpression of constitutive active Smad1 increased the transcriptional activity of αSMA, whereas that of an inactive form lacking phosphorylation sites abolished this increase. In conclusion, phosphorylation of Smad1 by BMP4 is a critical step and blocking this signal could be a novel therapeutic strategy for diabetic nephropathy.

6-OR

Glycemic Fluctuations and Progression of Diabetic Nephropathy (DN)

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It has been proposed that rate of progression of diabetic nephropathy (DN) is associated with glycemic fluctuations (GF), independent of overall glycemia. We have investigated the relationship between GF and five-year progression of biopsy measured DN in type 1 diabetic (T1D) subjects.

We studied 138 subjects participating in the Natural History of Diabetic Nephropathy Study (NHS) who performed a total of 160,591 self-glucose determinations over 5 years (for MAGE, minimum of 4/day for an average of 124 days). These mostly normoalbuminuric subjects were 16.8±6.2 (mean±SD) years old and had diabetes duration of 8.1±4.3 years at study entry. Statistical modeling and analyses was performed to estimate the effect of variation in glucose characteristics (mean, variance, Mean Amplitude of Glycemic Excursions [MAGE]) on DN progression. Progression was defined by 5-year change from baseline measurement of glomerular basement membrane (GBM) width* or mesangial fractional volume[Vv(Mes/glom)], measured in electron micrographs of research renal biopsies.

A relationship between mean glycemia and progression (Δ) of GBM thickening over 5 years was observed (R=0.199,P=0.020). When correlations between parameters relating to glycemic fluctuations and measurements of Δ GBM width, were calculated, MAGE, the primary measure of glycemic fluctuation, showed no significant correlation (R=0.069 and p=0.42). A similar lack of correlation was observed between variance of glucose levels and Δ GBM width (R=0.113, p=0.19). No significant correlations were observed between changes in Vv(Mes/glom), and any parameters of glycemic control.

We have previously shown in NHS that, early in DN, GBM width is the best structural predictor of DN clinical progression (Diabetes. 2005 :2164-71).These analyses suggest that glycemic fluctuations have less association with DN progression than mean glucose levels. At the same time, mean blood glucose levels account for only ~1% of the variation in DN progression, Thus, other variables not included in these analyses could be playing a role in early renal damage in diabetes.

* adjusted for the expected normal increase in GBM width with increasing age

Supported by: JDRF

7-OR

Pro12Ala Polymorphism in the PPARG Gene Contributes to the Development of Diabetic Nephropathy in Chinese Type 2 Diabetic Patients

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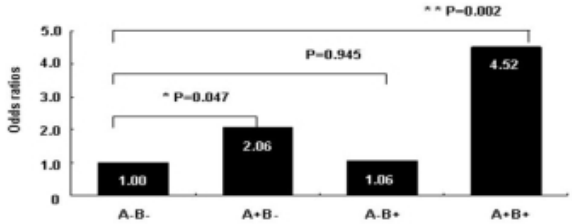
Oxidative stress is a major contributing factor in the development of diabetic nephropathy. Peroxisome proliferator-activated receptor gamma (PPAR-γ) heterozygous mice and Pro12Ala polymorphism in PPARG exhibited increased resistance to oxidative stress. Smoking increases the production of reactive oxygen species, which accelerates oxidative stress under hyperglycemia. To determine whether the Pro12Ala polymorphism, alone or in combination with smoking, contributes to the development of diabetic nephropathy, a case-control study was performed in 760 Chinese patients with type 2 diabetes. Among patients, 532 had diabetic nephropathy with microalbuminuria (n = 245) or overt albuminuria (n = 287), and 228 did not show either of these symptoms but had had diabetes for ≥ 10 years and were not undergoing antihypertensive treatment. After adjusting for confounders, the Pro/Pro genotype significantly associated with diabetic nephropathy (odds ratio 2.30 [95% CI 1.18-4.45], p=0.014); smoking was also an independent risk factor for diabetic nephropathy (1.99 [1.08-3.68], p=0.029). In addition, we identified possible synergistic effects, i.e., the high-risk group (smokers with the Pro/Pro genotype) showed 4.52 times higher risk (1.78-11.48; p=0.002) of diabetic nephropathy than the low-risk group (nonsmokers with Pro/Ala genotype) in a multiple logistic regression analysis controlled for the confounders.



[figure1]: Odds ratios of diabetic nephropathy versus no diabetic nephropathy in different combinations of PPARG Pro12Ala genotype and smoking status. A: Pro/Pro genotype. B: Smoker. A-B-: nonsmokers without the Pro/Pro genotype, who were considered as the reference group for determining P values and Odds ratios (95% CI).

Our results indicated that the Pro/Pro genotype and smoking were significant independent risk factors for diabetic nephropathy. The possible synergistic effects of genotype and smoking may aggravate oxidative stress and contribute to the development of diabetic nephropathy.

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8-OR

A Circulating Factor in Patients with Diabetic Nephropathy Causes Podocyte Insulin Resistance, Cytoskeleton Remodeling and Susceptibility to Apoptosis

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Insulin resistance correlates with microalbuminuria. Insulin receptor signaling is a key regulator of podocyte actin remodeling in vitro and in vivo. In fact, podocytes isolated from db/db mice prior to the onset of microalbuminuria are resistant to insulin action and are more susceptible to apoptosis. Based on these observations, we investigated if exposure of normal human podocytes to the sera of patients with diabetes with and without albuminuria causes cytoskeleton remodeling, cellular insulin resistance and susceptibility to apoptosis. We utilized serum pools collected from 10 diabetic patients with macroalbuminuria (D-MA) and 10 diabetic normoalbuminuric patients (D-NA) well matched for HgA_1C (7.9%). Serum pools from 10 healthy controls (NHC) were also utilized. Human podocytes

For author disclosure information, see page 785.

were exposed to 4% patient sera for 24 hours, and exposure to insulin (100ng/ml) was utilized in selected experiments. Actin remodeling was evaluated by confocal images of phalloidin stained cells and western blotting for synaptopodin and RhoA. Cellular insulin sensitivity was measured as insulin ability to phosphorylate AKT. Apoptosis was determined by quantification of cleaved caspase 3 and PARP. Changes in gene expression profiles were also evaluated using microarray analysis. Podocytes exposed to D-MA sera but not D-NA and NHC sera experienced actin cytoskeleton remodeling characterized by severe cell blebbing (90% of cells when compared to 20% in D-NA and 3% in NHC, $p<0.001$). Cell blebbing was associated with decreased synaptopodin and RhoA protein levels and increased cleaved caspase 3 and PARP ($p<0.01$) when compared to NHC. These findings were associated with a loss of the ability of insulin to phosphorylate AKT ($p=0.04$ in D-MA versus NHC) and with the significant modulation of several genes involved in cytokines action, actin remodeling and insulin signaling. In conclusion, our study suggests the presence of a circulating factor responsible for podocyte malfunction in patients with diabetes developing albuminuria.

GENETICS—TYPE 1, TYPE 2, AND COMPLICATIONS

9-OR

The Autoimmune Metagenome for Type 1 Diabetes Reveals Differences in the Metabolic Potential of an Aberrant Gut Microbiota

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The incidence of type 1 diabetes (T1D) has been increasing in the United States at a rate that suggests environmental determinants. Using high-throughput 16S rRNA sequencing, a previous study identified bacterial taxa that are negatively and positively correlated with the development of T1D-associated autoimmunity in young children at high risk for the disorder. In addition, a decrease in genetic diversity was observed in autoimmune subjects compared to age-matched, genotype-matched, nonautoimmune individuals. To determine the bacterial metabolic functions that may differ between autoimmune and healthy individuals, over 30 billion bases of Illumina metagenome data were analyzed from samples taken from the same subjects at the time of the development of autoimmunity. Contigs were built from approximately 90% of the trimmed, overlapping paired reads. From these, approximately one million open reading frames were predicted and compared to the SEED protein database. To analyze the metabolic potential of the microbiomes, subsystem hierarchies were used to make comparisons between the samples using a Poisson regression analysis. This revealed a significant increase in genes correlating to pathways for carbohydrates and stress responses in autoimmune subjects, and an increase in several other pathways in nonautoimmune subjects. Proteins that relate to virulence through cell adhesion were found to be more prevalent in autoimmune subjects, and, among other factors, could be involved in triggering the autoimmune response. Extensive differences in metabolic potential, that vary from broad functional categories, such as amino acid metabolism, to specific proteins, indicate that autoimmune subjects have a genetically and functionally aberrant microbiome. Most of the metabolic functions that differ in abundance between cases and controls are in higher levels in controls. This suggests that the case microbiomes have higher populations of fastidious bacteria. The relationship between these metabolic differences and the development of autoimmunity is still unknown but is under further investigation.

Supported by: General Diabetes Association

10-OR

Genomic Comparisons of *Lactobacillus* Strains Reveal Possible Contributions to the Onset of Diabetes in a Rat Model

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Lactobacillus species have been negatively correlated with diabetes onset. Recently the strains *Lactobacillus reuteri* TD1 and *Lactobacillus johnsonii* N6.2 were isolated from Biobreeding diabetes resistant rats (BB-DR). Feeding strain N6.2, but not strain TD1, to BioBreeding diabetes prone rats (BB-DP) slowed the onset of diabetes. Comparative genomic approaches were used to investigate the differences in these two genomes and how their unique characteristics relate to the onset of diabetes. Shotgun sequencing was performed using the Illumina Genome Analyzer IIx. De novo and reference assemblies were done using the CLC Genomics Workbench. Optical maps of both strains verified the accuracy of the de novo assemblies. Strain TD1 was missing approximately 300 genes from each of the reference strains DSM 20016 and JCM 1112. Strain TD1 lacked genes in two pathways, glycerol metabolism and vitamin B12 synthesis. Both of these pathways are necessary for the production of reuterin, a broad-spectrum bacteriocin thought to contribute to the probiotic activity of *L. reuteri* strains. Strain N6.2 was missing 247 genes, compared to the reference strain F19785, but no whole pathways were missing or significantly affected. However, in strain N6.2 known probiotic genes involved in bacteriocin production, such as those required for lactacin F and hydrogen peroxide production were found. Several stress resistance genes were also observed in strain N6.2, including those required for bile salt hydrolases. The probiotic factors present in strain N6.2 may contribute to its negative correlation with the onset of diabetes. The absence of reuterin production in strain TD1 may account for its ineffectiveness as a probiotic. Reuterin deficiency in strain TD1 could be responsible for the differential effectiveness seen in the two strains in mitigating the onset of diabetes.

Supported by: JDRF

11-OR

A Genome-Wide Meta-Analysis of Six Type 1 Diabetes Cohorts Identifies Multiple Associated Loci

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Diabetes impacts approximately 200 million people worldwide, of which type 1 diabetes (T1D) represents approximately 10%. The application of genome wide association studies (GWAS) has robustly revealed dozens of genetic contributors to the pathogenesis of T1D, with the most recent meta-analysis identifying in excess of 40 loci. To identify additional genetic loci for T1D susceptibility, we examined associations in the largest meta-analysis to date between the disease and ~2.54 million SNPs in a combined cohort of 9,934 cases and 16,956 controls. Targeted follow-up of 53 SNPs in 1,120 case-parent trios uncovered three new loci associated with T1D that reached genome wide significance. The most significantly associated SNP (rs539514, $P = 5.66 \times 10^{-11}$) resides in an intronic region of the *LMO7* (LIM domain only 7) gene on 13q22. The second most significantly associated SNP (rs478222, $P = 3.50 \times 10^{-9}$) resides in an intronic region of the *EFR3B* (protein EFR3 homolog B) gene on 2p23; however the region of linkage disequilibrium is approximately 800kb and harbors additional multiple genes, including *NCOA1, C2orf79, CENPO, ADCY3, DNAJC27, POMC*, and *DNMT3A*. The third most significantly associated SNP (rs924043, $P = 8.06 \times 10^{-9}$) lies in an intergenic region on 6q27, where the region of association is approximately 900kb and harbors multiple genes including *WDR27, C6orf120, PHF10, TCTE3, C6orf208, LOC154449, DLL1, FAM120B, PSMB1, TBP* and *PCD2*. These latest associated regions add to the growing repertoire of gene networks predisposing to T1D.

12-OR

New Genetic Loci Identified in a Genome-Wide Meta-Analysis of Diabetic Nephropathy

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The GENIE (GEnetics of Nephropathy: an International Effort) consortium was launched as a large study of diabetic nephropathy of 7,359 subjects with type 1 diabetes (T1D), with the aim to study the role of common genetic variation and the biological mechanisms contributing to diabetic nephropathy (DN).

All subjects had T1D diagnosed before 35 years of age, were at least 18 years old and provided written consent. Our primary analysis evaluated the

For author disclosure information, see page 785.

association of genetic loci with DN cases (n=2877), defined by the presence of macroalbuminuria or end-stage renal disease (ESRD). Controls in this analysis (n=3315) were individuals with no sign of DN despite 15 years of T1D. We further investigated the association of genetic loci with ESRD (n=1399) using different control group definitions.

Genome-wide association scans (GWAS) with our standardized quality control (QC) procedures were conducted separately for FinnDiane (3370 samples, 549,530 SNPs) and the All Ireland-Warren 3-UK GoKinD collection (1687 samples, 791,687 SNPs). The same QC steps were also applied to the US GoKinD data retrieved from dbGAP (1595 samples, 360,899 SNPs). Imputation (MACH, HapMapII CEU) resulted in ~2.4 million SNPs common to all cohorts. Association analysis was performed with PLINK and individual study results were meta-analyzed using METAL.

The primary DN analysis revealed five independent loci with $P<10^{-5}$ at 19q12, 20q11.21, 5q33.1, 4q34, and 2q33.3-q34. A genome-wide significant ($P<5x10^{-8}$) association was detected in the *AFF3* gene ($P=4.8x10^{-9}$, OR 0.74) when cases with ESRD were compared with all other subjects. This finding is further supported by an *in vitro* model of DN, where *AFF3* is downregulated in proximal tubular epithelial cells after TGF-β1 stimulation. Additionally, sex-stratified analysis of ESRD cases compared to normoalbuminuric controls revealed a significant association at 2q31.1 in women ($P=1.8x10^{-8}$, OR 1.9), with no association detected in men. Follow-up of our main findings for DN and ESRD-related analyses is completed for 15% of the total 4450 planned samples. In summary, we have identified loci associated with ESRD and expect to do likewise for DN in T1D.

13-OR

Expression-Based Genome-Wide Association Study Links *CD44* in Adipose Tissue with Diabetes Susceptibility

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Type 2 diabetes (T2D) is a complex, polygenic disease, and phenotypically characterized by insulin resistance. Growing evidence has indicated the causative link between adipose tissue inflammation and the development of insulin resistance. A number of genome-wide genetic association studies (GWAS) have revealed approximately 40 loci associated with susceptibility to T2D to date. However, these robust T2D-associated genes only explain ~10% of the heritability and pathogenesis of T2D. Here we report an alternative genome-wide approach to find additional potentially rarer T2D susceptibility genes. We performed a gene-expression based genome-wide association study (eGWAS) across 1,175 T2D case-control microarrays obtained from public data sources, and identified the immune-cell receptor *CD44* as our top candidate for T2D susceptibility (Figure: $P = 8.5 \times 10^{-20}$). We verified that *CD44* deficiency in a diabetic mouse model ameliorates insulin resistance and adipose tissue inflammation, and also found that anti-CD44 antibody treatment decreases blood glucose levels in a high-fat diet fed mouse model. Further, we discovered a genetic association of a *CD44* coding variant with T2D (rs1071695; OR = 1.43 [1.17-1.74], $P = 3.9 \times 10^{-4}$). The risk allele of *CD44* increased its mRNA expression in human adipose tissue, and serum CD44 levels were positively correlated with insulin resistance in humans. Our findings demonstrate that *CD44* is associated with T2D susceptibility, and eGWAS may yield novel susceptibility genes for complex diseases.

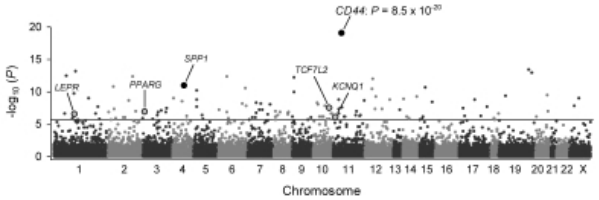

Figure: Expression-based genome-wide association study (eGWAS) for T2D.

Plot of -$\log_{10}$ (*P* value) (y-axis) by chromosomal position (x-axis). *P* values for each gene were calculated from our eGWAS across 1,175 T2D case-control microarray samples (591 T2D cases and 584 controls) as the likelihood of finding repeated differential expression compared to expected using a X^2 analysis. The black line indicates the Bonferroni threshold ($P = 2.0 \times 10^{-6}$). The highlighted grey dots indicate several well known T2D-susceptibility genes. *SPP1* (secreted phosphoprotein 1; also known as osteopontin (OPN)) encodes a ligand for CD44 receptor.

14-OR

Identification of Novel Type 2 Diabetes Susceptibility Loci by Large-Scale Replication Using the "Metabochip"

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There are more than 40 established type 2 diabetes (T2D) susceptibility loci, most identified through genome-wide association studies. However, replication efforts have focussed on only the strongest signals of association, and have failed to fully exploit large discovery meta-analyses. The "Metabochip", a custom iSELECT array containing ~195K SNPs, was designed to support large-scale follow-up of putative associations for T2D and other metabolic and cardiovascular traits.

We performed meta-analysis of Metabochip SNPs for 21,049 cases and 53,201 controls (23 cohorts of European descent). We considered 4,743 high quality, statistically independent SNPs which capture >95% of the ~5,000 strongest autosomal associations from the unpublished DIAGRAMv3 meta-analysis (12,057 cases and 56,071 controls of European descent). Association analyses in each cohort were performed assuming additive allelic effects and the results combined via fixed-effects meta-analysis.

We first compared patterns of replication between DIAGRAMv3 and Metabochip meta-analyses. After excluding SNPs at established loci, there was highly significant concordance in the direction of allelic effects (3,049 [65.0%] of 4,692 SNPs, binomial test $p=8.8x10^{-94}$). Combining the DIAGRAMv3 and Metabochip meta-analyses revealed 18 novel loci exceeding genome-wide significance, the strongest signals mapping to *GOLGA7* ($p=5.0x10^{-13}$), *SF4* ($p=5.9x10^{-12}$) and *HMG20A* ($p=7.4x10^{-12}$).

We also compared replication patterns between our European-ancestry meta-analysis and additional Metabochip data from 1,178 cases and 2,472 controls of Pakistani descent. The directional consistency was weaker, but still highly significant (2,321 [53.3%] of 4,354 SNPs, binomial test $p=6.8x10^{-6}$). Inclusion of these samples in the combined meta-analysis revealed two further novel loci at genome-wide significance. The concordance of risk alleles both within and between population groups is consistent with a long tail of common variants with modest effect on T2D susceptibility across ethnicities. Further Metabochip genotyping thus offers great promise for further discovery of T2D loci through inter- and intra-ethnic mapping studies.

15-OR

High-Throughput Sequencing and Dense Array Genotyping of Type 2 Diabetes Cases and Controls

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Type 2 diabetes (T2D) is a complex disorder of incompletely known genetic etiology. Although lower-frequency and rare variants likely contribute to T2D susceptibility, these variants have not yet been thoroughly investigated on a large scale.

In this study, we are attempting to identify additional variants contributing to T2D susceptibility in 1,325 T2D cases and 1,325 controls of Finnish, Swedish, and UK origin. We are using several approaches to target lower frequency variants, including low-pass (4x) whole-genome sequencing, deep exome sequencing, array genotyping of 2.5M SNPs, and genotype imputation using 1000 Genomes Project data. Analysis of data from each approach, both independently and jointly, will help elucidate the genetic architecture of T2D across a wider allele frequency spectrum as well as the most effective approaches to achieve this end.

Preliminary analysis of data from a first-term data freeze representing 179 low-pass genomes identified 11.3M SNPs, 40% of which are not present in public databases. Among novel, rare variants are several unique to cases that create or delete a stop codon (nonsense) in genes at loci involved in monogenic (*PAX4*-W91*, *WFS1*-E752*) and polygenic (*TSPAN8*-*238R) forms of T2D. In addition, several novel nonsense variants unique to cases implicate previously unreported genes. Variants were tested for T2D association after imputation into 4,100 case and 5,200 control samples with genome-wide array data from the DGI, FUSION and WTCCC studies. Patterns of association in common variants were consistent with previous results imputing HapMap data into the same samples, including three established T2D loci (*TCF7L2, CDKAL1, CDKN2A/B*) with $P < 5x10^{-8}$, suggesting that variants identified in low-pass sequencing can be effectively tested in larger sample sets to maximize risk allele discovery potential.

Data collection is ongoing with ~1000 whole-genomes, ~1500 exomes, and 2.5M array genotypes on all samples fully analyzed by June 2011, which

For author disclosure information, see page 785.

will greatly enhance statistical power for discovery and represents the first opportunity to gain insight into the contribution of variants across the allele frequency spectrum to T2D.

16-OR

Polymorphisms in the Selenoprotein S Gene Are Associated with Quantitative Measures of Subclinical Cardiovascular Disease in the Diabetes Heart Study

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The interaction between the genetic and environmental factors that contribute to risk for cardiovascular complications among individuals with type 2 diabetes mellitus (T2DM) is not well understood. Selenoprotein S (SelS), one member of the selenprotein family, has recently been investigated for its association with a range of inflammatory markers especially in the context of cardiovascular disease (CVD). The aim of the current study was to examine the role of *SELS* genetic variants in risk for CVD in T2DM.

The association of 10 single nucleotide polymorphisms (SNPs) tagging the *SELS* gene was evaluated with subclinical CVD phenotypes including coronary, carotid and aortic calcified plaque and intimal-medial thickness, C-reactive protein concentrations, and HbA1C in 1220 European Americans from the family-based Diabetes Heart Study (DHS).

Of the 10 SNPs examined, four showed significant association with at least one of the vascular calcification phenotypes (p=0.0044-0.0362). The strongest evidence of association was found for rs34713741 and rs12917258 which were associated with coronary ($p=1.25x10^{-5}$, β=-0.547 and $p=3.79x10^{-6}$, β=-0.542 respectively), carotid ($p=9.94x10^{-6}$, β=-0.561 and $p=1.75x10^{-7}$, β=-0.626) and aortic calcified plaque ($p=1.41x10^{-4}$, β=-0.501 and $p=5.68x10^{-6}$, β=-0.547) under an additive model of inheritance, as well as with both CRP (p=0.0279, β=-0.154 and p=0.0343, β=-0.142) and HbA1C (p=0.0022, β=-0.033 and $p=6.45x10^{-5}$, β=-0.041). Further, these two SNPs were also associated with all-cause mortality (p=0.0279, β=-0.154 and p=0.0343, β=-0.142 respectively) over the follow up period of 7.3 ± 2.3 years (mean ± SD).

These findings support a role of SelS in contributing to CVD development in T2DM. Understanding the interplay between glycemic control, inflammatory pathways and vascular calcification may prove important in predicting and managing the macrovascular complications of T2DM.

MECHANISMS REGULATING INSULIN SIGNALING AND GLUCOSE UPTAKE

17-OR

A Novel Akt Substrate Containing C2 Domain Regulates GLUT4 Insertion into the Plasma Membrane

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Insulin is the major hormone that regulates glucose disposal in humans through activating Glucose Transporter 4 (GLUT4) translocation from the intracellular compartments to the plasma membrane (PM) in the skeletal muscle and adipose tissues. Multiple studies have suggested that the activation of the phosphatidylinositol 3-kinase (PI 3-kinase) - PKBb/Akt2 pathway is necessary for the insulin-stimulated glucose transport and GLUT4 translocation. Here, we report that a novel Akt substrate containing C2 domain (ASC2D) regulates GLUT4 insertion into the PM linking Akt2 signaling to the mechanical process of GLUT4 translocation. ASC2D was identified, using SILAC-based quantitative proteomic approach, from the phospho-proteome enriched with the antibodies against phospho-Akt substrate motif (RXRXXpS/T). We further confirmed that ASC2D is phosphorylated directly by constitutively active Akt2 at Ser197 residue. RNAi-based functional analysis revealed that ASC2D is required for the insulin-stimulated glucose transport and GLUT4 translocation. Knockdown of ASC2D significantly blocks the insulin-stimulated fusion between GLUT4-containing vesicles and the PM whereas the vesicle docking at the PM is not affected in live adipocytes. Overexpression of ASC2D mutants lacking the C2 domain or the AKT2 phosphorylation site significantly inhibits the insulin-stimulated GLUT4 translocation to the cell surface. Biochemically, purified C2 domain from ASC2D interacts with calcium ion and lipid membranes. Furthermore, our data demonstrated that ASC2D co-localizes with phosphorylated Akt at the cell surface in response to insulin, and is required for GLUT4 translocation induced by constitutively active Akt2, suggesting that ASC2D function as the downstream of the Akt2 pathway. Taken together, these results show that the novel Akt substrate ASC2D is a key component of insulin signaling pathway involved in the regulation of GLUT4 fusion with the PM.

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18-OR

Overexpression of Myristoylated Akt1 Impairs Insulin-Mediated Glucose Uptake Via Mechanisms That Are Independent of Feedback Inhibition of Irs1 by S6k

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Hyperinsulinemia induces insulin resistance by activating negative feedback pathways. For example, activation of S6 Kinase (S6K) increases insulin receptor substrate 1 (IRS1) serine phosphorylation promoting its degradation in insulin responsive tissues such as liver and skeletal muscle. Whether or not these mechanisms exist in cardiac muscle is less understood. We therefore examined the impact of Akt activation in cardiac tissue using a doxycycline-inducible (tet-off) mouse model of transgenic Akt1 over-expression to determine if Akt-mediated insulin resistance is mediated by activation of mTOR. 10 days after doxycycline withdrawal, heart weight normalized by body weight was increased by 8.7% in Akt transgenic hearts, in parallel with increased phosphorylation of Akt at the Ser473 site and increased phosphorylation of the Akt target S6 at Ser235/236. Insulin-mediated 2-deoxy-glucose (DG) transport in isolated cardiomyocytes of tet-off Akt mice was decreased by 60% and pretreatment with rapamycin did not normalize glucose transport despite blocking S6 phosphorylation. Moreover, tyrosine phosphorylation of IRS1 remained normal, IRS1 Ser307 phosphorylation was not increased and IRS1 protein levels did not decline, suggesting that insulin resistance was not the result of defective IRS1 signaling. By contrast, 4 weeks of transgene activation blunted insulin-mediated Akt phosphorylation that was associated with decreased IRS2, but not decreased IRS1 content. Rapamycin treatment prevented the reduction of IRS2. Similarly, constitutive activation of PI3K in cardiomyocytes impaired 2-DG uptake prior to the decline in IRS1 and IRS2 content. Thus, chronic activation of PI3K or Akt in cardiomyocytes desensitizes insulin-mediated glucose uptake via mechanisms that are independent of and precede S6K-mediated inhibition of IRS1 signaling.

19-OR

Impaired Biogenesis of the Insulin-Responsive GLUT4 Storage Compartment under Insulin Resistance Revealed by Single Molecule Imaging

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It had long been believed that insulin resistance is caused by defects in insulin signaling. However, severe defects in insulin-dependent GLUT4 translocation were shown to be induced by independent or minor reductions of insulin signaling, suggesting that insulin resistance can occur via defects of GLUT4 trafficking itself. Recently, we established Qdot-based nanometrology for GLUT4 that can directly quantify "intracellular GLUT4 movements". With this approach, we reported 1) existence of "static GLUT4 retention" property in GLUT4 storage compartment and 2) importance of insulin-induced GLUT4 liberation from its static state, revealing a major functional aspect of insulin actions in 3T3L1 adipocytes. Here, we analyzed whether, and if so how, insulin resistance affects the "static GLUT4 retention" property in its storage compartment.

First, chronic endothelin-1 treatment, which evokes insulin resistance, resulted in disappearance of "static GLUT4 retention" property, and thereby Insulin failed to regulate proper GLUT4 trafficking, indicating biogenesis of insulin-responsive "static" GLUT4 storage compartment to be deranged. Based on our single molecule analysis combined with a molecular biological approach, we found that changes in sortilin expression, whether developmental, experimental or pathological, markedly alter intracellular trafficking activities of GLUT4.

Specifically, sortilin is critical for establishing the "static GLUT4 retention" property, which appears to be developed by sortilin-dependent endosomes-to-trans-Golgi network retrieval system for GLUT4 collaborating with retromers, golgin-97 and syntaxin-6.

Malfunction/reduction of sortilin under insulin resistant conditions thereby deranges biogenesis of the insulin-responsive GLUT4 storage compartment. Moreover, we found that AS160 plays a role in the insulin-induced GLUT4 liberation process from its static state. Together, these data demonstrate crucial role of sortilin-mediated retrograde trafficking of GLUT4 in generating "static GLUT4 retention" property, and suggest that "sorting defects" have one of important etiological roles of insulin resistance.

For author disclosure information, see page 785.

20-OR

RSK Is a Novel Feedback Regulator of Insulin Action in Hepatic and Muscle Cells through Promoting IRS-1 S1101 Phosphorylation

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It is critical to understand the molecular mechanisms that underlie insulin resistance to develop new therapeutic approaches. We previously demonstrated that the mTORC1-S6K1 pathway is activated by insulin and nutrient excess (e.g. amino acids [AA]), which leads to the inhibition of the PI3K-Akt pathway via inhibitory serine phosphorylation of IRS-1, notably on serine 1101 (S1101). However, even in the absence of AA, insulin still promote IRS-1 S1101 phosphorylation by other kinases that remain to be fully characterized. We have now uncovered a new negative feedback loop arising from the MEK-ERK pathway, which activates p90 ribosomal S6 kinase (RSK). Using an antibody that detects a phosphorylation site (S221) located in the activation segment of RSK, we found that insulin activate both RSK1 and RSK3 in L6 myocytes even in the absence of AA overload. Computational analyses revealed that S1101 within IRS-1 falls into the consensus motif of RSK. Furthermore, RSK inhibition using either the pharmacological inhibitor BI-D1870 or after adenoviral expression of a dominant negative (DN) RSK1 mutant showed that RSK promotes the phosphorylation of IRS-1 on S1101 but not on S636/639. Accordingly, expression of the DN-RSK1 mutant also improved insulin action on muscle glucose transport and glucose production in hepatic cells. Importantly, RSK inhibition did not impede insulin's ability to activate the mTORC1-S6K1 pathway indicating that RSK does not promote S1101 phosphorylation by activation of this pathway. Finally, we found a close correlation between the activation of RSK and the phosphorylation of IRS-1 S1101 in high-fat fed obese insulin-resistant mice. We propose that RSK is a novel regulator of insulin signaling and glucose metabolism by promoting IRS-1 S1101 phosphorylation. This work could lead to new therapeutic strategies to alleviate insulin resistance in obese patients.

Supported by: CIHR

21-OR

APPL1 Potentiating Insulin Signaling by Facilitating the Recruitment of IRS1 onto Insulin Receptor

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APPL1 is an adaptor protein mediating adiponectin and crosstalk between insulin and adiponectin signaling. APPL1 itself has also been shown as an essential mediator potentiating the insulin signaling, but the molecular mechanism remains unknown. In this study, we found that APPL1 can physically interact with the β-subunit of the insulin receptor (IRβ) and the interaction is stimulated by insulin and adiponectin. Disruption of APPL1 expression in cells and *in vivo* significantly diminished insulin signaling and action. On the other hand, overexpression of APPL1 potentiates insulin signaling downstream of the IRβ in an IRS1/2-dependent manner. APPL1 also binds with IRS1, which facilitates the recruitment of IRS1 onto IRβ and the tyrosine phosphorylation of IRS1. APPL1 undergoes insulin- and adiponectin-stimulated phosphorylation at Ser^{401} and Ser^{401} phosphorylation is essential for the promoting effect of APPL1 on IRβ-IRS1 interaction. Interestingly, Phosphorylation of APPL1 at Ser^{401} is greatly impaired in skeletal muscle tissues of high fat diet-fed mice and obese patients. Together, our results show that APPL1 promotes insulin signal by acting as a scaffolding protein to facilitate IRS1 interaction with IRβ. In addition, phosphorylation of APPL1 at Ser^{401} plays a critical role in regulating the interaction of IRS-1 with IRβ, thus uncovering a novel mechanism underlying the insulin-sensitizing role of APPL1.

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22-OR

Sequestosome 1/p62, a New Interacting Adapter Protein with IRS-1 in Insulin Signaling

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Skeletal muscle is the major site of insulin-stimulated glucose disposal and defects in skeletal muscle have been implicated in the development of insulin resistance but the precise abnormalities are largely unclear. Recent studies showed that gene targeted deletion of sequestosome 1/ p62 in mice results in mature-onset obesity and progressed to insulin, leptin resistance and type 2 diabetes. So far no study has shown the presence of p62 in insulin signaling pathway. p62 is a highly conserved cytosolic 62kDa protein, functions as a scaffolding protein that interacts with the atypical protein kinase Cs in several signaling pathways. p62 is involved in the receptor-mediated signal transduction; functions as an intracellular signal modulator or adaptor protein. Insulin receptor substrate-1 (IRS-1) plays a central role in transducing the insulin signal via phosphorylation, protein-protein interactions and protein modifications. We are the first to demonstrate that p62 interacts with IRS-1 on insulin stimulation in rat skeletal muscle cells by co-immunoprecipitation and immunostaining techniques. Mapping analysis indicates the SH_2 domain at the amino terminus of p62 interacts with IRS-1. p62 is found to interact with IRS-1 through YXXM motif present in the region between amino acids 524-698 and 861-1241 similar to other SH_2-domain containing proteins, such as phosphatidylinositol (PI) 3-kinase, Grb-2, SHP-2, Fyn, and Nck. These results suggest that p62 is a new binding protein with IRS-1 and its SH_2 domain interacts with YXXM motif of IRS-1.

23-OR

Hyperglycemia and Anorexia in Mice Following Ablation of Glut4-Expressing Neurons

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The central nervous system (CNS) plays an important role in metabolism, but neurons that relay metabolic signals are only partly known. We have previously mapped a sub-population of CNS neurons that express the insulin-responsive glucose transporter, Glut4. We now show that, in the mediobasal hypothalamus, Glut4 neurons show limited overlap with NPY and POMC neurons. In this study, we performed cell ablation experiments in the CNS of adult mice to examine the overall function of Glut4 neurons in metabolic control. We labeled Glut4 neurons with an inducible diphtheria toxin receptor allele, then administered diphtheria toxin by an ICV route. We observed selective loss of Glut4 neurons at appropriate anatomical sites. Before ablation, and for the first 48-hr thereafter, energy balance of control and Glut4-neuron-ablated mice were indistinguishable. Subsequently, Glut4-neuron-ablated mice showed significantly reduced spontaneous food intake, but preserved feeding response to NPY. Glut4-neuron-ablated mice also showed reduced RER, locomotor activity, and energy expenditure, associated with reduced body weight and fat mass, with preserved lean mass. In both fasted and refed conditions, Glut4-neuron-ablated mice showed significantly reduced locomotor activity, suggesting less food foraging. Despite reduced food intake and weight loss, Glut4-neuron-ablated mice showed significant fasting hyperglycemia and glucose intolerance, associated with decreased insulin levels during fasting and refeeding, and hepatosteatosis associated with elevated gluconeogenic genes. To map this complex phenotype to specific sites of Glut4 neurons, we stereotactically ablated Glut4 neurons in the mediobasal hypothalamus. This procedure recapitulated the metabolic phenotypes of reduced food intake, fasting hyperglycemia, and glucose intolerance. In summary, Glut4 neurons control various aspects of metabolism, including food intake, energy partitioning, glucose sensing, and peripheral metabolism. Ablating these insulin-sensitive neurons disrupts CNS integration of peripheral metabolism and causes diabetes.

24-OR

Development of Insulin Resistance by Chronic Hyperinsulinemia and Subsequent Reduction of Akt Signaling in Dorsal Root Ganglion Neurons

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Insulin resistance (IR) is the major feature of type 2 diabetes, glucose intolerance, obesity, dyslipidemia and hypertension; i.e. metabolic syndrome. IR studies are mainly focused on peripheral tissues such as muscle and liver. There is, however, little knowledge about IR in neuronal cells. In this study we examined whether neurons develop IR in response to hyperinsulinemia. As a first step we examined insulin signaling using adult mouse dorsal root ganglion (DRG) neurons as a model system. Acute insulin treatment (20 nM, 0-2 h) resulted in time- and concentration-dependent activation of the signaling cascade including phosphorylation of the insulin receptor (InsR), Akt, p70S6K and GSK-3b. To mimic hyperinsulinemia, cells were pretreated with 20 nM insulin for 24 h, and then stimulated with 20 nM insulin for 15 min. Chronic insulin treatment resulted in increased basal Akt phosphorylation. More importantly acute insulin stimulation after chronic insulin treatment resulted in blunted phosphorylation of Akt, p70S6K and GSK-3 b; the phosphorylation of InsR and ERK were not affected. Interestingly, when the cells were treated with PI3-K pathway inhibitor (LY294002), but not MAPK pathway inhibitor (U0126), chronic insulin treatment did not block acute insulin treatment-induced Akt phosphorylation.

Insulin-induced Akt phosphorylation was lower in DRG neurons from BKS-db/db compared to control BKS-db+ mouse.

For author disclosure information, see page 785.

This effect was age-dependent.

Our results suggest that hyperinsulinemia cause IR by disrupting the Akt-mediated pathway. Our results suggest a new theory for the etiology of diabetic neuropathy i.e. that, similar to metabolic tissues, neurons develop IR, and, in turn, cannot respond to the neurotrophic properties of insulin, resulting in neuronal injury and the development of neuropathy.

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OBESITY—ANIMAL

25-OR

CCR5 Plays a Critical Role in the Inflammatory Adipose Tissue Response to High Fat Feeding and Obesity by Regulating Both Macrophage Recruitment and M1/M2 Status

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Adipose tissue macrophage (ATM) accumulation through CCR2 and its ligand MCP-1 is considered pivotal in the development of insulin resistance. Recently, we demonstrated that loss of a different C-C chemokine receptor, CCR5, prevents insulin resistance induced by high fat (HF) feeding or leptin deficiency independently of MCP-1/CCR2. Expression of mRNA for CCR5 and its all ligands (MIP-1α, MIP-1β, RANTES, MCP-2) was markedly increased in stromal vascular (SV) fraction compared to adipocyte fraction of epididymal white adipose tissue (eWAT) of genetically (ob/ob) and diet-induced obese (DIO) mice. Immunohistochemistry on eWAT in DIO localized $CCR5^+$ cells to $F4/80^+$ macrophages in crown-like structure. To quantify $CCR5^+$ ATMs in lean and obese mice, fluorescence-activated cell sorter (FACS) analysis was performed on SV cells isolated from eWAT. ATMs identified as $CD45^+CD11b^+F4/80^+$ cells were increased in DIO mice by 12.2-fold compared with WT mice. Only a small percentage of ATMs coexpressed CCR5 in WT mice. However, DIO mice had 11.9-fold increase in $CCR5^+$ cells within ATMs, indicating that $CCR5^+$ macrophages accumulate in eWAT of obese mice. On a chow diet, no differences were observed in either $CD11c^+$ $MGL1^-$ (M1) or $CD11c^-$ $MGL1^+$ (M2) expression within ATMs from WT and *Ccr5*$^{-/-}$ mice. However, on a HF diet, in addition to reduction of total ATM content, *Ccr5*$^{-/-}$ mice had 39% fewer M1 ATMs whereas and 33% more M2 ATMs than WT mice, resulting in predominance of M2 over M1 ATM population. Importantly, chimeric mice lacking CCR5 only in myeloid cells (bone marrow transplant of *Ccr5*$^{-/-}$ into *WT*) were protected from HF diet-induced hyperinsulinemia and glucose intolerance. In conclusion, CCR5 deficiency causes M2 dominant phenotypic shift in ATM, which contributes to attenuation of obesity-induced insulin resistance.

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26-OR

Double-Stranded RNA-Dependent Protein Kinase (PKR) Acts as a Docking Protein and Is Necessary for Leptin Signaling in the Arcuate Nucleus

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PKR is a pathogen sensor and regulator of the innate immune response against viral infections. Recently it was shown that PKR responds to nutrient signals and regulates insulin action in peripheral tissues. It was also shown that PKR knockout (KO) mice on high fat diet (HFD) are lean and have lower fat mass. However, little is known about hypothalamic pathways involved in the regulation of energy balance in PKRKO mice. We investigated, herein, *in vivo*, the role of PKR in the modulation of leptin and insulin signaling in the hypothalamus. PKR is mostly expressed in arcuate nucleus (ARC). No significant changes on body weight (BW) or 24h food intake (FI) (g/g BW) was observed in the PKRKO on chow. However, intraperitoneal (IP) or intracerebroventricular (ICV) leptin injections induced lower suppression of BW and FI in PKRKO mice, suggesting that PKRKO are leptin resistant, despite normal BW. ICV leptin fails to increase STAT3 phosphorylation. In co-immunoprecipitation studies we demonstrated that after leptin injection PKR directly binds and form a complex with OBR/JAK2/ STAT3 in ARC, being an important docking protein. These results suggest that PKR is necessary to link OBR, JAK2 and STAT3 for normal leptin signaling. Differently, ICV insulin was able to reduce FI over 4-12h in the PKRKO mice. ICV insulin increases Akt/Foxo1 phosphorylation in ARC from PKRKO compared to PKR+/+. This suggests that the absence of PKR enhances insulin signaling in ARC. On HFD, PKRKO are lean and hypothalamic leptin resistant. However, PKRKO on HFD have higher hypothalamic insulin action and signaling that may contribute to the leanness. Furthermore, UCP-1 expression was increased in the brown adipose tissue (BAT) from PKRKO on HFD compared to PKR+/+, which may increase energy expenditure. In summary, the data provide evidence that PKR acts as a docking protein linking OBR, JAK2 and STAT3 and is necessary for leptin signaling in ARC and FI response. The enhanced insulin action/ signaling in ARC and higher UCP-1 expression in BAT may be a potential mechanism for leanness in PKRKO mice on HFD despite leptin resistance.

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27-OR

Induction of Autophagy by LKB1 Contributes to the Beneficial Effects of Metformin on Insulin Resistance and Glucose Homeostasis in ob/ob Mice

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Defective autophagic pathways have been implicated in the pathophysiology of type 2 diabetes (T2DM). We and others have demonstrated that LKB1/AMPK is required for the effects of metformin on glucose and lipid homeostasis in hatpocytes. However, the role of LKB1 in regulating autophagy and insulin resistance remains unclear. Here we report that LKB1 modulates hepatic autophagic pathway in insulin resistance *in vivo* and *in vitro*. We showed that autophagy markers, as indicated by LC3-II accumulation and p62 degradation, were impaired in the liver of genetically obese *ob/ob* mice. Consistently, autophagy-associated proteins such as Atg7, Beclin-1, and Atg5-Atg12 conjugation were downregulated. In contrast, administration of metformin (350mg/kg/day) in a liquid diet to ob/ob mice for 4 weeks significantly lowered fasting glucose levels by 40%, improved insulin resistance, and decreased S6K1-mediated serine phosphorylation of IRS-1. The insulin sensitizing effects of metformin were attributed to stimulated AMPK activity, inhibited mTORC1 activity and its downstream signaling, and induced autophagy in diabetic mouse livers. Moreover, AMPK activation by AICAR and metformin prevented the ability of excess amino acids to activate mTORC1 and suppress p62 degradation in human HepG2 hepatocytes. LKB1 activation by adenovirus-mediated overexpression of LKB1 complex including LKB1, STRADα and MO25α, also induced p62 degradation in HepG2 cells to the similar extent to that of metformin. The numbers of GFP-LC3 punctate in COS-7 cells, indicative of autophagosome formation, were increased by metformin. Importantly, enhanced autophagic signaling by metformin was completely abrogated in $LKB1^{-/-}$MEFs, suggesting that LKB1 is required for metformin to induce autophagic process. These findings indicate that LKB1-dependent activation of AMPK prevents hyperactive mTORC1 and impaired autophagy caused by excess nutrients in hepatocytes. The underlying mechanism may contribute to the beneficial effects of metformin to enhance insulin sensitivity and to restore glucose homeostasis in obesity and T2DM.

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Absence of dsRNA-Activated Protein Kinase (PKR) in Mice Improves Insulin Signaling in Lean and DIO Mice

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Double-strand RNA-activated protein kinase (PKR) is a protein related to recognition of viral patterns regulating innate immunity against virus, acting in conjunction with major inflammatory signaling, such as JNK and IKK, which are intimately involved with chronic inflammation in obesity status. In addition, PKR binds and activates the phosphatase PP2A, potentially modulating Akt phosphorylation. However, the role of PKR in the control of insulin sensitivity in physiological and pathological conditions was not yet investigated. In order to investigate the role of PKR in insulin sensitivity in physiological conditions and in obesity, we used a $PKR^{-/-}$ mice and evaluated metabolic parameters, PP2A phosphatase activity and protein signaling by western blotting in lean and high-fat fed mice. $PKR^{-/-}$ mice showed lower serum glucose and insulin indicating higher insulin sensitivity, corroborated by the increased glucose infusion rate, increased glucose tolerance and muscle glucose uptake and reduced hepatic glucose output, associated with an increase in insulin signaling pathway in liver and muscle. This observation was related to the striking reduction in PP2A phosphatase activity in liver and muscle, reducing the dephosphorylation of Akt, and improving insulin action. On high-fat diet for 12 weeks, $PKR^{-/-}$ mice did not gain weight, showing lower serum glucose and insulin levels and increased glucose infusion rate, improved glucose tolerance and reduced hepatic glucose

For author disclosure information, see page 785.

output, reduced activation of JNK and IKK and improved insulin signaling in liver and muscle compared to wild type DIO mice, without changes in PP2A activity. Based on these data, PKR is an important modulator of insulin signaling by a PP2A phosphatase mechanism in lean mice, but in DIO mice absence of PKR protects from obesity and insulin resistance by preventing the activation of JNK and IKK, indicating that PKR is an important modulator of insulin signaling in physiological conditions and in obesity, and a potential target for drugs to prevent obesity and insulin resistance. Financial support: FAPESP and CNPq

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Foxo1 in Adipose Tissue Macrophages Induces *Ccr2* Expression and Insulin Resistance

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Chronic inflammation and accumulation of adipose tissue macrophages (ATMs) play a pivotal role for generation of insulin resistance in obesity. Forkhead box-containing protein O subfamily 1(Foxo1) has important roles for metabolic regulation in several insulin-responsive tissues. However, little is known about physiological roles of Foxo1 in ATMs. Foxo1 expression is significantly increased in F4/80(+) CD11c (+) CD206 (-) macrophages in adipose tissues of mice under high fat-diet (HFD). Immunofluorescence of ATMs using both anti-F4/80 and anti-Foxo1 antibodies revealed that nuclear localization of Foxo1 in ATMs decreased at 16-week and increased at 24-week of HFD. In order to investigate roles of Foxo1 in ATMs, we generated macrophage-specific constitutively nuclear (*CNFoxo1*^{LysM}) or transactivation-defective Foxo1 (Δ256), which has no C-terminal domain after amino acid 256, transgenic (*Δ256*^{LysM}) mice using *Rosa26-CNFoxo1* or *Rosa26-Δ256* and M lysozyme Cre (*LysM-Cre*) mice. The *CNFoxo1*^{LysM} mice exhibited elevated insulin secretion during intraperitoneal glucose tolerance test, decreased insulin sensitivity in insulin tolerance test, and increased triglyceride accumulation in liver under HFD. FACS analysis revealed that F4/80(+) cells increased by about 50% and F4/80(+) CD11c (+) CD206 (-) cells increased by about 100% in stromal vascular fraction (SVF) from *CNFoxo1*^{LysM} compared with control mice. Analysis of gene expression of markers of M1 and M2 macrophages in SVF from epididymal fat and in ATMs collected by FACS revealed that *Ccr2* expression level was significantly increased in *CNFoxo1*^{LysM} mice. Transduction of adenovirus encoding CNFoxo1 in RAW264.7 cells also increased *Ccr2* expression. Luciferase assay demonstrated that CNFoxo1 increased *Ccr2* promoter activity and chromatin immunoprecipitation (ChIP) assay also demonstrated that Foxo1 bound to *Ccr2* promoter. In contrast, the *Δ256*^{LysM} mice exhibited significantly improved glucose tolerance and increased insulin sensitivity compared with control mice under HFD. These data suggest that Foxo1 induces *Ccr2* expression in ATMs, increases infiltration of macrophages to adipose tissue, and causes insulin resistance.

30-OR

Testosterone and Not Estrogen Causes the Sex-Specificity of the LepR L/I Phenotype

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Mice with a Tyr -> Leu mutation at residue 985 (homozygous denoted l/l) of the leptin receptor (LepRb) have been previously engineered to study leptin signaling through Tyr985 of LepRb.

Homozygous l/l female mice were found to be leaner than wild-type (wt) littermates, commensurate with an increase in leptin sensitivity, while the effect in male l/l mice was much more modest. As others have shown a relationship between gonadal-derived steroids and central leptin sensitivity, we hypothesized that sex hormone differences might influence the expression of the lean l/l phenotype. To investigate, circulating sex hormone concentrations were manipulated through castration (CAS), ovariectomy (OVX), and estradiol (E2) or testosterone (T) replacement via mini-osmotic pumps in 5-6 week old l/l males and females. OVX l/l females did not display a change in adiposity from intact l/l females. E2 replacement in OVX l/l females also did not affect the lean phenotype of OVX l/l females compared to mice receiving vehicle. CAS l/l males (n=9) were significantly leaner than sham-operated l/l males (n=18) (23.7% reduction in gonadal fat pad weight compared to sham-operated, p=0.05), and E2 administration (n=11) did not affect the lean CAS l/l male phenotype. T replacement, however, was able to partially reverse the loss of adiposity caused by castration in l/l males (n=8) in comparison to CAS l/l males receiving vehicle (n=8) (88% higher gonadal fat pad weight in mice receiving T compared to vehicle, p<0.02).

Overall, the data show that testosterone and not estrogen determines the sex-specificity of the expression of the lean phenotype in l/l mice, and that T must interact importantly with the feedback mechanisms engaged by Tyr985. This result gives insight into a possible mechanism of the long-noted sex differences in leptin sensitivity described in mammals as well as the sex-specificity of an increase in leptin sensitivity in SOCS3 (an important inhibitory binding partner of phospho-Tyr985) haploinsufficient mice.

ADA-Funded Research

31-OR

Improved Glucose Tolerance Despite Obesity in Neuron-Specific Lipoprotein Lipase Deficient Mice

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Lipoprotein lipase (LPL) is the rate limiting enzyme for the uptake of fatty acids from the hydrolysis of circulating triglyceride (TG)-rich lipoproteins for lipid storage and/or oxidation in peripheral tissues. LPL is also present throughout CNS neurons. On standard chow, mice with neuron-specific deletion of LPL (NEXLPL-/-) become obese by 16 wk, and then display reduced metabolic rate & physical activity. Substantial increases in AgRP mRNA (3.1 fold at 3 mo, p<0.01) occur in the hypothalamus before the onset of obesity and persist, suggesting this as a major mechanism for obesity development. A LPL-dependent decrease in TG-rich lipoprotein-derived lipid uptake in the hypothalamus and deficiency of specific polyunsaturated fatty acids suggest that modification of dietary lipid signal occurs before the onset of obesity (*Cell Metabolism*, 2011). Importantly, despite obesity, at 12 mo NEXLPL-/- mice display a normal fasting glucose and 30% reduction in glycemic excursion (p<0.02) after IP glucose. Marked increases in brown adipose tissue (BAT) mass and UCP-1 gene expression (56%-, p=0.001) also occur in these mice. The improved glucose tolerance (compared to relative control) is also observed in 6 mo NEXLPL-/- mice that are getting obese on a high fat diet and in 21 mo chow-fed heterozygous mice. Furthermore, only melanocortin-3 (Mc3r) not melanocortin-4 receptors are selectively up-regulated (2.4 fold, p<0.01) in the hypothalamus. This elevation is observed at 3 mo and persists. Taken together, these data suggest that the increased BAT mass/function in NEXLPL-/- mice serves as a potential compensatory mechanism to maintain whole body glucose tolerance as the mice become obese. This may be mediated through the persistent elevation in Mc3r gene expression as a result of the substantial early increase in AgRP gene expression in the hypothalamus. Thus, a putative role for Mc3r in energy balance and glucose metabolism has been suggested in response to modification of the dietary lipid signal in our mice. This proposed mechanism could lead to potential therapeutic application to maintain glucose tolerance in the setting of obesity.

ADA-Funded Research

32-OR

Gastrostomy Tube Placement in the Gastric Remnant at the Time of Gastric Bypass: A Rat Model for Selective Gut Stimulation

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Roux-en-Y gastric bypass (RYGB) surgery achieves high remission rates of type 2 diabetes mellitus in obese diabetic patients. It has been hypothesized that the changes in bowel nutrient exposure after RYGB results in altered release of gut hormones and improved glucose homeostasis. The aim of this study was to assess the feasibility and report our technique and initial experience with a model enabling selective gut stimulation in a gastric bypass rat model. We performed RYGB surgery with simultaneous placement of a gastric tube in the excluded gastric remnant of six obese Sprague-Dawley rats. Gastrostomy tubes were successfully inserted in all animals with no tube-related complications. The tubes remained patent throughout the study. Each rat was tested for oral glucose tolerance pre-operatively. On postoperative days 14 and 28, glucose tolerance was re-evaluated via oral and G-tube routes. Area under the curve (AUC) after oral glucose gavage decreased significantly after gastric bypass (p<0.001). Gastric remnant glucose gavage after RYGB essentially reversed the effects of surgery on glucose metabolism, and resulted in a glucose response AUC that significantly exceeded AUC for the oral route preoperatively and postoperatively (p<0.001). We conclude that placing a gastrostomy tube into the gastric remnant at the time of RYGB in a rat model is technically feasible. Our initial findings support the role of duodenal exclusion in improving glucose metabolism after RYGB. This innovative metabolic procedure provides access for selective gut stimulation and a new tool to better define the mechanisms of type 2 diabetes improvement after RYGB surgery.

For author disclosure information, see page 785.

ATHEROSCLEROTIC CARDIOVASCULAR DISEASE AND HUMAN TYPE 2 DIABETES

33-OR

Inflammation and Oxidative Stress in Type 2 Diabetes Vascular Complications: Predictive Power and Effects of Fenofibrate in the Fenofibrate Intervention and Event Lowering in Diabetes Study

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Inflammation and oxidative stress may promote diabetes vascular complications. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, a 5-year placebo controlled randomized trial evaluated co-micronized fenofibrate (200mg daily) in Type 2 diabetes and demonstrated benefit on secondary CVD end-points and pre-specified microvascular disease.

Aim of this project was to evaluate predictive power for complications of baseline measures of oxidative stress and inflammation and fenofibrate effects on biomarkers.

ELISAs and fluorescence spectroscopy were used to quantify serum biomarkers at baseline, after six weeks fenofibrate (n=9726) (in a 16 week run-in of diet-placebo-fenofibrate) and 1 year (n=1995) of fenofibrate vs. placebo: sVCAM-1, sICAM, sE-Selectin, Interleukin-6 (IL-6), interleukin-10 (IL-10), oxidized LDL (OxLDL), myeloperoxidase (MPO), Low Molecular Weight-Fluorophores (LMW-F) and leptin. Predictive power for a combined microvascular and a combined CVD end-point were determined.

All baseline biomarkers were positive (sICAM, sVCAM-1 and sE-Selectin, Ox-LDL, LMW-F, IL-6,), or negative (IL-10, leptin) predictors of microvascular disease in the combined fenofibrate and placebo groups; all $p<0.05$. CVD predictors in the combined groups were baseline Ox-LDL, LMW-F, IL-6, sICAM, sVCAM-1 and sE-Selectin, (positively related) and leptin (negatively related); all $p<0.05$. Biomarker variation was weakly related <7% to concurrent HbA1c and lipids. Except for sICAM and microvascular complications there were no statistically significant interactions between treatment group and prediction of diabetes complications by baseline biomarkers. Six weeks fenofibrate changed biomarker levels: Ox-LDL -17%, MPO -6%; LMW-F +21%, IL-6 -4%; sICAM +4%, sVCAM-1 +4%, sE-Selectin -6%, leptin +5%; all $p<0.001$. Effects at one year were similar.

In the FIELD study microvascular and CVD events were predicted by baseline inflammation and oxidative stress markers. Fenofibrate favorably affects some, but not all biomarker measures.

34-OR

Ability of Indices of Cardiovascular Disease (CVD) Risk and Comorbidity To Predict CVD Outcomes with Intensive Glucose Control in the VADT

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Recent large trials of intensive glucose control have demonstrated limited benefit of this approach on reduction of CVD complications in type 2 diabetes. However, several subanalyses of these studies suggested that patients with clinical histories indicative of less CVD burden and/or diabetes severity may respond better to intensive glucose control. Direct assessment of calcified coronary atherosclerosis (CAC) was also a potent indicator of response to intensive therapy (hazard ratio (HR) =0.08 for CAC ≤100). As CAC is expensive and requires radiation exposure, there is a need for effective clinical indices to predict response to intensive therapy. The aim of this study was to test the value of several validated CVD risk scores (Framingham and UKPDS) or comorbidity indices (Charlson's comorbidity index and a 4-year mortality prognostic index) in predicting primary CVD outcomes according to intensive vs. standard glucose control in the VA Diabetes Trial (VADT). The cohort included 1791 patients (mean age 60±9 yrs, HbA1c 9.4±1.5%). Unadjusted Cox proportional HR (95%CI) for intensive compared to standard therapy were determined within tertiles of each index (Table). Individuals in the upper tertile of each of the 4 indices showed no benefit from intensive therapy. In contrast, there was a modest to moderate benefit at lower levels of risk (either lower or middle tertile). Adjustment for prior CVD did not change results.

Index/Tertile	Lower	Middle	Upper
Framingham	0.85(0.58-1.25)	**0.66(0.48-0.91)***	1.06(0.82-1.37)
UKPDS	0.88(0.59-1.30)	0.77(0.51-1.05)	1.02(0.79-1.32)
Charlson's	0.99(0.72-1.36)	**0.57(0.39-0.84)***	0.94(0.73-1.21)
4-year mortality	**0.71(0.51-0.99)***	0.99(0.69-1.41)	0.92(0.71-1.19)

*p value <0.05

In conclusion, this study confirms that within the VADT, high levels of CVD risk or more extensive comorbidity at baseline were associated with poor response to intensive glucose control. The 4 clinical assessments may also identify individuals that benefit from intensive glucose control, however, they appear less effective than direct assessment of coronary calcified atherosclerosis.

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35-OR

The Predictive Ability of Retinol-Binding Protein 4 and High Molecular Weight Adiponectin with Respect to Incident Type 2 Diabetes Mellitus in the General Population

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The adipocytokines retinol-binding protein 4 (RBP-4) and adiponectin are potential mediators of insulin resistance and sensitivity, respectively through regulating glucose homeostasis and insulin production. In cross sectional studies high RBP-4 and reduced adiponectin levels have been reported in patients with type 2 diabetes mellitus. Therefore, the objective of this prospective study was to investigate the predictive ability of circulating levels of RBP-4 and high molecular weight (HMW) adiponectin with respect to incident type 2 diabetes mellitus. We measured biomarkers in 2444 persons, aged 30, 40, 50 or 60 years, mean BMI 25.9 kg/m^2 from the Danish WHO MONICA-10 cohort. After excluding cardiovascular disease (8 %) and diabetes mellitus (3 %) at baseline, 2189 subjects were included. Using the Danish National Diabetes database, which records all patients with a diagnosis of diabetes mellitus, the participants were followed for a median of 12.6 years and 176 incident cases of diabetes mellitus were registered. Using Kaplan-Meier analyses only plasma levels of RBP-4 in the lowest quartile (<31 mg/L) were associated with a reduced risk of 4.4 % for incident diabetes mellitus, as compared with subjects in the 3 upper quartiles of 8.7 % vs. 9.6 % vs. 8.9 %, respectively (P= 0.006). In multivariable Cox Proportional Hazard models, adjusting for risk parameters of type 2 diabetes (age, gender, BMI, waist circumference, HDL-cholesterol, triglycerides, fasting plasma glucose and fasting pro-insulin) RBP-4 levels below 31 mg/L were independently associated with a markedly reduced risk hazard ratio (HR) being 0.32 (95% CI: 0.29 – 0.59; P< 0.001). Interestingly, the HR was not attenuated after adjustment for HMW adiponectin levels, and both biomarkers remained independently associated with incident diabetes in the same model HR being 0.58 (95 % CI: 0.42- 0.92) for adiponectin values above 3,4 µg/mL (median level and optimal cut-off point). In conclusion, low circulating levels of RBP-4 may be protective of type 2 diabetes mellitus and a possible additive effect of high HMW adiponectin was observed.

36-OR

Pioglitazone (PIO) Reduces Progression of Carotid Atherosclerosis Independently of Improvement in Metabolic Risk Factors

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We previously reported that, compared with placebo, PIO therapy slowed carotid intimal-medial thickness (CIMT) progression in persons with IGT during the average 31± 10 month follow-up in the ACT NOW study. We now determined whether the protective effect of PIO on CIMT progression could be accounted for by changes in standard cardiovascular risk factors (CVRF), insulin levels, or insulin sensitivity. Of the 602 ACT NOW participants, 393 (PIO:194, placebo:199; age 53±12 yr; 54% female; 55% non-Hispanic White; BMI 33.2±5.3; HbA_{1c} 5.4 ± 0.4%), had 2-3 serial CIMT scans. Physical characteristics and CVRF did not differ between treatment groups at baseline. During the follow-up, HDL, total cholesterol to HDL ratio, fasting and 2 hour glucose and insulin values, and Matsuda insulin sensitivity index improved significantly in the PIO group compared with placebo group

For author disclosure information, see page 785.

and with baseline levels, despite increased BMI (Table). Annual rates of change in CIMT were determined from mixed models. After adjustment for age, gender, non-Hispanic White ethnicity, study sites, PIO reduced CIMT progression by 36%. After further adjustment for time dependent variables during the study (current smoking, BMI, SBP and DBP, total cholesterol to HDL ratio, Matsuda insulin sensitivity index, and statin and antihypertensive use), the annual rate of change in CIMT remained significantly lower (by 35%) in the PIO group (mean ± SE=0.0054 ± 0.0012 vs. 0.0089 ± 0.0011 mm/yr, $P < 0.01$). In conclusion, the effect of PIO on CIMT in IGT was not explained by concurrent improvements in metabolic risk factors, supporting a role for other vascular benefits of PIO.

	Baseline Placebo	Baseline Pioglitazone	Follow-up Placebo	Follow-up Pioglitazone
BMI(kg/m$^{2)}$	34±6	33±5	34±6	34±7
HDL(mg/dL)	41±10	40±10	45 ±11	47 ±12†
TC/HDL	4.4±1.2	4.3±1.2	3.9±1.1	3.7±1.2†
Fasting Glucose(mg/dL)	105±8	105±8	98.4±13	95±11‡
2 hour Glucose(mg/dl)	168±18	170±19	159±38	141±35‡
Fasting Insulin (µU/mL)	8.5 (4.5-14.1)	7.9(4.1-12.8)	7.5(3.8-12)	4.5(2.4-7.3)‡
2 hour Insulin (µU/mL)	89(58-132)	88(56-131)	74(45-118)	47(29-72)‡
Matsuda index	2.7(1.8-4.5)	2.8(1.9-4.4)	3.3(2.3-6.5)	5.6(3.3-9.8)‡

†P<0.04;‡P<0.01

Supported by: Takeda Pharmaceutical Co.

37-OR

Pioglitazone Prevents Carotid Atherosclerosis Progression in Persons with Impaired Glucose Tolerance and Hypertriglyceridemia

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Compared with placebo, randomization to pioglitazone (PIO) reduced atherosclerosis progression during 31±10 months in the ACT NOW study. We investigated whether the benefit of PIO might be greater for those with metabolic syndrome (MS) as defined by ATPIII criteria or individual MS components. Subjects were 392 ACT NOW participants with baseline and 1-3 follow up carotid (CIMT) scans (194 PIO, 198 placebo; age 53±12 yr; 54% female; 31% Hispanic; 16% nonwhite race; BMI 33.2±5.3 kg/m^2; HbA_{1c} 5.4±0.4%; 295 MS, 97 no MS). Annual rates of CIMT change were determined from mixed models. Models adjusted for study center, demographics, current smoking, and use of statins or antihypertensive agents and included PIO therapy, scan time, and MS. Interactions tested whether the effect of PIO on CIMT progression differed according to the presence of MS; in separate models within strata defined by presence or absence of MS, interactions tested whether PIO altered CIMT progression. Similar models were investigated for individual MS components. The effect of PIO on CIMT progression differed according to the presence of MS (p<0.05), central obesity (p<0.025) and hypertriglyceridemia (p=0.002). PIO reduced CIMT progression only in those with MS, central obesity or hypertriglyceridemia (p<0.001 for all, see Table) but not in those without those conditions. Strikingly, CIMT progression was absent in those with hypertriglyceridemia randomized to PIO. These data support the notion that the risk benefit ratio for PIO therapy to prevent atherosclerosis in persons with impaired glucose tolerance may be most favorable for those who have metabolic syndrome or central obesity and most particularly so for hypertriglyceridemic individuals.

	Annual Rates of CIMT Progression (mm/year, mean±SE)			
MS/MS Component	MS/MS Component Absent		MS/MS Component Present	
	Placebo	Pioglitazone	Placebo	Pioglitazone
MS	0.0075±0.0021	0.0088±0.0021	0.0090±0.0010	0.0039±0.0011
Central Obesity	0.0057±0.0023	0.0085±0.0022	0.0093±0.0010	0.0042±0.0011
Hypertriglyceridemia	0.0078±0.0012	0.0068±0.0011	0.0104±0.0015	0.00035±0.0017

Supported by: Takeda Pharmaceutical Co.

38-OR

Risk of Cardiovascular Disease Associated with Sulfonylurea or Metformin Use in Older Patients with Type 2 Diabetes

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Sulfonylurea (SU) therapies have been associated with the potential for increased risk of cardiovascular disease (CVD), but remain a commonly used antihyperglycemic medication particularly in the elderly. This retrospective cohort study examined the potential association between initial monotherapy with SU or metformin (MET) and subsequent CVD in older patients (pts) with T2DM. A cohort of pts who were ≥65 yrs old with T2DM and received their first prescription (Rx) of SU or MET monotherapy between 2003 and 2007 and remained on it for at least 90 days were selected from the GE electronic medical record database. Pts also had to have no antihyperglycemic Rx and CVD events (ischemic heart disease [IHD], myocardial infarction, stroke, transient ischemic attack, and peripheral arterial disease) within the prior year (baseline) and also had at least 2 yrs of follow-up after first Rx. Logistic regression evaluated the likelihood of having a CVD event. Cox regression estimated time to first CVD event. Differences in baseline characteristics (demographics, clinical and lab measures, and comorbidities) were controlled for using propensity score matching (PSM). Overall, 8,502 pts were included with 4,251/group. Mean age was 75 yrs and 49% were males. While controlling for differences in baseline characteristics using PSM, pts who initiated with SU had a significantly (p<0.001) higher incidence of CVD events (12.4% vs. 10.4%) compared to those initiated with MET after 2 years of follow-up. The difference was mainly driven by the increased incidence of IHD with SU (7.2%) compared to MET (5.5%) (p=0.002). The likelihood of having a CVD event was higher in pts initiated with SU than with MET (OR [95% CI] = 1.23 [1.08, 1.41]; p=0.002). SU use was associated with shorter time to first CVD event compared to MET (HR [95% CI] = 1.15 [1.03, 1.28]; p=0.004). Sensitivity analyses with 1 or 3 yrs of follow-up yielded similar results. In a cohort of older pts with T2DM initiating antihyperglycemic therapy, the likelihood of experiencing a CVD event was higher and these events occurred sooner in pts who started with SU monotherapy than those who started with MET.

39-OR

Placental Growth Factor Is Associated with Incident Type 2 Diabetes but Not Cardiovascular Disease in Otherwise Healthy Women

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Placental growth factor (PIGF) is a broadly expressed proangiogenic cytokine facilitating both macrophage recruitment within atherosclerotic plaque and adipose tissue expansion. Thus, elevated levels of PIGF may signal a shared biology in development of cardiovascular disease (CVD) and type 2 diabetes (T2D). Prior clinical data in healthy populations are sparse. We assessed whether baseline PIGF level is associated with incident T2D or CVD among otherwise healthy non-diabetic women (baseline HbA1c <6.5%) followed in the Women's Health Study (median follow-up 12.3 years). Using a prospective case-cohort study design, baseline levels of PIGF were measured among cases of incident T2D (n=491), incident CVD (n=474; myocardial infarction, stroke and CVD death) and a subcohort (n=561) serving as a common reference risk set. Hazard ratios (HRs) were estimated from Cox proportional hazard models, with inverse probability weighting and variance adjustment for the case-cohort design. In models that adjusted for age and race (Table), a marked increase in T2D risk across quartiles of PIGF (HRs 1.00, 1.13, 1.46, 2.13, P-trend <0.001) was noted. This relationship persisted in models additionally adjusting for BMI and other T2D risk factors including HbA1c and high-sensitivity C-reactive protein. No such relationship was evident for CVD; age- and smoking adjusted HRs were 1.00, 1.02, 1.02, and 1.15 (p-trend=0.47). These prospective data, if confirmed, support a unique role of angiogenic factors in T2D development distinct from that for CVD.

For author disclosure information, see page 785.

	Q1	Q2	Q3	Q4	
PlGF, range (pg/ml)	< 15.4	15.4-17.6	17.7-20.3	>20.3	P-trend
HRs for Incident T2D					
No. Cases	110	113	132	136	
M1	1.0	1.13	1.46	2.13	<0.001
M2	1.0	1.21	1.47	1.63	<0.001
M3	1.0	1.16	1.31	2.38	0.003
M4	1.0	1.13	1.61	2.70	<0.001
HRs for Incident CVD					
No. Cases	104	106	114	150	
M1	1.0	1.02	1.02	1.15	0.47
M2	1.0	0.99	1.02	1.14	0.52
M3	1.0	0.99	1.02	1.14	0.53
M4	1.0	0.99	1.05	1.17	0.46

M1: Adjusted for age, race, and WHS randomized treatments (aspirin, vitamin E)
M2: M1 plus BMI, HTN, hyperlipidemia, smoking, family history of T2D (for T2D models) or premature MI (CVD models), HRT, and exercise.
M3: M2 plus HbA1c
M4: M2 plus hsCRP

40-OR

Maturity Onset Diabetes of the Young (MODY) Is Characterised by Less Thrombotic Fibrin Network Structure and Reduced Inflammatory Environment Compared with Phenotypically Similar Type 2 Diabetes (T2DM) Subjects

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Cardiovascular risk in monogenic diabetes has not been well-studied. It is known that fibrin network structure determines predisposition to atherothrombotic disease. We investigated clot structure/fibrinolysis and inflammatory markers in subjects with MODY due to hepatocyte nuclear factor 1 αlpha (*HNF1A*) mutations and compared with T2DM matched for age, BMI, gender and HbA1c, as well as a healthy controls.

Thirteen HNF1A-MODY (35.3±3.2 yrs), 12 T2DM (35.5±3.2 yrs) and 13 healthy controls (29.3±1.2 yrs) were recruited. Fibrin clot structure was measured using a turbidimetric assay and clot final turbidity (FT), a measure of clot density, and time from full clot formation to 50% lysis, an indicator of lysis potential were recorded. Plasma fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and the inflammatory proteins complement-3 (C3) and C-reactive protein (CRP) were assayed.

HNF1A-MODY subjects had lower FT than T2DM (0.37±0.03 and 0.49±0.03 au, respectively; p=0.03), and both were significantly higher than controls (0.26±0.02 au). A trend towards shorter clot lysis was evident in HNF1A-MODY compared with T2DM group (456±350 and 588±55 sec, respectively; p=0.09) with the controls having shorter lysis time than T2DM, but not MODY, subjects (428±25 sec, p=0.03). MODY and T2DM subjects had similar fibrinogen, plasminogen activator inhibitor (PAI)-1 and CRP plasma levels. However, C3 levels were lower in HNF1A-MODY compared with T2DM (0.58±0.09 and 0.80±0.1 mg/ml, respectively; p<0.01). C3, but not CRP, levels correlated with clot lysis time (r=0.62, p=0.03).

Subjects with T2DM have tighter, more resistant to fibrinolysis, clot structure compared with phenotypically similar HNF1A-MODY. Similar plasma levels of fibrinogen and PAI-1 suggest this is due to post-translational modifications in fibrinogen, or to yet unidentified plasma factors, and may involve C3, a protein known to affect clot structure and fibrinolysis. Our data indicate decreased thormbotic tendency in HNF1A-MODY compared with T2DM, and hence lower cardiovascular risk.

Supported by: Oxford NIHR BRC

HEALTH OUTCOMES AND HEALTH CARE DELIVERY INNOVATIONS

41-OR

Successful Implementation of a Large, Community-Based Comparative Effectiveness Trial of the Diabetes Prevention Program (DPP)

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The DPP showed that an intensive lifestyle intervention reduces the development of type 2 diabetes in high risk adults, but this intervention was not designed for dissemination. We present the baseline characteristics and intervention attendance levels for participants of an ongoing randomized comparative effectiveness trial of a lower cost adaption of the DPP (PLAN4WARD or P4W) that is offered in partnership with the YMCA.

This RCT compares P4W versus standard advice control for 510 adult patients of 9 community primary care practices in Indianapolis, USA. Participants are 18 years of age or older with BMI ≥ 24 kg/m^2 and one of the following: 1) fasting plasma glucose 100-125 mg/dL OR 2) A_{1c} 5.7-6.9%. The primary outcome is change in body weight at 12 months; secondary outcomes include changes in weight, blood pressures, A_{1c}, blood cholesterol, and costs through 24 months. Baseline descriptive statistics and 6 month intervention attendance counts are summarized.

To date, 478 participants have been randomized and completed baseline data verification. Mean age is 52 years; 71% are women and 57% are African American. 58% report at least one family member with diabetes and 73% report an annual household income of less than $25,000 US. Mean BMI is 37.0 kg/m^2 (SD 8.8), A_{1c} is 6.0% (SD 0.3), systolic blood pressure is 132 mmHg (SD 14.4), total cholesterol is 186 mg/dL (SD 39.7), and HDL-C 42.7 is mg/dL (SD 14.6). Among 179 P4W group participants who have completed data collection and verification at 6 months, 65.4% have attended at least one P4W session at the YMCA. Among P4W attenders, 70% completed at least 9 of the 16 "core" P4W sessions, with a mean visit completion of 11.4 (SD 5.1) sessions.

This large comparative effectiveness trial of DPP delivery by the YMCA has overcome challenges to community-based implementation and has successfully randomized a high-risk population of middle-aged adults who are predominantly of minority race/ethnicity, low SES, and higher BMI. Despite these challenges, participation in a community prevention program following referral from primary care is very encouraging.

42-OR

Screening Detects Highly Prevalent Undiagnosed Diabetes and Prediabetes in Veterans Receiving Primary Care, but A1c Misclassifies Patients

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Screening to detect unrecognized dysglycemia is recommended, but the best methods to use and "real world" yield from screening in primary care are unknown. Since the quality of primary care in the VA is high, we examined the use of A1c for opportunistic screening at VA primary care visits.

Screening was offered to patients meeting NIDDK/ADA guidelines: without known diabetes, and with age ≥45 yr or BMI ≥25 kg/m^2 with another risk factor. An OGTT identified hyperglycemia classified by ADA criteria, and A1c findings were evaluated according to International Expert Committee (IEC, prediabetes 6.0-6.4%, diabetes ≥6.5%), ADA (5.7-6.4% and ≥6.5%), and new VA/Dept of Defense (VA, 5.7-6.9% and ≥7.0%) guidelines.

The 680 subjects were 96% male, 73% black, and 26% white, with average age 58 yr and BMI 30. By OGTT, 11% had diabetes (DIAB) and 41% prediabetes (PRE). In patients with DIAB by OGTT, A1c classification was incorrect in 76% by IEC criteria, 76% by ADA, and 87% by VA. With PRE by OGTT, A1c classification was incorrect in 66% by IEC, 41% by ADA, and 36% by VA. With NL by OGTT, A1c classification was incorrect in 21% by IEC, 56% ADA, and 57% VA. Weighted misclassification with A1c testing (DIAB*2 + PRE*1 + NL*0.5) averaged 56% with IEC, 54% ADA, and 54% VA.

OGTT	IEC	ADA	VA/DoD
Diabetes: % Correct	**24%**	**24%**	**13%**
False Neg NL	37%	24%	18%
False Neg PRE	39%	52%	69%
Prediabetes: % Correct	**34%**	**59%**	**64%**
False Neg NL	62%	37%	35%
False Pos DIAB	5%	5%	1%
Normal: % Correct	**79%**	**44%**	**43%**
False Pos PRE	19%	54%	57%
False Pos DIAB	2%	2%	0%

Conclusions: Many veterans targeted by ADA guidelines have unrecognized dysglycemia, showing the need for screening. However, screening such patients by measuring A1c would result in major misclassification – missing disease when it is present, and mislabeling normals as having disease. Consideration should be given to alternative

For author disclosure information, see page 785.

strategies that can be used opportunistically (during outpatient visits, without a fast) but are more accurate.

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43-OR

The Patient Centered Medical Home Improves Diabetes Care

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The Patient Centered Medical Home (PCMH), a new model for primary care, is being proposed to address the demands of the chronically ill, including diabetes. Eleven PCMH demonstrations that report quality of care data for diabetes were identified and 3 were excluded due to small size (<10 practices). The most frequently identified interventions to transform to a PCMH were care coordination, payment reform, patient registry implementation, learning collaboratives, and practice coaching. Overall, there were significant improvements in both process and outcome measures related to diabetes. Results from large integrated health systems include 1) Group Health (WA) with a 2 year improvement in its bundled "Composite Quality Score" (51 to 58.6%) for 9200 patients and lower costs with higher provider satisfaction 2) Geisinger Health System (PA) with first year improvements in A1C < 7.0 (32.2 to 34.8%, p<0.001), blood pressure (BP) <130/80 (39.7 to 43.9%, p<0.0001) and the "Diabetic Bundle" (2.4 to 6.5%) (n=20,000) with documented cost savings 3) Health Partners (MN) improved in the Diabetes Bundle (4 to 25%) over a 4 year period. Larger state lead initiatives have shown early results: Pennsylvania (North-Eastern Region) absolute change in A1C > 9 (-5.9%), systolic BP < 130 (+11.5%), and LDL<130 (+11.5%). Also improvements in setting self management goals (+38%), foot examinations (+24.7%), eye examinations (+18.2%) and diabetic nephropathy screening (+12.8%); Rhode Island reported improvements in A1C<7 (33 to 40%), BP < 130/80 (18 to 40%), LDL<100 (27 to 42%) after 2 years; Colorado and North Carolina have both successfully met NCQA quality benchmarks. Potential cost savings were evident in the majority of demonstrations thanks to reductions in ER visits and inpatient hospitalizations. In summary, based on early results of several initiatives, the PCMH holds significant promise to improve diabetes outcome and reduce costs. As a result, there are currently over 41 active PCMH demonstration projects in the US.

44-OR

Laboratory Calculated Diabetic Nephropathy Prevalence Trends Since the Introduction of the UK Pay-for-Performance Policy

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The UK Quality and Outcomes Framework (QOF) is a pay for performance system for achieving evidence based targets in chronic disease in family practice. Since its introduction in 2004, screening rates for diabetes complications have significantly increased. The aim of this study was to estimate its impact on the observed prevalence of diabetic nephropathy (DN) using all diabetes screening results for an entire UK region.

All HbA1c and Albumin Creatinine Ratio (ACR) results were extracted from the regional laboratory database in two cohorts (2001-2) and (2004-6) – before and after the introduction of QOF. All tests were matched to individual patients and then diabetes and DN patients determined, using thresholds of HbA1c ≥ 6.1% and 2 or more ACR results ≥ 3 mg/mmol (microalbuminuria). Diabetes prevalence was calculated as a proportion of the entire population and DN prevalence as a proportion of the diabetes cohort determined from laboratory results.

In the pre QOF cohort, 38,109 adults (2.15% prevalence) were identified from their laboratory results as having diabetes mellitus, rising to 51,973 patients (2.84% prevalence) in 2006-7. Pre QOF, the mean ACR testing rate for the diabetes population was 33.6%, almost doubling to 57.5% after the introduction of QOF, a significant increase of 71% in the first year of QOF(p<0.05). After the first year of pay-for-performance, this initial increase in testing rate plateaued in subsequent years. The laboratory prevalence of DN increased from 6.1 to 21.2% over the same period, with a breakdown of 57.9% persistent microalbuminuria to 42.1% proteinuria.

Pay-for-performance in UK family practice initially increased ACR testing rates in diabetes in the first year but significant increases were not observed in successive years. The observed DN prevalence rates have trebled from 6.1 to 21.2% since its introduction thus highlighting increased case finding with the increased testing. This model of care has lessons for all countries with rising diabetes and CKD prevalence rates.

Supported by: Northern Ireland Kidney Research Fund and the Metabolic Unit Research Fund

45-OR

A Decade of Changing Prescribing Patterns of Novel Type 2 Diabetes Medications: An EHR-Based Evaluation

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Electronic health records (EHRs) provide an opportunity to assess clinical responsiveness to a 2007 FDA advisory report linking rosiglitazone (ROSI) to adverse cardiovascular outcomes. Since 2001, a large multi-disciplinary group practice implemented and customized an EHR that included prescribing data. We evaluated 10 years of clinical data to describe changing patterns for thiazolidinediones (TZDs, ROSI and pioglitazone [PIO]), as well as uptake of novel drug classes (OTHER) approved between 2001-2010; OTHER included pramlintide, sitagliptin, saxagliptin, exenatide, liraglutide, and colesevelam. Eligible patients had a diagnosis of type 2 diabetes (T2D) based on an algorithm using ICD-9 codes (250.x0 or 250.x2) and a field specifying diabetes type (option "type 2"); these sources have 90% concordance. Earliest start date was used if a medication was discontinued and later resumed. Validation included manual review of 60 random records. From 2001-2010, 7846 T2D patients had 10539 new prescriptions for ROSI, PIO and OTHER (Panel A). After 2007, ROSI and PIO prescriptions declined dramatically; by 2010, new ROSI and PIO prescriptions were at 8% and 46%, respectively, of peak levels. The relative proportion of ROSI, PIO and OTHER medications was 44%, 50%, and 6% in 2005 and 2%, 18%, and 80% in 2010 (Panel B). Our findings suggest responsiveness of clinicians' prescribing patterns over a decade of changing safety profiles and approval of novel T2D therapies. However, the rapid adoption of OTHER drugs in the absence of long-term safety data merits further evaluation.

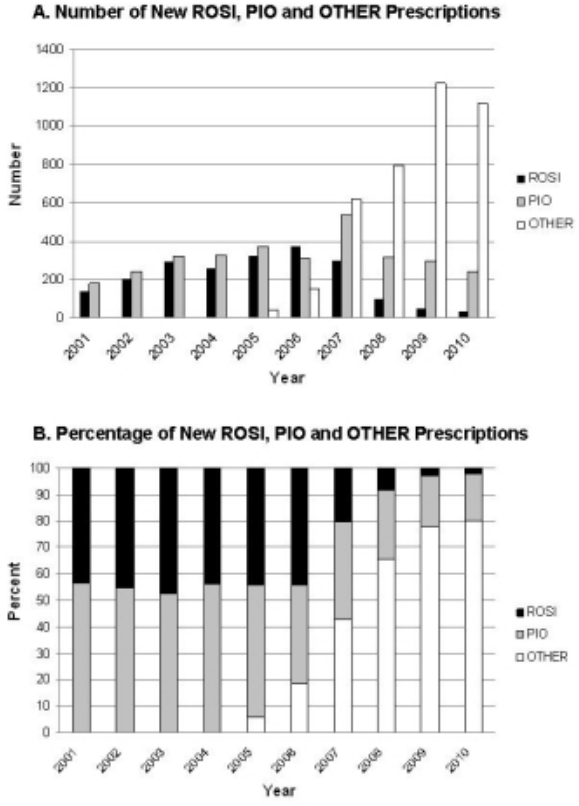

Figure. The numbers (Panel A) and relative percentages (Panel B) of new prescriptions of ROSI, PIO and OTHER from 2001-2010.

Supported by: Graetz Fund and the Diabetes and Endocrinology Research Center at Joslin

For author disclosure information, see page 785.

46-OR

Prioritization of Treatment and Its Consequences: Implications of Accountability Measures

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At a given office visit, well over half of adults with type 2 diabetes are above goal for glycemia, blood pressure, and lipids. A physician must often decide which clinical domain and action is most likely to lower the risk of complications. Physicians can prioritize treatment decisions at a given patient visit to: treat the condition that is (A) furthest from goal, or (B) closest to goal. Using a simulation model for diabetes management and a simulated population of 1000 patients (modeled on a real population of type 2 diabetes patients), we assessed the effect of these treatment strategies on long-term cardiovascular risk for patients.

We used a population percentile metric to determine the distance from goal for each clinical domain, e.g., if a patient is in the 95th percentile for SBP and 80th for LDL, SBP is assumed further from goal. We examined the impact of two diabetes treatment prioritization strategies, one that treats the condition with the highest percentile and the other the lowest percentile over 4 simulated encounters per year for 5 years. Outcomes assessed include (i) % of patients at goal, and (ii) 10-year cardiovascular (CVD) risk estimated using the UKPDS risk engine.

As shown in Table 1, rule (B) brings a higher percentage of patients to goal for blood pressure and lipids but not A1c. However, rule (A) achieves a greater reduction in projected CVD events.

Results indicate that, if physicians adopt strategies that target the clinical domain that seems easiest to achieve goal, it will maximize percent of patients at goal but may forego an opportunity to lower CHD risk. Diabetes accountability measures that incentivize providers to achieve thresholds for clinical goals, as opposed to lowering cardiovascular risk, may lead to unintended adverse consequences for patients with type 2 diabetes.

Table 1

		A:Furthest from Goal			B:Closest to Goal		
Outcome	Initial	Yr 1	Yr 3	Yr 5	Yr 1	Yr 3	Yr 5
Mean A1c (% *)	11.5	9.5(0)	8.0(6)	7.5(58)	10.5(0)	8.9(34)	8.0(57)
Mean SBP (% *)	171	155(0)	141(16)	136(50)	150(20)	142(60)	138(80)
Mean LDL (% *)	127	103(42)	93(57)	88(75)	106(72)	100(77)	90(84)
CVD Events **	508	368	292	267	394	325	288

*% at goal; **CVD events are based on 10-year risk.

47-OR

Effect of Intensive Versus Standard Blood Pressure Control on Health-Related Quality of Life in Type 2 Diabetes Mellitus: ACCORD Trial

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This study tested the hypothesis that among ACCORD participants with established type 2 diabetes, randomization to intensive (SBP < 130 mm Hg) versus standard (SBP 130-139 mm Hg) blood pressure (BP) control is related to significant improvement in health related quality of life (HRQL).

All ACCORD Blood Pressure HRQL sub-study participants who completed the baseline plus at least one of the 12, 36 or 48 month HRQL evaluations were included in this analysis. We used multivariate regression analysis to assess whether ACCORD BP treatment assignment (intensive versus standard BP control) was significantly related to baseline HRQL, or to change in HRQL from baseline to longest available follow-up time.

Among 1,028 eligible participants with analyzable data, we found no baseline differences in measures of HRQL between those in the intensive and standard BP treatment groups. At follow-up, 5 of 6 HRQL measures were no different between groups, but those assigned to intensive BP versus standard BP control had significantly worse SF36 Physical Component Scores (-0.8 versus -0.2; p=0.0195), with no variation across pre-specified patient subgroups.

Intensive control of BP in adults with type 2 diabetes did not affect, either positively or negatively, most pre-specified measures of HRQL. However, those randomized to intensive BP treatment had significantly worse physical function at the end of the trial. Further work is needed to elucidate the mechanism of this unexpected finding.

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48-OR

Major Depression Predicts Total Mortality in ACCORD Trial Participants

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Purpose: Depression affects up to 20-25% of adults with diabetes and has been shown to predict all-cause mortality in older patients with diabetes. However, few well-designed studies have examined effects of depression on composite CVD outcomes, macrovascular complications, or microvascular complications in those with type 2 diabetes.

Methods: The 2,053 participants in the ACCORD Health-Related Quality of Life (HRQL) investigation completed the 9-item depression measure from the Patient Health Questionnaire (PHQ-9) at baseline and at 12, 36 and 48 months. A score of ≥10 on the PHQ-9 has been shown to have 77% sensitivity and 94% specificity to the diagnosis of major depression by structured psychiatric interview. Cox proportional-hazards regression models were used to estimate hazard ratios and 95% confidence intervals for the time-varying impact of depression status on protocol-defined clinical endpoints with and without adjustment for age, gender, race/ethnicity, CHD and CHF status, HbA_{1c}, lipids, blood pressure, BMI, smoker, alcohol consumption, living alone, glucose, blood pressure and lipid medications, baseline microvascular complications, education, duration of diabetes, ACCORD clinical center, and all ACCORD study intervention arm assignments.

Results: In fully adjusted models, total mortality was significantly increased both in those with PHQ score of ≥10 (HR=1.84; 95% CI 1.17 to 2.89) and major depression (HR= 2.24; 95% CI 1.24 to 4.06). Major depression had a borderline impact on the ACCORD combined macrovascular endpoint (cardiovascular death, nonfatal heart attack or stroke, or congestive heart failure) (1.42; 95% CI 0.99 to 2.04). Major depression was not significantly related to the ACCORD primary composite outcome (cardiovascular death or nonfatal heart attack or stroke) (HR=1.53; 95% CI 0.85 to 2.73) or to the ACCORD microvascular composite outcome (HR=0.93; 95% CI 0.53 to 1.62).

Conclusion: Depression is a significant independent predictor of increased mortality and may increase risk of subsequent macrovascular events in adults with type 2 diabetes CV events. The impact of depression status on subsequent microvascular complications is less certain.

TREATMENT OF INSULIN RESISTANCE

49-OR

Identifying Candidate Genes for Thiazolidinedione-Induced Insulin-Sensitization Responsiveness by Expression Microarrays of Human Tissues

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Variable therapeutic efficacy is a shared feature of the thiazolidinediones (TZDs), a class of insulin sensitizers that includes troglitazone, rosiglitazone, and pioglitazone. In placebo-controlled studies, as many as 1/3 of TZD-treated insulin resistant patients do not improve their insulin sensitivity or HbA1c. In the everyday clinical setting, however, this non-responder population remains largely unidentified.

The purpose of this study was to find candidate genes that may harbor coding variants that determine variability in TZD-induced insulin sensitization. Subjects were 72 men and women, including both lean/obese and non-diabetic/type 2 diabetic individuals who were treated for 12 weeks with either troglitazone 600 mg/d, rosiglitazone 8 mg/d, or pioglitazone 45 mg/d. Before and after TZD treatment, hyperinsulinemic euglycemic clamp studies were conducted to measure insulin sensitivity, and muscle and fat biopsies were obtained to generate microarray expression profiles of >54,000 transcripts. Transcripts whose expression change correlated (positively or negatively) with insulin sensitivity change in insulin resistant subjects after TZD treatment were considered candidate genes for sensitization responsiveness. Insulin-sensitizing efficacy was similar for the three drugs. Candidate genes, i.e., those whose expression change was most strongly correlated with insulin sensitivity change (n~200), include CHST2 (r= 0.61, p<0.001) and PLXNA4 (r= 0.57, p=0.001) in adipose tissue, and TBC1D1 (r= -0.53, p<0.001), TBCA (r= 0.47, p=0.001) and DPH5 (r= 0.43, p=0.002) in muscle. At least one of these, TBC1D1, has biological plausibility as it encodes a protein associated with human obesity and inhibits GLUT4 translocation. Our future work aims to identify coding variants in our candidate gene set that correlate with insulin sensitization in response to TZD treatment. As pharmacogenomic markers, these variants could be used

For author disclosure information, see page 785.

to identify TZD non-responders prior to treatment and direct these patients to other classes of diabetes drugs, preventing wasted time, expense, and potential side effects.

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50-OR

A Targeting Inflammation Using Salsalate To Prevent Diabetes

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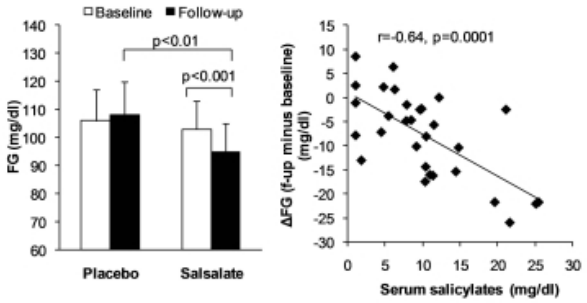
Chronic inflammation mediated by NF-κB may contribute to the pathogenesis of type 2 diabetes (T2D). Salicylates inhibit NF-κB activity and improve glycemia in T2D, although effects on insulin resistance are uncertain. 70 predominantly male veterans with IFG and/or IGT (mean age 58±8 yrs, BMI 32±6 kg/m^2, A1c 6.0±0.3%) were randomized in a double-masked 12 week trial of salsalate (SAL) up to 4.0 g/d vs placebo (P) to examine effects on glucose metabolism in "prediabetes". Baseline characteristics were generally similar between groups. The mean SAL dose was 3.7 ± 0.5 g/d (reduced for tinnitus in 32% SAL and 17% P treated patients). SAL significantly lowered fasting glucose (FG) by 8% (Figure) and total OGTT glucose area under the curve (AUC) by 5%, (p=0.05). Weight did not change during the study. No significant changes in fasting or AUC insulin, or AUC C-peptide occurred, although fasting C-peptide was reduced 20% (p<0.01), suggesting that FG was maintained in the setting of reduced insulin secretion. Notably, reductions in FG were strongly and inversely correlated (r= -0.64, p=0.004; Figure) with average serum salicylate levels. Glucose utilization (M) and insulin levels during euglycemic-hyperinsulinemic clamps were similar in SAL and P groups at baseline and did not change with therapy in either group; however, there was a modest positive association between end of study M values and salicylate levels. In summary, SAL lowers FG and C-peptide in patients with IFG/IGT, possibly indicating reduced beta cell burden. The modest effects on M suggest that improvement in FG may be due to reduced endogenous glucose production. Higher salicylate concentrations are associated with greater declines in FG, and may modestly enhance glucose utilization. Targeting inflammation may provide a new strategy for diabetes prevention.



Supported by: VA Clinical Sciences Research Program

51-OR

Peroxisome Proliferator Receptor delta Agonist Attenuates Hepatic Steatosis by Anti-Inflammatory Mechanism

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It is generally known that peroxisome proliferator-activated receptors (PPARs) involved in lipid and carbohydrate metabolism, inflammation, and cell differentiation. Currently, three genes in the PPAR family, PPAR-α, PPAR-γ, and PPAR-δ were discovered. Although PPAR-α (fibrate) and PPAR-γ (thiazolidinediones) have been used as chemical tools to uncover other biological roles for the PPARs, PPAR-δ has not been fully investigated. In this study, we examined the effects of the PPARδ agonist GW0742 on fatty liver changes in a type 2 diabetic rat model and HepG2 cells.

We investigated the effects of PPARδ agonist GW0742 on fatty liver changes in obese diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats and nondiabetic control Long-Evans Tokushima Otsuka (LETO) rats. Intraheptic triglyceride contents were investigated in the liver tissue of OLETF rats. Expression of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), monocyte chemo-attractant protein-1 (MCP-1), and peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α gene were evaluated in OLETF rats and HepG2 cells.

Rats treated with GW0742 (10 mg/kg/day) from 26 to 36 weeks showed low glucose levels, improved insulin sensitivity, and attenuated fatty infiltration of the liver. In liver tissues, mRNA expressions of TNF-α, MCP-1, and PGC-1α were significantly decreased in diabetic rats treated with GW0742 compared to diabetic control rats. We also observed that GW0742 had inhibitory effects on palmitate-induced fatty accumulation and inflammatory markers in HepG2 cells.

The PPARδ agonist (GW0742) may attenuate hepatic fat accumulation by anti-inflammatory mechanism and by reducing hepatic PGC-1α gene expression.

52-OR

Basal Insulin Secretion and Change from Baseline Predict Conversion from Impaired Glucose Tolerance to Type 2 Diabetes: Results from ACT NOW

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We examined metabolic characteristics that predict development of diabetes or reversion to NGT in persons with IGT participating in ACT NOW (n=602, FPG =105, 2-h PG [OGTT]=168 mg/dl), a randomized double-blind, placebo-controlled study to examine whether pioglitazone PIO (45 mg/day) can prevent/delay development of T2DM. Indices of insulin sensitivity (Matsuda index [MI]), insulin secretion (DI_{0-120}/DG_{0-120}), and beta cell function (DI/DG x MI) were derived from plasma glucose, insulin, and C peptide concentrations during the OGTT before randomization, at years 1 and 2 and at study end (median = 2.4 y). Dichotomous (NGT/IGT vs. T2DM and NGT vs. IGT/T2DM) and trichotomous (NGT, IGT, T2DM) logistic regression analysis were used to identify independent predictors of diabetes/reversion to NGT. 50 PLAC-treated subjects developed diabetes vs 15 PIO-treated subjects (odds ratio=0.27: 95% CI= 0.15-0.49; p<0.0001). 48% of PIO reverted to NGT versus 28% of PLAC (p<0.005). Higher baseline insulin secretion (OR [95% CI] = 0.76 [0.63-0.91] p=0.003) and higher baseline insulin secretion/insulin resistance index (IS/IR) (OR = 0.53 [0.44-0.65], p<0.0001) were associated with reduced risk of developing diabetes or remaining IGT. Baseline insulin sensitivity was not related to future diabetes risk. Improvement of each index was significantly associated (p<0.0001) with final glucose tolerance status: OR (95% CI)= 0.61 (0.54 – 0.80) for insulin sensitivity (MI), 0.61 (0.50 – 0.75) for insulin secretion (IS), and 0.26 (0.19 – 0.37) for beta cell function (IS/IR index). Trichotomous logistic regression of final glucose tolerance status predicted by change in index values (adjusted for baseline) provided an area under the receiver operating characteristic curve of 0.651 for IS, 0.652 for MI, 0.813 for IS/IR, and 0.820 for all indices combined. CONCLUSION: Improved in beta cell function (D IS/IR) is the greatest predictor of future glucose tolerance status. Addition of insulin secretion and/or insulin sensitivity to DIS/IR does not increase the predictive value.

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53-OR

Beneficial Effects and Signal Transduction Pathways of AdipoR1/AdipoR2 Activation in Muscle like Exercise, and in Liver, on Glucose/Lipid Metabolism and Anti-Inflammation

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The fat-derived hormone Adiponectin has been reported to produce a metabolic profile desirable for treating diseases of ageing such as type 2 diabetes and extend lifespan.

In skeletal muscle, by using muscle-specific AdipoR1 knockout mice, we showed that calcium signalling pathways as well as AMPK/SIRT1, and PGC-1alpha are principal modulators of pathways downstream of Adiponectin/AdipoR1, which increase mitochondrial content and function, and ameliorate insulin resistance and glucose intolerance, like exercise.

With regard to the liver, we show that hepatocyte-specific disruption of AdipoR1 results in increased molecules involved in gluconeogenesis, while hepatocyte-specific disruption of AdipoR2 results in decreased PPARalpha pathways such as decreased molecules involved in fatty-acid combustion including ACO, both of which are associated with insulin resistance.

We next try to identify and characterize orally active AdipoR agonists that are 1,000-fold more potent than adiponectin. One of these compounds, AdipoR agonist (ARA)-1 increases intracellular calcium concentration and PGC-1alpha, and also activates AMPK in C2C12 myocytes. On a high-fat diet,

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ARA-1 activates AMPK and increases molecules involved in mitochondrial biogenesis such as PGC-1alpha, Err alpha and Nrf1, molecules involved in fatty-acid combustion such as MCAD and oxidative stress-detoxifying enzymes in skeletal muscle, which are associated with increased insulin sensitivity, glucose tolerance and exercise endurance. In the liver, ARA-1 also activates AMPK and suppresses molecules involved in gluconeogenesis as well as activates PPARalpha pathways such as increased molecules involved in fatty-acid combustion including ACO. In white adipose tissues, ARA-1 suppresses MCP-1. Importantly, in AdipoR1 and AdipoR2 double knockout mice, all these effects are almost completely abolished.

These data suggest that ARA-1 may activate both AdipoR1 and AdipoR2, and also that orally active AdipoR agonists are promising new therapeutic approach for treating diseases of ageing such as type 2 diabetes as exercise-mimetics.

54-OR

PIOcomb Study: Combination Therapy of Pioglitazone with Insulin Glargine Improves the Composition of Lipid Subfractions in Patients with Type 2 Diabetes

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PPARγ agonists, such as pioglitazone, improve insulin sensitivity and glycemic control and appear to lower the concentration of atherogenic small dense LDL particles. We analyzed the efficacy of addition of pioglitazone (PI) (vs. metformin (MI) and vs. their combination (PMI)) to insulin glargine therapy in patients with type 2 diabetes on the LDL subfraction profile. In the double-blind, prospective PIOcomb study, 121 patients (47 women, 74 men, age: 63±8 yrs., disease duration: 11.1±6.2 yrs., BMI: 32.2±5.3 kg/m², HbA1c: 7.3±0.5 %) received an optimised and individualised insulin regimen with insulin glargine (titration) and were randomised to additional therapy with pioglitazone (2 x 15 mg/day) or metformin (2 x 850 mg/day), or a combination of both drugs. LDL subfractions were separated and analyzed by very-fast ultracentrifugation. Treatment with PI reduced the cholesterol concentration in the atherogenic LDL3 particles (density 1.040–1.066kg/l) in the PI-arm from 0.35±0.15 to 0.30±0.11 mmol/L ($p<0.05$) and with PMI from 0.30±0.18 to 0.27±0.11 mmol/L ($p<0.005$), while it increased with MI from 0.31±0.18 to 0.33±0.15 mmol/L (n.s.). The cholesterol content in the LDL1 particles, which are associated with reduced atherogenic risk, increased in both the PI arm (from 1.40±0.51 to 1.54±0.56 mmol/L, $p<0.05$) and the PMI arm (from 1.39 ±0.46 to 1.44±0.41 mmol/L, n.s.), while it decreased in the MI arm (from 1.60±0.60 to 1.43±0.51 mmol/L, $p<0.05$). The changes from baseline for LDL1 and LDL3 cholesterol content were significantly different between MI and PI (or PMI). Minor changes were also seen in the cholesterol content of the LDL2 subfraction (MI: +0.05±0.15 mmo/L; PI: -0.04±0.17 mmol/L ($p<0.05$ vs. MI); and PMI: -0.03±0.24 mmol/L). An increase in LDL1 and a decrease in small dense LDL2 and LDL3 have been related to a lower cardiovascular risk. Our data suggest that addition of pioglitazone to insulin glargine leads to a decrease in small dense LDL particles and modifies the LDL subfractions towards a less atherogenic risk profile.

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OBESITY—HUMAN

55-OR

Greater Metabolic Adaptation Despite Fat-Free Mass Preservation Following Dramatic Weight Loss Via Intensive Lifestyle Intervention Versus Bariatric Surgery

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Large weight losses often result in an undesirable decrease of fat-free mass (FFM). We hypothesized that an intensive lifestyle intervention incorporating resistance exercise will preserve metabolically active FFM and thereby attenuate the decrease in resting energy expenditure (REE) typically observed with weight loss. We compared changes in body composition (DXA) and REE (metabolic cart) in 13 pairs of subjects matched on sex (9 women, 4 men), body weight (135±28 kg vs.140±36 kg, P=0.68), BMI (47.0±7.6 kg/ m² vs. 47.6±9.5 kg/m², P=0.87), and age (39±9 yr vs.32±11 yr, P=0.14) that underwent either gastric bypass surgery (GB) or participated in "The Biggest Loser" television program (BL). Similar amounts of weight were lost 12 months post-surgery and after 7 months of lifestyle intervention (GB: 40.2±12.7 kg, BL: 48.8±14.9 kg; P=0.14). However, loss of FFM accounted for only 16±8% of the total weight loss in BL subjects compared to 30±12% for GB subjects ($P<0.01$). Baseline REE was higher in BL subjects than in GB subjects (2474±477 kcal/d vs. 2128±291 kcal/d, $P<0.05$); therefore, regression equations were developed that included terms for group and group*FFM interaction along with the usual independent variables of FFM, FM, age, and sex to predict the expected REE following weight loss.

The residual between the predicted and measured final REE defined the metabolic adaptation over and above the expected drop of REE due to weight loss. After weight loss, REE fell to similar levels in both groups (1857±463 kcal/d vs. 1832±196 kcal/d, P=0.83) because of a significantly greater metabolic adaptation in the BL group (409±96 kcal/d vs. 191±109 kcal/d, $P<0.001$). The degree of metabolic adaptation was correlated (r=0.531, $P<0.01$) with the magnitude of negative energy balance which was estimated by the average rate of change in FM over the weight loss period. In conclusion, despite significant preservation of FFM by intense exercise compared to bariatric surgery, REE was suppressed to similar levels due to greater metabolic adaptation which was related to the magnitude of negative energy balance.

56-OR

Striatal Dopamine Receptor Binding in Obese Women before and after Gastric Bypass Surgery

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In obese humans, a reduction in striatal D2 receptor(D2R) binding has been reported as well as a negative correlation between BMI and striatal D2R binding. It is not known whether this adaptation is a cause or consequence of the obese state. We hypothesized that inducing a hypocaloric metabolic state accompanied by weight loss would restore D2R in obese women undergoing bariatric surgery (Roux-en Y gastric bypass [RYGB]).

D2R SPECT studies were performed 2 weeks before and 6 weeks after RYGB using a brain-dedicated high-resolution scanner. 80 MBq of [^{123}I]IBZM was given intravenously as bolus, followed by continuous infusion of 20 MBq/h to achieve steady state regional brain activity levels. Acquisition of the images was started 2 h after the bolus injection. Dopamine receptor binding was expressed as the ratio of striatal to occipital binding (SOR), and compared within subjects.

We included 14 women (median age 37.6 [26-46] yrs, median BMI 47.3 [39-61] kg/m2). Compared to healthy historical controls (mean SOR 1.13 (0.75 – 1.78)), D2R was reduced pre-operatively significantly (mean SOR 0.83 (0.5-1.22)). Six weeks after RYGB, the median weight loss was 14 kg [8-24 kg] but SOR (0.81 (0.6-1.07)) did not change significantly.

Induction of a hypocaloric state and significant weight loss 6 weeks after RYGB in morbidly obese women did not upregulate striatal D2R binding. This finding does not support an important role for D2R in short-term changes in energy balance.

57-OR

Food Cues and Stress Preferentially Activate Limbic-Striatal Neural Pathways and Stimulate Food Craving in Obesity

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Obesity is associated with changes in neural motivation-reward pathways. These changes may be associated with altered food craving for highly palatable and calorie-dense foods, which could promote increased consumption of such foods. Stress may also increase food craving and consumption. We hypothesized that in response to stress and food cues obese as compared to lean individuals would have increased activation in motivation-reward brain regions and this activation would correlate with subjective food craving. We used functional magnetic resonance imaging (fMRI) to compare brain responses of obese (OB: BMI ≥30kg/m^2: N=25) vs. lean (normal weight) (NW: BMI 18.5-24.9kg/m^2: N=25) subjects during exposure to personalized food cue, stress, and neutral-relaxing situations using a validated, autobiographical, script-driven, guided-imagery paradigm. Food craving ratings were obtained to assess "desire" for a particular food with each imagery condition. In contrast maps of food cue vs. neutral-relaxing conditions, OB, but not NW, subjects had activation in the hypothalamus, putamen, insula, and thalamus ($p<.01$). In contrast maps of stress vs. neutral-relaxing conditions, OB, but not NW, subjects had activation in the putamen and insula ($p<.01$). In OB, but not NW, individuals food craving in the food cue condition correlated positively with activation in the thalamus, caudate, hippocampus, and parahippocampus ($p<.05$). In OB subjects, food

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craving in the stress condition correlated positively with activation in the caudate, putamen, insula, and parahippocampus; conversely, in NW subjects, food craving correlated inversely with activation in the caudate, putamen, and insula (p<.05). We conclude that in response to food cue and stress exposure, OB individuals have increased activation in limbic-striatal neural pathways; furthermore, activation in these regions is correlated with food craving. These findings suggest that food craving in OB individuals is linked to the activation of brain regions underlying motivation and emotion, and such activation may in turn contribute to over consumption of highly desirable foods.

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58-OR

Effects of Gatric Bypass Surgery on Glucose Metabolism Five Days and Three Months after Surgery in Subjects with Type 2 Diabetes and Normal Glucose Tolerance

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Roux-en-Y gastric bypass (RYGB) has profound effects on glucose metabolism very early after surgery leading to resolution of type 2 diabetes, however the mechanisms for this improvement remain uncertain.

In this study a group of 13 obese T2D (BMI: 43.1±1,4 kg/m2) and a group of 12 matched normal glucose tolerant (NGT) subjects were examined during a liquid meal 3 days before, 5 days and 3 months after RYGB. Three fasting blood samples were drawn before the meal (Fresubin Energy, 1260 kJ, 200 mL) followed by frequent blood sampling for 4 hrs.

In both groups fasting glucose (T2D: pre: 8.8±0.6, post: 7.0±0.3 p<0.01, 3 mths: 6.8±0.5 mM p<0.01; NGT 5.5±0.2, 5.0±0.2 p<0.01, 4.9±0.1 mM p<0.01) and insulin levels (T2D: 125±21, 73±9 p<0.01, 58±10 pM p<0.01, NGT: 82±8.2, 49±4 p<0.01, 43±4 pM p<0.01) decreased significantly compared to pre surgical levels. HOMA-IR was halved in both groups 5 days after RYGB (T2D: 6.6±1.0, 3.2±0.4 p<0.01; NGT: 2.9±0.3, 1.6±0.2 p<0.01), followed by a non-significant decrease by 3 months. Following meal ingestion, 120 min post prandial glucose levels decreased in T2D subjects (11.4±0.8, 8.2±0.7 p<0.01, 6.9±0.6 mM p<0.01), whereas levels were unchanged in NGT subjects immediately after RYGB but decreased by 3 months (5.6±0.3, 5.2±0.2, 4.2±0.3 mM p<0.01). IAUC C-peptide increased in both groups after RYGB, with a further increase in NGT after three months (T2D: 188±21, 254±28 p<0.05, 228±25 nM min; NGT: 168±17, 262±28 p<0.01, 329±41 nM min p<0.01). The insulinogenic index (IGI) increased after RYGB in T2D, but remained unchanged in NGT. Disposition index (IGI/HOMA-IR) increased in both groups with time from operation (T2D: 58±7, 170±32 p<0.01, 249±51 p<0.01; NGT: 415±68, 686±109 p<0.05, 848±107 p<0.01). GLP-1 iAUC were increased by a factor of 20 to 40 in both groups (T2D: 0.33±0.14, 6.0±1.0 p<0.01, 9.8±1.4 nM min p<0.01; NGT: 0.19±0.16, 7.8±1.2 p<0.01, 6.8±1.6 nM min p<0.01).

In conclusion, resolution of T2D after RYGB is explained by an early improvement of insulin sensitivity and insulin secretion. An exaggerated GLP-1 response may explain the potentiated insulin secretion after RYGB.

59-OR

Reduction in Plasma Concentrations of Endotoxin (LPS) and the Expression of Toll like Receptor-4 (TLR-4) and TLR-2 Following Roux-en-Y Gastric Bypass Surgery (RYGB) and Weight Loss

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We have previously demonstrated that the intake of a high fat high carbohydrate (HFHC) meal acutely induces an increase in plasma concentrations of endotoxin (LPS), and the expression of TLR-4 and CD-14 in peripheral blood mononuclear cells (MNC). This is associated with a comprehensive oxidative and inflammatory stress response. Since LPS and TLR-4 may have a role in the pathogenesis of insulin resistance, obesity and atherogenesis, we have now investigated whether the caloric restriction and the weight loss associated with RYGB results in the reduction of these inflammatory indices. Fifteen type 2 diabetic patients with morbid obesity underwent RYGB following which their caloric intake diminished and they lost 38.5±2.9Kg in weight over 6 months. BMI fell from 54.4±3.1 to 40.5±2.9Kg/m^2. There was a significant fall in plasma concentrations of glucose (from 148±8 to 101±4mg/dl), insulin (from 18.5±2.2 to 8.6±1.0mμU/ml) and HOMA-IR (from 7.1±1.1 to 2.1±0.3).There was a concomitant reduction in the plasma concentrations of LPS (from 0.567±0.033 to 0.443±0.022EU/ml, P<0.05). The mRNA expression in MNC of TLR-4 also fell by 25±9%, TLR-2 by 42±8% and CD14 by 27±10%. There was no significant change in MyD88 gene expression in MNC. In addition, there was a reduction in several pro-inflammatory mediators including CRP (from 10.7±1.6 to 5.8±1.0mg/L) and MMP-9 (from 492±42 to 356±26ng/ml). These data demonstrate that with the marked reduction in caloric and the weight loss that occur after RYGB there is marked reversal of the pro-inflammatory state which incorporates a comprehensive reduction in LPS and the facilitator receptor CD14 required for its ligation to its receptor, TLR-4. The reduction in the downstream signaling of LPS probably contributes to reversal of the inflammatory state and the restoration of insulin sensitivity as reflected in the reduction in plasma glucose and insulin concentrations and HOMA-IR.

Supported by: NIH

ADA-Funded Research

60-OR

Predictors of Hypoglycemia in Morbidly Obese Patients after Bariatric Surgery

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Postprandial hypoglycaemia is increasingly recognised as a potentially devastating complication of gastric bypass surgery. Since the predictors of hypoglycemia are widely unkown we performed a prospective study in a large cohort of patients with morbid obesity (MO). In total 789 patients with MO (mean BMI:43.8±9.6 kg/m^2; mean age:38 ±12 years; 80.7% females) were studied. In a longitudinal study 219 patients were evaluated before and 2 years after bariatric surgery. All patients underwent an oral glucose tolerance test (OGTT; 75g glucose) with measurements of blood glucose (BG) and insulin levels. Hypoglycemia was defined as a BG level ≤50 mg /dl. HOMA-insulin resistance was calculated.

Before surgery only 7 out of the 789 (0.9%) patients showed a hypoglycemic event during the OGGT. In patients included in the longitudinal study hypoglycemia was also rather low (0.9%) before surgery, but post-surgery 41 (18.7%) out of the 219 patients showed a post-challenge BG level ≤50 mg /dl (BG range 20-50mg/dl). Hypoglycemia was noted in 34% of patients after gastric bypass and in 18% after sleve gastrectomy, but in only 2% after gastric banding. Predicting factors in patients with hypoglycaemia versus those without were a greater change in BMI (15.0 vs 5.7 kg/m^2; p<0.001,) lower fasting levels for blood glucose (74±7 mg/dl vs 81±11 mg/dl; p<0.001), and insulin (7±3μU/ml vs 11±9μU/ml; p<0.001), but higher 1-h-post-challenge insulin values (155±103 μU/ml vs 94±81 μU/ml; p<0.001). Remarkably, HOMA-IR was significantly lower in the patients with hypoglycemia (0.2±0.3 vs 1.2±1.7; p<0.001)

This is the first prospective study indicating a much higher prevalence of severe hypoglycemia (18.7%) after bariatric surgery as previously assumed. The risk for hypoglycemia is particularly high in those patients with a greater weight loss associated with a low insulin-resistance state but still high post-challenge insulin levels. In conclusion, a systematic evaluation of glucose and insulin levels after an OGGT 2 years post-surgery may help to identify patients at increased risk for severe hypoglycemia.

61-OR

The Diagnostic Dilemma for Diabetes in Patients with Morbid Obesity

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The incidence of type 2 diabetes (T2DM) amongst patients undergoing bariatric surgery ranges from 15-23%. For the ongoing controlled outcome studies it is mandatory to use reliable criteria for the diagnosis of T2DM. In 2010 HbA1c was recommended for use as a diagnostic test for diabetes by the ADA. This is the first study comparing the different diagnostic criteria for diabetes in a large cohort of patients with MO.

In total 926 patients with MO (mean age 40±12 years) were included, of whom 14.2% (n=145) had a known T2DM and were therefore excluded. All patients with unknown T2DM (n=781) underwent an oral glucose tolerance test (OGTT; 75g glucose) and were included in our calculations. The thresholds of diagnostic criteria for diabetes were HbA1≥6.5%, fasting plasma glucose (FPG) ≥126mg/dl and 2 hour post-challenge glucose ≥200mg/dl. Insulin levels were assessed in the fasting state as well as post OGTT and HOMA-Insulin resistance (IR) was calculated. In addition, cardiovascular risk factors were evaluated in all patients.

The prevalence of undiagnosed T2DM was 6.9% (n=54) using the HbA1c criteria, 7.1% (n=55) using the 2-hour OGTT criteria ≥200mg/dl, but only 3.6% (n=28) using the FPG criteria. Remarkably, only 2.4% (n=19) of the patients had all three criteria for diabetes. Prevalence of the metabolic syndrome using the ATP III criteria was similar in patients diagnosed for diabetes by HbA1c (83%), FPG (79%) or 2 hour post-challenge glucose (87%) HOMA

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insulin resistance was 11±11 in the group defined by HbA1c, 11±7 in the 2 hour post-challenge glucose and 14±13 in the group defined by FPG.

This is the first study evaluating all three diagnostic criteria for diabetes in a large cohort of well defined patients with morbid obesity. In contrast to previous studies in patients without extreme obesity, the present studies indicate a significantly higher prevalence of diabetes when HbA1c or 2 hour glucose are used as diagnostic criteria in comparison with FPG indicating that the previously used FPG criteria underestimate the existence of diabetes in patients with morbid obesity.

62-OR

Diet Soft Drink Consumption Is Associated with Increased Waist Circumference in the San Antonio Longitudinal Study of Aging

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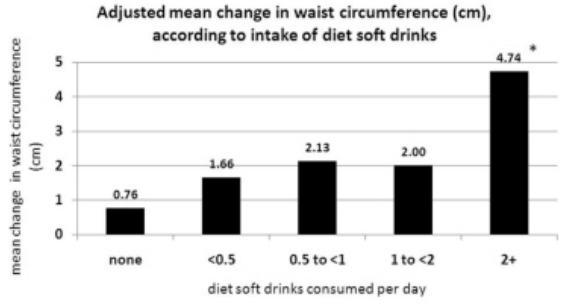
Consumption of diet soft drinks (DSDs) has been linked to increased incidence of obesity, metabolic syndrome, and diabetes. We examined the relationship between DSD consumption and long-term change in waist circumference (ΔWC) in 474 participants, aged 65-74 yrs at baseline, in the San Antonio Longitudinal Study of Aging (SALSA). Measures of height, weight, waist circumference (WC), and DSD intake were recorded at baseline and at each of 3 follow-up exams, for an average follow-up interval of 3.6 yrs (9.5 yrs total). Using repeated-measures ANCOVA, we compared mean ΔWC for DSD users vs. non-users in all follow-up periods, adjusted for sex; baseline WC, age, ethnicity, education, neighborhood, leisure physical activity, diabetes and smoking status; and length of follow-up.



* p<.001 vs. non-users

Overall, DSD users experienced 70% greater increases in WC compared with non-users: +2.11 ±0.33 cm vs. +0.78 ±0.24 cm, respectively ($p < 0.01$). A positive relationship emerged between DSD use and subsequent ΔWC ($p<0.001$ for trend). Point estimates for ΔWC were 63% higher in daily DSD users than in non-users, but this difference was not significant ($p=0.146$ for 1 to < 2 DSDs/day vs. none). Among frequent users (>=2 DSDs/day), however, mean increases in WC were 5 times greater than those in non-users ($p<0.001$).

WC is widely used as a proxy measure of visceral adiposity, a major risk factor for diabetes, cardiovascular disease, cancer, and other chronic conditions. These results suggest that – amidst the national drive to reduce consumption of sugar-sweetened drinks – policies which would promote the consumption of DSDs may have unintended deleterious effects. Data from this and other prospective studies suggest that the promotion of diet sodas as healthy alternatives may be ill-advised: they may be free of calories, but not of consequences.

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FOOT COMPLICATIONS

63-OR

The Association of Charcot Arthropathy with Postoperative Surgical Site Infections

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Surgical site infection (SSI), defined as a wound infection occurring within 30 days of surgery, is an inherent risk for all surgical procedures. This current prospective study aimed to determine if patients undergoing surgery on a Charcot foot (with and without diabetes mellitus) have an increased rate of SSI when compared with other foot and ankle surgical patients.

1423 consecutive orthopaedic foot and ankle surgical cases were prospectively evaluated with a standard data collection instrument. Of these, 77 (5.26%) involved Charcot feet (CF). Statistical analysis was performed and those variables which demonstrated significant associations with infection on univariate analysis ($p<0.05$) underwent multivariate analysis.

60 (78%) of the patients with Charcot foot had diabetes mellitus with a mean duration of 19±1.68 years. CF patients were significantly older than those without this deformity (55.9 ±1.17 vs. 47.74 ±.44 years, $P<0.000$). Significantly more men were affected as well (6.74% vs 4.3%, $P=0.041$). While the overall SSI rate in this study was 3.6%, 20.8% (16/77) of CF patients developed SSI compared with only 2.6% of non-Charcot patients ($P=0.000$). On univariate logistic regression, Charcot foot was found to impart a 10–fold risk for SSI (OR 9.8, 95% CI 5.15-18.69, $p<0.000$). Significant associations with SSI were found for both diabetic as well as non-diabetic Charcot patients. Of particular note, operating times were significantly longer in Charcot patients (143.89 ±9.08 vs 88.64 ±1.38 min, $p=0.000$). In the final multivariate analysis of the entire cohort, Charcot arthropathy, neuropathy, duration of surgery, and any smoking history all remained independently associated with SSI (OR=2.3, 3.6, 1.01, and 1.87, respectively). No amputations occurred in this cohort.

This study demonstrates a significant univariate and multivariate association between Charcot foot surgery and postoperative infection, irrespective of diabetes status. In this context, surgical management of this complex deformity (most frequently seen in diabetes mellitus) requires careful preoperative and postoperative evaluation to avoid potentially limb threatening complications.

64-OR

Comparison of Gastrocnemius Recession and Tendo-Achilles Lengthening in the Treatment and Prevention of Diabetic Forefoot Ulcerations

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Diabetes is the leading cause of non-traumatic foot amputations in the US. Foot ulcers are a major problem among diabetics, as they are a significant precursor for amputations. In fact up to 85% of amputations are preceded by foot ulcerations. Diabetics have the risk of limited joint mobility due to an Achilles-gastrocnemius-soleus contracture, a condition known as equinus, which further changes pressure distribution by increasing forefoot pressure. Two procedures, the tendo-Achilles lengthening (TAL) and the gastrocnemius recession, both of which are the focus of this study, can be utilized to decrease forefoot plantar pressure and subsequently decrease the risk of pedal ulceration. Despite the robust amount of research on effectiveness and uses of these procedures individually, there are no studies comparing the effectiveness between the two in the treatment of diabetic ulcers and effectiveness of preventing forefoot ulcerations in this patient population. The purpose of this study is to compare TAL to gastrocnemius recession for the treatment of forefoot ulceration with associated equinus in the diabetic population. A retrospective chart review of 100 charts was performed on patients that underwent either a TAL or gastrocnemius recession for diabetic forefoot ulcerations at Georgetown University Hospital. Patient information, comorbidities, healing times, return to ambulation times, and complications were noted for all patients. Of the charts reviewed, 64% of them had a TAL and 46% of them had a gastrocnemius recession. 80% of the patients with an ulceration that had a TAL healed their wounds, on average in 2.5 months. 45% of the patients with an ulceration that had a gastrocnemius recession healed their wounds on average in 4 months. Patients that underwent a TAL returned to ambulation on average one month after the procedure as compared to the patients with a gastrocnemius recession with an average return ambulation time of 5 months.

In conclusion, we found that patients that underwent a TAL had faster healing times and quicker return to ambulation times when compared to patients who underwent a gastrocnemius recession.

65-OR

Differences in the Coronary Calcium Score and Stenosis Comparing Health Subjects with Type 2 Diabetic Patients: The Prognostic Cardiovascular Role of Charcot Foot

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Type 2 diabetic subjects have an increased cardiovascular mortality. This elevated risk is linked to an accelerated vascular atherosclerosis, in particular coronary atherosclerosis. Coronary artery calcium (CAC) is an integral part of atherosclerotic coronary heart disease and there are several studies supporting the role of CAC score for prediction of myocardial infarction and cardiovascular mortality. The aim of our small cross-sectional study was to explore the differences in coronary calcium score (CCS, a marker of CAC) in four different population: 10 health subjects, 10 uncomplicated type 2 diabetic patients, 10 type 2 diabetic patients with autonomic neuropathy and 10 type 2 diabetic subjects with Charcot Osteoarthropathy. The

For author disclosure information, see page 785.

diagnosis of autonomic neuropathy was made with cardiovascular tests while Charcot foot was defined according to clinical signs and symptoms. CCS and CT Coronary Angiography were both performed by a 64-row multidetector CT scanner with ECG-gating. The three diabetic groups did not present significant differences about the variables known to influence coronary atherosclerosis (age, sex, disease duration, lipids, HbA1c, Waist-to-Hip ratio). Comparing diabetics with health controls, only HbA1c was significantly different (p<0.001). All Charcot and diabetic patients with autonomic neuropathy had a cardiovascular autonomic score >5 compared to controls and uncomplicated diabetics (all <2; p<0.001). With regard to total CCS, it was significantly higher in diabetic patients compared to controls (179 AS [112-862] vs 3.5 AS [3-4], respectively; p=0.009), without differences among the three diabetic groups. Notwithstanding this, the rate of Charcot patients with coronary stenosis >50% (clinically significant stenosis) was higher compared to no-Charcot patients (79 vs 52%; p=0.038). In conclusion, our study supports previous findings about CCS of diabetic subjects and underlines as Charcot Osteoarthropathy could be considered a prognostic marker of a more severe coronary atherosclerotic lesions. Further, larger and hard-points studies are necessary to confirm these data.

66-OR

Comparison of Transcutaneous Oxygen Tension after Autologous Stem Cell Therapy and Percutaneous Transluminal Angioplasty in Patients with Diabetic Foot Disease

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Cell therapy of peripheral arterial disease (PAD) could be effective in the treatment of diabetic foot disease (DFD) and is indicated mainly in patients with persistent ischemia after standard revascularization. A direct comparison of the effect of this new method and standard treatment by percutaneous transluminal angioplasty (PTA) is lacking. The aim of our study was to compare the effect of stem cell therapy and PTA on critical limb ischemia (CLI) measured by transcutaneous oxygen tension ($TcPO_2$) in patients with DFD.

Diabetic patients with CLI from our foot clinic treated by stem cell therapy (SCT group, n = 15) after unsuccessful standard revascularization or by angioplasty (PTA group, n = 21) between January 2008 and December 2009 were included into the study. CLI was defined by $TcPO_2$ < 30 mm Hg. 14/15 (93.3 %) patients in SCT group and 19/21 (90.5 %) patients in PTA group had a foot ulcer. Patients in SCT group did not significantly differ from patients in PTA group in mean age (63±7.8 vs. 63±11 years), gender (80 % vs. 75.6 % men), glycated hemoglobin (7.5±0.9 vs. 7.7±1.5 %) and in baseline $TcPO_2$ (10.7±8.5 vs. 14.7±8 mm Hg). Autologous stem cells were obtained from bone marrow or peripheral blood after stimulation by filgrastim and injected into muscles of affected lower limb. All patients were treated by standard podiatric methods. $TcPO_2$ was assessed after 6 and 12 months after the treatment.

$TcPO_2$ increased significantly from baseline in both SCT and PTA groups after 6 months (28.1±12.6 vs. 24.9±11 mm Hg; both $p < 0.001$) and after 12 months (28.9±8.1, $p < 0.01$ vs. 28.5±12.5 mm Hg, $p < 0.001$) with no significant difference between both groups. Number of healed patients up to 12 months was not significantly different between SCT group (9/14, 64.3 %) and PTA group (7/19, 36.8 %; p = 0.17).

Our study showed a comparable effect of stem cell therapy and PTA on CLI measured by $TcPO_2$ during a one year follow-up period. The question arising from our results is a revision of the indication for stem cell therapy in patients with less severe stages of PAD and DFD.

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67-OR

Offloading Adherence and Diabetic Foot Ulcer Healing

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While previous studies indicate that patient adherence to diabetic foot ulcer (DFU) offloading devices (OD) is low and may therefore result in delayed DFU healing, little empirical evidence exists to support the latter assumption. This study examined whether adherence to OD plays a role in DFU healing. Study participants (N=40) were type 2 DM patients (85% male; mean age 53 yrs) with active DFU (University of Texas Classification: 70% grade 1A; 17.5% 1B; 5% 2A; and 7.5% 2B). Adherence to offloading was assessed using a validated dual activity monitor method: a concealed activity monitor was attached to OD, and subjects were instructed to wear a second activity monitor at the hip. Activity data was uploaded to a centralized server via internet. The time stamped hip activity data was coded for compliancy using the synchronized OD activity data. DFU size was calculated in two ways, using: 1) ruler based: calculated as the manually measured length of the wound multiplied by the measured width and 2) Planimetric quantification of absolute DFU area using image analysis (image pro-plus software) of digital DFU photographs. DFU healing was defined as % reduction in wound size from baseline to 6 weeks. Initial findings indicate that OD were used during 62 ±18% of subjects' activity. The mean period of monitoring was 37±8 days, during which 13 DFU had completely healed. Multivariate regressions analyses controlling for the initial DFU size indicated that when DFU size was calculated by ruler measurements, the association between adherence and DFU healing was non-significant (beta= -.09; p=.44). However, when digital planimetry was used, there was a significant association between better adherence and faster healing (beta= -.40; p=.001) such that better adherence predicted more wound size reduction over 6 weeks. These preliminary findings 1) provide evidence that adherence to offloading predicts better DFU healing and 2) supports the need for more accurate methodologies than simple ruler measurements when assessing DFU healing.

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68-OR

Gait, Balance and Plantar Temperature Fluctuation in Charcot and Diabetes Patients with and without Active Foot Ulcer

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This study was performed to evaluate the impact of Charcot neuro-arthropathy (CN) on gait, dynamic balance and asymmetrical plantar temperature (PT) as a function of repetitive plantar stress. Fourteen CN (7 with active foot ulcer), 17 non-CN diabetic subjects with peripheral neuropathy (DPN) with (n=12) and without (n=8) active foot ulcer were recruited. A wearable technology was used to extract spatio-temporal parameters of gait. All subjects walked in the shod condition for two predefined routes of 50 and 150 steps respectively. Plantar foot thermal images were taken at baseline after foot acclimatization and immediately following each walking trial. A purpose-designed image processing toolbox was designed to extract plantar temperature in three anatomical regions of interest (ROI) including hind-, mid-, and forefoot. We estimated the 5^{th}, 50^{th}, and 95^{th} percentiles of measured temperature at each of the plantar regions. At baseline, CN patients demonstrated significantly higher temperatures in the clinical ROI (1.73±1.4°C ($p< 0.0001$)) compared to the contralateral as well as in all other ROI. No significant difference was observed in DPN subjects ($p>0.3$). In early steps, the plantar temperature in all subjects was reduced, but the drop in non-Charcot feet was significantly lower than Charcot feet. Interestingly, the plantar temperature in Charcot subjects *on both feet* was sharply increased after 200 steps while no difference was observed in non-Charcot subjects. Although, results suggest that both CN and foot ulcer alter gait velocity and gait steadiness, results were statistically significant only for gait steadiness values (64% and 300% increase in inter cycle gait variability respectively due to CN and foot ulcer, $p<0.05$). These results support modulating duration of continuous steps during daily activity could be helpful for reducing additional trauma in Charcot patients. Additionally, results suggest that both foot ulcer and CN increase the risk of falling by altering gait steadiness.

Supported by: Qatar National Research Fund

PHARMACOLOGIC TREATMENT OF DIABETES—INSULIN THERAPY

69-OR

Comparison of Human Hyaluronidase + Recombinant Human Insulin (RHI) vs. Insulin Lispro in a Basal-Bolus Regimen in Patients with Type 1 Diabetes (T1DM)

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Recombinant human hyaluronidase (rHuPH20) is FDA-approved to increase the dispersion and absorption of other injected drugs. rHuPH20 accelerates the exposure to and action of RHI and rapid insulin analogs. We compared glucose control in well-controlled T1DM using insulin lispro or RHI+rHuPH20 (INSULIN-PH20) as prandial insulin. After a 1 month run-in using bid insulin glargine and prandial lispro, 46 patients (age 42±13 years, BMI 26±4 kg/m^2, A1C=6.9±0.6 and 24 males) were randomized in an open-label crossover

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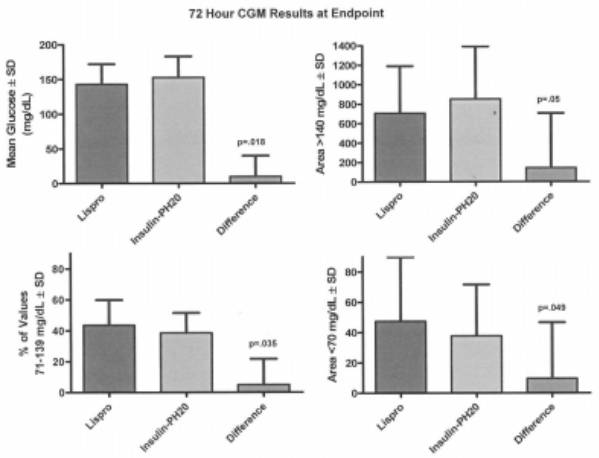
trial to INSULIN-PH20 or insulin lispro for 2 consecutive 12 week periods. Endpoint mean glucose excursion for 3 meals over 3 days for INSULIN-PH20 (17±36 mg/dl) was comparable to that for lispro (14±35 mg/dL) and met the prespecified primary endpoint noninferiority margin of 21.6 mg/dL. A1C was maintained for both groups: 7.0±0.5% for INSULIN-PH20 and 6.9±0.6% for insulin lispro, and met the commonly applied non-inferiority margin of 0.4% (upper 95% CI=0.23%). 72 hr continuous glucose monitoring (Figure) during the last 2 weeks of each treatment period showed similar mean glucose (153±30 vs 143±29 mg/dL), hyperglycemic excursions (Area>140mg/dL=852±539 vs 708±483 mg/dL/h), % of time in euglycemia (% of values 71-139 mg/dL=39±13 vs 44±16 %) and hypoglycemic excursions (Area≤70mg/dL=38±34 vs 47±42 mg/dL/h) for INSULIN-PH20 vs lispro, respectively.



Overall hypoglycemia (≤70 mg/dL) was 24.1vs 22.4 events per patient-month for INSULIN-PH20 vs lispro (NS). No significant changes in anti-insulin and anti-lispro antibodies were observed. Unlike commercially available regular human insulin, a formulation of RHI+rHuPH20 was comparable to insulin lispro for post-prandial glucose excursions with similar glucose control, safety and adverse event profiles in patients with type 1 diabetes.

70-OR

Insulin Degludec Improves Long-Term Glycemic Control with Less Nocturnal Hypoglycemia Compared with Insulin Glargine: 1-Year Results from a Randomized Basal-Bolus Trial in Type 1 Diabetes

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Insulin degludec (IDeg; former name SIBA), a new basal insulin, forms soluble multi-hexamers upon sc injection resulting in an ultra-long action profile. This 1-yr, open-label, treat-to-target trial compared the efficacy and safety of IDeg with insulin glargine (IGlar), both administered sc once-daily (OD) as basal-bolus (BB) treatment with mealtime insulin aspart. A total of 629 adults with type 1 diabetes (mean: 43.0 yrs, diabetes duration 18.9 yrs, A1C: 7.7%) treated with any BB insulin treatment for at least 1 yr were randomized (3:1) to IDeg or IGlar. Basal insulin was titrated to achieve FPG <90 mg/dl. A similar proportion of subjects completed the trial with IDeg (86%) and IGlar (87%). At 1 yr, IDeg and IGlar improved overall glycemic control (A1C) by 0.4% points (estimated treatment difference (ETD) IDeg-IGlar: -0.01% [95% CI: -0.14; 0.11]). A similar proportion of subjects achieved A1C <7% with IDeg and IGlar (40% vs. 43%, p=NS). Mean FPG was reduced by 23 mg/dl and 25 mg/dl for IDeg and IGlar, respectively (ETD: -5.9 mg/dl [95% CI: -18.6; 6.5] p=NS). The first time to meet titration target was shorter with IDeg (median of 5 vs. 10 wks, estimated hazard ratio: 1.37 [95% CI: 1.12; 1.67] p=0.002). Rates of nocturnal confirmed hypoglycemia (PG <56 mg/dl or severe episodes as per ADA definition) were 25% lower with IDeg (4.4 vs. 5.9 episodes/patient yr; estimated rate ratio (ERR): 0.75 [95% CI: 0.59; 0.96] p=0.021). Rates of overall confirmed hypoglycemia were similar for IDeg and IGlar (42.5 vs. 40.2 episodes/patient yr; ERR IDeg /IGlar: 1.07 [95% CI: 0.89; 1.28], p=NS). End-of-trial total mean daily insulin doses were 0.75 U/kg for IDeg and 0.82 U/kg for IGlar with an ~50:50 split of basal:bolus doses in both groups. Overall rates of adverse events were similar between groups, with no treatment-specific pattern or clustering. In conclusion, insulin degludec, given as basal-bolus treatment with insulin aspart in people with type 1 diabetes, improves long-term glycemic control with a significantly lower rate of nocturnal hypoglycemia compared with insulin glargine.

71-OR

Plasma Concentrations of Insulin Glargine and Its Metabolites after S.C. Injection of Glargine in Subjects with Type 1 Diabetes

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In vitro, glargine (GLA) exhibits higher affinity for IGF-1 receptors (IGF1R) than human insulin (HI), in contrast to its M1 and M2 metabolites that exhibit lower affinity. After s.c injection in vivo, GLA is enzymatically transformed into M1 and M2 with loss of the two arginines at B30a-30b but retention of the glycine for asparagine substitution at A21. Due to technical constraints few data exist on plasma concentrations of GLA, M1 and M2 after s.c. injection of GLA in humans. GLA, M1 and M2 plasma concentrations were determined from samples taken in a single-center, randomized, euglycemic glucose clamp trial where 12 male subjects with type 1 diabetes (BMI 25 kg/m^2; A1C < 8.0%) per group received single s.c. doses of 0.3, 0.6, or 1.2 U/kg GLA and were studied for >24 h. GLA, M1 and M2 were extracted using immunoaffinity columns and quantified by a specific LC-MS/MS assay, without cross-reactivity to endogenous HI or other insulins. LLOQ was 200 pg/ml. The areas under the GLA, M1 and M2 curves (PK-AUC$_{0\text{-}24h}$) were determined. The pharmacodynamic (PD) effect was determined from the AUC under the glucose infusion rate curve (PD-AUC$_{0\text{-}24h}$). GLA and M2 were detectable in only one third of subjects and at only a few time points. When detectable, GLA and M2 concentrations did not increase with increasing dose, and remained far below interprandial plasma insulin concentrations of nondiabetic subjects. M1 plasma concentration increased with increasing dose; geometric mean PK-AUC$_{0\text{-}24h}$ [%CV] was 7 [61], 18 [75], and 23 [24] ng·h/ml at doses of 0.3, 0.6, and 1.2 U/kg, respectively. The geometric mean PD-AUC$_{0\text{-}24h}$ [%CV] was 522 [64], 2330 [37], and 5231 [28] mg/kg, respectively.

After s.c. injection of GLA in subjects with T1DM, there was a rapid and dose-independent nearly total transformation of GLA into M1 that accounted almost totally for the PD effect of injected GLA. In vivo exposure to GLA, if any, appeared to be marginal. The rapid metabolism of GLA *in vivo* to active products that have lower affinity for IGF-1R than HI suggests it is highly unlikely that GLA *in vivo* binds to or activates IGF-1R or DNA synthesis more than HI.

Supported by: sanofi-aventis

72-OR

Early Basal Insulin Therapy Prevents New-Onset Diabetes after Transplantation by Improving Endogenous Insulin Secretion

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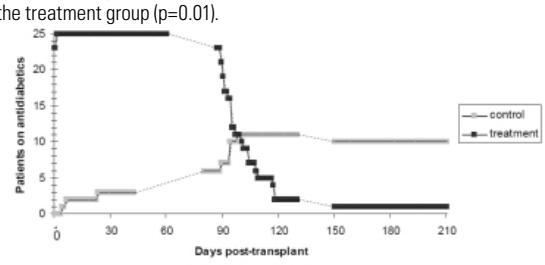
We tested efficacy and safety of early basal insulin therapy for post-transplant hyperglycemia and evaluated the development of new-onset diabetes after renal transplantation (NODAT). Fifty non-diabetic patients were randomly assigned to receive standard of care or basal insulin isophane treatment. HbA1c and OGTTs were obtained at 3 and 6 months post-transplant. Twenty-three of the 25 control patients had postoperative blood glucose >200 mg/dL, 18 required intermittent treatment with insulin and/or oral hypoglycemic agents. All 25 treatment patients had glucose levels >140 mg/dL by day 2, received and were discharged with insulin. Hypoglycemia of 41-60 mg/dL occurred once in the control versus 5 times in the treatment group (p=0.105), but was clinically mild. At 3 months, 5 control versus 2 treatment patients required antidiabetic therapy. NODAT was diagnosed by need for antidiabetic therapy or OGTT in 13 control versus 7 treatment patients (OR 2.8, p=0.083). HbA1c was 6.2±0.7% in the control versus 5.7±0.6% in the treatment group (p=0.005, primary endpoint). At 6 months, 10 control versus 1 treatment patients required antidiabetic therapy. NODAT was diagnosed in 13 control versus 3 treatment patients (OR 7.9, p=0.002). HbA1c was 6.3±0.7% in the control versus 5.8±0.6% in the treatment group (p=0.01).



		any antidiabetics	no antidiabetics	insulin alone	gliclazide alone	insulin + gliclazide	insulin + glimepiride
at discharge from ward	ctr	3	22	0	2	1	0
	treat	25	0	25	0	0	0
at 3-months OGTT (postop day 77-128)	ctr	5	20	0	1	4	0
	treat	2	23	2	0	0	0
at 6-months OGTT (postop day 170-209)	ctr	10	15	1	5	3	1
	treat	1	24	1	0	0	0

Solid lines represent patients on antidiabetic therapy. Dotted lines reflect periods of changes in treatment.

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Insulin resistance was similar in both groups at 3 and 6 months. Insulin secretion was significantly higher in the treatment group in comparison to the control group at 3 months, and remained at the same level in the treatment group at 6 months.

	3 mo	6 mo
HOMA: ctr	2.2±1.5	1.5±0.8
tr't	1.9±1.0	1.6±0.7
p	0.42	0.72
Ins'genic ind [nmol/mmol]: ctr	0.03±0.02	0.03±0.02
tr't	0.05±0.03	0.05±0.04
p	0.01	0.11

In conclusion, basal insulin treatment immediately following renal transplantation effectively prevents NODAT by providing beta cell rest.

73-OR

Time Course of Fasting Glucose, Hypoglycemia and Body Weight during Systematic Insulin Dose Titration: BID Aspart Premixed vs Glargine +1 Prandial Glulisine or Stepwise Addition of Glulisine to Glargine in Type 2 Diabetes Uncontrolled with Oral Agents

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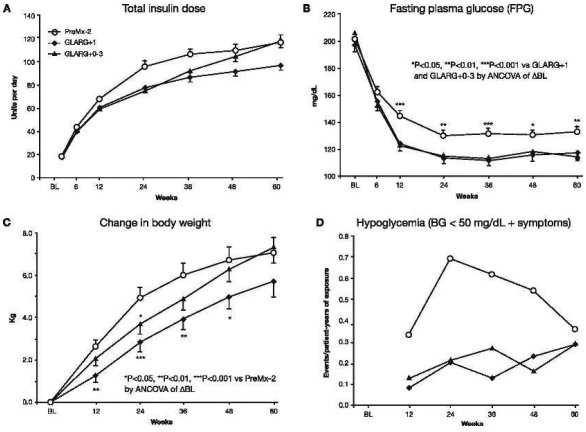
Several insulin regimens are proposed for uncontrolled type 2 diabetes patients (pts) on oral agents. This 60-wk randomized open-label study compared glycemic and non-glycemic effects of adding BID premixed biaspart 70/30 insulin (PM-2, n=192), basal insulin glargine+1 prandial insulin glulisine (G+1, n=189), or stepwise addition of prandial glulisine (G+0-3, n=191). Mean baseline A1C= 9.4% after 4-wk run-in on 2-3 oral agents; age 54 y, diabetes duration 9 y, BMI 33.2 kg/m^2. Insulin was titrated seeking fasting and preprandial glucose <100 mg/dL. At Wk 60 ΔA1C was -2.2%, -2.3% and -1.8% with G+1, G+0-3 and PM-2. Time courses of insulin dose, fasting glucose (FPG), body weight (BW) and confirmed symptomatic hypoglycemia <50 mg/dL (HYPO50) are shown below.



PM-2 insulin dose escalated earlier than basal/prandial regimens: by Wk 60, insulin units/d (A) were similar for PM-2 and G+0-3 and lower with G+1. FPG was similar with G+0-3 and G+1 and lower than PM-2 from Wk 12-60 (B) despite identical FPG goals. BW (C) increased in parallel with insulin dose and was similar among groups at Wk 60, despite less weight gain with G+1 or 0-3 than PM-2 in earlier wks. HYPO50 (D) had no relationship to insulin dose, FPG, or BW and was greatest with PM-2. Rate ratios vs PM-2 for HYPO50 were 0.43 (P<0.001) for G+1 and 0.46 (P<0.001) for G+0-3. More pts on PM-2 (27%) than G+0-3 (19%) or G+1 (21%) discontinued early. Conclusion: comparing 3 insulin regimens, BW increased in parallel with insulin dose; however, hypoglycemia was less frequent and decreases in FPG greater with glargine + prandial glulisine than premixed insulin, regardless of dose or duration of therapy.

Supported by: sanofi-aventis US

74-OR

Insulin Degludec Improves Long-Term Glycemic Control with Less Nocturnal Hypoglycemia Compared with Insulin Glargine: 1-Year Results from a Randomized Basal-Bolus Trial in People with Type 2 Diabetes

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Insulin degludec (IDeg; formerly named SIBA), a new basal insulin, forms soluble multi-hexamers upon sc injection resulting in an ultra-long action profile. This 1-yr, open-label (due to different insulin pen devices), treat-to-target trial compared the efficacy and safety of IDeg with insulin glargine (IGlar), both administered once daily in basal-bolus treatment with mealtime insulin aspart (IAsp) ± metformin ± pioglitazone. 992 subjects (mean: 58.9 yrs, diabetes duration 13.5 yrs, A1C 8.3%, FPG 166 mg/dl) with type 2 diabetes and A1C 7-10% after ≥3 months of any insulin regimen ± OAD(s), randomized (3:1) to IDeg or IGlar, were analyzed. Basal insulin was titrated to FPG <90 mg/dl. A similar proportion of subjects completed the trial with IDeg (83%) and IGlar (85%). At 1 yr, IDeg and IGlar improved overall glycemic control (A1C) by 1.2% and 1.3% points, respectively (estimated treatment difference (ETD) IDeg-IGlar: 0.08% [95% CI: -0.05, 0.21]). In both groups, 50% of subjects achieved A1C <7% (p=NS). FPG was reduced by 43 mg/dl with IDeg and 38 mg/dl with IGlar (ETD: -5.2 mg/dl [95% CI: -11.7, 1.1], p=NS). Rates of nocturnal confirmed hypoglycemia (defined as episodes with PG <56 mg/dl or considered severe per ADA definition, occurring between 00:00-05:59) were 25% lower with IDeg compared with IGlar (1.4 vs. 1.8 episodes/patient-yr; estimated rate ratio (ERR): 0.75 [95% CI: 0.58; 0.99] p=0.0399). Similarly, rates of overall confirmed hypoglycemia were lower with IDeg than IGlar (11.1 vs. 13.6 episodes/patient-yr; ERR IDeg/IGlar: 0.82 [95% CI: 0.69; 0.99], p=0.0359). At 1 yr, total mean daily insulin doses were 1.46 U/kg and 1.42 U/kg in the IDeg and IGlar groups, with an ~50:50 basal:bolus split for both groups. Rates of adverse events were similar between groups, with no treatment-specific pattern or clustering. In conclusion, insulin degludec, given as basal-bolus treatment with insulin aspart in people with type 2 diabetes, improves long-term glycemic control with significantly lower risk of overall and nocturnal hypoglycemia compared with insulin glargine.

75-OR

Time-Action Profile of Oral Enteric Insulin in Comparison with Subcutaneously Injected NPH Insulin in Healthy Volunteers

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Oral administration of insulin has the potential advantage of a more physiological action by its direct effect on hepatic glucose production. In this study, bio-adhesive calcium phosphate embedded insulin nano-particles with enteric coated capsules were used to facilitate gastrointestinal insulin absorption and overcome gastric acid damage. The aim of the study was to evaluate the pharmacodynamic profiles and duration of action for three oral doses of insulin (50,100 and 200 IU) and one subcutaneous dose of NPH insulin (6 IU). This single-center, randomized, four-period, cross-over study was carried out under euglycemic clamp conditions in 12 healthy volunteers. The result showed that administration of either NPH or enteric insulin capsules increased GIRs. The onset of action (Teffect) of insulin capsules was 38±10min (50 IU), 41±18 min (100 IU), 65±58 min (200 IU), which were comparable with NPH (35±8min, p> 0.05). The time for reaching a maximum values of enteric insulin capsules was 250±118 min(50 IU), 170±58 min (100 IU), 236±132 min (200 IU), respectively, versus 243±79 min for NPH (p> 0.05). The maximal metabolic activity (GIRmax) observed of enteric insulin capsules was lower compared with NPH (GIRmax 1.66±0.50, 1.61±1.00, 1.80±060, respectively, vs. 2.06±0.82mg/kg/min). The metabolic effect measured over 10 hours tended to be lower with enteric insulin capsules compared with NPH insulin (GIR-AUC0-600 min 428±269, 457±254, 421±332, vs. 658±405mg/kg). The relative effectiveness of enteric insulin capsules was 37.0±90.0, 13.0±27.2, 11.0±28.8 respectively when compared with NPH insulin. No dose-response relationship in the absorption and metabolic effect of the enteric insulin capsules was observed among 50IU, 100IU, and 200IU capsules. No safety concerns arose from this short-term study. We conclude that the oral enteric insulin capsules showed a similar time-action profile as NPH insulin but a rather high between-subject variability in absorption. Administration of oral enteric insulin demonstrated an obvious hypoglycemic effect with only a small increase in circulating plasma insulin concentrations.

For author disclosure information, see page 785.

76-OR

Metabolic and Mitogenic Signaling of AspB10 and Insulin Glargine In Vitro and In Vivo

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Treatment of diabetic patients with insulin analogs has been shown to provide a more efficient, reproducible, and convenient therapy than regular insulin. The analogs may vary from insulin with respect to metabolic potency, stability or onset and duration of action that is achieved by either sequence or secondary structural modifications. Since these changes may lead to an altered activation profile of insulin (IR) and/or IGF-1 (IGF1R) receptor signaling pathways and may change metabolic or mitogenic responses, a careful investigation of acute and long-term effects of insulin analogs has been a major focus.

[Asp^{B10}]Insulin (AspB10) is an insulin analog that was withdrawn from clinical development due to a higher incidence of breast cancer in rats. In vitro, AspB10 displays higher affinity toward both IR and IGF1R, a prolonged occupancy time at the IR and a higher proliferation rate in mammalian cell lines. This has led to the contention that insulin analogs with increased IGF1R affinity in vitro have increased growth promoting activity in vivo as well.

Insulin glargine (GLA) has an in vitro IR signaling and metabolic profile comparable to that of insulin (HI) and displays slightly greater IGF1R affinity. This long-acting analog undergoes rapid and significant metabolism in humans and animals, leading to early formation of two main metabolites (M1 and M2) that have in vitro metabolic and mitogenic profiles comparable with HI.

After injection of 1 or 12.5 U/kg subcutaneously in rats, neither HI nor AspB10 nor GLA induced IGF1R autophosphorylation in responsive tissues, whereas IGF1 injection produced a robust activation of the receptor. AspB10 induced an increased and prolonged phosphorylation of IR signaling molecules in several tissues. This ex vivo IR signaling pattern of AspB10 is distinctly different from that of HI and GLA and confirms earlier in vitro findings.

Therefore, we hypothesize that the carcinogenic effect of AspB10 is based on its altered IR activation profile and is independent of its slightly greater IGF1R affinity in vitro.

Supported by: sanofi-aventis

EPIDEMIOLOGY OF TYPE 1 DIABETES

77-OR

Misclassification of Type 1 Diabetes in a Large Pediatric Medicaid Population and Its Potential Clinical Implications

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The increasing prevalence of overweight and obesity is changing the demographics and manifestations of pediatric diabetes. The overlapping clinical picture between type 1 (T1DM) and type 2 (T2DM) diabetes in children could lead to confusion and inappropriate treatment. Our objective was to characterize rates of initial misclassification of T1DM as T2DM in a large cohort of children and adolescents; and to examine the impact of this misclassification on risk of diabetic complications. We analyzed a 10-year dataset (1996-2006) for medical and pharmacy claims to identify a cohort of patients age ≤17 enrolled in the South Carolina State Medicaid Program who had initial service encounter with an ICD-9 diagnosis of T2DM. We also evaluated ICD-9 codes for comorbid metabolic conditions such as obesity, dyslipidemia, and hypertension, and for vascular and other complications such as diabetic ketoacidosis (DKA). Of 4070 subjects who had a median follow-up of 7 years, 2489 (61%) maintained a diagnosis of T2DM, while more than one-third, 1581 (38.8%), were later reclassified as T1DM. After accounting for follow-up time, older age at diagnosis was associated with an increased risk (aOR 1.66; 95% CI 1.55-1.77) while obesity with a decreased risk (aOR 0.79; CI 0.68-0.91) of misclassification. The misclassified group was at significantly higher risk of developing neuropathic (aOR 3.75; CI 2.42-5.80) and renal (aOR 1.27; CI 1.05-1.54) complications. After controlling for duration of diabetes, those in the misclassified group had an almost 50 times higher risk of having at least one incidence of DKA (aOR 49.5; 95% CI 35.4-69.3). In conclusion, changes in pathophysiology and clinical picture of diabetes in children are impacting its manifestation, diagnosis, and management. There is a high rate of T1DM being misdiagnosed as T2DM early in management, increasing the chances of life-threatening, but potentially preventable, acute complications such as DKA. These findings have implications for patient care, health care utilization costs, the Medicaid program, and for society in general.

78-OR

Improvements in the Life Expectancy of Type 1 Diabetes: The Pittsburgh Epidemiology of Diabetes Complications Study

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Mortality in type 1 diabetes (T1D) has decreased over time, but formal estimates of improvement in life expectancy are lacking for T1D in the United States. We therefore estimate the all-cause mortality experience and life expectancy of the Pittsburgh Epidemiology of Diabetes Complications (EDC) study cohort and quantify improvements by comparing two subcohorts based on year of diabetes diagnosis (1950-1964, n=390 vs. 1965-1980, n=543).

Cumulative all-cause age-specific mortality was examined using Kaplan-Meier curves and the subcohorts were compared using the log-rank test. Abridged life tables were constructed and life expectancy was calculated using the cohort approach.

Chiang's maximum likelihood formula was used to estimate life table parameters for incomplete segments. Mortality was found to have declined significantly in participants diagnosed with childhood-onset T1D in 1965-80 compared to those diagnosed in 1950-64, with 30-year mortality being 11.6% and 35.6%, respectively ($p<0.0001$). Likewise, the life expectancy at birth for those diagnosed 1965-80 was estimated to be 68.8 years, approximately 15 years greater than that of participants diagnosed 1950-64 (53.4 yrs, $p<0.0001$), while an increase of less than 1 year was seen in the general US population over the same time period. This EDC 1965-1980 diagnosis cohort life expectancy is approximately 4 years less than that estimated for a comparable cohort of the US general population (72.4 years), while the 1950-1965 EDC diagnosis cohort had a life expectancy ~18 years less than the comparable US general population (71.5 years). The estimated 15 year improvement in life expectancy between the T1D diagnosis cohorts persisted regardless of sex or pubertal status at diagnosis of diabetes. Life expectancy for this Pittsburgh, hospital-based, childhood-onset T1D population has improved dramatically and the gap in survival between T1D and the US general population appears to be rapidly diminishing.

79-OR

Incidence of Multiple Autoimmune Phenotypes in a Population at High Risk of Type 1 Diabetes (T1D)

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HLA-DR3,DQ2 and DR4,DQ8 haplotypes are associated with multiple autoimmune diseases. However, little is known concerning the overall autoimmunity burden among children with these HLA markers.

The Diabetes Autoimmunity Study in the Young (DAISY) has followed for an average of 8 years 2542 children at risk for T1D, recruited by newborn screening for HLA DR3,DQ2 or DR4,DQ8, or as relatives of T1D patients. Participants were tested annually for autoantibodies to glutamic acid decarboxylase 65 (GAD), insulin (IAA), insulinoma associated antigen-2 (IA2) and transglutaminase (TG). The most recent sample was measured for cyclic citrullinated peptide (CCP), thyroid peroxidase (TPO), zinc transporter 8 (ZnT8) and 21-hydroxylase (21-OH); if positive, the entire subject sample series was tested. GAD, IAA, IA2 and ZnT8 are grouped as islet autoantibodies (IA). Positivity on 2 or more consecutive tests defined the specific autoimmune phenotype. Standard clinical criteria defined disease.

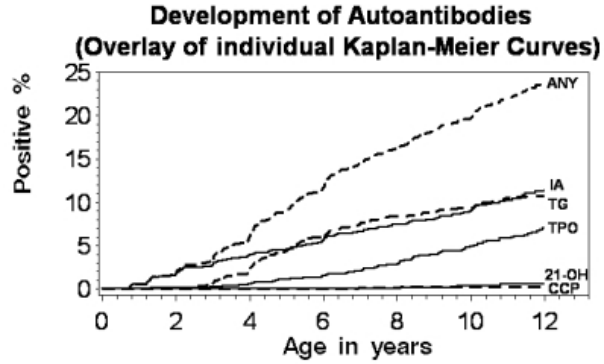
Cumulative incidences (95% CI) by age 12

Autoantibody	Cumulative Incidence %	Confirmed Disease
IA	11.3 (9.6-12.9)	69 T1D
TG	10.8 (9.1-12.4)	52 celiac disease
TPO	7.0 (5.4-8.6)	9 hypothyroidism
21-OH	0.5 (0.1-1)	1 Addison's disease
CCP	0.2 (0.0-0.5)	2 rheumatoid arthritis
Any antibody	23.8 (21.5-26.1)	129

By age 12, one in four children expressed an autoimmune phenotype and 50 (12.9%) had several phenotypes. The strongest relative risks (RR) for HLA genotypes were: DR3/4 for T1D RR=2.8 (2.0-3.9), DR3/3 for TG 2.4 (1.5-3.8), and DR4/4 for TPO 2.0 (1.1-3.5), adjusting for sex, ethnicity, and family history

For author disclosure information, see page 785.

of T1D. The high incidence of autoimmune phenotypes in children at genetic risk for T1D warrants a screening program and early intervention.



TG	2352	2123	1721	1383	1038	782	582
TPO	2195	1941	1647	1386	1064	798	597
IA	2542	2173	1804	1503	1151	882	662
21-OH	2195	1944	1658	1407	1093	841	637
CCP	2222	1959	1665	1411	1094	843	640
ALL	2542	2171	1761	1406	1045	781	575

80-OR

Elevated Uric Acid Levels Predict Multiple Type 1 Diabetes Complications

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Macro- and microvascular complications of type 1 diabetes (T1D) cluster. Improved control of blood pressure and hyperglycemia lowers but does not abolish the risk. We examined risk factors underlying both macro- and microvascular complications in a prospective cohort of adults with T1D (Coronary Artery Calcification in Type 1 Diabetes Study).

Study participants (N=540) were 19-56 yr old at baseline and re-examined six-years later. At both visits, urinary albumin excretion rate (AER) and albumin/creatinine ratio (ACR) were measured, and albuminuria was defined as AER ≥ 20 µg/min or ACR ≥ 30 mg/g. Serum creatinine was measured and estimated glomerular filtration rate (eGFR) was calculated using the CKD-Epi equation. Diabetic nephropathy (DN) was defined as new albuminuria, eGFR <60, dialysis or transplantation. Proliferative diabetic retinopathy (PDR) was defined as laser eye therapy; coronary artery calcium (CAC) was measured using electron beam computed tomography. CAC progression was defined as a change in the square root transformed CAC volume ≥ 2.5.

Incident DN occurred in 58 of 288 patients, proliferative DR in 34 of 392 patients, and CAC progression in 195 of 455 patients. Predictors of each complication were examined in stepwise logistic regression (Table). In addition to diabetes duration, hemoglobin A1c and hypertension, uric acid was an independent predictor of DN, PDR and CAC progression. LDL-cholesterol was entered into the model, but did not predict any of the outcomes.

Odds Ratios and 95% Confidence Intervals

	DN (n=288)	PDR (n=392)	CAC Progression (n=455)
Age (per 10 yrs)	-	0.6 (0.5-1.0)	1.9 (1.4-*2.6)
Diabetes duration (per 10 yrs)	-	2.4 (1.6-3.8)	2.2 (1.6-3.0)
Male sex	3.2 (1.5-7.2)	-	-
Hemoglobin A1c (per 1%)	1.3 (1.1-1.7)	1.5 (1.2-1.8)	1.3 (1.1-1.6)
Systolic BP (per 10 mmHg)	-	-	1.4 (1.2-1.7)
Diastolic BP (per 10 mmHg)	-	1.6 (1.1-2.2)	-
HDL-Cholesterol (per 10 mg/dL)	-	-	0.8 (0.7-0.9)
Uric acid (per 1 mg/dL)	1.5 (1.0-2.3)	1.4 (1.1-1.9)	1.5 (1.2-2.0)

This study demonstrated that uric acid is an independent, common risk factor for the development of both micro- and macrovascular complications of T1D, and may be an important therapeutic target in the quest to reduce T1D complications. **ADA-Funded Research**

81-OR

Interaction between *VDR* and *PTPN2* Predicts Progression to Type 1 Diabetes in Children with Islet Autoimmunity. The Diabetes Autoimmunity Study in the Young (DAISY)

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Vitamin D increases expression of protein tyrosine phosphatase, non-receptor type 2(*PTPN2*), a gene previously associated with type 1 diabetes(T1D) that contains a novel intronic binding site for the vitamin D receptor(VDR). We explored a potential gene-gene interaction between *VDR* and *PTPN2* polymorphisms on the risk of islet autoimmunity(IA) and T1D in a prospective cohort study.

DAISY is following children at increased risk of T1D for the development of IA (defined as presence of GAD-65, IA-2, or insulin autoantibodies on two or more consecutive visits), and then for progression to T1D, diagnosed by a physician. We genotyped 2007 DAISY children for rs1544410 in *VDR*, which represents the *BsmI* RFLP, and dichotomized it as AA/GA vs. GG. We genotyped rs1893217 in *PTPN2* and dichotomized it as GG/GA vs. AA. 143 children developed IA during follow-up. In Cox proportional hazards analyses, adjusting for HLA DR3/4 status, family history of type 1 diabetes, and ethnicity, neither *VDR* (HR: 1.23, 95% CI: 0.85-1.77) nor *PTPN2* (HR: 1.26, 95% CI: 0.88-1.81) were associated with development of IA and there was no evidence of a gene-gene interaction (p=0.38). We then examined whether these polymorphisms predicted progression to T1D, observed in 47 of the 143 IA positive children. While *VDR* and *PTPN2* alone did not predict progression to T1D, a significant gene-gene interaction was detected (p=0.0072). In children with the *PTPN2* AA genotype, the *VDR* AA/GA genotype was associated with a decreased risk of T1D (HR: 0.35, 95% CI: 0.17-0.70, p=0.003). In contrast, in children with the *PTPN2* GG/GA genotype, the *VDR* AA/GA genotype was marginally associated with an increased risk of T1D (HR: 3.46, 95% CI: 0.76-15.75, p=0.11). Similar results were seen when the cohort was limited to non-Hispanic whites.

The interaction between *VDR* and *PTPN2* polymorphisms in the risk of progression to T1D, if confirmed in other populations, may offer an insight concerning the role of vitamin D in the etiology of T1D and other autoimmune diseases associated with *PTPN2*, e.g., Crohn's disease.

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82-OR

Parietal Cell Autoantibodies (ATP4A) Are Associated with Islet Autoimmunity in Children at High Risk for Type 1 Diabetes

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We developed a radioimmunoprecipitation assay the H+/K+ ATP-ase autoantibody for ATP4A that is 95% sensitive and 100% specific in patients with autoimmune gastritis, surpassing the performance of previously used ELISA assays. We have previously reported that ATP4A is found in ~10% of children and 15-25% of adults with type 1 diabetes (T1D). Little is known concerning development of ATP4A in non-diabetic children with islet autoimmunity.

The Diabetes Autoimmunity Study in the Young (DAISY) has followed, for an average of 8 years, 2542 children at high risk for T1D recruited through newborn HLA screening or relatives of T1D patients. Serum samples were tested annually for islet autoantibodies (to glutamic acid decarboxylase 65 (GAD), insulin (IAA), insulinoma associated antigen-2 (IA2)) and transglutaminase (TG IgA). Autoantibodies to ATP4A were measured on the last available sample in 149 children with persistent GAD, IAA or IA2, 148 children persistently positive for TG IgA, and in 206 first degree relatives of T1D patients who were negative for GAD, IAA, IA2 and TG IgA.

Subjects persistently positive for islet autoantibodies were more likely to be positive for ATP4A than control children: 7.4% vs. 1% p=.003 (OR 7.1, 95% CI 1.5-34.2, adjusting for sex and HLA genotypes). ATPA4 was borderline significantly more frequent in females than males (4.9% vs. 1.3%, p=.033) and in children with the HLA-DR3/4,DQB1*0302 than in those with other genotypes (6.0% vs. 2.4% p=.097). In contrast, the prevalence of ATPA4 was not increased in children persistently positive for TG IgA, compared to controls (2.7% vs. 1% p=.241). ATP4A is expressed at a very low level in the islets. The specific association between the presence of ATP4A and islet autoantibodies, but not TG IgA, is independent of the HLA-DR,DQ genotype and may suggest a role for this antigen in the process leading to T1D.

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For author disclosure information, see page 785.

ADA-Funded Research

83-OR

Characteristics of Childhood Diabetes in a Norwegian Population-Based Registry

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Diabetes in childhood is classified into Type 1, Type 2 and others. An alarming increase of Type 2 diabetes in young people has been reported from some countries. Molecular genetic research has revealed causes of several subtypes of monogenic diabetes including neonatal diabetes and MODY.

We describe the epidemiology in an affluent society with a high incidence of childhood-onset diabetes ($32/10^5$/year) and organized screening for monogenic forms. We have a National Childhood Diabetes Registry with a biobank and documented completeness of 92%. In the period 2002-2008 hospital records and biological samples were collected from all new cases allowing genotyping, and testing of autoantibodies, serum insulin and non-stimulated c-peptide. HLA genotypes were categorized as: high-risk=DR3-DQ2/DR4-DQ8; intermediate risk=DR3-DQ2/ DR3-DQ2 or DR4-DQ8/ DR4-DQ8 or DR4-DQ8/X (X not DR3-DQ2 or DR4-DQ8 or DR2 (DQB1*0602)); low risk=all other genotypes. Relevant genes were screened in selected cases when monogenic diabetes was suspected on basis of clinical criteria.

During the period 2002-2008, 1608 new cases <15 years was reported, and we performed HLA-typing and autoantibody analysis in 1216 cases. Nine cases of monogenic diabetes were found. Of the ten cases diagnosed before 1 year of age, 2 were confirmed monogenic (Kir 6.2). Of the remaining 1207 cases, 1107 (91,7 %) could immediately be classified as Type 1A. Eighty antibody-negative cases (6.7 %) were classified as Type 1B based on HLA-genotype and low-c-peptide (< 0.4 nmol/l), confirmed by the clinical report stating that insulin treatment was started at diagnosis. Of the remaining 20 cases, 9 (0.7 %) were classified as Type 2 because they were on diet and/ or oral hypoglycemic agents and had BMI>25. Eleven cases (0.9 %) were not classified.

Type 1 diabetes is still the dominating type in children and adolescents in this nationwide study. Type 2 and monogenic forms each currently constitutes less than a percent of childhood onset diabetes in Norway.

84-OR

Temporal Trends in Recording of Diabetes on Death Certificates

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Our objective was to determine the frequency that diabetes is reported on death certificates of U.S. decedents with known diabetes and describe trends in reporting. Data were obtained from 11,927 participants with diabetes who were enrolled in Translating Research Into Action for Diabetes, a multicenter prospective observational study of diabetes care in managed care. Data on decedents (N=2,261) were obtained from the National Death Index from January 1, 2000 through December 31, 2007. The primary dependent variables were the presence of the ICD-10 codes for diabetes as a diagnosis listed anywhere on the death certificate or as the underlying cause of death. Diabetes was recorded on 41% of death certificates and as the underlying cause of death for 13% of decedents with diabetes. Diabetes was significantly more likely to be reported on the death certificate of decedents with diabetes dying of cardiovascular disease than all other causes. There was a statistically significant trend of increased reporting of diabetes as the underlying cause of death over time ($p<0.001$), which persisted after controlling for duration of diabetes at death. The increase in reporting of diabetes as the underlying cause of death was associated with a decrease in the reporting of cardiovascular disease as the underlying cause of death ($p<0.001$). Death certificates continue to substantially underestimate the prevalence of diabetes among decedents. The increase in reporting of diabetes as the underlying cause of death over the past 8 years will likely impact estimates of the burden of diabetes in the U.S.

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NEW CONCEPTS IN BROWN AND WHITE ADIPOCYTE BIOLOGY

85-OR

Global Mapping of Cell-Type-Specific Open Chromatin by Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE) in Adipocytes

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Identification of regulatory elements within the genome is crucial for understanding the mechanisms that governs cell-type-specific gene expression. We generated genome-wide maps of open chromatin sites using formaldehyde-assisted isolation of regulatory elements coupled with high-throughput sequencing (FAIRE-seq) in 3T3-L1 adipocytes (day 0 and day 8 of differentiation) and NIH-3T3 fibroblasts. FAIRE peaks at promoter were associated with active transcription and histone modifications of H3K4me3 and H3K27ac. Non-promoter FAIRE peaks were characterized by H3K4me1+, me3-, signature of enhancers and largely located in distal regions. The non-promoter FAIRE peaks showed dynamic change during differentiation while the promoter FAIRE peaks were relatively constant. Functionally, the adipocyte- or preadipocyte-specific non-promoter FAIRE peaks were associated with genes up- or down-regulated by differentiation, respectively. 51% of the adipocyte-specific FAIRE peaks overlapped with PPARγ binding sites. The adipocyte-specific FAIRE peaks were not evenly distributed in the genome but tended to form clusters. Genes highly up-regulated during differentiation were associated with multiple clustered adipocyte-specific FAIRE peaks. Neighboring genes in such regions were often co-regulated during differentiation. Finally, taking advantage of FAIRE-seq, an unbiased technique to identify potential regulatory elements without prior knowledge, we combined it with computational motif analyses and found that a motif for NF-I family transcription factors were specifically enriched in the adipocyte-specific FAIRE peaks. Nfia and Nfib were abundantly expressed in adipose tissue and up-regulated during adipocyte differentiation. Knockdown of Nfia or Nfib significantly suppressed the induction of adipogenic genes and lipid accumulation during differentiation. Our study demonstrates the utility of FAIRE-seq in providing a global view of cell-type-specific cis-regulatory elements in the genome and in indentifying transcriptional regulators of adipocyte differentiation.

86-OR

Adipose Tissue Extracellular Matrix and Vascular Abnormalities in Obesity and Insulin Resistance

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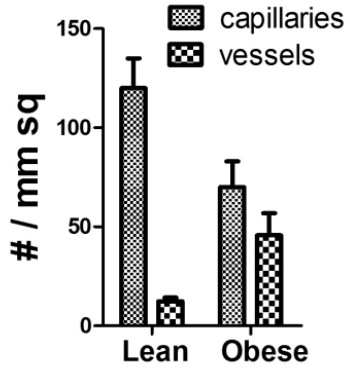
We hypothesized that adipose tissue (AT) blood vessel density and extracellular matrix (ECM) changes play a role in insulin resistance; we examined angiogenesis and ECM deposition in subcutaneous AT of non-diabetic subjects. Candidate gene expression was examined in 60 subjects (BMI 19-41 kg/m^2; S_I 0.47-9.5 $x10^{-4}$ $min^{-1}/\mu U/ml$) and immunohistochemistry (IHC) was performed in 8 lean-insulin sensitive (L/IS, BMI<25, S_I>2.65) and 8 obese-insulin resistant (O/IR, BMI>30, S_I<2.65) subjects. CD31 (an endothelial marker) mRNA showed little correlation with BMI or S_I. However, CD31 positive capillaries (by IHC) were decreased by 58% in O/IR compared to L/IS; in contrast, larger vessels, double stained for alpha smooth muscle actin (ASMA) and CD31, were increased by 70% in O/IR, accounting for the lack of change in CD31 expression with obesity.



For author disclosure information, see page 785.

IHC analysis of AT ECM components showed that both collagen (Col) V and VI were increased in O/IR AT but with different distributions. Col V was diffusely located in L/IS but mostly surrounded large ASMA-expressing blood vessels in O/IR. Elastin was reduced as a component of adipose ECM in O/IR (12% of area by IHC in L/IS, vs 5% O/IR, $p<0.05$). To examine adipocyte-macrophage interactions in vitro, adipocytes (from stem cells) were co-cultured with M1, M2a, and M2c macrophages. Col V and elastin were mostly expressed in adipocytes. In the presence of M2a and M2c alternatively activated macrophages, which account for 75% of macrophages in obese AT, adipocytes expressed 3-4x more Col V in vitro. These results suggest the ECM of O/IR subjects was more stiff and fibrotic, likely due to the increase in collagens and the decrease in elastin, with a decrease in capillaries and increase in larger blood vessels, which may promote hypoxia, infiltration of M2 macrophages, and further fibrosis.

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87-OR

Deletion of the Mitochondrial Transcription Factor A (TFAM) in Adipose Tissue Causes Mitochondrial Dysfunction and Protects Mice from Obesity and Insulin Resistance

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Obesity and type 2 diabetes are associated with mitochondrial dysfunction in adipose tissue, but whether this dysfunction participates directly in the development of these disorders remains an open question. We have generated a mouse model for mitochondrial dysfunction in adipose tissue by disrupting mitochondrial transcription factor A or Tfam (FAT-Tfam-KO). Tfam plays a key role in mitochondrial function by controlling both mtDNA stability and transcription. On normal chow diet, FAT-Tfam-KO mice are leaner, more insulin sensitive, protected from hepatosteatosis and have a higher energy expenditure in metabolic cages than control littermates. Brown adipose tissue (BAT) mass of FAT-Tfam-KO mice is decreased by 55%, and electron microscopy reveals many markedly enlarged mitochondria with distorted cristae. In BAT, Tfam deletion dramatically also reduces mtDNA copy number and mitochondrially-encoded gene expression while upregulating oxidative stress markers such as Gadd45. As a result, FAT-Tfam-KO mice show impaired thermogenesis in response to cold exposure. White adipose tissues mass in FAT-Tfam-KO mice is also reduced by 50-60% compared to control. Adipocytes are significantly smaller in subcutaneous fat, whereas perigonadal adipocyte size is unchanged, indicating a depot-specific reduction in adipocyte number. No change in adiponectin mRNA levels in adipose tissues was observed in FAT-Tfam-KO mice, while circulating adiponectin levels were much lower (55%), suggesting a mitochondrially-dependent defect in secretion. Thus, Tfam deletion leads to impaired mitochondrial activity and increased oxidative stress in fat, but clearly protects mice from obesity and insulin resistance. Our results suggest a novel paradigm of fat mitochondria dysfunction in development of type 2 diabetes and establish adipose tissue mitochondria as a potential therapeutic target for treatment of obesity.

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Ablation of *TRIP-Br2*, a Novel Regulator of Adipocyte Lipolysis, and Thermogenesis, Prevents Diet-Induced Obesity and Insulin Resistance

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Obesity results from an imbalance between energy intake and energy expenditure leading to the storage of excess energy as fat, primarily in adipose tissue. The intricate balance of energy homeostasis in mammals is tightly regulated by a complex network of metabolic genes under the control of transcription factors and co-regulators. Here we report using gene ablation to reveal a novel role for TRIP-Br2 (also known as SERTAD2), a previously characterized cell cycle transcriptional co-regulator, in the regulation of adiposity. *TRIP-Br2* null mice are highly resistant to the development of diet-, age- and genetic-induced obesity as well as obesity related insulin resistance. They exhibit markedly reduced adipose tissue mass, adipocyte cell size and triglyceride content. While adipogenesis and lipogenesis are normal, the adipocytes of *TRIP-Br2* null mice have a higher rate of stimulated lipolysis due to an increase in gene and protein expression of the hormone sensitive lipase (HSL) and b3-adrenergic receptors (Adrb3). Conversely, over-expression of TRIP-Br2 in adipocyte cell lines represses HSL and Adrb3 expression. We report, for the first time to our knowledge, that *HSL* and *Adrb3* are E2F-responsive genes and that TRIP-Br2 is a transcriptional co-repressor of the *Adrb3* and *HSL* genes in differentiated adipocytes. *TRIP-Br2* null mice also exhibit higher thermogenesis characterized by an increase in oxygen consumption, heat production and basal and cold-induced core body temperature. We report the identification of TRIP-Br2 as the first transcriptional co-regulator that functions physiologically to permit fat storage in adipocytes through a mechanism involving complementary regulation of lipolysis, thermogenesis and oxidative metabolism without affecting adipocyte differentiation in contrast to the direct effects on adiposity mediated by the well-characterized transcription factor PPARg. Our findings implicate TRIP-Br2 as a novel therapeutic target for counteracting the development of obesity and insulin resistance.

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Intra-Abdominal Transplantation of Subcutaneous Adipose Tissue Ameliorates High-Fat Diet-Induced Glucose Intolerance and Adiposity in Mice

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Relationships between intra-abdominal fat accumulation, glucose intolerance and adiposity were investigated in mice by transplanting additional inguinal (subcutaneous) and epididymal (visceral) fat into the abdomen. In 7-wk old male C57Bl6/J mice, additional subQ and vis depots were sutured onto the inner surface of the abdominal wall (subQ→vis and vis→vis mice, respectively). Recipients were then placed on a high-fat diet and sacrificed at 3, 6, and 17 wks afterwards.

At 3 wks, no differences in glucose tolerance or adiposity were observed between groups. From 6 wks onwards, improved glucose tolerance was observed in subQ→vis mice, and this difference was maintained at 10 and 14 wks post-transplant (see Table). Fasting glucose and insulin concentrations tended to be lowest in subQ→vis mice, and subQ→vis mice also gained less fat between 6 and 10 wks post-transplant ($P<0.05$ vs sham).

Examination of grafts at sacrifice showed that at 6 and 17 wks, but not at 3 wks, the mass of subQ grafts was reduced relative to time of transplant, while vis grafts had increased in mass ($P=0.0001$). Similarly, PPAR-γ and leptin protein expression increased with time in vis grafts, but not in subQ grafts. These time-course studies lead us to propose that the inability of subQ grafts to expand in their new anatomical location may underlie the beneficial effects of subQ→vis transplantation.

Variable	Sham mice (n)	SubQ→vis mice (n)	Vis→vis mice (n)	*P* (ANOVA)
6 wk GTT iAUC (mmol/l·min)	1434±67* (15)	1138±31 (14)	1362±66 (17)	0.024
10 wk GTT iAUC (mmol/l·min)	1651±153 (8)	1289±114 (6)	1766±95* (9)	0.045
14 wk GTT iAUC (mmol/l·min)	2006±79* (8)	1644±104 (7)	2012±87* (9)	0.015
14 wk fasting blood glucose (mmol/l)	7.8±0.8 (8)	7.4±0.8 (7)	8.2±0.6 (9)	0.73
14 wk fasting plasma insulin (ng/ml)	0.42±0.14 (8)	0.28±0.07 (6)	0.44±0.13 (7)	0.65
6 wk %body fat (%)	21.2±1.0 (15)	20.3±1.2 (13)	20.9±1.1 (17)	0.85
10 wk %body fat (%)	26.1±2.4 (8)	22.5±2.1 (7)	26.4±1.1 (9)	0.32
Δ %body fat (Wks 6-10)	5.6±1.1%* (8)	1.7±0.6% (7)	3.3±1.2% (9)	0.074
14 wk %body fat (%)	27.0±2.3 (8)	23.2±1.3 (7)	27.3±1.4 (9)	0.21

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Transplantation of Brown Adipose Tissue Exerts Beneficial Effects on Glucose Homeostasis

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Brown adipose tissue (BAT) functions to dissipate chemical energy in response to cold or excess feeding, and modulation of BAT metabolism influences energy balance. Thus, increasing BAT could be a potential treatment for obesity and metabolic disease. To determine the effects of increasing BAT content on glucose homeostasis, 0.1g of BAT from 12 wk old male donor mice was transplanted into the visceral cavity of age-matched recipient mice. To control for vascularization, separate mice were transplanted with 0.1g white adipose tissue (WAT) and a third group was sham operated. Mice were studied from 2 to 12 wks post-transplant and appeared healthy, with no differences in food intake or body weight throughout the study. Tyrosine hydroxylase staining showed sympathetic

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innervation of the transplanted BAT. Compared to controls, BAT-transplanted mice had significantly improved glucose tolerance (GTT) by 8 wks post-transplant, with maximal effects 12 wks post-transplant [GTT area under the curve (AUC) 42±2, 43±4, 33±2 arbitrary units; Sham, WAT, BAT respectively; P<0.01]. BAT-transplanted mice also had increased insulin sensitivity (assessed by glucose clamp and insulin tolerance tests), increased energy expenditure, decreased fat mass, increased lean mass, improved tolerance to cold exposure, and decreased circulating insulin, leptin, triglycerides, and cholesterol (P<0.05). To determine if the BAT effects were dose-dependent, additional mice received 0.1g or 0.4g BAT. Mice receiving 0.4g BAT showed a greater response to all parameters measured (e.g. GTT AUC 45±2, 35±3, 24±3 for Sham, 0.1g BAT, 0.4g BAT; P<0.01). Interestingly, both 0.1g and 0.4g BAT transplanted mice had increased circulating IL6 concentrations (1.3±0.5, 22±12, 33±8 pg/ml; P<0.05). To determine if IL6 was a mechanism for improved glucose homeostasis, 0.1g BAT from *IL6* $^{-/-}$ and *IL6* $^{+/+}$ mice was transplanted into wild-type animals. The improved glucose homeostasis was intact in the mice receiving BAT from *IL6* $^{+/+}$ mice, but not in mice receiving BAT from *IL6* $^{-/-}$ mice, suggesting that BAT-derived IL6 regulates systemic glucose homeostasis. Transplantation of BAT may be an effective treatment for metabolic disease.

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Changed to a poster presentation per author request. See abstract 1573(2)-P on page A429.

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Enhancement of Brown Adipose Tissue Development *In Vivo* by a Novel Insulin Sensitizer

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Interest in the role of brown adipose tissue (BAT) in metabolism and type 2 diabetes has been stimulated by the observation in man that BAT mass is inversely proportional to BMI. Thiazolidinediones that do not bind to or activate the PPARγ receptor at pharmacological concentrations (PPARγ-sparing, PsTZDs) elicit differentiation of BAT progenitor cells in tissue culture independent of PGC1α, potentially through a mitochondrial target of the TZDs. MSDC-0160, a PsTZD clinical candidate, produced insulin sensitizing pharmacology without weight gain or plasma volume expansion in a Phase 2A clinical trial. To determine if MSDC-0160 can increase BAT mass *in vivo* and/or increase "browning" of white adipose tissue (WAT), C57BL6 mice (WT) and C57BL6 mice in which the PGC1α gene was ablated (PGC1α KO) were exposed to MSDC-0160 (30mg/kg) for four weeks (standard chow diet). As predicted from the *in vitro* studies with committed BAT precursor cells, drug treatment increased the weight of the interscapular BAT pad in both WT and KO animals (>50% and >100%, respectively) and this was accompanied by a corresponding increase in expression of the BAT specific mitochondrial uncoupling protein, UCP1. In contrast to the findings in BAT, the mass of the epididymal WAT was significantly *decreased* (>25%) by drug treatment in the WT but not in the mice lacking PGC1α. There was also a mixed response in the perirenal fat pad, where treatment with MSDC-0160 produced an 8-fold increase in staining for UCP1 in WT cells. In contrast, treatment of KO mice produced a significant increase in the weight of the pad and there was no increase in UCP1 expression. In conclusion, we have demonstrated that MSDC-0160 increases functional BAT mass *in vivo* independent of PGC1α. Treatment with MSDC-0160 also increased "browning" of the perirenal fat, but this effect appeared dependent on PGC1α. These data suggest that insulin sensitizers that work through this mechanism may have positive effects on metabolism by selectively influencing cells resident in brown and white adipose stores.

THE ROLE OF THE LIVER IN OBESITY RESEARCH

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Liver-Derived Factor(s) Drive β-Cell Hyperplasia in Insulin Resistant Mice

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Islet β-cell hyperplasia occurs as a physiological or pathophysiological response to insulin resistant states in an attempt to normalize glycemia. To test the hypothesis that cross-talk between liver and pancreatic islets mediates β-cell growth in response to insulin resistance, we used the Liver-specific Insulin Receptor Knockout (LIRKO) mouse, a unique model that exhibits a dramatic islet hyperplasia. To explore whether circulating factors in LIRKO mice promote β-cell proliferation, we established *in vivo* transplantation (n=3-5) and parabiotic (n=6-8) mouse models and assessed β-cell replication by bromodeoxyuridine incorporation. We also cultured mouse (n=2-3) or human islets (control=1; type 2 diabetes=1) for 48 hours in serum from 3 month-old LIRKOs (LIRKO(s)) or controls (con(s)), followed by confocal microscopic evaluation of β-cell growth by Ki67 immunostaining. Our studies reveal that: (1) control mice parabiosed to LIRKOs show a 4-fold increase in β-cell mitosis (p<0.05); and (2) control islets transplanted under the kidney capsule in LIRKO mice exhibit 7-fold increased β-cell proliferation (control islets into LIRKO 0.14±0.03 vs. control islets into control 0.02±0.02%, p<0.05). Furthermore, LIRKO serum enhanced β-cell proliferation in primary mouse islets (LIRKO(s) 2.2±0.2 vs. control(s) 0.7±0.3% Ki67+β-cells, p<0.05), control human islets (LIRKO(s), 0.4% vs. con(s), not detected), and in islets from a patient with type 2 diabetes (LIRKO(s) 0.2% vs. con(s), not detectable). Finally, culturing mouse islets in conditioned media from liver explants (LECM) or hepatocytes (HCM) for 24 h enhanced β-cell growth (LIRKO-LECM, 0.5% vs. con-LECM, 1.6 %, n=2) and (LIRKO-HCM, 1% vs. con-HCM, not detected, n=2) respectively. Taken together, these data indicate the existence of circulating, non-neural and non-cell autonomous β-cell growth factors, and strongly implicate the liver as a critical source of these novel β-cell growth factor(s). The enhanced proliferation observed in β-cells from both control and diabetic humans underscores the significance of our model to identify the putative β-cell growth factor(s) with the long term goal of regenerating β-cells in both type1 and type 2 diabetes.

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Glucose and Mitochondrial Metabolism in Human Subjects with Nonalcoholic Fatty Liver Disease

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Individuals with non-alcoholic fatty liver disease have excess intra-hepatic triglycerides (TG). Several reports indicate that excess liver TGs may mediate insulin resistance secondary to mitochondrial dysfunction. In this study, subjects with metabolic syndrome and a range of liver TG were recruited to test the hypothesis that liver TG accumulation is associated with altered hepatic glucose and mitochondrial metabolism. Eleven subjects with a mean ±SD age of 46 ± 9.4 years, body mass index of 37 ± 8.4 and varied hepatic TG as assessed by proton MRS (range: 0-22 %) were infused

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with multiple ^{2}H and ^{13}C tracers after an overnight fast. NMR and GC-MS isotopomer analysis were performed on plasma metabolites to determine fluxes of glucose and mitochondrial metabolism in liver and peripheral free fatty acid (FFA) turnover (μmoles/kgLBM/min). Liver TG robustly correlated with hepatic mitochondrial TCA cycle flux (r = 0.67; P = 0.023), anaplerosis (r = 0.72; P = 0.016) and gluconeogenesis from 3-carbon substrates derived from the TCA cycle (r = 0.58; 0.05). There was also a significant correlation between FFA turnover and hepatic TCA cycle flux (r = 0.72; *P* = 0.013) and a positive relationship between FFA vs. ketone turnover (r = 0.51; P = 0.109), though the latter did not achieve statistical significance. When dichotomized by liver TG, subjects with elevated TG (17± 3.5%; n=5) had a ~2-fold increase in flux through the mitochondrial pathways of the TCA cycle (8± 3.8 vs. 17± 4.8; P = 0.010) and anaplerosis (45± 12.4 vs. 78± 24.3; P = 0.008) but not ketone turnover (2.1± 1.7 vs. 2.8± 0.8) compared to individuals with lower TG (3.9± 2.0%; n=6). The induction of liver mitochondrial metabolism in these individuals may occur as compensation for increased FFA delivery (4.3± 1.3 vs. 7.7± 1.3; P = 0.001). Surprisingly, acetyl-CoA is selectively partitioned through oxidation in the TCA cycle rather than ketogenesis, suggesting increased energy demand in fatty livers. We conclude that elevated liver TG is associated with an adaptive increase in both hepatic gluconeogenesis and mitochondrial metabolism, a factor that potentially predisposes the liver to oxidative damage.

ADA-Funded Research

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PEPCK-C Is Required for Normal Lipid, Glycerol and Cholesterol Synthesis

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Cytosolic Phosphoenolpyruvate carboxykinase (PEPCK-C) catalyzes the conversion of oxaloacetate to phosphoenolpyruvate. Although PEPCK-C is a gluconeogenic enzyme, it is also expressed in non-glucose producing tissues and is more generally a cataplerotic pathway required to match mitochondrial anaplerosis to biosynthesis and dynamic regulation of the TCA cycle. In addition to gluconeogenesis (GNG), PEPCK-C is important for glyceroneogenesis (GyNG), triglyceride esterification and mitochondrial function. Despite being required for GNG, we previously demonstrated that PEPCK-C expression does not strongly control the rate of GNG in liver, but that complete loss of liver PEPCK-C causes impaired mitochondrial function and fatty liver after fasting. GyNG is a pathway that shares most enzymatic reactions with GNG and is critical for triglyceride synthesis. The objective of this study was to determine the consequence of PEPCK-C loss of function on the regulation of GyNG and its broader impact on lipid synthesis. Mice with graded expression of whole body PEPCK-C and a liver specific PEPCK Knockout (KO) were administered deuterated water and food *ad libitum* for 4-days. Liver extracts were analyzed by deuterium nuclear magnetic resonance. Synthesis of the glycerol and fatty acid moieties of hepatic triglycerides, as well as flux through cholesterol synthesis were determined from incorporation of deuterium into lipid intermediates. As expected, there was a direct correlation between GyNG and whole body PEPCK-C expression. Remarkably, this correlation suggested a stronger control of fed state GyNG by PEPCK-C than fasted state GNG. Although there is no obvious direct requirement for PEPCK-C in lipogenesis or cholesterogenesis, the synthesis of saturated FFA, unsaturated FFA and cholesterol was suppressed in PEPCK-C deficient mice. This observation was most apparent in whole body PEPCK-C deficient mice and less evident in liver specific KO mice. These data confirm an important role for PEPCK-C in glycerol and triglyceride metabolism and reveal an unexpected role for PEPCK-C in FFA and cholesterol synthesis, perhaps secondary to disruptions in whole body triglyceride metabolism.

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ADA-Funded Research

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GLP-1-Derived Nonapeptide GLP-1(28-36)amide Targets Mitochondria, and Modulates Mitochondrial Oxidative Metabolism and Suppresses Glucose Production in Isolated Mouse Hepatocytes

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Metabolic syndrome is a disorder of nutrient metabolism due to the increasing prevalence of obesity and accompanying insulin resistance and systemic oxidative stress. Recently, we described that GLP-1(9-36)amide, the cleavage product of GLP-1(7-36)amide by Dpp4, inhibits weight gain and the development of metabolic syndrome (diabetes, hepatic steatosis) in diet-induced obese mice and suppresses glucose production independently of the GLP-1 receptor in isolated mouse hepatocytes suggesting potential insulin-like actions of GLP-1-derived peptides. Here we investigated whether the C-terminal nonapeptide FIAWLVKGRamide, GLP-1(28-36)amide, possibly derived by endopeptidase-mediated cleavage from the pro-peptide GLP-1(9-36)amide, is the active insulinomimetic peptide. In isolated hepatocytes treatment with GLP-1 (28-36)amide dose-dependently suppressed the increased glucose production in response to insulin resistance induced by cAMP, dexamethasone and lactate. These effects were independent of the GLP-1 receptor because co-incubation with the GLP-1 receptor antagonist, exendin (9-39), did not block GLP-1 (28-36)amide regulated gluconeogenesis. Moreover, GLP-1 (28-36)amide protected against the fall in ATP levels induced by either hydrogen peroxide in H4IIe hepatoma cells and hepatocytes or tert-butylhydroperoxide (t-BHP) in hepatocytes. Likewise, GLP-1(28-36)amide diminished oxidative stress in response to the addition of t-BHP both in hepatocytes isolated from normal and diet-induced obese mice. In H4IIe cells GLP-1(28-36)amide also decreased hyperglycemia-induced oxidative stress. A fluorescence-labeled nonapeptide (FAM-GLP-1(28-36)amide) readily enters hepatocytes and distributes in a pattern indistinguishable from that of the mitochondrial marker Mitotracker. These findings suggest that GLP-1(28-36) amide enters hepatocytes and directly modulates mitochondrial oxidative metabolism and suppresses glucose production raising the possibility that GLP-1(28-36)amide may be useful for the treatment of fasting hyperglycemia and metabolic syndrome in type 2 diabetes.

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Mice Lacking FGF21 Are More Insulin Resistant Than Wild-Type Mice When Fed a Low-Carbohydrate, High-Fat Ketogenic Diet

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Though fibroblast growth factor 21 (FGF21) has emerged as a novel treatment to improve insulin sensitivity, plasma FGF21 concentrations are also higher in conditions of insulin resistance (IR) such as nonalcoholic fatty liver disease (NAFLD), obesity and type 2 diabetes, suggesting a state of FGF21 resistance. Specifically, in mice fed a low-carbohydrate, high-fat ketogenic diet (KD), the development of hepatic IR is associated with a rise in FGF21. In order to address the role of FGF21 in hepatic IR, we assessed insulin action in KD-fed FGF21 knock-out (FGF21 KO) mice and their littermate wild-type (WT) controls using hyperinsulinemic [3 mU/(kg-min)]-euglycemic clamps. Though body weights of FGF21 KO mice tended to be lower, their percentage of fat mass, assessed by ^{1}H NMR, was ~36% higher (P<0.05), consistent with a ~16% reduction (P<0.05) in energy expenditure per mouse, without any differences in locomotor activity or caloric intake. Basal plasma glucose and glucagon concentrations were similar between groups but basal plasma insulin concentrations were ~56% higher (P<0.05) in FGF21 KO mice. Endogenous glucose production was higher in both the basal (WT: 13.3±1.3 versus FGF21 KO: 16.1+2.0 [mg/(kg-min)], P<0.05) and hyperinsulinemic-euglycemic (WT: 1.3±4.6 versus FGF21 KO: 10.1±4.6 [mg/(kg-min)], P<0.01) states in FGF21 KO mice, reflecting marked hepatic IR in FGF21 KO mice. There was no difference in insulin-stimulated whole-body glucose disposal, glycogen synthesis and glycolysis between groups. Consistent with hepatic IR, hepatic diacylglycerol (DAG) content was increased by ~140% in FGF21 KO mice (P<0.001) and insulin-stimulated AKT2 phosphorylation was decreased (P<0.05). Conclusion: These findings demonstrate that loss of FGF21 promotes hepatic steatosis and increases hepatic glucose production and hepatic IR associated with increased hepatic DAG content. Moreover, these data suggest that pharmacological administration of FGF21 in situations such as NAFLD, obesity and type 2 diabetes may lead to a reduction in hepatic DAG content with subsequent reversal of hepatic IR.

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Fructose-Induced AMPK Activation in the Hypothalamus Contributes to Increased Gluconeogenesis and Hepatic PEPCK Expression

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Fructose enriched diets are known to cause glucose intolerance. In addition, fructose-induced insulin resistance has been described to occur in the liver through mechanisms yet unsettled. Recently, it was demonstrated that intracerebroventricular (ICV) fructose injections activates AMPK and stimulates of food intake. Former studies have demonstrated that hypothalamic AMPK activation increases hepatic gluconeogenesis through activation of an inter-organ communication. The aim of the present investigation was to assess whether hypothalamic AMPK activation by fructose controls hepatic gluconeogenesis. Treatments were performed

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through a cannula implanted in lateral ventricle. Hypothalamus and liver were processed for western blot analysis of p-AMPK, p-ACC, PEPCK and G6Pase. Pyruvate tolerance test was performed to estimate gluconeogenesis. We have found that intraperitoneal 5-day treatment with fructose (IP-Fructose) increased gluconeogenesis and upregulated PEPCK expression in the liver. In parallel, IP-Fructose stimulated hypothalamic but not hepatic AMPK and ACC phosphorylation. ICV treatment for 5 days with fructose mimicked the hypothalamic, hepatic and metabolic changes induced by intraperitoneal treatment. Importantly, we have found that ICV AICAR (activator of AMPK) treatment efficiently increased whole-body gluconeogenesis and PEPCK expression in the liver with parallel activation of hypothalamic AMPK. In order to further strength the relationship between fructose-induced hypothalamic AMP activation and upregulation of gluconeogenesis we next adopted the following protocol: rats received IP-Fructose with or without ICV injections of Compound C (an inhibitor of AMPK). We found that Compound C suppressed IP-fructose-induced AMPK and ACC phosphorylation in the hypothalamus. Moreover, our data demonstrated that increase of gluconeogenesis and hepatic PEPCK expression induced by IP-fructose was suppressed by hypothalamic AMPK inhibition. The data presented herein demonstrate that hypothalamic activation of AMPK after short-term exposure to fructose is involved in hepatic upregulation of PEPCK and increase of gluconeogenesis.

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Dissociation of IRE1α Mediated JNK Activation from Hepatic Insulin Resistance in Conditional XBP1 Knockout Mice

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The pathogenesis of hepatic insulin resistance (HIR) is incompletely understood. Increases in endoplasmic reticulum (ER) stress and intrahepatic lipids, specifically diacylglycerol (DAG), have both been proposed to cause HIR. XBP1 was first described as a transcriptional regulator of the ER stress response and heterozygous XBP1 null mice are prone to ER stress and HFD-induced HIR. XBP1 also regulates hepatic lipogenesis and so HIR during HFD could result from accumulation of lipid as well. To assess the role of ER stress and lipids in the development of HIR, we fed fructose chow (FC) to conditional XBP1 knockout (XBP1Δ) mice. We hypothesized that XBP1Δ mice would be protected from FC-induced hepatic steatosis and HIR, despite increased activity of ER stress signaling pathways. XBP1Δ mice were protected from FC-induced HIR as reflected by a 50% improvement in suppression of hepatic glucose production ($P<0.05$) and no difference in whole-body or adipose glucose uptake during hyperinsulinemic-euglycemic clamps. HIR associated markers of ER stress were increased in FC fed XBP1Δ mice, including phosphorylated eIF2α protein and GRP78 mRNA. Both IRE1α mRNA and protein were elevated in XBP1Δ mice and hepatic JNK activity was 2-fold greater. There was no difference in plasma cytokines such as IL-1b, IL-6, IL-10 and IL-12, suggesting that JNK activation was unrelated to inflammation and potentially due to hyperactive IRE1α. Importantly, both hepatic triglyceride and DAG levels were reduced by 30% ($P<0.05$) and 50% ($P<0.05$) respectively in XBP1Δ mice, and were associated with a ~20% ($P<0.05$) reduction in PKCε activity. No difference in IRS1 serine phosphorylation was detected, while insulin-stimulated IRS2 tyrosine phosphorylation was increased in the XBP1Δ mice, consistent with the increased suppression of hepatic glucose production during the clamp. Conclusion: Despite increased IRE1α mediated JNK activation, XBP1Δ mice were protected from FC-induced hepatic steatosis and HIR. These data support the hypothesis that HIR associated with ER stress may result from defective lipid storage and not ER stress signals from the IRE1α pathway.

100-OR

Inactivation of Glucose-6 Phosphatase in the Liver Protects from Diabetes and Obesity

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The induction of endogenous glucose production is a major cause of fasting hyperglycemia in diabetics. This function is restricted to three organs: the liver, kidneys and intestine which express the key enzyme of gluconeogenesis: glucose-6 phosphatase (G6Pase). We aim to demonstrate the specific role of hepatic glucose production (HGP) in triggering insulin resistance and diabetes. For that, we developed an inducible and liver-specific G6Pase knock-out mouse model (Lg6pc-/-), normoglycemic in the fed state. First, we followed the development of diabetes in Lg6pc-/- mice fed on a deleterious high fat/high sucrose (HF/HS) diet. Secondly, loss of hepatic G6Pase was realized in obese diabetic mice to question the reversibility of diabetes by abolishment of HGP. After 21 weeks of HF/HS feeding, Lg6pc-/- mice resisted to the development of fasting hyperglycemia (95±10 mg/dL in Lg6pc -/- vs 166±20 mg/dL in controls, $p<0.05$) and hyperinsulinemia (0.8±0.2 ng/mL vs 3±0.5 ng/mL in controls, $p<0.05$). In addition, Lg6pc-/- mice ameliorated glucose tolerance (AUC 341 ± 22 mg/dL/h vs 521±54 mg/dL/h in controls, $p<0.01$), enhanced insulin sensitivity and muscle 2-deoxyglucose uptake. When liver *G6pc* deletion was realized in obese and diabetic mice, this resulted in a spectacular and early amelioration of glucose tolerance in Lg6pc-/- mice compared to that before liver *G6pc* deletion (AUC 358.2±13 mg/dL/h after vs 470.8±31 mg/dL/h before gene deletion, $p<0.01$) and a normalization of 16h-fasting glucose and insulin levels.

Interestingly, Lg6pc-/- mice were also resistant to diet induced obesity (weight gain of 9.0±1.2 g vs 20.9±1.9 g in controls after 21 weeks of HF/HS). This was associated to the induction of basal metabolism and muscle & brown adipose tissue thermogenesis machinery.

The role of several hepatic factors able to regulate peripheral energy metabolism and insulin sensitivity is being investigated to explain these beneficial modifications. This study underlines the specific deleterious role of HGP in the development of insulin resistance and diabetes, and provides a novel proof that hepatic glucose metabolism has the capacity to control both peripheral glucose and energy status.

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BETA CELL GROWTH AND MAINTENANCE

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Dedifferentiation, Not Loss of β-Cell Mass, Is the Main Mechanism of β-Cell Failure and Is Controlled by FoxO1

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Type 2 diabetes results from decreased β-cell mass and function. To evaluate the relative roles of these two processes in β-cell failure, we took advantage of previous work indicating that transcription factor FoxO1 regulates both. Under basal conditions, mice lacking Foxo1 in mature β-cells (Ins-cre:*Foxo1*$^{fl/fl}$, or IKO) feature normal metabolism and islet architecture. But when subjected to acute (low-dose Streptozotocin) and chronic stresses (crosses with insulin-resistant mice, aging, and multiparity), IKO mice showed accelerated β-cell loss and increased a cells, resulting in elevated blood glucose levels, hyperglucagonemia and hypoinsulinemia–similar to type 2 diabetes. Immunostaining with cell death markers failed to reveal differences between IKO and control mice. Lineage-tracing experiments to label β-cells *in vivo* demonstrated that Foxo1-deficient β-cells did not die of apoptosis, but rather lost their β-cell identity, based on gene expression profiling and immunohistochemistry. These "empty" β-cells appeared to be partly committed endocrine cells, and transdifferentiated with low frequency into α, δ and Pp cells. We next asked whether similar loss of differentiated b-cells occurred in other murine models of type 2 diabetes. We confirmed the presence of 'empty' endocrine cells in association with loss of FoxO1 in additional type 2 diabetes models, suggesting that this is a common mechanism of β-cell failure. Our results indicate that *Foxo1* is required to maintain β-cell identity and prevent β-cell dedifferentiation in response to pathophysiological stresses. Our findings should prompt a reevaluation of the relative roles of changes in β-cell mass *vs.* dedifferentiation in β-cell dysfunction. We suggest that treatment of β-cell failure should aim at restoring β-cell differentiation, and not at increasing β-cell mass.

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102-OR

Nkx6.1 Is Critical for Beta Cell Proliferation and Maintenance of Beta Cell Identity

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Previous studies from our laboratory have shown that the transcription factor Nkx6.1 is required for proper beta cell development.

However, because beta cells do not form in *Nkx6.1*-null mutant mice, specific roles of Nkx6.1 in mature beta cells cannot be dissected in this mouse model. In order to uncover beta cell-specific functions of Nkx6.1, we recently generated an *Nkx6.1* conditional mutant allele (*Nkx6.1*^{flox}) in mice. To define the role of Nkx6.1 during beta cell maturation, we ablated *Nkx6.1* in newly-generated beta cells using a rat *insulin II* promoter-driven Cre-recombinase expressing transgene (*RIP-Cre*). While *RIP-Cre*-mediated ablation of *Nkx6.1* did not affect beta cell expansion or survival during embryonic development, a marked decrease in beta cell mass was observed by six weeks of age. Compared to *wild type* beta cells, *Nkx6.1*-deficient beta cells exhibited a drastic reduction in their proliferative capacity, resulting in

For author disclosure information, see page 785.

reduced postnatal beta cell mass expansion. To examine whether beta cells continue to require Nkx6.1 for their proliferation later in life, we conditionally inactivated *Nkx6.1* in 6-week-old mice, using the islet-specific tamoxifen-inducible *Pdx1-CreER* transgene. Mirroring our observations in newly-generated beta cells, loss of Nkx6.1 in mature beta cells similarly resulted in decreased beta cell proliferation. In addition, our analysis of beta cell-specific *Nkx6.1* mutant mice also uncovered a critical requirement for *Nkx6.1* in maintaining beta cell identity. Using a beta cell-specific lineage tracing approach in *RIP-Cre; Nkx6.1$^{flox/-}$* mice, we found that beta cells adopted a delta cell identity by six weeks of age.

Moreover, deletion of *Nkx6.1* in adult mice resulted in a rapid loss in the expression of beta cell-specific genes, including insulin, MafA, and Glut2, culminating in the immediate onset of diabetes. Taken together, these findings demonstrate that Nkx6.1 is vital not only for beta cell development, but also for the maintenance of beta cell identity and function postnatally.

103-OR

K-Ras Signaling Paradoxically Reduces Pancreatic Beta-Cell Numbers

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KRAS encodes a regulated GDP/GTP molecular switch that controls several signaling networks. Although *KRAS* was first identified as an oncogene that causes abnormal cell proliferation in several human epithelial cancers, K-Ras signaling can elicit diverse, cell-type specific responses, and its function in beta-cell development and physiology remains unknown. By developing a novel imaging system that enables quantitative, wholemount visualization of the pancreas in mouse embryos, we screened for genetic mutations that perturb pancreatic endocrine formation. From this, we found that mice heterozygous for a *Kras* null allele have an abnormal increase in beta-cell mass. We show that the additional beta-cells come from two sources: an increase in Neurogenin3-expressing endocrine progenitors during embryogenesis, and an increase in beta-cell proliferation in the perinatal period. This increase in beta-cell mass results in improved glucose tolerance in adult animals. In contrast, expression of a constitutively active form of KRAS in the pancreas reduced endocrine differentiation and mass, demonstrating that K-Ras signaling antagonizes beta-cell expansion. Interestingly, either increasing or decreasing K-Ras signaling results in a normal sized pancreas, suggesting that beta-cell mass is particularly sensitive to this pathway. These studies provide new insight into K-Ras function and the unique pathways that drive beta-cell generation and regeneration and introduce an effective new method to discover genes and pathways that critically regulate beta-cell development and mass.

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104-OR

Improved β-Cell Proliferation and Function in *c-Kit$^{Wv/+}$;Fas$^{lpr/lpr}$ Double Mutant Mice*

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Understanding the molecular mechanisms that control the balance between proliferation and death of pancreatic β-cells has important implications for diabetes. c-Kit receptor and its ligand, stem cell factor, are important for β-cell survival and maturation while Fas (CD95) is a member of the tumor necrosis factor receptor family capable of triggering β-cell apoptosis. Our previous *in vivo* studies of c-Kit deficient (*c-Kit$^{Wv/+}$*) male mice have determined that disruption of c-Kit receptor signaling leads to a severe loss of β-cell mass and function with a significant increase in Fas expression and cell apoptosis. This led us to hypothesize that activation of the c-Kit receptor signaling regulates β-cell survival by down-regulating the Fas-mediated cell death cascade. Using a double (*c-Kit$^{Wv/+}$:Fas$^{lpr/lpr}$*) mutant mouse model, we found that *c-Kit$^{Wv/+}$:Fas$^{lpr/lpr}$* male mice displayed a significant improvement in fasting blood glucose levels ($p<0.01$) and glucose tolerance ($p<0.05$) when compared with *c-Kit$^{Wv/+}$:Fas$^{+/+}$* mice. Glucose-stimulated insulin secretion tests *in vivo* revealed that *c-Kit$^{Wv/+}$:Fas$^{lpr/lpr}$* mice also displayed improved β-cell function and increased β-cell proliferation ($p<0.001$) that was associated with increased islet number and β-cell mass. These *c-Kit$^{Wv/+}$:Fas$^{lpr/lpr}$* mice also showed a significant increase in insulin and Pdx-1 mRNA expression when compared to c-*Kit$^{Wv/+}$:Fas$^{+/+}$* mice. Taken together, these results demonstrate that down-regulation of the Fas pathway in c-Kit-deficient mice results in improved β-cell survival and function. This study shows that cross-talk between the c-Kit and Fas signaling pathways is critical for the regulation of β-cell mass and function.

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105-OR

Islet Microenvironment Modulates β Cell Proliferation and Regeneration

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Using a "tet-on" inducible, bitransgenic (BT) system (rat insulin promoter-reverse tetracycline activator and tet-operon-vascular endothelial growth factor, VEGF-A), we found that increased production of VEGF-A by islet β cells dramatically increased the number of intra-islet endothelial cells, but surprisingly resulted in a loss of the majority β cells within 1 wk of doxycycline (Dox) treatment. Six weeks after Dox withdrawal, islet morphology, vascularization, mass, and function showed near normalization with a transient burst in β cell proliferation 1 wk and 2 wks after Dox withdrawal. Lineage tracing studies confirmed that the new β cells during regeneration originated from pre-existing β cells. To determine whether β cell regeneration was stimulated by a factor derived from the pancreas or circulation, we transplanted 180 wild-type (WT) and BT islets beneath the right and left kidney capsule of BT recipients, respectively. Islets were allowed to engraft for 2 wks and then graft recipients were treated with Dox for 1 wk. Tissues were then analyzed at baseline without Dox (n=3), 1 wk after Dox treatment (n=3), and 2 wks after Dox withdrawal (n=5). After 1 wk of Dox treatment, VEGF-A was induced as effectively in BT islet grafts as in native BT islets with both sites showing an extensive increase in endothelial cell mass and β cell loss. The increased VEGF-A production in BT islet graft and native BT islets had no effect on vascularization or β cell mass of the WT islet graft in the contralateral kidney. The β cell proliferation rate was similar in native BT islets, WT islet grafts, and BT islet grafts without Dox at baseline (1.4±0.2 vs. 0.8±0.2 vs. 1.3±0.5%, $p>0.05$) and 1 wk after Dox treatment (0.10±0.05 vs. 0.4±0.2 vs. 0.4±0.1%, $p>0.05$). While this low proliferation rate continued in WT islet grafts (1.3±0.2%), the β cell replication index in BT islet grafts 2 wks after Dox withdrawal had increased by 4-fold (4.2±0.8%, $p=0.0018$) and was similar to that in native BT islets (6.2±0.7%). These data indicate that β cell replication in this regeneration model is modulated by the local microenvironment and is independent of the pancreatic site and a circulating soluble factor.

106-OR

Cytoplasmic-Nuclear Trafficking of G1/S Cell Cycle Molecules: A Critical Regulator of Adult Human β Cell Replication

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Proliferation is controlled by ~30 G1/S molecules, several of which (eg., cdks 2, 4, 6, cyclins D1-3, E) can induce adult human β cell replication. Although an islet G1/S molecule "roadmap" exists, it was derived from immunoblots of whole human islets, and does not document which, if any, of the G1/S molecules are actually present in the human β cell. Here, we present the first comprehensive "human β cell G1/S molecule atlas".

Using immunohistochemistry, we report for the first time that all 30 G1/S molecules, except cyclin D2, are present in the human β cell. Surprisingly, however, although we assumed they would be nuclear, all were cytoplasmic, with only three exceptions: pRb, p21 and p57. This was independently confirmed using subcellular fractionation of human islets.

We asked whether proliferation alters the subcellular localization of the 30 G1/S molecules in the β cell. Overexpression of cyclin D3 and cdk6 led to brisk increases in human β cell proliferation (from 0.3%, to 10-15%, 30-50x; Ki67). Nuclear appearance of cdk6 and cyclin D3 increased dramatically (from 0 to 35% of β cells). In parallel, the % of β cell nuclei containing cell cycle inhibitors also markedly increased (p16: 8% to 25%; p21: 8% to 35%, p27: 0 to 15% of β cells), whereas other G1/S members remained cytoplasmic. Importantly, nuclear entry of cdk6 and cyclin D3 occurred early (~24h) and remained constant for 72h, and co-localization of cdk6 with proliferating (Ki67$^+$) β cells remained constant. In contrast, co-localization of cyclin D3 with Ki67$^+$ β cells required longer (48h) and declined precipitously by 72h. Critically, proliferation occurred in cells that were positive for nuclear cyclin D3 and/or cdk6, and negative for cell cycle inhibitors.

We: 1) provide the first comprehensive "human β cell G1/S atlas"; 2) show that all G1/S molecules (except cyclin D2) reside in the human β cell; 3) show that G1/S molecules, widely assumed to be nuclear, are in fact cytoplasmic, but can traffic to the nucleus in association with replication; 4) define a previously overlooked level of obstruction to proliferation; and, 5) define a novel target for human β cell expansion.

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For author disclosure information, see page 785.

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The Role of Rb and Rb-Family of Proteins in Cell Cycle Regulation of Pancreatic β-Cells

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Pancreatic β-cells have the potential to re-enter the cell cycle in response to increased insulin demand resulting from metabolic disorders. Therefore, evaluation of cell cycle regulation in β-cells is essential for better understanding of pathogenesis and prevention of diabetes. The tumour suppressor, retinoblastoma protein (Rb), plays a critical role in cell cycle exit and differentiation of many tissues but seems dispensable in post-mitotic cells. Indeed, Rb plays a minor role in well differentiated β-cells as shown in Rb knockout islets driven by rat insulin promoter. However, the effect of Rb in proliferating β-cells, differentiation and islet homeostasis is not known. Here we addressed this issue by deleting Rb via Pdx1-Cre in proliferating β-cells. Pdx1-Cre:$Rb^{f/f}$ mice (RbKO) exhibited improved glucose tolerance without changes in insulin sensitivity compared to wild-type (WT) littermates, and this was associated with increased β-cell mass. Interestingly, when p107, an Rb family member, was concomitantly deleted by breeding RbKO mice with $p107^{-/-}$ knockout mice (p107KO), Rb/p107 double knock-out mice (DKO) showed similar degree of enhanced glucose tolerance. However, this improvement was abolished with aging (18-22 weeks). The older DKO mice showed an increase in both β-cell proliferation and apoptosis resulting in a net loss of β-cell mass. These data reveal that while Rb loss expands β-cell mess, combined loss of Rb and p107 leads to age-dependent depletion of β-cell by apoptosis. Further understanding of the complex cell cycle regulation in β-cells may lead to new strategies for prevention and treatment of diabetes.

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108-OR

Hepatocyte Growth Factor (HGF) Ameliorates Hyperglycemia in IRS2-Deficient Mice by Increasing β-Cell Mass and Maintaining Compensatory Hyperinsulinemia

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IRS2 deficiency leads to β-cell failure, uncompensated insulin resistance and diabetes. HGF activates the PI3K/PKC-zeta/Akt signaling pathways without the recruitment of IRS2. Several studies have shown that HGF increases β-cell proliferation, survival and function. In this study, we examined whether HGF could improve glucose and β-cell homeostasis in IRS2 deficient mice. For that purpose, we cross-bred transgenic (TG) mice overexpressing HGF in the β-cell with IRS2 knockout (KO mice) mice and generated four types of mice: wild-type mice (WT), TG mice, KO mice and both TG and KO mice (TG/KO mice).

Non-fasting blood glucose was reduced ($p<0.01$) [mg/dl: 147±5 (WT, n=9); 131±7 (TG, n=11); 500±1 (KO, n=4); 237±24 (TG/KO, n=5)] and plasma insulin increased ($p<0.01$) [ng/ml: 1.5±0.5 (WT); 2.5±0.4 (TG); 0.17±0.05 (KO); 5.5±1.0 (TG/KO)] in TG/KO mice compared with KO mice. In addition, glucose tolerance was significantly improved in TG/KO mice compared with KO mice. We next determine whether this glucose homeostasis improvement correlated with alterations in β-cell homeostasis. β-cell mass was increased ($p<0.05$) in TG/KO mice compared with KO mice reaching levels similar to WT mice [mg: 1.8±0.3 (WT); 3.5±0.3 (TG); 0.1±0.1 (KO); 1.7±0.5 (TG/KO)]. Interestingly, β-cell proliferation was increased ($p<0.05$) [-fold: 1±0.3 (WT); 2.6±0.4 (TG); 0.08±0.04 (KO); 1.7±0.4 (TG/KO)] and β-cell death was decreased ($p<0.05$) [-fold: 1±0.3 (WT); 1.2±0.3 (TG); 2.7±0.5 (KO); 0.9±0.2 (TG/KO)] in TG/KO mice compared with KO mice. Previous studies have shown that IRS2 KO mouse islets had increased p27 that was limiting for beta cell growth in these mice. Preservation of β-cell mass, proliferation and survival in TG/KO mice was accompanied by diminished expression of p27 and increased levels of skp-2, a key component in p27 degradation.

Taken together, these results indicate that HGF can compensate for the lack of IRS2 by normalizing β-cell mass, proliferation, survival and enhancing circulating insulin levels. HGF may be of value as a therapeutic agent for the treatment of diabetes.

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HOW TO EASE THE PAIN OF DIABETIC NERVE DISEASE

109-OR

Progression of Cardiovascular Autonomic Neuropathy in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications Study (DCCT/EDIC)

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Cardiovascular autonomic neuropathy (CAN) is associated with increased mortality in type 1 diabetes (T1DM). We reported that in subjects with T1DM participating in DCCT/EDIC, the benefits of former intensive therapy extend to measures of CAN up to 14 years after DCCT closeout. We now evaluate the effects of prior intensive diabetes therapy on the prevalence of CAN [assessed by R-R response to paced breathing (R-R), Valsalva maneuver, and the change in postural blood pressure] in former DCCT intensive (INT) and conventional (CONV) therapy subjects 16-to-17 years after DCCT closeout. CAN outcomes were defined as ordered categorical measures as in DCCT (R-R variation < 15, or R-R variation 15-19 and Valsalva ratio ≤ 1.5 or postural drop of 10 mm Hg in diastolic blood pressure), and as continuous measures with adjustments for age and sex. Normal errors linear models were used to assess treatment group differences in CAN measures at each time point adjusting for DCCT baseline age and sex. Preliminary data obtained in 971 DCCT/EDIC participants are shown in Table.

The benefits of former intensive insulin therapy on measures of CAN have diminished over time. The overall prevalence of CAN continued to increase through EDIC year 16/17 compared with year 13/14. The adjusted R-R variation (the most robust test) remained significantly higher in the former INT versus the former CONV group while other indices were no longer significantly different.

Test	Group	N	DCCT Baseline	DCCT Closeout	EDIC year 13/14	EDIC year 16/17
CAN N (%)	INT	493	21 (4.3)	36 (7.5)	137 (27.8) *	178 (36.2)
	CONV	478	25 (5.3)	48 (10.2)	169 (35.4)	191 (40.0)
R-R Variation < 15 N (%)	INT		18 (3.7)	32 (6.8)	109 (22.1) **	146 (30.0)
	CONV		20 (4.3)	45 (9.9)	143 (30.0)	163 (34.4)
***Adjusted* R-R Variation mean ± SE ^**	INT		48.4±1.0	41.6±0.9	29.6±0.8 **	26.4±0.8 *
	CONV		47.0±1.0	39.2±0.9	26.0±0.8	23.9±0.8
Valsalva Ratio ≤ 1.5 N (%)	INT		26 (5.5)	37 (8.2)	117 (26.2)	155 (33.1)
	CONV		23 (4.9)	40 (9.0)	121 (31.4)	151 (35.1)
***Adjusted* Valsalva Ratio mean ± SE ^**	INT		2.06±0.02	1.98±0.02	1.79±0.02 *	1.72±0.02
	CONV		2.06±0.02	1.99±0.02	1.74±0.02	1.70±0.0

*** p<0.05; ** p<0.01**
^ Means adjusted for baseline age and sex

110-OR

Transplantation of p75 Positive Cells Derived from Mouse iPS Cells Ameliorates Diabetic Polyneuropathy in Mice

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Recent studies have shown that transplantation of neural crest (NC) cells which are transient embryonic structure in vertebrates is effective on spinal cord injury. Here we investigated the therapeutic potential of transplantation therapy with p75, a marker of NC cells, positive cells (p75+) derived from mouse iPS cells on diabetic polyneuropathy (DPN) in mice. C57BL6/J mice were used in this study. Diabetes was induced by intraperitoneal injection of STZ. For differentiation, mouse iPS cells derived from aged mice carrying GFP were co-cultured with PA6 cells (day 0) and were treated with BMP4 (0.5nM) during day 5-9. After 12 days of co-culture with PA6 cells, p75+ were separated by MACS and assayed expression of NC markers and trophic factors by real-time PCR. p75+ (1×10^5 cells/limb) were

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transplanted into hindlimb skeletal muscles of 16-wk diabetic mice (DM) by unilateral intramuscular injection. Four wks after the transplantation, current perception threshold (CPT), motor and sensory nerve conduction velocity (MNCV and SNCV, respectively), plantar skin blood flow (PSBF), intraepidermal nerve fiber densities (IENFDs) and capillary densities (CDs) in soleus muscles were evaluated. p75+ expressed the markers of NC cells (Snail, Sox10 and PAX3), neurotrophic factors (NGF, NT3, GDNF and BDNF) and angiogenic factors (VEGF and bFGF). Neurotrophic factor and angiogenic factor mRNA levels in p75+ were significantly increased compared with undifferentiated iPS cells. p75+ resided in soleus muscle 2 wks after the transplantation. CPT in 16-wk DM was significantly increased compared with that in normal mice (N), indicating hypoalgesia, and this deterioration was normalized in the p75+-transplanted limbs. Delayed MNCV and SNCV in 16-wk DM were significantly ameliorated by p75+ transplantation (SNCV: N; 42.5 ± 4.1 m/s, DM; 31.7 ± 3.3, p75+-treated DM; 37.6 ± 1.5). PSBF, IENFDs and CDs in 16-wk DM were decreased compared with those in N, which were significantly improved by p75+ transplantation. These results suggest that transplantation of p75+ derived from iPS cells could have therapeutic effects on DPN through paracrine actions of growth factors secreted by p75+.

111-OR

Weight Loss and Lowered Triglycerides Improves Cutaneous Re-Innervation in Non-Neuropathic Subjects with Diabetes or Prediabetes

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Obesity and dyslipidemia have been identified as risk factors for neuropathy in diabetes, independent of glucose control We hypothesize they act in part by inhibiting nerve regenerative capacity. Topical capsaicin results in denervation into the dermis. Serial skin biopsies for intraepidermal fiber density (IENFD) can then measure nerve regeneration rate. We used this capsaicin axotomy model to compare re-innervation capacity between age matched cohorts of normal controls, subjects with metabolic syndrome, and subjects with type 2 diabetes. Subjects and controls have neither symptoms nor exam signs of neuropathy. IENFD at the patch site is compared immediately after 48 hours of capsaicin, then 30 days and 90 days later to calculate a regeneration rate (expressed in fibers/mm/day). In the second phase of the study, subjects participate in 6 months of nutritional counseling and twice weekly supervised exercise. During the second half of this regimen the re-innervation protocol is repeated.

A similar baseline reinnervation rate was observed for subjects with metabolic syndrome (0.083 +/- 0.047 f/mm/day) and diabetes (0.097+/- 0.067). These reinnervation rates are reduced compared to study controls (0.137 f/mm/day). No metabolic parameter (BP, BMI, lipid indices) significantly correlated with baseline re-innervation rate. Following 3 months of diet and exercise, there was a significant improvement in 30-day reinnervation rate for all subjects (0.092 +/- 0.47 before versus 0.133 +/- 0.042 post, p= 0.03). Baseline metabolic status (diabetes vs. metabolic syndrome) did not affect post-exercise re-innervation rate. There was significant improvement in re-innervation rate in subjects who reduced triglycerides (p<0.002) or BMI (p=0.002) during the lifestyle intervention compared to those who did not. These results suggest metabolic syndrome features suppress cutaneous re-innervation even before diabetes is present. With intensive diet and exercise, cutaneous re-innervation capacity increases, and this improved capacity appears to respond to weight loss and reduction in ectopic lipids more than to changes in glucose control.

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Neurotensin Improves Diabetic Wound Healing Via Inducible Nitric Oxide Synthase

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Peripheral neuropathy impairs wound healing in diabetes. We evaluated if neurotensin (NT) is promoting wound healing via iNOS by using the iNOS knockout (iNOSKO) mice or treating wounds with an iNOS inhibitor, 1400w.

We studied wild-type (WT) C57BL6/J, WT diabetic, iNOSKO, and diabetic iNOSKO mice. Diabetes was induced by streptozotocin (STZ, 150 mg/kg ip). After 8 weeks of diabetes, two 6 mm excision wounds were created in the dorsum of each mouse. One wound was treated daily with NT (50 μg) or 1400w (20 μg) and the other with saline. The wound healing process was monitored up to 10 days by acetate tracing. Inflammatory and angiogenic markers were quantified by q-RT-PCR.

NT improves wound healing in WT, WT diabetic and iNOSKO mice (p<0.05), but not in diabetic iNOSKO mice. Moreover, 1400w treatment impairs wound healing in WT mice (p<0.05), however, in combination with NT, wound healing do not change in WT or WT diabetic mice. iNOS, COX-2, TNF-alpha, MCP-1, KC and IL6 expression are increased after wounding (p<0.05) in WT mice, compared to baseline. In WT diabetic mice, COX-2 and TNF-alpha expression induced after wounding is significantly lower than in WT mice (p<0.01). NT increases iNOS (p<0.05), IL6 (p<0.05) and KC (p<0.05) in WT and WT diabetic mice, but not in iNOSKO mice. In addition, NT decreases TNF alpha (p<0.05) in WT and WT diabetic mice. VEGF expression increases after wounding in WT and WT diabetic mice (p<0.05) but not in iNOSKO mice. NT promotes VEGF expression in WT and WT diabetic mice (p<0.05), but not in iNOSKO mice. VEGFR2 expression increases after wounding in WT (p<0.05), WT diabetic (p<0.05) and iNOSKO mice (p<0.01), and NT treatment increases VEGFR2 expression (p<0.05) at day 10 only in WT and WT diabetic mice.

These results show that NT has a pro-inflammatory effect by increasing iNOS, IL6 and KC expression and an anti-inflammatory effect by decreasing TNF alpha expression. Moreover, NT has an angiogenic effect increasing the expression of VEGF, VEGFR2 in normal and diabetic mice but not in iNOSKO mice. These results suggest that the effects of NT in wound healing can be via iNOS signaling.

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113-OR

Painful Diabetic Neuropathy in a Large Community-Based Diabetes Population in the UK—The Size of the Problem

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Diabetic painful neuropathy causes significant morbidity and has a detrimental effect on quality of life. Population-based studies defining the prevalence and natural history of this condition are limited to a few small studies of several hundred patients. We have had the unique opportunity to assess in a large, community-based diabetic population: a) prevalence of painful neuropathic symptoms; b) relationship between symptoms and clinical severity of neuropathy; c) the role of diabetes type, gender and ethnicity. Over 4 years, trained podiatrists examined 15,692 diabetic patients attending primary and secondary health care clinics in northwest England. Neuropathy severity was assessed using the Neuropathy Disability Score (NDS) and symptoms using the Neuropathy Symptom Score (NSS). The prevalence of painful diabetic neuropathy (NSS≥5) was 34%. Whilst 60% of patients with severe clinical neuropathy (NDS>8) had painful symptoms, 26% without clinical neuropathy (NDS≤2) also had painful symptoms. Worsening neuropathic severity was associated with increasingly severe neuropathic symptoms (p<0.001). The age- and diabetes duration-adjusted risk of painful neuropathic symptoms in Type 2 diabetes was double that of Type 1 (OR=2.1 [1.7-2.4, 95% CI], p<0.001) and not affected by severity of neuropathy, insulin use, foot deformities, smoking or alcohol. Women had 50% increased risk of painful symptoms compared with men (OR=1.5 [1.4-1.6], p<0.0001) after adjustments for age, diabetes duration and severity of neuropathy. Despite less clinical neuropathy in Asians (14%) compared with Europeans (22%) and African-Caribbeans (21%), p<0.0001, painful symptoms were greater in Asians (38%) than Europeans (34%) and African-Caribbeans (32%), p<0.0001. Our data indicate a large morbidity from neuropathic pain in the diabetes community with one-third of patients having painful neuropathy, regardless of degree of neuropathic deficit. Painful neuropathy was more prevalent in patients with Type 2 diabetes, in women and in people of South Asian origin. These areas demand further investigation and highlight key groups who may warrant screening for painful diabetic neuropathy.

114-OR

Abnormal Central Pain Processing in Painful Diabetic Neuropathy: A Functional Magnetic Resonance Imaging Study

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Painful diabetic neuropathy (DN) is a distressing diabetic complication associated with a high degree of suffering. Using functional magnetic resonance imaging (fMRI) we sought to investigate regional brain activation during pain processing.

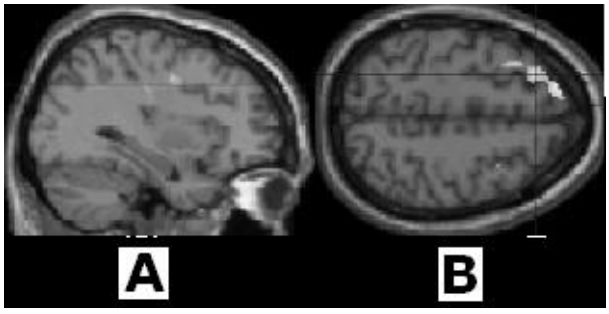
Methods: 15 painful-DN and 12 healthy volunteers (HV) underwent neurophysiological assessment. DN subjects had neuropathic pain below the knees. Mood disorders were assessed using the Hospital Anxiety and Depression Scale (HADS). All subjects underwent box car fMRI whilst heat pain was applied to the anterior thigh (non-neuropathic region) at a pain level of at least 7 on an 11-point Likert scale. Images were analysed using SPM5.

For author disclosure information, see page 785.

ADA-Funded Research

Results: In painful-DN, there was significantly (p<0.001, uncorrected) *greater* activation in the ventromedial prefrontal cortex (Brodmann's area (BA) 9, stereotactic coordinates: x=28, y=34, z=24; peak t[5]=3.15; Figure 1B) and cingulate gyrus (-8,2,30;3.09; Figure 1A) compared with HV. Subjects with painful DN were then divided into two groups based on HADS-D. Painful-DN subjects with depression (HADS-D>8,n=9) had significantly *greater* activation (p<0.001, uncorrected) in the prefrontal cortex (BA 9, 8,46,52;3.72) compared with those without (HADS-D<8,n=6).

Discussion: BA9 and the cingulate gyrus are involved in emotional pain processing. Increased activation in these regions suggests a greater perception of suffering in painful-DN. Increased activation in BA9 in subjects with depressive symptoms suggests a neurocortical link between mood disorders and abnormal acute pain processing. Using this novel technique, we are gaining new insights into brain regions involved in abnormal pain processing which maybe amenable to targeted treatments.



Supported by: Juvenile Diabetes Research Foundation

115-OR

Corneal Confocal Microscopy Detects Neuropathy in Impaired Glucose Tolerance

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Recent studies employing skin biopsies have demonstrated a higher than expected prevalence of impaired glucose tolerance (IGT) in patients with idiopathic small fibre neuropathy leading to the suggestion that IGT may cause neuropathy. However, the prevalence of neuropathy in IGT per se is not established.

We have undertaken detailed assessment of neuropathy (neuropathy symptom profile (NSP), neuropathy disability score (NDS), large fibre (peroneal and sural nerve electrophysiology, vibration perception threshold (VPT)) and small fibre (warm and cold thresholds (WT/CT) (°C), sudomotor function (Neuropad), Corneal confocal microscopy (CCM) and non-contact corneal aesthesiometry (NCCA) fibre function in 32 subjects with IGT and 10 healthy controls (C).

In subjects with IGT v C; age was (58.5±12.1 v 52.7±3.5 yrs), HbA1c (%) (6.3±0.4 v 5.8±0.2), total cholesterol (mmol/l) (5.1±1.1 v 5.3±0.8), HDL (1.2±0.3 v 1.5±0.5) and triglycerides (2.3±1.3 v 1.5±0.9). The IGT group had a significantly higher NSP (3.9±4.1 v 0, P<0.0001), NDS (2.4±2.8 v 0, P<0.0001), VPT (14.7±10.7 v 7.3±5.7, P<0.0001), but no difference in sural nerve amplitude (μV) (13.7±9.0 v 16.4±9.1), sural NCV (m/s) (48.2±5.8 v 49.9±5.9) and peroneal NCV (45.5± 4.5 v 47.2±3.9). However, CT (26.6±5.5 v 29.2±2.5, P=0.02) was significantly increased and % colour change of Neuropad (68.7±36.2 v 100, P<0.0001) was decreased, with no difference in WT (40.0±5.1 v 37.9±3.4). Furthermore, corneal sensitivity (mBar) (1.1±1.0 v 0.6±0.3, P=0.08), corneal nerve fibre density (no.mm2) (30.2±6.4 v 37.2±4.4, P=0.005), branch density (no.mm2) (61.8±43.2 v 108.3±44.3, P=0.003) and length (mm/mm2) (24.6±6.4 v 28.5±2.9, P=0.04) were decreased with an increase in tortuosity (20.6±6.0 v 14.9±2.1, P=0.001).

We demonstrate evidence of significant small but not large fibre neuropathy in subjects with IGT which is readily detectable with CCM compared to standard methods.

Supported by: NIH

116-OR

Small Fibre Function Assessed Using a New LDI Flare Technique in Type 1 Diabetes: Importance of Microvascular Disease and Duration-Averaged HbA1c

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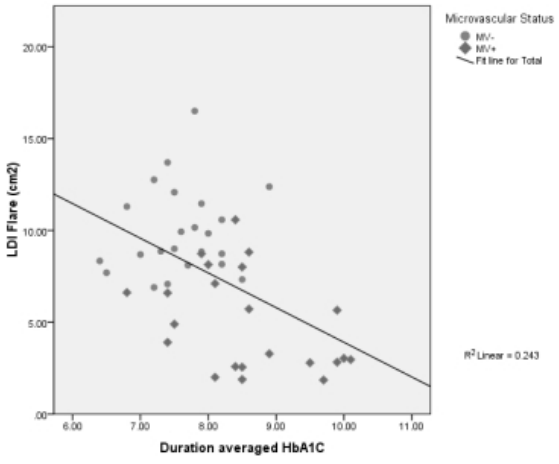
Studies in IGT and Type 2 diabetes suggest small fibre neuropathy occurs early in the disease and precedes clinical neuropathy. It is unclear if this is true for Type 1 diabetes. Using the axon-flare reflex as a marker of small fibre function we found preserved function in long duration Type 1 subjects free from clinical neuropathy and microvascular disease. Given this surprising finding, we further explore the impact of microangiopathy and diabetes control on small fibre function in Type 1 subjects without clinical neuropathy. Groups- Type 1 with [microalbuminuria and/or retinopathy] (MV+, n=24) and without microangiopathy (MV-, n=24), and healthy controls (HC, n=24); matched for age, sex, height, diabetes duration and all with a NDS ≤2. Axon reflexes were elicited using a new technique involving foot skin heating to 47^0C for 3min (our previous method used 44^0C for 20 min) and measuring the induced flare using laser Doppler Imagery (LDI Flare).LDI Flares were reduced in MV+ compared with HC (5.0±1.8 cm^2 v 10.0±3.0, p<0.0001) and MV- groups (9.9±2.9, p<0.0001). MV- and HC groups did not differ. There was no difference in diabetes duration between MV- and MV+ (17.5±5.7 and 20.8±5.2yr, p=0.06) nor current HbA1c (8.0±1.2 v 8.0±0.9, p=0.53); neither parameters correlated with flare size. In contrast, duration-averaged HbA1c was higher in MV+ group (8.6 v 7.6, p<0.001) and correlated with LDI Flare size; r=0.49, p<0.001.



The presence of microvascular disease and high duration-averaged HbA1c are associated with small fibre dysfunction in Type 1 patients. In contrast function is preserved in those free from microvascular disease despite the duration of the hyperglycaemia. This differs from IGT and Type 2 diabetes suggesting different aetiologies in the two forms of diabetes.

NUTRITION—CLINICAL

117-OR

Vitamin D Status and Progression to Diabetes in Patients at Risk for Diabetes: An Ancillary Analysis in the Diabetes Prevention Program Randomized Controlled Trial

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The objective of the study was to investigate the association between vitamin D status and risk of incident diabetes in the Diabetes Prevention Program (DPP), a 3-arm trial comparing intensive lifestyle modification or metformin vs. placebo for prevention of diabetes in patients with pre-diabetes.

Over a mean 3.2-year follow-up period, we assessed the association between plasma 25OHD, measured at yearly intervals, and incident diabetes in the cohort of 2,039 participants randomized to either intensive lifestyle (n=1,017) or placebo (n=1,022). Analyses were adjusted for age, gender, BMI, race, UV index, family history of diabetes, hypertension, smoking, alcohol consumption, C-reactive protein, kidney function, physical activity

For author disclosure information, see page 785.

and intervention. Variables measured at multiple study time points (25OHD, BMI and physical activity) entered the analyses as time-varying "lagged" covariates.

After multivariate adjustment, participants in the highest tertile of 25OHD (median 25OHD 30.1 ng/mL) had a hazard ratio of 0.74 (95%CI, 0.59 to 0.93) for developing diabetes compared to participants in the lowest tertile (median 25OHD 12.8 ng/mL). When analyses were repeated by categories of 25OHD based on cut-points suggested by the 2010 Institute of Medicine Dietary Reference Intake report on calcium and vitamin D, there appeared to be a 'dose-effect' with the hazard ratio for incident diabetes being lowest (0.46; 95%CI, 0.23 to 0.90) in the highest category (25OHD ≥50 ng/mL) compared to the lowest category (25OHD <12 ng/mL) with no evidence of a threshold. In subgroup analyses by tertiles, the association was in the same direction in placebo (0.72; 95%CI 0.53, 0.96) vs. lifestyle arm (0.80; 95%CI 0.54, 1.14) (p=0.67 for interaction).

Higher vitamin D status, assessed repeatedly during the follow-up period, is associated with lower risk of diabetes among persons at high risk for diabetes, even after adjusting for lifestyle interventions known to decrease diabetes risk. The role of vitamin D in reducing diabetes risk needs to be confirmed in vitamin D supplementation trials.

Supported by: R01DK79003

118-OR

Common Variant Near *GIPR* Gene Modifies Fasting Glucose and Related Traits Response to Weight-Loss Diets in a Two-Year Randomized Trial

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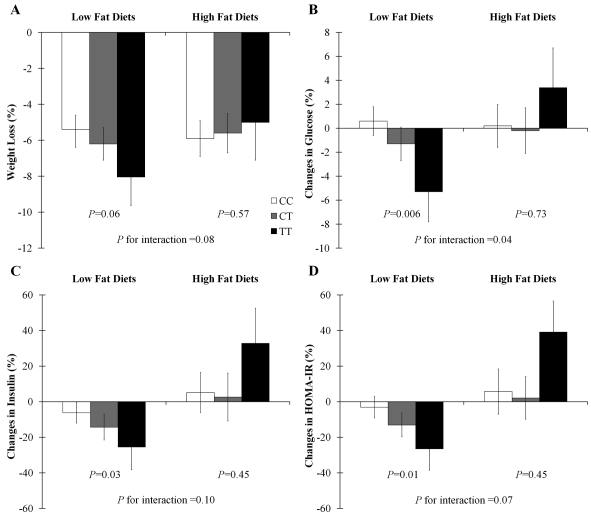
Gastric inhibitory polypeptide receptor (*GIPR*) was suggested to play roles in glucose and energy metabolism. Mice with disruption of *Gipr* are resistant to high-fat diet-induced obesity. Recent genome-wide association studies idenftified genetic variants near *GIPR* associated with obesity risk and glucose. We examined whether the *GIPR* rs2287019 modifies the changes in body weight and fasting glucose and related traits in response to weight-loss diet interventions in the Pounds Lost Trial. We genotyped this variant in 763 overweight adults, who were randomly assigned to one of four diets for 2 years; the targeted percentages of energy derived from fat, protein and carbohydrate in the four diets were 20, 15 and 65%; 20, 25 and 55%; 40, 15, and 45%; 40, 25 and 35%. We assessed the progress in weight loss, fasting glucose and related traits by genotypes at 6 months and 2 years in comparison of low (20%) vs. high (40%) fat diets. At 6 months, the nonrisk-conferring T-allele of rs2287019 was associated with more weight loss (β=-1.05±0.56, *P*=0.06), and more decreases in glucose (β=-2.33±0.86, *P*=0.006), insulin (β=-8.76±4.13, *P*=0.03) and HOMA-IR (β=-10.52±4.39, *P*=0.01) among participants assigned to the low-fat diets, while no significant genotype effect on changes in these traits was observed in those assigned to the high-fat diets (β=0.34±0.59, 0.31±0.92, 3.44±4.52 and 3.82±5.10; all *P*>0.44; *P* for interaction=0.08, 0.04, 0.10 and 0.06; respectively).



At 2 years, most participants regained body weight, probably due to diminished adherence that occurred between 6 months and 2 years in the intervention, and the genotype effects were significantly attenuated. In conclusion, we found that the T-allele carriers of *GIPR* rs2287019 might obtain more weight loss and improvement of glucose metabolism than non-carriers with a low-fat diet.

119-OR

Lifestyle Change and Metformin Improve Biomarkers of Inflammation, Endothelial Dysfunction and Coagulation in the Diabetes Prevention Program (DPP)

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Alterations in inflammation, endothelial dysfunction and coagulation biomarkers in participants at high risk for diabetes provide an opportunity for early use of lifestyle or pharmacologic intervention to lower the activity of these processes. However there have been few large studies of these interventions' effects on multiple biomarkers in multiethnic, high risk cohorts over an extended period. We measured a group of markers at baseline and after 1 year of lifestyle (LS) and metformin (MET) intervention compared to placebo (PLAC) in samples from 3194 DPP participants. All biomarker levels were lowered by both LS and MET except adiponectin, which was raised (Table). When the intervention effects were assessed by demographic subgroups using interaction terms in regression models, age (in 1 year units) attenuated the change in leptin (β=-0.08, p=0.03) in LS and in tPA in both LS (β=-0.04, p<0.01) and MET (β=-0.04, p<0.01), whereas it enhanced adiponectin change (β=0.02, p=0.02) in LS. Women had a greater E-selectin (β=3.1, p<0.01) and tPA change (β=0.68, p=0.016) but a smaller leptin change (β=-2.2, p<0.01), and Blacks had more robust E-selectin and sICAM1 changes (β=2.6, p=0.04; β=12.6, p=0.01) in LS only. These findings demonstrate that both LS and MET have favorable effects on markers of inflammation, endothelial function and coagulation, but that these effects may be influenced by age, sex and race/ethnicity.

	PLAC (Baseline)	PLAC (1yr change)	LS (Baseline)	LS (1yr change)	MET (Baseline)	MET (1yr change)
LogCRP (mg/L)	.6	-0.00	.58	-.14*[2,3]	.58	-.06*[1]
IL-6 (pg/ml)	2.50	.04	2.50	-.26*[2]	2.46	-.18*[1]
Fibrinogen (mg/dl)	385.9	3.79	384.6	-9.75*[2,3]	379.2	-.54
sICAM1 (ng/m	248.4	-4.86*	250.9	-22.4*[2,3]	248.8	-17.3*[1]
sE-Selectin (ng/ml)	46.8	.08	45.3	-4.8*[2,3]	46.1	-.25
tPA (ng/ml)	11.39	-.71	11.34	-2.52*[2,3]	11.26	-2.12*[1]
Adiponectin (µg/ml)	7.78	.09	7.97	.83*[2,3]	8.07	.22*
Leptin (ng/ml)	24.5	.51	23.6	-3.65*[2,3]	23.9	-1.76*[1]
MCP-1 (pg/ml)	150.1	-9.3*	148.6	-12.9*	154.4	-8.05*

*p<0.05 vs. baseline; P-values are adjusted for multiple comparisons: [1]p<0.05 for PLAC vs. MET; [2] p<0.05 for PLAC vs. LS: [3] p<0.05 for MET vs. LS

Supported by: NIH

120-OR

Accuracy of Carbohydrate Counting Does Not Relate to Post-Prandial Excursion in Young Children with Type 1 Diabetes

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In type 1 diabetes (T1DM), carbohydrate counting is a common strategy used to estimate the carbohydrate content of meals. Research has found that greater accuracy in carbohydrate counting is associated with lower HbA1c levels in school-age youth with T1DM. However, it is not known if carbohydrate counting accuracy also has an effect on post-prandial excursion. We examined parents' accuracy in carbohydrate counting and post-prandial glucose levels in young children with T1DM. Parents' carbohydrate estimations were extracted from the insulin pumps of 8 young children (5.1±1.4 years). In addition, parents kept a 3-day record of all foods and beverages consumed by their child which were analyzed using computer analyses. Young children's post-prandial glucose was recorded using continuous glucose monitoring. Glucose excursion was calculated based on the percent of readings greater than 10 mmol/l within 2 hours of the start of the meal. Group data are presented first, followed by data pairing meals with children's corresponding post-prandial excursion. Overall, there were 55 meals recorded by parents and paired with post-prandial glucose levels. Average meal carbohydrate content as estimated by parents was

For author disclosure information, see page 785.

45±27 grams compared to 54±26 grams according to computer analyses. Thus, parents' mean carbohydrate estimates were 91±55% of the computer calculated intake, suggesting a tendency to underestimate carbohydrate intake overall. To examine the effect of carbohydrate counting accuracy on corresponding post-prandial excursion, carbohydrate ratios were grouped by quartile according to accuracy (quartiles 2-3 most accurate). ANOVA found no differences in young children's post-prandial excursions [F(3,51)=0.38, p= 0.76] based on quartiles of accuracy. While accuracy in carbohydrate counting may relate to lower HbA1c levels in school-age youth with T1DM, in young children there does not appear to be a difference in children's immediate post-prandial excursion based on accuracy. When working with families of young children, clinicians may want to focus on other factors affecting glucose excursion than parents' accuracy in carbohydrate counting.

121-OR

Neither Short Nor Six Week Feeding of an Isocaloric High Saturated Fat Diet Alters Insulin Sensitivity in Healthy Humans

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High fat diets can induce adiposity and diabetes mellitus type 2. Studies in humans showed rapid development of insulin resistance when a hypercaloric high-fat diet was applied. This study investigated the effects of a sudden switch from healthy low fat to isocaloric high-fat diet on body weight (BW), insulin sensitivity (S_I) and serum lipids in healthy humans.

42 healthy women and men, age 18 - 70, were investigated for 12 weeks. An isocaloric diet rich in carbohydrates (55% carbohydrates, 15% protein, 30% fat) was applied with dietary counselling for 5 weeks and afterwards nutrients were supplied for 6 days. Then an isocaloric diet rich in saturated fat (40% carbohydrates, 15% protein, 45% fat) was applied for 6 days with nutrients supplied, followed by 4 weeks with dietary counselling and for another 6 days when nutrients were supplied again. A frequently sampled iv glucose test (FSIGT), anthropometry and blood tests for total Cholesterol, LDL and Triglycerides (TG) were performed after the period of diet rich in carbohydrates (*carb*), after the first 6 days (*short HF*) and at the end of the period of diet rich in fat (*long HF*). The FSIGT was analyzed according to the Minimal Model.

Over the time of intervention BW and Body Mass Index (BMI) was constant: Carb 69.83±2.08 kg (BMI 23.28±0.49), short HF 69.82±2.05 kg (BMI 23.38±0.48) and long HF 70.16±2.09 kg (BMI 23.50±0.49). The S_I index obtained from modelling of the FSIGT did not change: Carb 13.14±1.52 [(mu/l)^-1.min^-1], short HF 15.09±3.07 [(mu/l)^-1.min^-1] and long HF 12.74±1.33 [(mu/l)^-1.min^-1]. Total Cholesterol was 4.70±0.12 mmol/l for carb, 4.79±0.13 mmol/l for short HF and 5.00±0.13 mmol/l for long HF. There was also no change observed in LDL (2.88±0.11 mmol/l for carb, 2.95±0.11 mmol/l for short HF and 3.07±0.12 mmol/l for long HF) and TG (1.01±0.07 mmol/l for carb, 1.18±0.13 mmol/l for short HF and 1.15±0.10 mmol/l for long HF). P=NS by oneway ANOVA for all parameters tested.

These results show that high saturated fat diets do not induce insulin resistance in the absence of caloric excess in healthy humans indicating that energy balance may be more important than diet composition.

122-OR

Daily Flaxseed Consumption Improves Glycemic Control in Individuals with Pre-Diabetes: A Randomized, Controlled Study

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Flaxseed is a good source of omega-3 fatty acids and viscous fiber. Consuming flaxseed on a regular basis may reduce the risk of progressing from pre-diabetes to type 2 diabetes and heart disease through various mechanisms. The purpose of this randomized, cross-over study was to determine the effect of consuming 0, 13 or 26 g of ground flaxseed daily in addition to habitual diet, on glycemic control and inflammation in 25 overweight or obese men and postmenopausal women with pre-diabetes. Each diet treatment period lasted 12 weeks. Blood samples collected at the beginning and end of each diet treatment period were analyzed for fasting glucose, insulin, homeostatic model assessment (HOMA), fructosamine, high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), adiponectin, and fatty acid composition. Data were analyzed by comparing the mean change for each endpoint for each diet treatment period. Glucose, insulin, HOMA and normalized percent of α-linolenic fatty acid (ALA) showed a significant difference by treatment (MANOVA, p=0.036, p=0.013, p=0.008 and p=0.024 respectively). Paired t-tests showed change in glucose for the 13 g intervention was significantly different than the 0 g period [13g = -2.10 ± 1.66 mg/L (mean ± SEM), 0g = 9.22 ± 4.44 mg/L, p=0.036]. The change in insulin for the 13 g intervention was significantly different than the 26 g (p=0.021) and 0 g (p=0.013) periods (13g = -2.12 ± 1.00 mU/L, 26g = 0.67 ± 0.84 mU/L, 0g = 1.20 ± 1.16 mU/L). The change in HOMA for the 13 g period was significantly different than the 26 g (p=0.012) and 0 g (p=0.008) periods (13g = -0.71 ± 0.31, 26g = 0.27 ± 0.24, 0g = 0.51 ± 0.35). The change in normalized percent of ALA for the 0 g period was significantly different than the 13 g (p=0.024) and the 26 g (p<0.001) periods (13g = 0.20 ± 0.04, 26g = 0.35 ± 0.07, 0g = -0.01 ± 0.07). Mean changes in fructosamine, hs-CRP, adiponectin and IL-6 were not significantly different between treatment periods. Flaxseed intake can decrease glucose, insulin, and improve insulin sensitivity as part of a habitual diet in individuals with pre-diabetes.

ADA-Funded Research

123-OR

Liver Steatosis Evaluated through Chemical-Shift Magnetic Resonance Imaging and Liver Enzymes; Effect of Weight Loss Obtained with Intragastric Balloon and Gastric Banding

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Liver steatosis (LS) is frequent in morbid obesity. Liver biopsy is required for definite diagnosis of LS, but can not be used for follow-up studies. Spectroscopy-magnetic resonance imaging (MRI) and chemical-shift MRI are accurate indices of LS, more than computerized scans or echography, and can be used in follow-up studies. We evaluated the effect of weight loss on LS, evaluated through chemical-shift MRI (MRI-LS) and liver enzymes (AST, ALT, gGT, ALP). 18 obese subjects (16 W/2 M, BMI 43.7±6.6 kg/m^2) underwent intragastric balloon (BIB) or gastric banding (LAGB), and 12 obese subjects (7 W/5 M, BMI 41.1±9.1 kg/m^2) received hypocaloric diet; all subjects were re-evaluated after 6 months. At baseline, MRI-LS correlated with echography-LS (p=0.006), ALT (p=0.0004), AST (p=0.006), gGT (p=0.01). After 6 months, subjects undergoing BIB or LAGB had significant changes of BMI (p=0.0001), ALT (p=0.0001), AST (p=0.0001), GGT (p=0.04), ALP (p=0.0001), and of MRI-LS (P=0.003). Diet-treated obese subjects had no significant change of any parameter under study. Also after 6 months, MRI-LS correlated with ALT (p=0.001), AST (p=0.006), gGT (p=0.01), and ALP (p=0.001). These data indicate that significant weight loss is associated with decrease of LS, and suggest that chemical-shift MRI is adequate to evaluate LS in follow-up studies.

Supported by: Progetto PRIN, Ministero Italiano dell' Universitàe della Ricerca

124-OR

Dietary Intake in Japanese Patients with Type 2 Diabetes: Japan Diabetes Complication Study (JDCS)

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Although there have been many observational studies of dietary intake and eating patterns among populations with diabetes in Western countries, there have been no such large-scale studies from Asian regions. Therefore, the dietary situation among Asian subjects with diabetes has not been clarified. This study aimed to elucidate the actual dietary intake among Japanese with type 2 diabetes who participate in the Japan Diabetes Complication Study (JDCS). The JDCS is a nationwide cohort of Japanese patients with type 2 diabetes aged 40–70 years from outpatient clinics in 59 universities and general hospitals. Data on nutritional and food intake from 1516 participants who completed the food frequency questionnaire based on food groups (FFQg) were analyzed. Mean energy intake for all participants was 1737 +/- 412 kcal/day, and mean proportions of total protein, fat, and carbohydrate comprising total energy intake were 15.7%, 27.6% and 53.6%, respectively. Results showed that the proportion of fat consumption was similar to that reported in the general Japanese population and was considerably lower than that reported among diabetic subjects in many Western countries, which ranged from 35 to 45%. The proportion of fat consumption in the JDCS patients was also within the suggested range (generally 25-35% fat) of a 'low-fat energy-restricted diet', which has been traditionally recommended in Western countries. In addition, JDCS patients were found to consume large amounts of grain (191g daily), fruits (133g), green-yellow vegetables (138g), and other vegetables (186g). As a protein source, consumption of fish (100g) and soybean products (71g) was larger than that of meat (50g) and eggs (29g). These results imply that dietary

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content and patterns among Japanese patients with type 2 diabetes are quite close to those reported as decreasing the risk of diabetes in Europe and America. These basic dietary features of Asians with diabetes, together with future study results regarding their associations with glycemic and other control parameters, would contribute to establishing ethnic-specific medical nutrition therapy for diabetes.

STUDIES IN CHILDHOOD OBESITY AND TYPE 2 DIABETES

125-OR

Effects of an Intravenous Lipid Challenge and FFA Elevations on Intramyocellular Lipid (IMCL) Content and *In Vivo* Insulin Sensitivity in African-American (AA) Versus Caucasian (C) Adolescents: Are There Ethnic Differences?

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This investigation tested the hypothesis that:1) in both AA and C adolescents, acute elevations in plasma FFA levels result in increased IMCL and decreased insulin sensitivity; and 2) the increase in IMCL will be significantly greater in AA than in C youth potentially explaining race-related differences in insulin sensitivity. Nine AA (14.2 ± 1.7 yrs) and 14 C (14.4 ± 1.7 yrs) normal weight (BMI≤85th) adolescents underwent a 3-h hyperinsulinemic-euglycemic clamp, on two occasions in random order, with overnight 12-hr infusion of: 1) 20% intralipid (IL) and 2) normal saline (NS). IMCL was quantified by ^{1}H-magnetic resonance spectroscopy in tibialis anterior muscle before and after IL infusion. Age, tanner stage, BMI and body fat (%) were similar between AA and C, but triglyceride (TG) was lower in AA than in C. During IL infusion, plasma TG and FFA levels increased significantly (P<0.01) with no differences between AA and C, however, the increase in fat oxidation was greater in AA than in C (interaction, P=0.016). IL infusion compared with NS was associated with increases (P<0.01) in IMCL which were comparable between AA (122%, NS: 1.8 ± 0.9 vs. IL: 4.0 ± 1.2 mmol/kg ww) and C (96%, NS: 2.6 ± 2.1 vs. IL: 5.1 ± 2.4 mmol/kg ww), and similar reductions (P<0.01) in insulin sensitivity between AA (37%, NS: 9.3 ± 2.5 vs. IL: 5.9 ± 1.5 mg/kg/min per µU/ml) and C (39%, NS: 13.9 ± 6.4 vs. IL: 8.5 ± 3.8 mg/kg/min per µU/ml). Our findings demonstrate that acute elevations in plasma FFA levels are accompanied by a significant increase in IMCL and reduction in insulin sensitivity in healthy adolescents without ethnic differences in either.

Supported by: 2R01-HD-27503, UL1 RR024153 CTSA

126-OR

The Triglyceride–HDL Cholesterol Ratio: A Potentially Useful Marker of Insulin Resistance in Obese Children and Adolescents of Different Racial/Ethnic Background

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We evaluated whether the triglyceride/HDL-Cholesterol ratio or triglycerides levels alone represent a reliable marker of Insulin Resistance in a large multiethnic cohort of obese youths.

1452 obese adolescents had an OGTT and a fasting lipid profile. Insulin sensitivity was estimated using the Whole Body Insulin Sensitivity Index (WBISI), HOMA-IR, and evaluated in a subgroup of 146 obese youths by the Hyperinsulinemic-euglycemic clamp. The cohort was divided by ethnicity (612 Whites, 357 Hispanics and 483 African Americans) and then stratified into ethnicity specific tertiles of TG/HDL-ratio. Differences across tertiles were evaluated and the association between the TG/HDL ratio and insulin sensitivity was defined by a multiple stepwise linear regression analysis. The area under the ROC curve (AUC) was determined to calculate the TG/HDL-ratio cutoff to identify insulin resistant subjects in each ethnic group.

In each ethnic group and across rising tertiles of TG/HDL, insulin sensitivity (WBISI), progressively decreased. The cutoffs for TG/HDL-ratio were 2.27 in Whites, 2.98 in Hispanics and 1.96 in African Americans. The odds of presenting with IR in youths with TG/HDL ratio higher than the cutoff, was 6.023 (95% CI 2.798–12.964; p<0.001) in Whites, 2.998 (95% CI 1.525–5.891; p<0.001) in Hispanics and 7.943 (95% CI 3.698–17.06; p<0.001) in African Americans.

In obese youths, the TG/HDL ratio is strongly associated with IR and represents an acceptable marker of insulin sensitivity. TG/HDL ratio could be a simple and practical tool to identify obese children at increased risk of Type 2 Diabetes.

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Ethnic Differences in Hepatic Fat Deposition in Pre-Diabetic Overweight Latino and African-American Youth

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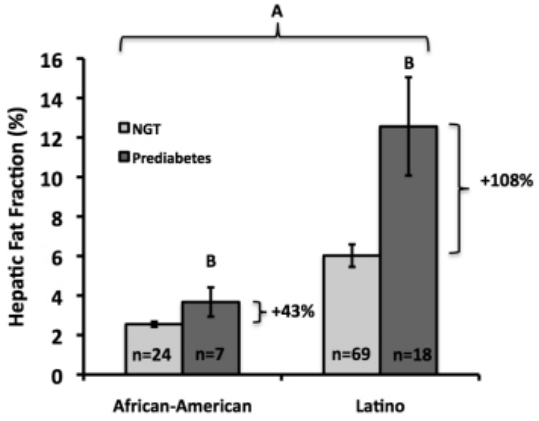
The role of hepatic fat in the progression to pre-diabetes in overweight minority youth is unclear. Our objective is to examine ethnic differences in hepatic fat deposition by pre-diabetic status. We hypothesized that the pattern of hepatic fat deposition in prediabetic youth would differ in Latino (LA) vs African American (AA) youth. From 118 healthy overweight children and adolescents (31 AA/87 LA, 77M/40F; age 14.3±2.4 yrs; BMI %tile: 96.7±3.3), we defined 2 groups: Normal Glucose Tolerant (NGT, n=93; fasting glucose (FG) <100mg/dL, 2hr glucose <140mg/L, and A1C <6.0%);and Prediabetic (PD, n=25: FG ³100mg/dL and/or 2hr glucose 140-199mg/dL and/or A1C 6.0-6.4%). Measures included A1C; OGTT glucose and insulin; visceral adiposity & hepatic fat fraction (HFF) by MRI; body fat by DEXA. The PD group had higher BMI %tile and body fat than NGT group (p<.01) but there were no differences in gender, age, or Tanner stage. Compared to NGT group, PD group had higher A1C (6.12±0.10 vs. 5.57±0.29%, p<.001), FG (89.0±6.5 vs. 85.4±6.3 mg/dL, p<.01), and fasting insulin (20.6±16.3 vs. 12.6±6.9mU/mL, p=.02). Compared to AA, LA had higher percent body fat (36±0.8 vs 32±1.8%, p=.03) and 2hr glucose (117±2.4 vs 106±6.9mg/dL, p=.04), but A1C and FG did not differ. An ethnicity by PD interaction was marginally significant independent of age, gender and Tanner stage (p=.06). When stratified by ethnicity, PD AAs had 43% higher HFF than NGT AAs (3.7±1.5 vs. 2.6±0.6%, p<.01), while PD LAs had 108% higher HFF than NGT LAs (12.6±2.5 vs 6.0±0.8%, p<.01). In summary, HFF is higher in PD youth of both ethnic groups. However, the relative increase in HFF in PD vs NGT is greater in LA than AA children. This may suggest a difference in the role of hepatic fat deposition in promoting diabetes risk in the different ethnic groups.



Legend
A: Ethnicity x Prediabetes interaction p=0.06 after adjusting for gender, age and Tanner stage.
B: Within ethnicity differences in HFF p<0.01 after adjusting for gender, age, Tanner stage, total fat and visceral adiposity.

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128-OR

Metabolic Effects of Antipsychotics in Children (MEAC): Primary Endpoint Results

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Mental health conditions are associated with higher risk for obesity and diabetes, in part related to adverse effects of psychotropic treatment. The effect of antipsychotic treatment on metabolic risk in children has received limited study. The NIMH-funded MEAC study characterized the effects of 12 weeks of randomized treatment with aripiprazole, olanzapine or risperidone on direct measures of adiposity and insulin sensitivity in children ages 6-18 with disruptive behavior disorders, not previously treated with antipsychotics. Metabolic assessments at baseline and 12 weeks included body composition analysis with Dual Energy X-ray Absorptiometry (DEXA) and abdominal magnetic resonance imaging (MRI), hyperinsulinemic-euglycemic clamps with stable isotopomer tracing, and fasting plasma analyses. ANCOVA was used to test the interactive effects of time and treatment

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condition on treatment outcome; primary endpoints included change in whole-body adiposity and insulin sensitivity. Antipsychotic treatment was associated with adverse changes in adiposity and insulin sensitivity in all treatment groups (N=125). In addition, medication-specific differences in the magnitude of change from baseline were observed, for example, for increases in DEXA %fat (F[2,119]=8.98, p<0.0001) and decreases in whole-body insulin sensitivity (F[2,94]=2.99, p=0.055). Importantly, treatment also resulted in marked improvement in Aberrant Behavior Checklist irritability/aggression subscale scores for all groups with a pooled mean decrease of 16.64 (SD 7.98) points (F[2,120]=0.658, p=0.520).

These results indicate that 12 weeks of initial antipsychotic treatment is associated with increases in adiposity and insulin resistance, changes known to be associated with long term increases in cardiovascular and diabetes risk. This underlines the importance of careful attention to the balance of potential risks and benefits during use of antipsychotics in pediatric populations.

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129-OR

Increased Anti-Oxidant Effect of Exercise in Obese Children, Despite Greater Baseline Oxidative Status

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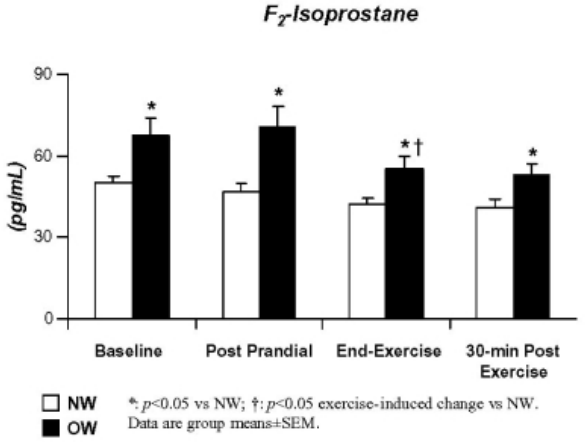
Obesity (OW) and hyperlipidemia are often associated with oxidative stress (OS) and inflammation, now identified as pathogenetic mechanisms increasing cardiovascular (CV) risk in these conditions. Excessive dietary lipid and exercise have been shown to separately exert opposite effects on long-term CV risk, i.e respectively to worsen and reduce it. Very little is known, however, on the simultaneous effect of high-fat feeding and exercise, especially in obese children, a vulnerable, understudied and alarmingly growing population. We therefore measured F_2-Isoprostanes (F_2-IP), reflecting systemic lipid peroxidation, in 15 healthy (NW, 12±1 yrs, 8F, BMI% 53±7), and 19 obese (OW, 12±1 yrs, 13F, BMI% 97±0.4) children, who ingested a high-fat meal 45-min before exercise (ten 2-min cycling bouts @ 80% VO_2max, with 1-min rest intervals). Samples: baseline, 45-min after fat feeding, end- and 30-min post exercise. In OW, F_2-IP (pg/mL) were consistently greater than NW; baseline (68±7 vs 50±2, p<0.05), 45-min after fat ingestion (71±7 vs 47±3, p<0.01), end-exercise (56±5 vs 42±2, p<0.05); 30-min post (53±4 vs 41±3, p<0.05). Importantly, while exercise had little effect on F_2-IP in NW, in OW F_2-IP decreased significantly at end-exercise (-15±4 vs -5±2, p<0.05 vs NW). Our data indicate that despite a consistently elevated oxidative status (increase F_2-IP at baseline, after feeding and end-exercise) when compared to healthy controls, obese children may be more responsive to interventions aimed at reducing oxidative stress. These results stress the vital necessity to identify and implement, in obese children, optimal preventive and therapeutic strategies, including customized exercise regimens aimed towards specific pathogenetic mechanisms of long-term cardiovascular complications.



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Surrogate Indices of β-Cell Function in Obese Youth with NGT, IGT and Diabetes

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Assessment of insulin secretion is crucial in research settings evaluating β-cell function and interventions that modify it. The hyperglycemic clamp gold standard is not universally applicable, particularly in large scale trials. This investigation aimed to compare insulin and C-peptide secretion measured by the hyperglycemic clamp with simple estimates of β-cell function in obese youth across the spectrum of glucose tolerance. Obese youth (age 10-<20 yrs) with normal glucose tolerance (NGT=94), impaired (IGT=45), type 2 diabetes (T2DM=22) and type 1 diabetes (OT1DM=13) underwent a 2-hr hyperglycemic clamp (225 mg/dl) to determine first phase insulin (1^{st}PI) and C-peptide (1^{st}PC), and on a separate occasion a 2-hr oral glucose tolerance test (OGTT). Fasting (insulin/glucose [I_F/G_F], C-peptide/glucose [C_F/G_F], HOMA%β) and OGTT-derived (ratio of incremental change 0-30 min of insulin and C-peptide to glucose [$\Delta I_{30}/\Delta G_{30}$, $\Delta C_{30}/\Delta G_{30}$] and the ratio of insulin and C-peptide area under the curve to glucose [I_{AUC}/G_{AUC}, C_{AUC}/G_{AUC}]) indices were calculated. Differences (P<0.05) across glucose tolerance groups were determined using ANOVA. Hyperglycemic clamp 1^{st}PI and 1^{st}PC were higher in NGT and IGT compared to T2DM and OT1DM. Among the different indices, only I_{AUC}/G_{AUC} and C_{AUC}/G_{AUC} showed similar statistical differences between each of the groups as the clamp measures.

Table 1. Correlations between clamp and surrogate indices of β-cell function by glucose tolerance groups,*P<0.05.

	NGT	IGT	T2DM	OT1DM	Total
Clamp first phase insulin vs.					
I_F/G_F	0.55*	0.65*	0.76*	0.91*	0.57*
HOMA%β	0.57*	0.70*	0.82*	0.86*	0.67*
$\Delta I_{30}/\Delta G_{30}$	0.65*	0.50*	0.64*	0.76*	0.74*
I_{AUC}/G_{AUC}	0.58*	0.60*	0.78*	0.69*	0.72*
Clamp first phase C-peptide vs.					
C_F/G_F	0.56*	0.85*	0.69*	0.97*	0.61*
$\Delta C_{30}/\Delta G_{30}$	0.71*	0.62*	0.54*	NS	0.80*
C_{AUC}/G_{AUC}	0.61*	0.79*	0.61*	0.75*	0.78*

Correlations between surrogate indices and clamp measures of β-cell function varied depending on glucose tolerance status, and correlations were overall higher with measures of C-peptide than insulin. Therefore, while surrogate estimates of β-cell function could be useful, attention should be paid to the glucose tolerance status of the population studied.

ADA-Funded Research

131-OR

Markers of Platelet Activation in Adolescents with Diabetes Mellitus

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Although type 1 diabetes mellitus (DM) predominates in children and adolescents, the increase in type 2 DM in recent years has paralleled the increase in obesity in youth. In adults with DM, poor glycemic control, insulin resistance and dyslipidemia are associated with markers of endothelial dysfunction and platelet hyperactivity, and with macrovascular complications including atherosclerosis and cardiovascular disease (CVD). Adolescents with type 2 DM may also be at risk for early onset atherogenesis and CVD associated with platelet hyperactivity. Markers of *in vivo* platelet hyperactivity have not been reported in youth with DM. The objective of this pilot study was to compare markers of *in vivo* platelet activation in adolescents with type 1 DM, type 2 DM and with age-matched controls with no DM (n=15 per group, ages 12-18 years). Clinical data obtained were BMI, blood pressure, HbA1c, and fasting lipids. Expression of platelet activation markers CD63, CD62P, and PAC1-binding was quantified by flow cytometry of whole blood samples. Activated platelet-derived soluble markers, sCD62P, sCD40L and platelet factor 4 (PF4), were measured in plasma by ELISA. Statistical comparisons, performed using the Mann Whitney U test, demonstrated increased expression of CD63, CD62P and sCD62P in type 2 DM subjects vs. controls (p<0.01); increased expression of CD63 and PAC1-binding in type 1 DM subjects vs. controls (p<0.05); increased expression of PF4 and sCD62P in type 2 DM vs. type 1 DM subjects (p<0.01). Relationships between platelet activation markers and HbA1c, BMI, blood pressure and lipids were investigated using the Spearman rank correlation coefficient. Positive associations included CD63 and CD62P with HbA1c (p<0.01), and

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PAC1-binding, PF4, and sCD62P with BMI z-score ($p<0.05$). In this study, adolescents with DM had increased expression of markers of platelet hyperactivity compared to controls, which was more evident in subjects with type 2 DM. Longitudinal studies will be required to determine whether platelet activation markers in adolescents with DM are prognostic for early onset CVD.

132-OR

Predictors of Increased Carotid intima Media Thickness in Youth with Type 2 Diabetes

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Dyslipidemia is an independent risk factor to develop cardiovascular (CV) disease. The ability of specific lipid parameters to predict worse intermediate CV outcomes in youth with type 2 diabetes (T2D) is unclear. Thus, we examined the relationship between lipid parameters and carotid intima media thickness (IMT) in 244 adolescents with T2D (mean age 18.1 ± 3.1 yrs, 65% female, 44% Caucasian, duration of T2D 3.2±2.6 years). Clinical, biochemical data and carotid intima media thickness by B mode ultrasound were collected. Lipid parameters included LDL, HDL, TG, non HDL, TG/HDL and LDL/HDL ratio. General linear models were constructed to determine which lipid parameter best predicted a thicker carotid IMT. Models were then adjusted for potential confounders to determine whether lipids remained a significant independent predictor of an increased carotid IMT. LDL/ HDL ratio was the strongest independent predictor of a thicker carotid for the internal, common and bulb segments ($p<0.02$). However, after adjusting for confounders (age, race, sex, BMIz, BP, HbA1c, duration of T2D), LDL/HDL ratio was no longer significant. Age and BMIz were the most consistent determinants for a thicker carotid artery (model R^2: common= = 0.19, internal =0.15, bulb =0.13, all $p<0.01$). We conclude: 1) lipids do not appear to contribute significantly to the early intermediate CV outcomes in youth with T2D; 2) despite the predominance of age and adiposity in our models, age and adiposity do not contribute a large percentage to the development of carotid IMT (all R^2 <0.19). Thus, further investigation is needed to examine the role of potential non-traditional risk factors on intermediate CV outcomes in youth with T2D.

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OCULAR COMPLICATIONS

133-OR

Identification of Activating Transcription Factor 4 as a Key Coordinator in Retinal Inflammation and Vascular Damage in Diabetic Retinopathy

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Inflammation plays a key role in the development of vascular damage in diabetes. The purpose of this study is to examine the role of activating transcription factor 4 (ATF4), a major downstream effector of endoplasmic reticulum (ER) stress response, in retinal inflammation and vascular injury in diabetic retinopathy. In streptozotocin (STZ)-induced diabetic mice, retinal ER stress markers, including expression of GRP78, phosphorylation of IRE1alpha and eIF2alpha, and ATF4 expression were significantly increased, concomitant with elevated levels of inflammatory factors (ICAM-1, VEGF and TNF-alpha) in the retina. Intensive expression of ATF4 was observed in the inner retina and partially colocalized with Müller cell markers (glutamine synthase, CRALBP and GFAP), suggesting a potential role of ATF4 in retinal Müller cell dysfunction and inflammation in diabetes. In cultured retinal Müller (rMC-1) cells, high glucose (30 mM) induced a rapid increase in ER stress and activation of ATF4. Pharmacological induction of ER stress or overexpression of ATF4 was sufficient to increase ICAM-1 and VEGF expression in rMC-1 cells. Conversely, inhibition of ER stress by chemical chaperone taurine-conjugated ursodeoxycholic acid or 4-phenylbutyrate significantly attenuated high glucose-induced ICAM-1 and VEGF upregulation. In addition, blockade of ATF activity by adenoviruses expressing a dominant negative mutant of ATF4 also inhibited ICAM-1 and VEGF expression in rMC-1 cells exposed to high glucose. Furthermore, intravitreal injection of adenovirus expressing ATF4 induced a potent inflammatory response in the retina in normal mice. Genetic depletion of ATF4 using ATF4 knockout mice or inhibition of ATF4 activity by dominant negative mutant of ATF4 successfully ameliorated retinal inflammatory factor expression and mitigated retinal vascular leakage in STZ-induced diabetic mice. Taken together, these results provide the first evidence that activation of ATF4 by ER stress plays a critical role in retinal inflammation and vascular damage in diabetic retinopathy.

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134-OR

Oxidized LDL Immune Complex and the Progression of Retinopathy in Type 1 Diabetes

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Immune complexes (IC) target important components in retinopathy development: lipids, oxidative stress and induction of a pro-inflammatory humoral immune response. We report the effect of IC containing oxidized LDL (oxLDL) on retinopathy development over a 16-year period in subjects with type 1 diabetes. Levels of oxLDL-IC were measured on 517 subjects of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications cohort at DCCT baseline. General characteristics of the 517 subjects were similar to those of the whole cohort. Retinopathy was assessed by stereoscopic fundus photography periodically throughout the study. The effect of oxLDL-IC on the risk of retinopathy progression was assessed by Cox Proportional Hazard models. Of the 517 patients, 27% progressed to severe non-proliferative retinopathy (SNPDR), 22% developed clinically significant macular edema (CSME) and 63% experienced a 3-step progression beyond their DCCT baseline ETDRS score. One standard deviation (SD) increase in baseline oxLDL-IC was associated with 30% increased risk of developing CSME (HR=1.3; 95% CI:1.1-1.6), 42% increased risk of progressing to SNPDR (HR=1.4; 95% CI:1.2-1.7) and 14% increased risk of experiencing a 3 step worsening in ETDRS score (HR=1.1; 95% CI:1.0-1.3). All the above analyses were adjusted by treatment group, lipid levels, HbA1c, blood pressure, smoking, albumin excretion rate, age, gender and diabetes duration. There was a significant interaction between retinopathy cohort (primary vs secondary prevention) and oxLDL-IC for progression to severe retinopathy (p=0.05), thus the analyses were stratified by baseline retinopathy cohort. In the primary prevention cohort, HR associated with one SD increase in oxLDL-IC was 2.2 (95% CI:1.3-3.8) while the secondary prevention cohort experienced an attenuated hazard (HR=1.3; 95% CI:1.1-1.6). In summary, increased levels of oxLDL-IC at DCCT baseline are associated with an increased risk for progression of retinopathy in type 1 diabetes indicating that the humoral immune response to modified LDL is a significant factor in the progression of retinopathy.

Supported by: NIH, JDRF and the Dept of Veterans Affairs

135-OR

Increased Risk of Diabetic Macular Edema (DME) among Type 2 Diabetes [T2D]Patients Treated with PPAR-G Agonists: Results of a Large Cohort Study

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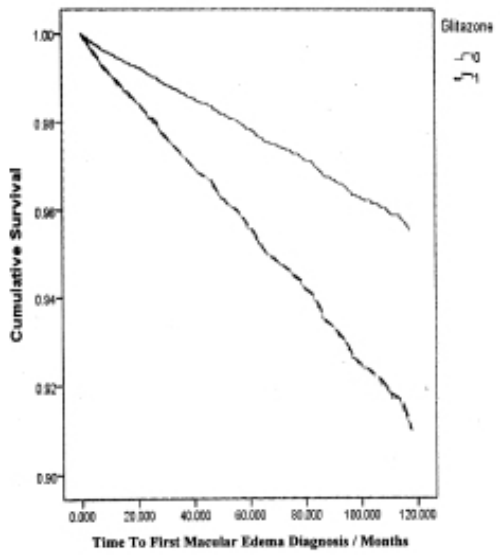
We have previously shown that a direct hyper-permeability effect on human capillary endothelial cells may contribute to the development of peripheral and pulmonary edema in patients treated with glitazones [1], but the possibility that TZDs (and new PPAR-g agonists) could have similar effects in the eye has not been investigated in large populations. Using a UK database of Primary Care, The Health Improvement Network (THIN), we undertook a retrospective cohort study to investigate the risk of developing DME among patients treated with a glitazone. A total of 103,368 T2D patients without DME at baseline were divided according to whether they received TZD therapy or not. The primary outcome was the development of DME at 1-yr and at 10-yrs follow-up. At 1-yr, the incidence of DME was 1.3% and 0.2% among users vs non-users of glitazones (odds ratio [OR]: 5.7; 95%CI 4.1-7.9). After Cox multiple regression analysis to adjust for potential confounders (e.g HbA1c, age, SBP, lipids, aspirin, fibrates, insulin, oral antidiabetic drugs, renin-angiotensin-system [RAS] blockers), TZD use was still associated with a significantly increased risk of DME (OR 3.3; 95%CI 2.2-4.9). This effect was also present after 10-yrs (hazard ratio [HR] 5.2; 95%CI 4.3-6.3), even after adjustment for confounders (HR 2.3; 95%CI 1.8-3.0) (Figure). Using an interaction model, combination therapy with insulin + TZD increased the risk of DME (HR 3.2, 95%CI 1.7-6.1) while RAS blockade was protective (HR 0.7; 95%CI 0.5 -0.9). In conclusion, TZDs are associated with a 3-6 fold increased risk of DME, even after adjustment for confounding variables. Patients at high risk of sight-threatening DME should avoid TZDs, and the ocular safety of newer PPAR-g agonists merits prospective evaluation.

For author disclosure information, see page 785.

Figure: Kaplan-Meier graph showing the development of DME among users vs non-users of TZD over a 10 year period (N=103,368)



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136-OR

Per2 Mutation Recapitulates Diabetes Like Phenotype and Accelerates Progression of Diabetic Vascular Dysfunction

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Previously, we reported that the development of the diabetic vascular complications is associated with endothelial progenitor (EPC) dysfunction, downregulation of clock genes in retina and EPCs, bone marrow (BM) neuropathy and an altered circadian release of EPCs from the BM. In this study, we hypothesized that clock gene (*Per2*) mutant mice will possess a vascular phenotype similar to diabetes. Per2 ($Per2^{tm1Brd}$/J) (n= 3) or wild type (WT) control mice (n=4) or STZ DM mice (n=4) were utilized and $lin^{-}Sca1^{+}ckit^{+}$ (LSK) cells (mouse EPCs) were quantified and proliferation, mRNA expression was evaluated. In parallel studies retinas were processed for mRNA expression of by qRT-PCR. BM-neuropathy was assessed by staining of femurs with tyrosine hydroxylase (TH) and neurofilament 200 (NF-200) while BM fat content was determined using quantitative magnetic resonance spectroscopy.

Per2 mutant mice were similar to WT control mice in terms of their body weight, blood glucose and HbA_{1c}. At ZT-5, EPC numbers in BM of Per2 mutant mice were reduced by 57 % ($p<0.05$) as compared to WT controls, however there was no change in EPC number for peripheral blood. Proliferation of Per 2 mutant EPC was reduced 3-fold as compared to control mice. BM fat content of Per2 mutant mice was reduced by 50 % as compared to control animals, but was similar to type 1 diabetic mice. There was significant decrease ($p<0.05$) in TH positive nerve processes and NF-200 staining in Per2 mutant mice as compared to WT controls but similar to diabetic mice. mRNA expression of endothelial function associated genes (eNOS, VEGFR2, FLT1) was downregulated (1.5-2 fold, $p<0.05$) in per2 mutant retinas while there was an upregulation of inflammatory and senescent genes (iNOS; 1.5 fold, TGFb1 2 fold, $p<0.05$) in retinas of Per2 mutant mice as compared to WT controls.

In conclusion Per2 mutant mice possess phenotype similar to diabetes as indicated by reduced EPC number, dysregulated vascular function and bone marrow neuropathy. Thus our study further validates that circadian alterations play a crucial role in the progression of diabetic complications

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137-OR

Diabetic Retinopathy Screening: Implications with Exenatide Treatment

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Diabetic retinopathy (DR) could deteriorate significantly with rapid improvement in glycemic control. Exenatide treatment has been demonstrated to cause rapid and significant lowering of HbA1c. The aim of our study was to assess the impact of tight glycemic control achieved with Exenatide therapy on the status of DR.

A retrospective analysis was conducted on all patients who had continued on Exenatide 10mcg twice a day beyond 6 months. We included only patients who had DR and HbA1c status available before and after the initiation of Exenatide. DR information was collected from the community retinal screening programme database and the lowest HbA1c achieved was used for analysis.

165 patients were included in the analysis. Mean baseline HbA1c: 9.6%. The lowest HbA1c achieved was 8.1%, with a reduction of -1.8% (range 0 to -6.4). The lowest HbA1c was achieved after 176 days (54-747); 19.4% patients worsened their HbA1c despite continued Exenatide treatment. Repeat DR screening was done at 216 days (90-617).

49 of the 165 patients (29.7%) had progression of DR (new onset 16: progression of pre-existing DR 33) compared to 19.4% of the patients whose DR improved ($p<0.005$). 47 of the 49 patients had a significant reduction in HbA1c based on the lowest value achieved (baseline 10.1%; lowest 7.7%; mean reduction 2.1%; range 0 to -6.4%; duration 268 days). The proportion of patients with progression of DR was higher with greater reductions in HbA1c (0 to -2%: 30.1%, -2 to -4%: 43.6%, >-4%: 45.5%). Of the 133 patients who had a reduction in HbA1c, 47 (35.3%) had progression of DR compared to 17.3% whose DR improved ($p<0.001$). The proportion of patients with progressing DR was significantly higher compared to patients who did not have an HbA1c improvement (35.3 vs 6.2%; $p<0.001$). The progression of DR was negatively correlated to improvement in HbA1c ($r=-0.22$).

In conclusion, significant reduction in HbA1c with Exenatide treatment is associated with progression of DR. More frequent monitoring of DR status may need to be recommended, especially in patients with significant reduction of HbA1c. Annual screening will not necessarily promptly identify this well recognised, but less commonly perceived complication.

138-OR

Association of Increased Retinal Thickness and Systemic Complications in Patients with 50 or More Years of Type 1 Diabetes

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Optical coherence tomography (OCT) is a widely used noninvasive imaging technique which assesses diabetic macular edema (DME) and other ocular pathology in diabetes (DM). However, the association of OCT-derived retinal central subfield thickness (CST) with non-ophthalmic diabetic complications has not been fully explored. Since the macula is composed of neural and vascular tissues, diabetes induced changes in CST might reflect systemic changes in similar tissues and correlate with non-ocular pathology, making OCT a potential noninvasive quantitive biomarker for systemic complications.

We evaluated time domain OCT-derived CST, retinal edema risk factors, and extent of diabetic retinopathy (DR), nephropathy, neuropathy and cardiovascular (CV) disease in 557 patients with $\geq$50 years of type 1 DM (Medalists). DR severity was assessed by ETDRS-protocol fundus photographs, nephropathy by ACR$\geq$ 30 mcg/mg creatinine, neuropathy by the Michigan Neuropathy Screening Instrument, and CV disease by historical self-report. Current A1C, blood pressure and lipid levels were also obtained. Age (mean$\pm$SD) was 67$\pm$8 yrs, DM duration 55$\pm$6yrs, age at DM onset 11$\pm$7 yrs, current A1c 7.3$\pm$1.0% and 49% were male. Retinal thickening (CST$\geq$250 µm) occurred in 20% of eyes, 56% of which had no detectable DME on photographs. PDR was present in 48%, nephropathy in 13%, neuropathy in 35%, and CV disease in 43% of patients. CST$\geq$250 µm was associated with older age (70 vs 66 yrs, $p<0.0001$), longer DM duration (57vs 55 yrs, $p=0.0004$), older age at DM onset (13 vs 11yrs, $p=0.0006$), and male gender (25 vs 15%, $p=0.001$). CST$\geq$250 µm was also associated with the presence of complications (DR: $p<0.001$; nephropathy: $p=0.0006$; CV disease: $p=0.04$; neuropathy: $p=0.23$). In a multivariable model adjusting for age, DM duration and DR severity, nephropathy ($p=0.01$) was significantly associated with CST$\geq$250 µm.

These data suggest that OCT retinal imaging could be a sensitive quantitive biomarker for the severity of diabetic retinopathy, nephropathy and cardiovascular diseseases.

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For author disclosure information, see page 785.

139-OR

Downregulation of Mitochondrial Transport Protein ABC-me Promotes Retinal Vascular Lesions

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ABC-me is a novel ATP binding cassette transporter protein that has been shown to play a protective role against oxidative stress. We have previously shown that retinal endothelial cells express ABC-me and that under high glucose condition, a significant downregulation of ABC-me occurs. Additionally, a downward trend in ABC-me expression was previously observed in retinas of diabetic rats. While these studies suggest that high glucose condition reduces ABC-me expression, the consequence of such reduced ABC-me expression is currently unknown in the retinal vascular network.

Retinas isolated from ABC-me heterozygous knockout mice and age-matched, gender matched, control mice, were subjected to trypsin digestion technique for isolation of retinal capillary networks. The retinal capillary networks were stained with hematoxylin and PAS and analyzed for acellular capillaries and pericyte loss. To determine if reduced ABC-me expression influenced apoptosis, TUNEL assays were performed in the retinal capillary networks of wildtype and knockout mice. Digital images of ten random fields from each retina of wildtype and knockout mice were photographed and analyzed. TUNEL positive cells in the retinal trypsin digests were counterstained to confirm endothelial cell/pericyte localization.

A significant increase in the number of acellular capillaries and pericyte ghosts was detected in the retinal capillaries of ABC-me knockout mice compared to those of wildtype mice (200±77%, p=0.04; 222±66%, p=0.04, respectively). Data from TUNEL assays confirmed that the reduced number of endothelial cells and pericytes in the ABC-me knockout mice was due to apoptosis. The number of TUNEL positive cells was significantly increased (approximately three fold) in the ABC-me knockout mice compared to those of the wildtype mice.

The findings from this study indicate that ABC-me expression protects retinal vascular cells from apoptosis. A strategy to upregulate ABC-me expression may be useful in preventing oxidative stress-related vascular lesions in diabetic retinas.

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140-OR

Dysregulation of Circadian Pattern of MiRNA 146a and 200b Expression in Diabetic Retina

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There is a growing body of evidence that circadian and metabolic systems are coordinated and loss of circadian regulation plays vital role in the pathogenesis of diabetes and diabetic complications. We have previously demonstrated that diabetic retinopathy (DR) is associated with a dysfunctional peripheral clock. In addition, miRNAs have been identified as important post-transcriptional regulators of circadian rhythmicity, lipid metabolism, and inflammation. A number of miRNA were found to be downregulated in DR, including a negative regulator of inflammation miR-146a, and a negative regulator of angiogenesis miR-200b. The purpose of this study was to establish the relation between circadian clock, key metabolic genes and miRNA in the retina and determine whether this regulatory mechanism is impaired in diabetes.

Type 1 diabetic rats and age matched controls were used. All rats were kept in standard 12/12 light/dark cycle conditions. Rats were sacrificed and their retinas were harvested for RNA and protein analysis every 2 hrs for a 24 hr period. The expression levels of clock genes (*Clock, Bmal-1, Per-1, Per-2, Cry-1, Cry-2 and Erb)* and lipid metabolic genes (*PPARα, PPARγ, SREBP-1c, ELOVL4 and ELOVL2*), and the expression of miRNA 146a and 200b was examined by quantitative real-time PCR.

Circadian expression of *Clock, Bmal-1, Per-1,* and *Per-2* was significantly affected by diabetes. In addition, lipid metabolic genes *SREBP-1C and PPARα* displayed a significant circadian pattern in retina (p<0.001). Lipid metabolic genes, *SREBP-1C*, *PPARγ* and *ELOVL2* also showed differential expression profiles in the diabetic versus control rats (p<0.01). Interestingly, miRNA 146a and 200b were expressed in a clear circadian pattern and the circadian expression of miRNA 146a and 200b was significantly affected by diabetes. Our results demonstrate that dysregulation of miRNA circadian expression in diabetic retina could lead to changes in light/dark pattern of retinal lipid metabolism with potential implications for the development of diabetic retinopathy.

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EXERCISE AND DIABETES

141-OR

Muscle Energy Reserves Changes during Exercise

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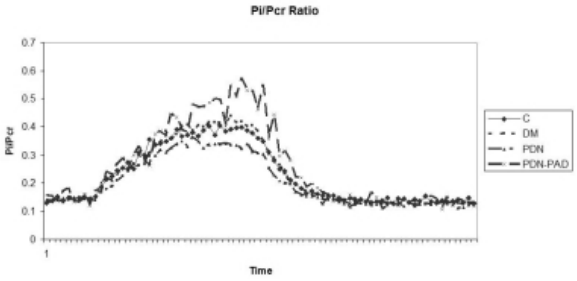
We employed Magnetic Resonance Spectroscopic (MRS) measurements to identify changes in the calf muscle energy reserves during graded exercise in diabetes (DM). We studied healthy control subjects [group C, n=12, age 54 ± 11 yrs (mean ± sd)], DM patients without neuropathy (DM, n=11, 63 ± 9), DM patients with neuropathy (PDN, n=7, 57 ± 10) and DM patients with both neuropathy and peripheral arterial disease (PDN-PAD, n=3, 61 ± 16). We performed continuous measurements of phosphorous metabolites (PCr and Pi) during a 3-minute graded exercise at the level of the posterior calf muscles (gastrocnemius soleus muscles). The intensity of the exercise was quantified and standardized for all participants.



The resting Pi/PCr ratio at baseline and the maximum reached during exercise was similar in all groups. The post-exercise time required for recovery of Pi/Pcr ratio to resting levels increased gradually (C: 59 ± 29 seconds, DM: 61 ± 15, PDN: 79 ± 21, PDN-PAD 86 ± 12, p<0.05). The ratio of Pi/Pcr area under the curve to the applied force during exercise (Pi/Pcr/AUC) also increased gradually (C: 0.33 ± 0.23, DM: 0.53 ± 0.38, PDN: 0.61 ± 0.41, PDN-PAD 0.82 ± 0.31, p=0.11). When PDN and PDN-PAD were considered as one group, the above ratio was statistically higher than the C group (p <0.05). Strong correlations were observed between the Pi/Pcr/AUC and measurements of neuropathy such as the neuropathy disability score (NDS, r=0.41, p<0.02) and vibration perception threshold (VPT, r=0.38, p<0.05) and between the post-exercise time required for recovery of Pi/Pcr ratio to resting levels and NDS (r=0.41, p<0.02) and VPT (r=0.47, p<0.01). No associations were observed between MRS measurements and skin blood flow and measurements of oxy- and deoxyhemoglobin. We conclude that diabetes adversely affects the muscle energy reserves during exercise and that neuropathy and PAD further aggravate this impairment.

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142-OR

Neither Aerobic Exercise Nor Resistance Exercise Improves Glycemic Control in Type 1 Diabetes: A Randomized Controlled Trial

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In most previous trials, aerobic exercise did not improve glycemic control (A1C) in type 1 diabetes (T1D), but 2 very small studies (n<10) found resistance exercise (weight lifting) in T1D reduced A1C. Most previous trials were done prior to availability of short-acting insulin analogs and 5-second glucose monitors. The T1-DARE trial was undertaken to determine the effects of aerobic exercise and resistance exercise on A1C, hypoglycemia, and lipids in previously sedentary T1D adults receiving modern diabetes care.

Methods: Before randomization, we stabilized subjects on therapy including multiple-dose insulin or insulin pump, carbohydrate (CHO) counting and frequent glucose monitoring. 66 subjects (28M/38F, age 18-68 yr, A1C 6.6%-9.9%,) were randomized to 6 months of: A: Aerobic exercise, progressing to 45 min 3X/wk at 75% of max. HR; R: Resistance exercise, 3 sets of 8 exercises at 8 rep max 3X/week; AR: both aerobic exercise as in A, and resistance exercise as in R; or C: sedentary control. Exercise training took place at local YMCAs, supervised by personal trainers. Pre-exercise CHO supplements and post-randomization insulin adjustments were done according to pre-specified protocols. Analyses were intention-to-treat, using linear mixed modeling.

For author disclosure information, see page 785.

Results: There were no significant intergroup differences in A1C change, or in frequency of hypoglycemia. There were 3 cases of hypoglycemia requiring assistance (1 each in A, C, and R; none in AR). Compared to control, the AR group had significant increase in HDL-C (+0.25 mmol/L, p=0.026), and reduction in triglycerides (-0.40 mmol/L, p=0.011). Total cholesterol/HDL-C ratio decreased significantly in AR compared to C (-0.66, p=0.012) and compared to R (-0.51, p=0.043). Other intergroup differences for lipids were nonsignificant.

Conclusions: Neither aerobic nor resistance exercise improved glycemic control in previously-sedentary patients with type 1 diabetes, in marked contrast to previous findings in type 2 diabetes. However, the combination of aerobic and resistance exercise had significant favorable effects on HDL-C, triglycerides and total cholesterol/HDL-C ratio.

Supported by: Canadian Diabetes Association

143-OR

Exercise Induced Improvements in Insulin Sensitivity Are Related to Changes in Muscle Ceramides and Sphingosine 1-Phosphate in Obesity and Diabetes

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Ceramides are signaling lipids involved in cellular stress and insulin resistance. Conversely, sphingosine 1-phosphate (S1P), a ceramide metabolite, promotes growth and survival, and stimulates glucose uptake. We examined the effect of exercise training on insulin sensitivity, and skeletal muscle ceramide and S1P content in 23 obese subjects (13 men, 10 women; age 63±1 years; BMI 33.6±1.2 kg/m^2) with either type 2 diabetes mellitus (T2DM, N=9), or normal glucose tolerance (NGT, N=14). Subjects were evaluated before and after a 12-week supervised aerobic exercise program, 5 days/wk, for ~1 hr/day, at 80-85% of maximum heart rate. Insulin sensitivity was assessed by 40mU/m2/min hyperinsulinemic euglycemic clamp, muscle ceramides (C14:0, C16:0, C18:0, C18:1, C24:0 and C24:1) and S1P were quantified by tandem mass spectrometry after separation with HPLC. Before the intervention, insulin sensitivity (glucose disposal rate, GDR) was lower in subjects with T2DM (1.9±0.5 mg/kg/min) compared to controls (2.6±0.2 mg/kg/min). Subjects with T2DM also had higher levels of muscle C16:0 (18.2±1.7 nmol/g ww) and total ceramide (54.0±3.2 nmol/g ww) compared to controls (C16:0, 12.2±0.6 and total ceramide, 43.8±7.2 nmol/g ww). Exercise significantly improved insulin sensitivity in both groups (P=0.0001), however changes in GDR in the NGT group (1.5±0.4 mg/kg/min) were higher than the T2DM (0.6±0.2 mg/kg/min). After the intervention, muscle C16:0, C24:1, and total ceramide levels were paradoxically increased in both groups combined (C16:0, P=0.005; C24:1, P=0.01; and total ceramide, P=0.001). Independently, these changes reached significance only in the NGT group (P=0.002). Exercise significantly increased S1P in both groups (P=0.04 for control and P=0.05 for T2DM) and reduced C14:0/S1P (P=0.01) and C20:0/S1P (P= 0.05) ratios in the T2DM group. Because of the opposing effects of ceramides and S1P on insulin resistance, we conclude that the reduction in the muscle ceramide/S1P ratio contributes to the increase in insulin sensitivity after exercise training.

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144-OR

Six Months of Endurance Type Exercise Training Increases Skeletal Muscle Lipid Content, Mitochondrial Density, and Perilipin 2 Expression in Obese Type 2 Diabetes Patients

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Oxidative capacity and intramuscular lipid metabolism can be enhanced following prolonged exercise training. In the present study we assessed intramuscular lipid and mitochondrial content as well as the expression of the lipid droplet protein perilipin 2 (also termed ADRP/adipophilin) before and after 6 months of endurance type exercise training in type 2 diabetes patients. A total of 10 obese, male type 2 diabetes patients (age 62±1 years, BMI 31±1 kg/m^2) completed 3 sessions per week for a period of 6 months, consisting of 40 min of continuous endurance type exercise at 75% $VO_{2\ peak}$. Muscle biopsies collected at baseline and after 2 and 6 months of intervention were analysed using quantitative fibre type specific immunofluorescence microscopy. The training intervention induced a 6% reduction in trunk and leg body fat (P< 0.05) and a 13% increase in maximal oxygen consumption (P< 0.05). Intramuscular lipid content increased two-fold in response to the 6 month training period in both type I and type II fibres (P< 0.05). A three-fold increase in perilipin 2 expression was observed from baseline to 2 and 6 months of the intervention in type I fibres only (Fig. 1, P< 0.05). Mitochondrial content increased after 6 months of training (P< 0.05) and the increase was most prominent in the subsarcolemmal region. This study demonstrates for the first time that prolonged endurance type exercise training increases both intramuscular lipid content and the expression of the lipid droplet protein perilipin 2 in skeletal muscle in type 2 diabetes patients. These adaptations are likely instrumental to the improvements in lipid metabolism observed with prolonged exercise training, and may be of key importance for the clinical benefits of exercise training in type 2 diabetes patients.

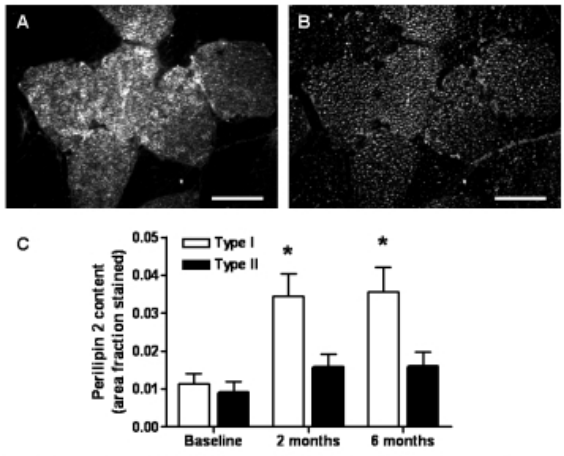

Figure 1. Immunofluorescent staining of type I muscle fibres (A) and perilipin 2 (B). Perilipin 2 expression in skeletal muscle was significantly elevated in type I fibres after 2 and 6 months of exercise training (C, P < 0.05).

145-OR

NFATc1 Activity in Human Insulin Resistant Skeletal Muscle

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Nuclear Factor Activated T-Cells isoform c1(NFATc1) is a transcription factor in the Ca^{2+}/calmodulin/calcineurin pathway and has a major role in activating the oxidative gene program in type I muscle fibers. Aerobic exercise (EX) induces Ca^{2+} influx in skeletal muscle and activation of calcineurin, which dephosphorylates NFAT and triggers its translocation to nuclei. Our recent results show a lack of up-regulation of key genes after a single bout of EX in insulin resistant (IR) subjects relative to insulin

sensitive (IS) controls, leading to the hypothesis that transcriptional activity of the NFAT pathway in response to EX is reduced in skeletal muscle of IR subjects. To test if altered NFATc1 activation is involved in the decreased transcription response to exercise in IR muscle, we performed muscle biopsies(BX) before, 30 minutes and 5 hours after a single bout of moderate intensity EX in 4 IR and 5 IS subjects. A euglycemic hyperinsulinemic clamp was performed to assess insulin action. mRNA isolated from BX was analyzed using Agilent 44K Microarrays. Glucose disposal was 9.4±1.3mg/kg/min in IS vs. 5.4±1.0mg/kg/min in IR. No difference in Basal NFATc1 levels was observed in IR or IS. However, a 45% and 21% increase in NFATc1 transcripts was observed post EX in the IR and IS groups respectively. To assess NFATc1 activity, we looked at downstream targets of NFATc1, particularly, Troponin 1 Fast (TNNI2) and Myosin Heavy Chain 1 (MYH1), genes that activated NFATc1 inhibits and activates, respectively. TNNI2 transcripts increased 28±0.5% in the IR but only 1±0.6% in the IS. Additionally, protein abundance of MYH1 increased 6±0.06% in IS but decreased 18±0.126% in IR. Although, NFATc1 is activated by dephosphorylation, the specific residues modified in response to EX remains unknown. To assess NFATc1 phosphorylation, human NFATc1 overexpressed in HEK293A cells was analyzed by mass spectroscopy, and 12 unique phosphorylation sites, predominantly located in the transactivation domain, were identified. Taken together, the data suggest activity of NFATc1 is compromised in IR without a change in NFAT expression. Analysis of NFATc1 phosphorylation will lead to new understanding of Ex-induced transcription in IR muscle.

For author disclosure information, see page 785.

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Hepatic Glucagon Action Is Essential for Exercise Training-Induced Reversal of Mouse Fatty Liver

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Lipid infiltration in hepatocytes is a component of the Metabolic Syndrome. Exercise intervention has been implicated as protective against fatty liver; however, the mechanism responsible for this reversal has not been elucidated. Glucagon stimulates glucose production and fat oxidation during exercise. The hypothesis that reductions in high fat diet (HFD)-induced fatty liver require hepatic glucagon receptor activation was tested. 6wk-old glucagon-receptor null mice (*grcr*$^{-/-}$) and littermates (*grcr*$^{+/+}$) were fed a HFD (60kcal/g of fat) for 6wks followed by 10wks voluntary wheel running (RW) or 6wk treadmill exercise (30min at 20m/min, 5days/wk). Exercised mice remained on the HFD during training and HFD sedentary mice (SED) were studied in parallel to each training regimen. Liver lipids were assessed by thin layer chromatography and magnetic resonance. After 8wks on a HFD, fatty liver developed in WT mice (9.7±1.6 vs 3.6±0.3% fat in chow fed mice, $p<0.05$) with increases in liver triglycerides (TG), diglycerides (DG), and cholesterol esters (CE). RW mice of both genotypes had 42% less body fat versus SED. However, only RW *grcr*$^{+/+}$ had significant ($p<0.05$) reductions in liver mass (1.1±0.1 vs1.7±0.1g for SED), total liver fat (3.9±1.0 vs 14.0±0.9% for SED), TGs (37.4±1.0 vs 58.0± 3.0mg/g for SED), and DGs (0.40±0.02 vs 0.60±0.05mg/g for SED). The fatty liver phenotype in RW *grcr*$^{-/-}$ mice was indistinguishable from SED. Exercised *grcr*$^{+/+}$ had a 29% drop in hepatic energy charge [ATP+(ADP/2)/ATP+ADP+AMP], increased activation of AMPK, and an induction of mRNAs involved in fat oxidation (i.e. FGF21 and PPARα). In contrast, changes in hepatic energy charge, AMPK activation, and gene expression were attenuated in RW *grcr*$^{-/-}$. Treadmill exercise caused similar changes in the liver of *grcr*$^{+/+}$ that were absent in *grcr*$^{-/-}$. Thus, glucagon receptor activation is required for the exercise-induced reductions in fatty liver, activation of AMPK, and induction of the oxidative program. These findings highlight the essential role of glucagon in the hepatoprotective benefits of regular physical exercise.

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147-OR

Acute Exercise Induces Phenotypic Switching from M1 to M2 Polarization of Macrophages in White Adipose Tissue of Diet-Induced Obesity Rats

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Macrophages infiltrated in white adipose tissue (WAT) have been identified as an important source of inflammatory cytokine production and a key component in the progression to insulin resistance in a state of obesity. In obese animals, macrophages assume a proinflammatory classical activation profile also known as M1, in contrast to lean conditions with an alternative activation state (M2) and production of immunosuppressive factors, such as IL-10. Although physical exercise might decreases proinflammatory status in WAT, it remains uncertain whether exercise affects the adipocytes or the infiltrated macrophages. Thus the present study aimed to analyze the effects of acute swimming exercise on the inflammatory status and insulin signaling of the tissue fractions, stromal-vascular fraction (SVF) and adipocytes. The effect of exercise was investigated on circulating IL-6 and TNF-α

levels by an enzyme-linked immunosorbent assay, insulin signaling proteins and cytokine production by *Western blotting*, and also macrophage infiltration and polarization in WAT fractions of diet-induced obesity (DIO). The results showed that acute exercise reduced the proinflammatory status mainly in SVF evidenced by reduced expression of MCP-1, TNF-α and IL-1β, without changing WAT macrophage infiltration. In parallel it also increases the IL-10 and IL-13 protein content. The exercise promotes reduction only in circulating TNF-α levels. We also observed higher insulin-induced phosphorylation of IRβ, IRS-1 and Akt in adipocytes in exercised animals. Our results show that exercise in DIO rats induces not only an important suppression in proinflammatory cytokines, but also increases anti-inflammatory cytokines secretion. Taken together these alterations indicate that the exercise can induces the phenotypic switching from M1 to M2 macrophages and that this condition contributes to exercise-induced improved insulin signaling in WAT of DIO rats.

These data provide considerable progress in our understanding of the molecular events that link physical exercise to an improvement in inflammation and insulin resistance in WAT.

Supported by: FAPESP and CNPQ

148-OR

Sucrose Nonfermenting AMPK-Related Kinase (SNARK) Regulates Exercise- and Ischemia-Stimulated Glucose Transport in the Heart

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The signaling mechanisms mediating myocardial glucose transport are not fully understood. SNARK is an AMPK-related protein kinase that is expressed in the heart and has been implicated in contraction-stimulated glucose transport in mouse skeletal muscle. To study SNARK in the heart, we first determined if SNARK is phosphorylated on Thr^{208}, a site critical for SNARK activity. Mice were treated with exercise (treadmill running, 0.6 mph, 12.5% grade for 10, 30, or 60 min), ischemia (global, 3 min), submaximal insulin (induced by i.p. injection of 1 g glucose/kg bw), and maximal insulin (1 U/kg bw, i.p.), and the hearts were dissected and processed. Treadmill exercise slightly but significantly increased SNARK Thr^{208} phosphorylation (10 min:12%, 30 min:15%, 60 min:14%; all $P<0.05$). Ischemia also increased Thr^{208} phosphorylation (35%; $P=0.03$), but there was no effect of submaximal or maximal insulin. HL1 cardiomyocytes were used to overexpress wild type SNARK (2-3 fold) and to knockdown endogenous SNARK (by shRNA, 50-60%). Glucose transport and glycogen concentrations were measured in cells in the basal state and in response to ischemia (acidic N_2 gassed buffer, 45 min) and insulin (100 nM, 20 min). Overexpression of wild type SNARK increased basal glucose transport by 36% ($P<0.05$) and glycogen by 52% ($P<0.02$). Overexpression of wild type SNARK had no effect on ischemia-stimulated glucose transport and glycogen; however, SNARK knockdown impaired ischemia-stimulated glucose transport by 29% ($P<0.05$). SNARK overexpression or knockdown did not alter insulin-stimulated glucose transport or glycogen concentrations. To study SNARK function *in vivo*, SNARK heterozygous knockout mice (SNARK+/-) and wild type littermates performed treadmill exercise (0.6 mph, 12.5% grade, 30 min), and glucose transport was measured. Exercise-stimulated glucose transport was decreased by 51% in hearts from SNARK+/- mice ($P=0.04$). In summary, exercise and ischemia increase SNARK Thr^{208} phosphorylation in the heart and SNARK regulates exercise- and ischemia-stimulated glucose transport. SNARK is a novel mediator of insulin-independent glucose transport in the heart.

AUTOMATED INSULIN DELIVERY SYSTEMS—ARE THEY READY FOR PRIME TIME?

149-OR

Automated Adaptive Closed Loop Insulin Delivery for Stress Hyperglycemia in Type 1 Diabetes

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Stress hyperglycemia from acute medical problems, emotional stress, or drugs is difficult to manage and can increase morbidity. We hypothesized that an automated sensor-controlled algorithm based on continual measurement of insulin sensitivity would reduce the magnitude of stress hyperglycemia. The goal is to detect and adapt to drug-induced reductions in insulin sensitivity by the use of a bihormonal closed loop algorithm.

This is an interim analysis (Jan 2011) of the first 8 human inpatient experiments in persons with type 1 diabetes. Each subject was given hydrocortisone, 40 mg Q 4 hours orally for 7 doses. Every 30 minutes, an adaptive algorithm measured insulin sensitivity over the prior 90 minutes and adjusted proportional and derivative gain factors to control subcutaneous (SC) insulin delivery. The signal from the more accurate of two SC glucose sensors (Dexcom 7+) served as the afferent input. Each study lasted 33 hours, with 3 meals per day.

Initial pre-hydrocortisone venous glucose was 174 $\pm$ 19 mg/dl and initial basal insulin delivery was 1.6 $\pm$ 0.2 units per hour. Thirteen hours after beginning the study (10 hours after the first cortisol dose), toward the start of the adaptation scheme, pre-prandial glucose level had risen to 244 $\pm$ 11 and 0-3 hr mean post-prandial glucose had risen to 288 $\pm$ 17 mg/dl. Within a few hours, the model responded to the reduction of insulin sensitivity. Toward the end of the study, at hr 28, the algorithm had markedly increased the aspart insulin infusion rate to 4.1 $\pm$ 0.8 u/hr. At this time, pre- and post-prandial glucose levels had declined substantially to 165 $\pm$ 19 and 191 $\pm$ 14 mg/dl ($p = 0.01$ and 0.005 vs start of adaptation).

In response to steroid-induced hyperglycemia, an automated model-based algorithm detected reduced insulin sensitivity and markedly increased the infusion rate of SC insulin. These adaptations led to marked reductions in

For author disclosure information, see page 785.

pre- and post-prandial glucose levels despite continued high dose steroid administration. Automated, adaptive closed loop SC insulin delivery appears to be a promising treatment for stress hyperglycemia in type 1 diabetes.

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150-OR

The Low Glucose Suspend (LGS) Function in Sensor-Augmented Pump Therapy Prevents Hypoglycaemia in Children

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The sensor-augmented insulin pump (SAP) "Paradigm®VEO" system offers a novel automatic insulin shut-off mechanism (LGS) possibly preventing severe hypoglycaemia. The present two-phase study (SAP only (2 weeks) vs. SAP plus LGS (6 weeks)) investigated a potential reduction in the frequency of hypoglycemic episodes by using the "low glucose suspend" function.

Data analysis was possible in 21 of 24 patients from 3 pediatric centers (age 10.8±3.8years, diabetes duration 5.9±3.0years, CSII experience 3.7±1.7years). Baseline A1C level was 7.8±1.1% (DCA 2000). A total of 1298 LGS alerts occurred, 853 were shorter than 5 minutes as patients reacted immediately and no interruption of insulin delivery took place. The frequency of LGS alerts was 2.56±1.86 per patient/day (6am-10pm: 76%). Of all LGS episodes, 42% lasted less than 30 min while 24% took more than 120 min, respectively. LGS >120 min was more frequent in the night (84%) [fig.1]. The AUC<70 mg/dl was decreased by using LGS (SAP vs. SAP+LGS: 0.76 mg/dl x day vs. 0.53 mg/dl x day, p=0.05) as well as the time spent in hypoglycemia (average minutes/day: 101±68min vs. 58±33min, p=0.002). Also, the number of hypoglycemic excursions was significantly reduced during SAP+LGS (excursions < 70mg/day 1.27±0.75 vs. 0.95 ± 0.49, p=0.01, excursions≤40 mg/dl: 0.28±0.18 vs. 0.13±0.14mg/dl/day, p=0.005) with no difference in the mean glucose level (145±23 vs. 148±19mg/dl). Regarding safety, no episodes of severe hyperglycemias or DKA were observed following LGS.

The present study provides evidence for reducing the risk for hypoglycemia with LGS without compromising the safety of CSII therapy.

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151-OR

Modular Advisory/Automated Control (AAC) Reduces Glucose Excursions out of a Safe Range and Hypoglycemia in Adults & Adolescents with Type 1 Diabetes

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Closed-loop delivery from insulin pumps informed by continuous glucose monitoring (CGM) enable automated safety and advisory features engaging patients in their treatment optimization, while maintaining the standard CSII therapy framework. Modularity is a key to such designs, combining patient interaction with automated closed-loop control.

Fourteen adults and 11 adolescents, all with Type 1 diabetes (T1D), participated in randomized cross-over protocols in Montpellier and at UVA using CGM (Navigator® or Dexcom 7®) and Omnipod® insulin pump. Admissions included meal challenges, moderate exercise, and overnight stay. The advisory module used metabolic state estimation to predict hyperglycemia and to suggest conservative correction boluses with a target of 150mg/dl (limited to 1/hr, none for 2hrs after meals). The safety module reduced basal rate automatically using predicted risk of hypoglycemia which was updated every 15 min. Repeated measures ANOVA with age group as a factor was used for analysis.

Compared to the patient's own CSII therapy, the AAC:

(i) reduced out-of-range glucose deviations as indicated by increased percent time within the target range of 70-180mg/dl: 60% vs. 75% (p=0.003), and by reduced risk of extreme glucose excursions (blood glucose risk index of 8.4 vs. 4.9, p=0.003);

(ii) decreased hypoglycemic events (BG<70mg/dl) over two-fold - from 26 to 10 episodes with dramatic reductions post exercise (6 vs. 1) and overnight (11 vs. 2).

While age was a significant factor with adolescents generally running higher and more variable glucose levels, all within-/between-subject interactions were non-significant implying similar effects of the AAC on both age groups.

In conclusion, a hybrid AAC system combining automated safety with advised corrections provides a feasible alternative, or replacement, for standard open loop insulin pump therapy.

Supported by: JDRF AP Consortium, UVa K12 VICTR

152-OR

Initial Evaluation of a Fully Automated Artificial Pancreas

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The goal of the JDRF Artificial Pancreas Project to produce an autonomous artificial pancreas that can safely regulate glycemia in people with type 1 diabetes mellitus. This fully automated closed-loop system combines one or more subcutaneous continuous glucose monitors (CGM), a continuous subcutaneous insulin infusion(CSII) pump with a sophisticated control algorithm. Once the system is initiated, all insulin calculations and delivery are done automatically. An artificial pancreas system that aims to replicate normal b-cell function by using the sc-sc route needs to address inherent delays in both glucose sensing and insulin delivery. It should operate safely without any knowledge of meals or other disturbances. We have developed the Artificial Pancreas System(APS©) and used it to clinically evaluate a control strategy that allows efficient glycemic control without any *a priori* meal information using multi-parametric model predictive control(mpMPC) with an insulin-on-board(IOB) safety constraint. The clinical evaluation of the system involves a two step procedure; the first step is the development of both a personalized model and control algorithm based on three days of ambulatory CGM, CSII and meal information. The next step is the actual evaluation of thesystem during a clinical day. The controller is challenged to restore euglycemia and then to overcome an unannounced meal (30-40 g CHO).

Five fully automated closed-loop control sessions were conducted using the APS© with mpMPC and an IOB controlalgorithm. The controller successfully brought the subjects back to the euglycemic range (110mg/dl +/- 30) for all of the subjects. The system recognized all of the unannounced meals and gave appropriated meal boluses. The average percent time in range (80 – 180 mg/dL) was 77% with one mild hypoglycemia episode(YSI=75). This one episode occurred five hours after the meal and was to the consequence of insulin given in response to an elevated sensor signal that was due to a sensor drift (peak postprandial Sensor=283 and YSI=200).

All reported results are within the A+B zones of the Control Variability Grid Analysis. The mpMPC controller safely regulated glycemia despite initial hyperglycemia and unannounced meals.

Supported by: JDRF

153-OR

Day-and-Night Closed-Loop (CL) Glucose Control in Adolescents with Type 1 Diabetes (T1D)

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We evaluated closed-loop (CL) insulin delivery during a 36h period replicating normal daily activities. Twelve adolescents with T1D (M 5; age 15.0±0.9years; A1C 7.9±0.7%; BMI 21.4±2.6kg/m^2; duration of diabetes 6.1±2.8years; total daily dose 1.0±0.4U/kg/day; mean±SD) were studied at a clinical research facility on two occasions. Subjects were randomly allocated to receive either CL or open-loop (OL) (conventional CSII treatment) from 19:30 on day 1 for 36h. During CL, basal rates on insulin pump were manually adjusted every 15min as per advice of a model-predictive-control algorithm informed by real-time continuous glucose monitor. On each occasion, subjects engaged in normal daily activities (e.g. playing computer games, walks). They consumed meals (50-80gCHO), accompanied by self-calculated insulin boluses, and snacks (15-30gCHO). Moderate-intensity exercise on a stationary bicycle at 140bpm heart-rate was performed at 10:40 (40min) and at 17:30 (20min). Overall mean plasma glucose levels were 130±22mg/dl during CL versus 162±52mg/dl during OL (p=0.009). Time spent in target glucose range 70-180mg/dl was 82±10% vs 55±29% (p=0.002). Time above 180mg/dl was 13±12% vs 37±33% (p=0.005) and time spent below 70mg/dl was 5±4% vs 7±11% (p=0.50). Overnight, plasma glucose levels were in target for 97±7% vs 51±38% (p=0.02) and for 95±11% vs 45±45% (p=0.001) during the first and second night, respectively. Hypoglycemia occurred on 11 occasions during OL vs 9 during CL (5 episodes were exercise-related, 4 occurred within 2 hours after meals). In conclusion, day-and-night CL may improve glucose control significantly compared to conventional CSII. Further

For author disclosure information, see page 785.

adjustments are needed to optimise insulin delivery to minimise risk for hypoglycemia after exercise and around meals.

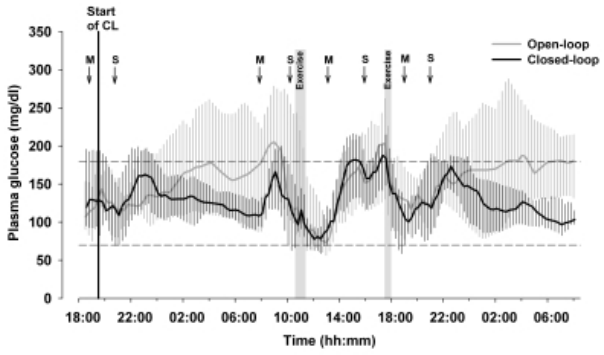

Figure. Plasma glucose (median, IQR) during OL (grey line) and CL (black line). Meals (M) and snacks (S) are shown by vertical arrows.

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154-OR

Glucose Control Using Closed Loop Insulin Delivery during Nights with or without Antecedent Afternoon Exercise

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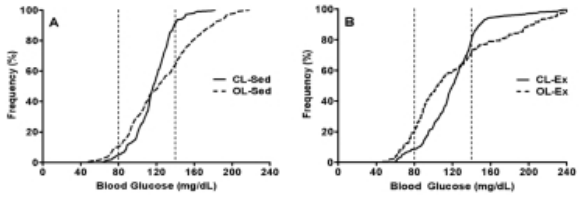
The aim of this study was to determine whether a closed-loop (CL) system utilizing subcutaneous glucose sensing and insulin delivery improves overnight glucose control following afternoon exercise compared to usual open-loop (OL) pump use in adolescents and young adults with type 1 diabetes (T1D). Subjects completed two separate 48-hour inpatient periods of glucose control, in random order: usual OL, and CL control using a PID+IFB algorithm. Each admission included a sedentary day and an "exercise day", with a standardized protocol of four 15-minute periods of brisk treadmill walking to 65-70% HR_{max} starting at 3PM. During CL control, target glucose was set at 120 mg/dl; nocturnal hypoglycemia (NH) was defined as venous blood glucose (BG) level < 60 mg/dl between 10PM-6AM. Twelve subjects (7 female, age 12-26 y, A1C 7.4±0.6%) were studied. During nights following sedentary days, 86% of BG values were within target (80-140 mg/dl), 5% were below target (<80 mg/dl), and 9% were above target (>140 mg/dl) during CL control, compared to 54%, 10%, and 46% during OL ($p<0.0001$). During nights following daytime exercise, 72% of BG levels were within, 8% below, and 20% above target during CL control, compared to 52%, 21%, and 27% during OL control ($p=0.006$). One episode of NH after daytime exercise occurred during CL control compared to 14 during OL ($p=0.06$); following sedentary days, 2 episodes of NH occurred during CL compared to 8 during OL (p=ns).



In conclusion, compared to OL pump therapy, the CL system was associated with greater percentage of nocturnal BG values within target and fewer episodes of NH following sedentary or exercise days. Such systems, even if used at night only, may be useful to improve glycemia and minimize the risk of NH in people with T1D in the home setting.

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155-OR

The Multi-Modular Model Predictive Control-to-Range (MPC2R) Allows Simultaneous Improvement in Safety and Efficacy of Closed-Loop Insulin Delivery in Type 1 Diabetes (T1D)

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Our international Artificial Pancreas study group (iAP) proposes a multi-modular MPC2R for closed-loop insulin delivery, including a safety supervision and an open-loop informed control module, that aims at combined safe and normal range glucose control in T1D. We assessed the ability of MPC2R to improve % time spent in the 3.9-10 mmol/l glucose range vs. patient-driven continuous subcutaneous insulin infusion (CSII). Secondary endpoints included % time spent in the tighter 4.4-7.8 mmol/l range overnight and intra-/inter-subject glucose variability.

Eight (eleven studied, 3 discarded due to software problems) T1D patients (5M, 3F; age =37±2 yrs [mean±SE]; BMI=23.6±0.9 kg/m^2; duration =24±3 yrs; HbA1c=7.4±.0.3%) underwent two clinical trials with MPC2R or CSII in randomized order. Admission was at 10am, lunch at noon, exercise (30 min at 50% VO2max) at 4pm, dinner at 7pm and snack at 10.30 pm. Trials ended the next morning at 8am. All patients wore Dexcom7Plus™ or FreeStyle Navigator™ glucose monitoring devices and OmniPod™ insulin pump. MPC2R run on APS-UCSB platform and was switched on at 2 pm. Overall and overnight controls were assessed on 4pm-8am and 0-8am intervals, respectively.

MPC2R improves overall % time spent in 3.9-10 mmol/l range and mean YSI-glucose levels with no increased % time <3.9 mmol/l (Table 1). Moreover, MPC2R improves % time spent in tighter 4.4-7.8 mmol/l range, mean YSI-glucose levels and intra-/inter-subject glucose variability overnight.

MPC2R will be employed in the forthcoming JDRF multicenter trial.

Table 1: Glucose control with MPC2R vs CSII.

	Overnight			Overall		
	CSII	MPC2R	p-value	CSII	MPC2R	p-value
% time [4.4 7.8] mmol/l	43%	79%	0.040	47%	64%	ns
% time [3.9 10] mmol/l	80%	98%	ns	77%	93%	0.039
% time <3.9 mmol/l	1.5%	1.8%	ns	1.5%	2.6%	ns
mean G (YSI) [mmol/l]	7.74	6.17	0.0004	8.10	7.15	<0.0001
intra-subject G variability [mmol/l]	3.76	2.01	0.011	3.40	2.96	ns
inter-subject G variability [mmol/l]	2.53	0.94	0.016	3.21	2.25	ns

ns : not significant

Supported by: JDRF

156-OR

Conforming to the New Consensus Guidelines for ICU Management of Hyperglycemia: The Updated Yale Insulin Infusion Protocol

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Based on recent clinical trial data, a 2009 AACE-ADA consensus statement stresses the use of intravenous (IV) insulin in the intensive care unit (ICU), but with a less stringent blood glucose (BG) target (140-180 mg/dl) than previously endorsed. Since 2003, our 966-bed tertiary care hospital has utilized a standardized IV insulin infusion protocol (IIP), initially targeting 100-140 mg/dl, and subsequently (2005) revised to 90-120 mg/dl. Both have been validated and published and are now used in many US hospitals either in their original forms or after local adaptation. In response to the new guidelines, we revised our IIP to a BG target of 120-160 mg/dl - chosen with the intent of achieving an average BG toward the lower end of the recommended 140-180 mg/dl range, as intimated in the consensus statement.

To validate the new IIP, we tracked clinical responses with the initial 115 patients in our medical ICU (mean age 62±14 years, 52% male, 35% ethnic minorities, 66% with a history of diabetes). The 3 most common admission diagnoses were acute repiratory failure (29%), pulmonary sepsis (25%) and bacterial pneumonia (11%). The mean admission APACHE III score was 62±27. The median length of ICU stay was 9.50 days (IQR, 4.25-19.75) and duration of insulin infusion, 58.5 hours (IQR, 25.0-127.5.) The mean baseline BG was 365±81 mg/dl, with the BG target achieved after a median of 7.0 hours (IQR, 5.0-11.5). Once the target was reached, the mean BG on the IIP was 155.3±21.7 mg/dl (median, 150 [IQR, 127-180] mg/dl.) The mean insulin

For author disclosure information, see page 785.

ADA-Funded Research

infusion rate required to reach and maintain the target range was 3.9±2.2 units/hour. The mean nadir BG was 93±26 mg/dl. Hypoglycemia was rare, with only 0.3% of hourly BGs recorded <70 mg/dl and just 0.02% <40 mg/dl. In all cases hypoglycemia was rapidly corrected using intravenous dextrose with no evident untoward outcomes.

The updated Yale IIP provides effective and safe targeted BG control in the critically ill, in compliance with current national guidelines. It can be easily implemented by hospitals who are now utilizing the original Yale IIP.

FACTORS RELATED TO ONSET AND MANAGEMENT OF TYPE 1 DIABETES FROM INFANCY THROUGH ADOLESCENCE

157-OR

Heterogeneity of Islet Autoimmunity Developing in Children 0-12 Years Old

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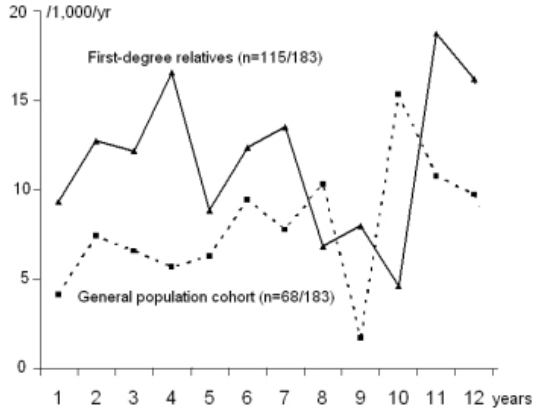
One of the paradigms of the natural history of type 1 diabetes (T1D) has been that islet autoimmunity (IA) rarely develops beyond early childhood. The Diabetes Autoimmunity Study in the Young (DAISY) has followed 2,542 children at increased genetic risk of T1D for a median of 9 years, allowing testing of the hypothesis that IA may develop in older children/adolescents. DAISY subjects are tested annually for autoantibodies (AB) to glutamic acid decarboxylase (GAD65), insulin (IAA), insulinoma associated antigen-2 (IA-2), and zinc transporter 8 (ZnT8). Persistent IA has developed in 183 children; of those 69 have progressed to T1D. Surprisingly, the incidence of IA appears to be bimodal, with the second peak beginning at ages 10-11, both in the general population and first-degree relatives (FDR).

Figure. Age-specific incidence of persistent IA by family history of T1D



Children who developed IA after age 7 were more likely than those with earlier IA to belong to ethnic minorities or have a high-risk MIC-A 5.1 allele (Table, X^2 test). They were more likely to first develop GAD65, but less likely to initially present with or quickly develop multiple ABs. Interestingly, both groups were similar in terms of having an FDR with T1D (63% vs 63%), or HLA-DR3/4,DQB1*0302 (34% vs 33%), PTPN22 (28% vs 21%), INS (10% vs 11%) or CTLA4 (67% vs 74%) high-risk genotypes.

Characteristic	Early Onset (≤7yrs)	Late Onset (>7yrs)	p-value
	n=140	n=43	
Non-Hispanic white	86%	67%	0.0070
MICA 5.1	62%	81%	0.0491
GAD65+ first	41%	58%	0.0445
Spreading to other ABs	48%	16%	0.0002
IAA+ first	45%	30%	0.0856
> 1 AB at the first test	18%	7%	0.0830

At least a third of the IA cases develop after age 7 as a slowly progressing phenotype. The role of nutrition, puberty, and minor genetic factors, which are more frequent in minority populations, in slowly progressing IA in older children remains to be explored.

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158-OR

The Use of the Diabetes Prevention Trial-Type 1 Risk Score (DPTRS) for Identifying Normoglycemic Individuals at High Risk for Type 1 Diabetes

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Autoantibody positive individuals with dysglycemia (impaired fasting glucose, a 30, 60 or 90 minute glucose value ≥200 mg/dl, and/or impaired glucose tolerance) are considered to be at high risk for developing type 1 diabetes (T1D) within 5 years. However, there has not been a definitive means for identifying autoantibody positive individuals at high risk when glucose tolerance is normal. We assessed the use of the Diabetes Prevention Trial-Type 1 Risk Score (DPTRS) for identifying such individuals. The DPTRS is based on proportional hazards regression estimates for age, log BMI, log fasting C-peptide, and glucose and C-peptide sums (30, 60, 90 and 120 minutes) during oral glucose tolerance tests. Analyses were performed in two cohorts: the Diabetes Prevention Trial-Type 1 (DPT-1; n=670) and the TrialNet Natural History Study (TNNHS; n=991). Participants in both cohorts were autoantibody positive relatives of T1D patients. The 5-year risks for T1D for normoglycemic individuals with a DPTRS≥7.00 were 0.74 in DPT-1 (n=117) and 0.73 in the TNNHS (n=62). In a comparison with dysglycemic individuals (n=138 in DPT-1; n=221 in the TNNHS), those normoglycemic with a DPTRS≥7.00 were much younger, both in DPT-1 (9.5±4.5 yrs vs. 15.2±10.9 yrs; p<0.001) and in the TNNHS (8.1±4.9 yrs vs. 19.6±14.3 yrs; p<0.001). Although both cohorts included individuals up to 46 years, all of those normoglycemic with a DPTRS≥7.00 were <25 years; over 85% were <15 years. First-phase insulin responses (FPIR) from intravenous glucose tolerance tests were measured in DPT-1 (normoglycemic: n=190 for FPIR<10th percentile and n=49 for FPIR<1st percentile, respectively). The T1D risk was higher (p<0.001) for those with a DPTRS≥7.00 than for those in either FPIR group (5-year risks for FPIR<10th and FPIR<1st percentiles: 0.41 and 0.48, respectively). In conclusion, the DPTRS effectively identifies normoglycemic, autoantibody positive individuals at high risk for T1D, and appears to be better at identifying such individuals than the FPIR. The DPTRS is particularly useful for identifying normoglycemic children who are at high risk for T1D.

159-OR

Adiposity at Diagnosis of Childhood Type 1 Diabetes—Have We Reached a Plateau?

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Increased adiposity has been implicated as an accelerator for type 1 diabetes (T1D), however findings have been inconsistent across different populations. We examined anthropometric trends in children aged 0-16 years (n=2025) with T1D, diagnosed between 1976-2009, from Sydney, Australia. Height, weight and BMI standard deviation scores (SDS) were examined after initial stabilization, 1-3 months after diagnosis. Multiple linear regression was used to examine factors associated with BMI SDS, including time period (T1 to T5), age, gender and birth weight SDS. Patient characteristics (mean ± SD or %) are shown in the table.

Characteristic	T1 1976-89	T2 1990-94	T3 1995-99	T4 2000-04	T5 2005-09	*P* value
Number	50	271	481	689	534	
Males (%)	42	47	42	53	55	0.001
Age at diagnosis (yr)	8.09 ± 3.50	8.97 ± 3.38	8.52 ± 3.63	9.20 ± 3.64	9.04 ± 3.73	0.11
Height SDS	0.04 ± 1.00	0.22 ± 0.97	0.34 ± 1.01	0.58 ± 1.24	0.61 ± 1.14	<0.001
Weight SDS	0.09 ± 1.13	0.37 ± 0.98	0.63 ± 0.97	0.79 ± 1.04	0.83 ± 1.01	<0.001
BMI SDS	0.05 ± 1.47	0.41 ± 0.96	0.62 ± 0.99	0.67 ± 0.97	0.71 ± 0.95	<0.001
BMI > 85th centile (%)	22	28	35	37	37	0.03
Birth weight SDS	-0.14 ± 1.10	-0.25 ± 1.10	-0.07 ± 1.14	0.19 ± 1.21	0.06 ± 1.43	0.004

BMI SDS increased from T1 to T3 (p<0.001) but remained unchanged from T3 to T5 (p=0.17). Similarly, overweight/obesity (BMI >85th percentile) increased from T1 to T3 (p=0.03), with a plateau from T3 to T5 (p=0.67). Higher BMI SDS was associated with younger age at diagnosis (p<0.001),

For author disclosure information, see page 785.

however in children aged < 5 years, BMI SDS did not increase over time. Birth weight SDS increased over time, but was unchanged from T3 to T5 (p=0.98). Factors associated with BMI SDS in multiple linear regression analysis were time period (p<0.001), age (p<0.001) and male gender (p=0.02). In conclusion, Australian children with T1D are not heavier at birth compared with reference standards. BMI SDS at diagnosis of T1D has followed secular trends, with a plateau since 1995. Therefore, whilst postnatal weight gain may be an accelerator for T1D, increased adiposity does not explain the rising incidence of T1D in recent years.

160-OR

Preschool Children with Type 1 Diabetes Are Less Physical Active Than Non-Diabetic Children

FRIDA SUNDBERG, GUN FORSANDER, ULF EKELUND, *Gothenburg, Sweden, Cambridge, United Kingdom*

Physical activity reduces insulin resistance, lowers plasma glucose and HbA1c. Children with diabetes are recomended to be at least as physically active as other children.The aim of this study was to investigate if children younger than seven years with type 1 diabetes are less physically active than non-diabetic children. 24 children with diabetes (12 boys, mean age 4.5 years, duration 2.0 years) where compared with controls. Physical activity was measured with a combined accelerometer and heart rate monitor (Actiheart, Cambridge Neurotechnology Ltd, UK) applied on the child's thorax. The children wore the monitors continuously during one week in the autumn and one week in the spring. Sickdays were reported and excluded from the analyses.Children with diabetes were found to be less physically active than the control group, expressed both as time spent in Moderate to Vigorous Physical Activity (p<0,05) and total physical activity, counts per minute (p<0,05), but the difference in time spent sedentarily was not significant. Reduced physical activity during childhood may have negative consequences. The reasons for the observed differences needs further research, since diabetes in itself does not impair the ability to be physically active.

	Diabetes			Controls		
	Total n=24	Boys n=12	Girls n=12	Total n=26	Boys n=12	Girls n=14
Age (years)	4.5 (1.7)	4.3 (1.6)	4.7 (1.9)	4.6 (1.6)	4.9 (1.4)	4.4 (1.8)
Diabetes duration (years) *	2.0 (1.6)	2.1 (1.9)	1.8 (1.3)			
HBA1c NGSP (%), *	7.8 (0.64)	7.8 (0.58)	7.8 (0.72)			
HbA1c IFCC (mmol/mol), *	60 (7.0)	60 (6.4)	60 (7.9)			

	Diabetes			Controls			p
	Total n=24	Boys n=12	Girls n=12	Total n=26	Boys n=12	Girls n=14	
Registrations, number of	43	20	23	46	21	25	
Number of days per registration *	6.7 (0.87)	6.7 (0.75)	6.7 (0.98)	6.9 (0.58)	6.9 (0.44)	6.8 (0.69)	ns
Registration mins./day *	1230 (43)	1235 (44)	1226 (43)	1223 (51)	1231 (26)	1217 (64)	ns
MVPA/time registered *	0.038 (0.023)	0.041 (0.024)	0.034 (0.021)	0.050 (0.024)	0.060 (0.021)	0.041 (0.024)	0.017
cpm *	68 (23)	73 (26)	65 (21)	81 (22)	90 (19)	73 (21)	0.011
Sedentary time/ registered *	0.60 (0.065)	0.59 (0.076)	0.61 (0.050)	0.58 (0.053)	0.57 (0.051)	0.58 (0.054)	0.077

*mean (SD)

161-OR

Dietary and Physical Activity Patterns in Adolescents with and without Type 1 Diabetes

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Diet and physical activity (PA) are fundamental aspects of care in type 1 diabetes (T1D), but scant data exist on diet and PA behaviors of T1D adolescents, especially compared to non-diabetic (non-DM) controls. The AAP recommends eating 3 balanced meals/day, eating breakfast daily, and limiting sugary beverages and energy dense foods. The ADA recommends a diet that includes carbohydrates from fruits and vegetables as well as moderate-intensity PA for at least 150 min/week.

Data in 302 T1D (age=15.4±2.1 yrs, T1D duration=8.8±3.0 yrs, A1c=8.9±1.6, 50% male) and 100 non-DM youth (age=15.4±2.1 yrs, 47% male) were collected. Diet data (eating out/week, meals/day, snacks/day, and weekly consumption of sugary beverages, breakfast, fruit, vegetables, sweets, and fried foods), PA, screen time (TV, computer, video games), and alcohol and tobacco use were collected using interviewer administered questionnaires.

Overall, no significant differences in diet were found between adolescents with and without T1D; both groups did not meet the nutrition guidelines of eating breakfast daily or fruit and vegetable intake. Both groups met the PA guidelines. T1D youth were more physically active, but had higher BMI than non-DM controls (Table). Screen time did not differ between groups (4.3±3.2 v. 4.0±2.5 hrs/d, p=0.39) and was double the AAP recommended amount (1-2 hrs/d). More T1D youth reported living with someone who smoked (21% v. 11%, p=0.03), however; there was no difference in smoking and alcohol use.

Adolescents with and without T1D have similar poor dietary patterns despite T1D adolescents receiving dietary education and support through their diabetes care team. Adolescents with T1D had higher BMI than non-DM controls despite increased PA. Improving diet in T1D youth remains an unmet need.

	T1D	non-DM	p-value
BMI, kg/m^2	22.8±3.9	21.9±4.3	**0.04**
#Meals/d	3.1±0.6	3.0±0.8	0.29
#Snacks/d	2.3±1.2	2.2±1.1	0.56
Breakfast*	3.3±1.2	3.4±1.1	0.42
Vegetables*	2.8±1.2	3.0±1.2	0.07
Fruit*	2.9±1.2	2.8±1.3	0.67
PA d/wk	5.0±1.8	4.9±1.6	0.63
PA min/wk	560.6±456.8	455.5±301.7	**0.02**
PA level†	1.9±0.6	2.0±0.5	0.40

*Times per week

†PA level: 1=mild, 2=moderate, 3=strenuous

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162-OR

The Use of Diffusion Tensor Imaging (DTI) in Young Children with Type 1 Diabetes

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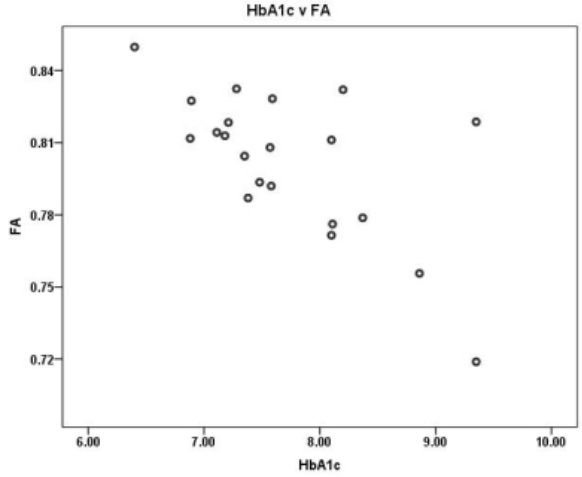
DTI is used to investigate white matter (WM) structure in the brain. DTI has shown deficits in WM structure in adult subjects with T1DM that correlated with reduced neurocognitive function. However, there are no published DTI studies in children with T1DM. Young children, ages 3 to 10 years, with TIDM and matched healthy controls (HC) completed age appropriate batteries of neuropsychological tests and MRI scans of the brain. Twenty-one DTI scans from children with T1DM (mean age 7.9 ± 1.6) and 12 scans from HC (mean age 7.3 ± 1.6) were analyzed using Tract-Based Spatial Statistics (TBSS). Voxel-wise between-group comparisons of Fractional Anisotropy (FA, degree of diffusion anisotropy), Radial Diffusivity (RD, representing diffusion perpendicular to the fiber axis) and Axial Diffusivity (AD, representing diffusion along the fiber axis) were performed using Threshold-Free Cluster Enhancement in a fully corrected analysis. We used TBSS to conduct correlation analysis between WM structure and HbA1C levels, duration of T1DM, and neuropsychological test scores. Using a whole-brain analysis, there were no significant differences between T1DM and HC. Negative correlations of FA (p = 0.053) and RD (p = 0.055) with increased HbA1c in the internal capsule and splenium of the corpus callosum approached significance.



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ADA-Funded Research

A negative trend was seen between FA and NEPSY attention scores (0.09) in the fronto-striatal tracts which are known to be related to attention. These tracts were used in a post-hoc analysis as a region of interest to investigate whether attention-related circuits are affected in T1DM. In this analysis, significantly reduced AD was seen in T1DM when compared to HC (p = 0.04). This is the first study to suggest early signs of WM variation in children with T1DM. Larger studies of WM structure are needed to define the impact of T1DM on the developing brain.

163-OR

Psychological Screening in Adolescents with Type 1 Diabetes Predicts Outcomes One Year Later

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Adolescents with type 1 diabetes are at increased risk for depression and anxiety. Symptoms of depression and anxiety adversely affect diabetes management, glycemic control, and quality of life (QOL). Despite these findings, systematic psychological screening is rarely employed in pediatric diabetes centers. The aim of this study was to assess the ability of psychological screeners to predict adolescents' blood glucose monitoring (BGM) frequency, A1c values, and QOL over one year. 150 adolescents with type 1 diabetes completed the Children's Depression Inventory (CDI) and State-Trait Anxiety Inventory for Children (STAIC) screeners at baseline. One year later, meters were downloaded to assess BGM frequency, A1c values were obtained, and parents rated teens' diabetes-related quality of life on the Pediatric Quality of Life Inventory™ (PedsQL). Adolescents had a mean age of 15.5±1.4 years and diabetes duration of 6.0±3.9 years. Mean A1c at baseline was 8.8±1.9%, mean BGM frequency was 3.8±1.7 checks per day, and 63% of the teens received insulin via continuous subcutaneous insulin infusion (CSII). The predictive utility of the screener scores was examined using separate regressions for each outcome at 12 months. Each analysis included nine demographic and medical covariates. In three separate regressions, higher depression scores predicted less frequent BGM (b=-0.05, p<.05) and poorer diabetes-related QOL (b=-0.71, p<.01), and higher state anxiety scores predicted higher A1c values (b=0.07, p<.05). In addition, older age and single caregiver marital status predicted lower BGM. Receiving insulin via multiple daily injections (MDI), single caregiver marital status, and private insurance predicted higher A1c. Continuous screener scores identified risk for poor 12-month outcomes more robustly than did clinical cut-off scores. Adolescents' psychological screener scores predict critical diabetes outcomes one year later. Results support recommendations for annual psychological screening of adolescents with type 1 diabetes with the goal of identifying symptoms of depression and anxiety and intervening to prevent suboptimal diabetes outcomes.

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164-OR

Measuring Resource Use of Children with Type 1 Diabetes

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To understand the burden of disease and its treatment from a societal perspective, costs of care including those incurred by patients and families must be assessed with psychometrically sound instruments. The purpose of this study was to assess the psychometric properties of a 20 item resource utilization questionnaire (RUQ) modified for use in families of children with type 1 diabetes (T1DM) using insulin pumps.

We established content validity in a pediatric T1DM population from 2 centers using an iterative process that included: (1) initial RUQ modification by the research team in consultation with a health economist, (2) review by an expert panel of parents and health professionals, and (3) discussion with a parent focus group. To establish criterion validity, parents completed the modified RUQ (mRUQ) and participated in an individual interview to compare mRUQ responses with actual healthcare bills. All groups/interviews were audio-taped and field notes taken.

Two health professionals and 16 parents (age 46.9±5.8 yrs, 88% female, 94% Caucasian, 63% ≥college degree) of 14 T1DM children (age 13.6±4.0 years, T1DM duration 7.7±4.1 yrs, pump duration 4.6±3.1 yrs) participated in an expert panel, focus group, or interview. The expert panel and focus group recommended stratification of mRUQ direct healthcare costs by provider type, addition of parent and child time and productivity items (e.g. time for diabetes self-care, school absences), and decreased recall timeframe from 6 to 3 months. The finalized mRUQ contained 25 items: 5 direct and 3 non-direct healthcare, 8 time, and 9 productivity costs. On average parents completed the mRUQ in <10 minutes. Criterion validity of direct healthcare costs was high (TIDM appointments 100%; supplies/medications 67%); time and productivity costs were not able to be verified.

Assessment of the costs of pediatric T1DM interventions from a societal perspective enables broader understanding of disease and treatment and provides stakeholders with information on intervention value and efficiency. The mRUQ validly measures cost incurred by T1DM children and families, is easily completed, and can be used in future comparative effectiveness research to inform decision-making.

EXPERIMENTAL ISLET TRANSPLANTATION/ GLYCEMIC CONTROL AFTER KIDNEY TRANSPLANTATION

165-OR

Comparison of Exendin-4 on Beta-Cell Replication in Mouse and Human Islet Grafts

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Enendin-4 can stimulate β-cell replication in mice. Whether it can stimulate β-cell replication in human islet grafts is unknown. Therefore, we compared the effects of exendin-4 on β-cell replication in mouse and human islet grafts in diabetic mice. Mouse islets were isolated from young (8 week-old) and old (>40 week-old) C57BL/6 mice. Human islets from 9 pancreatic donors were obtained from the JDRF and NIH Islet Cell Resource Centers. They are divided into ≤22 year-old (n=4) and ≥35 year-old (n=5). Islets were transplanted into streptozotocin-induced diabetic mice or immunodeficient nude mice. All recipients were given BrdU daily with or without exendin-4 (10nM/kg daily). At 4 weeks posttransplantation, islet grafts were removed for insulin and BrdU staining. Diabetes was reversed in all mice transplanting 100 syngeneic mouse islets from young or old donors. However, normoglycemia was achieved significantly faster in exendin-4 treated mice (P<0.01). The percentage of insulin$^+$/BrdU$^+$ β cells was 7.2±1.8% in untreated islet grafts from younger donors and 16.2±5.2% in exendin-4 treated islet grafts from younger donors (P<0.01); 3.6±3.1% in untreated islet grafts from old donors and 16.7±5.8% in exendin-4 treated islet grafts from old donors (P<0.01). Normoglycemia was achieved in 50% of untreated and 94% of exendin-4 treated nude mice that received human islets from donors ≤22 years-old; and in 52% of untreated and 74% of exendin-4 treated nude mice that received islets from donors ≥35 years-old. Human islet grafts from young donors had more insulin$^+$/BrdU$^+$ β cells in exendin-4 treated mice than in untreated mice. The percentage of insulin$^+$/BrdU$^+$ β cells was 4.5±2.4% in islet grafts from untreated nude mice and 9.9±3.9% in islet grafts from exendin-4 treated nude mice (P<0.01). However, human islet grafts from donors ≥35 years-old contained few insulin$^+$/BrdU$^+$ β cells. Our data demonstrated that exendin-4 stimulated β-cell replication in mouse islet grafts from both young and old donors and in human islet grafts from donors ≤22 years-old, but not from donors ≥35 years-old. Our studies indicated that GLP-1 agonists can be used to stimulate β-cell replication in human islets from young donors.

166-OR

Stimulating Beta Cell Replication and Improving Islet Graft Function by AR231453, a GPR119 Agonist

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G protein-coupled receptor 119 (GPR119) is predominantly expressed in β cells and intestinal L cells. AR231453 is a GPR119 agonist that can enhance glucose-dependent insulin secretion and GLP-1 release. In this study, we investigated whether AR231453 can directly stimulate β-cell replication and improve islet graft function in diabetic mice. Diabetes in C57BL/6 mice was induced by streptozotocin rejection. To determine islet graft function, 100 syngenic C57BL/6 mouse islets were transplanted under the left kidney of each diabetic C57BL/6 mouse. These recipient mice were given BrdU daily and with or without AR231453 at 10 mg/kg/day, starting from the day of islet transplantation. Islet graft function was monitored by measuring blood glucose level. At 4 weeks, left nephrectomy was performed to remove the kidney bearing islet grafts to determine β-cell replication in islet grafts. Insulin and BrdU immunofluorescence staining was performed and insulin$^+$ and BrdU$^+$ β cells in islet grafts were accounted using confocal microscope. To determine whether AR231453 increases plasma GLP-1 level, we collected

For author disclosure information, see page 785.

plasma from AR231453 treated mice at 30 minutes after treatment and measured plasma active GLP-1 by ELISA. Although all recipient mice achieved normoglycemia at 4 weeks with or without treatment, normoglycemia was achieved in significantly fewer days in AR231453 treated mice. The vehicle treated mice achieved normoglycemia in 16 ±6 days (N=7), while AR231453 treated mice only required only 8±3 days (N=7, P<0.01). The percentage of insulin$^+$ and BrdU$^+$ β cells in islet grafts were significantly higher in AR231453 treated mice than in vehicle treated mice. The mean percentage of insulin$^+$ and BrdU$^+$ β cells in islet grafts was 21.5±6.9% in AR231453 treated mice and 5.6±3.7% in vehicle treated mice (P<0.01). The plasma active GLP-1 levels were also significantly higher in AR231453 treated mice than in control mice (9.0±2.2 pM vs. 4.8±1.1 pM, P<0.05). Our data demonstrated that GPR119 agonist can stimulate β-cell replication and improve islet graft function. Targeting GPR119 is a novel therapeutic approach to stimulate β-cell replication in islet grafts.

167-OR

Variants of the Adiponectin and Adiponectin Receptor 1 Genes Are Associated in a Gender Specific Manner with Posttransplantation Diabetes Mellitus in Renal Allograft Recipients

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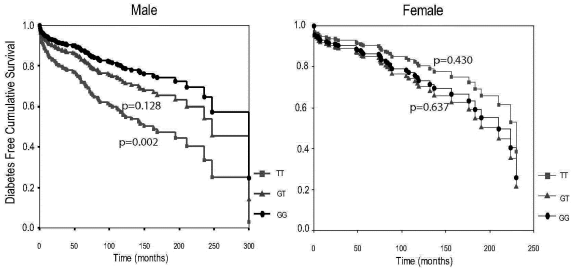
Posttransplantation diabetes mellitus (PTDM) is a major metabolic complication in renal transplant recipients. A number of risk factors for PTDM have been reported and genetic factors are also considered to play an important role in PTDM development. We examined the association between PTDM and 10 single nucleotide polymorphisms (SNPs) of adiponectin gene (*ADIPOQ*) *and* adiponectin receptor-1 gene (*ADIPOR1*) in a cohort of renal allograft recipients.

A total of 575 patients who received kidney transplants between 1989 and 2007, without a previous history of diabetes and had a pretransplant fasting glucose<5.5 mmol/L were included. We analyzed the association between the PTDM development and the following SNPs: *ADIPOQ* rs266729, rs822395, rs822396, rs2241766, rs1501299 and *ADIPOR1* rs2232853, rs12733285, rs1342387, rs7539542, and rs10920531.

Patients with the CG-heterozygote of *ADIPOQ* rs266729, TT-homozygote of *ADIPOQ* rs1501299 and CC-homozygote of *ADIPOR1* rs10920531 were associated with PTDM after adjustment for age, gender, amount of weight gain and type of immunosuppressant (HR=1.70; P=0.015, HR=1.70; P=0.032, and HR=1.96; P=0.039, respectively). There was an interaction between gender and *ADIPOQ* rs1501299 genotype (P=0.037). In male patients, those with the TT-homozygote in *ADIPOQ* rs1501299 were significantly more likely develop PTDM (HR=2.50, P=0.002) than those with the wild GG-homozygote and subjects with the GT-heterozygote showed a tendency to develop diabetes (HR=1.41, P=0.128) comparing with those with the wild GG-homozygote.

These data suggest that genetic variations in *ADIPOQ* rs1501299 is associated with PTDM in a gender specific manner.



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168-OR

Glycemic Control after Kidney Transplantation: Does It Matter?

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There is conflicting data as to whether maintaining near-normal glucose levels during the immediate post-transplant hospitalization or during the first year post-transplant may result in decreased episodes of rejection, readmission or infection. We conducted a one-year multicenter retrospective review of all adults undergoing renal transplantation. We captured all blood glucose (BG) values for the initial peri-operative (PO) hospital stay, as well as HBA1C levels, GFR, therapies for immunosuppression and diabetes, all rejections, infections, and readmissions for 1 year following transplant. We excluded patients receiving a simultaneous liver or pancreas transplant, and patients with primary graft failure. We studied 202 patients (70% male), mean age 52±13 years, 68% Hispanic/African American, 25% Caucasian; 59% had pre-existing diabetes. 59% received cadaveric, 28% living-related, and 12% living-unrelated transplants. 98% received glucocorticoid therapy in the immediate post-operative period. Mean PO BG was 157.6±34.5 mg/dl, and 82% received insulin during the PO period. Mean length of stay was 8.3±4.3 days. Mean HBA1C for the first year post transplant was 6.8±1.5%. Initial immunosuppression was: mycophenolate-97%, tacrolimus-74%, cyclosporine-23%, and prednisone-55%. 74% of patients were hospitalized at least once in the next year, 27% were treated for acute or chronic rejection, and 44% were treated for an infection. There was no significant association between mean PO BG and the rates of rejection (p=0.59), readmission (p=0.74) or infection (p=0.22). There was also no significant association between mean HBA1C in the first year post transplant and the rates of rejection (p=0.11), readmission (p=0.2), or infection (p=0.12). These results were unchanged after adjustment for age, sex, race, and a history of pre-existing diabetes. 2 patients required re-initiation of dialysis within the first year post-transplant. The 1-year mortality was 3%. Conclusion:In the current era of renal transplantation, we found no association between the glycemic control at the time of transplantation or during the first year post-transplant and the rates of graft rejection, infection or hospital readmission.

169-OR

The Incretin Response Post-Islet-Transplantation

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While little is known of the incretin response in intraportal allogeneic islet-recipients, the incretin response is reportedly normal in whole-pancreas transplant- recipients. The incretin response might be altered post-islet-transplantation because of extrinsic islet denervation, reduced engrafted islet-mass and β-cell exposure to higher levels of GLP1 in the portal vein. We aimed to evaluate the incretin response in islet-recipients and compare it with that in controls and type 2 diabetics. 3 islet-recipients in their first year of insulin-independence, 3 type 2 diabetics and 10 non-diabetic controls underwent a 4-hour 75g OGTT and an isoglycemic IVGTT on 2 separate occasions. Plasma glucose, insulin and C-peptide were measured at 0,30,60,90,120,150,180 and 240min during both procedures. Percent incretin response was calculated with the following formula:

[incAUCins(OGTT) –incAUCins(isoglycemic IVGTT)]/incAUCins(OGTT) x 100

We found that the mean incretin response in the islet-recipients and the type 2 diabetics was significantly lower when compared with controls(recipients: 48.7 $\pm$ 1.9, T2DM: 47.4 $\pm$ 4.6, controls: 80.1 $\pm$ 2.8 SEM%; recipients vs controls, T2DM vs controls, p<0.05).To conclude, this is the first report on the incretin response in intra-portal allogeneic islet-recipients. The incretin effect while impaired when compared with controls, was still present post-islet –transplantation. Possible reasons for the impairment include a low engrafted beta-cell mass, GLP1-resistance and loss of neurogenic reflexes. These findings indicate that while extrinsic innervation plays a negligible role in the incretin response in whole-pancreas-recipients, neurogenic mechanisms have a greater role post-islet-transplantation where β-cell mass is greatly reduced. The fact that the incretin response is not totally abolished however implies the ability of circulating gastrointestinal hormones to augment insulin secretion during the OGTT is partially preserved in islet-recipients. These findings indicate a role for supplemental exenatide in islet-recipients early rather than late post-transplant.

170-OR

Coating of Porcine Islets with Endothelial Progenitor Cells Prevented IBMIR and Improved Graft Survival after Intraportal Transplantation

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Composite islet-endothelial cells have been known to reduce instant blood-mediated inflammatory response (IBMIR) in islet transplantation. Endothelial progenitor cells (EPCs) seem to have lower profile of procoagulation in some

For author disclosure information, see page 785.

environment compared to mature endothelial cells. Recently, composite islet-EPCs were reported to inhibit IBMIR effectively *in vitro.* EPCs can be isolated from peripheral blood, and be expanded ex vivo, which is important as for clinical application. These suggest that EPC might be a better source to protect islet grafts from early graft loss. Therefore, we tested effects of composite islet-EPC grafts *in vivo* using porcine islets.

Human EPCs were isolated from cord blood, and expanded *in vitro.* Porcine islets were co-cultured with EPCs and were infused through portal vein of streptozotocin (STZ)-diabetic athymic mice. Body weight and blood glucose were monitored, and liver tissues were harvested within 48 hours after transplantation to examine the effects of EPCs on the grafts using immunohistochemical staining and RT-PCR.

Coating islets with EPCs significantly improved blood glucose levels compared to islet-only grafts immediately after transplantation, which suggested better graft survival and function. On morphologic examination of liver tissues, those with EPC-coated grafts showed about 2-times more insulin-positive area than those with islet-only grafts. CD11b+ cells around grafts, TUNEL-positive beta cells and inflammatory markers were diminished in the liver with EPC-coated grafts, compared to the liver with islet-only grafts.

From these results, EPC-coating has potential as a candidate that can relieve primary failure of islet grafts infused to portal vein, and clinical islet transplantation would be more feasible strategy for the cure of diabetes.

Supported by: The grant from IRICT, by Ministry of Health and Welfare, Republic of Korea

171-OR

A Randomized Controlled Trial To Evaluate the Effect of Glycemic Control on Renal Transplantation Outcomes

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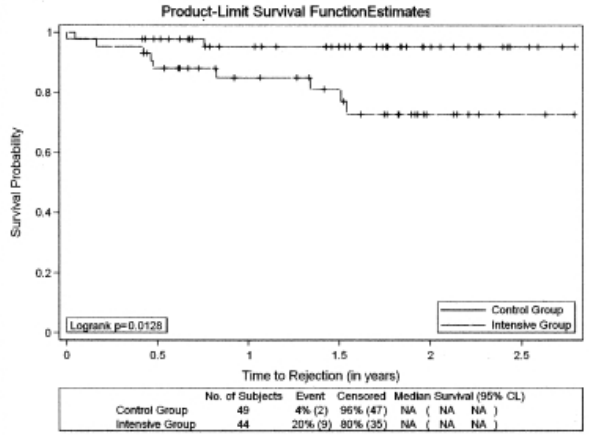
Purpose: This study was a randomized controlled clinical trial which examined two methods of blood glucose (BG) control in patients undergoing renal transplantation.

Methods: Participants were randomized to an intensive treatment arm (intensive, with IV insulin infusion calculator treatment to maintain the BG 70-110 mg/dL for 3 days, followed by subcutaneous insulin to maintain BG 70-140 mg/dL) or a control group (control, with SC insulin to keep BG 70-180 mg/dL while hospitalized, followed by SC insulin to maintain BG 90-180 mg/dL). The primary endpoint was delayed graft function (DGF).

Results: A total of 104 people were screened and the intention-to-treat analysis set consisted of 93 participants (n=44 intensive, n=49 control). DGF rates were 18% (8/44) in the intensive group and 24% (12/49) in the control group (p=0.46). There were 4424 BG readings during inpatient hospitalization and the occurrence of severe hypoglycemia (BG<40 mg/dL) and severe hyperglycemia (BG>350 mg/dL) were the primary safety outcome measures. There were 10 events of hypoglycemia identified, 8 of which (80%) were in the intensive treatment group. There were 30 instances of hyperglycemia with 5 (11%) participants in the intensive group and 12 (24%) participants in the control group (p=0.10). The group-specific mean BG level during the inpatient stay for the intensively treated participants was 122.5 mg/dL, which was lower than the 177.3 mg/dL for the control group (p<0.001). There were 11 rejection episodes, 9 were in the intensive treatment group with a statistically significant increase in the risk for rejection in the intensive group (p=0.013, Figure).



Conclusions: The primary outcome measure of delayed graft function was not statistically different for the two treatment groups, but the intensively treated participants were at higher risk for a rejection episode.

ADA-Funded Research

172-OR

Combination Therapy with Induction of Chimerism and Administration of Growth Factors Reversed Late-Stage Autoimmune Diabetes

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Type 1 diabetes (T1D) results from autoimmune destruction of insulin-secreting β cells. At present, there is no effective therapy for late-stage T1D. Although islet transplantation can provide insulin-independence, the therapeutic effect lasts only for approximately 3 years, due to chronic rejection of the islet graft. Therefore, a therapy that can reverse autoimmunity and augment β cell regeneration simultaneously is required for the cure of late-stage T1D. We have recently developed a radiation-free and graft versus host disease preventative anti-CD3-based conditioning regimen for induction of chimerism and reversal of autoimmunity. With this new regimen, although induction of chimerism did not reverse hyperglycemia in late-stage diabetic NOD mice, there were clusters of insulin-secreting β cells juxtaposed to pancreatic duct in the chimeric recipients, indicating there may be β cell neogenesis in the late-stage T1D mice after induction of chimerism. Since epidermal growth factor (EGF) and gastrin have been reported to augment β cell regeneration, we treated late-stage diabetic NOD mice with combination of induction of chimerism and administration of EGF+gastrin, and we found that this treatment led to reversal of late-stage T1D in about 60% of recipients, but 0% in single therapy control mice. In addition, compared with control mice, the mice given combination therapy had more than 10-fold higher β cell mass and 3-fold higher serum insulin levels after glucose stimulation, indicating that the combination therapy augments β cell regeneration in late-stage T1D mice. Additionally, induction of chimerism led to increased β cell survival and insulin sensitivity as indicated by TUNEL assay and insulin tolerance test respectively. We are now using Rip-CreER-Rosa26-YFP mouse model to trace the origin of new-formed β cells to determine whether the combination therapy augments β cell neogenesis, replication or both. In conclusion, combination therapy with induction of chimerism under a non-toxic anti-CD3-based conditioning regimen and administration of growth factors may result in a cure of late-stage T1D.

Supported by: Iacocca Family Foundation

MACRONUTRIENTS, FOOD INTAKE, AND THE GUT

173-OR

Synergistic Effect of Obesity and Fat Ingestion in Blunting the GH Response to Exercise in Children

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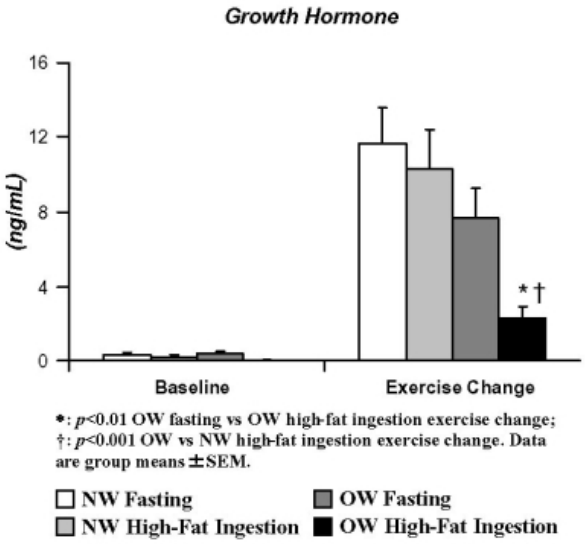
Exercise is a key modulator of growth and development in children via stimulation of the growth hormone (GH)→insulin-like growth factor-I axis. Several factors may alter normal physiological exercise responses such as acute stress, chronic exercise training and diet. We have previously reported that the normal GH response to exercise in children is reduced by: a) obesity (in a dose-dependent fashion); b) ingestion of a high-fat meal. The combined effect of these two conditions, however, has not yet been studied in children. We therefore measured the GH response to exercise in fasting conditions or 45 min after ingestion of a high-fat meal in 16 healthy (NW, 12±1 yrs, 9F, BMI% 51±7), and 19 obese (OW, 12±1 yrs, 13F, BMI% 97±0.4) children. Samples were drawn at baseline and at the end of a 30-min intense, intermittent exercise challenge (ten repeats of 2-min cycling @ ~80% VO_2max, separated by 1-min rest). GH (ng/mL) increased significantly in all groups at the end of the exercise challenge. In NW children, ingestion of a high-fat meal caused a small, non significant blunting of the GH response. In OW children, the GH response to exercise was lower than in NW already in fasting conditions. The GH response in OW was then further reduced after ingestion of a high-fat meal (2.3±0.6), which was significantly lower that in both NW (10.3±2.1, $p<0.001$) and OW (7.7±1.6, $p<0.01$). Our data show that in obese children, who already display reduced GH adaptation to exercise, ingestion of fat nutrients shortly before exercise greatly amplifies this blunting effect. As pediatric obesity is largely due to high-fat content diets, this may reflect a commonly

For author disclosure information, see page 785.

occurring situation in obese children, with a potentially negative impact in growth factor homeostasis, and possible long-term effects on growth and development.



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Accelerated Gastric Emptying but No Carbohydrate Malabsorption One Year after Gastric Bypass Surgery

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Gastric bypass surgery (GBP) results in 30-50% excess weight loss and type 2 diabetes (T2DM) remission in 70-80% of cases. Following GBP, post prandial incretin levels increase with normalization of the incretin effect on insulin secretion, in parallel with improved glucose homeostasis. The mechanisms of the increased incretin release after GBP is not elucidated, and the role of altered gastric emptying and gut nutrient sensing is unclear.

Morbid obese subjects were studied before and 1 year with after GBP with a D-xylose test (25 g of D-xylose in 200 ml water) after an overnight fast. Blood was collected at frequent time intervals for 3 hours to determine gastric emptying (time to appearance of d-xylose in blood). Carbohydrate malabsorption was determined using standard criteria: D-xylose levels < 20 mg/dL at 60 minutes and <22.5 mg/dL at 180 minutes. D-xylose levels were measured by colorimetric assay. Ten subjects, age 35.6±11.3 years, BMI 46.8±6.3 kg/m^2, were studied at baseline. Four subjects had T2DM, diagnosed within 1 year of GBP, well controlled with baseline HbA1c of 6.0±1.0%. One year after GBP, body weight decreased by 51.2±14.3 kg (p<0.001), BMI was 27.6±5.7 kg/m^2 and fasting glucose decreased from 105.2±17.0 mg/dl to 83.5±8.2 mg/dL (p=0.005). Gastric emptying was accelerated after GBP. The mean time to first appearance of D-xylose in serum decreased from 25.0±18.7 min prior to GBP to 9.2±5.8 min after GBP (p=0.039). However, there was no carbohydrate malabsorption either before, as expected (serum D-xylose levels = 32.8±17.9 mg/dL at 60' and 34.8±8.5 mg/dL at 180'), or 1 year after (serum D-xylose levels= 33.0±19.2 mg/dL at 60' and 31.3±8.9 mg/dL at 180') GBP. Circulating D-xylose concentrations were not significantly different before and after GBP at 60 min (p=0.971) and/or at 180 min (p=0.538).

These data demonstrate that accelerated gastric emptying (GE) for liquid, but no carbohydrate malabsorption is present one year after GBP. Rapid GE, but not altered nutrient sensing, may play a role in the incretin response after GBP and the resulting improved glucose homeostasis. **ADA-Funded Research**

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Variability of the GLP-1 Response after Gastric Bypass Surgery in Patients with Type 2 Diabetes

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We have previously shown that post-prandial total glucagon-like peptide-1 (GLP-1) levels increase and glucose homeostasis improves after gastric bypass surgery (GBP) in morbidly obese patients with type 2 diabetes (T2DM). After oral glucose, the GLP-1 peak levels increase and glucose levels decrease. The effect is robust with 100 % responders, however significant variability exists between subjects. Here we studied the variance (σ^2) of the magnitude of peak plasma levels of glucose and GLP-1, and σ^2 of the time to reach peak levels (time-to-peak), during a 50g 3-h oral glucose tolerance test (OGTT) before and 1 month after GBP. Data are given as mean±SD. Paired Student t-test and Pitman tests were used to assess changes in the mean and σ^2 of outcome variables, respectively. Eighteen morbidly obese subjects with T2DM, age 44.5±10.1 y, BMI 44.5±6.9 kg/m^2, T2DM duration 36.8±36.2 months, HbA1c 6.9±1.0 %, were studied. One month after GBP, subjects lost 11.9±5.7kg (P<0.001). The glucose peak decreased from 245±59mg/dl to 207±45mg/dL (P=0.026), with no change in its σ^2 (P_{σ}^2>0.10). The time-to-glucose-peak decreased from 65.8±15.6min to 45.0±14.6min (P=0.002), also with no change in variance (P_{σ}^2>0.10). In contrast, GLP-1 peak increased from 18.7±11.2pM/L to 89.7±51.3pM/L (P<0.001), with a marked increase in σ^2) (P_{σ}^2<0.005). The time-to-GLP-1-peak did not change after GBP (21.1±14.8 vs. 20.0±9.1min, P=0.784) but σ^2 decreased (P_{σ}^2<0.02). 72% of patients reached GLP-1 peak levels 10 to 20 minutes after glucose ingestion post-GBP versus only 39% pre-GBP. A strong correlation appeared between the time-to-peak of glucose and of GLP-1 (r = 0.601, P = 0.008) post-GBP. In summary, although the glucose peak decreased significantly and appeared sooner after GBP, the variance of the glucose response did not change after GBP. In contrast, the GLP-1 peak was greater, with increased variance in the response and with a less variable time-to-peak after GBP. The strong correlation between the peak time of glucose and GLP-1 may possibly be in relation with accelerated gastric emptying. These data reflect the complex relationship between GLP-1 release and glucose homeostasis after GBP. **ADA-Funded Research**

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Metabolic Surgery as a Treatment for Non-Obese Type 2 Diabetic Patients: Incretins, Adipocytokines and Insulin Secretion/Resistance Changes in a One-Year Interventional Clinical Controlled Study

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To compare duodenal-jejunal bypass (DJB) with standard medical care in non-obese type 2 diabetes and evaluate surgically induced endocrine and metabolic changes. Eighteen patients submitted to a DJB procedure met the following criteria: overweight, diabetes diagnosis less than 15 years, current insulin treatment, residual beta-cell function, and absence of autoimmunity. Patients who refused surgical treatment received standard medical care (control group). At baseline, 3, 6, and 12 months after surgery, insulin sensitivity and production, glucagon-like peptide-1(GLP-1) and glucose-insulinotropic polypeptide (GIP) were assessed during a meal tolerance test. Fasting adipocytokines and dipeptidyl-peptidase-4 (DPP-4) concentrations were measured. The mean age of the patients was 50 (5) years, time of diagnosis: 9 (2) years, time of insulin usage: 6 (5) months, fasting glucose: 9.9 (2.5) mmol/dl, and HbA_{1C} was 8.9 (1.2) %. DJB group showed greater reductions in fasting glucose (22% vs. 6% in control group, P< 0.05) and daily insulin requirement (93% vs. 15%, P< 0.01). Twelve patients from DJB group stopped using insulin and showed improvements in insulin sensitivity and beta-cell function (P< 0.01), and reductions in GIP (P< 0.001), glucagon during the first 30 min after meal (P< 0.05), and leptin (P< 0.05). DPP-4 increased after surgery (P< 0.01), but GLP-1 did not change. Improvements in glycemic control were also observed in the control group due to the optimization of current medical therapy, but DJB was still superior to standard therapy. In addition, we determined that the effect on glucose metabolism was a direct consequence of DJB rather than a secondary effect from weight loss. DJB improved insulin sensitivity and beta-cell function and reduced GIP, leptin and glucagon production. In clonclusion, DJB was an effective treatment for non-obese type 2 diabetic patients resulting in better glycemic control and reduction in insulin requirement, but DJB did not result in remission of diabetes.

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For author disclosure information, see page 785.

ADA-Funded Research

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Ileal Interposition Surgery Increases Energy Expenditure, Improves Glucose and Lipid Metabolism and Preserves β-Cell Mass in the UCD-T2DM Rat Model of Type 2 Diabetes Mellitus

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We have previously reported that IT surgery delays diabetes onset in UCD-T2DM rats. IT surgery involves interposing a 10 cm segment of ileum into the proximal jejunum. In the present study IT, sham (S) or no surgery (C) was performed on 2 mo old weight-matched male UCD-T2DM rats. Animals were euthanized 1.5 (n=12 per group) or 4.5 mo after surgery (n=16 per group). Animals underwent an oral glucose tolerance test (OGTT) at 1, 3 and 4 mo after surgery. Indirect calorimetry was performed at 1.5 mo after surgery. An oral fat tolerance test (OFTT) was preformed at 2 mo after surgery. Diabetes onset was determined by weekly non-fasted blood glucose measurements (<200 mg/dl). Circulating glucose concentrations were lower in IT-operated animals at multiple time points (P<0.05) despite similar body weight and food intake between groups. EE did not differ between groups in the light phase, but was 5% higher in IT-operated animals compared with sham during the dark phase (C=67±7, S=67±5, IT=70±5 kcal/min/kg x 585min; P<0.05), without a difference in physical activity. The glucose AUC was lower and insulin secretion was higher in IT-operated animals during all 3 OGTTs (P<0.05). Peak active GLP-1 values were 2-fold greater in IT-operated animals at 1 and 4 months after surgery (P<0.05). The TG AUC during the OFTT was 60% lower in IT-operated animals, suggesting improved lipid clearance (C=12426±1944, S=10921±1168, IT=4514±913 mg/dl x 180min; P<0.01). β-cell mass was lower in IT-operated animals at 1.5 months after surgery, indicative of improved insulin sensitivity (C=12.7±0.6, S=12.2±1.2, IT=9.0±1.0 mg, P<0.05). β-cell mass was 20-30% higher in IT-operated animals at 4.5 months after surgery, suggesting compensatory β-cell proliferation, possibly mediated by increases of GLP-1 (C=10.1±0.8, S=9.0±0.8, IT=12.1±1.0 mg, P<0.05). Thus, IT surgery increases EE and delays diabetes onset by improving insulin sensitivity, lipid metabolism, and islet function, effects which are present up to 4 months after surgery. Increased nutrient-stimulated GLP-1 secretion could contribute to all of these effects.

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A Mixed Meal Challenge Exposes Defects in the GI Tract, Beta Cell and Liver after Chronic Consumption of a High-Fat/High-Fructose Diet

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Previously we demonstrated that consumption of a high-fat/high-fructose (52/17% of energy) diet (HFFD) significantly impairs net hepatic glucose uptake (NHGU) in response to a glucose challenge, but factors other than glucose influence gastric emptying (GE), insulin secretion and NHGU. Therefore, the aim of this study was to elucidate the impact of a HFFD on the response of the gastrointestinal (GI) tract, beta (β) cell and liver to a mixed meal challenge. Adult male dogs were fed a standard chow diet (CTR; n=5) or a HFFD (n=5) for 8 wks. Dogs underwent hepatic/portal vein catheterization at 6wks and an oral mixed meal tolerance test 2wks later. The HFFD group displayed markedly impaired glucose tolerance vs CTR, (peak arterial plasma glucose concentrations of 262±36 vs 166±10 mg/dl, respectively [P<0.05]). This was due in part to relative β cell failure, given that augmented arterial plasma insulin levels in HFFD vs CTR (84±17 vs 41±5 μU/ml; P<0.05) were insufficient to normalize postprandial hyperglycemia (PPHG). However, PPHG was also attributable to accelerated GE, given that net gut glucose output (mg/kg/min) and arterial plasma acetaminophen levels (μg/ml; an index of the GE rate) peaked at 12.4±2.1 and 11.4±1.0, 1.5h post-meal in HFFD (P<0.05 vs CTR) vs 9.7±0.2 and 8.0±1.9, 3h post-meal in CTR. In support of accelerated GE, arterial blood alanine (μmol/L) and GLP-1 (pM) concentrations were significantly increased in HFFD (422±21 and 8.9±2.5, respectively; P<0.05 vs CTR) vs CTR (350±20 and 4.3±0.5, respectively) 1h post-meal. HFFD feeding also impaired the response of the liver to the meal, as indicated by a significant decrease in maximal NHGU (mg/kg/min) and glycogen synthesis (GLY; mg/kg/min; HFFD: 0.9±0.9 and 1.3±0.8, respectively [P<0.05 vs CTR]; CTR: 4.8±1.3 and 5.3±1.3, respectively). In conclusion, HFFD feeding accelerates meal macronutrient absorption, impairs glucose tolerance and β cell function, and diminishes NHGU and GLY in the context of a mixed meal challenge. These data reveal novel metabolic consequences of a HFFD on the function of the GI tract, endocrine pancreas and liver in the PP state.

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Pharmacological Inhibition of Diacylglycerol Acyltransferase 1 Alters the Lipid Metabolism Transcriptome and Associated Lipid Species in the Rat Jejunum

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Triglyceride accumulation is associated with obesity and type 2 diabetes. Genetic disruption in mice of Diacylglycerol acyltransferase 1 (DGAT1), which catalyzes the final reaction of triglyceride synthesis, confers dramatic resistance to high-fat diet induced obesity. Hence, DGAT1 is an attractive therapeutic target for treating obesity and related metabolic disorders. In an effort to elucidate the molecular events shaping the mechanism of action following pharmalogical inhibition of DGAT1, we utilized a combination of gene expression and lipomic profiling to analyze the effects of a DGAT1 inhibitor over time (1, 3, and 7 days) in a high fat habituated Sprague Dawley rat. For the gene expression analysis we applied a Causal Reasoning Engine (CRE) inference algorithm that integrates GeneChip transcriptomic data with peer reviewed literature to infer upstream molecular events (hypotheses) driving transcriptional changes measured in the rat jejunum. Following a single dose of DGAT1 inhibitor, robust reductions were observed in mRNA abundance of key lipid metabolism and transport genes: SCD-1, Insig-1, SREBP-1c, LXR, PCK1, CD36, Scarb1, and apolipoproteins A1, A4, B and CIII. The CRE analysis identified hypotheses of decreased nuclear transcriptional regulators of lipid metabolism, including SREBP-1c, HNF4a, PPARg, PPARa, PGC1a, and RXR. CRE generated hypotheses accurately predicted the measured reductions in triacylglycerol, diacylglycerol and free fatty acid levels in the jejunum of DGAT1 inhibitor treated rats. Another prediction confirmed by lipomics analysis was an enrichment in long chain poly unsaturated fatty acids, that can act as ligands for the nuclear hormone receptors indicated above. Thus our analyses support the notion that DGAT1 inhibition in the jejunum prevents the metabolic derangements of high fat diet on lipid metabolism.

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Role of Fatty Acid Transport Proteins in Oleic Acid-Induced Secretion of Glucagon-Like Peptide-1

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Glucagon-like peptide-1 (GLP-1) is an intestinal L cell hormone that is released in response to nutrient ingestion and enhances glucose-dependent insulin secretion. We have previously demonstrated that the long-chain monounsaturated fatty acid, oleic acid (OA), is a particularly effective GLP-1 secretagogue, in accordance with clinical studies showing that ingestion of OA-rich olive oil increases GLP-1 release and improves glucose homeostasis. We have also shown that the actions of OA on GLP-1 release are mediated via the atypical protein kinase C isozyme PKCζ. However, the mechanism by which OA crosses the plasma membrane (e.g. diffusion or transport) is not known. Previous studies in the murine GLUTag L cell model have demonstrated expression of mRNA transcripts for the fatty acid-transport proteins CD36 and FATP1, 3 and 4. We hypothesized that one of these proteins plays an essential role in OA-induced GLP-1 secretion. Immunoblotting demonstrated the presence of CD36 and FATP1, 3 and 4 proteins in the murine GLUTag L cell line. GLUTag cells demonstrated specific ^{3}H-OA uptake for up to 60 min, and this was competitively inhibited in a dose-dependent manner by 0.5 and 1.0 mM unlabeled-OA (P<0.001). Furthermore, different mechanisms appear to be involved in OA uptake by the L cell, as indicated by an increase in the slope of the uptake curve at t=45 min (0-45 min: 0.55±0.06; 45-60 min: 1.77±0.17; P<0.001). Phloretin (200 μM), a non-specific inhibitor of carrier-mediated transport, significantly decreased ^{3}H-OA uptake at early time points (5-15 min, P<0.001), whereas sulfo-*N*-succinimidyl oleate (SSO; 400 μM), a specific inhibitor of CD36, significantly decreased uptake at 60 min (P<0.001). FATP4 siRNA knockdown (by 20±3%) also decreased ^{3}H-OA uptake at 60 min (P<0.05). Finally, treatment of GLUTag cells with 0.5 and 1.0 mM OA increased GLP-1 secretion by 24±9% and 59±9%, respectively (P<0.05-0.001), while phloretin (P<0.01), SSO and FATP4 knockdown (P<0.05) decreased OA-induced GLP-1 secretion by up to 46±6%. Together, these findings indicate an important role for FATP4 and CD36 in the regulation of OA-induced GLP-1 secretion.

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For author disclosure information, see page 785.

BETA CELL SURVIVAL IN DIABETES

181-OR

Deficiency in the Nuclear Factor E2-Related Factor 2 Renders Pancreatic β-Cells Vulnerable to Oxidative and ER Stress-Induced Cell Damage

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Oxidative and endoplasmic reticulum (ER) stress is implicated in the pancreatic β-cell dysfunction that occurs in both Type 1 and Type 2 diabetes. Nuclear factor E2-related factor 2 (NRF2) is a CNC-bZIP transcription factor that is well established as a master regulator in the cellular adaptive response to oxidative stress. Our previous study proposed that chronic activation of NRF2 in response to low-level oxidative stress blunts glucose-triggered reactive oxygen species (ROS) signaling and impairs glucose-stimulated insulin secretion (GSIS). The current study found that islets isolated from global *Nrf2*-knockout (*Nrf2*-KO) and β-cell-specific *Nrf2*-KO mice and MIN6 cells with stable *Nrf2* knockdown (*Nrf2*-KD) exhibit reduced induction in many antioxidant and phase 2 detoxification enzymes in response to various oxidative stressors. *Nrf2*-KO islets and *Nrf2*-KD MIN6 cells were more susceptible than wild-type/scramble controls to oxidative and ER stress-induced cell damage as evaluated by decrease in cell viability, impairment of ATP production and/or increase in apoptosis. In wild-type islets and scramble MIN6 cells, oxidative stressors such as hydrogen peroxide and arsenite, ER stress inducers such as thapsigargin and tunicamycin, and nitric oxide donor SNAP caused cell damage in a dose-dependent and time-dependent manner, whereas disruption of *Nrf2* sensitized the islets or MIN6 cells to the damage. In contrast, pretreatment of cells with NRF2 activators, such as t-butyl hydroquinone and sulforaphane, protected the cells from oxidative and ER stress-induced cell damage in a NRF2-dependent fashion. These findings demonstrate NRF2-mediated antioxidant response plays an important role in the pancreatic β-cell defense mechanism against oxidative damage. Considering the potential inhibitory effect of chronic NRF2 activation on ROS signaling in GSIS, the current study suggests a paradoxical role of NRF2 in oxidative stress-related β-cell dysfunction.

182-OR

Stem Cell Based Strategies To Enhance Pancreatic Islet Survival in Type 1 Diabetes Mellitus

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Islet transplantation is a potentially curative treatment for Type 1 diabetes mellitus, however disappointing long-term outcomes have limited the widespread application of this procedure. Adipose stromal cells (ASCs) are multipotential mesenchymal progenitor cells that can be differentiated into adipoctyes. ASCs have been shown also to have functional and phenotypic overlap with pericytes and function to support the growth of new vasculature. Importantly, ASCs also secrete a number of paracrine factors, including HGF, FGF, and VEGF, each of which have been shown in isolation to enhance pancreatic islet survival in models of Type 1 diabetes. To investigate the ability of ASCs to support islet survival and function *in vitro*, we co-cultured mouse islets with ASCs for 7 days. Islets exposed to ASC-conditioned media via a transwell system demonstrated improved survival and preserved glucose-stimulated insulin secretion following prolonged culture. To characterize the *in vivo* effects of ASCs on cytokine-mediated islet death, NOD-SCID mice were treated with multiple low doses of streptozotocin to induce inflammatory insulitis. Following confirmation of glucose intolerance in streptozotocin-treated mice, 2 x 10^6 ASCs were administered via tail vein injection. GFP-labelled ASCs were shown to home to the pancreas. Intraperitoneal glucose tolerance tests performed 11 days after injection demonstrated significantly improved glucose tolerance in ASC-treated mice. $AUC_{glucose}$ in ASC-treated mice was measured to be 25,285 ± 1975 compared to a value of 37,875 ± 2623 in mice treated with saline following streptozotocin injection. ASC-treated mice had preserved β cell mass (1.533 ± 0.09 mg vs. 0.7193 ± 0.02 mg in saline-treated controls), and showed increased rates of proliferation compared to controls. Together, these data suggest that ASCs have potential as a cell-based treatment strategy in Type 1 diabetes mellitus. Further studies are underway to characterize the specific ASC-derived factors responsible for these observed effects and to investigate the utility of combining ASCs with islets in models of islet transplantation.

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Metabolite Profiling by Tandem Mass Spectrometry Reveals Differences between Pro-Inflammatory Cytokines and Genuine Inducers of Apoptosis in Rat β-Cell Lines

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Immune cell-derived pro-inflammatory cytokines (e.g., IL-1β + γ-IFN) contribute significantly to losses in functional β-cell mass during the autoimmune process of Type 1 diabetes. However, controversy persists over the mechanisms underlying β-cell death in response to cytokines. Thus, we tested the hypothesis that pro-inflammatory cytokines produce a metabolic profile distinct from bona fide inducers of apoptosis. Using 832/13 rat insulinoma cells, an 18h exposure to 1 ng/mL IL-1β +100 U/mL γ-IFN produced a 15-fold increase in nitrite accumulation, an index of nitric oxide production, which correlated with a reduction in cellular viability by 52% with no attendant increase in caspase-3 enzyme activity. By contrast, an 8h exposure to 1 mM camptothecin, a topoisomerase I inhibitor and accepted inducer of apoptosis, increased caspase-3 enzyme activity by 10-fold, producing a 40% decrease in viability. Using mass spectrometry to profile metabolite changes that occur prior to cellular death in response to these effectors, we screened over 150 metabolites in 832/13 cells exposed to IL-1β + γ-IFN or camptothecin. Citrulline, a product of the inducible nitric oxide synthase reaction, was elevated 8-fold over control following exposure to IL-1β + γ-IFN, but was unchanged by camptothecin. By contrast, dATP, a critical metabolite involved in apoptosome formation, is increased by camptothecin, but not IL-1β + γ-IFN.

Additionally, methylthioadenosine (MTA) increased approximately 5-fold in response to camptothecin, but was unchanged by cytokines. Direct addition of 1, 2, or 4mM MTA inhibited the 30-fold increase in NF-κB reporter gene activity by IL-1β in a dose-dependent manner (50, 72, and 85%, respectively). To our knowledge, this is the first evidence that metabolite accumulation incurred during induction of the apoptotic pathway inhibits signaling through NF-κB in pancreatic β-cells.

We conclude that the β-cell metabolic signature in response to pro-inflammatory cytokines is distinct from true induction of apoptosis; moreover, MTA, generated by induction of apoptosis, functions to inhibit signaling through NF-κB.

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Using Differentially Methylated Circulating DNA for In Vivo Detection of β Cell Death in Diabetes

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In diabetes mellitus, β cell destruction is largely invisible and can only be detected after significant loss of insulin secretory function when only replacement therapy is available. We have developed a method for the in vivo detection of β cell death by amplifying and measuring the relative proportion of β cell derived DNA in the serum of prediabetic and diabetic mice. We have identified β cell specific methylation patterns in the insulin 1 gene by analysing the DNA of the murine βTC3 cell line. Methylation specific primers were designed to distinguish hypomethylated DNA of β cell origin from hypermethylated DNA of a non-β cell origin. These primers were capable of detecting β cell derived DNA from purified murine islets and cell sorted insulin positive cells ex vivo. Injection of BALB/c mice with streptozotocin resulted in a ~4 fold increase in the ratio of hypomethylated (β cell derived) to hypermethylated (non-β cell derived) DNA in the sera of diabetic mice when compared with untreated controls. Moreover, 14 week old non-obese diabetic mice showed a significant ~68 fold increase in the ratio of hypomethylated to hypermethylated DNA in their serum when compared with 7 week old mice. This increase was detected prior to the development of frank hyperglycemia and was sustained following the development of hyperglycemia. In summary, our novel biomarker assay provides a non-invasive method for the detection of β cell death in the serum that may be used to determine progression and guide treatment of diabetes.

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185-OR

Protective Effects of Glucokinase Activator on the Onset of Diabetes and β Cell Apoptosis in Akita (*Ins2*) Mice

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Endoplasmic reticulum (ER) stress plays a crucial role in β cell apoptosis and the development of diabetes. In this study, we evaluated the impact of glucokinase activator on ER stress by using Akita (*Ins2*) mouse, an animal model of ER stress-mediated diabetes. After weaning at postnatal day (P)

For author disclosure information, see page 785.

19, wild-type mice (WT) and Akita mice (Akita) were given a standard diet for 14 days (until P33). Akita mice were also challenged with diet containing 0.01% glucokinase activator CpdA (GKA), 0.4 % sitagliptin (STG), or 0.5% phlorizin (PHZ) for 14 days. Body weight gain, serum cholesterol levels, serum triglyceride levels and serum free fatty acid levels showed no significant differences among the five groups. All of the mice in GKA and PHZ groups showed causal plasma glucose below 200 mg/dl until P33, suggesting the prevention from the onset of diabetes. By contrast, all the animals in Akita and STG groups developed diabetes. Oral glucose tolerance test (OGTT) following a 20-24 h fast at P33 revealed that Akita had impaired glucose tolerance and severely impaired insulin secretion compared to WT. Of note, neither GKA, STG nor PHZ restored insulin secretion during OGTT, suggesting that these agents were still insufficient to improve β cell function in Akita mice. In Akita at P33, β cell mass and β cell ratio in islet were significantly decreased, islet morphology was abnormal, and CHOP- and TUNEL-positive apoptotic β cells were significantly increased, compared to WT. GKA and STG, but not PHZ, restored β cell mass, normalized islet morphology, and significantly decreased CHOP- and TUNEL-positive apoptotic β cells. These results indicated that GKA and STG protected from β cell ER stress and apoptosis, which resulted in increased β cell mass. GKA significantly decreased the mRNA expression of CHOP, CEBP-β and significantly increased the mRNA expression of IRS-2 in islet, compared to Akita. Furthermore, in isolated islets from wild-type mice under tunicamycin-induced ER stress, the mRNA expression of CHOP was decreased by treatment with GKA. In conclusion, GKA was able to prevent ER stress-induced apoptosis.

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Cytokine-Induced Beta-Cell Death Is Mediated through Class I Lysine Deacetylases

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Interleukin (IL)-1β and interferon (IFN)γ are candidate mediators of beta-cell demise in Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D). Cytokine-induced beta-cell death is abrogated by pan-inhibitors of the classical histone deacetylases (HDACs), now termed lysine deacetylases (KDACs). There are three KDAC classes: Class I: HDAC1,-2,-3 and -8; class II: HDAC4,-5,-6,-7,-9 and -10; and class IV: HDAC11, all expressed in the beta-cell. It is unknown which subtype(s) mediate(s) cytokine-induced beta-cell death.

We examined the protective effect of several KDAC inhibitors (KDACi) on IL-1β and IFNγ-exposed INS-1 cells to identify targets for future selective KDACi as T1D and T2D therapeutics.

We first examined the protective effects of an HDAC1,-2 and -3 selective KDACi

(MS-275) and a class II selective KDACi (MC1568) on cytokine-induced INS-1-cell apoptosis (Cell death ELISA) (n=3). MS-275 abrogated the toxic effects of cytokines ($p<0.01$) whereas MC1568 had no effect, indicating class I KDACs as the mediators of cytokine-induced beta-cell death. To verify this finding we screened 3 different commercially available HDAC1,-2 and -3 selective inhibitors (CI-994, HC toxin and MGCD0103), and 1 inhibitor selective for HDAC8 (PCI34051), using the xCELLigence System for real-time monitoring of well-matrix surface impedance, a surrogate of total cell-area that correlated well with IL-1β and IFNγ-induced INS-1-cell apoptosis determined by Cell death ELISA. Inhibitors of HDAC1 and -2 yielded the highest rescue scores followed by inhibitors of HDAC3 (n=2-5). Inhibition of HDAC8 was ineffective. Based on this screen we knocked down HDAC2 in INS-1 cells by Lentiviral transduction of 3 different specific shRNAs. The specific, but not empty vector, constructs effectively reduced HDAC2 protein, but failed to prevent cytokine-induced apoptosis (n=6). A shRNA which abrogated HDAC1 protein halved cytokine-induced beta-cell death and no further protection was conferred by adding a pan-KDACi (n=2).

These data suggest that HDAC1 is important for cytokine-induced beta-cell death and that HDAC1 is a novel therapeutic target for the therapy of T1D and T2D.

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Physiological Expression of cMyc Is Responsible for Proliferation in Rodent β Cell Lines, and Drives Human β Cell Replication

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Human β cell replication has been difficult to achieve, and human β cell lines do not exist. In contrast, rodent β cell lines have been proliferating continuously for decades. To define the mechanisms underlying sustained rodent β cell proliferation, we examined cell cycle proliferation and found that Ins1, RIN, BTC3 and MIN6 cells all display an identical cell cycle "signature" comprised of increases in cdks 1,2,4,6 and cyclins A, E and D3. Here, we screened potential candidate upstream regulatory pathways that might drive these "7 signature G1/S molecules".

We screened 15 candidate regulatory pathways, including MAPK, STATs, SMADs, Src, Cbl, PKCs, PAX4, GSK3a/b, β-catenin, and found no reproducible activation vs. normal rat islets. In contrast to these 15 pathways, cMyc was reproducibly increased (5-7x) in Ins1 and RIN cells vs. rat islets. Silencing cMyc (by siRNA) and pharmacological inhibition (with 1RH) markedly reduced both proliferation by 70%, as well as the 7 G1/S molecules. Conversely, modest cMyc overexpression in rat β cells activated the 7 G1/S molecules, and also activated proliferation 50x (from 0.2% to 10%, BrdU), without activating cell death. To determine if cMyc might activate human β cell proliferation, cMyc was expressed at low levels in isolated human β cells. This increased human β cell proliferation from 0.2% to 1.4% (7x) without inducing cell death.

cMyc is a normal β cell growth mediator, and is activated 1.5-2-fold in response to nutrients and mitogens. Despite its reputation for inducing β cell de-differentiation and death, this has only occurred when cMyc has been overexpressed at extreme levels (200-300x). Here we report that low level (7x) overexpression of cMyc is the proximal cause of sustained proliferation in Ins1 and RIN β cell lines, and is the upstream driver of the 7 signature G1/S molecules. Notably, low level cMyc expression also can markedly accelerate human β cell proliferation, without inducing cell death. Further, cMyc is positioned at the farthest upstream level in pathways that lead to human β cell replication. The cMyc pathway is attractive for driving human β cell replication.

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188-OR

Newly Identified LP Analogue Stimulates the In-Vivo-Regeneration of β-Cell through Regulation of Cyclin D3 and $p57^{Kip2}$ Leading to Diabetes-Free in db/db Mice

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Loss of insulin producing β-cell mass is one of the hallmarks of type 2 diabetes in humans. Pancreatic β-cells are dynamic cells that are able to modulate their mass in response to a variety of physiological (pregnancy) and pathophysiological (obesity) states. Currently there are few effective therapeutic approaches targeting β-cell regeneration in clinical practice although some anti-diabetic drugs may positively affect β-cell mass. Lysophospholipids (LPs) including lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are membrane-derived bioactive factors acting on G-protein-coupled receptors (GPCRs) and their levels are increased during pregnancy and obesity. We have screened several analogues of LPs by treatment of islets ex vivo or intraperitoneal injection of *db/db* mice, in which the insulin-producing β-cells are gradually depleted after diabetes onset. Here we show that oral administration of LP analogue (LP2008) can normalize fasting blood glucose in the diabetic *db/db* mice by increasing β-cell mass and blood insulin levels without affecting insulin sensitivity. Fasting blood glucose remained normal in the *db/db* mice even after the drug was withdrawn after 18-weeks of treatment. Stereological analysis of islets showed that the areas of islets in the pancreas of the LP2008-treated *db/db* mice were two-fold larger than that of the untreated db/db mice. BrdU incorporation and Ki67-staining showed cell proliferation within the islets and in pancreatic duct area. We further demonstrated that CDK inhibitor $p57^{kip2}$ was decreased and cyclin D3 was increased significantly in the islets isolated from LP2008-treated *db/db* mice. Our data demonstrated that LP analogue may be a new reagent to prevent and/or cure type 2 diabetes by promoting the *in-vivo*-regeneration of β-cells in patients with diabetes and revealed a novel network that controls β-cell regeneration in the setting of obesity-diabetes through regulating cyclin D3 and $p57^{kip2}$ expression by GPCR signaling pathway.

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LIPIDS AND LIPOPROTEINS

189-OR

NPY Acts Primarily Via the Y1 Receptor To Induce Hyperlipidemia

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Concomitant with the parallel obesity and diabetes epidemics, an increasing healthcare burden is due to the complications of hyperlipidemia (atherosclerosis). Elevated very-low density lipoprotein (VLDL)-triglyceride

For author disclosure information, see page 785.

(TG) production from liver contributes to atherogenic dyslipidemia (elevated TGs, small dense low-density lipoprotein, and low high-density lipoprotein).We sought to identify novel mechanisms accounting for obesity dyslipidemia. Neuropeptide Y (NPY) expressing neurons, found in the mediobasal hypothalamus (as well as numerous other brain regions), are an important regulator of feeding and energy homeostasis, and obesity is characterized by elevated NPY tone. We have previously demonstrated that intracerebroventricular (ICV) administration of NPY doubles hepatic VLDL-TG production, suggesting that the same neural circuits that control feeding are involved in the regulation of lipoprotein metabolism. We hypothesized that NPY action contributes both to the pathogenesis of obesity, *per se,* and to the pathogenesis of obesity dyslipidemia, but does so via distinct mechanisms. In this study, we set out to determine which of multiple NPY receptors (Y1, Y2, Y4, or Y5) affect feeding behavior and/or lipoprotein metabolism. In the lean 4-h fasted rat (n=4-7/group), the TG secretion rate was estimated by serial blood sampling after intravascular tyloxapol pretreatment; CNS NPY signaling was modulated by ICV injection of selective NPY receptor agonists. We found that Y1 ([F7, P34]-NPY; 1nmol), Y4 (hPP; 1nmol), and Y5 agonists ([Ala31, Aib32]-NPY; 2nmol) all potently induced hyperphagia in satiated, lean rats, with the Y2 agonist (hPYY (3-36); 1nmol) having the most robust effect. Conversely, neither the Y4 nor the Y5 agonist had an effect on TGs (Vehicle (Veh), 3.6 ± 0.1 mg/dl/min vs. Y4, 3.0 ± 0.6 mg/dl/min, p=ns; Veh vs. Y5, 4.2 ± 0.3 mg/dl/min, p=ns) whereas the Y1 (Veh vs. Y1, 6.8 ± 0.5 mg/dl/min, p<0.001) and to a lesser extent, the Y2 agonist (Veh vs. Y2, 4.8 ± 0.3 mg/dl/min, p<0.01) stimulated VLDL-TG production. These findings indicate that NPY regulates feeding and lipoprotein metabolism largely via separate NPY receptors and, therefore, different pathways.

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Adenosine Triphosphate Binding Cassette Transporter G1-Mediated Cholesterol Efflux to Serum Is Impaired in Type 2 Diabetes Mellitus

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Cholesterol efflux from cells is an early step of reverse cholesterol transport and is mediated by regulated transporter-facilitated processes and aqueous diffusion. Adenosine triphosphate binding cassette transporter G1 (ABCG1) has recently been identified as an important cholesterol transporter that mediates cholesterol efflux from cells to mature HDL. Our aim is to evaluate the capacity of serum to induce ABCG1-mediated cellular cholesterol efflux in patients with type 2 diabetes mellitus and its relationship to glycemic control and inflammation.

60 diabetic patients and 50 age-matched healthy controls were recruited. Serum capacity to induce cholesterol efflux was determined by measuring the transfer of [3H]cholesterol from human ABCG1-transfected CHO-K1 cells to the medium containing the tested serum. Plasma high sensitivity C-reactive protein (hs-CRP) was measured by an immunoturbidimetric assay and HbA1c by ion-exchange high performance liquid chromatography.

The diabetic patients had significantly higher plasma triglyceride and hs-CRP, and lower HDL than controls. Serum cholesterol efflux capacity was reduced in diabetic patients (2.6 ± 1.4% vs 4.1 ± 1.8%, p<0.01) and remained significant even after adjusting for HDL level. Serum cholesterol efflux capacity correlated with HDL only in the controls (r=0.32, p=0.03) but not in the diabetic patients. There was no significant association between serum cholesterol efflux capacity and HbA1c but an inverse correlation between serum cholesterol efflux capacity and log(hs-CRP) was seen in the diabetic patients (r=-0.42, p=0.01).

In conclusion, the capacity of whole serum to stimulate cholesterol efflux from cells provided integrated information on lipoproteins and serum components involved in promoting cholesterol efflux from cells. We have shown that the capacity of serum to induce ABCG1-mediated cholesterol efflux was impaired in diabetic patients and this process was related to the degree of subclinical inflammation.

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191-OR

The Association between Liver Fat, Retinol Binding Protein 4 (rbp4) and Plasma Triglycerides Is Different According to the Adiponutrin Polymorphism, in Type 2 Diabetes

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Hypertriglyceridemia has been shown to be associated with liver fat content and increased plasma rbp4 levels, in type 2 diabetes. It has been shown that the minor allele G in the adiponutrin gene was associated with increased liver fat content. However, the influence of adiponutrin polymorphism on the association between liver fat content, rbp4 and triglycerides (TG) remains unknown in type 2 diabetes.

This prompted us to perform a study in 194 patients with type 2 diabetes, without diabetic nephropathy or liver fibrosis, including liver fat measurement by proton spectroscopy, intra-abdominal and subcutaneous fat content assessment by MRI, plasma lipid, adiponectin and rbp4 measurements and genotyping of the adiponutrin gene by PCR.

Among the 194 patients, 90 were carriers of the minor G allele of adiponutrin (G group) and 104 were C allele homozygote carriers (C group). The only difference between the 2 groups was a higher fat content in the G group (p=0.02). For the whole diabetic patients, TG were, in multivariate analysis, associated positively with rbp4, fasting glycemia, liver fat content, male gender and negatively with HDL-C and age. In the adiponutrin G group, TG were associated positively with rbp4, liver fat content, fasting glycemia, LDL-C and negatively with HDL-C. In the adiponutrin C group, TG were associated negatively with HDL-C and positively with rbp4 and fasting glycemia, but not with liver fat content. In the adiponutrin G group, liver fat content was associated negatively with adiponectin and positively with TG, whereas in the C group, it was associated negatively with adiponectin and positively with visceral fat, subcutaneous fat, rbp4 and fasting glycemia, but not with TG.

In conclusion, in type 2 diabetes, plasma TG are associated with liver fat content only in patients with the minor G allele of adiponutrin but not in the C allele homozygotes. Our data indicate that adiponutrin influences significantly the association between liver fat content and plasma TG, in type 2 diabetes, but not the association between TG and rbp4.

192-OR

Visceral Adipose Tissue Has Greater Influence on Triglyceride and ApoB and ApoCIII Levels Than Insulin Resistance: Study of Black and White Women

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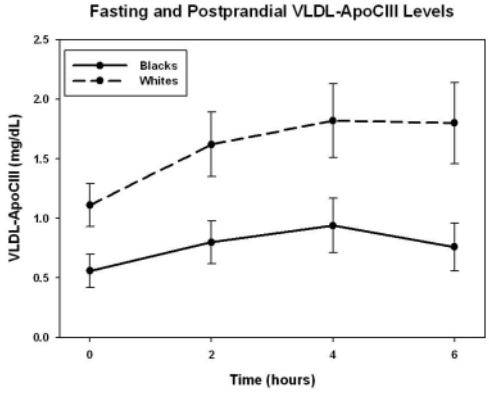
Compared to white women, black women are more insulin-resistant but have less visceral adipose tissue (VAT). Due to greater insulin resistance, black women would be expected to have higher levels of triglyceride (TG), apolipoproteins B (apoB) and CIII (apoCIII). Yet the opposite is true, suggesting that lower VAT in black women might be a dominant protective influence. We aimed to determine whether race differences in very low density lipoprotein (VLDL) composition (i.e. VLDL-TG, VLDL-ApoB and VLDL-ApoCIII levels) in black and white women are related to VAT or insulin resistance. In 31 age and BMI-matched women (16 B, 15 W, age 37±10y, mean±SD, BMI 30.9±6.7kg/m2) the insulin sensitivity index (S_I) was obtained by the minimal model. After 7 days of a eucaloric standard diet, a high fat breakfast was given (FAT 40%, CHO 40%, PRO 20%). TG, apoB and apoCIII were measured at 0, 2, 4 and 6h. As expected, blacks were more insulin-resistant than whites (S_I: 3.6±1.3 v 5.6±2.9, P<.01) with less VAT (75±59 v 103±71, P<.01). VLDL-TG, VLDL-apoB and VLDL-apoCIII levels were lower in blacks than whites (all P≤0.01) (Figure for VLDL-apoCIII). In 3 separate random effects models with VLDL-TG, VLDL-B or VLDL-apoCIII as the dependent variable, the race difference decreased by 50% after adjustment for VAT. S_I did not affect the race difference in VLDL levels when included with VAT (Table for VLDL-apoCIII). Hence, lower VAT in black women partially explains race differences in VLDL-TG, apoB, and apoCIII levels. Yet, determinants of at least 50% of the race difference remains unidentified.



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ADA-Funded Research

Table. Random Effects Model for ApoCIII after Meal in Black and White Women

	VLDL-ApoCIII AdjR^2=41%		
Variables	**B-coef**	**SE**	**P-value**
Race*	-0.99	0.35	**<0.01**
VAT	0.51	0.18	**<0.01**
S_I	-0.09	0.08	**0.25**

*Black women as referent

193-OR

Re-Evaluation of the Association between HDLc and Its Subfractions with Coronary Artery Disease Risk in Type 1 Diabetes

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Individuals with type 1 diabetes exhibit increased cardiovascular disease risk, despite the generally elevated mean HDL cholesterol (HDLc) concentrations compared to the general population. Moreover, we have previously reported increased coronary artery disease (CAD) risk among women with HDLc >80 mg/dl. To further investigate the HDL-CAD association in type 1 diabetes, we studied HDL subspecies by nuclear magnetic resonance spectroscopy (NMR) and the incidence of CAD among individuals with type 1 diabetes.

The Epidemiology of Diabetes Complications study is a historical, prospective, cohort based on incident cases of childhood onset type 1 diabetes. Lipoprotein subfractions by NMR were measured using the earliest available stored sample (244 men, 248 women; mean age, 27.7 years and diabetes duration, 19.5 years). CAD was defined as angina, ischemic ECG changes, confirmed MI, angiographic stenosis ≥50%, revascularization, or CAD death.

During 20 years of follow-up, CAD developed in 29% of men and 25% of women. Women had a greater number of total, large and medium HDL particles and a lower number of small HDL particles compared to men (all p-values<0.05). Incidence rates, however, were very similar by gender at any specific HDL particle concentration. In both genders, a low number of large HDL particles (<3.0 μmol/L) and a high number of small HDL particles (>20 μmol/L) was associated with increased CAD risk. In multivariable analyses using Cox proportional hazard models adjusting for LDL particle number and standard CAD risk factors, only large HDL particle number showed an independent effect (HR=0.92, 95% CI=0.85-0.99), largely reflecting its effect in women (p=0.02) rather than in men (p=0.38).

Despite differences in the absolute number of HDL particles between men and women, CAD incidence appeared similar by gender for the same level of HDL subfraction. However, only large HDL particles were an independent predictor of CAD incidence, an association that appeared stronger in women.

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194-OR

Increases in Adiponectin Resulting from Lifestyle and Metformin Interventions Are Strongly Linked to Increases in HDL-C in the Diabetes Prevention Program (DPP)

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Interventions that slow progression from impaired glucose tolerance (IGT) to diabetes such as intensive lifestyle (ILS) and metformin (MET) treatments have been shown to increase HDL-cholesterol (HDL-C), a major inverse risk factor for cardiovascular disease. However the factors that affect HDL-C concentration and its treatment-associated change are poorly characterized. To investigate this we measured demographic, anthropometric, and metabolic variables as well as markers of inflammation, endothelial function and coagulation in samples from 3191 DPP participants at baseline and after 1 year of intervention. Mean [95% CI] HDL-C at baseline was 40.2 [39.4-40.6] mg/dl in men and 48.0 [47.6-48.3] mg/dl in women, and low HDL-C (<40/<50 mg/dl in men/women) was present in 51% and 59% respectively, with significant differences between intervention groups among women (p=0.01). At baseline, BMI (ρ, Spearman correlation= -0.12), fasting glucose (ρ= -0.18), 1/fasting insulin (ρ=0.30), adiponectin (ρ=0.42), tPA (ρ=-0.32) sE-selectin (ρ= -0.16), sICAM1 (ρ= -0.12) and leptin (ρ=0.18) were correlated with HDL-C values. After 1 year of intervention, the mean increases in HDL-C and adiponectin were respectively 1.3 [0.89-1.68] mg/dl and 0.83 [0.73-0.92] μg/ml in the ILS and 0.74 [0.41-1.2] mg/dl and 0.22 [0.13-0.32] μg/ml in the MET groups, which were all significantly different from the placebo group. In a multivariate regression model with adjustment for demographics and treatment, changes in adiponectin (β=1.6, R^2=13%), leptin (β=0.05, R^2=0.5%), and BMI (β=-0.24, R^2=0.5%) were significantly associated with changes in HDL-C and these associations did not differ among treatment groups. However, the effects of the ILS and MET groups on HDL-C were no longer significant in the model suggesting that there was no independent effect of interventions on HDL-C change. Conclusion: These findings demonstrate that the adiponectin level and uniquely, the change in adiponectin resulting from diabetes prevention treatments in the DPP, are strongly linked to improvement in HDL-C.

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195-OR

Overestimation and Underestimation of Cardiovascular Risk by Apolipoprotein-B (Apo-B) Measurements, Compared to Currently Recommended Non-HDL-Cholesterol Goals in Patients with Diabetes and Hypertriglyceridemia

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Increased triglyceride-rich particles are associated with compositional changes in LDL particles, leading to misinterpretation of LDL-cholesterol (LDL-C). The current lipid guidelines recommend targeting non-HDL-cholesterol in patients with hypertriglyceridemia with TG levels 200-499 mg/dl(HTG). However, whether non-HDL-C is as good a surrogate for LDL particle number, as apo-B, remains controversial. The ADA/ACC recommend achieving apo-B levels of < 80 for atients with CHD or high risk diabetes, and < 90 mg/dl for diabetes alone, in addition to non- HDL-C goals of < 100 and < 130 mg/dl respectively.

We analyzed data from 1430 samples from 1083 patients with diabetes and HTG (mean age 54.9 yr, BMI 32.5, 58% male). Table 1 presents the distribution of non-HDL-C < or ≥ 130, and the corresponding distributions of apo-B < or ≥ 90.

Non-HDL-C (mg/dl)	Apo B < 90 mg/dl	ApoB >=90 mg/dl	ApoB < 80 mg/dl	ApoB>=80 mg/dl	Total
< 100	130	1	123	***8***	131
>=100	572	727	***232***	1067	1299
< 130	607	***127***	339	395	734
>=130	***95***	601	16	680	696
Total	702	728	355	1075	1430

There was good concordance between non-HDL-C < 100 and Apo-B, with only 6.1% of apo-B values ≥ 80. However, of the 1299 samples with non-HDL-C ≥130, 232(17.9%) had apo-B at < 80, i.e an overestimate of risk by non-HDL-C. Moreover, in case of non-HDL-C < 130, 127 of 734(17.3%) had apo-B ≥90, whereas 95 of 696(13.6%) with non-HDL-C ≥130 had apoB < 90, i.e. an underestimate and overestimate of risk respectively. Identical results were seen with n of patients, rather than n of samples. The regression line between non-HDL-C and apo-B showed significant differences between genders (slope, males,1.23; females, 1.10, p< 0.05), indicating an underestimate of risk by non-HDL-C in females, compared to males.

In conclusions, considerable discordance exists between risk estimates by non-HDL-C and apo-B determinations in patients with diabetes and HTG. In addition, the gender differences we observed may partly contribute to the relatively worse atherogenic profile previously reported in diabetic women.

196-OR

Oxidized-LDL Immunocomplexes Are Implicated in Diabetic Retinopathy

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Extravasated, then oxidized, low-density lipoproteins (ox-LDL) have been identified in the human diabetic retina and are implicated in diabetic retinopathy (DR). Ox-LDL is immunogenic; we previously reported that ox-LDL immunocomplexes (ox-LDL-IC) are present in diabetic retina but not in normal retina, and that human ox-LDL-IC cause pericyte loss in cell culture.

In the present study, we aim to investigate potential pathways for this ox-LDL-IC-induced pericyte loss. *In vitro*, human retinal capillary pericytes (HRCPs) were exposed to human native (N)-LDL (50 mg protein/L), human

For author disclosure information, see page 785.

ox-LDL (50 mg/L) and human ox-LDL-IC (50 mg/L) for 6 or 24h. Expression of CD64 (FcγRI, IgG high affinity receptor), CD32 (FcγRII, IgG low affinity receptor), and CD36 (scavenger receptor for ox-LDL) were detected by Flow Cytometry. In addition, indices of oxidative stress, mitochondrial dysfunction, ER stress, and apoptosis were measured by multiple techniques. Our results showed that at these low doses, neither N-LDL nor ox-LDL had any effect on measured outcomes. In contrast, ox-LDL-IC reduced retinal capillary pericyte viability($p<0.05$, n=3), increasing CD64, but not CD32 and CD36, expression. It induced oxidative stress (increased ROS; decreased Gpx-1), mitochondrial dysfunction (decreased mitochondrial membrane potential, increased Cyt-C and Bax/Bcl2 ratio), ER stress (increased levels of GRP78, splicedATF6, CHOP and ATF6 translocation to nucleus) and apoptosis (increased annexin V, PI double positive cells and TUNEL positive cells). Pretreatment with NAC (an inhibitor of oxidative stress) or 4-PBA (an inhibitor of ER stress) partially attenuated the apoptosis, oxidative stress, mitochondrial dysfunction and ER stress induced by ox-LDL-IC. The data suggest a potentially important role for ox-LDL-IC, formed after extravasation and oxidation of LDL, in the early stages of diabetic retinopathy. At very low concentrations, below those at which ox-LDL has discernable effects, ox-LDL-IC promoted an early feature of DR, pericyte loss, and this involves activation of the CD64 receptor, increased oxidative stress, mitochondrial dysfunction, and ER stress.

EVERY BITE YOU TAKE—INFLUENCE OF DIET ON METABOLISM IN PREGNANCY AND GESTATIONAL DIABETES MELLITUS

197-OR

Changes in Glucose Turnover and during Pregnancy in Women with Type 1 Diabetes

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Postprandial hyperglycaemia is associated with fetal growth acceleration and increased risk of large for gestational age in T1DM pregnancy. Little is known either about the impact of advancing gestation (early vs late) or different meals (dinner vs breakfast) on the rate of appearance (Ra) or rate of disposal (Rd) of glucose in T1DM pregnancy. We quantified Ra and Rd of glucose in 10 women with T1DM during a 24h clinical research facility visit in early (12-16 weeks) and in late gestation (28-32 weeks). Ra and Rd was measured using dual tracer approach with stable-label glucose tracers, standardised dinner (80g carbohydrate at 18.00h), overnight fast (18.30-07.00h) and breakfast (57g carbohydrate at 07.00h). Variable continuous subcutaneous delivery of rapid acting insulin analogue (Aspart) maintained glycaemic control between meals with prandial insulin boluses given immediately pre-meal. There were no changes in fasting Ra (10±2 vs 11±2μmol/kg/min; p=0.32) or fasting Rd (11±2 vs 11±1μmol mol/kg/min; p=0.77) in early vs later gestation. Gestation did not affect bioavailability of simple carbohydrates (102±6 early vs 97±9% late; p=0.43) or timing of postmeal Ra ($T_{50\%}$ 58 ± 18 early vs 52 ± 33min late after breakfast; 109±24 early vs 97±39min late after dinner; p=0.61). Glucose Rd was delayed ($T_{50\%}$ 125±21 late vs 103±17min early after breakfast; $T_{50\%}$ 142±34 late vs 112±22min early after dinner) in late gestation (p=0.003). Peripheral insulin sensitivity was similarly reduced (0.07±0.03 late vs 0.11±0.05 μmol/kg/min per pmol/l early after breakfast; 0.05±0.02 late vs 0.09±0.04 umol/kg/min per pmol/l early after dinner) in late gestation (p=0.002). Prandial insulin absorption was delayed (time-to-peak 78±34min late vs 46±10 early after breakfast; 79±33min late vs 53±13 early after dinner) in late gestation (p=0.0002). In conclusion, postprandial glucose rate of appearance is unchanged in late pregnancy but postprandial glucose disposal is slowed down because of delayed insulin absorption and decreased insulin sensitivity. Earlier prandial insulin administration may be required for optimal postprandial glucose control in women with T1DM during late pregnancy.

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The Effect of Diet on Endogenous Glucose Production and Lipolysis in Obese Pregnant Women

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Maternal obesity is associated with obstetric and metabolic complications but the role of dietary factors in developing these complications is not clear. We explored the relationship between diet composition and maternal endogenous glucose production (EGP) and lipolysis in obese pregnant women at 34 weeks gestation.

Obese (>30% body fat) women (n=19) were recruited at 20 wks gestation and randomized to a low fat or low glycemic load diet for the remainder of pregnancy. Dietary intake, monitored via periodic, random 24-hr recalls, was analyzed using the Univ of Minnesota Nutrition Data System. At 34 wks gestation, basal EGP and lipolysis were measured after an overnight fast (4-hr primed infusion of 6,6-[$^2H_2$]glucose 4.7 mg/min and [$^2H_5$]glycerol 0.88 mg/min).

EGP was 2.93±0.35 mg/kg FFM per min and lipolysis was 0.73±0.19 mg/kg FM per min. Consumption of added sugars and discretionary calories (as % calories) correlated negatively with EGP ($r = -0.80$, $p=0.0014$; $r= -0.81$, $p=0.0013$, respectively), whereas consumption of protein, and more specifically gluconeogenic amino acids (as % calories), positively correlated with EGP ($r=0.69$, $p=0.011$; $r=0.72$, $p=0.0083$, respectively). There was, however, an inverse correlation between total protein and added sugars ($r= -0.59$, $p < 0.03$). Glycemic index, analyzed as a continuous variable, positively correlated with lipolysis ($r=0.58$, $p=0.045$) but not with EGP ($r= -0.40$, $p=0.19$).

These results suggest that dietary macronutrient composition may, in part, determine EGP in pregnancy. Added sugars and discretionary calories may be associated with decreased EGP due to increased carbohydrate availability for the fetus from these dietary sources. Conversely, increases in dietary protein and gluconeogenic amino acids may stimulate EGP in late pregnancy, when the fetal need for glucose peaks, consistent with previous data showing an increased EGP response to acute amino acid infusions in pregnancy. Given the inverse relationship between dietary protein and added sugar, further studies will be required to determine which dietary factor is the primary driver of EGP in pregnancy, in addition to studies to more clearly explore dietary relationships to lipolysis.

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Continuous Glucose Profiles in Obese and Lean Pregnant Women on Controlled Diet: Metabolic Determinants of Fetal Growth

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It is unknown whether obese pregnant women without GDM have higher glucose levels than lean when diet and gestational age are controlled and whether indices of glycemia correlate with infant adiposity. In this prospective study, we used continuous glucose monitoring (CGMS) to measure glycemia in early (14-16 wks) and late (26-28 wks) pregnancy in lean (L: n=22) and obese (O; n=16) non-diabetic pregnant women for 2 days on an ad libitum and 2 days on a control diet. Fasting glucose (FBG), triglyceride (Tg, early only), free fatty acid (FFA) and insulin were measured. Skin fold measures were used to calculate infant % body fat (%BF). The 24-hr glucose area-under-the-curve (AUC) was higher in O vs L mothers both early and late ($p< 0.05$) despite controlled diets and nearly all fasting and postprandial glycemic indices were higher in the O women late in pregnancy. Postprandial glucoses were significantly higher in the O women late in pregnancy on a controlled diet compared to L (mean±SEM; 1-hr 115±2 vs 102±2 mg/dl and 2-hr 107±2 vs 96±2 mg/dl; $p<0.001$) and mean nocturnal glucoses were also higher (96±4 vs 80±2 mg/dl $p<0.01$). Fasting Tg (early, 152±14 vs 85±6 mg/dl; $p<0.001$), FFA (late, 547±58 vs 336±31 uEq/L; $p=0.001$) and insulin (late, 15±2 vs 5.0±1 ng/ml; $p<0.001$) were also higher in O mothers. Infants born to O mothers had more %BF compared to L (9.2% vs 7.3%; $p<0.01$). Maternal BMI ($r=0.55$), mean daytime glucose (late; $r=0.48$) mean glucose (late; $r=0.44$), fasting insulin (late; $r=0.49$), and FFA (late; $r=0.54$) correlated with infant %BF ($p<0.05$). However, Tg (early; $r=0.67$) was the most powerful predictor in a stepwise regression analysis. This is the first CGMS study to show that non-diabetic O mothers have higher levels of glycemia than L when diet and gestation are controlled. Infant %BF was related to maternal BMI, glucose, insulin, and FFA but the strongest predictor was Tg measured early in pregnancy. These data support that O non-diabetic mothers have relative hyperglycemia and hyperlipidemia compared to L. These factors may explain higher macrosomic rates in O women and can be targeted therapeutically to prevent excess fetal growth.

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Race/Ethnicity Disparities in the Prevalence of Gestational Diabetes by Body Mass Index

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Race/ethnicity and obesity are independent risk factors for gestational diabetes mellitus (GDM). However, the demographic distribution of obesity (highest among African Americans and lowest among Asians) does not mirror the demographic distribution of GDM (lowest among African Americans and highest among Asians). Therefore, the risk of GDM may be related to different BMI thresholds across race/ethnicity groups. Within each race/ethnicity group we examined the prevalence of GDM across BMI categories in a cohort of 125,682 women without recognized overt diabetes, screened for GDM who delivered between 1995 and 2006 at Kaiser Permanente of Northern California. GDM was defined by the ADA plasma glucose cut-offs. Among all race/ethnicities, the age-adjusted prevalence of GDM increased with BMI (kg/m2) category. However, Asian and Filipina women had the highest prevalence of GDM and they showed a prevalence of 9.9% and 8.5%, respectively, at a BMI of 22-24 kg/m2. In Hispanic and white women, the prevalence was >8.0% at a BMI 28-30 kg/m2 and 34-36 kg/m2, respectively. Whereas, African American women had the lowest prevalence of GDM in every BMI category, the prevalence reached 9.3% only at a BMI > 37 kg/m2. Clinicians should be aware that the BMI threshold for increased GDM risk vary by race-ethnicity, and that the risk is very high even at BMI <25 kg/m2 in Asian and Filipina women.

	Ethnic Group				
	White	Asian	African American	Hispanic	Filipina
BMI (kg/m²)	Age-adjusted Prevalence % (95% Confidence Interval)				
<19	3.3 (2.1, 4.4)	4.7 (3.5, 5.8)	4.3 (0.2, 8.9)	3.5 (1.2, 5.8)	1.9 (0.7, 3.1)
19-21	1.8 (1.6, 2.1)	6.2 (5.6, 6.8)	1.3 (0.5, 2.1)	3.2 (2.5, 3.9)	6.3 (5.4, 7.3)
22-24	2.3 (2.1, 2.5)	9.9 (9.1, 10.7)	1.8 (1.2, 2.4)	3.8 (3.4, 4.2)	8.5 (7.6, 9.5)
25-27	3.9 (3.5, 4.2)	12.4 (11.2, 13.5)	3.8 (3.0, 4.6)	5.9 (5.4, 6.4)	11.9 (10.5, 13.3)
28-30	5.7 (5.1, 6.3)	15.7 (13.7, 17.7)	5.6 (4.5, 6.7)	8.8 (8.1, 9.5)	15.2 (13.0, 17.4)
31-33	7.8 (6.9, 8.6)	14.5 (11.7, 17.4)	6.3 (4.9, 7.6)	10.9 (9.9, 12.0)	18.2 (14.7, 21.7)
34-36	9.1 (8.0, 10.3)	15.7 (11.4, 20.0)	5.6 (4.0, 7.1)	13.2 (11.8, 14.7)	15.2 (10.2, 20.2)
37+	12.9 (11.6, 14.1)	16.4 (11.5, 21.3)	9.3 (7.8, 10.9)	14.6 (13.1, 16.0)	17.8 (11.9, 23.8)

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Free Fatty Acids (FFA) Cause Increased Inflammatory Response Via Toll-Like Receptor 4 (TLR4) in Adipose Cells of Obese Pregnant Women

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Obese women have dyslipidemia and low grade inflammation during pregnancy, suggesting a link between lipid metabolism and immune regulation. We have shown that TLR4, the obligatory receptor for bacterial lipopolysaccharide (LPS) is activated in adipose tissue of obese women. The aim of this study was to investigate the role of endogenous TLR4 in mediating lipid induced inflammation in adipose tissue of obese pregnant women.

Subcutaneous abdominal adipose tissue (SAAT) was obtained from 10 obese (BMI>30) pregnant women recruited at term elective CS delivery. Stromal vascular cells (SVF) were isolated from the SAAT and cultured with oleic and palmitic acid (1000 μM), the two most abundant nutritional FA, or LPS (100 ng/ml) as a positive control. Cytokine expression in adipose cells was monitored by realtime PCR.

LPS treatment resulted in enhanced gene expression of IL-6, IL-8 and TNF-α (12-, 16- and 4 fold over control respectively; $p < 0.001$). The unsaturated oleic acid induced a similar stimulation with 2- to 12 fold increase in MCP-1, TNF-α, IL-6 and IL-8 gene expression ($p < 0.001$). The saturated palmitic acid induced an even more pronounced activation in cytokine expression with 12 - 25 fold increase for MCP-1 and TNF-α and 200 fold for IL-6 and IL-8 ($p < 0.001$). The stimulatory effect of LPS and fatty acids on IL-6 expression was reduced by 75% and 50% after pre-treatment with an inhibitor of the TLR4 signaling pathway and by 60% and 50% with an inhibitor of NF-*k*B respectively.

These data indicate that both saturated and unsaturated FFAs are capable of recruiting TLR4 signaling to induce an inflammatory response. The magnitude of the fatty acid induced pro-inflammatory effect is of the same order (oleic acid) or higher (palmitic acid) than that of LPS.

These data suggest that the dyslipidemia of obese women may enhance the low grade inflammatory environment of late pregnancy and potentially aggravate the resistance to the insulin action.

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202-OR

Do Overweight Pregnant Women with Glucose Intolerance Require Additional Energy Intake during Pregnancy?

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Strict glycemic control is required for pregnant women with glucose intolerance to prevent various maternal or fetal complications during pregnancy and after delivery.The ADA recommendation is 30%-33% calorie reduction for obese women with GDM, nothing minimum 1,800 calories. It remains unknown whether or not additional energy intake is necessary for overweight women during pregnancy who are glucose intolerant. We examined the resting energy expenditure (REE) of overweight pregnant women with glucose intolerance to determine whether or not additional dietary energy is necessary for each trimester. Ten glucose intolerant overweight pregnant women (age 33±7, BMI before pregnancy 31.7 kg/m^2, 2 with gestational diabetes, 1 type 1 and 7 type 2 diabetes). Seven women were treated with insulin and 3 with dietary management alone. Daily energy intake was set at 25-30 kcal/kg×IBW with no additional energy during pregnancy. The REE was measured at 22±4 weeks (second trimester) and 37±2 weeks (third trimester) using a metabolic analyzer (MedGem). We simultaneously examined hemoglobin A1c, dietary energy intake, urinary ketone bodies, and body composition, including body fat mass. There was no significant difference of REE between second (32.1±3.3 kcal/kg×IBW) and third (33.0±4.1) trimesters. Energy intake was almost the same and maternal body fat mass showed a 2.1±0.7 kg decrease from the second to the third trimester. Hemoglobin A1c showed good glycemic control levels throughout the whole pregnancy and 5.7±0.7 and 6.0±0.5 for the second and third trimesters, respectively. Urinary ketone bodies in all the patients stayed negative during throughout pregnancy. Body weight increased 2.5 kg in the third trimester compared to that before pregnancy. Delivery outcomes were: heavy for date, 2; and appropriate for date, 8. No infant had any deformities. Dietary energy set in the present study resulted in reducing body fat mass, maintaining good glycemic control, almost appropriate birth weight and no untoward delivery outcomes. These results suggested that additional energy intake is not necessary for overweight pregnant women with glucose intolerance during pregnancy.

ISLET TRANSPLANTATION

203-OR

Accumulation of Intrahepatic Islet Amyloid in a Non-Human Primate Transplant Model

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Islet amyloid is hypothesized to play a role in non-immunologic transplanted islet graft loss. Islet amyloid is composed of amylin fibrils deposited within and surrounding β-cells where they exhibit direct toxicity. Amylin is co-secreted from β-cells with insulin, but normally does not form fibrils. We performed a quantitative histologic analysis of intrahepatic islet grafts transplanted in streptozotocin-induced diabetic cynomolgus macaques during a preclinical trial. Liver biopsies were performed at variable times post-transplant, and stained for insulin and glucagon to detect islet β- and α-cells, for CD3 and CD20 to detect lymphocytes, and with thioflavin S to detect amyloid. All 7 animals treated with anti-thymocyte globulin (ATG) + rapamycin or ATG + rituximab experienced islet graft rejection with lymphocytic infiltrates present on islet graft biopsies. Except for one case involving the largest (and presumably oldest) donor where amyloid was present on initial biopsy within one month post-transplant, none of the 6 other cases with lymphocytic infiltrates contained amyloid, including one case biopsied serially to 25 months. In contrast, none of 6 animals treated with ATG + rituximab + rapamycin had evidence of lymphocytic infiltrate at the time of biopsy, but all 4 cases followed > 4 months demonstrated amyloid deposition at subsequent time points. Amyloid severity increased with time post-transplant ($r=0.68$, $p<0.05$) and with decreasing islet β-cell area ($r=-0.68$, $p<0.05$). In 2 islet recipients still normoglycemic and insulin-independent at the first detection of amyloid, β-cell secretory capacity declined over time

For author disclosure information, see page 785.

coincident with increasing amyloid severity and decreasing β-cell area, with both animals becoming hyperglycemic and insulin-dependent. These results indicate that in cynomolgus macaques amyloid deposits may accumulate over time in intrahepatic transplanted islets, and its accumulation may be associated with subsequent decline in β-cell mass and function with eventual recurrence of hyperglycemia. This supports the development of intrahepatic islet amyloid as a potential mechanism for non-immunologic islet graft loss.

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Liver Fat Accumulation and Islet Graft Survival

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This study aimed at determining the fat content of the liver of type 1 diabetes mellitus (DM) patients after islet transplantation (ITx) and to evaluate its association with islet graft survival. The liver fat content was evaluated by MRI [fat liver score = $(SI_{in} - SI_{opp})/SI_{in}$ times 100, where SI is average liver signal intensity divided by the average spleen signal intensity, SI_{in} is signal intensity on in-phase images, and SI_{opp} is signal intensity on opposed-phase images] 16 months (interquartile range: 11-31) after graft failure in 33 type 1 DM ITx recipients. Correlations between clinical and laboratorial variables and fat liver score were done by Spearman test. Comparisons were performed between subjects below and above the median value of the fat liver score. Differences in outcomes (time-to-graft dysfunction or failure) were compared by Kaplan-Meier curves. The only baseline variable associated with the fat liver score was the waist circunferece (r: 0.64; p = 0.044). Regarding the islet function outcomes, an inverse correlation was observed between time-to-graft failure and fat liver score (r: - 0.39; p = 0.026). Patientes with a longer islet survival (define as functional islets for more than 40 months) had lower fat liver scores (1.68±0.50 vs. 2.01±0.38; p = 0.046) in comparison with those with shorter functional grafts. As well, a tendency towards longer islet survival was observed among subjetcs with liver fat score below the median (63.6±6.7 vs. 43.9±6.9 months, p = 0.053). In conclusion, a shorter islet graft survival was associated with increased liver fat after graft failure. Prospective clinical trials may confirm the cause-effect association between liver fat accumulation and islet failure and evaluate if strategies focusing its reduction may prolong islet graft survival.

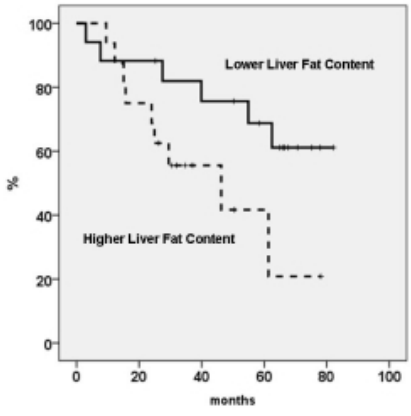

Figure: Time-to-graft failure in patients with lower fat liver content (fat liver score bellow median) and higher fat liver content (fat liver score above median).

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205-OR

Positive Effects of Clinical Islet Transplantation on Diabetic Retinopathy over 5 Years

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Successful clinical islet transplantation (CIT) results in improved glycemic control in subjects with brittle type 1 diabetes. Its impact on microvascular complications of diabetes is less well defined. Previous short term studies on limited numbers of patients suggest stabilization or improvement in retinopathy.

To determine the effect of CIT on retinopathy, we compared 7-field fundal photographs (graded according to ETDRS classification by two reviewers blinded to transplant status) at baseline and annually in 94 subjects who received CIT at our centre vs. 17 subjects receiving standard medical therapy (subjects eligible for CIT who had declined to proceed).

Proliferative retinopathy was more common in CIT subjects (35.9% of eyes vs. 18.2%, p=0.047) at baseline. No significant differences were found in retinopathy status change between CIT subjects and controls at 1, 3 or 5 years (Table 1).

Change in retinopathy grade	1 year		3 years		5 years	
	CIT subjects (n=143)	Controls (n=33)	CIT subjects (n=81)	Controls (n=17)	CIT subjects (n= 63)	Controls (n=9)
Improvement by ≥ 3 steps	2 (1.4)	1 (3.0)	2 (2.5)	0	2 (3.2)	0
Improvement by < 3 steps	35 (24.5)	5 (15.2)	19(23.5)	2 (11.8)	16 (25.4)	4 (44.4)
No change	71 (49.7)	18 (54.5)	40(49.4)	11 (64.7)	28 (44.4)	4 (44.4)
Worsening by <3 steps	30 (21.0)	7 (21.2)	17(21.0)	3 (17.6)	12 (19.0)	0
Worse by ≥3 steps	5 (3.5)	2 (6.1)	3 (3.7)	1 (5.9)	5 (7.9)	1 (11.1)

For CIT subjects, no differences in diabetes duration, pre- and post-transplant HbA1c, blood pressure, eGFR and post-transplant C-peptide were found between patients who had improved/stable retinopathy vs. those who with worse retinopathy at 3 or 5 years. At 3 years, there were more insulin independent subjects with stable/improved retinopathy than with worse retinopathy (38.5% vs. 6.7%, p=0.033); while at 5 years, lower baseline retinopathy grade was found in those with worse retinopathy (p = 0.034).

Improved glycemic control following CIT is associated with stabilization or improvement of retinopathy in most subjects. Progression in a minority highlights the role of non-glucose factors in the progression of retinopathy in those with longstanding brittle diabetes.

206-OR

Reduction in Carotid Intima Media Thickness after Pancreatic Islet Transplantation in Patients with Type I Diabetes

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Mortality from ischaemic heart disease in those with type 1 diabetes is substantially higher than the general population. We sought to determine the impact of islet transplantation on carotid intima-media thickness (CIMT), a marker of atherosclerosis, in healthy individuals with type 1 diabetes. This case-series consists of 14 consecutive Caucasian adult patients who underwent allogeneic pancreatic islet transplant(s) as part of an ongoing phase III clinical trial to achieve insulin independence. Patients had type 1 diabetes for more than five years, presented with hypoglycemic unawareness despite optimal insulin management efforts, displayed normal kidney function, and received a total of 23 islet transplants (1-3 transplants each). Three patients withdrew 13-22 months after first transplant and one died 19 months after transplant. Current follow-up of the rest ranges from 1-4.5 years after first transplant (2005-2010). CIMT was assessed prior to and every 12-16 months following first transplant (totaling 2-5 CIMT assessments over 4.5 years). Outcomes of interest were change from baseline to 12 and 50 month follow-up in mean common and internal carotid artery IMT. A combined CIMT score was defined as the sum of the standardized IMT measurements (standard deviation units (SDs)) of both the common and internal arteries. Multivariable mixed-effects linear regression was utilized. All patients achieved insulin independence. There was a significant decrease in CIMT at 12 months (n=14) for the common carotid (-0.058 mm, p=0.009) and combined score (-1.31 SDs, p=0.007). For those with 50 month follow-up (n=7), the significant decrease in the combined score continued from 12 months (-1.59 SDs, p=0.047) to 50 months (-0.77 SDs, p=0.04). During follow-up, the decreasing slope of change in CIMT was associated with a decreasing slope of change in HbA1c and cardiovascular/inflammatory markers. Islet transplantation is associated with a reduction in CIMT. Therefore, islet transplantation not only leads to insulin independence but may also ameliorate atherosclerosis caused by type 1 diabetes.

For author disclosure information, see page 785.

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Immunosuppressive Therapy May Promote Development of Diabetes and Dyslipidemia Via Alterations in Adipose Tissue Metabolism

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Immunosuppressive agents (IA), such as cyclosporine (CsA), tacrolimus (FK) and rapamycin (Rap) can cause dyslipidemia as well as new-onset diabetes (NODAT) in solid organ-transplantation patients. The aim of this study was to investigate whether adipose tissue plays a role in the perturbations of glucose and lipid metabolism caused by IAs.

This was evaluated in abdominal subcutaneous adipose tissue obtained from healthy volunteers (24F/15M, 23-72 yrs, BMI: 21-36 kg/m^2). Adipose tissue and isolated adipocytes were incubated in the absence and presence of IA: CsA (0.01μmol/l), FK (0.1μmol/l) and Rap (0.01μmol/l) and glucose uptake, lipolysis and protein and gene expression were measured.

All three IAs inhibited insulin-stimulated ^{14}C-glucose uptake in adipocytes: CsA 25%, FK 35% and Rap 30% ($p<0.001$) compared to baseline. The western blot analysis showed that Rap decreased IRS2 protein levels and also insulin-stimulated phosphorylation of PKB Ser473 by 20% ($p<0.05$), compared to control. No effects were observed on the level of other proteins (IRS1, GLUT4 and PKB). Exposure of adipocytes to CsA, FK and Rap increased isoprenaline-stimulated lipolysis (30% $p<0.01$; 20% $p<0.05$ and 20% $p<0.05$, respectively, compared to control), but only Rap increased basal lipolysis (40% $p<0.05$). In addition treatment with Rap was associated with changes in expression of some genes that are important for metabolic regulation: an increase of perilipin and IL6 ($p<0.05$) and a decrease of lipin1 and leptin ($p<0.05$). Paradoxically, IRS2 gene expression was increased after Rap treatment ($p<0.05$). No effects on IRS1, LPL and adiponectin gene expression were observed.

In conclusion, these results demonstrate that CsA, FK and Rap, at therapeutic concentrations, can impair insulin action on glucose uptake and increase lipolysis in the subcutaneous adipose tissue. In addition Rap also promotes altered gene expression of important lipolysis regulators and adipokines that can promote insulin resistance. These effects of IAs in the adipose tissue may potentially contribute to the development of diabetes and dyslipidemia in organ-transplant patients.

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The Role of Islet Autotransplant in Patients with C-Peptide Positive Diabetes Undergoing Total Pancreatectomy for Chronic Pancreatitis

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When total pancreatectomy (TP) is performed to treat painful chronic pancreatitis(CP) in non-diabetic patients, a simultaneous islet autotransplant (IAT) can prevent or minimize post-surgical diabetes mellitus(DM). The role of IAT in preserving residual beta cell function in CP patients (pts) who already have DM but are C-peptide positive prior to TP has not been described.

Herein we report islet yield from pts with CP and preexisting DM undergoing TP/ IAT, and assess the preoperative variables that may predict islet yields in such pts. We reviewed the medical records and metabolic testing results of 23 such pts from 1985 to 2010. Pts in the modern era (9/2006- 2010) had hemoglobin A1c (HbA1c), glucose, and fasting and stimulated C-peptide levels (from mixed meal tolerance test) measured preoperatively.

The 23 diabetic IAT recipients were predominately female (n=20); mean age was 43 ± 15 yrs; mean BMI was 27.3 ± 7.1 kg/m2; 12 were on insulin. All pts tested (n=12) were C-peptide positive before surgery. Mean islet yield was 1565 ± 1524 islet equivalents (IE)/kg vs 3775± 2531 in non-diabetic IAT recipients (n= 321) ($p<0.001$). In the pre-TPIAT diabetic pts, mean IE/kg was significantly lower in insulin-dependent pts (952 ± 1507) vs those not on insulin (2316 ± 1259, p=0.01), but there was overlap (range 9-4858 vs 517-4588 IE/kg). Fasting and stimulated C-peptide correlated with islet yield ($p<0.01$); in pts with stimulated C-peptide≥6.0 ng/ml yield was >2,000 IE/kg in all, while the 2 with stimulated C-peptide <3.0 had low yields (37 & 191 IE/kg). There was no relationship between fasting glucose or HbA1c and islet yield. All patients required insulin postoperatively, but 6/8 tested maintained C-peptide positivity (>0.3 ng/mL).

In conclusion, these results suggest that moderate islet yields can be achieved in some patients with pre-existing DM who undergo TP for pain relief. Thus, DM alone should not exclude pts from undergoing an IAT to preserve beta cell mass. C-peptide may be one important predictor of islet yield. Future follow up will determine the long-term benefit of IAT in these recipients.

209-OR

Optimal Initial Islet Mass in Islet Transplantation: Single or Multiple Transplantations?

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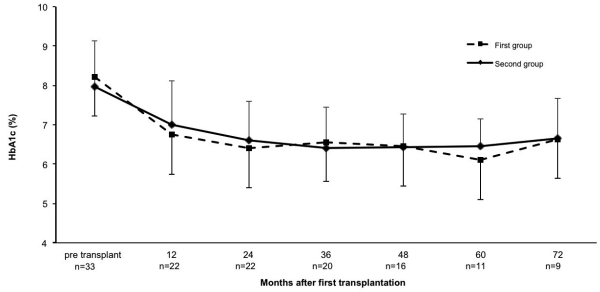
The main goal of islet transplantation in patients with type 1 diabetes mellitus has changed over the last years from insulin independence to good glycemic control and avoidance of severe hypoglycemia. No data exists about the optimal initial islet mass. In this study we compared patients who underwent combined islet-kidney or islet after kidney transplantation at our institution with multiple initial islet transplantations (11 patients with maximal 5 transplants) as compared to single initial islet transplantation (22 patients who were retransplanted only if good metabolic control was no longer achieved). The time between the first and second transplantation was 2.4±2 and 25.8±9 months, respectively. Age was 50.8±8.5 and 53.6±7.4 y with 45.5% and 72.7% male patients. Total follow-up (FU) was 78 and 51 months. Islet retransplantation was performed 0.35 times per patient-year of FU in the 1st group and 0.11 times in the 2nd group. HbA1c decreased significantly in both groups after transplantation (p=0.017 and p=0.026) but did not differ between the groups during FU.



Frequency of hypoglycaemia was comparable in the two groups after transplantation (0.16 vs 0.13 hypoglycemia/patient-year, p=0.88). Insulin dosage decreased significantly in the 1st group (from 0.62 IE/kg to 0.25 IE/kg, p= 0.017) during the first year after transplantation, but not in the 2nd group (from 0.54 IE/kg to 0.4 IE/kg, p=0.38). Similarly, C-peptide was higher one year after transplantation in the 1st group (1454±554 pM) as compared to 655±443 pM (p=0.028) in the 2nd group (single transplants).This study demonstrates that a single initial transplantation with retransplantation only if glycemic control deteriorates, uses less pancreas donors as compared to multiple initial transplantations, but results in equal glycemic control and rate of severe hypoglycemic episodes.

210-OR

High Islet Yield from Pre-Teenage Patients Undergoing Pancreatectomy and Islet Autotransplantation for Chronic Pancreatitis Compared to Teen-Age and Adult Patients

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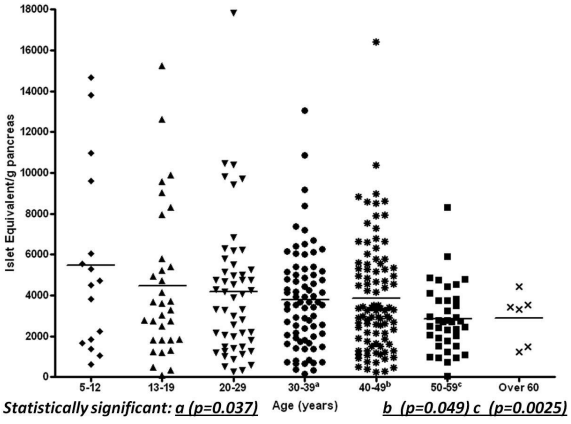
Chronic pancreatitis (CP) in children is associated with intractable abdominal pain, total pancreatectomy (TP) may be required for pain relief, as well as an islet auto-transplant (IAT) to prevent the diabetes. Our series of TP-IAT includes both adults and children, some of whom are very young (5-12 years). We have compared islet yield in the young pediatric (YP) group (n=17) to the older patients (OP) in our series (n=345).The donor and isolation characteristics are summarized in the table below. Four different isolation enzymes were used for all isolations. In the YP cases the average BMI and pancreas weight were 18.9±4.3 and 36.8±25.3 grams, respectively. The average tissue volume obtained in YP isolations was 5.8±5.2ml and islet purification was not required. Mantle islets were present in most cases. The average islet yield/gm pancreas in the YP group was 5,476±4,498 IEQ, higher than in the other age groups: 12-19 yrs (4,456±3,676), 20-29 (4,164±3,324), 30-39 (3,789±2,451), 40-49 (3,848±2,716), 50-59 (2,843±1,623), 60-69 (2,894±1,262) and statistically significant compared to the 30-39, 40-49 and 50-59 age groups. Islet isolation in YP with CP is associated with results in an islet yield/gram comparable or higher than average islet yields of all other

For author disclosure information, see page 785.

age groups including our cadaveric clinical islet preparations (n=48; 4072 IEQ/g). This data also indicate that YP pancreases may be suitable for use in allogenic islet transplantation using multiple donors.

Donor and isolation characteristics	
Age Range (yrs)	5-12
Gender (M/F)	6/11
BMI	18.9±4.3
Pancreas Wt. (g)	36.8±25.3
Total Islet Equivalent (IEQ)	155,803±118,057
Average IEQ/gm Pancreas	5,476±4,498
Transplanted IEQ/kg	4,817±4,013
Tissue Volume (mL)	5.8±5.2
Severity of fibrosis	Severe=9 Moderate=3 Mild=5
Isolation Enzymes	Liberase-HI=9 Serva GMP=3 Serva Premium=4 Vitacyte=1



IT'S NOT ALL ABOUT INSULIN— OTHER HORMONES AFFECTING INSULIN SIGNALING AND ENERGY METABOLISM

211-OR

Activation of Central Glucagon-Like Peptide 1 Receptors (Glp1r) Improves Glucose Tolerance and Hepatic Insulin Action in High Fat (HF)-Fed Mice

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Liver insulin action is impaired in chow-fed Glp1r knockout mice, but it is improved in these mice when they are fed a HF diet. This difference is due to secondary adaptations to chronic loss of Glp1 action in the HF-fed state. Here we tested the hypothesis that acute inhibition of Glp1r exacerbates hepatic insulin resistance in already insulin resistant mice. We also tested whether Glp1r activation enhances liver insulin action in HF-fed mice. We targeted central Glp1r, which acutely regulate hepatic insulin action in chow-fed rodents. Oral glucose tolerance tests (OGTTs) and hyperinsulinemic-euglycemic clamps (HECs) were performed in 5h-fasted, 18-wk old male mice fed a HF diet for 12 wks. Intracerebroventricular infusions of ACSF as vehicle (V), the Glp1r antagonist Exendin-3 (E) or Glp1 (G) were administered 1h prior to and throughout OGTTs and HECs. HECs (4 $mU \cdot kg^{-1} \cdot min^{-1}$ insulin; ~150 mg/dL glucose) were performed in conscious, unrestrained mice using jugular vein and carotid artery catheters for infusions and sampling, respectively. Rates of endogenous glucose appearance (EndoRa) and glucose disappearance (Rd) were assessed using [3-^{3}H]glucose. 2[^{14}C]deoxyglucose was used to assess muscle glucose uptake (MGU). Oral glucose tolerance was unaffected by (E) but improved by (G) (AUC: V=1391±185, E=1446±158, G=980±73 $mg \cdot min \cdot dL^{-1}$). For HECs, fasting and clamp glucose were not different between groups. Fasting insulin was lower with (E) but higher with (G) (V=2.2±0.3, E=1.4±0.2, G=3.5±0.7ng/mL). Percent suppression of endoRa by insulin was insignificantly impaired by (E) but enhanced by (G) (V=54±8, E=42±7, G=76±5%). There was a reciprocal tendency for Rd to be improved by (E) and impaired by (G) (V=13±1, E=17±2, G=11±1 $mg \cdot kg^{-1} \cdot min^{-1}$). There were no differences in glucose infusion rates or MGU. In sum, acute inhibition of central Glp1r does not affect glucose flux in HF-fed mice, but central activation of Glp1r improves oral glucose tolerance in HF-fed mice due to enhanced suppression of endoRa. This expands the glucoregulatory role of Glp1 and shows central Glp1 action to be a viable target for diabetic therapies. **ADA-Funded Research**

212-OR

Fibroblast Growth Factor-21 Promotes Glucose Uptake through Activation of the Serum Response Factor/Ets-Like Protein-1 Signaling Cascade in Adipocytes

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Fibroblast Growth Factor-21 (FGF-21) is a liver-secreted endocrine factor with multiple beneficial effects on obesity-related disorders. Several previous studies indicated that FGF-21 induces glucose uptake by inducing the expression of glucose transporter-1 (GLUT1) in adipocytes. However, the molecular events underlying its actions in adipocyte remain poorly characterized. Here we investigated the signalling pathways that mediate FGF21-induced GLUT1 expression in vitro and in animals. Quantitative real-time PCR and luciferase reporter assay showed that FGF-21 induced GLUT1 expression through transcriptional activation. Suppression of the protein kinase ERK1/2 by its pharmacological inhibitor PD98059 abolished FGF21-induced glucose uptake and transactivation of the GLUT1 gene, indicating that FGF-21 transactivates GLUT1 through ERK1/2. The luciferase reporter assay demonstrated that truncation of -3174 bp to -3105 bp of the GLUT1 promoter, which contains two highly conserved Serum Response Element (SRE) and E-Twenty Six (ETS) binding motifs, dramatically decreased the promoter activity of the GLUT1 gene. Furthermore, mutations in either of these two core elements completely abolished the promoter activity of GLUT1. Chromatin immunoprecipitation (ChIP) assay demonstrated that the transcription factors Serum Response Factor (SRF) and Ets-like protein-1 (Elk-1), both of which are the downstream targets of ERK1/2, were recruited to the GLUT1 promoter upon FGF-21 stimulation. Explant studies on epididymal fats showed that FGF21-evoked phosphorylation of ERK/12, expression of GLUT1 and glucose uptake in high fat diet-induced obese mice were significantly attenuated compared to lean littermates, implying the presence of a FGF-21 resistant state in obese adipose tissue. In conclusion, FGF-21 induces GLUT1 expression through sequential activation of ERK1/2 and SRF/Elk-1, which in turn triggers the transcription of GLUT1 to enhance glucose uptake in adipocytes. The present findings highlight the potential of FGF-21 as a drug target for obesity-related metabolic diseases.

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213-OR

The Novel Adipokine Dipeptidyl Peptidase 4 (DPP4) Is a Potential Risk Factor for Insulin Resistance and Cardiovascular Disease

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DPP4 is a ubiquitously expressed cell-surface protease, which selectively cleaves N-terminal dipeptides from a variety of substrates including growth factors, hormones, neuropeptides and chemokines. Due to the degradation of the incretin glucagon-like peptide (GLP)-1, DPP4 has gained considerable interest as a therapeutic target for type 2 diabetes mellitus treatment. Comprehensive proteomic profiling of the human adipocyte secretome identified DPP4 as a novel adipokine. The aim of this study was to characterize DPP4 as an adipokine and to investigate its direct effects on human primary adipocytes, skeletal muscle cells (hSkMC) and smooth muscle cells (hSMC).

The expression as well as the release of DPP4 increased significantly during adipocyte differentiation (4 and 5-fold, respectively). DPP4 release is not only substantially higher in adipocytes compared to preadipocytes but also compared to adipose tissue-derived macrophages (3-fold). Furthermore the release of DPP4 from adipocytes is significantly upregulated by TNF-α and insulin. The treatment of adipocytes, hSkMC and hSMC with DPP4 in a concentration of 500ng/ml, which reflects serum levels of DPP4 in healthy subjects, induced insulin resistance in all three cell types at the level of

For author disclosure information, see page 785.

insulin-stimulated Akt phosphorylation. Using 20-500ng/ml of DPP4, we could impair insulin-stimulated Akt phosphorylation in a dose dependent manner with the most prominent effect in hSMC (65% of control). This effect could be completely abrogated by the specific DPP4 inhibitor K579. Additionally, DPP4 induced a 1.6 fold increase in hSMC proliferation, which could be completely blocked by K579.

In conclusion, these data suggest that DPP4 is a novel adipokine which may exert auto- and paracrine effects leading to insulin resistance in adipocytes, hSkMC and hSkMC and an increased proliferation of hSMC. Due to increased circulating levels of DPP4 in obesity this novel adipokine may play a role in linking obesity to the metabolic syndrome and cardiovascular disease.

214-OR

Insulin Crosstalks with the Wnt Signaling Pathway in Hepatocytes Via Upregulating TCF7L2 Expression and β-Catenin Phosphorylation at Serine 675

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Certain single nucleotide polymorphisms (SNPs) in TCF7L2 confer high risk for T2D. TCF7L2 and β-cat are major downstream effectors of the canonical Wnt signaling pathway. In response to Wnt ligands, β-cat enters the nucleus and forms a bipartite transcription factor with TCF7L2, leading to transactivation of Wnt downstream targets. Although previous studies on TCF7L2 and Wnt have established their role in embryonic development and cell proliferation, mechanisms by which these SNPs confer T2D risk remains largely unknown. While certain studies have shown that TCF7L2 plays a role in β cell proliferation and insulin secretion, we and others presented conflicting results. TCF7L2 expression could not be appreciably detected in rodent islets and canonical Wnt activity was barely detectable in TOPGAL mouse pancreas. Here we investigated TCF7L2 expression by immunostaining, RT-PCR and Western blotting in organs other than the pancreas where it may contribute to metabolic homeostasis. We revealed TCF7L2 expression in zebrafish and mouse liver and brain. Also, canonical Wnt activity was detected in both liver and brain of TOPGAL mice. Since individuals with TCF7L2 risk SNPs may also exhibit abnormal hepatic glucose production, we examined Wnt pathway in the rodent liver. TCF7L2 expression in the mouse liver could be upregulated by insulin injection, consistent with our previous report that insulin and Wnt crosstalk in the gut. In addition, in the mouse Hepa1-6 cell line, insulin upregulated TCF7L2 mRNA and protein expression, as well as TCF7L2 promoter activity. Furthermore, insulin upregulated Wnt activity via stimulating β-cat Ser675 phosphorylation. This stimulation was not blocked by Akt inhibition, although it was attenuated by PI3K or MEK inhibition. We suggest that hyperinsulinemia leads to increased Wnt activity via upregulating TCF7L2 expression and β-cat Ser675 phosphorylation in an Akt-independent but PI3K/MEK-dependent manner in the liver. Whether TCF7L2 and Wnt regulate the hepatic expression of important metabolic genes is under further investigation.

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215-OR

Hsp60, a Leptin-Induced Mitochondrial Chaperone, Impacts on Central Insulin/IGF-1 Signaling

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Leptin is secreted from white adipose tissue and acts on the hypothalamus to control energy metabolism by increasing energy expenditure and promoting satiety. In addition, leptin has a neuroprotective effect by stabilizing mitochondrial membrane potential, enhancing cell viability and mitochondrial function. Heat shock protein (Hsp) 60, a nuclear encoded mitochondrial chaperone, is crucial for proper folding of mitochondrial proteins and plays a pivotal role in maintaining mitochondrial function and cell viability. Here we show that in leptin-resistant db/db mice and leptin deficient ob/ob mice there is a 50% decrease in the level of Hsp60 in the hypothalamus at both the mRNA and protein level. This is due to direct regulation of Hsp60 expression by leptin, which can be observed in the cultured murine hypothalamic N25/2 cell line using luciferase promoter assay, chromatin immunoprecipitation assays and western blots. siRNA knockdown of Hsp60 in N25/2 cells results in increased reactive oxygen species as measure by lipid peroxidation using the TBARS assay. In addition, knockdown of Hsp60 *in vitro* results in a defect in the electron transport chain activity due to a decrease in the protein levels of several subunits of complex I, II, III and IV. *Ex vivo* analysis of mitochondria isolated from db/db hypothalami shows a similar defect in the electron transport chain activity when compared to control hypothalami. Knockdown of Hsp60 in cultured cells also leads to increased sensitivity to a variety of stress stimuli, including oxidative stress such as paraquat, rotenone and 6-OH-dopamine. In addition, knockdown of Hsp60 in N25/2 cells leads to insulin and IGF-1 resistance, in part by enhancing the ubiquitination of the IGF-1 receptor. Conversely, overexpression of Hsp60 leads to increased insulin and IGF-1 signaling and increased mitochondrial capacity. Together these data show that leptin plays an important role in regulating mitochondrial function in the hypothalamus by regulating the mitochondrial chaperone Hsp60. This provides a novel pathway of leptin/insulin crosstalk in the brain and may impact on hypothalamic control of energy balance.

216-OR

Phosphatidylcholine Transfer Protein (PC-TP) and Thioesterase Superfamily Member 2 (Them2) Exacerbate Endoplasmic Reticulum (ER) Stress: Pathogenic Role in Insulin Resistance

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PC-TP is a phosphatidylcholine binding protein, which activates the long chain acyl-CoA thioesterase Them2 to convert fatty acyl-CoAs to free fatty acids. Both proteins are highly expressed in oxidative tissues, and mice lacking expression of either exhibit improved hepatic glucose homeostasis and are protected against diet-induced diabetes. In cell culture systems, knockdown of PC-TP or Them2 activates insulin signaling. Because free fatty acids promote ER stress, which in turn diminishes insulin responsiveness, this study was designed to explore whether PC-TP and Them2 may regulate insulin signaling by modulating ER stress. We utilized siRNA targeting constructs to knock down endogenous PC-TP or Them2 expression in a mouse hepatoma cell line (Hepa 1-6). Scrambled siRNA served as the control. ER stress was induced by addition of tunicamycin to the media. Markers of ER stress were assayed by immunoblot analysis and quantitative real-time PCR. Under basal serum starved conditions, knockdown of PC-TP or Them2 expression did not alter expression of the ER stress markers PKR-like ER kinase (PERK), activating transcription factor 6 (ATF6) and C/EBP homologous protein (CHOP). Whereas tunicamycin increased phosphorylation of PERK and expression of ATF6 in the scrambled siRNA-treated cells, siRNA-mediated knockdown of PC-TP or Them2 expression led to marked reductions. Tunicamycin failed to elevate CHOP protein or mRNA expression in the setting of PC-TP or Them2 knockdown, and mRNA levels of glucose-regulated protein 78 (GRP78)/BiP were lower following knockdown of either protein, both in serum-starved and tunicamycin treated cells. We conclude that the induction of ER stress is reduced in the absence of PC-TP or Them2 expression. We speculate that PC-TP-stimulated, Them2-mediated conversion of long chain fatty acyl-CoAs to free fatty acids may play a pathogenic role in the development of insulin resistance and type 2 diabetes by exacerbating ER stress.

217-OR

Alteration in Gut Microbiota Leads to Insulin Resistance in Toll-Like Receptor 2 Knockout Mice

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Recent findings indicate that Toll-like Receptors (TLRs) can mediate crosstalk between the immune systems and whole body metabolism. Mice genetically deficient for TLR2 are protected from high fat induced insulin resistance. However, the studies that have characterized the role of TLR2 in animal models were performed in a germ-controlled environment and, thus, cannot predict the influence of the microbiota. Therefore, the goal of the present study was to investigate the role of the microbiota in the insulin sensitivity of TLR2 knockout (KO) mice in a non-germ free facility. We investigated weight gain, the insulin sensitivity and signaling in liver, muscle and white adipose tissue of TLR2 KO mice fed a standard chow. Stool microbiota analysis was studied by 16S rRNA sequencing. TLR2 KO mice and their controls were similar concerning the weight gain at 8 weeks old, on standard chow, but, at the age of 16 weeks, TKR2 KO mice were significantly heavier than their controls. Moreover, at 8 weeks old, with similar weight, TLR2 KO mice presented decreased glucose tolerance and decreased insulin sensitivity and signaling. In TLR2 KO mice, the phosphorylation of JNK was increased; in synergy, there was increased activation of endoplasmic reticulum stress. TLR2 KO mice presented 3-fold increase of Firmicutes and similar composition of Bacteroidetes comparing with their controls. The proportion of Firmicutes was reduced to the same level of the controls after the use of a mixture of antibiotics, leading to increased insulin sensitivity and signaling in these animals.

TLR2 KO microbiota transplantation into wild type (WT) mice mono-associated with Bacillus conferred many aspects of TLR2 KO phenotype,

For author disclosure information, see page 785.

such as the impairment of the insulin signaling and sensitivity. Therefore, we can suggest that the loss of TLR2 result in a phenotype reminiscent of metabolic syndrome, characterized by a clear differences in gut microbiota, which induces insulin resistance, subclinical inflammation associated with ER stress, glucose intolerance and later obesity, which is reproduced in WT by microbiota transplantation and can be reversed by using antibiotics.

Supported by: FAPESP

218-OR

Hepatocyte Growth Factor Is Required for Angiogenesis in Developing Adipose Tissue

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Hepatocyte growth factor (HGF) is an angiogenic factor produced by preadipocytes and adipocytes. We have previously shown that transient knockdown of HGF in preadipocytes attenuates fat pad development 3 d following injection under the skin of nude mice. In this study, we used lentiviral-delivered small interfering RNA to induce stable HGF knockdown in 3T3-F442A preadipocytes and examined the long-term consequences to fat pad development. Knockdown of HGF (siHGF) reduced (P<0.05) HGF mRNA expression 98.5 ± 6.5% and protein 62 ± 2%. At 3 and 14 days post-injection, harvested fat pads from HGF knockdown cells weighed less (P<0.05) than fat pads from control cells (13.5 ± 1.8 vs. 22.5 ± 2.0 mg and 5.8 ± 4.3 vs. 28.4 ± 4.0 mg, siHGF vs. control, 3 and 14 d). Expression of PECAM-1, VEGF, and TIE-1 mRNA was increased (P<0.05) at 14 d compared to 3 d in fat pads from both control and HGF knockdown cells (226.5 ± 35.5 vs. 90.6 ± 35.5, 116.0 ± 26.3 vs. 78.4 ± 26.3, 48.4 ± 5.6 vs. 8.1 ± 5.6 relative units, 14 vs. 3 d). Expression of PECAM-1, VEGF, and TIE-1 mRNA post-injection was decreased (P<0.05) in fat pads with HGF knockdown (228.2 ± 28.2 vs. 102.9 ± 28.2, 78.1 ± 21.8 vs. 157.3 ± 21.8, 20.66 ± 4.40 vs. 38.66 ± 4.40 relative units, control vs. siHGF). Similarly, mRNA expression of the lipogenic gene lipoprotein lipase was decreased (P<0.05) in fat pads with HGF knockdown (50.3 ± 55.0 vs. 271.0 ± 60.5 relative units, siHGF vs. control). 3T3-F44A preadipocytes express the HGF receptor, cMET; therefore cMET knockdown cells were also examined to evaluate the role of HGF in cell survival in developing fat pads. Knockdown of cMET receptor had no effect on fat pad weight at 3 d post injection (18.4 ± 3.8 vs. 15.8 ± 3.8 mg, siCMET vs. control). Decreased expression of both endothelial and lipogenic genes in fat pads developed from cells with HGF knockdown suggests that HGF secretion from adipocytes plays a critical role in the coordination of endothelial cell migration and growth in developing fat pads. An autocrine effect for HGF in preadipocyte survival in developing fat pads does not appear to be required.

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MUSCLE-RELATED STUDIES IN OBESITY RESEARCH

219-OR

Insulin-Induced Microvascular Recruitment in Skeletal Muscle Is Paralleled by Capillary Recruitment in Skin and Both Are Associated with Whole-Body Glucose Uptake

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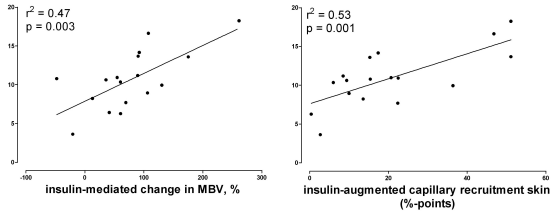
Insulin-induced microvascular recruitment is considered a significant determinant of insulin-mediated glucose uptake in muscle. The study of insulin's microvascular actions has been hampered by a perceived lack of appropriate techniques for its study in humans. Studies examining microvascular action of insulin examined the skin microcirculation, however, there is concern whether the vascular responses observed in skin reflect those in muscle. Recently, Contrast Enhanced UltraSound (CEUS) emerged as a novel tool to study muscle microcirculation. In this study, we compared insulin's vascular effects in skeletal muscle and skin in a group of subjects with a wide variation in insulin sensitivity. Eighteen healthy volunteers (21-55 years) underwent measurements of capillary/microvascular recruitment with capillary videomicroscopy in the nailfold and CEUS. Microvascular measurements were performed before, and at the end of euglycemic hyperinsulinemic clamping. Capillary recruitment was assessed during reactive hyperemia after four minutes of arterial occlusion. Microvascular recruitment is the difference between saline microvascular blood volume (MBV) and hyperinsulinemic MBV. During hyperinsulinemia, capillary recruitment in skin increased by a median 15 %-points (range 0 – 51) and microvascular recruitment increased by a median 69 % (range -47 – 261) and correlated with an r^2=0.32, p=0.02. Moreover, both capillary recruitment and microvascular recruitment were highly correlated with insulin sensitivity; r^2=0.53, p=0.001 and r^2=0.47, p=0.003, respectively. The present study shows that insulin-induced capillary recruitment in skin is paralleled by microvascular recruitment in muscle and both are directly associated with whole-body glucose uptake.



Supported by: The Netherlands Heart Foundation Grant #2009B098

220-OR

Dysregulation of Acetylation of Mitochondrial Proteins after Exercise in Insulin Resistance

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Acetylation (Ac) of non-histone proteins is an important regulator of metabolism and mitochondrial biogenesis. To provide the first human muscle and mitochondrial acetylome and define its relationship to insulin action and exercise (EX), we used mass spectrometry and normalized spectral abundance factor (NSAF) quantification in lysates of muscle biopsies and mitochondria (Mito) isolated from muscle biopsies (Bx) of 3 insulin sensitive (IS) and 2 insulin resistant (IR) nondiabetics. Glucose clamps measured insulin action (Rd); Bx were taken at rest and 24 hours after one EX bout (70-90% VO2max). Rd was 8.7±0.6 vs 5.5±0.6 mg/(kg.min) in IR vs IS. We found 70 unique Ac sites in proteins including adenine nucleotide translocase 1 (ANT1), malate dehydrogenase, superoxide dismutase and others. Basal Ac did not differ between groups. Ac of Mito but not non-Mito proteins was decreased by EX (1.91±0.06 to 1.22±0.04; NSAFX10^6, P<0.05). The decrease in Ac was correlated with Rd (R^2=0.84, P<0.01), indicating greater post-EX de-Ac in IS. ANT1 Ac (Lys 10,23 92) decreased in IS but not IR. ANT1 controls efflux of ATP from Mito and is a key point of metabolic regulation. Modeling dynamics of crystal structure of ANT1 revealed these lysine Ac sites affect ANT1 molecular motion. To determine if Ac of ANT1 affects function, isolated Mito were incubated with saturating substrates (reducing, high NADH) or without substrates (oxidizing, high NAD, activating de-AC). Carboxyatractyloside titration in respiring Mito was used to determine if ANT1 activity increased in NAD-loaded mitochondria (de-Ac proteins). ANT1 activity increased under de-Ac conditions. The results provide the first human muscle acetylome and evidence that EX-induced de-Ac of Mito proteins is defective in IR. We speculate that dysregulated EX-induced deacetylation of Mito proteins is involved in decreased Mito function in IR.

221-OR

Adiponectin Enhances Skeletal Muscle Oxidative Metabolism by Suppressing MAPK Phosphatase-1

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Skeletal muscle (SM) is a major tissue for energy expenditure. Oxidative metabolism in SM is closely related to insulin sensitivity. The main objective of this project is to investigate the underlying mechanisms through which adiponectin enhances oxidative metabolism in SM. Male mice with PGC-1α or adiponectin gene knockout (AdnKO) and differentiated C2C12 myotubes were used. Our results showed that PGC-1α expression and mitochondrial contents were significantly lower in both soleus and gastrocnemius muscle from AdnKO mice compared to wild-type mice. Adiponectin treatment increased not only PGC-1α expression and mitochondrial content but also oxidative myofibrillar proteins such as MHCIIx in C2C12 myotubes. Interestingly, remarkably elevated MAPK phosphatase-1 (MKP1) expression was detected in SM of AdnKO mice, while significantly reduced MKP1 protein was observed in adenovirus-mediated adiponectin-reconstituted mice and adiponectin-treated C2C12 myotubes. These results indicate that adiponectin suppresses MKP1 gene expression in SM. MKP1 is a MAPK specific phosphatase that dephosphorylates and inactivates MAPKs such as p38. MKP1 reduces PGC-1α activity by reducing its protein stability. Therefore, we hypothesized that decreased MKP1 expression might mediate adiponectin-enhanced mitochondrial biogenesis and oxidative metabolism in SM. In line with this hypothesis, decreased PGC-1α, mitochondrial contents and significantly increased glycolytic marker MHCIIb were observed in Ad-MKP1 transduced C2C12 myotubes. Most importantly, overexpression of MKP1 attenuated adiponectin-induced PGC-1α expression and mitochondrial biogenesis in C2C12 myotubes. MKP1 overexpression

For author disclosure information, see page 785.

suppressed p38 phosphorylation, which regulates PGC-1α gene expression and protein stability. Furthermore, in vivo adiponectin overexpression failed to increase mitochondrial biogenesis in SM of PGC-1α gene knockout mice, despite the fact that adiponectin overexpression suppressed MKP1 expression. Our study indicates that adiponectin enhances mitochondrial biogenesis and oxidative metabolism in SM by suppressing MKP1 expression and then increasing p-38/PGC-1α activation.

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222-OR

Hypothalamic Response to GLP-1 Agonist Reduces Energy Intake in Humans

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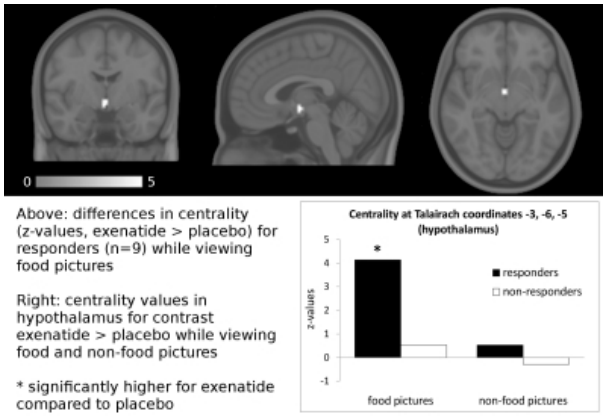
The glucagon-like peptide-1 (GLP-1) agonist exenatide is used in clinical practice to enhance insulin secretion in type-2-diabetes. In some, but not all patients, it also decreases energy intake (EI) and reduces body weight. In animals, intracerebral injection of GLP-1 causes weight loss probably by binding to hypothalamic neurons. We studied the relationship between changes in EI during exanatide infusion and changes in hypothalamic function.

In a cross-over, placebo-controlled, double-blinded study with 16 obese, otherwise healthy male subjects (BMI 32-46 kg/m²) we performed functional magnetic resonance imaging (fMRI) during continuous iv exenatide administration with 0.12 pmol/kg/min. Inside the scanner subjects rated food and non-food pictures for tastiness/valence, and after the scan consumed an ad libitum meal to assess EI.

In line with clinical observations we found that in some subjects exenatide had an effect on EI (i.e., responders: >10 % kcal reduction compared to placebo, n=9, mean -418 kcal, -27 %, p<.001) while in others not (i.e., non-responders: <10% kcal, n=7, mean +137 kcal, +15 %, p=ns).

We analysed fMRI data with a model-free method called 'eigenvector centrality mapping' (ECM), that attributes a centrality value to each brain voxel reflecting its degree of connectedness to all other brain voxels. Only responders showed a significantly higher centrality of the hypothalamus in the exenatide condition compared to placebo when rating food pictures (z-value 4.13, uncorrected, max at Talairach coordinates -3, -6, -5) (see Fig. 1).



Here, we demonstrate for the first time that peripherally administered exenatide in humans alters both, hypothalamic connectivity and EI. Non-responders did not show changes in hypothalamic connectivity supporting the assumption that absence of anorectic effects is related to an absent hypothalamic response.

Supported by: Lilly Deutschland GmbH

223-OR

LKB1 and AMPK Regulate Glucagon Secretion in Pancreatic α-Cells To Modulate Whole Body Glucose Metabolism

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Normal secretion of glucagon is essential for the maintenance of blood glucose and responses to acute hypoglycaemia. The tumour suppressor LKB1 (STK11), and the downstream kinase AMPK, are critical regulators of cellular metabolism in a range of tissues. Here, we show by conditional deletion of the corresponding genes in the α-cell that each is required for the control of glucagon secretion *in vivo*.

LKB1 (αLKB1 KO) or the α1 catalytic subunit of AMPK (αAMPK α1 KO) were ablated selectively in islet α-cells by breeding LKB1 or AMPK α1 *flox*'d mice with mice expressing *Cre* recombinase under the pre-proglucagon promoter. Male αLKB1 KO mice displayed improved glucose (1g/kg) tolerance (peak value: 16.5±1.3 mmol/l in wild type *vs* 13.3±0.6 mmol/l in KO, p<0.05) and reduced fasting plasma glucagon levels (61.9±8.6 pg/ml in wild type *vs* 38.2±8.4 pg/ml in KO, p<0.05) at 8 weeks of age. Moreover, aminoimidazole carboxamide ribonucleotide (AICAR) tolerance tests showed increased AICAR sensitivity and profound hypoglycaemia in knock-out mice after intraperitoneal injection of 0.25g/kg AICAR. Correspondingly, LKB1 deletion in pancreatic α-cells decreased glucagon secretion from isolated islets incubated with 3 mmol/l glucose by 60.2±6.8% (p<0.05) and increased the secretion from islets incubated with 17 mmol/l glucose 3.1-fold without altering secretion at 0.1 mmol/l glucose, or insulin secretion at all glucose concentrations tested.

Similarly, αAMPK α1 KO mouse islets showed a 39.3% decrease of AMPK activity, comparable to that in islets from αLKB1 KO (39.5% decrease) and these animals displayed decreased fasting plasma glucagon levels (58.8±11.9 pg/ml in wild type *vs* 39.0±1.4 pg/ml in KO) despite normal glucose tolerance. Correspondingly, αAMPK α1 KO mouse islets displayed a 54.9±6.9 % (p<0.05) decrease in glucagon secretion at 3 mmol/l glucose with no changes at 0.1 or 17 mmol/l glucose.

LKB1 thus appears to serve as a positive regulator of glucagon secretion, possibly acting upstream of AMPKα1. Defects in α-cell LKB1 or AMPK signalling may contribute to abnormal glucagon release in some forms of diabetes.

Supported by: EU (IMIDIA) and the Wellcome Trust

224-OR

Combined Long-Acting PYY and GLP-1 Agonism Synergistically Normalizes Weight and Glucose in Obese and Diabetic Mice

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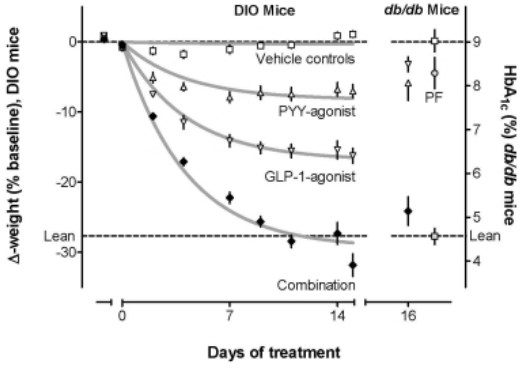
Increases in the L-cell hormones, GLP-1 and PYY, are a feature of Roux-en-Y bariatric surgery (RYGB), which results in both long term weight loss and resolution of T2DM. We generated long-acting agonists of PYY and GLP-1 to investigate the effects of this combination on these activities in models of diabetes (*db/db* mice) and obesity (diet-induced obese (DIO) C57BL/6 mice). In DIO mice, 2-week co-administration of these long-acting agents resulted in a 31.8±1.8% loss from pre-treatment weight (return to lean control body weight) and a 19.2% inhibition of food intake, reflecting supra-additive effects (see Fig). Combination therapy dropped body fat from 39.5% to 18.9% (equaling lean controls). In *db/db* mice, 2-week combination therapy resulted in a significant 43% decline in HbA1c (by 3.6% units) to levels observed in lean controls (see Fig, gray square). Pair-fed mice showed no such effect (gray circle). In both the *db/db* and DIO models, combination therapy returned clinical chemistry parameters (*e.g.* glucose, HbA1c, cholesterol, triglycerides, aspartate aminotransferase, alanine transaminase) to lean control values. Histopathological analysis of livers from combination treated groups confirmed amelioration of the hepatic steatosis exhibited by both models. In summary, we report that long-acting PYY and GLP-1 agonism can act synergistically to recapitulate RYGB-like efficacy for weight loss, control of glucose, and modulation of other metabolic parameters.



For author disclosure information, see page 785.

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Role of Endotoxin in Insulin Resistance in Human Muscle

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Emerging evidence has implicated the gastrointestinal flora in the pathogenesis of insulin resistance by causing chronic, low-grade endotoxemia. The goals of this study were to determine: (i) whether obese nondiabetic and obese diabetic subjects have abnormal endotoxin [lipopolysaccharide (LPS)] concentrations in plasma; (ii) whether LPS promotes an inflammatory response in human muscle, employing a primary muscle cell culture system; and (iii) whether this inflammatory response is mediated by toll-like receptor 4 (TLR4). We measured plasma LPS concentrations and insulin-stimulated glucose metabolism (M) with the euglycemic insulin clamp (160 mU/m^2.min) in 13 lean [BMI=25±1 kg/m^2, age=39±2 y, fasting plasma glucose (FPG)=92±3 mg/dl, M=10.4±0.7 mg/kg.min], 9 obese nondiabetic (BMI=32±1, age=48±3, FPG=92±2, M=8.5±0.7), and 11 obese diabetic (BMI=34±1, age=48±3, FPG=151±13, M=4.2±0.5) subjects. Obese nondiabetic and diabetic subjects had elevated LPS concentrations in plasma by 2.5- and 2.8-fold, respectively (P<0.05 vs. lean). There was an inverse correlation (r=-0.46, p=0.005) between LPS concentrations and insulin sensitivity (M value). Incubation of human myotubes derived from lean nondiabetic subjects with 200 ng/ml LPS for 12 h caused an inflammatory response, as evidenced by increased p38 MAPK (by 88%) and JNK (by 54%) phosphorylation and increased IL-6 (by 56-fold) and MCP1 (by 41-fold) mRNA expression (P<0.05). Further, knocking down TLR4 using siRNA reduced the LPS-induced increases in p38 (by 71%) and JNK (by 22%) phosphorylation and IL-6 (by 57%) and MCP1 (by 67%) mRNA expression (P<0.05). SUMMARY: (i) Obese nondiabetic and obese diabetic subjects have increased concentrations of endotoxin in plasma, and this could play a role in the pathogenesis of insulin resistance; (ii) LPS directly causes an inflammatory response in human muscle; and (iii) downregulation of TLR4 blunts the LPS-induced inflammatory response in human muscle, suggesting that pharmacologic blockade of TLR4 could be a useful strategy for the treatment of insulin resistance.

226-OR

Glucagon-Like Peptide 1 (GLP-1) Increases Muscle Microvascular Recruitment and Glucose Uptake Via a Nitric Oxide-Dependent Mechanism

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GLP-1 agonists/analogs have been widely used in the management of diabetes. In addition to its effect on glucose-mediated insulin secretion, GLP-1 has been shown to increase tissue glucose uptake and cause vasodilation in conduit arteries independent of insulin. Whether GLP-1 exerts a vasodilatory effect on microvasculature and subsequently affects glucose uptake in muscle remain unknown.

Two groups of adult male Sprague-Dawley rats were studied after an overnight fast. One group received continuous GLP-1 (7-36) amide infusion (30 pmol/kg/min) for 2 hrs. The other group received 2 hrs of GLP-1 infusion in the presence of the nitric oxide (NO) synthase (NOS) inhibitor L-NAME (50 µg/kg/min) which started 30 min prior to the GLP-1 infusion. Hindleg muscle microvascular blood volume (MBV), a measure of microvascular surface area and perfusion, and microvascular blood flow velocity (MFV) were determined using contrast-enhanced ultrasound. Muscle microvascular blood flow (MBF) was calculated (= MBV x MFV). Plasma NO levels and muscle interstitial oxygen saturation were quantified.

GLP-1 infusion acutely increased muscle MBV (p<0.05) within 10 min without altering MFV, blood pressure or femoral artery blood flow. This effect persisted throughout the 120 min infusion period, leading to a > 2-fold increase in muscle MBF (p<0.05). This microvascular response was paralleled with increases in plasma NO levels (~2-fold, p<0.05), muscle interstitial oxygen saturation (p<0.01), and hindleg glucose extraction measured as hindleg arterio-venous [glucose] differences (> 2-fold, p<0.05). Systemic infusion of L-NAME blocked GLP-1-mediated increases in muscle MBV, glucose disposal and NO production.

We conclude that GLP-1 acutely increases muscle microvascular recruitment and basal glucose uptake via a NO-dependent mechanism. Thus, GLP-1 may afford potential to improve muscle insulin sensitivity and decrease the cardiovascular complications associated with diabetes by expanding microvascular endothelial surface area thereby increasing tissue delivery of substrates, oxygen and insulin. **ADA-Funded Research**

DIABETES EDUCATION—LOOKING THROUGH THE KALEIDOSCOPE

227-OR

Comparitive Effectiveness of Multiple Modalities of Lifestyle Intervention in the Community: Results of the Rethinking Eating and ACTivity Study (REACT)

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Interventions adapted from the DPP are effective at weight loss and diabetes risk reduction in community settings (CS). To impact public health policy and clinical care, understanding the comparative effectiveness of multiple prevention modalities in CS is critical to assist consumers, clinicians, and policy makers in making informed decisions. We aimed to determine if three Group Lifestyle Balance (GLB) intervention modalities were effective in decreasing risk for diabetes. 555 individuals (86.1% female, 95.1% white, 55.8% obese) from 8 underserved, rural communities, in southwestern, PA were screened for BMI ≥ 25 kg/m^2 and waist circumference ≥ 102 cm in men and 88 cm in women. Communities and their eligible participants (n=493; mean age: 51 yrs, 87.6% female, 94.1% Caucasian, 86.8% BMI ≥ 30 kg/m^2) were assigned to 4 GLB groups: Face to Face (FF) (n=119), DVD (n=113), internet (INT) (n=101), and self-selection (SS) (n=101). Participants in SS were empowered to select their GLB modality (60% chose FF, 40% INT, 0% DVD). Outcomes for these analyses included improvement in impaired fasting glucose (IFG), defined by ADA criteria and meeting a 5% weight loss goal following the GLB intervention. Improvements in the proportion of participants with IFG were observed in all groups (FF: 20.8% vs. 14.8%, p=0.17; DVD: 48.4% vs. 34.6%, p=0.11; INT: 32.6% vs. 27.8%, p=0.65), however improvement was statistically significant only in SS (62.3% vs. 41.5%, p=0.002). SS participants were 1.5x (95% CI: 1.1-1.9) less likely to have IFG following the GLB intervention, compared to other groups. Greater than 50% of participants in all groups met the 5% weight loss goal (FF: 57.6%, DVD: 57.8%, INT: 66.75, SS: 68.5%). Of those who met the goal, > 90% of participants, across all groups, sustained the 5% weight loss at 6-month follow-up, with 100% in SS sustaining their weight loss. Despite the modality, GLB was effective at improving IFG and achieving 5% weight loss. SS participants experienced greater improvements in both outcomes compared to other groups. Indeed, the importance of patient-centered decision-making in healthcare is paramount.

228-OR

Technology-Supported Diabetes Self-Management Intervention Improved Weight Loss through Enhancing Self-Efficacy

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Achieving weight loss has been a challenge for adult patients with type 2 diabetes. Enhancing self-efficacy is essential to improving diabetes self-management skills, yet no study has examined its role when technology is used to support the intervention. Mediation analysis is widely used to explore the mechanism of the intervention. Thus, we conducted a mediation analysis to examine the role of self-efficacy in a technology-supported diabetes self-management intervention using data from a 6-month randomized clinical trial. Participants were randomized to 1) attention control or 2) intervention using a personal digital assistant (PDA) for self-monitoring diet as part of a Social Cognitive Theory-based behavioral intervention. Self-efficacy, defined as the patients' confidence in their ability to manage diabetes, was measured by the Multidimensional Diabetes Questionnaire self-efficacy subscale. Mediation analysis was performed using bootstrap resampling to build a 95% confidence interval of the indirect effect. The sample included 296 adult patients with type 2 diabetes, they were 69% White, 67% female, had an average age of 55.6 ± 10.9 years and a mean BMI of 34.6 ± 7.4. Retention at the 6-month assessment was 83.1%. Compared to the attention control group, there was a significant increase in self-efficacy in the intervention group (p=.004). Self-efficacy improvement was significantly associated with weight loss at 6 month (rho=.164, p=.014). Mediation analysis indicated a significant indirect effect of the intervention on weight loss through self-efficacy improvement (95% CI: .04, .62). These findings suggest that the effect of the PDA-supported behavioral intervention on weight loss was through self-efficacy improvement. Diabetes self-management education programs using emerging mobile technology tools may be more effective in helping patients achieve successful weight loss when clinicians and educators focus on increasing patients' self-confidence in their ability to manage diabetes. Additional research is warranted to confirm our results.

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For author disclosure information, see page 785.

ADA-Funded Research

229-OR

A1c, BP and LDL Goals: Successful Use of Telemedicine (DTMS™) in 1000 Compliant T2DM Subjects over 6 Months

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The major barriers to reach treatment targets in diabetes are with fear of hypoglycemia and non adherence to instructions on drug dosages and life style modifications.

Diabetes Tele-Management System (DTMS™) is one approach which combines slow, steady titration of drugs with repeated tele counselling.

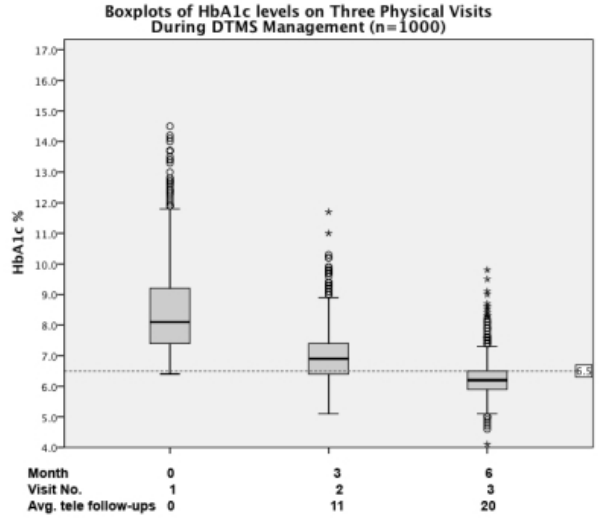
DTMS comprises of a multi-disciplinary team of doctors, dieticians, educators, nurses, pharmacists & psychologist, aided with customised electronic medical records. Patients report 4-point SMBG values (fasting and 2hrs after each main meal) and 3am and other special values as and when needed, through phone, email or website, with individualized frequency. DTMS team follows up after each report/query on modifications of drug dosages, diet, exercise & offers continuing patient education.

Dosage titration with DTMS minimises risk of low sugars and also saves cost and time of frequent physical visits.

This retrospective study of 1000 compliant patients, age 30-75 yrs with no severe co-morbidities, shows DTMS effective, with highly statistically significant reductions of glycemic and cardiovascular risk parameters from baseline to 6th month.

Variables	Physical Visit 1 Mean(SD)	Physical Visit 2 Mean(SD)	Physical Visit 3 Mean(SD)	Difference (Physical Visit 1- Physical Visit 3) (*p-value =0.000)
BMI	25.4 (3.79)	25.3 (3.51)	25.1(3.48)	0.3*
Systolic BP	132 (19)	126 (15)	122 (12)	9.6*
Diastolic BP	81 (10)	77 (8)	77 (7)	4.5*
Total Cholesterol	194 (42)	154 (24)	138 (17)	56*
LDL	126 (40)	95 (26)	82 (20)	44.1*
HDL	42 (6)	45 (6)	46 (4)	-3.4*
Triglycerides	137 (62)	112 (36)	102 (28)	35*
Creatinine	0.9 (0.11)	0.8 (0.09)	0.8 (0.09)	0.06*
FBS	174 (59)	120 (25)	107 (19)	67*

Average A1c at baseline was 8.5% (1.4) compared to 6.2% (0.6) at 6 months.



DTMS based tele follow up in selected compliant diabetes patients, help achieve all treatment targets.

Supported by: LifeScan

230-OR

Impact of a Computer-Based House-Staff Training Program on the Management of Inpatient Diabetes

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Hospitals are struggling to implement ADA guidelines for the management of hospitalized patients with diabetes; inadequate house-staff training may be one of the reasons for this difficulty. We hypothesized that computer-based house-staff training could improve the management of hospitalized patients with diabetes. We designed a case-based computer training program that all internal medicine house-staff at the Brigham and Women's Hospital were required to complete during the month of September 2009. House-staff confidence and knowledge for managing inpatient diabetes were assessed via questionnaire before and after the training program. Medical records were reviewed to assess the management of diabetes patients treated by house-staff before (August 2009) and after the training program (December 2009). Medical records were also reviewed for the months of August 2008 and December 2008, when no training program was enforced, to serve as a control group. Out of 182 house-staff, 165 (90.7%) completed the training program. House-staff confidence increased from 31.6 ± 7.2 to 39.2 ± 6.3 (arbitrary units, 0- 45; p<0.0001), and knowledge increased from 16.4 ± 3.0 to 19.8 ± 2.9 (arbitrary units, 0 - 22; p<0.0001). Use of sliding scale insulin decreased and use of basal-bolus insulin regimens increased after the training program, but not in the control group (Table 1, *P<0.05). Episodes of hyperglycemia or hypoglycemia were unchanged. We conclude that computer based house-staff training focused on inpatient diabetes improves the implementation of basal-bolus insulin regimens for the management of diabetes among hospitalized patients.

	Control Group		Intervention Group	
	August 2008	December 2008	August 2009	December 2009
HbA1c obtained unless unavailable within 3 months of admission (% of patients)	57	64	55	58
Sliding scale insulin used alone (% of patients)	35	34	28	16*
Basal bolus insulin (% of patients)	65	66	72	84*
Average number of days per patient with one blood glucose > 250 mg/dL	0.87	1.09	1.00	1.06
Average number of days per patient with one blood glucose < 70 mg/dL	0.18	0.29	0.32	0.31
Average hospital days/patient	3.79	4.16	3.81	4.04

231-OR

Multisite Community Diabetes Self-Management Education in a Low Socio-Economic Latino Population

AMPARO GONZALEZ, ALICIA GORDON-THOMAS, ROBERTO MEJIA, GUILLERMO UMPIERREZ, *Altanta, GA, Medellin, Colombia*

Recent projections continue to predict an increase in prevalence of diabetes in Latinos, and the quality of diabetes care among this group lags behind that of non-Latino whites. Linguistic, cultural and socioeconomic barriers present significant challenges to Latino patients with diabetes and their healthcare providers. Several studies have shown that in order to be successful, immigrant minorities require culturally appropriate interventions and modifications to the standard U.S. healthcare delivery system. We previously showed the success of the Emory Latino Diabetes Education program (ELDEP) in the Grady Healthcare System in Atlanta.We expanded the ELDEP program "*Viva más y mejor … con su diabetes bajo control*" to provide diabetes education to community sites and primary care settings in Georgia and Alabama. A total of 1076 individuals (age 50±0.7 yr, female 61.3%, unemployed 45.8%, low income <$15,000/yr 76.1%, uninsured 74.95%, unable to read Spanish 55.8% or English 93.1%) participated between 2006 and 2010.Participants attended 2.5 hr initial diabetes class followed by 1.5 hr club meetings held monthly with an open invitation to attend.Curriculum is based on the AADE 7 self-care behavior framework. Data collected revealed a baseline A1C of 9.0±3.15%, improved to 7.97±2.06% at follow up (p<.001). In addition, self-care behavior practices improved including:daily foot exam from 48.9% to 67.3%, flu vaccination from 39.3% to 51.6%, engaged in physical activity from 53.6% to 82.7%, blood glucose monitoring from 63.2% to 85.4%, keeping a blood glucose log from 46.6% to 64.5%, and knowing type of diabetes from 57.1% to 82%. In conclusion, our results show that a diabetes education program from an academic institution can be successfully exported to diverse community programs in low-socioeconomic populations. We observed that diabetes self-management education (DSME) programs in community centers achieved similar glycemic control and adherence with clinical practice recommendations achieved in academic centers. Dissemination of DSME programs in Latino populations are needed to improve diabetes care and reduce chronic complications in underserved Latino population.

For author disclosure information, see page 785.

232-OR

A Comparison of Group and Individual Education for Patients with Sub-Optimally Controlled Type 2 Diabetes: A Randomized Controlled Trial

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The study objective was to determine whether group education is more beneficial than either individual education or usual care for patients with sub-optimally controlled diabetes.

In a multisite randomized controlled trial that took place in Minnesota and New Mexico from 2008-2009, 623 adults with type 2 diabetes (T2DM) and glycosylated hemoglobin (A1c) ≥7% were assigned to (1) individual education, (2) group education, or (3) usual care (no education). Education was delivered through the American Diabetes Association accredited program of the patient's care system. The individual education group received three 1-hour sessions. The group education group received four 2-hour sessions using the U.S. Diabetes Conversation Map® program. Main outcomes were change in A1c, general health status (SF-12™), diabetes distress assessed by Problem Areas in Diabetes (PAID), Diabetes Empowerment Scale (DES), Diabetes Care Profile (DCP), nutrition assessed by Recommended Food Score (RFS), and Behavioral Risk Factor Surveillance System (BRFSS) physical activity score. General and linear mixed-modeling methods assessed patient-level changes in outcomes from baseline to follow-up.

Four months after completing education, mean A1c decreased in all groups, but significantly more in the individual education group than group education (-.25%, p=.01) and usual care (-.27%, p=.02). Compared to usual care, diabetes distress was reduced with individual education (-.37, p=.02) and group education (-.30, p=.05). Individual education (but not group education) improved SF-12 physical component score (+1.98, p=.03), physical activity (+41.17 minutes/week, p=.05), and nutrition score (+.66, p=.06). Empowerment was unchanged with either educational intervention.

In the short-term, individual education for this patient population resulted in better A1c levels than did usual care and group education. Diabetes-specific distress was reduced with both educational interventions, but individual education was superior to group education for A1c improvement and other pre-specified outcomes.

Supported by: Merck

233-OR

Impact of a Lay-Led Diabetes Self Management Program in Community Settings

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The Diabetes Network of Saint Louis (DNSL) aims to enhance diabetes self management by employing a community-based model for chronic disease management support. Lay volunteers are recruited and trained to lead diabetes self-management education groups. These groups are held in the communities where diabetic patients in need of services reside. Intervention components included structured physician communication, self-empowerment, and goal setting through a succession of six themed sessions. Final results of a two year evaluation of this program are presented. Participants (n=377) were recruited representing 68 zip codes in the Saint Louis region, 52% were African American (AA), while 44% were Caucasian and 4% were other races. Gender distribution showed 73% female with 59% AA female. Incomes less than $30,000 were seen in 56% and the average age was 63. Support group sessions were held at 33 different community sites. The average number of sessions attended out of a possible 6 was 2.8 and 52% of the participants attended at least half of the sessions.

Participants showed improvements in both behavioral and clinical outcomes. The average number of days per week that participants followed an eating plan increased from 2.6 at baseline to 3.5 (t-test=3.502, p=.001). The weekly number of days that 5 or more fruits or vegetables were consumed increased from an average of 3.5 days per week to 4.1 (t-test=2.641, p=.010). The average days of weekly physical activity increased from 3.0 to 3.5 (t-test=2.319, p=.023). Weekly days of blood glucose monitoring increased from 3.5 to 4.7 (t-test=3.946, p<.001). BMI decreased from 34.0 at baseline to 33.7 (t-test=2.390, p=.018). Systolic blood pressure decreased from 141.3 to 137.5 (t-test=2.818, p=.005) while diastolic blood pressure decreased from 78.3 to 76.1 (t-test=2.680, p=.008). HbA1c decreased from 7.40% at baseline to 7.19% (t-test=3.491, p=.001).

The DNSL was successful in enhancing diabetes self-management and hard clinical metrics in community setting using lay leaders. This is a sustainable model that addresses the disparity in care and the impact of the impending metabolic tsunami.

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Impact of Diabetes Counseling and Education on Diabetes Clinical and Cost Outcomes

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The impact of diabetes counseling and education (C/E) on patients with type 2 diabetes mellitus (T2D) has not been well studied. Using a national managed care database (i3 Innovus) from Jul 1, 2006 to Dec 31, 2008, this retrospective study compared clinical and economic outcomes in a large group of T2D patients who received C/E vs. those who did not (non-C/E). T2D adult patients were included in the study group if they had received ≥1 outpatient C/E, as identified by Current Procedural Terminology (CPT) codes in the medical claims, had ≥6 month pre-C/E and ≥1-year post- C/E continuous health plan coverage, and evidence of T2D during the study. The first evidence of C/E was identified as their index date. Those who had not received outpatient C/E served as the control group with a randomly assigned index date. Hypoglycemic events and health care costs were determined during follow-up. A1C values were identified on the index date and at 3 and 6 months after the index date. A total of 272,397 eligible patients were identified with only 6.4% receiving ≥1 outpatient C/E. Significant differences existed between the C/E vs. non-C/E groups (mean age 53.3 vs. 55.7 years; male 49.5% vs. 55.8%; mean Charlson Comorbidity index 1.55 vs. 1.40). Diabetes education was the most common C/E (65.6%), followed by nutrition/diet C/E (36.0%). After adjustment using multivariate analyses, compared with the non-C/E group, the C/E group had significantly lower A1C at 6 months post-index date (6.8% vs. 7.3%, $P<0.01$), were more likely to achieve A1C <7.0% (odds ratio [OR] 1.64; 95% confidence interval [CI] [1.48, 1.82]; $P<0.01$), and have hypoglycemic events (OR 1.14; 95% CI [1.02, 1.29]; $P=0.03$) during the follow-up period. Overall and diabetes-related 1-year adjusted follow-up costs were higher in the C/E vs. non-C/E group (Overall, $14984 vs. $12596; Diabetes-related: $5504 vs. $4677, both $P<0.01$). Additional analyses to further control for selection bias are being undertaken. This study suggested that outpatient C/E may be associated with improved glycemic control, but also an increased likelihood of hypoglycemia and higher healthcare costs.

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CONTINUOUS GLUCOSE MONITORING—HOW CAN WE USE IT MOST EFFECTIVELY?

235-OR

The Use of Continuous Glucose Monitoring (CGM) To Evaluate Performance of Closed-Loop Insulin Delivery Systems

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CGM devices measure glucose in the interstitial tissue. We investigated whether CGM-based assessment of closed-loop insulin delivery systems can be used instead of gold standard plasma glucose (PG) assessment. Data were obtained from 33 subjects with type 1 diabetes treated by conventional subcutaneous insulin infusion (9 adolescents 12 to 18 year old, and 24 adults 18 to 65 year old) attending clinical research facility on two occasions, in random order, to undergo overnight closed-loop or conventional insulin pump treatment. During closed-loop, basal rates on insulin pump were manually adjusted every 15min using a model-predictive-control algorithm and real-time CGM levels. Reference PG levels were measured every 15minutes to assess closed-loop performance. Time when glucose was in the target range from 70 to 145 mg/dl was overestimated by CGM during closed-loop nights (86 [65 - 97] vs 75 [60 – 91]%; CGM vs PG; P=0.027; median [IQR]) but not during conventional treatment (57 [32 - 74] vs 51 [29 - 73]%; P=0.36). When CGM was interpreted as stochastic, normally distributed variable to account for the measurement error of 15% derived from matched PG CGM pairs (N = 4345), the adjusted CGM gave unbiased estimate of time in target during both closed-loop (79 [60 - 86] vs 75 [60 – 91]%; P=0.15) and conventional treatment (53 [32 - 66] vs 51 [29 – 73]%; P=0.19). Additionally, treatment effect calculated as differences between time in target during closed-loop and conventional treatment was nearly identical with the use of adjusted CGM (22 [14 - 30] vs 22 [15 - 29]%; mean [95% confidence interval]; P=0.91). We conclude that CGM without adjustment may overestimate the benefit of closed-loop insulin delivery. Once adjusted, CGM provides unbiased estimate of time when glucose is in target and the treatment effect. Adjusted CGM may be acceptable for assessment of closed-loop performance in outpatient settings.

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For author disclosure information, see page 785.

ADA-Funded Research

236-OR

Patient- or Physician-Driven Continuous Glucose Monitoring (CGM) Improves Control and Quality of Life (QoL) in Poorly-Controlled Type 1 Diabetic Patients on Intensified Insulin Therapy: A One-Year Multicenter Study

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Benefits of real-time CGM have been clearly shown in patients with T1D in 3 to 6-month studies. The aim of the present study is to assess the effect of two approaches of 1-year use of CGM in poorly-controlled patients with T1D.

The study protocol was designed to a 1-year, open, muticenter trial. Patients were randomly assigned into 3 groups (1:1:1). Inclusion criteria were age ≥8yrs, T1D for ≥ one year, use of either multiple insulin injections or an insulin pump and HbA1c level ≥8%. Two modes of using CGM (FreeStyle Navigator™) (Group 1: on patient demand, Group 2: physician-prescribed) were compared to a conventional Self Monitoring of Blood Glucose practice (Group 3: control). The primary outcome was the change in the HbA1c level at one year. The secondary outcomes were SD of glucose levels, hypoglycaemia and QoL.

In total, 178 patients completed the study: age: 36±14 yrs, T1D duration: 17±10 yrs, A1c: 8.9±0.9 %, SD glucose: 70 [52; 84] mg/dl (mean±SD, or mean [95% CI]). At one year, the HbA1c level similarly improved in both CGM groups, and was significantly reduced when compared to the control group: G1 vs G3:-0.52 %, p = 0.0006, G2 vs G3:-0.47 %, p = 0.0008, G1+G2 vs G3: -0.50 %, p < 0.0001). SD glucose was also reduced by 11.9 [0; 2.6] mg/dl in G1+G2 vs G3 (p = 0.018) and by 15.1 [0; 3.5] mg/dl in G2 vs G3 (p=0.049). Occurrence of hypoglycaemia was similar in the 3 groups. As far as QoL is concerned, patient satisfaction (DQoL) and physical health (SF36) scores improved in both CGM groups at one year (respectively p=0.004 and p=0.04). The frequency of the CGM system use is significantly correlated to the improvement of HbA1c (p = 0.05). The HbA1c level is more improved in patients on pump (G1+G2 vs G3: -0.67 %) than on multiple injections (G1+G2 vs G3: - 0.23 %).

A long-term use of CGM resulted in a sustained and significant improvement of metabolic control and QoL in poorly-controlled T1D patients on intensified insulin therapy. The use of CGM "on patient demand" is as effective as a "physician-prescribed" use strategy.

237-OR

Long Term Clinical Evaluation of a New Subconjunctival Glucose Sensor

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An ocular mini insert (OMI) able to monitor glucose concentrations has been recently developed by Eysesense company. It is subconjunctivaly implanted and contains a glucose binding lectin dispersed thoughout a hydrogel as well as competitive binding fluorophore fluorescing near infrared. The glucose dependant fluorescence is measured by a small handheld fluorophotometer. We investigated accuracy of glucose measurement as well as tolerability and safety of the OMI in a long term study over 9 months.

Study was performed in 28 insulin dependent diabetic patients (Type 1 n=20; Type 2 n= 8). The OMI was inserted subconjunctival into the right eye under local anaesthesia. We investigated: correlation between capillary glucose measured by laboratory method and interstitial glucose measured by the OMI while inducing an increase and decrease of glucose values in a range between 60 – 300 mg/dl.; mean average relative error (MARE) of glucose readings made by OMI; local tolerability and safety by ophtalmological examination.

After implantation most patients exhibited postop. a small subconjunctival hemorrhage and minor foreign body sensation which disappeared within few days. The implants were overall well tolerated : 8 pat. showed a mild conjunctivitis and 1 pat. a moderate prolonged wound healing which could be successfully treated with local agents; 6 patients spontaneously lost the OMI without any local complications. The prevalence of working OMIs 1, 3, 6 and 9 months after implantation was 100%, 88%, 74% and 50%. Corresponding MARE were 18%, 27%, 28%,28%. High accuracy, i.e. MARE < 20% as well as fair accuracy, i.e. MARE < 30% were found in 40 % and 70% of the working OMI at the end of observation period. The reason for the time-dependent decrease of working OMIs is a thin encapsulation of the implant as detected by ophthalmological and confirmed by histological examination.

The study shows good tolerability and safety of the implant. Measurement performance was high in working OMIs even 9 months after implantation.

Encapsulation of the OMI may be prevented by special coatings.

238-OR

Is the Accuracy and Lag of Continuous Glucose Monitoring Systems in Measuring Physiological Changes in Blood Glucose Levels Affected by Sensor Life?

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The potential for continuous glucose monitoring systems (CGMSs) to improve glycemic management depends, in part, upon the maintenance of its accuracy in measuring blood glucose level over time. No study to date has examined how both CGMS accuracy and lag respond to physiological changes in blood glucose levels over extended periods of wear. To address this issue, 8 non-diabetic participants (7 females and 1 male; aged 30.2 ± 8.3 years; BMI of 24.3 ± 2.5 kg/m2) were fitted with 2 CGMSs (Paradigm®722-Real-Time, Medtronic Diabetes, Northridge, CA; abdominal and triceps regions), and after an overnight fast completed an oral glucose tolerance test (OGTT) on 6 occasions over 9 days while the CGMS was worn without removal. Only non-diabetic participants were tested as this allows for reproducible basal blood glucose levels and OGTT responses over consecutive days. Arterialized blood samples were collected for comparison to CGMS values. Across all OGTTs, there were significant mismatches between blood glucose and CGMS values, most notably during the first 30 minutes where blood glucose levels increased rapidly. However, CGMS accuracy did not significantly deteriorate in response to consecutive OGTTs using either the abdominal (mismatch at peak blood glucose on day 1 = 3.7 ± 0.6 mmol/l vs day 9 = 1.8 ± 0.6 mmol/l; p = 0.1) or triceps sensor (day 1 = 2.8 ± 0.2 mmol/l vs day 9 = 3.0 ± 0.5 mmol/l; p = 0.8). Similarly, the CGMS lag time to peak glucose levels did not significantly change using either the abdominal (day 1 = 26.3 ± 5.5 vs day 9 = 18.8 ± 3.8 min; p = 0.4) or triceps sensor (day 1 = 18.8 ± 3.8 vs day 9 = 10.9 ± 2.4 min; p = 0.09). All participants found the triceps sensor site more comfortable than the abdominal site (p = 0.005). For the first time, we show that the accuracy and lag of CGMS readings during physiological changes in blood glucose levels are not negatively affected by sensor life over 9 days of repeated glycemic challenges *in vivo*. Thus, despite issues of accuracy and lag, the CGMS sensor may be used for extended periods, providing added economic and health benefits for the wearer.

239-OR

Prediction of Short-Term Glucose Trends for Type 1 Diabetes Using Empirical Models and Frequency-Band Separation

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The prediction of short-term glucose dynamics (0-60 min) is a key element of the model predictive control strategies that are being evaluated in the quest for the Artificial Pancreas. Accurate glucose predictions can be used to calculate insulin administration, to provide early warnings of impending hypoglycemia, and to monitor overall performance of the Artificial Pancreas. In this research, data-driven models are developed based on CGM data and frequency band filtering of the glucose measurements. A key issue is whether a fixed "universal model" can be used for all patients or whether patient-specific models would be significantly more accurate.

High-order autoregressive models are developed for 23 ambulatory clinical subjects using 4 days of CGM data (5 minute sampling) for each subject. Predictive models are identified for each subject using three approaches for filtering the glucose data: (i) a single, low frequency band; (ii) two frequency bands, high and low; and (iii) no frequency band filtering. Model accuracy is evaluated using two well-known metrics, the coefficient of determination (R^2) and Error Grid Analysis (EGA). The results for the 23 ambulatory clinical subjects are summarized in Table 1.

Type of Model	R^2 (%)	EGA (% in zone A)	EGA (% in zones A and B
Universal low-frequency	58±20	70±11	95±4
Subject-dependent low-frequency	60±21	71±12	95±4
Universal two-frequency	66±20	77±11	96±3
Subject-dependent two-frequency	63±22	76±12	97±3
Subject-dependent, no frequency band filtering	63±23	76±11	97±3

The two universal models were identified from the glucose data for subject #1; similar results were obtained when the universal models were identified based on other subjects.

For author disclosure information, see page 785.

The prediction accuracies of the five models are quite similar with over 95% of the predictions in zones A and B for the EGA. The universal two-frequency model provides the best results; universal models based on a single subject are as accurate as subject-dependent models. The practical implication for the Artificial Pancreas is that the effort required to develop the empirical model for MPC can be significantly reduced by using a universal model.

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240-OR

Towards a Closed Loop System: Effects of Activities of Daily Living on Glucose Variability in T1D and Healthy Subjects

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Moderate to high intensity physical activity (PA) changes insulin action with increased risk for hypoglycemia in Type 1 Diabetes (T1D). However, the effect/s of low intensity PA, mimicking activities of daily living (ADL), measured with precise accelerometers, on glucose variability (GV) in T1D has not been examined. Quantifying the effects of PA on GV would substantially improve Artificial Endocrine Pancreas (AEP) algorithms. Fourteen nondiabetic (7 males; age 29.6±2.0yrs.; BMI 25.1±1.0kg/m^2; fasting plasma glucose {FPG} 4.8±0.1mM; HbA1c 5.3±0.1%) and 7 T1D subjects (3 males; age 43±5yrs.; BMI 27±1.6 kg/m^2; FPG 7.6±1.1mM; HbA1c 7.2±0.3%) were studied in the Clinical Research Unit at Mayo Clinic, Rochester for a 88 hour period. The participants underwent a planned PA protocol, adherence to which was captured using the physical activity monitoring system (PAMS). Walking was at 1.2mph totaling 3.5 to 4.2 miles/day; 1.7METS during the active periods. Interstitial fluid glucose concentrations were captured with Dexcom Seven® Plus continuos glucose monitoring system (CGM). Three identical meals/day were provided during the study (10 cals/kg, 50 grams carbs; 35% carbs, 30% protein, 35% fat). In random latin square order, 1 meal/day was followed by subjects lying in bed for 6 hrs while the other meals were followed by participants undergoing activity protocol. CGM and PA data for total of 189 meals were analyzed from T-30 minutes prior to meal ingestion up to T+270 minutes post meal. The area under curve (AUC) calculations were computed over the basal PA & CGM levels. The basal glucose levels and PA levels were similar in both nondiabetic and T1D participants (P<0.24). In nondiabetic subjects, the incremental glucose AUC was 3.50±0.29mM/270 min for meals followed by activity whereas it was 8.29±2.87mM/270min (p=0.02) for meals followed by inactivity. The corresponding glucose excursions for those with T1D were 3.82±5.82mM/270 min and 13.53±6.04 mM/270 min (p=0.03). Our preliminary results show that low grade PA mimicking ADL impacts postprandial glucose excursions in T1D and utilized in real time would substantially enhance AEP algorithms.

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241-OR

Association of SMBG with Medication Adherence and Glycemic Control

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There is debate about the value of SMBG in non-insulin type 2 diabetes necessitating further research. This study examines the association between SMBG presence (i.e. evidence of SMBG prescription fills), medication adherence, and improved glycemic control.

We used a US claims database (i3 Innovus) to identify 5172 patients who began non-insulin diabetes medications (including injectables) between 10/1/06 and 3/31/09. Patients also had an A1C within the 3 months before and 4-12 months after medication initiation. Most patients were started on metformin (69%), sulfonylurea (10%), TZDs (10%), or DDP-4 inhibitors (6%). 2,744 patients (53.1%) had SMBG available post-medication start and 2,428 (46.9%) did not. We calculated a medication possession ratio (MPR) for each patient and defined medication adherent patients as having an MPR ≥ 80% (an accepted measure of medication adherence).

Using logistic regression, we found that SMBG presence was associated with higher likelihood of being medication adherent (OR 1.5, 95% CI 1.4-1.7). Medication adherent patients had a significantly larger decline in A1C in the post-medication period (-1.35 vs. - 0.82 for non-adherent patients, p < 0.001). ANCOVA controlling for age, gender, and prior A1C demonstrated a significant interaction among SMBG presence, medication adherence, and prior A1C (p<0.0001). Higher baseline A1C was associated with greater change in A1C post-medication. Both SMBG presence and medication adherence were associated with a similar degree of change in A1C. For example, when prior A1C is 9% and other factors are held at mean values, among nonadherent patients the change in A1C was -2.00 for SMBG and -1.36 (p<0.0001) for no SMBG; and for medication adherent patients, -2.37 for SMBG and -2.08 for no SMBG (p<0.0001).

SMBG presence was associated with greater medication adherence. Additionally, SMBG presence was associated with improvement in glycemic control for both medication adherent and nonadherent patients. This suggests an association of SMBG presence with glycemic control independent of medication adherence.

242-OR

The Benefit of Multiple Glucose Sensors in Type 1 Diabetes: Implications for Artificial Pancreas Design

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The development of an artificial pancreas device that is suitable for outpatient use has been slowed by suboptimal glucose sensor accuracy. We asked whether sensor accuracy could be improved by utilizing multiple glucose sensors. We also asked whether positioning the sensor wires very close, as may occur in a future artificial pancreas, would negate the benefit of redundancy.

Thirteen adult subjects with type 1 diabetes participated in 24 nine hour studies. Subjects wore four DexCom 7+ sensors during each of two studies. One pair of sensors were worn on each side of the abdomen, with each sensor pair placed at a pre-determined distance apart on the skin surface and 20 cm away from the pair on the other side of the abdomen. Venous blood was drawn every 15 min via an indwelling IV catheter for measurement of glucose for comparison to sensed glucose values. Sensors were calibrated once at the study start. Subjects were given their typical insulin doses and two meals. Mean inter-sensor distances, measured between the sensor tips by x-ray, from lowest to highest inter-sensor distance, were 6 ± 1, 12 ± 1, 17 ± 2, and 27 ± 2 mm.

As compared to the use of a single sensor, there was a clear benefit of redundancy. Use of the median glucose value significantly improved accuracy as compared to a single randomly chosen sensor (mean ARD 13.1 ± 1.2 vs. 15.6 ± 1.2%, p < 0.05). Use of the median values led to a greater accuracy benefit than use of the mean. Inter-sensor distance did not affect the function of sensor pairs. There was no interaction between the distance between sensors and the difference between the sensor readings of each pair (R^2 = 0.004). If sensors positioned close together were entrained, the differences would be lower for closely-positioned pairs.

We conclude that when four sensors are used simultaneously, there is an accuracy benefit compared to use of a single sensor. The benefit of redundancy is present even when sensors are positioned very close together (6 mm apart). These findings are useful in the design of an integrated artificial pancreas device.

THE COSTS AND COST-EFFECTIVENESS OF DIABETES PREVENTION AND CARE

243-OR

Alternative Fasting Glucose (FG) Cutoffs To Identify Adults Eligible for Diabetes Prevention: Health and Economic Implications

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Although FG is used to identify persons eligible for diabetes (DM) prevention programs, the cost-effectiveness of alternative FG cutoffs is not known.

We used a simulation model to assess the health implications, costs and cost-effectiveness of progressive 5 mg/dl decrements in FG cutoffs from 120 to 90mg/dl among the U.S. non-DM adult population under 2 scenarios of lifestyle intervention: "High-Cost" (HC)- a $1200 per person-year intervention that reduces incidence by 55.8%, as in Diabetes Prevention Program; and "Low-Cost"(LC) - $300 per person-year intervention that reduces incidence by 25%, as in PLAN4WARD, a community program. We used data from the National Health and Nutritional Examination Survey to estimate FG prevalence and data from Atherosclerosis Risk in Communities study to estimate DM incidence by FG values. Outcomes were measured by percent of DM cases prevented, number of DM-free years, medical cost, quality-adjusted life-year (QALY), and incremental cost-effectiveness ratio (ICER) defined as the cost per QALY associated with each 5 mg/dl reduction in the FG cutoff. We examined 10-year and lifetime horizons from a healthcare system perspective.

The percent of cases prevented, number of DM-free years and costs continuously increased with decreasing FG cutoff, but were all less under

For author disclosure information, see page 785.

the LC scenario. The cutoffs corresponding to an ICER of ≤$50,000/QALY, a common cost-effective benchmark, were ≥ 100 mg/dl under HC scenario and ≥ 95 mg/dl under LC scenario. We conclude that the recommended cutoff of 100 mg/dl is cost-effective, but slightly lower cutoffs provide greater health benefits and thus may be preferred if resources were less constrained. If the cost of preventive interventions can be reduced, lower cutoffs may also be preferable.

FG Cutoff, mg/dl	High-risk Population, %	HC Scenario			LC Scenario		
		10-Year DM Prevented,%	Lifetime DM-free Years,Millions	Lifetime ICER, $1000/QALY	10-Year DM Prevented,%	Lifetime DM-free Years, Millions	Lifetime ICER, $1000/QALY
120	1	1	14	14	0.3	5	27
115	4	2	56	15	1	19	22
110	8	5	143	18	2	53	21
105	16	9	278	27	4	108	25
100	29	14	461	41	6	185	34
95	49	19	691	57	8	285	46
90	70	24	930	81	11	390	63

244-OR

Cost-Effectiveness of Multiple Modalities of Lifestyle Intervention in the Community: Projections from the Rethinking Eating and ACTivity Study

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To impact public health policy and clinical care, understanding the cost-effectiveness (CE) of multiple prevention modalities in community settings is critical to inform medical decisions. We aimed to determine the CE of 3 Group Lifestyle Balance (GLB) modalities (Face to Face [FF], DVD, Internet [INT]) in reducing risk for diabetes in 493 overweight, abdominally obese subjects in southwestern PA. Eight rural communities, along with the eligible subjects, were randomized to one of 4 groups: FF, DVD, INT, or Self-Selection (SS). Subjects in SS selected their preferred modality (48% FF, 0% DVD, 52% INT). We constructed a Markov model to estimate the CE of the 3 GLB modalities and no GLB. Model time horizon for base case analysis was 3 years, taking a health care system perspective and discounting costs and benefits at 3% annually. Study-based costs and outcomes were used; other costs, disease progression data, and mortality from diabetes, and utilities were from published literature. Sensitivity analyses were conducted to assess effects of varying model assumptions and parameters. In base case analysis, INT and SS were strictly dominated by FF, while DVD was weakly dominated by a blend of no GLB and FF; FF produced the greatest effectiveness. Compared to DVD and no GLB, FF cost $26,573 and $178,607/QALY. Over 5 years, INT, SS, and DVD were dominated; compared to DVD and no GLB, FF cost $6,065 and $63,377/QALY. Increasing weight loss effects on reducing diabetes risk showed that INT, SS, and DVD were dominated; compared to DVD and no GLB, FF cost $2,700 and $53,088/QALY. Increasing intervention effects on reducing weight resulted in FF costing $972 and $106,064/QALY compared to DVD and no GLB. In probabilistic sensitivity analysis, DVD, INT, and SS were unlikely to be favored over FF or no GLB. Using an acceptability threshold of $100,000/QALY, FF was favored in 12% of model iterations (compared to 86% for no GLB and 2% for DVD); with a $300,000/QALY threshold, FF was favored in 62%. FF appears to be a sound investment among the GLB modalities, and may be economically reasonable compared to no GLB.

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245-OR

Impact of Diabetes and Prediabetes on the VA and Veterans in the Southeastern U.S.

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Screening for diabetes or prediabetes is uncommon in the VA, the largest healthcare system in the US. Since screening would be more justified if negative effects of diabetes occurred early in the natural history, we asked when the adverse impact begins.

Using a database of veterans from SC, GA, and AL, diabetes pts with continuous primary care (≥5 visits over ≥4 yr before and ≥4 visits over ≥3 yr after diagnosis) were matched 1:2 with controls with similar follow-up, age, race/ethnicity, gender, and VA facility. The fiscal year including the diagnosis was assigned as yr 0, "unrecognized diabetes" included yr -1 and -2 (from previous analyses), and "prediabetes" included yr -3 and previous. Costs and resource use were obtained from VA datasets, and cardiovascular disease (CVD) from ICD-9 codes.

2062 diabetes pts and 4124 controls averaged age 63 yr and were 99% male, 29% black, 39% white, and 33% unknown race/ethnicity; BMI was 30.8 diabetes vs. 27.8 controls ($p<0.001$). Total VA costs/yr were higher in diabetes than control patients from yr -4 ($4,083 vs. $2,754) through yr +5 ($8,347 vs. $5,700), all $p<0.003$, reflecting underlying increases in outpatient, inpatient, and pharmacy costs (all $p<0.05$). The pattern was similar for outpatient contacts in yr -4 through yr +4 (all $p<0.01$), and CVD was also more prevalent in yr -4 through yr +4 (all $p<0.001$). Regression analysis showed that diabetes and prediabetes contributed an average of $1666/year to costs independent of CVD ($p<0.001$).

Thus, VA costs per veteran are increased over $1,000/yr before and over $2,000/yr after diagnosis, reflecting underlying increases in outpatient, inpatient, and pharmacy costs, increased outpatient visits, and increased CVD. Moreover, adverse impact on both the VA (resource use and costs) and on veterans' health (CVD) occurs early in the natural history – several yr before diabetes diagnosis. Conclusion: Since the adverse impact of diabetes begins during periods of prediabetes and unrecognized diabetes, greater consideration should be given to systematic screening – to reduce costs and permit early detection and initiation of preventive management.

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Outpatient Resource Utilization of Type-2 Diabetes Patients: New Evidence from the UKPDS

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Reliable estimates of the impact of diabetes related complications on resource use and healthcare costs are important for researchers studying cost-effectiveness of interventions. The costs of outpatient care are especially poorly understood. Previous cost estimates based on a cross-sectional study of patients enrolled in the UKPDS have been widely used. We report updated results that incorporate 10 years post UKPDS follow-up, with a larger number and wider range of complications that allow us to observe the impact of changing best practice protocols on resource utilization over time.

We report results from six questionnaires administered between 1997 and 2007, resulting in 3,589 responders and 10,920 questionnaires. We recorded information on all home, clinic, and telephone contacts with general practitioners, nurses, podiatrists, opticians, and dieticians, and with eye and other hospital out-patient clinics over recall periods varying from 4 months to one year, depending on the questionnaire issue date. Completed questionnaire response rates varied from 71% to 76%.

We estimated the immediate (within the recall period) and long term (prior to the recall period) impact on costs of myocardial infarction, ischemic heart disease, stroke, heart failure, amputation, blindness in one eye, retinal photocoagulation, cataract extraction and vitreous haemorrhage, controlling for patient specific characteristics.

The largest average annual outpatient cost was £1,153 (£907 - £1,465) for amputation, followed by heart failure and vitreous haemorrhage. The immediate (within-recall period) and longer-term cost impact of the complications examined did not significantly differ, apart from MI. The average annual costs for those with complications increased significantly over time, from £484 in 1997 to £838 by 2007.

For patients having no complications, routine outpatient care costs increased by 54% over the 10-year follow-up, and particularly between 1997 and 2003. This period corresponded with the publication of the UKPDS results that demonstrated the benefits of intensive therapy and the subsequent changes in treatment guidance and protocols.

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For author disclosure information, see page 785.

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Costs Associated with Delivering a Structured Lifestyle Intervention and Diabetes Education Program in Overweight and Obese Adults with Type 2 Diabetes: Year 4 Results from the Action for Health in Diabetes (Look AHEAD) Study

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Structured lifestyle interventions are effective for preventing and managing type 2 diabetes (T2DM), yet the costs of delivering such programs are not well documented. We describe these costs for the lifestyle and standard care interventions in Look AHEAD, a multicenter randomized clinical trial designed to examine the long-term effects of an intensive lifestyle intervention (ILI), compared to diabetes support and education (DSE), for reducing cardiovascular events in 5,145 obese/overweight adults with T2DM.

We estimated intervention costs associated with the first four years of ILI and DSE for participants. Study staff completed questionnaires that described the types of personnel and time involved in group sessions (ILI and DSE), individual sessions (ILI), and reminders for all sessions, separate from research costs.

We calculated the average clinic-specific personnel cost of a typical visit (ACPCTV) by multiplying reported times by salary data. The annual intervention personnel cost for each individual participant was estimated as the product of ACPCTV and the number of visits attended per year. The total personnel intervention cost for each participant was the sum of the cost components for that participant.

Personnel costs in 2007 US dollars associated with delivering the Look AHEAD interventions by year are below. Combined with the effectiveness of ILI, these and other cost estimates will be used to assess cost-effectiveness of the ILI.

Intervention years	Year1	Year2	Year3	Year4
ILI				
Individual visits				
Average cost per visit ($)	143	147	111	97
Average number of visits per participant	12.8	10.0	8.7	8.5
Average cost per participant ($)	1,831	1,466	956	825
Group visits				
Average cost per participant per visit ($)	53	42	40	51
Average number of visits per participant	25.5	7.0	6.4	6.9
Average cost per participant ($)	1,344	294	258	351
Total average cost per participant ($)	3,175	1,760	1,214	1,176
DSE				
Average cost per visit ($)	31	28	31	36
Average number of visits per participant	2.7	2.3	2.1	2.0
Total average cost per participant ($)	83	64	65	72

Supported by: NIH,CDC

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Initial HbA1c and HbA1c Lowering: Implications for Model-Based Economic Evaluations in Type 2 Diabetes Mellitus (T2DM)

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Most economic models of T2DM are based on a core relationship between HbA1c and the risk of developing micro- and macrovascular complications. HbA1c efficacy assumptions have been sourced from trial data and have not routinely adjusted for differences in important patient characteristics such as baseline HbA1c. If starting HbA1c values in the trial population differ from those of the simulated population, using reductions unadjusted for baseline HbA1c may systematically bias treatment effects. The objective of this study is to assess the potential bias associated with using unadjusted HbA1c reductions by comparing economic evaluations of Metformin (MET) versus Glimiperide (SU) in the treatment of individuals newly-diagnosed with T2DM. We used the Economic and Health Outcomes (ECHO)-T2DM model to estimate the cost-effectiveness of MET versus SU over 20 years under both a traditional approach that assumes unadjusted HbA1c reductions and an alternative with reductions adjusted for baseline HbA1c. These reductions were taken from a recently published meta-analysis. Results were generated for two different cohorts with mean baseline HbA1c of 7% and 8%, respectively. For both cohorts and for both unadjusted and adjusted HbA1c reductions, MET compared favorably to SU, partly attributable to the assumed slower rise in HbA1c over time with MET. The magnitude of benefit differs by type of treatment effect, however. Unadjusted reductions lead to cost savings of $6,416 and $4,550 and quality-adjusted life year (QALY) gains of 0.125 versus 0.102 for the 7% and 8% cohorts, respectively. The cost savings and QALY gains for MET versus SU decrease using adjusted HbA1c reductions ($4,609 versus $3,999 and 0.108 versus 0.091 for the 7% versus the 8% cohorts, respectively). Moreover, the differences between these two cohorts narrow, since with adjusted estimates, the HbA1c lowering effect is smaller in the 7% versus the 8% cohort. These results confirm the need for model-based economic evaluations to consider this type of bias, especially in cases where the assumed initial HbA1c differs appreciably from the mean reported in the clinical trial setting.

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Comparative US Claims Data in Type 2 Diabetes Mellitus Patients Who Have Undergone Bariatric Procedures

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Bariatric surgery is an effective weight-loss option for morbidly obese patients with type 2 diabetes (T2D). This longitudinal study measures the intermediate-term health care consumption and costs associated with the restrictive procedure, laparoscopic adjustable gastric banding (LAGB), and the malabsorptive Roux-en-Y bypass (RYGB) procedure in patients with T2D, whose medical and prescription claims may also serve as proxy measures to disease state severity. Utilizing Source® Lx integrated claims repository data from 2002-09, physician practice, pharmacy, and hospital claims identified RYGB and LAGB patients by CPT or ICD-9 code. Patients were screened for T2D using ICD-9 code and evaluated up to 4 years(yr) before and after their initial procedure. Care consumption was analyzed across prescription claims, physician office visits, and hospital visits. Costs of care were measured through out-of-pocket prescription expenses and total cost, submitted physician office and hospital charges, and total healthcare charges. All subjects experienced an increase in metrics of care consumption from 4 years pre-procedure up to the procedure date. Differences were observed in consumption and costs between RYGB and LAGB post-procedure:

Average Per-Patient Utilization & Cost	1 Yr Pre		1 Yr Post		4 Yrs Post	
	RYGB	LAGB	RYGB	LAGB	RYGB	LAGB
Diabetes Rx Claims	10.5	9.5	5	4.5	6.5	4
Rx Claims (total cost $)	27(2200)	22(2280)	20(1530)	16(1730)	20(1540)	13(1230)
HCP Office Visits (charges $)	10(2590)	8.5(2110)	8(1790)	8(1770)	6(2480)	4(970)
Hospital Visits (total charges $)	4.5(6060)	4(6160)	5(7730)	4.5(7180)	5(11370)	3.5(6690)
Total Healthcare charges $	7743	7630	7245	7832	9254	4287

These data demonstrate that both surgical procedures are effective at reducing both care consumption and cost in T2D patients in the short-term. However, longer-term costs significantly increased in patients undergoing RYGB, whereas patients with LAGB had a reduction in cost and utilization. It is hypothesized that the increased 4 year costs of RYGB are related to surgical complications and to micronutrient-related morbidity caused by malabsorption. These differences require further study.

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Medical Expenditures Associated with Major Depression among Private Insured Working Age Adults with Diabetes in the US, 2008

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Among persons with diabetes, major depression (MD) is associated with greater medical expenditures. Previous research quantifying this association relied on small samples, self-reported data or focused on the elderly. We estimated excess medical expenditures associated with MD among working age adults (18-64 years) with diabetes. We used claims data from over 500,000 individuals with diabetes continuously enrolled in fee-for-service health plans in the US MarketScan database in 2008. We used one and two-part regressions to estimate medical expenditures controlling for demographics, diabetes treatment regimes, health plans and other comorbidities.

The prevalence of MD was 2.9%. Younger age, being female, urban residence, using insulin only or both insulin and oral medications, having a higher Charlson comorbidity score and enrolling in non-preferred provider organization plans were significantly positively associated with MD. Predicted mean annual medical expenditure, both in total and by component, were significantly greater among diabetic patients with MD than that among those

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without (Table). Predicted mean total medical excess expenditure associated with MD was $8,912. Of this, excess outpatient and inpatient expenditures each accounted for 34% and prescription drugs accounted for 32%.

Among working age adults with diabetes, MD is associated with substantial excess medical expenditures: those with comorbid MD had total medical expenditures 1.6 times greater than those without. A substantial reduction in medical expenditures for people with diabetes would number among many benefits of effective management of MD.

Table 1. Predicted mean annual medical expenditures (US $) associated with MD among adults with diabetes (n=553,613).

Expenditure	With MD		Without MD		Excess with MD		Expenditure ratio: MD to without MD
	Mean	SE	Mean	SE	Mean	SE	
Outpatient	9723	128	6655	18	3068	128	1.46
Inpatient	6712	199	3671	23	3041	198	1.83
Drug	6640	52	3837	6	2803	52	1.73
Total	23075	242	14163	30	8912	241	1.63

SEs are bootstrap standard errors with 100 replications. All means are significantly greater for diabetic persons with MD than for those without MD (p<0.001).

TYPE 2 DIABETES PREVENTION, COMPLICATIONS, AND MEDICATIONS

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Impact of Alternative Prevention Policies on Future Diabetes Prevalence in the United States

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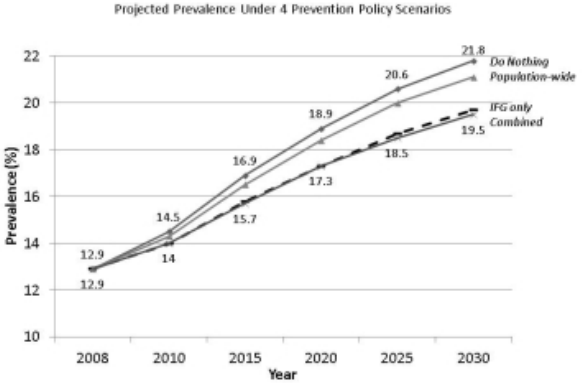
Diabetes is common, costly and has increasing prevalence in the United States. However, questions remain about what impact various prevention scenarios will have on diabetes incidence and prevalence. We developed a dynamic model that considers five diabetes status states and incorporates national data on prevalence, incidence, migration, and mortality. We estimated the long-term impact of four prevention scenarios: 1) Do nothing; 2) A "moderate" risk strategy, in which only adults with impaired fasting glucose (IFG) (FPG ≥ 100, 26% of the population) are given structured lifestyle intervention; 3) A "population" strategy in which the entire population is exposed to broad policies aimed at reducing obesity; 4) A "combined" strategy which combines both the moderate risk and population-wide strategies. We assume that the moderate risk approach achieves a 25% reduction in annual diabetes incidence among the sub-population identified and the population-wide approach reduces annual diabetes incidence by 4%. Compared to the "do nothing" strategy (21.8% prevalence, 58.4 million cases in 2030), the moderate risk strategy results in a 2.2 percentage point lower diabetes prevalence (19.6%) and reduces the number of prevalent cases by 5.7 million (52.7 million) in 2030. The population-wide strategy had a smaller effect, reducing prevalence by 0.7 percentage points and 1.7 million cases in 2030. The combined strategy results in a slightly greater reduction (-2.3 percentage points and 6.1 million fewer cases) than the moderate risk strategy. Organized prevention policies can prevent millions of prevalent cases. However, future diabetes prevalence remains high even under optimal intervention. Demand for management, treatment and prevention of diabetes complications and disability will continue to grow, along with the need for primary prevention resources.



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Time-Varying Incidence of Cancer after the Onset of Type 2 Diabetes: Evidence of Potential Surveillance Bias

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We examined the risk of site-specific cancers in people with incident type 2 diabetes, during different time windows following diabetes index.

Using linked health databases from British Columbia, Canada, we identified incident diabetes and non-diabetes cohorts, matched on age, sex, and index year (1996-2006). Following a minimum two-year cancer washout period, first cancers were identified prospectively in both cohorts. Cox regression was used to estimate the hazard ratio (HR) and 95% confidence intervals (CI) for incident cancer during early and late time windows adjusting for age, sex, socioeconomic status, and frequency of physician visits in the two years prior to cohort index.

Among the 185,100 subjects in each cohort, the mean (SD) age was 60.7 (13.5) years and 54% were men. Within 3 months following index, subjects with diabetes had significantly increased risks of colorectal (HR: 2.89, 95%CI: 2.22-3.75), lung (HR: 2.70, 95%CI: 2.09-3.47), liver (HR: 2.53, 95%CI: 1.93-3.31), endometrial (HR: 2.43, 95%CI: 1.26-4.68), pancreatic (HR: 13.84, 95%CI: 7.49-25.58), and prostate (HR: 1.98, 95%CI: 1.57-2.48) cancers. The risks of incident cancers were greatest for those with the fewest number of previous physician visits. After the initial 3-month period, colorectal (HR: 1.15, 95%CI: 1.05-1.25), liver (HR: 2.53, 95%CI: 1.93-3.31), and endometrial (HR: 1.58, 95%CI: 1.28-1.94) cancers remained significantly elevated compared to those without diabetes. The diabetes cohort remained at increased risk of pancreatic cancer in later years, but followed a different pattern: HR 2.94 (95%CI: 2.00-4.33) at 1-2 years, 1.78 (95%CI: 1.14-2.77) at 2-3 years, and 1.65 (95%CI: 1.28-2.13) at 3-10 years. After the initial period of elevated risk, men with type 2 diabetes had a decreased risk of prostate cancer (HR: 0.82, 95%CI: 0.76-0.88).

People with type 2 diabetes are at increased risk of select cancers; this risk is particularly elevated at the time of diabetes onset, which is likely due to increased surveillance. Further evaluations of these patterns are warranted to address issues related to surveillance bias and reverse causality.

Supported by: CIHR reference #MOP=82737 & #OTG=88588

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Domain-Specific Cognitive Function and Fine Motor Speed in Women 65 Years and Older with Type 2 Diabetes Mellitus: Results from the Women's Health Initiative Study of Cognitive Aging

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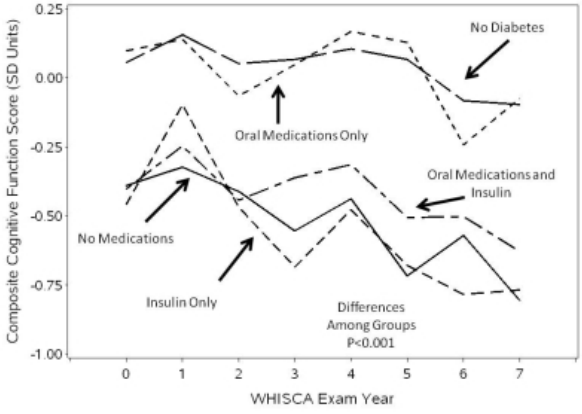
We examined whether Type 2 diabetes mellitus (T2DM) was associated with accelerated decline in global cognitive function, six domain-specific measures of cognitive function, and fine motor speed. Women aged 65-80 years who were enrolled in a clinical trial of postmenopausal hormone therapy were grouped as having T2DM (N=179) or not (N=1,984) and followed with annual standardized assessments of domain-specific cognitive function for an average of 5 years. Mean patterns of cognitive measures over time were contrasted between groups using general linear models and Wald tests, with varying levels of covariate adjustment. The influences of age at onset, use of oral medications, and use of insulin were also examined. T2DM was associated with mean deficits of 0.2 to 0.4 standard deviations across follow-up in most cognitive domains.



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Consistent evidence that rates of decline were accelerated among women with T2DM was evident only for verbal knowledge and verbal memory ($p<0.05$). Decrements in fine motor speed, but no measure of cognitive function, were greater for women with earlier onset T2DM. Among women with T2DM, treatment with oral diabetes medications alone was associated with better overall cognitive function, with scores similar to those for women with no T2DM. In sum, T2DM was associated with lower cognitive performance in most domains. Group differences in rates of change suggest that relative deficits in verbal knowledge and verbal memory may continue to increase after deficits in other domains have stabilized.

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Long Term Tolerability and Safety of Metformin in IGT Participants in the Diabetes Prevention Program (DPP) and Its Outcomes Study (DPPOS)

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The DPP and DPPOS reported benefits of metformin (MET) compared to placebo (PLA) in the prevention of type 2 diabetes. We also assessed the long-term tolerability and safety of MET as used in participants with IGT in the double-blind placebo-controlled DPP (average 3.2 years follow-up) and through the open-label DPPOS (additional 6 years follow-up). During DPP, all adverse events (AEs) were collected, and because of reports that MET may be associated with vitamin B12 deficiency, hemoglobin (Hgb) and hematocrit (Hct) were measured annually in both groups. MET was well tolerated during DPP and DPPOS; no safety issues were identified. During DPP, self-reported GI problems and GI symptoms attributed to study medication were more common in MET than PLA participants (average 28% vs. 16%, $p=0.01$ and 9.5% vs. 1.1%, $p<0.001$, respectively). Both GI problems and GI symptoms attributed to study medication declined over time, and GI problems were similar between groups by the latter years of DPPOS. Non-serious AEs for hypoglycemia and anemia during DPP were similar in MET and PLA (hypoglycemia: 7 participants in MET and 8 in PLA; anemia: 50 in MET and 38 in PLA). Throughout DPP and DPPOS, only 2 MET and 1 PLA participant had a serious AE for anemia and no serious AEs were reported for lactic acidosis or hypoglycemia during nearly 18,000 patient-years. During DPP, average Hgb and Hct levels were slightly lower in the MET group than in the PLA group (Hgb: 13.6 vs. 13.8; Hct: 40.6 vs. 41.1; $p<0.001$ for both), and the percent of participants with low Hgb and Hct was higher in MET than PLA (Hgb: 11.2% vs. 7.6%, $p=0.17$; Hct: 12.6% vs. 8.4, $p=0.035$). Among MET participants, changes in Hgb and Hct occurred during the first year following randomization, with no further changes observed over time. We conclude that MET used in individuals with IGT is safe and well tolerated over many years. The slight decrease of Hb/Hct during MET use is of uncertain significance and unlikely to be of clinical consequence, although it may require further evaluation in MET users.

Supported by: NIH

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Medication Use in Elderly US Adults with Diabetes and the Potential Practice Ramifications of Raising the Glycemic Target, NHANES 2003–08

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The optimal strategy for glycemic control in elderly adults with diabetes is uncertain, because of competing risks from non-diabetic conditions and higher risk of adverse drug effects and polypharmacy. We sought to characterize drug usage by diabetic elders in the US and to quantify the number who might benefit from reduced diabetes drug exposure if the target A1c were increased from 7% to 8% in this age group. We analyzed data from NHANES 2003-2008, a nationally representative sample of US adults. Analyses were weighted to represent the US population and to account for the complex survey design. A total of 756 adults 65 yrs and older with diagnosed diabetes were included in the analysis, representing 6.4 million individuals. Mean age was 73 (SE 0.3), 46% were men, and 12% were non-Hispanic blacks. Mean HbA1c was 6.8% (SE 0.06). More than 80% of elders were treated with diabetes medications: 39% with sulfonylureas, 37% with metformin, 19% with thiazolidinediones (TZDs), and 17% with insulin. Most elders also used multiple non-diabetes prescription medications, with mean of 5.4 (SE 0.2) for total numbers of medications. Despite contraindications, 2% of elders used metformin (despite renal dysfunction or age ≥80), and 3.4% used a TZD (despite heart failure). The Table displays medication use by HbA1c level. If the glycemic target for diabetic elders were less than 8%, rather than less than 7%, then about 50% to 67% (corresponding to bold/shaded area in table) of diabetic elders (3.1 to 4.3 million adults) might be able to simplify their diabetic regimen and thereby reduce the potential for adverse effects of polypharmacy in general and non-metformin diabetes drugs in particular. A trial that demonstrates that the A1c target in diabetic elders could be safely moved to 8% could have a substantial effect on clinical practice in the US.

HbA1c, %	N of Diabetes Medications				
	None	1 drug		2 or more drugs	Total
		metformin	non-metformin		
<7.0	13.5%	**7.5%**	**21.5%**	**20.9%**	63.4%
7.0 to 7.9	3.2%	3.5%	**5.3%**	**11.9%**	23.9%
>=8.0	1.8%	2.0%	2.7%	6.3%	12.8%
Total	18.4%	12.9%	29.6%	39.2%	100%

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Hemoglobin A1c and Patient-Reported Limitations Due to Urinary Incontinence in Older Women with Diabetes: The Diabetes and Aging Study

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Previous studies have found no association between Hemoglobin A1c and the presence of urinary incontinence. It is unknown whether higher A1c is associated with more severe incontinence-related limitations among women already living with incontinence.

We examined the cross-sectional relationship between A1c and patient-reported limitations due to incontinence in an ethnically stratified random sample of women from the Northern California Kaiser Diabetes Registry who responded to the DISTANCE study survey (response rate=62%) and reported urinary incontinence (n=3916). Mean A1c for 12 months before the survey was obtained from the medical record. Limitations due to incontinence were determined by asking "During the past 12 months, how much did the leakage of urine affect your day-to-day activities?" Multivariate ordinal logistic regression was used to determine the predicted probability of limitations across A1c levels.

Average age was 59 (±10) years with an A1c of 7.5 (±1.5). Women who had higher A1c (p for trend=0.04), more comorbidities (p for trend<0.001) and a longer duration of diabetes (p for trend<0.001) reported more severe limitations due to incontinence. Multivariate adjustment did not substantially alter our results: Women with poor control (A1c= 9+) were likely to report more severe limitations due to incontinence than women with excellent control (A1c <6) (p=0.02).

In women with diabetes and urinary incontinence, higher A1c levels were associated with patient reports of more severe limitations due to incontinence.

Predicted Probabilities of Patient-Reported Limitations due to Incontinence by Hemoglobin A1c

A1c (%)	No Limitations (95% CI)	Slight Limitations (95% CI)	Moderate Limitations (95% CI)	Quite a Bit or Extreme Limitations (95% CI)	p-value
<6	49 (41-58)	33 (29-37)	10 (7-12)	8 (5-11)	Ref
6-6.9	40 (37-44)	36 (33-39)	12 (10-14)	11 (9-13)	0.06
7-7.9	45 (40-49)	35 (32-38)	11 (9-13)	10 (8-12)	0.36
8-8.9	43 (37-50)	35 (32-38)	11 (9-14)	10 (7-13)	0.29
9+	37 (31-43)	37 (34-39)	14 (11-16)	13 (10-16)	0.02

Adjusted for age, ethnicity, education, income, parity, diabetes duration, diabetes treatment, comorbid conditions, and BMI

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Rotating Night Shift Work and the Risk of Type 2 Diabetes in Women

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Rotating night shift work disrupts circadian rhythms, and has been associated with obesity, metabolic syndrome, and glucose dysregulation. However, very few studies have prospectively examined the relationship between shift work and type 2 diabetes mellitus (T2DM). We aimed to evaluate this association within two large cohorts: the Nurses' Health Study (NHS) I and II. We followed 69269 women aged 42-67 from 1988 to 2008 in NHS I, and 107915 women aged 25-42 from 1989 to 2007 in NHS II. At baseline, participants were asked how

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long they had worked rotating night shifts (defined as at least 3 nights/month in addition to days and evenings in that month), with responses in 7 prespecified categories: never, 1-2, 3-5, 6-9, 10-14, 15-19, ≥20 years. The information was updated every 2-4 years in NHS II, but queried only at baseline in NHS I. Self-reported T2DM was confirmed by medical record. We used Cox proportional hazards models to calculate hazard ratios (HRs) and 95% confidence intervals (CIs), adjusting for potential confounders. A total of 6165 (NHS I) and 3961 (NHS II) incident T2DM cases were documented. In the age-adjusted models, duration of shift work was monotonically associated with an increased risk of T2DM in both cohorts (P_{trend} <0.001), and this association was slightly attenuated after controlling for other covariates except body mass index (BMI). Further adjustment for BMI largely attenuated the association, and the HRs (95%CIs) for participants with 1-2, 3-9, 10-19, and ≥20 years of shift work were 1.00 (0.94-1.07), 1.06 (0.99-1.13), 1.09 (1.00-1.20), and 1.20 (1.08-1.34) in NHS I, as well as 1.04 (0.96-1.14), 1.05 (0.96-1.13), 1.10 (0.98-1.24), and 1.34 (1.02-1.76) in NHS II, compared with women who reported no shift work. In the model without BMI, every 5-year increase of shift work was associated with an 11% (9%-14%) and 17% (13%-21%) increased risk of T2DM in NHS I and II, respectively, and this estimate was reduced to 5% (2%-7%) and 4% (0%-8%) after adjustment for BMI. In conclusion, our results suggest that women have a modestly increased risk of T2DM after extended period of shift work, and this association appears to be largely mediated through BMI.

258-OR

Dynamics of Body Mass Index from Adolescence to Adulthood and Risk of Diabetes Versus Coronary Heart Disease

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The relationship between changes in body mass index (BMI) from adolescence to adulthood and the risk of obesity-related diseases in young adulthood is unclear.

Apparently healthy 37,674 young men were followed prospectively for incidence of angiography-proven CHD and type 2 diabetes through the Israeli-army examination center.

Height and weight were measured at age-17 and repeatedly during adulthood.

Mean age-17 (adolescence) BMI ranged between 17.3kg/m^2 (first decile) to 27.6kg/m^2 (top decile). During ~650,000 person-years of follow-up (17.4 years) we documented 1,173 incident cases of type 2 diabetes and 327 incident cases of CHD. In a multivariate model adjusted for age, family history, blood pressure, life-style parameters, and blood biomarkers, increased adolescence BMI was a significant predictor of diabetes (HR=2.76;95%CI:2.11-3.58; extreme deciles), and angiography-proven CHD (HR=5.43;95%CI:2.77-10.62; extreme deciles). Further adjustment to BMI at early adulthood completely attenuated the association between adolescence BMI and diabetes (HR=1.01;95%CI:0.75-1.37), but not with CHD (HR=6.85;95%CI:3.30-14.21). Consistent were findings with adjusting both BMI values as continuous predictor variables (Diabetes: $\beta_{BMI\text{-}17}$=1.05;p=0.28, $\beta_{BMI\text{-}30}$=1.12;p=0.003, p-interaction=0.885; CHD: $\beta_{BMI\text{-}17}$=1.36;p=0.004, $\beta_{BMI\text{-}30}$=1.21;p=0.029, p-interaction=0.048). In a multivariate model, adjusted for age-17 BMI, each incremental unit of BMI *gain* between adolescence and adulthood was associated with 8% increase in diabetes risk (HR=1.08 (95%CI;1.06-1.11) but not with CHD (HR=1.01(95%CI;0.97-1.05)).

In conclusion, diabetes risk mainly associates with more recent weight gain and with increased BMI closer to the time of diagnosis, whereas a longer 'BMI memory' exists for CHD risk. Increased BMI at adolescence, well within the currently-considered normal range, constitutes a significant risk factor for CHD at early adulthood.

Supported by: The Talpiot Medical Leadership Program, Chaim Sheba Medical Center

MECHANISMS AND TREATMENT OF TYPE 1 DIABETES

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Lymphocyte Infiltration in Pancreatic Islets Mediates β-Cell Proliferation in NOD Mice

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Type 1 Diabetes (T1D), is a T-cell mediated disease characterized by selective destruction of β-cells. While leukocytes are thought to negatively impact beta-cells by promoting their death, recent data argue they may enhance their replication. To identify the immune cell-type(s), and dissect the mechanism(s) promoting β-cell replication in the NOD mouse model of T1D, we performed adoptive transfer of splenocytes derived either from 20 week-old diabetic or 8 week-old pre-diabetic female NOD mice into 6 week-old NODRag1$^{-/-}$ female mice (n= 9-25). We observed higher β-cell proliferation (BrdU, Ki67 or pHH3) in NOD.RAG1$^{-/-}$ mice that received splenocytes from diabetic mice (pre-diabetic, 0.46±0.09; diabetic, 2.2±0.29%; p<0.001) and were independent of blood glucose (94±12 vs 96±6 mg/dl) or apoptosis. Liver and adipose tissues were free of immune-cells indicating pancreas-specific infiltration. To assess the role of B- versus T-cells we used *in vitro* and *in vivo* depletion approaches. Transfer after negative selection for B-cells showed high proliferation of β-cells, suggesting a B-cell independent process (n=6-9). To evaluate the relative importance of the T-cell compartments we depleted donor splenocytes of CD4+ or CD8+ cells via magnetic sorting and/or monoclonal antibodies. NOD.RAG1$^{-/-}$ mice receiving CD8+ cell-depleted splenocytes showed a 2-fold increase in β-cell proliferation compared with CD4+ cell-depleted splenocyte recipients (CD4+, 0.14±0.09; CD8+, 0.3±0.1%; n=3-6). Finally, we examined the direct impact of infiltration in NOD mice given streptozotocin (100 mg/kg/BW; n=3/day up to 7 days). β-cell proliferation occurred soon after immune-cell infiltration, before the onset of diabetes and was independent of glucose/insulin (Correlation between single islet proliferation and insulitis, R^2=0.74). Our results indicate that a subtype(s) of T- but not B-cells, has a dominant effect in the initiation phase of T1D and potentially stimulates β-cell proliferation. Identifying the cell type and dissecting the mechanism(s) underlying the replication would be an important advance in treating T1D.

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Zinc Transporter-8 Autoantibodies (ZnT8A) Increase Type 1 Diabetes (T1D) Risk in Single Autoantibody Positive Relatives

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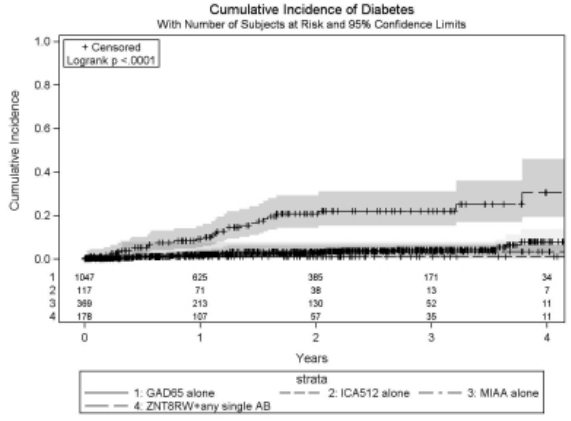
The risk of T1D in relatives of patients with TID who are positive for ZnT8A and one other biochemical autoantibody (Ab) is unclear. In the TrialNet Natural History Study, we assessed T1D risk by ZnT8A in a large cohort screened for Abs to insulin (mIAA), ICA-512 (ICA-512A), and glutamic acid decarboxylase (GADA). Baseline samples from 2293 Ab positive relatives and 811 randomly chosen Ab negative relatives were tested for ZnT8A by a fluid phase radioassay using construct JH5.2. Ab positive relatives underwent biannual oral glucose tolerance tests. ZnT8A were found in 8/811(1%) Ab negative, 178/1716 (10%) single Ab positive, and 373/577 (65%) multiple Ab positive relatives. ZnT8A positivity rates in single Ab positive relatives were: mIAA, 19/388 (5%); GADA, 109/1156 (9%); and ICA-512A 50/167 (30%). The figure shows the cumulative diabetes incidence in single Ab positive relatives by ZnT8A. ZnT8A positivity raised diabetes risk (4 yr. estimate [95% CLs] 31% [19,46%] vs. 7% [4,11%] if ZnT8A negative, p < 0.0001). In relatives positive for ZnT8A and one other Ab, the 3 year diabetes risk differed according to the other Ab (GADA, 9%/3 yrs; mIAA, 41%/3 yrs; ICA-512A, 45%/3 yrs; p = 0.0002). We conclude that isolated ZnT8A positivity is rare in T1D relatives but ZnT8A occur in 10% who have one other biochemical Ab (a group comprising ~ 70% of all Ab positive relatives). ZnT8A identify relatives at much higher T1D risk. Secondary ZnT8A testing in relatives positive for one other biochemical Ab better defines single and multiple Ab positivity and can be used to adjust the intensity of follow-up in Ab positive participants in T1D prediction studies.



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For author disclosure information, see page 785.

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Novel Inflammatory Pathways Linked to Type 1 Diabetes Development

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Type 1 Diabetes (T1D) is an autoimmune disease resulting from the immune-mediated destruction of insulin producing beta (β) cells in the pancreatic islets. The inflammatory pathways causing β-cell damage are not known. One candidate pathway involves metabolism of arachidonic acid to 12-hydroxyeicosatetraenoic acid (12-HETE) via 12/15-lipoxygenase (12/15-LO). We previously showed that female congenic NOD mice deficient in 12/15 LO (NOD-Alox15^{null}) are almost completely protected from T1D development. However, the mechanism linking 12/15LO to T1D is unknown. 12/15-LO action generates IL-12, and IL-12 in turn leads to T cell activation and phosphorylation of signal transducers and activators of transcription 4 (STAT4). Phosphorylated STAT4 contributes to the production and activation of proinflammatory cytokines involved in T1D autoimmunity. The goal of these studies was to characterize the IL-12 axis in order to understand its role in subsequent β-cell damage. Islets from 8 wk old female NOD and NOD-Alox15^{null} mice (n=8/group), islets from human T1D (n=3), and human pancreatic tissues from the Network for Pancreatic Organ Donors with Diabetes (nPOD) were used to determine the expression of mediators in the 12/15-LO pathway within islets. Results of a Th17 PCR array showed that IL-12 increased 3-fold and STAT4 increased >100 fold in the NOD versus NOD-*Alox15*^{null} mice islets, suggesting that deletion of 12/15-LO decreased expression of the IL-12 axis pathway. Also, IL-12 and its receptor subunits, IL-12RB1 and IL-12RB2, are highly expressed in NOD mice islets. IL-22 and its receptor, IL-22R, were also detected in the islets of NOD mice at significantly higher levels than NOD-*Alox15*^{null} mice. Islets from human T1D showed a 4-fold increase in STAT4, a 12-fold increase in IL-12RB2, a 2-fold increase in IL-12RB1, and a 4-fold increase in IL-12p35 over normal controls. IL-12RB2 staining of pancreatic tissues from nPOD showed distinct differences between normal, auto-antibody$^+$, and T1D patients. In auto-antibody$^+$ and T1D, IL-12B2 staining co-localized with islets. These data suggest that the IL-12 axis may be a promising therapeutic target to prevent β-cell damage in T1D.

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Non-Genetically Determined Factors as Additive Predictors of Type 1 Diabetes: A Twin and Population Study

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Type 1 diabetes mellitus is caused by destructive innate and adaptive immune responses. Serum diabetes-associated autoantibodies, to glutamic acid decarboxylase (GADA), insulinoma-associated antigen-2 (IA-2A) and zinc transporter-8 (ZnT8A), reflect adaptive immunity, while carboxy-methyl-lysine (CML), an advanced glycation end-product, is associated with proinflammation and innate immunity. We assessed whether genetic factors determine serum CML levels and autoantibodies in type 1 diabetes.

Serum CML and autoantibodies were determined in two prospective studies: a classical twin study of twins discordant for type 1 diabetes (32 monozygotic (MZ), 32 dizygotic (DZ) pairs) and a population study of 7,287 normal subjects. CML levels were determined by ELISA, autoantibodies by radio-immunoprecipitation, HLA class II genotyping by sequence-specific oligonucleotides.

CML levels were increased in diabetic and non-diabetic twins, population-based autoantibody positive (n=115) and pre-diabetic (n=33) children (all $p<0.001$). Twin CML correlations (r) were strong, irrespective of zygosity: model-fitting showed familial shared environmental, factors explained 66% variance. Autoantibodies were more frequent in diabetic than non-diabetic twins ($p<0.001$) and non-shared environment explained all variance. Baseline HLA, CML and autoantibodies, predicted diabetes, the latter two additively and quantitatively, with CML the major single predictor; during the prediabetic period, CML remained abnormal ($p<0.002$) and autoantibodies persisted.

Environmental, factors were strong determinants of two diabetes-associated features, which predicted type 1 diabetes additively and quantitatively. We postulate that distinct, additive, environmental events are associated with the natural history of type 1 diabetes. The familial environmental origin of the novel, potent predictor, serum CML, could have therapeutic implications.

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Thymic Dominant Tolerance of Type 1 Diabetes: Essential Roles of Insulin Expression in Bone Marrow-Derived Antigen Presenting Cells in Maintaining Peripheral Self-Tolerance to Islet Beta-Cells, but Not for Central Negative Selection

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Extrapancreatic insulin expression in subsets of antigen presenting cells (APCs) within the stroma of immune organs has been implicated in modulating immune tolerance towards pancreatic beta cells. While the indispensable roles of insulin expression in medullary epithelial cells of the thymus (mTECs) in establishing central tolerance of beta cells was demonstrated conclusively, the immunologic function of insulin production in APCs of bone marrow (BM) origin remains elusive.

To clarify their potential tolerogenic role, we have generated a novel animal model in which insulin expression was abrogated in all the hematopoietic compartments (Vav-DIns mice). Vav-DIns mice remain euglycemic throughout life, displaying no signs of glucose intolerance or defective islet function. Immunohistochemical analysis of pancreata revealed healthy insulin-producing islets with no immune cell infiltration. In an effort to further elucidate the potential roles of BM-derived insulin, we generated Aire-Cre:RosaYFP mice, in which all Aire-expressing cells were labeled with the yellow fluorescent protein (YFP), and subsequently identified the insulin-expressing APCs as a population of Aire-expressing, CD11c^{int}B220$^+$ plasmocytoid dendritic cells (pDCs). To investigate their role under defective central negative selection conditions, animals with insulin deletion specifically in CD11c+ DCs were generated (CD11c-DIns mice) and crossed to B6.H2g7 mice to carry the diabetes-prone MHC alleles (H2g7). Although CD11c-DIns-H2g7 mice can effectively maintain glucose homeostasis, increased levels of both CD4+ and CD8+ T cell infiltration were observed in the pancreas, implicating a partial breakdown of peripheral tolerogenic mechanisms to beta cells. While insulin expression in mTECs is essential to establish a self-tolerant T cell repertoire to pancreatic beta cells, its production in BM-derived CD11c^{Int}B220$^+$Aire$^+$ tolerogenic pDCs might play essential maintenance roles to prevent uncontrolled activation and clonal expansion of islet-specific autoreactive T cells in the periphery.

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Tim4 Targeting in Allo- and Autoimmune Anti-Islet Response

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Tim1-Tim4 is a new costimulatory pathway, which has been related to Th1 and Th17 activation and Tregs inhibition. Targeting TIM1 promotes a regulatory network in the contest of allogenic heart transplantation. We now tested the use of a new anti-TIM4 antibodies (RMT4-53) in the context of alloimmune (BALB/c islets transplanted under the kidney capsule of C57BL/6 mice rendered diabetic trough streptozotocin-1), autoimmune (diabetes prevention in NOD mice, a model of autoimmune diabetes-2) and mixed allo- and autoimmune anti islet response (BALB/c islet transplanted in diabetic NOD mice-3).

(1) In the alloimmune model, TIM4 was found expressed on Dendritic cells (DCs) with significant downregulation during rejection (TIM4$^+$ DCs day 0: 11.1 ± 0.7%; day 14: 0.7 ± 0.1%; n=3, $p<0.01$); in this model the use of RMT4-53 (500ug day 0, 250ug day 2,4,6,8,10) significantly increased islet graft survival (MST ctrl: 12 days; RMT4-53 treated: 21 days, n=5, $p<0.05$ vs. ctrl); the ELISPOT assay at day 14 revealed increased Th2 and reduced Th1 response. Differently, in a context of absent Th1 response (BALB/c islet transplanted into Tbet$^{-/-}$ C57BL/6 mice), the delay in islet rejection produced by inactivation of the Th1 response (indefinite survival in 40% of the mice) was reversed by the use of RMT4-53 (MST Tbet$^{-/-}$ RMT4-53 treated: 10 days, n=5, $p<0.05$ vs. Tbet$^{-/-}$), probably due to increased Th2 response. (2) In autoimmune anti-islet response 10wks old mice were treated with RMT4-53 or left untreated. No difference was seen in the 2 groups. (3) Finally, we tested the efficacy of anti-TIM treatment in allo- and autoimmune anti-islet response. The use of RMT4-53 alone did not have any effect; however the combination with Rapamycin (1mg/kg day 1 to 10) suggested a possible synergistic effect between the two compounds (MST Rapamycin: 19 days n=5; RMT4-53+ Rapamycin: 50 days, n=3, p=ns).

From these data we can conclude that targeting the Tim1-Tim4 axis is effective in downregulating alloimmune anti-islet response, while it has no effect on autoimmune anti-islet response. Finally, Rapamycin synergizes with Tim1-Tim4 mAb in downregulating anti-islet response.

For author disclosure information, see page 785.

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Multi-Drug Combination Therapy Reverses Diabetes in NOD Mice with Established Disease

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Combination therapy with drugs selected given their potential for mechanistic synergy offers the most promising approach to preventing and reversing T1D. We tested the hypothesis that a 4 drug combination, using agents that would (1) attenuate autoimmunity (anti-thymocyte globulin [ATG], and granulocyte colony stimulating factor [GCSF]), and (2) enhance islet regeneration and/or preservation (dipeptidyl peptidase-4 inhibitor [DPP-4i] and a proton pump inhibitor [PPI]), would improve T1D reversal in NOD mice with new onset and established disease. In this study, female NOD mice were treated with drug combinations either at onset or 14 days post onset (established disease). All mice received one insulin pellet at onset, which was sufficient to maintain euglycemia for 14 days. New onset and established mice were each divided into 4 treatment arms: vehicle only (n=12), ATG+GCSF (n=12), DPP-4i+PPI (n=12), ATG+GCSF+DPP-4i+PPI (n=12-13). The drug regimen consisted of 2 doses of ATG (day 0 and day 3) (250μg/dose), 2 doses of GCSF (day 0 and day 15) (120μg/dose), 84 days of DPP-4i (200μg/dose/day), and/or 84 days of PPI (600μg/dose, 2 doses/day). Diabetes reversal was considered successful in the absence of hyperglycemia 120 days post onset. All animals treated with insulin plus vehicle eventually reverted to diabetes. In new onset mice, 50% of ATG+GCSF, 58% of DPP-4i+PPI, and 84% of ATG+GCSF+DPP-4i+PPI (P<0.0001) treated mice maintained euglycemia through 120 days post treatment. Surprisingly, in animals with established disease, 33% of ATG+GCSF, 42% of DPP-4i+PPI, and 50% of ATG+GCSF+DPP-4i+PPI (P<0.0001) treated mice remained euglycemic to the 120 day end point. Multi-drug combination therapies with approaches designed to attenuate autoimmunity while preserving and possibly regenerating beta cells may lead to reversal of T1D, both at disease onset and in established disease.

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A Subset of Highly Affinity Maturated Regulatory B-Cells Prevents the Onset of Diabetes in NOD Mice

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B-cells play a central role in the pathogenesis of type 1 diabetes (T1D). For instance, B-cell-depletion prevents the onset of T1D in NOD mice. This effect was linked to the emergence of B-cell subsets with immunoregulatory features.

We characterized the pancreatic B-cell repertoire in the subpopulation of female NOD mice that is naturally protected from T1D. We hypothesized that a pool of IL10-producing regulatory B-cells (Bregs) mediates protection against autoimmune destruction of pancreatic islets in these mice. Long-term normoglycemic (Lnglc) NOD mice exhibited reduced lymphoid infiltration of islets and preserved islet morphology compared to prediabetic (Nglc) and hyperglycemic (Hglc) NOD mice. Immunoglobulin heavy chain variable region (IgV_H) libraries were constructed from intra-islet B-cell repertoires of Nglc, Hglc and Lnglc NOD mice and 415 sequences were analyzed for affinity maturation and clonal expansion. Comparison of the IgV_H transcripts to the germline revealed significantly higher amino acid mutation frequencies in Lnglc (6.2±0.1) compared to Hglc NOD mice (3.0±0.3, P<0.001). Analysis of IgV_H libraries from Lnglc mice identified 41.0±3.6 clones per 100 sequences compared to 58.3±7.7 within libraries from Hglc animals. Interestingly, the clonal population of Lngl mice demonstrated higher percentages of clonal variants and intraclonal isotype switch compared to Hglc mice. Thus, islet-harbored B-cells of Lnglc NOD mice proved to be highly affinity maturated and clonally expanded towards the auto Ag, yet, failed to promote destruction of islets. Phenotypical analysis revealed significantly higher frequencies of $B220^+IL10^+$cells (Bregs) in the pancreas of Lnglc (4.9%±1.8) vs. Hglc mice (1.2%±0.2, P<0.05). Similarly, increased frequencies of anergic B-cells ($B220^+CD93^+CD23^+IgM^{low}$) were seen in the pancreata of Lnglc (2.1±0.2%) compared to Hglc animals (0.1% ±0.1, P<0.001). We suggest that pancreas-specific subsets of highly maturated regulatory and apoptotic B-cells protect NOD mice from the onset of diabetes. Expansion of Bregs within the pancreas could induce tolerance towards autoimmune destruction of pancreatic islets in patients with T1D.

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ATHEROSCLEROTIC CARDIOVASCULAR DISEASE AND HUMAN TYPE 1 DIABETES

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Loss of Female Cardiovascular Protection in Type 1 Diabetes May Begin in Adolescence

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A four-fold increased risk of cardiovascular disease (CVD) and the loss of premenopausal CVD protection in women with Type 1 Diabetes (T1D) are well documented, but it is unknown how early in life these risks begin. This study examined gender differences in CVD risk factors among adolescents with T1D and without diabetes (Non-DM).

Study participants were youth with T1D (age 15 ± 2 yrs, duration 9 ± 3 yrs) and Non-DM (age 15 ± 2 yrs). Tanner stage of puberty was determined by physician (n=350) or self-report (n=52). Examined risk markers included hemoglobin A1c (HbA1c), LDL-cholesterol, systolic blood pressure (SBP), body mass index (BMI), and C-reactive protein (CRP).

Girls with T1D had higher levels of all age- and Tanner stage-adjusted CVD risk factors when compared to Non-DM girls, and had higher CRP, LDL, HbA1c and BMI compared to T1D boys. Boys with T1D had similar CVD risk factors compared with Non-DM boys. When further adjusted for BMI, differences in all risk factors for girls with T1D compared to Non-DM girls remained significant. Increased CRP and LDL in girls compared to boys with T1D remained when further adjusted for BMI and HbA1c. Additionally, T1D had a more detrimental effect in girls than in boys for LDL and SBP (interaction p-values, p=0.02 and p=0.05, respectively).

Least Square Means and 95% Confidence Intervals for CVD Risk Factors

	Boys		Girls	
CVD Risk Factor*	**T1D (n=152)**	**Non-DM (n=47)**	**T1D (n=150)**	**Non-DM (n=53)**
HbA1c (%)	8.7(8.5-8.9)	5.3(4.9-5.7)[d]	9.1(8.9-9.4)[a]	5.2(4.9-5.6)[d]
LDL (mg/dL)	82(78-86)	81(74-89)	95(90-99)[b]	80(73-87)[d]
SBP (mmHg)	115(113.6-116.0)	113(110.7-115.1)	111(110.2-112.7)[b]	106(104.3-108.5)[d]
BMI (kg/m²)	22.1(21.5-22.7)	21.6(20.6-22.6)	23.6(22.9-24.0)[b]	22.1(21.2-23.1)[c]
CRP¥ (mg/dL)	0.15(0.098-0.24)	0.07(0.03-0.16)	0.86(0.55-1.37)[b]	0.15(0.07-0.32)[d]

*Age and Tanner stage adjusted ¥Geometric Mean
a p≤ 0.05, b p≤0.001 for gender differences within T1D status
c p≤ 0.05, d p≤0.001 for differences by T1D status within gender

Striking gender differences in CVD risk factors among T1D patients are present in adolescence. This age may be a critical period for CVD prevention in girls with T1D.

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WITHDRAWN

For author disclosure information, see page 785.

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Relationship between Carotid Intima-Medial Thickness and Left Ventricular Mass in Type 1 Diabetes: Results from the Epidemiology of Diabetes Interventions and Complications (EDIC) Study

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While carotid intima-medial thickness (IMT) is a marker of generalized atherosclerosis and increased left ventricular (LV) mass is a recognized risk factor for future cardiovascular events, the relationship between carotid IMT and LV mass in type 1 diabetes (T1D) has not been established.

EDIC is a multicenter observational study designed as a follow up to the Diabetes Control and Complications Trial (DCCT). LV mass was measured in 2008 (EDIC year 15) with cardiac MRI (CMRI) and IMT was assessed using B-mode ultrasonography in 2005 (EDIC 12).

A total of 896 participants had both LV and IMT measures. Mean age was 49 years; mean T1D duration was 28 years; 52% were males. After adjusting for basic covariates (machine, reader, age and gender), a significant association between LV mass and common IMT (estimate 1.95 g/m^2 per 0.1 mm common IMT increment, $P < 0.0001$) was observed. The association between common IMT and LV mass was diminished but remained statistically significant after adjusting for basic covariates and cardiovascular risk factors (T1D duration, current smoker, mean blood pressure, LDL, and HbA1c). The association also remained after adjusting for an additional variable, macroalbuminuria (ever albumin excretion rate >300 mg/24 hour) (estimate 1.05 g/m^2 per 0.1 mm common IMT increment, $P < 0.001$).

Attained Age (years)	-0.22 ± 0.06	0.0020
Gender (Females vs. Males)	-10.29 ± 0.72	0.0020
Attained T1D Duration (years)	-0.25 ± 0.07	0.0085
Current Smoker (yes vs. no)	4.07 ± 1.09	0.0036
Mean systolic blood pressure (mmHg)	0.34 ± 0.05	0.0020
Mean LDL cholesterol (mg/dl)	-0.06 ± 0.02	0.0160
Mean HbA1c (%)	0.22 ± 0.38	0.9698
Ever albumin excretion rate ≥ 300 mg/24 hour (yes vs. no)	9.50 ± 1.68	0.0020
Common IMT (year 12) (0.1 mm)	**1.05 ± 0.28**	**0.0036**

*Model was adjusted for IMT machine and reader, and CMRI machine.

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Effect of Type 1 Diabetes on the Carotid Artery Thickness and Stiffness—The SEARCH CVD Study

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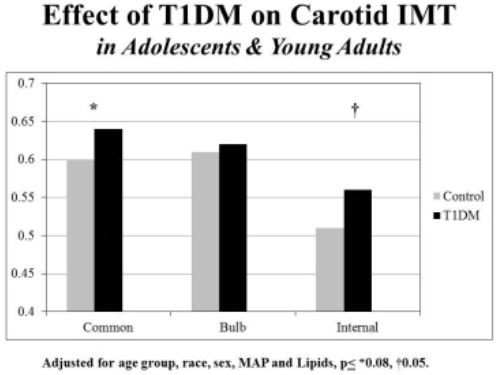
Increased carotid intima-media thickness (cIMT) is an independent risk factor for adverse cardiovascular (CV) events. cIMT increases with age and adults with diabetes mellitus have more rapid cIMT progression. Therefore, we examined cIMT to determine if age and type 1 diabetes (T1DM) had a similar effect on cIMT in adolescents and young adults. A total of 162 subjects (age 20 ± 3 years, 51% male, 56% Caucasian, 78% T1DM) were examined. Body mass index (BMI), mean arterial pressure by mercury (MAP), and fasting lipids and A1c were obtained. Ultrasound was used to measure cIMT with M-mode for calculation of Petersons Elastic Modulus (PEM). T-tests or Chi square were performed to evaluate differences in CV risk factors, cIMT and PEM among healthy controls (C) and T1DM. General linear models were constructed to determine if the differences remained after adjustment for covariates. There were more males and Caucasians in the T1DM group but they did not differ from controls in other covariates. After adjustment for age group (< or ≥20 years), race and sex, T1DM subjects had thicker internal cIMT ($p \leq 0.04$) with a trend for thicker common cIMT ($p \leq 0.09$). PEM was stiffer in T1DM among youth age ≥ 20 years ($p \leq 0.02$). These differences persisted after further adjustment for MAP and lipids (see figure) but only retained significance for PEM in older group after adjusting for BMI. We conclude that T1DM has an adverse effect on internal carotid thickness and common carotid stiffness that is measurable by the time patients reach young adulthood. Better glycemic control and reduction in CV risk factors especially obesity, in adolescents may improve CV outcomes in T1DM.



Supported by: NIH R01- DK078542

271-OR

The Relationship between Hemoglobin and CAD Differs by Haptoglobin Genotype in Type 1 Diabetes

TINA COSTACOU, TREVOR JOHN ORCHARD, *Pittsburgh, PA*

Haptoglobin (Hp) is a plasma protein that binds free hemoglobin, thereby inhibiting hemoglobin-induced oxidative damage. There are three major Hp genotypes in humans, Hp 1-1, Hp 2-1 and Hp 2-2 and we have previously shown an increased coronary artery disease (CAD) incidence among individuals with childhood-onset type 1 diabetes and either the Hp 2-1 or Hp 2-2 compared to those with the Hp 1-1 genotype. We thus examined whether hemoglobin relates to CAD incidence and if so, whether this association differs by Hp genotype in the same Epidemiology of Diabetes Complications (EDC) study cohort.

Participants from the EDC study who were free of CAD at study entry and had DNA available were selected (n=450; at study entry, mean age, 27 years and diabetes duration, 18.7 years). CAD was defined as EDC clinic physician diagnosed angina, ischemic electrocardiogram, myocardial infarction confirmed by Q-waves on electrocardiogram or hospital records, angiographic stenosis ≥50%, or revascularization.

During 18 years of follow-up, 15.1% of individuals with Hp 1-1 versus 31.5% of those with the Hp 2-1 or Hp 2-2 genotype developed CAD (p=0.01). There were no differences in age, age at diabetes onset or diabetes duration between individuals with the Hp 1-1 and those with the Hp 2-1 or Hp 2-2 genotype. Hemoglobin levels were similar between those with (15.0 g/dl) and without (15.2 g/dl) subsequent CAD. However, among those with the Hp 1-1 genotype, both hematocrit (p=0.05) and hemoglobin (p=0.02) were modestly increased in incident CAD cases. Conversely, both hematocrit (p=0.02) and hemoglobin (p=0.02) were decreased in incident CAD cases among those with the Hp 2-1 or the Hp 2-2 genotype. This interaction between Hp type and hemoglobin in relationship to CAD incidence was highly significant (p=0.006).

In this cohort of individuals with childhood onset type 1 diabetes, a strong interaction was observed between hemoglobin and the Hp genotype in terms of CAD incidence, which merits further exploration.

Supported by: NIH Grant DK34818

272-OR

Are Endothelial Progenitor Cells Protective Factors on the Development of Cardiovascular Disease in Type 1 Diabetic Patients?

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Identification of protective factors against diabetic complications is difficult as it requires a population of patients who have survived diabetes for extreme duration. We have characterized 600+ patients who have had T1DM for more than 50 years, the Joslin 50 Year Medalist study. The study shows that only 50% have advanced retinopathy, 26% have nephropathy, and 43% have cardiovascular disease (CVD=coronary artery disease and/or peripheral vascular disease). In Medalists, there is a significant correlation

For author disclosure information, see page 785.

of CVD with age, duration of disease, lower HDL, higher LPa, higher CRP, and renal dysfunction. Recently, we have analyzed the differences of circulating and endothelial progenitor cells (CPC and EPC) in the Medalists in comparison to age-matched controls and other T1DM and T2DM patients since CPC/EPC have been related to risk of developing CVD. We prospectively enrolled 88 Medalists, 49 non-DM controls, 14 T2DM age>50y, and 10 T1DM age<50y. EPC and CPC were quantified from peripheral blood by flow cytometry with EPC defined as positive for CD34/CD133/VEGFR2, and CPC identified as CD34/CD133 positive. Surprisingly, analysis of the results found similar levels of CPC and EPC between the Medalists and healthy age-matched controls, with CVD-free Medalists having even higher EPC than controls ($p<0.05$). CPC levels are reduced in T1DM and T2DM compared to controls ($p<0.05$) as previously observed. Medalists have significantly higher CPC levels than both T1DM and T2DM patients ($p=0.03$, $p=0.009$, respectively). In Medalists with CVD, the decrease in CPC/EPC is independent of age, duration of disease, HbA1c, BMI, blood pressure, eGFR ($p>0.1$). Increased EPC are associated with statin use. EPC/CPC levels are not associated with retinopathy or nephropathy. In summary, Medalists have EPC/CPC similar to a healthy aging population and higher than other diabetic patients. The novel finding of high EPC in Medalists without CVD compared to controls suggest that EPC/CPC could be protective for cardiovascular disease in diabetes and have therapeutic potential in lowering cardiovascular risk in diabetes.

Supported by: NIDDK, JDRF **ADA-Funded Research**

273-OR

Association between Red Blood Cell Distribution Width (RDW) and Macrovascular and Microvascular Complications in Diabetes

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Red blood cell distribution width (RDW) is a measure of variability in size of circulating erythrocytes obtained in standard automated complete blood counts. Increased RDW has been reported as a risk marker of morbidity and mortality for cardiovascular disease (CVD). However, no studies have investigated the relationship between RDW and diabetes complications. The aim of the present study was to evaluate RDW as a marker of macrovascular and microvascular complications in a nationally representative sample of the US adult diabetes population. A cross-sectional study was performed using the 1988-1994 data set from the National Health and Nutrition Examination Survey (NHANES III). The association between RDW quartiles and vascular complications was evaluated in 2,497 adults aged 20 years and older with diabetes (including type 1 and type 2), identified among 18,825 NHANES III participants. Logistic regression modeling was used to adjust for potential confounding. Higher RDW values related to older age, less education, current smoking and decreased hemoglobin levels. Increasing RDW quartiles were also related to non-Hispanic Black racial origin, obesity, systolic hypertension, nutritional deficiencies and CRP > 3 mg/l. The prevalence of subjects with HbA1c < 7% differed among RDW quartiles, but this did not follow a linear trend. Compared to the lowest RDW quartile, higher RDW values (3rd and 4th quartiles) were associated with increased adjusted risk of myocardial infarction (OR 4th quartile = 2.37 [95% CI 1.05-5.35]), heart failure (OR 4th quartile = 4.19 [95% CI 1.80-9.78]), stroke (OR 4th quartile = 3.03 [95% CI 1.33-6.92]) and nephropathy (OR 4th quartile = 2.32 [95% CI 1.39-3.89]). The risk of diabetic retinopathy was not significantly increased across RDW quartiles. In conclusion, higher RDW values are associated with an increased risk for CVD and nephropathy in a nationally representative sample of US adults with diabetes. RDW may represent an important clinical marker for diabetes complications independent of traditional risk factors and disease duration.

274-OR

Stem Cell Growth Factors in Patients with Late Autoimmune Diabetes of the Adults in Comparison to Type 1 or Type 2 Diabetes

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Late Autoimmune Diabetes of the Adults (LADA) is a unique form of diabetes. LADA is mostly disguised among type 2 diabetes (T2D) despite its common features with type 1 diabetes (T1D). T1D and T2D patients suffer from macro- and microangiopathic complications. In T1D and T2D diminished stem cells and their responsible growth factors have been demonstrated to be involved in the latter complications. We investigated the expression of stem cell growth factors in subjects with different diabetes entities but comparable glucose control.

Serum levels of Vascular Endothelial Growth Factor (VEGF), Angiogenin (ANG), Brain Derived Neurotrophic Factor (BDNF) and Interleukin-18 (IL-18) were measured by ELISA in 303 subjects: 40 LADA, 73 T1D, 100 T2D, and 90 age and gender matched healthy controls (CO). Controls and patients did not differ for age, gender, body-mass-index (BMI), weight, total cholesterol and HDL-cholesterol. Data is displayed as median (25,75 percentile).

Serum ANG and IL-18 levels were different between patients and CO: 350 (216,401) vs. 537 (258,615) ng/ml; $p=0.004$ and 330 (214,528) vs. 295 (219,436) pg/ml; $p=0.031$. VEGF ($p=0.01$), ANG ($p<0.001$), IL-18 ($p=0.003$), but not BDNF ($p=0.181$) were different in between CO, LADA, T1D, and T2D (ANOVA). To exclude age-and gender influence, paired comparisons were investigated: LADA to matched CO: ANG ($p=0.001$); T1D to CO: ANG ($p<0.001$); T2D to CO: ANG ($p=0.027$) and IL-18 ($p=0.013$). In order to identify the significant predictors for LADA a logistic regression analysis was applied in which LADA and the other forms of diabetes were the binary duo. Univariate logistic regression revealed that log10-VEGF ($p=0.017$) and log10-ANG ($p=0.002$) were significant in this model. A combination model demonstrated that log10-VEGF ($p=0.039$) was not confounded by log10-ANG ($p=0.004$).

We are first to show that stem cell growth factors are differentially expressed in LADA versus T1D and T2D.

PHARMACOLOGIC TREATMENT OF DIABETES—INCRETIN MIMETICS

275-OR

Administration of Intravenous Exenatide to Patients with Sustained Hyperglycemia in the Coronary ICU

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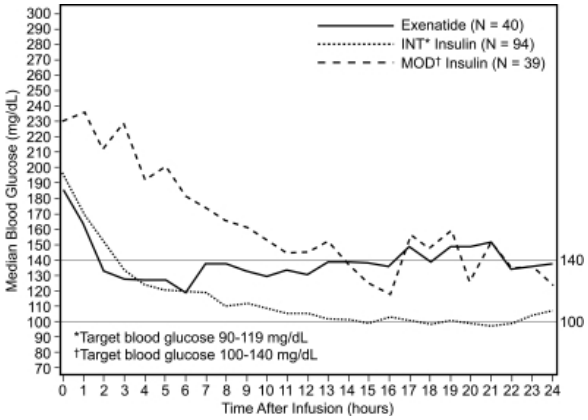
Persistent hyperglycemia is associated with increased mortality and complications in critically ill patients. Intravenous (IV) insulin is current standard of care but requires frequent monitoring and causes excess hypoglycemia. We performed a pilot study to determine the feasibility, efficacy and safety of IV exenatide in hyperglycemic cardiac ICU patients.

A prospective, single-center, open-label, non-randomized study comparing IV exenatide to insulin controls. Eligible patients had a primary cardiac diagnosis and admission blood glucose (BG) 140-400 mg/dL. Exenatide was infused at fixed dose 0.05 mcg/min (30 min bolus) then 0.025 mcg/min continuously for 24-48 hrs. Exenatide was benchmarked to 2 insulin control groups 1) intensive (INT) (target BG 90-119 mg/dL) and 2) modified (MOD) (target BG 100-140 mg/dL).

Exenatide was infused in 40 patients (age 65 years, 83% male, 63% acute coronary syndromes, 75% T2DM). Admission BG was 199 $\pm$ 53 mg/dL in exenatide patients and 240 $\pm$ 44 mg/dL in MOD ($P=0.02$). Time to target BG was lower in exenatide than MOD (4 $\pm$ 4 vs 9 $\pm$ 7 hours, $P<0.001$). Drug-related nausea occurred in 8 (20%) exenatide patients; 5 (13%) discontinued use early. Exenatide was associated with lower BG than MOD (Figure). Of 668 and 745 glucose observations in the exenatide and MOD groups, respectively, there were numerically fewer overall hypoglycemic episodes in the exenatide group (0.9% vs 1.2%, $P=0.57$) including severe events (0% vs 0.3%, $P=0.18$). BG in exenatide treated patients was more frequently within target (100-140 mg/dL; 37% vs 29%, $P<0.001$) and within 71-140 mg/dL (48% vs 39%, $P<0.001$) compared with MOD.

Our findings suggest that fixed-dose IV exenatide is feasible in hyperglycemic ICU patients, achieves similar efficacy compared with IV insulin, and does not cause severe hypoglycemia.



Supported by: Amylin Pharmaceuticals

For author disclosure information, see page 785.

276-OR

A New Type 2 Diabetes Treatment Paradigm: Sequential Addition of Liraglutide to Metformin and Then Basal Insulin Detemir

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Emerging publications report on GLP-1 receptor agonists (GLP-1RA) added to insulin therapy, but there are no data on starting insulin in GLP-1RA-treated insulin-naïve patients with T2DM. This randomized study evaluated the efficacy and safety of a novel T2DM treatment intensification sequence starting with liraglutide 1.8 mg added to metformin (MetLira), followed by adding insulin detemir (IDet) systematically titrated from 10 U/day for those unable to achieve A1c <7.0% on MetLira. The 38-week trial comprised a 12-week run-in with MetLira and a 26-week main period, where those achieving A1c <7.0% remained in an observational (O) arm continuing on MetLira, while those with A1c ≥7.0% were randomized to either MetLira+IDet or continuing MetLira (Control; C). Patients enrolled using Met or Met+SU, but SU was stopped upon entering run-in. Following the 12-week run-in with MetLira, 61% of patients reached A1c <7.0% with decreases in A1c (1.3%) and bodyweight (4.4 kg). After the 26-week main period, patients randomized to MetLira+IDet had a further 0.5% A1c reduction, while A1c remained stable in the MetLira (C) group. 43% of patients randomized to MetLira+IDet reached target A1c <7% at 26 weeks vs. 17% remaining on MetLira (C). Bodyweight decreased in all groups, mainly during run-in (Table). Minor hypo rates were low (0.116, 0.029, 0.247 events/subject-year for MetLira (O), MetLira (C), MetLira+IDet, respectively), with no major hypoglycemic events during the main period. Transient nausea occurred in 21% during run-in, while 4% reported nausea during the main period. In conclusion, IDet added to MetLira was safe, well tolerated, and further improved glycemic control with low risk of hypoglycemia and sustained weight loss in subjects not reaching target with MetLira.

	Randomized		Observational
	MetLira (C) n=161	MetLira+IDet n=162	MetLira (O) n=498
A_{1c}; Week -12, %	8.3	8.2	7.7
ΔA_{1c}; Week -12 to Week 0, %	-0.66	-0.60	-1.34
A_{1c}; Week 0, %	7.6	7.6	6.4
ΔA_{1c}; Week 0 to Week 26, %	-0.04	-0.51	0.24
Overall ΔA_{1c}; Week -12 to Week 26, %	-0.76	-1.13	-1.12
A_{1c}; Week 26, %	7.5	7.1	6.6
Weight; Week -12, kg	98.8	99.5	99.0
Δweight; Week -12 to Week 0, kg	-3.46	-3.53	-4.35
Δweight; Week 0 to Week 26, kg	-1.13	-0.31	-0.37
Overall Δweight; Week -12 to Week 26, kg	-4.74	-4.00	-4.78

Data=means (summary statistics). Week -12 to Week 0=run-in, Week 0 to Week 26=main period (completers); 167 of 988 subjects are not included in the results presented as they withdrew during run-in

277-OR

DURATION-3: Efficacy of Exenatide Once Weekly (EQW) and Insulin Glargine QD (IG) after 84 Weeks in Patients with Type 2 Diabetes (T2D)

MICHAELA DIAMANT, LUC VAN GAAL, STEPHEN STRANKS, BRUNO GUERCI, LEIGH MACCONELL, MICHAEL TRAUTMANN, *Amsterdam, The Netherlands, Antwerp, Belgium, Adelaide, Australia, Toul, France, San Diego, CA, Indianapolis, IN*

Recently we reported that after 26 weeks, EQW vs IG resulted in superior A1C reduction, reduced risk of hypoglycemia, and progressive weight loss in patients with T2D on metformin (MET) or MET+sulfonylurea (MET+SU). This open-label, comparator-controlled extension of that study reports results of continued therapy for a total of 84 weeks. Of the 456 patients randomized to EQW or IG, 196 (EQW) and 192 (IG) entered the extension study, and 173 (each group) completed 84 weeks. Between Weeks 26 and 84, groups did not differ in the number of discontinuations for any reason (most common reason: patient decision [EQW 9; IG 11]). The daily IG dose continued to be titrated from 31.1 (SE 1.4) IU at 26 weeks to 34.8 (1.9) IU at 84 weeks. A1C reduction from baseline to 84 weeks was significantly greater for EQW compared with IG (-1.2% [SE 0.1]) vs -1.0% [0.1], treatment difference: (-0.2%, CI: -0.3% to 0.0%; p=0.029), consistent with a lower endpoint A1C for EQW (7.1% [SE 0.1]) than for IG (7.3% [0.1]), p=0.029. From Week 26 to 84 A1C increases were observed in both treatment groups (EQW 0.4%; IG 0.3%). A similar proportion of patients on EQW vs IG achieved endpoint A1C <7.0% (45 vs 37%, p=0.084), yet a higher proportion of patients on EQW vs IG achieved A1C ≤6.5% (31 vs 20%, p=0.009). At endpoint patients on EQW lost weight (-2.1 kg [SE 0.2]), while those on IG gained weight (2.4 kg [0.2]); treatment difference: [EQW – IG] -4.5 kg (CI: -5.0 kg to -3.9 kg, p<0.001). Within the subgroup on MET+SU, the overall incidence of minor hypoglycemia (BG <3.0 mmol/L) was 24% for the EQW group compared with 54% for IG (p<0.001). For the subgroup on MET alone 8% of patients on EQW vs 32% on IG reported minor hypoglycemia (p<0.001). Among adverse events occurring in ≥5% in a treatment group, diarrhea, nausea, vomiting, and injection site nodules occurred more frequently in the EQW group than the IG group (p<0.05 for each AE). Patients treated 84 weeks with EQW continued to experience better glycemic control and sustained overall weight loss, with a lower risk of hypoglycemia than patients treated with IG.

Supported by: Eli Lilly and Company

278-OR

Lixisenatide Significantly Improves Glycemic Control in Asian Patients with T2DM Insufficiently Controlled on Basal Insulin±SU

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This 24-wk, randomized, double-blind (db), placebo(pbo)-controlled, 2-arm, multicenter, Ph 3 study assessed the efficacy and safety of lixisenatide in Asian patients with T2DM insufficiently controlled on basal insulin±SU. 311 patients (mean age 58.4 yrs, diabetes duration 13.9 yrs, BMI 25.3 kg/m^2, from Japan, South Korea, Taiwan, Philippines) previously treated with basal insulin±SU were randomized to OD 20 µg lixisenatide (n=154) or pbo (n=157). Groups were generally comparable at BL. After screening and a 1-wk, single-blind, pbo run-in, followed a 24-wk, db, pbo-controlled treatment period. The primary endpoint was change in A1C from BL to wk 24. Lixisenatide OD significantly improved A1C *vs* pbo (LS mean difference -0.9%). Significantly more lixisenatide patients achieved A1C ≤6.5% (17.8%) and <7.0% (35.6%) *vs* pbo (1.3% and 5.2%; p<0.0001). Lixisenatide also significantly improved 2-h PPG, glucose excursion and average 7-point SMPG. Lixisenatide was well tolerated and 86% of patients on lixisenatide completed the study *vs* 92% on pbo. 9 pbo (5.7%) and 10 lixisenatide (6.5%) patients experienced a serious TEAE. More lixisenatide patients (14 [9.1%]) discontinued due to TEAEs than pbo patients (5 [3.2%]), mainly due to GI AEs. Nausea and vomiting were reported in 39.6% and 18.2% of the patients in the lixisenatide group *vs* 4.5% and 1.9% in the pbo group. As expected in an insulin±SU-treated population, the % of patients with symptomatic hypoglycemia was higher with lixisenatide (42.9%) *vs* pbo (23.6%), but the rate decreased to 31.8% with lixisenatide *vs* 28.3% with pbo in those not receiving SU. There were no cases of severe hypoglycemia. In this Asian T2DM population insufficiently controlled by basal insulin±SU, lixisenatide OD significantly improved glycemic control with a pronounced FPG and PPG effect, and was well tolerated.

Mean baseline and 24-week changes in efficacy parameters in Asian patients with T2DM insufficiently controlled by basal insulin±SU

Parameter		Lixisenatide (n=153)	Placebo (n=157)	LS mean difference [95% CI]	p-value
HbA_{1c} (%)	Baseline±SD LS mean± SE change from baseline	8.53±0.73 -0.77±0.137	8.53±0.78 0.11±0.131	-0.88 [-1.116 to -0.650]	p<0.0001
2-hour postprandial plasma glucose (mmol/L)*	Baseline±SD LS mean± SE change from baseline	17.88±3.27 -7.96±0.598	17.99±3.66 -0.14±0.563	-7.83 [-8.887 to -6.769]	p<0.0001
Glucose excursion (mmol/L)*	Baseline±SD LS mean± SE change from baseline	9.72±3.22 -7.09±0.576	9.94±4.00 0.14±0.542	-7.22 [-8.245 to -6.204]	p<0.0001
Fasting plasma glucose (mmol/L)	Baseline±SD LS mean± SE change from baseline	7.64±2.31 -0.42±0.314	7.75±2.25 0.25±0.302	-0.67 [-1.225 to -0.112]	p=0.0187
Average 7-point SMPG (mmol/L)	Baseline±SD LS mean± SE change from baseline	11.56±2.54 -1.91±0.272	11.44±2.45 -0.56±0.271	-1.35 [-1.843 to -0.861]	p<0.0001
Body weight (kg)	Baseline±SD LS mean± SE change from baseline	65.99±12.94 -0.38±0.284	65.60±12.47 0.06±0.271	-0.43 [-0.925 to 0.061]	p=0.0857

*After standardized meal

For author disclosure information, see page 785.

ADA-Funded Research

279-OR

Combination of Linagliptin and Metformin Improves Glycemic Control in Type 2 Diabetes: A Randomized Trial with an Open-Label Arm in Patients with Poor Glycemic Control

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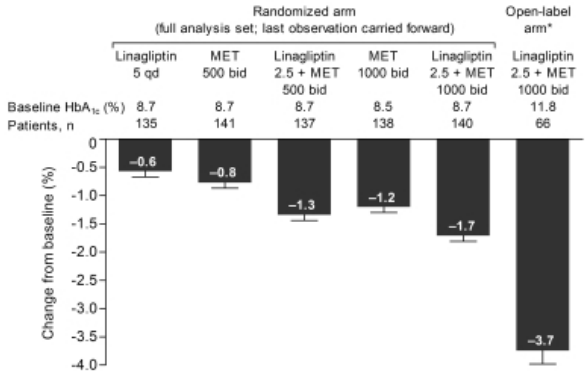
Progression to combination of oral glucose-lowering drugs in patients with type 2 diabetes mellitus (T2DM) is recommended when monotherapy fails to reach treatment targets. This 24-week, double-blind, placebo-controlled study randomized 791 T2DM patients. The 6 treatment groups included 2 arms receiving free combinations of linagliptin 2.5 mg bid + either low- or high-dose (500 or 1000 mg) metformin (MET) bid. Four monotherapy arms received linagliptin 5 mg qd, MET 500 or 1000 mg bid, or placebo. Patients with a baseline HbA_{1c} ≥11% received open-label combination therapy with linagliptin 2.5 mg bid + MET 1000 mg bid (n=66). Mean baseline HbA_{1c} was between 8.5% and 8.7%, and 11.8% in the open-label arm. Placebo-corrected, adjusted mean HbA_{1c} changes after 24 weeks are shown in the figure.

Placebo-corrected, adjusted mean-SE HbA_{1c} changes after 24 weeks



*Open-label arm in patients with poor glycemic control; data are mean-SEM (full analysis set; observed cases, n=48)

For the combination of linagliptin 2.5 + MET 500 or 1000, the placebo-corrected reduction in HbA_{1c} was -1.3% and -1.7%, respectively. Both combination regimens were superior to the monotherapy arms. In patients with poor glycemic control, mean change in HbA_{1c} from baseline was -3.7%. Adverse event rates were similar across treatment arms. The total number of hypoglycemic events during combination treatment was low (in total, 5 [1.8%] randomized patients receiving linagliptin 2.5 + MET 500 or 1000). The difference in body weight after treatment with linagliptin 2.5 + MET 1000 compared with MET 1000 was -0.23 kg. The combination of linagliptin and MET was well tolerated and improved glycemic control more than either monotherapy. Combination of linagliptin with MET significantly improves glycemic control towards treatment targets without weight gain and with a very low risk of hypoglycemia. [ClinicalTrials.gov, NCT00798161]

Supported by: Boehringer Ingelheim Pharmaceuticals, Inc.

280-OR

Efficacy and Safety of Exenatide Once Weekly Versus Metformin, Pioglitazone, and Sitagliptin Used as Monotherapy in Drug-Naïve Patients with Type 2 Diabetes

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This 26-wk multinational, double-blind study is the first to evaluate efficacy and safety of exenatide once weekly (EQW) monotherapy, vs. metformin (MET), pioglitazone (PIO), and sitagliptin (SITA) monotherapy in drug-naïve patients with type 2 diabetes (T2D). Patients were randomized to subcutaneous (SC) EQW 2.0mg + daily oral placebo (PBO, N=248), MET + weekly SC PBO (N=246), PIO + weekly SC PBO (N=163), and SITA 100mg/d + weekly SC PBO (N=163). MET and PIO dosages were increased up to 2500 and 45 mg/day, or maximum-tolerated doses, respectively. The primary endpoint was change in A1C after 26 wks. Baseline characteristics were similar across groups: male, 59%; Caucasian, 67%; age, 54 y; A1C, 8.5%; fasting serum glucose (FSG), 178 mg/dL; body weight, 87.0 kg; and T2D duration, 2.7 y (means). Over 26 wks, all treatments resulted in improvements in A1C, with many patients achieving A1C target <7.0% (Table). Of these treatments, EQW and MET, and to a lesser extent SITA, provided the additional benefit of weight reduction. The most common treatment-emergent adverse events were: EQW, nausea (11.3%) and diarrhea (10.9%); MET, diarrhea (12.6%) and headache (12.2%); PIO, nasopharyngitis (8.6%) and headache (8.0 %); and SITA, nasopharyngitis (9.8%) and headache (9.2%). Minor (confirmed) hypoglycemia reports were rare and no major hypoglycemia occurred. EQW provides a once weekly dosing option for the initial treatment of T2D in drug-naïve patients given its A1C reduction, associated weight loss, and low risk of hypoglycemia.

	EQW (N=248)	MET (N=246)	PIO (N=163)	SIT (N=163)
Change A1C (%)	-1.53 (0.07)	-1.48 (0.07)	-1.63 (0.08)	-1.15 (0.08)*
Endpoint A1C (%)	6.94 (0.07)	6.99 (0.07)	6.84 (0.08)	7.32 (0.08)
A1C<7.0%	62.9	54.6	60.5	43.2*
A1C≤6.5%	49.2	36.0†	42.2	25.5*
Change FSG (mg/dL)	-40.5 (2.5)	-35.7 (2.5)	-46.3 (3.2)	-20.4 (3.2)*
Change Weight (kg)	-2.0 (0.2)	-2.0 (0.2)	+1.5 (0.3)*	-0.8 (0.3)*

Data are least-squares mean (SE) from repeated-measures ANCOVA model or percentage of patients achieving A1C targets at 26 wks. *p<0.001 vs. EQW. †p=0.004 vs. EQW.

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SIGNAL TRANSDUCTION

281-OR

Alpha Cells Secrete Acetylcholine as a Non-Neuronal Paracrine Signal Priming Human beta Cell Function

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Acetylcholine (ACh) is a neurotransmitter that plays a major role in the function of the insulin secreting pancreatic beta cell. Parasympathetic innervation of the endocrine pancreas, the islets of Langerhans, has been shown to provide cholinergic input to the beta cell in several species, but the role of autonomic innervation in human beta cell function is at present unclear. We performed immunohistochemistry on human and mouse pancreatic sections and found that, in contrast to mouse islets, human islets are scarcely innervated by cholinergic (vAChT-immunoreactive) parasympathetic nerve fibers. Instead, most human alpha cells were strongly vAChT immunoreactive. The presence of vAChT in isolated, denervated human islets was confirmed by Western blot analysis. Using a novel method to detect transmitter release - ACh biosensors - we found that human islets release ACh when stimulated with kainate or a lowering in glucose concentration, two specific stimuli for alpha cells. Thus, in marked contrast to the parasympathetic cholinergic control of insulin secretion described for rodents and other species, alpha cells of the human islet may provide paracrine cholinergic input to surrounding endocrine cells. To infer the role of ACh as a paracrine signal we subjected isolated human islets to an experimental protocol in which beta cells and alpha cells were stimulated intermittently while modulating the intrinsic cholinergic signaling.

Blocking acetylcholine degradation with the acetylcholinesterase blocker physostigmine increased insulin release during repeated exposure to high glucose (11 mM), whereas adding the M3 receptor-specific antagonist J-104129 reduced insulin responses. Thus, in the absence of any influence from the autonomic nervous system, endogenously released acetylcholine in human islets is able to sensitize the beta cell to subsequent increases in glucose concentration. Our results demonstrate that in human islets ACh is a paracrine signal that primes the beta cell to respond optimally to subsequent increases in glucose concentration. We anticipate these results to revise models about neural input and cholinergic signaling in the endocrine pancreas.

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For author disclosure information, see page 785.

282-OR

Synaptotagmin-7 Phosphorylation by Protein Kinase A and Insulin Secretion Regulation

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Type 2 diabetes results from relative insulin deficiency, which may be due to impaired insulin secretion and peripheral insulin resistance. A primary approach in treating diabetes is to increase glucose-stimulated insulin secretion (GSIS). Insulin secretion is a highly regulated process. Like synaptic vesicles in neurons and large dense core vesicles in neuroendocrine cells, insulin-containing secretory granules undergo exocytosis in response to elevated intracellular calcium levels. We previously showed that synaptotagmin-7 is a positive regulator of calcium-dependent insulin secretion, and functions as a calcium sensor for insulin granule exocytosis. In this study, we investigated whether synaptotagmin-7 undergoes post-translational modification in response to signaling pathways that potentiate insulin secretion. We found that synaptotagmin-7 showed a minor molecular weight shift in response to forskolin treatment. The shift occurred within minutes of forskolin treatment and was reversible after forskolin washout. Furthermore, the effect of forskolin on synaptotagmin-7 could be mimicked by a membrane-permeable cAMP analog, and inhibited by a PKA-specific inhibitor or by protein phosphatase treatment. Truncation and site-directed mutagenesis analysis revealed that Serine103 in the linker region of synaptotagmin-7 was the phosphorylation site. Together, these results show that synaptotagmin-7 is a downstream target of cAMP-pathway in regulating insulin secretion, and suggest that synaptotagmin-7 phosphorylation may be involved in the regulation of cAMP-potentiated insulin secretion. As synaptotagmin-7 regulates insulin granule exocytosis only in elevated intracellular calcium levels, downstream of high glucose-induced metabolic and membrane events, synaptotagmin-7 may represent an ideal target in enhancing GSIS in diabetes treatment.

283-OR

Evidence for Immune Pathway Activation in Islets from Humans with Type 2 Diabetes

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Loss of β cell function and mass is a key feature of type 2 diabetes (T2DM). Immune responses in adipose tissue play a critical role in insulin resistance in T2DM. However the role of immune cells and related cytokines in the decline of β cell function and viability in T2DM remains unclear.

Human islets from T2DM (n=8) and non-diabetic (n=6) donors were obtained through the Integrated Islet Distribution Program and Beta-Pro. After overnight culture, islets were processed for following 4 assays. 1. RT-PCR. 2. Western blot followed by densitometry. 3. Glucose-stimulated insulin secretion (GSIS) by perifusion. 4. FACS analyses of human islets dispersed to single cell suspensions and stained for immune cell markers. RT-PCR showed 4.1 fold increase in TNFα in T2DM islets compared to non-diabetic islets along with increases in MCP-1 (2.7 fold) and heterodimeric cytokine IL-12 (3.9 fold, all $p<0.05$). Western blot confirmed increases in IL-12 p35 and p40 protein expression in T2DM islets (2.2 and 2.7 fold of non-diabetic islets, $p<0.05$). Importantly Western blot showed a 1.9 fold increase in active (phosphorylated) form of STAT4 in islets from T2DM compared with non-diabetic controls ($p<0.05$). STAT4 is an IL-12-activated transcription factor mainly expressed in T helper 1 cells (Th1). Therefore, activation of STAT4 indicates Th1-related response and T cell activation *in situ* in islets. Contribution of cytokines on islet function was implicated by the negative correlation between GSIS at perifusion and mRNA expression levels of MCP-1 and TNFα in T2DM islets ($p<0.05$ for both cytokines). To further investigate the involvement of immune response in inflammatory process within T2DM islets, we performed flow cytometry analysis of the immune composition of human T2DM islets. For the first time, a distinct population of lymphocytes that included T and B cells was found within T2DM islets. Moreover, about 35% of total $CD45^+$ cells were of myeloid origin that consisted of 60% $CD11b^+$ cells.

These results support the hypothesis that local immune cell activation contributes to dysfunction and loss of viability in islets from humans with T2DM.

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Conjugated Linoleic Acids Stimulate Insulin Secretion through G-Protein Coupled Receptor FFA1/GPR40

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Conjugated linoleic acids (CLAs) are widely used neutraceuticals in the western world. Beside anti-cancer properties favorable body weight management has been observed such as a reduction in fat stores and an increase lean muscle mass. However, a number of adverse effects are also ascribed to the intake of CLAs such as aggravation of insulin resistance and the risk of developing diabetes. Yet, a mechanism accounting for the adverse effects remains to be established. To elucidate a possible link between CLAs and glucose homeostasis, both CLA isomers, 9c,11t-CLA and 10t,12c-CLA, were tested for their ability to stimulate FFA1 in 1321N1 and HEK cells engineered to stably express FFA1 but also in insulin-producing INS-1E cells which endogenously express FFA1 and in pancreatic islets from wild type and *FFA1*$^{-/-}$ mice. Using biochemical second messenger assays, both CLAs dose-dependently between 0.01 mM and 1 mM increased cytosolic $[Ca^{2+}]_i$ in 1321N1 cells overexpressing FFA1. The effect was inhibited by a selective antagonist for FFA1 and was absent in control 1321N1 cells which do not express FFA1. The effect was specific for FFA1 as HEK cells which overexpress FFA1 but not control cells or cells that overexpress FFA2 or FFA3 responded to CLAs when examined by the novel, label-free non-invasive dynamic mass redistribution technology (Corning Epic® biosensor). To analyse functional consequences of FFA1 activation by CLAs their effects on insulin secretion was examined. In INS-1E cells and in isolated islets of wild type but not of *FFA1*$^{-/-}$ knockout mice both CLAs enhanced glucose-stimulated insulin secretion with minor effects on basal secretion. The stimulation of secretion was inhibited by a selective FFA1 antagonist. The concentration of 100 µM which maximally stimulated insulin secretion *in vitro* is sufficient to account for FFA1 activation *in vivo*. Our findings, thus, reveal that CLAs are functional FFA1 agonists and identify these dietary supplements as insulinotropic components.

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Regulation of Islet β Cell SERCA2 Expression in Type 2 Diabetes Mellitus

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The maintenance of intracellular Ca^{2+} homeostasis in the β cell plays a vital role in insulin secretion and ER function. β cell Ca^{2+} levels are closely regulated by activity of the sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump, which hydrolyzes 1 ATP in order to move 2 Ca^{2+} molecules across the ER membrane. Our data demonstrate a significant loss of β cell SERCA2 expression in several models of Type 2 diabetes (T2DM), including islets from db/db mice, diabetic cadaveric human islets, and human islets and rat INS-1 cells treated with 25 mM glucose and the pro-inflammatory cytokine IL-1β (HG+IL-1β). Loss of SERCA2 correlated with elevated basal $[Ca^{2+}]$, impaired Ca^{2+} response to glucose, secretory defects, and activation of ER stress pathways, while overexpression of SERCA2 in INS-1 cells following treatment with HG+IL-1β rescued insulin secretion. Treatment with the PPAR-γ agonist, pioglitazone (pio), was also able to restore SERCA2 expression in diabetic mouse and human islets and led to improved β cell function and survival. To determine if PPAR-γ was a direct transcriptional regulator, luciferase assays were performed in INS-1 cells using fragments of the SERCA2 promoter, in which 5 putative PPAR responsive elements were identified. Results show that the -259 bp region is sufficient to confer PPAR-γ transactivation; mutagenesis, ChIP and EMSA confirmed that PPAR-γ directly binds a PPRE in this proximal region. To characterize the mechanisms by which SERCA is downregulated in T2DM, PPAR-γ expression was measured and found to be decreased under conditions of stress and restored with pio. Further, inhibition of cyclin dependent kinase 5 (CDK5), which has been shown to phosphorylate and inactivate PPAR-γ in adipose tissue, led to restoration of SERCA2 expression and improved β cell survival under diabetic conditions. Taken together, these data suggest that dysregulation of SERCA2 is a prominent component of the β cell dysfunction observed in T2DM. Our results also suggest that PPAR-γ directly regulates transcription of the SERCA2 gene, which may explain key aspects of the beneficial islet-specific effects of PPAR-γ agonists.

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For author disclosure information, see page 785.

ADA-Funded Research

286-OR

Functional Muscarinic Receptors in Human Islet of Langerhans

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Acetylcholine is thought to promote beta cell function and survival in rodent islets via signaling through muscarinic receptors, which have been proposed as an intervention point in the treatment of diabetes. However, the role of acetylcholine in the human islet has not been fully elucidated. Here we first examined the expression of acetylcholine muscarinic receptors using Western blotting and found that all muscarinic receptor subtypes (M1-M5) were expressed in human islets. We next performed in vitro and in vivo functional studies to determine the role of muscarinic receptors in hormone release. Perifusion assays showed that acetylcholine stimulated insulin and somatostatin secretion, but not glucagon secretion in human islets. Insulin responses to acetylcholine were completely inhibited by the general muscarinic antagonist atropine.

Insulin secretion was stimulated by the muscarinic agonist oxotremorine, but not by nicotine, ruling out a contribution of nicotinic receptors. The muscarinic receptor M3 antagonist J-129104 reduced insulin responses to acetylcholine by 70% and the presence of the M4 antagonist MT-3 increased insulin secretion. Calcium imaging showed that mainly beta cells, but not alpha cells, responded to acetylcholine with increases in cytoplasmic [Ca^{2+}].

The M3 receptor antagonist inhibited [Ca^{2+}] responses to acetylcholine in beta cells. To study the effects of cholinergic signaling in vivo, we transplanted human islets into the eyes of diabetic nude mice and performed glucose tolerance tests. Topical application of oxotremorine to the transplanted eyes reduced the glucose excursion, indicating an improvement in glucose control. We are currently addressing which receptor subtype is responsible for this effect in vivo. Our results demonstrate that M3 is present in human beta cells and that its activation promotes insulin secretion, in line with what has been shown for rodent beta cells. Our results further suggest that additional muscarinic receptors are present in human beta cells. Detailed knowledge of the cholinergic mechanisms in human islets will help identify target signaling pathways to promote beta cell function in the treatment of diabetes.

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287-OR

Negative Regulation of B-Cell Function by Grb10 *In Vivo*

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Growth factor receptor binding protein 10 (Grb10) is a pleckstrin homology and Src homology 2 domain-containing protein that interacts with several receptor tyrosine kinases including the insulin and IGF-1 receptors in several tissues. We previously found that Grb10 is expressed in insulin target tissues such as fat and muscles, but the highest expression is in the pancreas (Wang et al. (2007) Mol. Cell. Biol. 27, 6497-6505). To elucidate the in vivo role of Grb10 in pancreatic function, we generate pancreas-specific Grb10 knockout mice. Pancreatic-specific disruption of the Grb10 gene enhanced insulin/IGF-1 signaling in islets, increased beta-cell mass and insulin content, and elevated resistance to STZ-induced beta cell apoptosis and body weight loss. The pancreas-specific Grb10 knockout mice also showed enhanced insulin secretion and improved glucose tolerance in response to high fat diet-induced obesity. Taken together, our results indicate that Grb10 is an important negative regulator of beta-cell proliferation and insulin secretion in vivo. Reducing the expression levels of Grb10 in beta cells could thus provide a novel approach for developing therapeutic interventions for treating type 1 and type 2 diabetes.

288-OR

iNOS Promotes Proteasome-Dependent Degradation of IRS-2 and Suppresses PDX-1 Expression by Activating GSK-3β in Pancreatic β-Cells

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Nitric Oxide (NO) and inducible NO synthase (iNOS) play a pivotal role in dysfunction and destruction of pancreatic β-cells in type 1 and type 2 diabetes. The underlying mechanisms, however, remain to be determined. Treatment with IL-1β or IL-1β-plus-IFN-γ for 24 h induces iNOS expression and suppresses expression of IRS-2 and PDX-1 along with decreased phosphorylation of Akt and GSK-3β, and increased phosphorylation of glycogen synthase in cultured β-cells (INS-1, βTC-6 cells) and mouse isolated islets. These alterations were reverted by inhibition of iNOS by gene ablation or an inhibitor, L-NIL. NO-mediated suppression of PDX-1 expression was associated with reduced expression of its downstream genes, insulin, glucokinase, and Glut-2. Likewise, NO donors (GSNO, SNAP, 30-200 μM) induced these changes in β-cells and mouse islets. NO-mediated reduction in IRS-2 protein expression was accompanied by increased ubiquitination of IRS-2, while IRS-2 mRNA levels were not decreased. Proteasome inhibitors, MG132 and lactacystin, blocked GSNO-induced decreased IRS-2 protein expression in β-cells. In contrast, both protein and mRNA levels of PDX-1 were reduced by iNOS and NO donor, and proteasome inhibitors failed to ameliorate NO donor-induced reduction in PDX-1 expression. The inhibition of GSK-3β by inhibitors (SB216763, SB415268) and siRNA-mediated knockdown almost completely prevented NO-mediated protein degradation of IRS-2 and reverted suppression of PDX-1 at protein and mRNA levels in β-cells. If any, there was little effect of JNK inhibitor (SP600125) on IRS-2 and PDX-1 expression in NO donor-treated β-cells. These findings demonstrate that iNOS promotes proteasome-dependent degradation of IRS-2 and suppresses mRNA and protein expression of PDX-1 by activating GSK-3β in β-cells. These data suggest that GSK-3β may be a major regulator of PDX-1 transcription and protein stability of IRS-2 in β-cells. Our results indicate that iNOS expressed in β-cells and/or intraislet macrophages contributes to the development of β-cell failure by suppressing the expression of IRS-2 and PDX-1 via GSK-3β activation in diabetes.

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HYPOGLYCEMIA UNAWARENESS—POSSIBLE MECHANISMS

289-OR

Cerebral Inefficiency in Type 1 Diabetes during Hypoglycemia

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Abnormal brain activity patterns in the default mode network (DMN) have been linked to several diseases of the brain that affect cognition. The DMN, which consists of the posterior cingulate and temporoparietal cortex, is most active at rest and deactivates during tasks. Because of its high level of metabolism, it is vulnerable in diseases like Alzheimer's and may be prone to the glycemic abnormalities of diabetes. Since hypoglycemia (HYPO) places stress on the brain and is common in T1DM, we studied the effects of HYPO on DMN activity relative to non-diabetic controls (ND). All participants underwent fMRI during an insulin clamp while performing a working memory task (WMT) at 90 and 50 mg/dl. We used a whole brain analysis to compare brain activity during the WMT relative to a rest task and determined which regions were deactivated during the task at both euglycemia (EU) and HYPO. We studied 16 patients with T1DM (8 males; mean±SD: age = 33±10 years, education = 16±5 years, FPG = 107±25 mg/dl, HbA1c = 7.4±1.1%, disease duration = 16.7±3 years) and 16 ND (13 males; mean ± SD: age = 31±10 years, education = 17±3 years, HbA1c = 5.2±0.3%). Both groups deactivated the DMN (e.g., posterior and anterior cingulate) during WMT equally during EU, but during HYPO, the T1DM patients did not deactivate the DMN. Both groups performed equally well on the WMT at both EU and HYPO. Previously, we reported that T1DM patients showed hyperactivity in the prefrontal cortex during a WMT as well as structural changes (reduced gray matter density) in several DMN regions compared to ND. This pattern of intact cognition with reduced deactivation of the DMN during rest and hyperactivity of task-relevant regions during tasks has been termed cerebral inefficiency, and has been found in neurologic diseases such as mild cognitive impairment or Alzheimer's disease. Our findings regarding DMN activity in T1DM suggest that greater brain activity is needed to maintain cognitive function during HYPO and may be an early marker of later clinically evident cognitive dysfunction that may emerge with longer diabetes duration.

290-OR

Hypoglycemia Induced Increases in Cerebral Blood Flow (CBF) Are Blunted in Subjects with Type 1 Diabetes (T1D) and Hypoglycemia Unawareness (HU)

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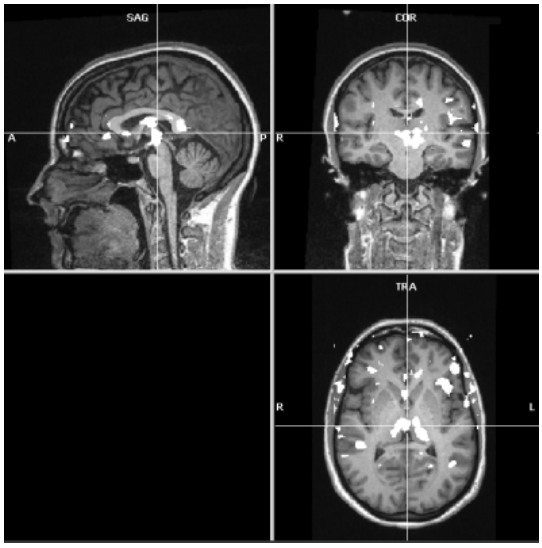
Recurrent hypoglycemia (HG) reduces the threshold at which the counterregulatory response (CR) is elicited during subsequent HG. In patients

For author disclosure information, see page 785.

with insulin treated diabetes, this can lead to HU, which has been attributed at least in part to altered cerebral glucose sensing. Previous studies in humans have demonstrated an increase in hypothalamic and thalamic blood flow—a marker of neuronal activation—in healthy controls (CON) during insulin-induced HG. Other studies have found that the glucose threshold for hypothalamic activation does not differ between subjects with T1D and CON. In this study, we use the sensitive technique of pulsed arterial spin labeling (PASL) to test the hypothesis that subjects with T1D and HU have reduced CBF in cerebral areas involved in glucose sensing and coordination of CR as compared to CON. 6 T1D and 6 CON were studied in a 3 tesla scanner using PASL. A 2-step hyperinsulinemic clamp was performed: blood glucose was first maintained at 100 mg/dL (NG) and then at 50 mg/dL (HG). CBF was measured when glycemia reached target levels. Maps of t-test values were generated to compare CBF in T1D vs. CON, in conditions of NG and HG, and to compare the CBF variations induced by HG (ΔCBF) in T1D vs. CON. During HG, CBF increased significantly in the thalamus and hypothalamus of CON, but this hemodynamic response was largely reduced in T1D (Fig. 1). These findings suggest that there is reduced neuronal activation in critical regions that participate in glucose sensing and/or coordination of CR in subjects with T1D that likely contributes to the development of HU.

Fig. 1: t-test maps comparing ΔCBF induced by HG between T1D and CON. White pixels indicate regions of the brain where CBF response to hypoglycemia was higher in CON as compared to T1D, with t-test values >3.5.



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291-OR

Magnitude of Exercise-Induced β-Endorphin Response Determines Subsequent Development of Hypoglycemia Associated Autonomic Failure

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Hypoglycemia (HYPO)-associated autonomic failure (HAAF) increases the risk of severe HYPO in intensively treated diabetes. Antecedent HYPO or exercise induce HAAF, providing an experimental model of this disorder, whose mechanism remains largely unresolved. Beta-endorphin (β-E) is released in response to various stressors and modulates the autonomic response to HYPO *via* opioid receptor activation, suggesting that this system could play a critical role in HAAF. We examined the effects of β-E release during exercise on the development of HAAF during subsequent HYPO. Sixteen healthy subjects (10 M, 6 F, age 26±4.3 yr, BMI 26.1±5.6 kg/m^2) were studied with 3 experimental paradigms on 2 consecutive days. Day 1 consisted of either 1) two 90-min hyperinsulinemic HYPO clamps (60 mg/dl), HD1; 2) two 90-min hyperinsulinemic euglycemic clamps while subjects exercised at 60% VO_{2max}, ED1; or 3) two 90-min hyperinsulinemic euglycemic clamps (Control). Day 2 followed with stepped hyperinsulinemic (57±1 μU/ml) HYPO clamps (90, 80, 70 and 60 mg/dl plasma glucose steps), using plasma hormone concentrations and glucose turnover ([3-^{3}H]-glucose) as indicators of HYPO counterregulation (CR). As expected, HD1 resulted in marked deterioration of the Day 2 CR to HYPO compared to Control. ED1 was also associated with lower levels of epinephrine (EPI) and norepinephrine (NE) compared to Control, but the difference was not significant. However, analysis of subjects with significant exercise-induced rise in β-E levels (>25 ng/dl, N=7, ED1 β-E), revealed significant decreased levels of EPI and NE, comparable to HD1. These findings demonstrate that the development of HAAF, as a result of antecedent exercise, may be dependent on the magnitude of the increase in β-E, further emphasizing the central role that the endogenous opioid system plays in HYPO CR.

	EPI (pg/ml)	NE (pg/ml)	Glucagon (pg/ml)	EGP (mg/kg/min)
Control	881±113	489±64	96±6	1.9±0.2
ED1	512±74*	309±39*	47±8*	0.8±0.2*
HD1	691±422	436±78	104±8	1.3±0.4
ED1 β-E	457±58*	328±53*	88±9	0.8±0.2*

Day 2 responses averaged during 60 mg/dl plasma glucose step.
* Minimum significance level p<0.05 vs. Control and ED1.

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292-OR

Opioid Receptor Blockade during Antecedent Exercise Prevents Exercise Associated Autonomic Failure in Humans

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Both hypoglycemia (HYPO) and exercise induce release of β-endorphin (β-E), which plays an important role in the modulation of the autonomic response during subsequent HYPO. Since opioid receptor blockade during antecedent HYPO prevents HYPO associated autonomic failure (JCEM 94(9):3372-80, 2009), we hypothesized that opioid receptor blockade during vigorous exercise (associated with significant increase in β-E levels) can also prevent exercise associated autonomic failure (EAAF). We studied 8 healthy subjects (5 M, 3 F, age 28±5.3 yr, BMI 25.2±5.7 kg/m^2) on 2 consecutive days, with each participating in 3 different studies in random order. Day 1 consisted of either A) two 90-min hyperinsulinemic euglycemic clamps (90 mg/dl) plus naloxone infusion (0.4 μg/kg/min [Control]); or B) two 90-min hyperinsulinemic euglycemic clamps while the subjects exercised at 60% VO_{2max}, plus naloxone infusion (0.4 μg/kg/min, [N+]); or C) same protocol as in N+ group, but with saline infusion replacing naloxone [N-]). Day 2 followed with stepped hyperinsulinemic (55±5 μU/ml) HYPO clamps (90, 80, 70 and 60 mg/dl plasma glucose steps), using plasma hormone concentrations and glucose turnover ([3-^{3}H]-glucose) as indicators of HYPO counterregulation. N- studies resulted in a blunted counterregulatory response to subsequent HYPO compared to Control. Conversely, the N+ group exhibited normalization of HYPO counterregulation, characterized by an appropriate increase in epinephrine (EPI), norepinephrine (NE), and endogenous glucose production (EGP). Plasma glucagon and cortisol levels increased similarly in all studies. These results demonstrate that blockade of β-E receptors with naloxone during antecedent vigorous exercise prevents the development of EAAF by improving the EPI and NE responses, and restoring EGP to near-normal levels.

	EPI (pg/ml)	NE (pg/ml)	Cortisol (μg/dl)	Glucagon (pg/ml)	EGP (mg/kg/min)
Control	877±136*	512±47*	21±1.6	118±13	1.8±0.2*
N-	489±71	284±79	23±1.7	104±16	0.9±0.2
N+	836±152*	545±102*	18±1.2	97±11	1.9±0.3*

Day 2 responses averaged during the 60 mg/dl plasma glucose step.
* Minimum significance level p<0.05 vs. N-.

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293-OR

Improvement of Glucose Counterregulation Following Human Islet Transplantation in Long-Standing Type 1 Diabetes: Preliminary Results

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Patients with long-standing type 1 diabetes (T1D) and absolute insulin deficiency exhibit defective glucose counterregulation and impaired hypoglycemia symptom recognition that substantially increase their risk for experiencing severe hypoglycemic events. Reported cross-sectional data on counterregulatory hormonal and symptom responses to hypoglycemia in islet transplant recipients are conflicting. To date, we have studied glucose counterregulation and hypoglycemia symptom recognition longitudinally in the same 6 subjects with T1D prior to and 6 months after undergoing intrahepatic islet transplantation. Subjects had 33±5 years of T1D and

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received 9,978±750 islet equivalents/kg by portal vein infusion resulting in 5/6 insulin-independent with islets from one donor and 1/6 remaining insulin-dependent despite receiving islets from two donors. Glucose counterregulation was assessed using paired hyperinsulinemic (1 mU/kg/min) hypoglycemic (hourly steps at ~80, ~65, ~55 and ~45 mg/dl) and euglycemic (~90 mg/dl) clamps with infusion of 6,6-$^2H_2$-glucose (0.05 mg/kg/min) for measurement of endogenous glucose production (EGP) by the isotopic dilution method. During the final hour of hypoglycemia, plasma glucagon was 30±4 pg/ml pre-transplant and increased to 56±12 pg/ml post-transplant ($p<0.05$), plasma epinephrine was 107±28 pg/ml pre and increased to 235±28 pg/ml post ($p<0.05$), EGP was 0.47±0.10 mg/kg/min pre and increased to 1.20±0.15 mg/kg/min post ($p<0.05$), and the autonomic symptom response was 3.3±1.0 pre and increased by trend to 6.8±0.9 post ($p<0.1$). Pre-transplant, the results obtained during the final hour of hypoglycemia were not different from the results obtained during the final hour of euglycemia, while post-transplant the levels of glucagon, epinephrine, EGP, and the autonomic symptom response were all significantly greater in the final hour under hypoglycemic vs. euglycemic conditions ($p<0.05$ for all comparisons). These results indicate that intrahepatic islet transplantation can improve glucose counterregulation and hypoglycemia symptom recognition in patients with long-standing T1D.

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Deaths Due to Severe Hypoglycemia Are Exacerbated by Diabetes and Ameliorated by Hypoglycemic Pre-Conditioning

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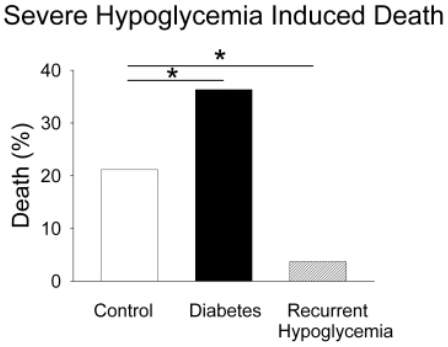
Severe hypoglycemia is lethal and associated with the "dead in bed" syndrome. In previous experiments we noted a highly variable risk of death associated with severe hypoglycemia. We have examined our pooled data to test the hypothesis that uncontrolled diabetes increases mortality associated with severe hypoglycemia and antecedent recurrent moderate hypoglycemia affords a survival benefit. Nine week old Sprague-Dawley rats were divided into non-diabetic controls (CON, n=123), uncontrolled diabetic (DIAB, 65 mg/kg streptozotocin; n=95), and non-diabetics exposed to recurrent insulin (4-6 U/kg) induced hypoglycemia (~40mg/dl) for 3 days (RH, n=27). All rats were subjected to hyperinsulinemic (0.2 $U \cdot kg^{-1} \cdot min^{-1}$) severe hypoglycemic (10-15 mg/dl) clamps for 60-90 minutes. Mortality and witnessed seizures were measured. Basal glucose levels for CON, DIAB, and RH were 117±10, 463±43, and 74±4 mg/dl, respectively. During the severe hypoglycemic clamp, blood sugar was 12±0.3 mg/dl. Of all hypoglycemia induced deaths, 86% were preceded by visible seizures, but the number of seizures did not correlate with death ($r^2 = 0.74$). Mortality rate due to severe hypoglycemia in CON was 21%. Mortality rate increased to 36% in DIAB, whereas RH decreased mortality to 4% ($p=0.0007$, Fisher exact test; see figure). Ventricular tachycardia and fibrillation were witnessed before deaths. In summary, diabetes worsens, while recurrent antecedent hypoglycemia protects against severe hypoglycemia-induced mortality. Seizures appear to be mostly necessary, but not sufficient for hypoglycemic deaths. It is concluded that, in our model, surviving an episode of severe hypoglycemia is dependent on antecedent blood sugar control. Pre-conditioning by recurrent moderate hypoglycemia provides a survival advantage against the lethal effect of severe hypoglycemia.



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Microinjection of a Constitutively-Active AMPK to the Ventromedial Hypothalamus Amplifies the Counterregulatory Response to Hypoglycemia of Both Normal Rats and Rats Exposed to Antecedent Hypoglycemia

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AMP-activated protein kinase (AMPK) is a highly conserved serine/threonine kinase that is activated during cellular energy depletion; acting to suppress ATP-consuming, while activating ATP-generating pathways. Recent *in vivo* rat studies suggest AMPK within the ventromedial hypothalamus (VMH) plays an important role in glucose sensing during hypoglycemia, where AMPK activation or inhibition, respectively, amplifies or suppresses the counterregulatory response to acute hypoglycemia. In this study we examined whether the defect in hormonal counterregulation that develops following recurrent hypoglycemia could result from reduced VMH AMPK activation. Male Sprague-Dawley rats received bilateral VMH microinjections under general anesthesia of an adeno-associated virus containing a constitutively-active (CA)-AMPK (n=18) or control RNAi (n=18), as well as having vascular catheters inserted. 7 days later both groups of rats underwent 3 consecutive days of insulin-induced hypoglycemia (10U/kg) or saline control injections before undergoing a hyperinsulinemic (20mU/kg/min) hypoglycemic (~60 mg/dl) clamp study on the 4th day (10-days post surgery). Peak hormonal responses during the clamps are shown in the table below: (*= $P<0.05$).

	Control	AMPK-CA	Control-RH	AMPK-CA-RH
Glucagon (ng/l)	305±42	435±63	282±37	480±90
Epinephrine (pg/ml)	2383±291	3103±357	1805±226	2664±319

The presence of CA-AMPK in the VMH amplified both glucagon and epinephrine responses to hypoglycemia in both normal rats and rats exposed to prior repeated hypoglycemia; with a more pronounced effect on glucagon. We conclude that maintaining VMH AMPK activity during repeated hypoglycemia, through delivery of CA-AMPK to the VMH, prevented, at least in part, the development of defective hormonal counterregulation during subsequent hypoglycemia. These findings suggest that reduced activation of AMPK in the VMH contributes to the development of defective hypoglycemia-induced hormonal counterregulation.

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Activation of Glucose Counterregulation by VMH Glutamate Neurons Is Mediated by Kainic Acid Receptors

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Glutamatergic neurotransmission in the ventromedial hypothalamus (VMH) plays an important role in stimulating counterregulatory responses to hypoglycemia. Mice with a VMH-specific knockout of the vesicular glutamate transporter, VGLUT2, are incapable of releasing glutamate and exhibit counterregulatory failure. Glutamate acts through a number of different ionotropic and metabotropic receptors to elicit its effects and the specific receptor subtype in the VMH that is responsible for modulating counterregulatory responses has never been identified. Therefore, this became the focus of the current study. To address this question, we locally delivered compounds into the VMH that either activated or blocked one of two forms of ionotropic glutamate receptors, the N-methyl-D-aspartate (NMDA) or kainic acid (KA) receptors and measured the counterregulatory hormone responses to hypoglycemia. In the first set of studies, we assessed the role of the NMDA receptor by microinjecting either artificial extracellular fluid (aECF), NMDA or the NMDA receptor antagonist, AP5, into the VMH of Sprague-Dawley rats just prior to subjecting them to a hypoglycemic (~45mg/dl) glucose clamp. In these studies, we noted that neither activating nor blocking VMH NMDA receptors affected glucose counterregulatory responses to hypoglycemia. We then proceeded to examine the role of KA receptors in the VMH by directly microinjecting either: 1) aECF (n=6), 2) KA (n=7) or 3) DNQX (n=5), a KA receptor antagonist, into the VMH before inducing hypoglycemia with an insulin clamp. Activation of KA receptors in the VMH amplified the glucagon response by 58% ($P<0.01$) and the epinephrine response by 46% ($P<0.05$), whereas blocking KA receptors with DNQX suppressed the glucagon and epinephrine responses by 45% and 40%, respectively ($P<0.02$).

Our data suggests that 1) glutamate in the VMH exerts its stimulatory effects through KA receptors to enhance the counterregulatory responses to

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hypoglycemia and 2) that the VMH glutamatergic system may be a potential target for the therapeutic treatment or prevention of hypoglycemia.

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NEW DEVELOPMENTS IN BEHAVIORAL RESEARCH

297-OR

Improved Functioning and Well Being Following a Multi Modality Group Lifestyle Balance Program Intervention: Results of the Rethinking Eating and ACTivity Study (REACT)

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When individuals are empowered to make informed decisions regarding their healthcare, psychosocial outcomes (PO) are likley to improve. Interventions adapted from the DPP demonstrate the effectiveness of weight loss and diabetes risk reduction in "real world" settings; however it is unknown if individuals who are empowered to select an intervention modality experience larger improvements in PO compared to those who have the modality dictated to them. We aimed to determine if 3 Group Lifestyle Balance (GLB) intervention modalities were effective in improving functioning and well being in overweight individuals from 8 rural communities in southwestern, PA. Communities and their eligible participants (n=493; mean age: 51 yrs, 87.6% female, 94.1% Caucasian, 86.8% BMI ≥ 30 kg/m^2) were assigned to 4 GLB groups: Face to Face (FF) (n=119), DVD (n=113), internet (INT) (n=101), and self-selection (SS) (n=101). Participants in SS were empowered to select the modality (60% chose FF, 40% INT, 0% DVD). Functioning and well being was defined as the physical and mental component summary (PCS & MCS) scores of the SF12. Data were collected at baseline and three months. Significant improvements were observed in PCS within all groups, except for INT (FF: 51.2 vs. 53.2, p=0.0005, DVD: 50.6 vs. 52.9, p=0.0007, INT: 51.6 vs 52.6, p=0.26, SS: 51.5 vs 54.7, p=0.0002). Change in MCS by group was (FF: 49.7 vs. 53.3, p=0.0002, DVD: 52.3 vs. 54.7, p=0.02, INT: 51.8 vs. 52.2, p=0.78, SS: 52.9 vs 57.3, p=0.0003). After controlling for baseline PCS and MCS values, age, number of healthcare provider visits, poverty, GLB group, and the clustering of communities within GLB group, particpants in SS experienced the largest improvements in PCS (p=0.02) and MCS (p=0.03) compared to other groups. These results demonstrate that GLB is effective in improving functioning and well-being when delivered through FF and DVD, but not INT. The largest improvements were observed when individuals were given the opportunity to choose the modality. This concept supports empowerment, which prioritizes patient choices to achieve personal goals.

298-OR

Patient-Reported Outcomes in the Sensor-Augmented Pump Therapy (SAPT) for A1c Reduction (STAR) 3 Trial

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In STAR 3, the first large RCT comparing sensor-augmeted pump therapy (SAPT) to optimal conventional therapy (multiple daily injection [MDI] therapy with SMBG) in adults and children (N=485) over 12 months, we examined patient-reported outcomes (PRO) in study participants and in childrens' caregivers. Within and between treatment arm PRO changes from baseline were assessed.

Adult Health-related quality of life (HRQOL): (SF-36 Physical Component Summary [PCS] and Mental Component Summary [MCS]): PCS scores improved in the SAPT arm only (p<0.05), with no MCS change in either arm and no between-arm PCS or MCS difference in change (all p>0.05).

Child and caregiver HRQOL: PedsQL Physical Health Summary [PhysHS] and Psychosocial Health Summary [PsycHS]): PhysHS child and caregiver scores did not change in either arm (p>0.05) with no between-arm difference in change. Child PsycHS scores improved in both arms (p<0.05), with no between-arm difference in change. Caregiver PsycHS scores improved in the SAPT arm only (p<0.05), with greater improvement in SAPT than MDI (p<0.05).

Fear of hypoglycemia: (Hypoglycemia Fear Survey Worry [HFSW] and Behavior [HFSB] subscales): HFSW and HFSB scores declined in adults, children, and caregivers in the SAPT arm only, with greater improvement in SAPT than MDI in adults and caregivers (all p<0.05).

Treatment satisfaction: (Insulin Delivery System Rating Questionnaire [IDSRQ]): All IDSRQ subscale measures improved in SAPT adults, and key measures (Convenience, Efficacy, and Overall Preference) also improved in SAPT children and caregivers (all p<0.001). All significant between-arm differences in IDSRQ subscale change favored SAPT; SAPT arm improvement was greater for key measures (Convenience, Efficacy, Overall Preference) in adults, children, and caregivers (all p<0.001).

In summary, in the first large study of SAPT compared to optimal conventional therapy SAPT had significant PRO advantages over MDI with SMBG, especially for treatment satisfaction in adults, children, and caregivers, hypoglycemia fear in adults and caregivers, and HRQOL in caregivers.

299-OR

Diabetes Prevention Program for Native American Youth: The JOURNEY to Native Youth Health Feasibility Study

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The purpose of this study was to modify the adult-based Diabetes Prevention Program (DPP) for Native American youth and determine the feasibility of implementing the lifestyle change program titled "JOURNEY to Native Youth Health," in a small number of youth. The general content and behavioral goals in the JOURNEY DPP were based on the original DPP model. Using an iterative process, community members and project staff modified the program to be developmentally and culturally appropriate for Native youth. The diabetes prevention strategies targeted healthy weight maintenance, lowering fat intake, and increasing physical activity. Cultural aspects were fused within the JOURNEY program and included berry picking, horseback riding, dancing, use of storytelling to convey information, sessions led as talking circles, and participation of elders. Native youth 10-14 years old, living on two Montana Indian reservations were recruited for the feasibility study (n=64). The participants were randomized to treatment (Tx) or comparison (C) group. Pre- and posttest measures included weight, height, dietary intake, physical activity, and knowledge, attitudes and beliefs (KAB) about nutrition. All data were collected at baseline (e.g., prior to delivery of the program) and at the end of the 9-session program. At end-of-program measures, the Tx group had 54% more minutes of moderate and vigorous activity than the C group. Energy (kilocalories) expended in posttest measures were 27% higher in the Tx group vs the C group. The average decline in percent of kilocalories from fat intake was four times greater in the Tx group vs the C group. At posttest, the Tx group increased their nutrition KAB score by 8% while the C group had no change in their score. In the short pilot study, differential effects were observed for the Tx and C group among children who were overweight or obese at baseline. Among these at risk children, BMI growth was 40% greater in the C group vs the Tx group. This study suggests the Tx curricula can impact important indicators of future diabetes risk and sets the stage for a full scale trial of the JOURNEY to Native Youth program.

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Long-Term Effects of READY-Girls on Intentions and Behaviors for Family Planning and Preconception Counseling in Teens with Diabetes

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ADA recommends preconception counseling (PC) at routine clinic visits starting at puberty for all women with diabetes of child-bearing potential. *READY-Girls* is a self-administered PC program for teen girls with diabetes. We examined the long-term (6-12mons) effects of *READY-Girls* on intentions and behaviors for family planning and PC in teen girls with type 1 and 2 diabetes (T1D, T2D).

In a multisite RCT with 109 teens [mean age=15.8yrs (13-19yrs)], 58 were randomized to a standard care control group (CG) to receive March of Dimes pamphlets and 51 to an intervention group (IG) to receive the READY-Girls over 3 clinic visits (baseline DVD, boosters: 3-mo DVD, 6-mo book). Data were collected by validated measures at pre- and post-intervention sessions, 17% were African American and 11% had T2D. At baseline, 20% (n=11) of each group was sexually active with mean sexual debut of 15.4yrs (12-18yrs); 64% IG had unsafe sex vs. 36% CG (p=0.2).

PC intentions showed significant group by time effects. IG had greater intentions to discuss PC (F=2.23, p=.04) and birth control (F=3.00, p=.007) with health professionals, and to use family planning and seek PC (F=2.31, p=.033). IG showed trends toward lower rates of becoming sexually active (33% vs 45%) and higher overall rates of abstinence (43% vs 35%) than CG, findings were not sustained (X^2=4.51, p=.212). No pregnancies were reported in either group during the study.

Significant and sustained positive changes in intentions occurred following a booster. Behavioral outcomes improved with READY-Girls, but were not significantly sustained. IG had higher PC scores and were sustained

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the longest. READY-Girls had no effect on seeking PC behavior over time; although it may be too early to detect (68% were <17yrs).

Boosters appeared to be an important component of the intervention. A stronger, internal, more permanent booster maybe warranted. Providing knowledge and skills to mothers and daughters to initiate dialogue and boost support could have long-term behavioral effects.

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301-OR

Long-Lasting Effects of a 2-Year Diabetes Self-Management (DSMS) Intervention: Outcomes at 1-Year Follow-Up

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This study examined the diabetes-related health impact of a 2-year empowerment-based, diabetes self-management support (DSMS) intervention designed for African Americans at 1-year follow-up. The 2-year DSMS intervention consisted of 88 weekly sessions conducted in a group setting and participants were invited to attend weekly education/support groups as frequently as needed. The DSMS intervention was led by two health care professionals and emphasized experiential learning, emotional coping, problem solving, goal setting, and action planning. Following patient empowerment principles, discussion was guided by participant-identified priorities and concerns. Outcomes of interest included metabolic and cardiovascular measures, self-care behaviors, and diabetes-specific quality of life (QOL). The study followed a longitudinal prospective design and recruited 77 African-American adults with type 2 diabetes at baseline. Fifty-two participants completed the 3-year study yielding an attrition rate of 32% (results based on sample size n=55). Mean age was 65 years (SD = 10.3); 73% (n=38) were women. Baseline, 24-month (post-intervention), and 36-month (1 year follow-up) assessments measured A1C, weight, body mass index, serum cholesterol, HDL, LDL, systolic and diastolic blood pressure, self-care behaviors, and diabetes-specific QOL. Following the 2-year DSMS intervention, significant improvements were found for following a healthy diet (p=0.034), spacing carbohydrates evenly across the day (p=0.005), using insulin as recommended (p=0.047), and diabetes-specific QOL (p<0.022). At 1-year follow-up, not only did participants sustain the improvements made in the 2-year DSMS intervention, but they also demonstrated additional improvements in A1C (p<0.001), serum cholesterol (p<0.001), and LDL (p=0.001). Findings suggest participation in an empowerment-based DSMS intervention has a positive and enduring effect on diabetes-related health outcomes and that metabolic and cardiovascular improvements continue to occur 12 months post-intervention.

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302-OR

Cognitive Behavioral Therapy vs. Sertraline in Patients with Depression and Poorly Controlled Diabetes: A Multicenter Randomized Controlled Trial

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Depression is associated with adverse outcomes in patients with diabetes. No study exists on the direct comparison of pharmacological to psychological treatments for this patient group.

We compared the efficacy of 10 sessions (20 hrs.) of diabetes-specific cognitive behavioral group therapy (CBT) to sertraline (SER) in 251 adult patients with depression and poorly controlled diabetes who were treated with insulin. The diabetes treatment was not part of the study protocol ("treatment as usual"). After 12 weeks of open-label therapy, only treatment-responders (50% reduction of depression, Hamilton Depression Rating Scale, HAMD) were included in the 1-year phase of the study. CBT-responders received no further treatment, while SER-responders obtained a sustained SER regimen as relapse prevention. Group differences in HbA1c (primary outcome) and HAMD between 1-year follow-up and baseline were analyzed by ANCOVA controlling for baseline values. Subgroup analyses were conducted for type of diabetes.

After 12 weeks 115 (45.8%) patients responded to the treatments (CBT 53, SER 62). In the 1-year follow-up HbA1c changed from 9.3±1.6 to 9.2±1.7 after CBT and from 9.2±1.4 to 9.4±1.4 under SER with no significant treatment difference (p= 0.129). HAMD scores improved after CBT from 18.0±4.6 to 7.8±6.5 and from 18.9±5.1 to 5.5±5.7 under SER; the difference was significant (p=0.020). Subgroup analyses revealed significant differences only within the CBT group and only regarding HbA1c (difference 0.73) favoring type 2 diabetes (HbA1c reduction: -0.40 vs. +0.324 for type1, p=0.0036).

Both treatment groups showed sustained reduction of depression with a small but significant advantage of SER vs. CBT. But, no substantial improvement could be obtained for glycemic control independently of the type of treatment. Even though patients with type 2 diabetes achieved better glycemic control after CBT the results still remained largely above the recommended limits. The results point towards the tailoring of new specific treatment modules to the individual patient rather than recommending 'established' general strategies.

Supported by: Competence Network Diabetes

303-OR

Depressive Symptoms and Suboptimal Glycemic Control: A Key Role for Anhedonia?

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Recent studies examining the relationship between depression and glycosylated haemoglobin (HbA_{1c}) concentrations in patients with type 2 diabetes have yielded mixed findings. One explanation may lie in the heterogeneity of depression, as core characteristics range from depressed mood to a loss of interest or pleasure (anhedonia), and additional symptoms may even include anxiety. Therefore, our aim was to explore whether distinct features of depression were differentially associated with suboptimal glycemic control. In this substudy of the DIAZOB Primary Care Diabetes study (covering a dynamic cohort of primary care patients with type 2 diabetes from the Eindhoven region, The Netherlands), 5772 individuals completed baseline measurements of demographic, clinical, lifestyle and psychological factors between 2005 and 2009. The Edinburgh Depression Scale was used to assess symptoms of depressed mood, anhedonia and anxiety. Suboptimal glycemic control was defined as HbA_{1c} values ≥ 7%, with 30% of the sample (n=1718) scoring above this cut-off. In univariate logistic regression analyses, anhedonia was significantly associated with suboptimal glycemic control (OR 1.29, 95% CI 1.09 – 1.52), while both depressed mood (OR 1.04, 0.88 – 1.22) and anxiety (OR 0.99, 0.83 – 1.19) were not. The association between anhedonia and glycemic control remained after adjustment for the other depression measures (OR 1.33, 1.11 – 1.59) and increased in size when higher cut-off values for suboptimal HbA_{1c} were used. Alcohol consumption and physical activity met criteria for mediation, but did not attenuate the association between anhedonia and glycemic control by more than 5%. Although diabetes duration was identified as a confounder and controlled for, the association was still significant (OR 1.20, 1.01 – 1.43). These findings suggest that studying different symptoms of depression, in particular anhedonia, may add to a better understanding of the relationship between depression and glycemic control. Prospective studies are needed to clarify the directional nature of this relationship and to test mediation through other behavioral or biological pathways.

304-OR

Increased Mortality Risk after Myocardial Infarction in Patients with Diabetes and Depression

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Both diabetes and depression are independently related to an increased risk of mortality in patients who had a myocardial infarction (MI). Studies in the general population suggest that having both diabetes and depression results in an increased mortality risk, beyond that of having diabetes or depression alone. We studied the possibility of a synergistic effect on mortality in a cohort of MI patients.

Data were derived from two multicenter studies in the Netherlands, comprising 2704 patients who were hospitalized for MI. Depression, defined as a Beck's Depression Inventory (BDI) score ≥10, and diabetes were assessed during hospitalization. Hazard ratio's (HR) were calculated using Cox proportional hazards models. Patients were divided into four groups: 1) patients without diabetes and without depression (reference group), 2) patients with diabetes only, 3) patients with depression only, and 4) patients with both diabetes and depression.

During an average follow-up of 6.3 years, 405 patients died. The mortality rate was 13% (212/1630) in the reference group, 21% (42/198) for patients with diabetes only, 21% (109/531) for patients with depression only, and 45% (42/93) for patients with both diabetes and depression. In unadjusted analyses, the HR for mortality was 1.93 (95% CI 1.42 – 2.61) for patients with diabetes only, 1.71 (95% CI 1.36 – 2.14) for patients with depression only, and as much as 4.29 (95% CI 3.10 – 5.94) for patients with both diabetes and depression, compared to the reference group. After adjustment for age, sex,

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smoking, hypertension, left ventricular ejection fraction, prior MI, and Killip class, the HR attenuated but remained significantly elevated for patients with both diabetes and depression (HR 2.70, 95% CI 1.91 – 3.83). Adjusted HR for diabetes only and depression only were 1.40 (95% CI 1.03 – 1.90) and 1.38 (95% CI 1.09 – 1.74), respectively.

We observed an increased mortality risk in patients with both diabetes and depression after an MI, beyond the effect on mortality of diabetes and depression alone. This increased risk can be partly explained by the increased comorbidity and disease severity associated with diabetes and depression.

PHARMACOLOGIC TREATMENT OF DIABETES—NOVEL THERAPIES

305-OR

Short-Term Treatment with Glucagon Receptor Antagonist LY2409021 Effectively Reduces Fasting Blood Glucose (FBG) and HbA1c in Patients with Type 2 Diabetes Mellitus (T2DM)

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Glucagon levels are elevated in many patients with T2DM and contribute to hyperglycemia. LY2409021 is a potent, selective glucagon receptor antagonist. This Phase 1, randomized, double-blind, placebo (PBO)-controlled study examined the safety, tolerability, pharmacokinetics (PK), and short-term (28-day) efficacy of once-daily doses of LY (5, 30, 60, or 90mg) in patients with T2DM treated with diet and exercise or metformin (N=47; mean FBG 148 mg/dL; HbA1c 8.0%).

Across LY dose levels, PK parameters t_{max}, $t_{1/2}$, and apparent clearance (CLss/F) ranged from 6 to 8 h, 56.1 to 61.9 h, and 0.263 to 0.345 L/h, respectively, and C_{max} increased in proportion to dose. Accumulation (R_A) ranged from 3.74 to 4.5.

LY produced clinically significant reductions in FBG by Day 2; by Day 14, least squares (LS) mean changes from baseline vs. PBO were -25.74, -39.96, and -37.44 mg/dL ($p< .01$ for all) in the 30, 60, and 90mg dose groups, respectively. By Day 28, LS mean changes in HbA1c were statistically significant vs. baseline in all treatment groups, including PBO (-0.49%; 90% confidence interval: -0.70 to -0.28), such that significant reductions vs. PBO were seen only at 60- and 90-mg LY, respectively: -0.53% (p=.0117); -0.43% (p=.0391). ED_{50} for glucose-lowering was 5mg. Fasting glucagon significantly increased by 0.6-, 1.5-, 2.5-, and 4.2-fold vs. baseline across LY dose levels, and fasting active glucagon-like peptide-1 (GLP-1) by 59% at 90mg. Glucagon and GLP-1 returned to baseline levels during follow-up. No significant effects of LY on fasting or postprandial insulin and C-peptide were seen.

LY was generally well tolerated. Hypoglycemia was infrequent and mild to moderate in intensity (4 events at 90mg; minimum glucose 62mg/dL). Reversible elevations in hepatic transaminases > 3x ULN were seen in 5 of 9 patients in the 90mg dose group, with no clinical signs or significant elevations in bilirubin or alkaline phosphatase.

The potent glucose-lowering observed during short-term treatment with LY supports the continued development of this glucagon receptor antagonist as a once-daily treatment for T2DM.

306-OR

The Novel GPR119-Receptor Agonist PSN821 Shows Glucose Lowering and Decreased Energy Intake in Patients with T2DM after 14 Days Treatment

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GPR119 is a G protein-coupled receptor expressed predominantly in pancreatic β-cells and enteroendocrine cells in humans. PSN821 is a potent, selective GPR119 agonist shown in animal disease models to substantially lower blood glucose and reduce body weight. This randomized, double-blind, placebo-controlled assessment investigated the safety, tolerability and PD effects of PSN821 in overweight/obese patients with T2DM following washout of either all antidiabetic medication or with continuation of a stable dose of metformin only.

Three cohorts of patients dosed twice-daily with either PSN821 250mg monotherapy [n=7], PSN821 (250mg [n=6] or 500mg [n=7]) added to metformin therapy or placebo [n=5] were evaluated. After 14 days treatment median T_{max} of PSN821 in plasma ranged from 2 to 5 hours and geometric mean $t_{1/2}$ ranged from approximately 5.5 to 7 hours post-dose; co-administration with metformin did not affect PK parameters of either. Changes in fasting plasma glucose levels from baseline were apparent in all active treatment groups (-2.0, -2.3, -2.1 mmol/L respectively; placebo -0.7 mmol/L). Following meal challenge, reductions in glucose exposure (E_{max}, $AUC_{0\text{-}5hr}$, reactive $AUC_{0\text{-}5hr}$) were greater for all doses of PSN821 than placebo. PSN821 500mg showed substantial reductions from baseline in energy intake (-40%) after 14 days treatment, with weight changes from baseline apparent in all active treatment groups (-2.4, -1.8, -2.1 kg respectively; placebo -1.1 kg). Cardiovascular risk markers showed neutral to modestly positive changes in all actively treated groups. PSN821 was well tolerated with a low incidence of nausea or gastrointestinal upset and without any adverse effects on labs, vital signs or ECG. There were no SAEs or discontinuations due to AEs.

Alone or in combination with metformin PSN821 has a PK profile consistent with regular oral dosing and showed fasting and post-meal glucose lowering in patients with T2DM. The higher PSN821 dose also substantially reduced energy intake with weight changes apparent across active treatments. PSN821 thus holds potential as a novel therapy for the treatment of T2DM and obesity.

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Dapagliflozin, Metformin-XR, or Both Together as Initial Therapy for T2DM

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Combining metformin-XR (MET) with another antidiabetic agent as initial therapy for T2DM may be advantageous in patients with high baseline HbA1c. We report data from 2 randomized, double-blind, active-controlled, 24-wk trials of the selective SGLT2 inhibitor dapagliflozin (DAPA) and MET, combined or alone, in treatment-naïve patients with T2DM. Study 1 (MB102021) compared once daily DAPA 5mg + MET, DAPA 5mg, and MET. Study 2 (MB102034) compared once daily DAPA 10mg + MET, DAPA 10mg, and MET. In each trial MET was titrated to 2000mg (majority at 2000mg), and patients with age range 18-77 years and HbA1c 7.5–12% were eligible for inclusion. The primary endpoint was change from baseline in HbA1c at Wk 24. Secondary endpoints included changes in FPG and weight. In both trials DAPA+MET was more effective than either drug alone in reducing HbA1c and FPG, and more effective than MET in reducing weight (Table). In a pre-specified comparison, DAPA 10mg was non-inferior to MET in reducing HbA1c, and superior in reducing FPG and weight. Events suggestive of genital infection were reported in 6.7%, 6.9%, 2.0% (Study 1) and 8.5%, 12.8%, 2.4% (Study 2) of patients in respective DAPA+MET, DAPA and MET arms; events suggestive of urinary tract infection were reported in 7.7%, 7.9%, 7.5% (Study 1) and 7.6%, 11.0%, 4.3% (Study 2) of patients. No event of major hypoglycemia was reported. In conclusion, DAPA+MET as initial therapy for T2DM was generally well tolerated and effective in reducing hyperglycemia and weight.

Wk 24 Results (LOCF)

	Study 1 (n=598)			Study 2 (n=638)		
Adj. mean change from baseline (SE)	**MET**	**DAPA 5mg**	**DAPA 5mg + MET**	**MET**	**DAPA 10mg**	**DAPA 10mg + MET**
HbA1c, %	–1.35 (0.09)	–1.19 (0.09)	–2.05* (0.09)	–1.44 (0.08)	–1.45¶ (0.07)	–1.98* (0.08)
FPG, mg/dL	–33.6 (2.7)	–42.0 (2.7)	–61.0* (2.8)	–34.8 (2.5)	–46.4§ (2.5)	–60.4* (2.5)
Weight, kg	–1.29 (0.24)	–2.61 (0.24)	–2.66 (0.24)	–1.36 (0.24)	–2.73§ (0.23)	–3.33§ (0.24)
% Patients with HbA1c<7.0%, adj. for baseline (SE)	35 (3)	23 (3)	52† (4)	35 (3)	32 (3)	47‡ (3)

Significant at: *P<0.0001, †P≤0.0003, and ‡P≤0.02 *vs* both DAPA and MET; §P<0.002 *vs* MET. ¶Non-inferior to MET.

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A Randomized, Double-Blind, Placebo-Controlled Study for Diacerein in Patients with Inadequately Controlled Type 2 Diabetes Mellitus

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Type 2 diabetes mellitus is linked to chronic inflammation. Recent studies suggest that therapy targeting interleukin 1 (IL-1) pathway may be efficacious in type 2 diabetes. We evaluated the efficacy and safety of diacerein (diacetylrhein, 4,5-Bis(acetyloxy)-9,10-dihydro-9,10-dioxo-2-anthracenecarboxylic acid), an IL-1β inhibitor, in type 2 diabetes patients

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as add-on therapy. During a 24-week, double-blind, placebo-controlled trial, 76 patients were randomized to placebo (n=38) or 100 mg/d (n=38) of diacerein. All patients were maintained with prior existing anti-diabetic medication. The primary efficacy endpoint was a change in the level of glycosylated hemoglobin (HbA1c), and secondary endpoints were changes in beta-cell function, insulin sensitivity, and inflammatory markers. There was a significant difference in HbA1c level reduction between diacerein and placebo groups at week 24 (0.63% vs. 0.00%; p-value = 0.0158). A significant improvement in fast plasma glucose (FPG) was observed in diacerein group at week 24 (vs. placebo; p-value = 0.0372). Diacerein significantly reduced the level of IL-6 and BMI within the groups. No statistical significant difference in postprandial plasma glucose (PPG), beta-cell functions, hs-CRP, IL-6 and BMI was observed between the groups. Excellent safety and tolerability profile was demonstrated in this trial and a sister trial in type 2 diabetes patients with microalbuminuria (N=146). Adverse events were reported by similar percentages in diacerein patients (52.1%) and placebo patients (47.9%). Diarrhea was a possible drug-related AE which was only observed in 5 diacerein patients (6.8% vs. placebo 2.7%). This study demonstrated that diacerein is well tolerated and add-on diacerein to existing anti-diabetic medications may offer therapeutic benefit in glycemic control in type 2 diabetes patients.

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Efficacy and Tolerability of MK-0893, a Glucagon Receptor Antagonist (GRA), in Patients with Type 2 Diabetes (T2DM)

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Excessive hepatic glucose production is a major feature of the pathophysiology of hyperglycemia in T2DM and increased glucagon action has been implicated in this process. Thus, treatments targeting glucagon action are currently being explored for T2DM. The present study evaluated MK-0893, an oral, highly-selective GRA, in a 12-wk, double-blind, parallel arm study. Eligible patients (pts) with baseline (BL) A1C of 7-11% were randomized equally to once-daily MK-0893 20, 40, 60 or 80 mg, metformin (MF) 1000 mg bid or placebo (PBO). Change in FPG from BL was the primary endpoint. At BL, randomized pts (N=342, 51% males) had a mean age of 55 yrs, mean A1C of 8.4% and median duration of T2DM of 4 yrs. At 12 wks, treatment with MK-0893 led to significant, dose-dependent reductions in FPG and A1C from BL compared to PBO (Table). Changes from BL in 2-hr postmeal glucose ranged from -65 to -110 mg/dL with MK-0893 compared to -69 mg/dL with MF and -8 mg/dL with PBO. Relative to MF, glycemic improvements were numerically greater with MK-0893 at doses ≥40 mg. The incidence of adverse events including hypoglycemia was generally similar across groups. LDL-C increased from BL with MK-0893 compared to PBO (Table). MK-0893 was also associated with an increase in ALT, with median changes from BL of 17-31% compared to 0% with MF and 12% with PBO. MK-0893 significantly increased body weight (BW) in a dose-dependent manner (LS mean change from BL = 0.5, 0.2, 1.5, 2.3 for MK-0893 20, 40, 60 and 80 mg, respectively) compared to a decrease of 1.0 kg with MF and 0.9 kg with PBO. In summary, 12-wk treatment with MK-0893 improved glycemic control in a dose-dependent manner in pts with T2DM but was associated with increases in LDL-C, ALT and BW.

	PBO	MK-0893 20 mg	MK-0893 40 mg	MK-0893 60 mg	MK-0893 80 mg	MF 2000 mg
BL FPG, mg/dL	184	180	192	193	183	186
Δ FPG, mg/dL	-2	-32*	-48*	-53*	-63*	-37*
BL A1C, %	8.3	8.3	8.5	8.4	8.5	8.5
Δ A1C, %	0.5	-0.6*	-1.0*	-1.1*	-1.5*	-0.8*
BL LDL-C, mg/dL	115	116	118	111	113	109
% Δ LDL-C	-3	8**	8**	12**	15*	1

*p<0.001 or **p<0.05 vs PBO, BL data are means, change from BL data are LS means

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Oral Chemokine Receptor 2 Antagonist CCX140-B Shows Safety and Efficacy in Type 2 Diabetes Mellitus

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CCX140-B is an oral, specific CCR2 antagonist expressed on monocytes/macrophages. Increased adiposity leads to elevated recruitment of inflammatory monocytes/macrophages into adipose tissue. We have shown that treatment of obese, diabetic mice with a CCR2 antagonist significantly improved glucose homeostasis.

This is a multinational, randomized, double-blind, placebo- and active-controlled clinical trial in 159 subjects with type 2 diabetes on stable metformin. HbA1c was 6.5 to 10% and fasting plasma glucose [FPG] 135 to 270 mg/dL at study entry. Randomized subjects received double-blind placebo QD (N=32), 5mg CCX140-B QD (N=32), 10mg CCX140-B QD (N=63), or open-label pioglitazone 30mg QD (N=32) for 4 weeks. The primary objective of the study was to evaluate the safety and tolerability of CCX140-B. Secondary objectives included assessment of glycemic parameters and CCX140-B pharmacokinetics.

Baseline characteristics, mean (SD), were: Age 59 (7) yrs, 64% male, BMI 32 (4) kg/m^2, median diabetes duration 5.8 yrs, HbA1c 7.5 (0.8) %, FPG 170 (41) mg/dL. CCX140 was well tolerated by study subjects. No serious adverse events were observed in the CCX140-B groups (one syncope in placebo). There were no safety concerns regarding laboratory hematology, chemistry, or urinalysis. FPG showed a CCX140-B dose-dependent decrease through week 4. HbA1c least-squares mean changes from baseline to week 4 for the placebo, 5 mg CCX140-B, 10 mg CCX140-B, and pioglitazone groups were -0.09%, -0.09%, -0.23% (p=0.045 vs. placebo), and -0.13% (NS vs. placebo), respectively. Unlike other CCR2 antagonists, no significant changes were seen in plasma MCP-1 (CCL2) or blood monocyte count with CCX140-B treatment. Mean trough plasma levels of CCX140 were 1269 and 2355 ng/mL for the 5mg and 10mg CCX140-B groups, respectively.

A statistically significant decrease in HbA1c was observed after only 4 weeks of treatment with CCX140-B 10mg QD compared to placebo. No detrimental effects were observed on plasma MCP-1 or blood monocyte levels, and once daily oral CCX140-B provided excellent plasma coverage. The novel oral CCR2-specific antagonist CCX140-B was effective, safe and well tolerated in this study.

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Inhibition of Glucagon-Induced Hyperglycemia Predicts Glucose Lowering Efficacy of a Glucagon Receptor Antagonist, MK-0893, in Type 2 Diabetes (T2DM)

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MK-0893 is an orally active glucagon receptor antagonist being developed for treatment of T2DM. To determine appropriate doses for evaluation of proof of concept, a double-blind, placebo-controlled, glucagon challenge study was performed to assess the pharmacological activity and safety of single oral doses of MK-0893. In Part I (N=12), subjects were randomized to a sequence of 3 out of 4 possible treatments: placebo, 10-, 40-, and 200 mg doses of MK-0893. In Part II (N=6), subjects were randomized to receive a sequence of 3 possible treatments: placebo, 200- and 1000-mg doses of MK-0893. At 24 hours and at 72 (Part I) or 120 hours (Part II) post-dose, IV infusion of glucagon, somatostatin, and basal insulin were administered simultaneously and blood glucose excursion was measured over a 4-hour period. Treatment-related reduction of glucose excursion compared to placebo was assessed as a measure of functional glucagon receptor blockade. Results of this study demonstrated that MK-0893 dose-dependently blocks glucagon-induced glycemic excursions. Single doses of 200 mg and 1000 mg achieved ~59% and near maximal blockade of glucagon-induced glucose excursions, respectively.

Based on these results, two daily doses of MK-0893, modeled to achieve steady state exposures associated with ~50% and ~100% blockade of the glucagon response, were tested as monotherapy in a 4-week Phase IIa proof-of-concept study in T2DM. Change in 24 hour weighted mean glucose (WMG) was the primary endpoint. Seventy-four patients were randomized equally to placebo, MK-0893 40 mg q.d., MK-0893 120 mg q.d., or metformin 1000 mg b.i.d. Changes from baseline difference in LS mean 24 hour WMG vs pbo were 25.9 mg/dl (40mg MK-0893), 53.6 mg/dl (120mg MK-0893), and

For author disclosure information, see page 785.

26.0 mg/dl (metformin). All values had p-value <0.001. Trends for increases in LDL-cholesterol, LFTs and blood pressure were noted with 120mg MK-0893. These results suggest that glucagon receptor blockade has the potential for antihyperglycemic efficacy substantially exceeding that observed with metformin. Efficacy and potential dose-dependent safety signals were explored in larger phase 2 studies.

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TAK-875, a Novel GPR40 Agonist, Improves Both Postprandial and Fasting Hyperglycemia in Japanese Patients with Type 2 Diabetes

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GPR40 (G-protein-coupled receptor 40), mainly expressed in pancreatic β cells, mediates free fatty acid-induced insulin secretion. TAK-875, a novel and highly selective GPR40 agonist, demonstrated glucose-lowering effects in animal models of type 2 diabetes.

In this randomized, double-blind, placebo-controlled study, the pharmacological effects of once-daily dosing of TAK-875 for 2 weeks were evaluated in 65 Japanese subjects with type 2 diabetes (n=21 for placebo, n=22 for TAK-875 100 mg, and n=22 for TAK-875 400 mg). The change in $AUC_{0\text{-}3hr}$ of plasma glucose (PG) from baseline at week 2 following 75 g OGTT was significantly decreased in the TAK-875-treated groups compared with placebo ($p<0.0001$). In addition, a significantly larger effect was observed in the TAK-875 400 mg group compared to the 100 mg group ($p<0.05$) after OGTT.

After the OGTT, PG at 120 minutes was decreased in the TAK-875 treatment groups (-59.0±31.68 mg/dL for 100 mg and -85.0±52.84 mg/dL for 400 mg), vs placebo (2.0±44.17 mg/dL). Fasting PG was also significantly decreased following TAK-875 administration (-33.9±17.98 mg/dL for 100 mg and -45.6±19.55 mg/dL for 400 mg), with no change after placebo (0.0±24.20 mg/dL). Statistically significant differences in the PG level at 120 minutes and the fasting PG were observed between the placebo and TAK-875 treatment groups ($p<0.0001$). The $AUC_{0\text{-}3hr}$ of insulin was significantly increased by TAK-875 administration for 2 weeks in a dose-dependent manner ($p<0.05$). No serious adverse events occurred. Most adverse events in the TAK-875 groups were mild and not dose-dependent. No hypoglycemic episodes were observed during the study, with marked glycemic improvement in the TAK-875 groups.

These results demonstrate that TAK-875 rapidly and effectively improves both postprandial and fasting hyperglycemia with a low risk of hypoglycemia in patients with type 2 diabetes. These clinical findings are consistent with observations from nonclinical studies, suggesting that TAK-875 acts as a glucose-dependent insulinotropic agent that may be beneficial in the treatment of type 2 diabetes.

TRANSCRIPTIONAL, EPIGENETIC, AND PHYSIOLOGIC INTEGRATION DURING PREGNANCY

313-OR

Exposure to a Maternal High Fat Milieu Alters Hepatic Epigenome in Mice

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Exposure to a high fat (HF) diet in utero is associated with increased incidence of cardiovascular disease (CVD), diabetes and metabolic syndrome later in life. However, the molecular basis of this enhanced susceptibility for metabolic disease is poorly understood. We used gene expression microarray and genome-wide DNA methylation analysis to examine mRNA expression and DNA methylation patterns in liver of offspring exposed to a Control (C) or HF maternal diet. WT mice were fed a C (9.5% fat, 3.59 kcal/g) or HF (35.5% fat, 5.29 kcal/g) diet for 2 wk before mating, throughout pregnancy and lactation. Offspring were weaned to a low fat (5.6% fat, 3.4 kcal/g) diet and were sacrificed at 5wks of age. Exposure to a maternal HF milieu activated genes of immune response, inflammation, and hepatic dysfunction. Conversely genes of lipid metabolism and biogenesis were down regulated especially in the cholesterol biosynthesis pathway. DNA methylation analysis revealed 3360 differentially methylated loci, the majority of which (76%) were hypermethylated in HF liver. The distribution of DNA hypermethylation was similar in promoters, gene bodies and intergenic regions (75%, 82% and 72%, respectively). Interestingly, less hypermethylation was noted at CpG islands particularly those present in promoters (51% hypermethylation). Chromosomal distribution analysis showed hypermethylation hot spots on chromosomes 4 (atherosclerosis susceptibility QTL1) and 18 (insulin dependent susceptibility 21). Most of the hypermethylated genes in these hot spots are associated with cardiovascular system development and function. 140 differentially methylated genes showed a 1.5 fold increase or decrease in mRNA levels.

Many of these genes play a role in cell signaling pathways associated with metabolic disease. Of these metalloproteinase 9 (MMP9), whose dysregulation plays a key role in diabetes, obesity and CVD, was up-regulated 1.75 fold and hypermethylated in the gene body. In summary, exposure to a maternal HF diet significantly alters the DNA methylation and gene expression patterns in the liver of exposed offspring and contributes to programmed development of metabolic disease later in life.

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Regulation of β-Cell Mass in Pregnant Mice on a Background of Insulin Resistance

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An adaptive expansion of β-cell mass is critical for regulating glucose homeostasis in both physiological and pathophysiological states (e.g. pregnancy and insulin resistance). Recent studies report a role for serotonin in regulating β-cell mass during pregnancy. We aimed to define whether pathways active in pregnancy are independent of those induced by insulin resistance in promoting islet compensation. We used pregnant and non-pregnant, liver-specific insulin receptor knockout (LIRKO) mice, a model that shows robust insulin resistance-induced β-cell proliferation, and wild type (WT) mice at gestational (G) days 15.5, 17.5, and postpartum (P) days 0 and 4 (n=3-6). Insulin levels were consistently higher in LIRKO mice during and after pregnancy ($p<0.0001$), while glucose levels were elevated in LIRKOs only on days 15.5 to P0. To assess proliferation we immunostained pancreases for Ki67+/insulin+ cells and analyzed β-cell mass. Ki67+ β-cells were higher in non-pregnant and pregnant LIRKO mice with a 4-fold increase at G15.5 and G17.5. The proliferation reduced to control levels post-partum. Islet hyperplastic responses were different between groups with the LIRKOs showing a 2-fold increase in the basal state and peaking at P0 (LIRKO, 2.16%; WT 0.69%; n=3-4). While the number of serotonin+/insulin+ cell were 2-fold greater in non-pregnant LIRKOs (LIRKO, 0.12%; WT, 0.28%; n=6), during pregnancy, the double+ cells reached a peak at G15.5 (1.6 fold greater in LIRKOs, $p<0.05$; n=3-4) and remained significantly high at P4 (LIRKO, 62.9%; WT 8.0%; n=3-4). Consistently plasma serotonin levels were elevated in LIRKOs (159.1±36.1 vs 48.2±6.4 ng/ml; n=2). These data support a β-cell proliferation reserve even in models that exhibit insulin resistance-induced hyperplasia, and suggest that serotonin and insulin resistance act additively to promote β-cell hyperplasia in the LIRKOs during pregnancy. Interestingly, α-cells showed low proliferation in the LIRKOs during pregnancy. Our data indicate that the serotonin pathway is involved in β-cell proliferation that is independent of insulin resistance and has important therapeutic implications for enhancing β-cell mass in states of β-cell deficiency.

315-OR

Differential Expression of Placental 11β-Hydroxysteroid Dehydrogenases as a Key Determinant of Fetal Environment in Pregnancies with Gestational Diabetes Mellitus

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Fetus exposure to excess glucocorticoid is one of critical etiological factors for fetal origins of adult diseases. However, the mechanism of the local regulation of glucocorticoid activity in human placenta of pregnancies complicated with gestational diabetes mellitus (GDM) has not been fully understood. In this study, we aimed to investigate placental 11β-hydroxysteroid dehydrogenases (11β-HSDs) expression, and analyze their relationship with maternal and fetal cortisol levels in pregnancies of GDM. Age matched pregnant women with GDM or normal glucose tolerance were recruited for this community-based study. We collected maternal and umbilical venous cord blood and placental tissues from both groups. Explanted placentas from NGT were cultured with palmitic acid, dexamethasone, insulin or their mixture for 24-h. We examined cortisol and insulin levels, homeostasis model assessment of insulin resistance index (HOMA-IR) and insulin secretion index. Quantitative real-time PCR, Western blot and immunohistochemical assay were applied for measurement of 11β-HSD1 and 11β-HSD2 mRNA and protein. GDM had higher maternal cortisol levels, HOMA-IR and insulin secretion index. No significant change in fetal cortisol levels and newborn weight was found. GDM placental

For author disclosure information, see page 785.

11β-HSD1 was reduced while11β-HSD2 was increased. Treatment of placenta explants from NGT with palmitic acid, dexamethasone, insulin or their combination resulted in a significant drop of 11β-HSD1 and increase in 11β-HSD2. Our studies demonstrated that differential expression of 11β-HSDs in GDM placenta provides a protective mechanism for fetus to prevent against adverse maternal environment during pregnancy by limiting excessive exposure of fetus to glucocorticoids.

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Placental Phospholipid Transfer Protein Is Expressed by Fetal Endothelial Cells and Activated by Gestational Diabetes Mellitus

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Gestational diabetes mellitus (GDM) has been associated with fetal hypercholesterolemia as well as changes in fetal lipoprotein composition. The main lipoproteins in the fetal circulation are, in contrast to adults, high density lipoproteins (HDL). Many key genes involved in transplacental cholesterol transfer and HDL metabolism are under transcriptional control of nuclear liver X-receptors (LXRs). Phospholipid transfer protein (PLTP) enhances cholesterol efflux to HDL, contributes to HDL remodeling, is an LXR target gene and is highly expressed in the placenta. We hypothesized that oxysterols, as key regulators in chosterol metabolism and endogenous LXR ligands, are elevated in GDM thus resulting in an upregulation of LXR target genes such as PLTP in the placenta.

GC-MS measurements of different oxysterol species demonstrated a significant (1.5-fold, $p < 0.01$) elevation of enzymatically produced 27-hydroxycholesterol in GDM offspring. Additionally, a pronounced elevation of oxysterols formed by reactive oxygen species such as 7α-, 7β-hydroxycholesterol, 7-ketocholesterol (> 1.7-fold, $p < 0.05$) was detected in fetal plasma of GDM subjects. These may have been generated *in vivo* but may also reflect a greater antioxidative potential of sera of non-diabetic offspring. As measured by qPCR, PLTP was 1.8-fold ($p < 0.05$) upregulated in total placental tissue of pregnancies complicated by GDM. Its predominant vascular location as identified by immunohistochemistry suggests that diabetes associated changes occur in the placental endothelial cells. High insulin and/or glucose treatments of isolated placental endothelial cells of the fetal surface resulted in increased PLTP protein levels (> 1.8-fold, $p < 0.01$) and PL transfer activity (1.2-fold, $p < 0.01$) in cell supernatants.

We conclude that elevated fetal plasma oxysterol levels may increase maternal-to-fetal transfer of cholesterol in maternal diabetes. In addition, local cholesterol homeostasis in the placental vasculature may be modified in the wake of the fetal diabetic environment where PLTP may play an important role.

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Novel Vascular Effects of IGF2 on Human Fetoplacental Endothelium

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Gestational diabetes is associated with increased insulin and insulin-like growth factor 2 (IGF2) levels in the fetal compartment. IGF2 is an important growth factor for fetal development. Its signaling receptors, i.e. the insulin receptor isoform A and the IGF1-receptor are expressed on the placental endothelium but the role of IGF2 on the fetoplacental vasculature is not yet understood. We hypothesized that IGF2 exerts pro-angiogenic effects on the fetoplacental endothelial cells by activating a range of intracellular processes.

IGF2 (10nM) and insulin (10nM) effects were analysed using primary placental arterial endothelial cells (PLAEC). Whole genome microarray determined their effect on gene expression. eNOS phosphorylation and VEGF expression were measured by immunoblot and sqRT-PCR, respectively. As functional endpoint *in vitro* angiogenesis was assayed by 2D-network formation assay. Endothelial permeability was assessed by measuring electrical impedance using an ECIS (Electric Cell-substrate Impedance Sensing) electrode assay.

IGF2 significantly ($p<0.05$) regulated expression of 373 genes. Among these, phospholipase A2 (PLA2), responsible for prostacyclin production leading to vasodilation, was down-regulated. Conversely, insulin up-regulated PLA2 expression. IGF2 treatment of PLAEC in matrigel significantly increased total tube length and the number of branching points of the network by 20% each, similar to insulin. Moreover, IGF2 induced an increase in eNOS phosphorylation (+17%, $p<0.01$) and in VEGF mRNA expression (+15%, $p<0.035$). Electrical impedance and, hence, permeability of PLAEC monolayer was decreased by the presence of IGF2. Insulin also leads to an increase in eNOS phosphorylation and VEGF expression as well as to a decrease in endothelial permeability but not as pronounced as IGF2.

We conclude that IGF2 is a potent regulator of vascular functions in placental endothelial cells. This includes angiogenesis stimulation and decrease of vascular permeability, the last has not been described in any endothelial cell type before. Elevated fetal IGF2 levels in pregnancies complicated by diabetes may hence cause abnormal vascular development and function of the placenta.

318-OR

Type 1 Diabetes Is Associated with Impaired Placental MMP Expression in Early Pregnancy

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In the first trimester of pregnancy placental trophoblast cells invade the maternal uterine wall in order to anchor the placenta and to establish adequate utero-placental blood flow by remodelling of the uterine arteries. Therefore, trophoblasts are well equipped with matrix degrading enzymes i.e. matrix metalloproteinases (MMPs). Maternal T1D is associated with a higher risk for early pregnancy loss, pre-eclampsia and fetal growth restriction. As underlying reason we hypothesise impaired trophoblast invasion. Because placental changes in early gestation are difficult to determine, indirect evidence is needed to demonstrate changes in trophoblast invasion. The aim of the present study was to identify changes in MMP expression in first trimester placentas from healthy vs. T1D pregnancies. Additionally, the effect of low oxygen on MMP expression was determined in a first trimester trophoblast cell line (ACH-3P).

First trimester placentas from normal (n=15) vs. T1D (n=12) pregnancies between gestational week 6 and 10 were obtained after pregnancy interruption for psycho-social reasons and immediately frozen in liquid nitrogen. Total RNA was isolated and mRNA expression of all 23 human MMPs was measured using sqRT-PCR and analyzed according to the gestational weeks. Expression signals were normalized to the trophoblast marker cytokeratin 7. ACH-3P cells were cultured under various oxygen concentrations (2.5, 5, 12, 21) for seven days and expression of MMPs was measured similarly.

In the first trimester, the placenta expresses 14 MMPs. T1D was associated with a reduced expression of 11 MMPs in weeks 8-10. Hypoxia reduced expression of various MMPs in ACH-3P cells.

This is the first study to demonstrate altered placental MMP expression during early human pregnancy as a result of maternal T1D. T1D is associated with a decrease of MMP expression which may hence impair the process of trophoblast invasion. Lower uterine oxygen concentrations as a result of diabetes may be involved in the impaired MMP expression. These results may explain the higher incidence of spontaneous abortions in T1D women and their higher risk for pre-eclampsia and IUGR in late pregnancy.

Supported by: OeNB 13307

TYPE 2 DIABETES ETIOLOGY

319-OR

Risk of Diabetes with Tamoxifen Treatment in Older Breast Cancer Survivors

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Tamoxifen is used as adjuvant treatment for estrogen-receptor positive breast cancer and has been shown to reduce breast cancer recurrence. Tamoxifen may promote the risk of diabetes via estrogen-receptor antagonism and decreased insulin secretion. This study aimed to determine whether the risk of diabetes is increased in breast cancer survivors treated with tamoxifen.

Using population-based anonymized health care databases in Ontario, Canada, we identified women over age 65 years with invasive breast cancer who underwent breast surgery between April 1994 and March 2007. We compared the risk of diabetes between women treated with and without tamoxifen using a nested case control approach. Cases were defined as cohort members diagnosed with diabetes from cohort entry until March 2008, and each case was age-matched with up to 5 controls who did not

For author disclosure information, see page 785.

develop diabetes. Exposure to tamoxifen was based on prescription records at diabetes diagnosis (index) date, and current users (prescription within < 14 days of index) were compared to non-users (no prescription for > 5 years). Adjustments were made for other risk factors. We also compared risk of diabetes between current aromatase inhibitor users versus non-users.

Of 8,665 women breast cancer survivors identified, 1,445 (17%) developed diabetes over a mean (SD) follow-up of 3.46(2.56) years. Of these cases, 531 (37%) were prescribed tamoxifen and 127 (9%) were prescribed an aromatase inhibitor. Current tamoxifen therapy was associated with a significantly higher risk of diabetes compared to no tamoxifen therapy (adjusted odds ratio, aOR 1.23, 95% confidence interval, CI 1.08-1.41, P=0.0027). There was no association between aromatase inhibitor therapy and diabetes.

Current tamoxifen therapy was associated with an increased risk of diabetes in older breast cancer survivors. These findings suggest that tamoxifen treatment may exacerbate an underlying risk of diabetes in susceptible women, and further studies are needed to better explore this association.

Supported by: Ontario Institute for Cancer Research

320-OR

Risk of Incident Diabetes on Intensive Compared to Moderate Dose Statin Therapy: A Collaborative Meta-Analysis of Randomized Trials

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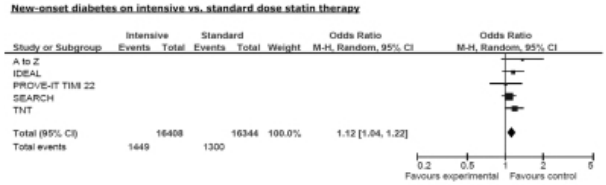
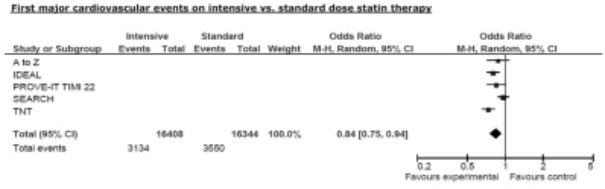
A recent meta-analysis demonstrated that statin therapy is associated with an excess risk of developing diabetes. Whether any such relationship exists between intensive statin therapy and new-onset diabetes compared to moderate dose therapy is unclear.

We searched Medline, Embase and the Cochrane Central Register of Controlled Trials from 1996 to 2010 for randomized controlled endpoint trials including more than 1000 patients with identical follow-up in both arms and duration of more than 1 year. Using published and unpublished data, we calculated trial-specific risk estimates for patients developing diabetes and experiencing major cardiovascular events (cardiovascular death, non-fatal myocardial infarction or stroke, coronary revascularization), and combined these using random-effects model meta-analysis. Between-study heterogeneity was assessed using the I^2 statistic.

We identified five statin trials with 32,752 participants without diabetes, of whom 2,749 (1,449 assigned intensive therapy, 1,300 assigned standard therapy) developed diabetes and 6,684 (3,134 and 3,550 respectively) experienced cardiovascular events over an average follow-up of 4.9 years. Intensive statin therapy was associated with a 12% higher risk for new-onset diabetes (odds ratio [OR] 1.12; 95% CI 1.04–1.22; I^2=0%) and 16% reduction in cardiovascular events (OR 0.84; 95% CI 0.75-0.94; I^2=74%). For every three fewer patients experiencing major cardiovascular events on intensive therapy, one additional case of diabetes occurred.





There is a dose-dependent relationship between statin therapy and new-onset diabetes.

While intensive statin therapy reduces cardiovascular events in high risk patients, the associated risk of new-onset diabetes suggests that such patients should be monitored for the development of diabetes.

321-OR

Gender Differences in the Association of Liver Markers with Incident Type 2 Diabetes (T2DM): The IRAS Family Study

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The liver markers alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT) are increased in those with central obesity, insulin resistance and hepatic steatosis, and they have been prospectively associated with increased risk of T2DM. However, limited data are available from Hispanic (HA) and African American (AA) cohorts, and few studies have evaluated the association of these markers with incident T2DM after adjustment for direct measures of visceral adipose tissue (VAT) or insulin sensitivity (S_I). Our objective, therefore, was to study the association of ALT, AST and GGT with incident T2DM after adjustment for confounders including directly measured VAT and S_I in 1070 HA and AA subjects free of DM at baseline (2000-02). S_I was determined from frequently sampled IV glucose tolerance tests with minimal model analysis. VAT was determined by CT. T2DM was defined using 2003 ADA criteria, and liver markers were assayed using standard methods. Generalized estimating equations were used to account for familial correlations. Eighty five subjects had incident T2DM after 5 y. After adjustment for age, sex, ethnicity, and alcohol, liver markers were not significantly associated with T2DM (ALT: OR x 1SD unit difference=1.12 (95% CI 0.92–1.36); AST: OR=1.06, (0.89-1.27); GGT: OR=1.06, (0.88-1.27)). There were no significant interactions of ethnicity, sex or impaired fasting glucose (IFG) on these associations, except for an interaction of ALT with sex (p<0.05), indicating a stronger association of ALT with T2DM in women vs. men (OR=1.64 (1.03-2.60) vs. OR=0.95 (0.62-1.47)). The association in women was significant after additional adjustment for IFG, triglyceride, HDL and systolic BP (OR=1.87 (1.13-3.10)), as well as S_I and VAT (OR=1.77 (1.02-3.07)). In conclusion, ALT, AST and GGT were not associated with incident T2DM in HA and AA subjects. A gender-ALT interaction was present, however, and ALT was associated with T2DM in women after adjustment for confounders including insulin resistance and visceral obesity. This finding suggests that gender differences may exist in the impact of liver fat on diabetes pathogenesis.

322-OR

Proinsulin-to-C-Peptide Ratio Versus Proinsulin-to-Insulin Ratio in the Prediction of Incident Type 2 Diabetes (T2DM): In the Insulin Resistance Atherosclerosis Study (IRAS)

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Most previous studies assessing proinsulin (PI) levels and incident T2DM have used PI-to-insulin ratios, which may be affected by variations in the hepatic clearance of insulin resulting from insulin resistance (IR). C-peptide is not similarly impacted by differences in hepatic clearance, although few studies have investigated the association of PI-to-C-peptide ratios with incident T2DM. Our objective, therefore, was to compare fasting intact and split PI-to-insulin ratios (PI/I, SPI/I) with intact and split PI-to-C-peptide ratios (PI/C-pep, SPI/C-pep) in the prediction of incident T2DM. The study included 818 subjects who were non-diabetic at baseline. Insulin sensitivity (S_I) and acute insulin response (AIR) were determined from frequently sampled intravenous glucose tolerance tests, and fasting intact and split PI were measured using highly specific two-site monoclonal antibody-based immunoradiometric assays. Associations of PI ratios with T2DM were determined using logistic regression and differences in prediction were assessed by comparing areas under the receiver operating characteristic curves (AROC). PI/C-pep and SPI/C-pep were more strongly associated with incident T2DM than PI/I and SPI/I and were significantly better predictors of T2DM in AROC analyses (PI/C-pep=0.662 vs. PI/I=0.603, p=0.02; SPI/C-pep=0.690 vs. SPI/I=0.631, p=0.01). Both PI/C-pep and SPI/C-pep were associated with T2DM after adjustment for age, sex, ethnicity, waist, impaired glucose tolerance (IGT) and S_I. Differences in associations between the two sets of ratios were especially pronounced in subjects with IR. Finally, both PI/C-pep and SPI/C-pep were significantly associated with incident T2DM in models that included AIR (OR=1.46 (1.17-1.82) and OR=1.73 (1.36-2.21), respectively, both p<0.001). In conclusion, PI/C-pep ratios were stronger predictors of T2DM in comparison with PI/I ratios and the differences were most profound in subjects with IR. These findings support the use of C-peptide as the denominator for PI ratios to more accurately reflect the degree of disproportionate hyperproinsulinemia.

For author disclosure information, see page 785.

323-OR

Age at Menarche and Physician-Diagnosed Type 2 Diabetes Mellitus in Chinese Singaporeans: The Singapore Chinese Health Study

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The association between age at menarche and type 2 diabetes (T2D) remains unclear, especially in Asian populations. We examined the association between age at menarche and T2D within a prospective cohort study of Singaporean Chinese women aged 45-74 at baseline in 1993-1998. After excluding participants who had missing information on age at menarche (n=6), were free of diabetes at baseline but did not complete follow-up interview (n=5,677), reported having physician-diagnosed diabetes at less than 30 years of age (n=19), or had an unclear diabetes status based on a validation study (n=11), we included 28,315 women in the present analyses. There were 4,398 cases of diabetes (3,090 prevalent and 1,308 incident). Odds ratios and 95% confidence intervals for T2D were computed across the age range of menarche and adjusted for baseline factors: age, year of interview, dialect, physical activity, alcohol use, smoking, educational level, oral contraceptive use, age at first delivery, parity, menopause status, and use of hormone replacement therapy. Median age at menarche was 13.5 years. Report of menarche at age 12 or younger was associated with increased odds of developing T2D as an adult. Alternatively report of menarche at age 15 or older was associated with reduced odds of developing T2D (see table). The association was only partially mediated by adjustment for baseline adult body mass index. These data suggest that age at menarche is an indicator of diabetes risk as an adult. Life-course studies with measures of reproductive hormones, body composition, and related biologic measures will best elucidate the potential causal mechanisms.

Odds ratios (ORs) and 95% confidence intervals (CIs) for type 2 diabetes by age at menarche in Chinese Singaporeans

	Age at Menarche (year)				
	≤12	13-14	15-16	≥ 17	*P* for Trend
n	4,181	10,928	9,665	3,541	
No. of cases	598	1,698	1,479	623	
Age at diabetes diagnosis, years[a]	52.0 (9.19)	54.3 (9.28)	56.3 (9.13)	57.8 (9.22)	
Multivariable OR (95% CI)	1.21 (1.08-1.35)	1.00	0.80 (0.73-0.86)	0.80 (0.72-0.89)	<.0001
Multivariable (w/BMI) OR (95% CI)	1.17 (1.05-1.31)	1.00	0.82 (0.76-0.89)	0.83 (0.74-0.92)	<.0001

[a] Mean (standard deviation)

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324-OR

WITHDRAWN

325-OR

Prospective Association of Vitamin D with Beta-Cell Function and Glycemia

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Emerging evidence suggests that vitamin D (25-hydroxyvitamin D, 25(OH)D) may be involved in the development of type 2 diabetes (T2DM). However, few studies have assessed the prospective association of 25(OH)D with insulin resistance or beta(β)-cell function. Our objective, therefore, was to examine the association of baseline 25(OH)D with insulin resistance, β-cell function and glucose homeostasis longitudinally in subjects at high T2DM risk.

We followed 483 individuals, age 50±10y, for 3 years. Fasting blood samples were collected, and 75g oral glucose tolerance tests (OGTT) were administered at baseline and follow-up. Insulin sensitivity was measured using the Matsuda index (IS_{OGTT}) and HOMA-IR. β-cell function was determined using both the insulinogenic index divided by HOMA-IR (IGI/IR) and the Insulin Secretion Sensitivity Index-2 (ISSI-2). Glycemia was assessed using the area under the glucose curve ($AUC_{glucose}$) during the OGTT. Regression models were adjusted for age, sex, season, ethnicity, baseline level of the outcome variable, PTH, and baseline and change in physical activity, vitamin D supplement use, and BMI.

The baseline mean serum 25(OH)D concentration was 57.75 ± 23.12 nmol/L. Multivariate linear regression analyses indicated no significant association of baseline 25(OH)D with IS_{OGTT} or HOMA-IR at follow-up (β=0.001, p=0.46 and β=-0.001, p=0.54 respectively). There were, however, significant positive associations of baseline 25(OH)D with both IGI/IR (β=0.004, p=0.037) and ISSI-2 (β=0.002, p=0.054) at follow-up, and a significant inverse association of baseline 25(OH)D with $AUC_{glucose}$ at follow-up (β=-0.0009, p=0.026) after multivariate adjustment. Progression to dysglycemia (impaired fasting glucose, impaired glucose tolerance or T2DM) occurred in 115 subjects. There was a significant reduced risk of progression with higher baseline 25(OH)D (OR=0.72, 95% CI 0.55-0.95, p=0.02), but this association was attenuated after adjustment for baseline and change in BMI (OR=0.80, 95% CI 0.60-1.07, p=0.13).

In conclusion, higher baseline 25(OH)D independently predicted better β-cell function and lower $AUC_{glucose}$ at follow-up, supporting a potential role for vitamin D in T2DM etiology.

326-OR

Impaired Glucose Tolerance and Obesity as Modifiers of the Excess Risk of Diabetes in Mexican Americans: The San Antonio Heart Study (SAHS)

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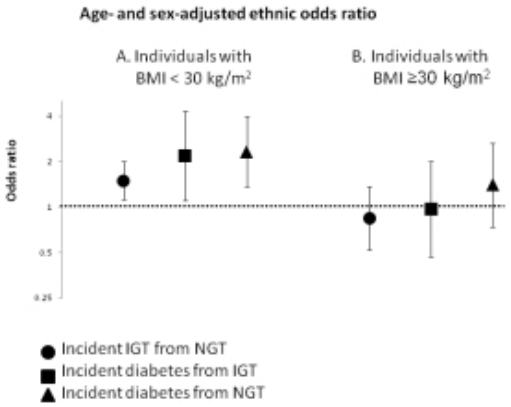
Mexican Americans have a 2.5-fold increased incidence of diabetes relative to non-Hispanic whites. Studies among individuals at high risk of diabetes (those with impaired glucose tolerance [IGT] and increased adiposity) have reported that Hispanics and non-Hispanic whites have similar incidence of the disease. Thus, we hypothesized that the excess incidence of diabetes in Mexican Americans could be more pronounced in the early (from normal glucose tolerance [NGT] to IGT) than in the latter stages of the conversion process (from IGT to diabetes) or more among the lean than among the obese. We examined risk of conversion to diabetes in 3,228 participants in the SAHS (aged 40 - 65 years). The 2003 ADA criteria were used to define NGT, IGT, and diabetes. During the 7.7-year follow-up period, 226 of 1,996 (11.3%) Mexican Americans and 69 of 1,232 (5.6%) non-Hispanic whites developed diabetes. The age- and sex-adjusted difference in the ethnic odds of developing diabetes was 2.42 (1.81 – 3.21), but was 2.64 (1.79 – 3.91) among non-obese individuals, and 1.42 (0.92 – 2.20) among obese individuals. Ethnic differences in developing IGT from NGT, diabetes from IGT, and diabetes from NGT were 1.41 (1.10 – 1.80), 1.61 (1.01 – 2.58), and 2.51 (1.51 – 3.36), respectively. These ethnic differences were statistically significant among non-obese individuals but not among obese counterparts.

For author disclosure information, see page 785.



In summary, among non-obese individuals, Mexican Americans have an excess risk of diabetes. This ethnic difference is not more pronounced in the early stage of the disease development. Among obese individuals, non-Hispanic whites lose much of the ethnic advantage. These findings have important implications for clinical practice and genetic and epidemiological studies.

Supported by: NIH NHLBI (RO1-HL24799 and RO1-HL36820)

NEW THERAPIES FOR TYPE 1 DIABETES

327-OR

WITHDRAWN

328-OR

WITHDRAWN

329-OR

Teplizumab Treatment Induces Migration of Human T Regulatory Lymphocytes to the Small Intestine In Vivo

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Trials of Teplizumab(anti-CD3 mAb) in type 1 diabetes have provided proof of concept of this approach for immunotherapy. To determine the mechanism of action of Teplizumab we have utilized a humanized mouse model to simulate a human clinical trial.

CD34+ stem cells were isolated from human umbilical cord blood and engrafted into neonatal NOD/SCID/IL2g^{null} mice to reconstitute human immune systems. Mice were treated with a single dose of Teplizumab or human Ig as control. After treatment, peripheral blood, lung, spleen, bone marrow and gut were analyzed to determine the effects of Teplizumab treatment on migration and function of cells.

Prior to treatment with Teplizumab, the proportion of hCD45+ cells engrafted in circulation was(N=22) 33.72%±24.71(Mean±SD). After a single dose of Teplizumab there was a decrease in circulating CD4+ T cells in the peripheral circulation(N=11) 11%±18.75 vs (N=11)26.19% ± 28.1 in hIg treated mice($p<0.05$) and spleen (12.74%±18.1 vs 30.1%±28.42;$p<0.05$). This was associated with migration of hCD45+ cells to the small intestine in the Teplizumab group only in which 4.39%$\pm$3.92 of the cells were hCD45+ vs 0.44%$\pm$0.33 in controls($p<0.01$). No migration of hCD45+ cells to lung or bone marrow was observed. Ex vivo and in vitro studies demonstrated that Teplizumab treatment induces expression of the gut homing chemokine receptor CCR6 on T cells which migrate to the gut in response to increased expression of CCL20 by the small intestine. Quantitative PCR analysis of hCD4+ and hCD8+ T cells in the small intestine demonstrated that they have increased expression IL-10 and FoxP3 as compared to controls($p<0.05$). In patients with T1DM, treated with Teplizumab, we found a decrease in the number of circulating CD4CD25^{high}CD127^{low}CCR6+ cells that rapidly recover post treatment, suggesting that these Tregs transiently migrate to the GI tract during treatment.

These findings suggest that the transient lymphopenia observed in clinical trials of Teplizumab may be due to the margination of CCR6+ lymphocytes and Tregs to the small bowel. They indicate that the gastrointestinal tract in humans may play a role in tolerance induction by Teplizumab.

For author disclosure information, see page 785.

330-OR

Multi-Drug Combination Therapy Increases the Frequency of Regulatory T- and B-Cells in NOD Mice with Established Disease

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There is a growing list of agents capable of not only preventing, but also reversing recent-onset diabetes in NOD mice. However, outside of islet transplantation, few if any protocols can effectively reverse established disease in this animal model. We hypothesized that a combination therapy of agents (1) modulating immunological tolerance [anti-thymocyte globulin (ATG), granulocyte colony stimulating factor (GCSF)] and (2) enhancing β-cell replication, [dipeptidyl peptidase-4 inhibitor (DPP-4i) and a proton pump inhibitor (PPI)] would reverse T1D in new onset and established diabetes. Female NOD mice followed for T1D onset (BG >240 mg/dL on 2 consecutive d) were randomized into either (1) new onset or (2) established disease (14 days post diagnosis on insulin pellet) groups. Mice were randomized to receive concomitantly either ATG+GCSF, DPP-4i+PPI, or ATG+GCSF+DPP-4i+PPI (n=12-13/group). Appropriate control groups were utilized (n=11-12/group). Animals that remained euglycemic were followed up to 120 d post therapy. All three combination therapies led to beta cell preservation in both new onset and established mice (increased beta cell mass and reduced insulitis vs. controls). All treatment groups also showed significant increases in regulatory cells of various phenotypes when compared to controls: an average 1.9 fold increase in Tregs (CD4+CD25+FoxP3+) (P<0.0001), 3 fold in CD8 Tregs (CD8+CD25+FoxP3+) (P=0.0015), 1.9 fold in regulatory B cells (B220+CD5+CD1dhiCD21+, Breg) (P= 0.0008), and 1.7 fold in CD4 memory T cells (CD4+CD44hiCD62Llo, Tm) (P=0.0007). Furthermore, an average 1.7 fold increase in CD4 Tregs, 5.36 fold in CD8 Tregs, 2.24 fold in CD4 Tm, and 1.68 fold in Bregs was observed in mice that reversed compared to those that did not. No significant differences in marginal zone B cells were seen between treated and untreated mice. In conclusion, these results suggest that this multi-drug combination therapy is a robust approach for attenuating autoimmunity leading to beta cell preservation/replication.

331-OR

Combination Therapy with Anti-IL1β Antibodies and Islet Auto-Antigen GAD65 Reverts Recent-Onsent Diabetes in the RIP-GP Mouse Model

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Type 1 diabetes (T1D) is an autoimmune condition in which self-reactive T cells attack pancreatic β cells secreting insulin. Since interleukin (IL)-1β is a proinflammatory cytokine produced by several cell types, including β cells when exposed to high glucose concentrations, one therapeutic avenue for treating T1D would be to neutralize this cytokine temporarily. This could be combined with islet autoantigen specific approaches to increase efficacy and maintain tolerance to β cell antigens, as our laboratory recently showed using combination therapies with anti-CD3 and glutamic acid decarboxylase of 65 kd (GAD65)-expressing plasmids.

Presently, we investigated whether treatment for 5 weeks with anti-IL1β antibodies alone or in combination with GAD65 DNA vaccines could reverse disease development in rat insulin promoter-glycoprotein (RIP-GP) transgenic mice that turned diabetic upon lymphocytic choriomeningitis virus (LCMV) infection (RIP-LCMV-GP model for T1D). Our results show that anti-IL1β monotherapy could protect 25% of mice from T1D, but that combination-therapy with neutralizing IL-1β antibodies and GAD65 DNA could reverse disease in all animals. Reversion of hyperglycemia was associated with preservation of insulin-producing islets and decreased production of IFN-γ and TNF-α by GP33 peptide-specific ('autoreactive' in the RIP-LCMV model) CD8 effector T cells in the spleen and pancreatic lymph nodes. Our findings suggest that IL-1β blockade alone can reverse recent-onset diabetes to a certain extent, but that combination therapy with GAD65 DNA vaccines permits a marked improvement of clinical and immunological aspects of the disease. These data hold promise for the treatment of new-onset T1D patients with combination therapies involving blockade of IL-1β.

332-OR

Rapamycin Plus IL-2 Combination Therapy in Subjects with T1D Results in a Sustained Increase in IL-2 Responsiveness and a Transient Decrease in C-Peptide Levels

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Treatment of NOD mice with combination of rapamycin and IL-2 prevents spontaneous and recurrent autoimmune diabetes through selective inhibition of effector responses and promotion of Treg. Correlative immune phenotyping and functional studies were performed as part of a rapamycin plus IL-2 Phase I clinical trial in T1D subjects to determine the impact on Treg and effector populations. T1D subjects (n=9) were antibody positive adults within 4 years of diagnosis who had peak C-peptide after MMTT ≥ 0.4pmol/ml. The IL-2 was administered as a SC injection $4.5x10^6$ IU three times weekly for a total of 12 doses and rapamycin given orally for three months to achieve trough blood levels between 5-10 ng/ml. Composition of hematopoietic populations in peripheral blood and functional phenotypes of T cells were assessed by multi-color surface, intracellular and phospho flow cytometry. Treg and NK cell numbers transiently increased in peripheral blood following treatment, as has been observed in other settings upon IL-2 therapy.

% in PBMC	CD25+ Treg	CD56+ NK
Pre-treatment	4.05+/-4.15	4.82+/-4.15
1 month	14.32+/-2.41	11.25+/-5.16
2 months	3.53+/-2.74	5.01+/-1.27

We have previously shown that samples from subjects with T1D have impaired responsiveness to IL-2 as compared to controls. Interestingly, response to IL-2 as measured by phosporylation of STAT5 increased upon treatment and was sustained even a year following therapy as compared to pre-treatment in 5 out of 6 patients tested. This persistent enhancement of IL-2 responsiveness occurred in the $CD25^+$ Treg population, but also in the NK and $CD25^-$ CD4 and CD8 T cell populations. C-peptide levels measured at 3 months after initiation of therapy dropped in all subjects. These data suggest that rapamycin plus IL-2 combination therapy as given in this Phase 1 study does not promote Treg in isolation, but also increases NK and effector T cell responsiveness to IL-2.

Supported by: Immune Tolerance Network, N01-A1-15416 (to J.B.)

METABOLIC REGULATORS OF INSULIN SENSITIVITY

333-OR

Impaired Insulin Signaling in the Endothelial Cells Reduces Insulin-Induced Glucose Uptake by the Skeletal Muscle

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In subjects with type 2 diabetes and obesity, insulin delivery to and insulin-dependent glucose uptake by the skeletal muscle are known to be delayed and impaired. However, the mechanisms involved in such delay and impairment are not yet clearly understood. To clarify this issue, we generated mice with endothelial-cell-specific knockout of Irs2 (ETIrs2KO mice), which is one of the major Irs isoforms expressed in the endothelial cells. Irs2 deletion in the endothelial cells causes an insulin signaling defect in the cells that results in impairment of insulin-induced eNOS phosphorylation, capillary recruitment and increase of the interstitial concentrations of insulin, consequently impairing the SKM glucose uptake. We next investigated whether an insulin signaling defect in the endothelial cells may also be implicated in the high-fat (HF) diet-induced, obesity-linked SKM insulin resistance. The expression levels of Irs1 were modestly reduced and those of Irs2 were markedly reduced in the endothelial cells of the HF diet-fed obese mice. Insulin-induced eNOS phosphorylation, capillary recruitment and insulin delivery were markedly impaired in the HF diet-fed obese mice, similar to the observation in the ETIrs2KO mice.

To determine whether restoration of insulin-induced eNOS phosphorylation in the endothelial cells might restore glucose uptake by the SKM, we administered a stable prostaglandin $(PGI)_2$ analogue to the ETIrs2KO and HF diet-fed mice; this agent has been reported to increase the expression levels of eNOS mRNA and protein. Restoration of the insulin-induced eNOS phosphorylation in the endothelial cells completely reversed the reduction in the capillary recruitment and insulin delivery in the ETIrs2KO and HF diet-fed mice, and as a result, significantly restored glucose uptake by the SKM. Taken together, insulin signaling in the endothelial cells plays a pivotal role

For author disclosure information, see page 785.

in the regulation of glucose uptake by the SKM. Improving endothelial insulin signaling may serve as a novel therapeutic strategy for ameliorating SKM insulin resistance.

334-OR

Knockdown of HIF-1alpha Abrogates Insulin-Stimulated Glucose Uptake in the Skeletal Muscle Cells

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Background and aims: Insulin resistance in the skeletal muscle is known to be associated with functional defects in insulin signaling. Molecular mechanism determining glucose uptake by skeletal muscle in response to insulin, however, remains largely unknown. The hypoxia-inducible factor-1α (HIF- 1α) is a transcription factor operating in the cellular homeostatic regulation under hypoxic conditions. Recent studies have uncovered pleiotropic actions of HIF-1α in response to various cellular stimuli including insulin under normoxic condition and we thus examined the role of HIF-1α in regulation of glucose uptake by the skeletal muscle cells. To this end we generated C2C12 myocytes in which HIF-1α is knocked-down by a stable transfection with HIF-1α shRNA expression plasmids (ΔHIF-1α myocytes) and determined the glucose uptake rate of the cells treated with insulin by monitoring the influx of 2-deoxy glucose. Results: Upon the treatment with insulin, C2C12 wild type myocytes increased uptake of 2-DG by 2-fold compared to the basal level. On the other hand, knockdown of HIF-1α expression in the myocytes resulted in abrogation of insulin-dependent glucose uptake. Accordingly, in ΔHIF-1α myocytes translocation of GLUT4 to plasma membrane was severely inhibited even under treatment with insulin. Furthermore, *ex vivo* glucose uptake by the skeletal muscles of heterozygote of HIF-1α gene knock-out mice was significantly reduced compared to wild type animal. The Akt substrate of 160 kDa (AS160) is known to be responsible for regulation of GLUT4 vesicle transport. In the wild type myocytes efficient phosphorylation of AS160 by insulin was observed. Contrary, in ΔHIF-1α myocytes phosphorylation of AS160 by insulin treatment was significantly reduced. On the other hand, the cells expressing constitutively-active HIF-1α showed phosphorylation of AS160 and upregulation of GLUT4 translocation even in the absence of insulin. Of interest, insulin enhanced HIF-1α expression in the wild type myocytes. Conclusion: we propose a novel aspect of HIF-1α as a determinant for insulin sensitivity in the skeletal muscle thus as a possible target to alleviate insulin resistance in type 2 diabetes.

335-OR

AMPK Directly Phosphorylates SREBP-1c at Ser 372, Suppresses Hepatic *De Novo* Lipogenesis, and Prevents Hepatic Steatosis in Diet-Induced Insulin Resistant Mice

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The dysregulation of hepatic SREBP-1c, a key lipogenic transcription factor, has been implicated in the pathogenesis of hepatic steatosis and type 2 diabetes. We have previously demonstrated that resveratrol and the synthetic polyphenol S17834 persistently activate AMP-activated protein kinase (AMPK), which may explain their beneficial effects on diabetic dyslipidemia *in vivo*. However, little is known about the downstream signaling of AMPK in regulating lipogenesis and type 2 diabetes. Here, we characterize SREBP-1c as a new target of AMPK via phosphorylation. *In vitro* kinase assays, mutagenetic studies, and immunoblots with a newly developed phospho-specific SREBP-1c antibody indicated that Ser372 of SREBP-1c was directly phosphorylated by purified active AMPK *in vitro* and by AMPK activators in cultured cells. Ser372 phosphorylation of SREBP-1 was stimulated by resveratrol and metformin in $AMPK^{+/+}$MEFs, and these effects were abrogated in $AMPK^{-/-}$MEFs. AMPK activation by polyphenols inhibited the cleavage processing of myc-tagged wild type SREBP-1c in HEK293T cells. Ectopic expression of SREBP-1c mutant, in which Ser372 was substituted by alanine, abolished polyphenol-mediated inhibition of its cleavage. Moreover, overexpression of dominant negative AMPK diminished the ability of polyphenols to enhance phosphorylation of SREBP-1c, to decrease its nuclear active form and to lower lipid accumulation in primary hepatocytes and in HepG2 cells exposed to high glucose and insulin. In the liver of high fat/high sucrose diet-induced insulin resistant mice, phosphorylation of AMPK and SREBP-1c was enhanced by S17834, leading to decreased nuclear SREBP-1c, lowered hepatic triglycerides, and improved hepatic steatosis. These findings 1) characterize AMPK as an upstream kinase regulator of SREBP-1c; 2) AMPK-dependent phosphorylation of SREBP-1 is required for AMPK activators to suppress hepatocyte lipogenesis; and 3) reveal that phosphorylation and inactivation of SREBP-1c by pharmacological AMPK activators is therapeutically important in preventing hepatic lipogenesis and steatosis association with type 2 diabetes.

336-OR

The Protectin D1 Analog 10(S),17(S)-DiHDoHE Prevents Lipid-Induced Inflammation and Insulin Resistance

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We recently reported that reduced biosynthesis of n-3-derived docosanoids in adipose tissue and skeletal muscle is associated with inflammation and insulin resistance in high fat-fed obese mice. Here we studied whether the docosanoid lipid mediator and Protectin D1 analog, 10(S),17(S)-DiHDoHE, possesses therapeutic potential for lipid-induced inflammation and insulin resistance. Using first *in vitro* experiments, we found that 10(S),17(S)-DiHDoHE (10-100nM) significantly blunted lipid-induced cytokine secretion and activation of iNOS and JNK in J774A.1 macrophages treated with 400uM palmitate for 16h. Next we examined whether 10(S),17(S)-DiHDoHE could prevent lipid-induced inflammation and insulin resistance *in vivo* using a paired lipid-infusion hyperinsulinemic-euglycemic clamp model. Compared to control saline infused mice, 6h of intralipid infusion reduced insulin-mediated glucose disposal by ~70% and this could be explained by impaired insulin-mediated suppression of hepatic glucose production and insulin-stimulated glucose utilization by peripheral tissues. These effects were associated with elevated circulating inflammatory cytokines and greater activation of JNK and iNOS in insulin target tissues. Remarkably, i.v. administration of 10(S),17(S)-DiHDoHE (2ug/mouse) completely reversed the insulin-resistant effect of lipid infusion on glucose metabolism. The restored insulin sensitivity was associated with complete suppression of plasma cytokine release and inhibition of lipid-induced JNK and iNOS activation in liver and muscle. Accordingly phosphorylation of Akt on S473 was restored in liver and muscle of 10(S),17(S)-DiHDoHE treated mice. These data are the first to demonstrate the important therapeutic potential of n-3 derived docosanoids for obesity-related metabolic disorders and support a mechanistic role for this class of lipid mediator in the beneficial actions of long-chain n-3 fatty acids in inflammatory cells and metabolic tissues.

337-OR

The Role of TRIB3 in Regulation of Insulin Sensitivity and Glucose Oxidation under Short-Term Nutrient Excess and Fasting

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Tribbles homolog 3 (TRIB3) is a pseudokinase that has been identified as a negative regulator of Akt activity in insulin sensitive tissues. Our laboratory has shown TRIB3 can impair insulin-stimulated glucose transport in L6 myocytes and muscle levels are elevated in T2DM patients. In the current study utilizing *Sprague Dawley* rats, we found fasting enhanced insulin sensitivity, and led to decrements in TRIB3 mRNA (66.2%; $p<0.05$) and protein (81.4%; $p<0.05$) in muscle, while TRIB3 mRNA was increased in adipose tissue (96.3%; $p<0.05$), when compared to non-fasted controls. In rats fed a high fat diet for 7 days, the nutrient excess induced insulin resistance, and produced increments in TRIB3 mRNA (155.5%; $p<0.05$) and protein (69%; $p=0.0567$) in muscle and a decrease in the mRNA in adipose (76.6%; $p<0.05$). In lentiviral stably-transfected L6/L6-GLUT4*myc* myotubes, we found TRIB3 hyperexpression impaired insulin-stimulated glucose uptake, and increased both basal glucose oxidation and maximal oxidative capacity of mitochondria as reflected by the uncoupled oxygen consumption rate (OCR). In L6/L6-GLUT4*myc* myotubes with stable shRNA suppression of TRIB3, basal and insulin stimulated glucose uptake were increased while basal glucose oxidation and the maximal uncoupled OCR were decreased. In conclusion: 1) An increase in TRIB3 expression above baseline induced insulin resistance in both L6 myotubes and skeletal muscle from high fat-fed rats, and a decrease in TRIB3 resulted in enhanced insulin sensitivity in both L6 myotubes and in muscle from fasted rats; 2) In L6 myocytes, TRIB3 hyperexpression increases and TRIB3 suppression decreases basal glucose oxidation and maximal mitochondrial oxidative capacity. These data indicate that muscle TRIB3 is a potent physiological regulator of insulin sensitivity under conditions of nutrient deprivation and excess. Further, with nutrient excess, the TRIB3 elevation inhibits glucose uptake but promotes oxidation of intracellular stored fuel, and in fasting TRIB3 suppression would promote glucose uptake and limit fuel oxidation allowing for repletion of fuel stores.

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For author disclosure information, see page 785.

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Regulation of Skeletal Muscle Oxidative Metabolism by the Co-Lipase CGI-58

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Adipose triglyceride lipase (ATGL) regulates neutral lipid stores in multiple tissues notably cardiac and skeletal muscle. Recent studies indicate that ATGL activity is tightly regulated by CGI-58 (comparative gene identification 58). We investigated here the specific role of CGI-58 in the regulation of energy metabolism in skeletal muscle.

We first examined CGI-58 protein expression in various skeletal muscle types in relation to triglyceride hydrolase (TAGH) activity in mice. We next modulated CGI-58 expression during overexpression and knockdown studies in human primary myotubes and evaluated the consequences on lipid and glucose metabolism.

We observed a preferential expression of CGI-58 in oxidative muscles (soleus and heart) in mice consistent with TAGH activity. We next showed in human primary myotubes that CGI-58 overexpression increased intracellular fatty acid (FA) release (210%, p=0.01), increased endogenous FA oxidation (220%, p=0.01), and reduced fatty acid incorporation into triglyceride (-56%, p<0.05) measured by pulse-chase. Oppositely, CGI-58 silencing reduced intracellular FA flux (-37%, p=0.05), DAG production (-50%, p<0.05), and endogenous FA oxidation (-77%, p<0.001), while it increased glucose oxidation and glycogen synthesis. Interestingly, we also show that CGI-58 overexpression enhanced mitochondrial and β-oxidation gene expression while CGI-58 silencing induced opposite effects. This regulation could involve PPARδ activation.

Altogether, these data reveal that CGI-58 plays a limiting role in the control of oxidative metabolism by modulating substrate fluxes and the expression of PPARδ target genes, and highlight an important metabolic function of CGI-58 in skeletal muscle.

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339-OR

CYP2E1 Role in Insulin Resistance and Weight Control: From GLUT4 to Energy Expenditure

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Impaired glucose uptake and GLUT4 function in insulin target tissues is a major defect in obesity and diabetes. Applying DNA microarray analyses to untreated and insulin-treated streptozotocin-induced diabetic rats, we identified cytocrome P450 (CYP)-2E1 as potential modulator of glucose homeostasis. Opposite to GLUT4 gene repression, CYP2E1 mRNA levels were 22- and 35-fold enhanced in white adipose tissue and skeletal muscle in diabetes, and restored to control level upon insulin treatment.

To study CYP2E1 contribution to overall glucose homeostasis *in vivo,* we used CYP2E1-/- null mice. When challenged with high fat diet for 8 weeks, CYP2E1-/- null mice were protected against diet-induced obesity in spite of greater food intake, compared to wild type littermates. CYP2E1-/- null mice also displayed improved glucose tolerance, and had enhanced energy expenditure.

To investigate the underlying molecular mechanisms *in vitro,* we studied CYP2E1 regulation in rat skeletal muscle-derived L6 cells and primary adipocytes (PRA). CYP2E1 overexpression significantly decreased 2-deoxyglucose uptake rates and GLUT4*myc* protein levels at the cell surface of insulin-stimulated L6-GLUT4*myc* cells (by 25% and 60%, respectively), while having minor effects in resting cells. CYP2E1 silencing by siRNA had opposite effects. In both cell types, CYP2E1 overexpression repressed GLUT4 promoter (GLUT4-P) activity by 33%-70%, whereas CYP2E1 silencing enhanced endogenous GLUT4 mRNA expression by 1.6-1.8 fold. The antioxidant N-acetylcysteine (20mM) as well as CYP2E1-specific inhibitor chlormethiazole (20μM) alleviated CYP2E1 repression of GLUT4-P in PRA. Further promoter studies suggested that NRF2 is the nuclear mediator of CYP2E1-induced GLUT4 gene repression.

These data demonstrate that at the cellular level CYP2E1 induces insulin resistance via oxidative stress and NRF2-mediated specific inhibition of GLUT4 gene expression and its translocation machinery. Further, CYP2E1 has important role in weight control, energy expenditure and overall glucose homeostasis. Thus, CYP2E1 is suggested as a new molecular target for diabetes and obesity therapy.

340-OR

Effect of Mitochondrial DNA Damage on Glucose Tolerance and Insulin Sensitivity

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The mitochondrial genome encodes 37 genes. Damage to mitochondrial (mt) DNA has been hypothesized to play a role in the pathogenesis of diabetes- and aging-related insulin resistance. To test this hypothesis, mtDNA damage was induced in mice through mitochondrial-specific transgenic expression of a restriction nuclease (Eco-R1) which cuts mtDNA. The Eco-R1 gene was placed under the control of a Tet-off system and therefore expressed in the absence of tetracycline. Mice fed with standard (Eco R1 positive) or isocaloric doxycycline-complemented chow (Eco R1 negative) for 16 weeks were evaluated with an intraperitoneal (I.P.) glucose tolerance test (GTT) and a euglycemic, hyperinsulinemic (5 mU/kg.min) clamp. RESULTS: Eco R1 positive mice were leaner (weight in Eco R1 neg=41±3 g; Eco R1 pos=26±1 g; P<0.05), despite having higher food intake (daily intake in Eco R1 neg=5.1±0.05 g; Eco R1 pos= 6.65±0.10 g; P<0.05). EcoR1 positive mice had better glucose tolerance during the GTT (glucose AUC in Eco R1 neg=808±92; Eco R1 pos=556±26; P<0.05). Consistent with the GTT, insulin-stimulated total glucose disposal (TGD) measured with the insulin clamp was 2.6-fold higher in the EcoR1 positive mice (TGD in Eco R1 neg=15±3 mg/kg.min; Eco R1 pos=39±3 mg/kg.min; P<0.05). SUMMARY/CONCLUSION: Contrary to our hypothesis, mtDNA damage induced leanness, improved glucose tolerance, and enhanced peripheral insulin sensitivity. Our findings do not support a role for mtDNA damage in the pathogenesis of insulin resistance.

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INSULIN SECRETION AND ISLETS—CLINICAL AND PRECLINICAL STUDIES

341-OR

Early Improvement in Insulin Secretion and Insulin Sensitivity Predicts Conversion from Impaired (IGT) to Normal Glucose Tolerance (NGT): Results from ACT NOW

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Studies have shown that thiazolidinediones delay/prevent onset of diabetes, yet the physiologic mechanisms explaining their salutary effect remain unknown. Persons with IGT are near maximally insulin resistant and have lost 70-80% of beta-cell function. This study examined factors that determine IGT reversion to NGT. Subjects were participants in ACT NOW, a randomized double-blind, placebo-controlled study to test whether pioglitazone (PIO, 45 mg/day) prevents development of diabetes in persons with IGT (n= 602, FPG =105, 2-h PG =168 mg/dL). Insulin secretion and insulin sensitivity were derived from plasma glucose, insulin, C-peptide during OGTT before, at years 1, 2 and at study end (median 2.4 y). Diabetes developed in 50 PLAC-treated subjects vs. 15 PIO (hazard ratio=0.28: 95% CI= 0.16,-0.49; p<0.001). 48% of PIO reverted to NGT (responders) vs 28% of PLAC (p<0.005). After one year, responders had 47% greater Matsuda index (MI) of insulin sensitivity (6.57±0.4 vs. 4.47±0.2, p<0.001) and 64% greater insulin secretion/insulin resistance (IS/IR) index ($\Delta I_{0\text{-}120}/\Delta G_{0\text{-}120}$ x MI) (5.9±0.4 vs. 3.6±0.4, p<0.001) versus those who remained IGT or converted to diabetes (non-responders). The increment in IS/IR index was much greater in responders than non-responders (2.03±0.30 vs. 0.14±0.15, p<0.001) and persisted to study end. Change in IS/IR index at 1 year correlated with improvement in FPG (r=0.384, p<0.001) and 2-h PG (r=0.578, p<0.001). Improvement in beta-cell function (IS/IR index) for responders was similar, irrespective of whether they were treated with PIO or PLAC. However, among responders after 1 year, improvement in MI was greatest in PIO (p =0.03). Improved insulin sensitivity and glucose tolerance in PIO occurred despite weight gain of 2.7 kg, while persons treated with PLAC who reverted to NGT lost 1.6 kg.

Conclusion: Early (1 year) improvement in β-cell function (IS/IR index) characterizes reversion in glucose tolerance from IGT to NGT. In IGT intervention trials, non-responders after year 1 should be considered for more intensive pharmacotherapy.

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Diurnal Pattern in Insulin Action, Secretion and Hepatic Extraction in Healthy Humans: Implications for Artificial Pancreas Software

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To determine the presence/absence of a diurnal pattern to post prandial insulin action and secretion in response to mixed meals, we studied 14 nondiabetic subjects (7 men, BMI 25.1±1.0 kg/m^2, age 29.6±2.0 yrs) with normal fasting glucose (4.8±0.1mM) and HbA1c (5.3±0.1%). Identical mixed meals (10 cal/kg, 50 g carbs; 35% carbs, 30% protein, 35% fat) containing [1-^{13}C]glucose were ingested during either breakfast (B), lunch (L) or dinner (D) at 0700, 1300 and 1900 in randomized Latin Square order on three consecutive days. Physical activity measured with accelerometers was equal everyday. At time of labeled meal ingestion, [6-^{3}H]glucose and [6,6-$^2H_2$]glucose were infused to enable concurrent measurement of rates of meal appearance (MRa), endogenous glucose production (EGP), and glucose disappearance (Rd). Postprandial glucose excursion was significantly lower at B than L (296±51 vs. 431±52 mM/6 hours;p=0.025) and D (296±51 vs.524±60 mM/6 hrs;p=0.002) and lower at L than D (p=0.03). Postmeal insulin concentrations were similar at B, L and D (33±4 vs. 32±4 vs. 30±3 nM/6 hrs respectively) implying a diurnal decline in insulin action. C-peptide concentrations tended to be lower at B than L and D (333±25 vs. 376±28 vs. 367±28 nM/6 hrs) suggesting a decrease in insulin secretion normalized to glucose. Hepatic insulin extraction was significantly lower at B than D (48±3 vs. 54±3%;p=0.01) and L (53±3%;p=0.04). While MRa did not differ between meals, % suppression of EGP was lower (36±4 vs. 45±3%; p=0.04) at B than D while % increase in Rd higher (100±12 vs. 74±9%; p=0.04) at B than L. We conclude that under controlled conditions of identical mixed meals and physical activity, there is a progressive decline in meal tolerance from B to D. Hepatic insulin extraction increased during the day resulting in slightly higher insulin levels and a higher Rd at B than D. The results demonstrate a diurnal pattern to insulin action, secretion and extraction in healthy humans. Studies in type 1 diabetes are needed to explore diurnal changes in insulin action and, if present, will need to be incorporated into future Artificial Pancreas software systems.

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GLP-1 Enhances Insulin Biosynthesis in Type 2 Diabetic Subjects during Hyperglycemia

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Insulin Biosynthesis is known to be upregulated *in vitro* and *in vivo* by glucose and GLP-1. We recently reported that glucose and GLP-1 significantly increased insulin biosynthesis in normoglucose tolerant (NGT) and Type 2 diabetic (DM) subjects *in vivo.* Prior studies in rat islets have suggested that the GLP-1 analog exendin-4, has additional stimulatory effects on insulin biosynthesis in the presence of a maximal stimulatory concentration of glucose (Alarcon Diabetol 2006). At present there is no data in vivo as to whether GLP-1 provides additional stimulation to insulin biosynthesis in the presence of a maximal stimulatory concentration of glucose.

Methods: Insulin fractional synthesis rate (FSR) was monitored in vivo using a primed constant infusion of $^2H_7$ leucine (1.2 mg/kg; 1.2 mg/kg/hr) into DM, obese (OB) and nonobese (nonOB) NGT controls (C) for 6-7 hrs under conditions of hyperglycemia (HG, 250 mg/dl) or hyperglycemia + GLP-1 infusion (0.75 pmol/kg/min; 250GLP). Incorporation of d-leucine into M1 and M2 C-peptide species was monitored by high resolution 2D LC-MS between 120 and 420 minutes (plasma), and peak enrichment (urine). FSR was calculated by two isotopomer analysis of C-peptide (M1, M2) as previously described by our group.

Results: 24 hour FSR, monitored in plasma, for combined hyperglycemia with GLP-1 infusion (250GLP) was greater in diabetic subjects compared to either OB or nonOB controls (1.547 ± 0.200, 1.050 ± 0.052, 1.090 ± 0.037; ANOVA p<.03; SNK DM vs OB p<.05, DM vs nonOB p<.05). For further analysis NGT groups were pooled. 24h FSR was increased by GLP-1 during HG in NGT subjects (1.085 ± .041 vs 0.872 ± 0.082, 250GLP vs HG; p<.05). Similarly in DM subjects GLP-1 also increased FSR during HG (1.547 ± 0.200 vs 0.969 ± 0.146, 250GLP vs HG; p<.05). We document for the first time that GLP-1 stimulates the fractional insulin biosynthesis rate during hyperglycemia in normal (24%) and Type 2 diabetic subjects (60%). These and our prior results suggests that GLP-1 action is important for optimal insulin biosynthesis at all levels of glycemia in normal and diabetic subjects in vivo.

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Xenin-25 Increases Insulin Secretory Responses to GIP in Humans with Impaired Glucose Tolerance and Type 2 Diabetes Mellitus

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Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are incretin peptides produced by intestinal K and L cells, respectively. Both are released into the blood immediately after meal ingestion and potentiate glucose-stimulated insulin secretion (GSIS). Agents that increase GLP-1 action are now being used to treat type 2 diabetes mellitus (T2DM).

Insulin secretory responses to GIP are blunted in T2DM precluding its use for treating this disease. We earlier showed that xenin-25, a peptide also produced by the K cell, amplifies GIP-mediated GSIS via a cholinergic neuronal relay in hyperglycemic and other mice with reduced GIP responses. This study determined if Xen amplifies GIP-mediated GSIS in humans with normal glucose tolerance (NGT; n=10), impaired glucose tolerance (IGT; n=10) and/or mild T2DM (n=9). Each subject received graded intravenous glucose infusions (GGIs) on 4 separate occasions after a 10-h fast by increasing glucose infusion rates every 40 min. During these 4 studies, subjects were also infused with either Albumin alone (Alb; No Peptide) or pharmacologic doses of GIP, Xen, or GIP plus Xen (G+X). Plasma glucose, insulin, and C-peptide levels were measured and insulin secretion rates (ISRs) calculated by deconvolution of C-peptide levels. Mild diarrhea was the only side effect of peptide administration. Infusion of GIP, but not Xen, caused a rapid and transient (0-40 min) increase in ISR in all subjects which was further amplified when Xen was infused with GIP (~2.3-fold increase with G+X vs Alb). Over the entire 4-hour GGI, Xen amplified the effects of GIP on ISRs in humans with IGT [1.85-fold with G+X (p<0.001) vs 1.43-fold with GIP alone (p<0.05)]. Diabetics exhibited a statistically significant increase in ISR in response to G+X (2.24-fold; p<0.01) but not to GIP alone. In conclusion, Xen improves but does not normalize the stimulatory effect of GIP on GSIS in subjects with IGT and T2DM. Studies are underway to determine if GIP action can be further normalized by optimizing peptide doses.

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ADA-Funded Research

345-OR

GLP-1 Receptor Signaling Is Required for Improvement in Glucose Tolerance after Roux-en-Y Gastric Bypass in Diet-Induced Obese (DIO) Mice

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Remission of type 2 diabetes mellitus (T2DM) after Roux-en-Y gastric bypass (RYGB) is associated with a higher nutrient-stimulated secretion of glucagon like peptide-1 (GLP-1). Given its known actions on insulin secretion, GLP-1 has been postulated as a key mediator of the antidiabetic effects of RYGB. To determine whether GLP-1 action is required for the effects of RYGB on glucose homeostasis, we chronically blocked peripheral GLP-1 action in RYGB-treated or sham-operated (SO) DIO C57BL/6 male mice. Mini-osmotic pumps with the GLP-1 receptor antagonist Exendin 9-39 (Ex9) or vehicle (Vh) control solution were subcutaneously implanted for 6 weeks. At 6 weeks, we measured fasting glucose (FG), oral glucose tolerance (OGT), and glucose stimulated insulin secretion (GSIS). Results: RYGB improved FG by 30% compared to SO (RYGB-Vh, 117.8±3.6 vs. SO-Vh, 164.7±6.8; p<0.05). Compared to Vh controls, Ex9 increased FG by 18% in both RYGB and SO mice groups (RYGB-Ex9, 137.6±11.2 vs. RYGB-Vh, 117.8±3.6; SO-Ex9, 195.8 ±6.4 vs. SO-Vh, 164.7±6.8; p<0.05 for each). RYGB decreased the area under the curve of glucose excursion after OGT by 57% compared to SO (RYGB-Vh, 7697±512 vs. SO-Vh, 17830±1856; p<0.05). In SO mice, Ex9 treatment led to a 9% increase in glucose AUC of OGT. In contrast, after RYGB, Ex9 caused a 87% increase in glucose AUC, corresponding a 65% reversal of the effect of RYGB on OGT (RYGB-Ex9, 14296±882 vs. RYGB-Vh, 7697±512; SO-Ex9, 19468±1514 vs. SO-Vh, 17830±1856; p<0.05 for each). Ex9 decreased GSIS by 70% and 40% in RYGB and SO mice compared with their respective Vh controls (RYGB-Ex9, 7.1±1.9 vs. RYGB-Vh, 23±3; SO-Ex9 23±4.8 vs. SO-Vh, 39±7; p<0.05 for each). Conclusions: These results demonstrate that the chronic enhancement in GLP-1 secretion is essential for the profound improvement in OGT in DIO mice after RYGB. In contrast, GLP-1 appears to make only a modest contribution to the improvement in FG after this procedure. The observed effect of Ex9 on GSIS suggests that GLP-1 improves glucose homeostasis after RYGB primarily through its well described incretin action.

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ADA-Funded Research

346-OR

Inhibition of NADPH Oxidase Prevents Fat-Induced β-Cell Dysfunction *In Vivo*

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NADPH oxidase activation has been implicated in free fatty acid (FFA)-induced β-cell dysfunction *in vitro*; however, the *in vivo* role remains unclear. We here investigated whether the inhibition of NADPH oxidase, using either pharmacological or genetic models, prevents FFA-induced β-cell dysfunction *in vivo*. Normal rats were infused intravenously for 48h with either saline or oleate (1.4μmol/min) with or without the NADPH Oxidase inhibitor Apocynin (APO, 0.5μmol/kg/min). This was followed by the assessment of β-cell function in isolated islets. 48h intravenous oleate infusion increased total but not mitochondrial superoxide in islets, consistent with increased NADPH Oxidase activity, and impaired β-cell function *ex vivo* at 13 (33% reduction in insulin secretion with oleate, $p<0.05$) and 22mM glucose (38% reduction with oleate, $p<0.05$). Co-infusion of APO with oleate normalized NADPH Oxidase activity and total superoxide and partially prevented FFA-induced β-cell dysfunction at both 13mM and 22mM glucose (16% and 20% reduction from saline, respectively). Additionally, NADPH Oxidase subunit p47-null mice (KO) and their littermate controls (WT) were infused with saline or oleate (0.3μmol/min) for 48h, after which β-cell function in isolated islets was assessed at 22mM. 48h FFA elevation in WT mice impaired β-cell function compared to saline infused WT mice (48% reduction in insulin secretion with oleate; $p<0.01$). In contrast, KO mice subjected to 48h elevation of FFA had no significant impairment in β-cell function (23% reduction from KO saline). These data show that NADPH oxidase plays a causal role in FFA-induced β-cell dysfunction *in vivo*, and is thus a potential therapeutic target for type 2 diabetes.

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Nicotinamide Mononucleotide Protects Against Cytokine-Mediated Islet Dysfunction

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Nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme for NAD^+ biosynthesis, is secreted from adipose tissue and can regulate pancreatic insulin secretion. Administration of nicotinamide mononucleotide (NMN), a product of the NAMPT reaction, corrects impaired insulin secretion in NAMPT+/- mice. NMN may affect islet function through induction of SIRT1, an NAD^+ -dependent metabolic regulator that has anti-inflammatory effects in islets. Chronic inflammation is common in insulin resistance and exposure to pro-inflammatory cytokines impairs islet insulin secretion. We investigated whether NMN could correct islet dysfunction in a model of diet-induced insulin-resistance and in islets from mice on standard diet (CON) exposed to IL1β and TNFα.

Mice fed a high-fructose (60%; FF) diet for 16 weeks were administered NMN (500 mg/kg; IP) or equal volume of water 16 h prior to islet isolation. Islets isolated from CON mice were incubated with IL1β and TNFα (both 5 ng/ml) +/- NMN (100 μM) for 48 h. Insulin secretion was measured following 1h incubation with 3 or 17 mM glucose (GSIS). Gene expression was measured by qPCR.

IL1β and TNFα gene expression was increased in white adipose tissue (3 fold; $P<0.01$), whilst IL1β gene expression was raised in islets (1.8 fold; $P<0.001$) of FF mice. Raised IL1β/TNFα was associated with lowered GSIS (71%; $P<0.01$) in FF mice. IL1β/TNFα incubation reduced GSIS (25%; $P<0.001$) in CON islets. These changes were associated with suppressed gene expression of PDX1 and GLUCOKINASE, key markers of islet function. Impaired GSIS and gene expression were reversed by NMN in both models.

FF and IL1β/TNFα exposed islets displayed decreased gene expression of SIRT1 and also SIRT3, an NAD^+ dependent positive regulator of ATP production with no prior reported function in islets. NMN restored SIRT1 and SIRT3 to basal levels. In contrast, FF-mediated increased IL1β expression was reversed by NMN administration.

NMN protects against cytokine-mediated islet dysfunction in models of diet-induced insulin-resistance and may represent an effective treatment strategy for T2DM.

The effects of NMN likely occur through SIRT1 mediated resolution of islet inflammation.

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Pancreatic Progenitors Derived from Human Embryonic Stem Cells Produce Functional Islets and Correct Diabetes in Athymic Nude Rats

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Human embryonic stem cells (hESC) are an attractive source material for the generation of a universal, renewable cell therapy for diabetes as they can be expanded virtually indefinitely and can be differentiated to almost any cell type. Previously we have described a differentiation protocol to efficiently generate pancreatic progenitor cells that upon engraftment in immune-compromised mice develop into fully functional, glucose-responsive islet tissue. Moreover, once matured, these hESC-derived grafts were shown to protect recipient mice from streptozotocin (STZ) induced hyperglycemia. Here we describe further transplantation studies of hESC-derived pancreatic progenitor cells into a larger immune-compromised rodent model, the athymic nude rat. Pancreatic progenitors were transplanted into the epididymal fat pad, and glucose stimulated insulin secretion assays were performed to monitor graft development and function. By 15 weeks post implant, glucose stimulated serum c-peptide levels reached greater than 1ng/ml and by 25 weeks post implant levels were greater than 3ng/ml. We subsequently treated the engrafted rats with STZ to ablate their endogenous beta cells. Engrafted STZ-treated rats did not become hyperglycemic and in fact maintained blood glucose levels similar to the human blood glucose set-point. Pancreatic progenitor cells were also transplanted into diabetic STZ-treated rats into the omentum and epididymal fat pad sites. Diabetic rats were maintained with insulin pellets and supplemental long-acting insulin injections while the grafts matured. Graft function developed in a similar time frame to that observed previously, and STZ-treated rats became insulin independent by approximately 17 weeks post implant and maintained euglycemia for up to another 360 days. These studies demonstrate for the first time that hESC-derived pancreatic progenitors develop and maintain long term function in the athymic nude rat model.

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BETA CELL STIMULUS-SECRETION COUPLING

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Protein Kinase D Mediates Fatty-Acid Potentiation of Insulin Secretion Via GPR40

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Fatty acids do not initiate insulin secretion at low glucose concentrations, but greatly amplify glucose-stimulated insulin secretion (GSIS) from the pancreatic beta-cell. This effect is, in part, mediated by the G protein-coupled receptor 40 (GPR40). As such, GPR40 is actively pursued as a potential therapeutic target to enhance insulin secretion in type 2 diabetes. However, the mechanism of action of GPR40 are still poorly understood. The goal of this study was to characterize the signalling cascade by which GPR40 regulates insulin secretion. We performed dynamic perifusions and static incubation to measure insulin secretion in response to stimulation by various secretagogues from wild type (WT), GPR40 knock-out (GPR40-KO) and protein kinase C δ knock-out (PKCδ-KO) isolated islets. In perifusion experiments, addition of glucose (16.7 mmol/l) elicited a biphasic release of insulin of similar magnitude in both GPR40-KO and WT islets. Oleate (0.5 mmol/l) potentiated predominantly the second phase of GSIS, and this effect was reduced by approximately 50% in GPR40-KO mice. Exogenous diacylglycerol (DAG; 100 μmol/l), a second messenger generated by phospholipase C-mediated hydrolysis of membrane phospholipids, mimicked the potentiating effect of oleate on GSIS, and the effects of DAG were not additive with those of oleate. In addition, the loss of secretion in response to oleate observed in GPR40-KO mouse islets was completely restored by exogenous DAG. Since PKCδ and protein kinase D (PKD)-1 are activated by DAG and control insulin secretion, we evaluated the potential implication of these proteins in mediating the effect of GPR40 signalling on GSIS. The potentiating effect of oleate on GSIS was unaltered in islets from PKCδ-KO mice. In contrast, oleate rapidly induced PKD1 phosphorylation at the plasma membrane in a GPR40-dependent but PKCδ-independent manner. Importantly, oleate potentiation of GSIS was greatly reduced in islets infected with lentivirus encoding a shRNA against PKD1. We conclude that FA-potentiation of second-phase GSIS via GPR40 involves the generation of DAG and downstream activation of PKD1.

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For author disclosure information, see page 785.

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Inhibition of Small-Maf Factors in Pancreatic Beta Cells Is Associated with Improvement in High-Fat Diet-Induced Glucose Intolerance in Mice

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MafA, one of the major regulators of insulin gene expression and beta-cell function, is a member of the large Maf family of leucine zipper transcription factors and positively regulates insulin expression. We previously demonstrated small-Maf factors (MafF, MafG and MafK), that lack an N-terminal transactivation domain present in the large-Maf factors, are also expressed in beta cells and thus, can function as a negative regulator. Both oxidative stress and lipotoxicity reduced insulin gene expression and concomitantly increased both small-Maf mRNA and protein expressions in a beta-cell line. Here, to evaluate the effect of small-Maf factors on glucose tolerance in vivo. transgenic (Tg) mice with beta-cell specific expression of dominant-negative small-Maf factor were generated. Three Tg mouse lines were established on the C57BL/6J background, which showed normal size and growth. Both wild type (Wt) and Tg male mice were fed a regular-chow (R) or high-fat (HF) diet respectively from 6 weeks of age. At 20 weeks, glucose tolerance test was performed. Islets were isolated and analyzed to determine expressions of MafA and small-Mafs. Pancreatic sections were obtained for immunohistochemistry to evaluate the beta-cell mass and expressions of those proteins. The results showed no significant differences in body weight and food intake between Wt and Tg mice. Feeding HF diet resulted in higher levels of serum insulin and blood glucose, and increased beta-cell mass. Small-Maf and MafA protein expressions were significantly increased. Interestingly, despite similar MafA expression pattern, glucose tolerance in HF diet-fed Tg (HFD-Tg) mice was significantly improved as compared with HF diet-fed Wt (HFD-Wt) mice. Moreover, HFD-Tg mice showed higher serum insulin level after a glucose load than HFD-Wt mice. These data suggest that small-Maf factors are important for the regulation of insulin expression in vivo and inhibition of small-Mafs in beta cells leads to increment of capability to secrete insulin on demand. Therefore, preventing small-Maf function in beta cells may represent a novel therapeutic target to improve beta-cell function.

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Gelsolin Knockout Impairs Insulin Secretion Independently of Actin Polymerization

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The actin severing protein gelsolin is implicated in the control of insulin secretion by mediating actin remodeling during glucose-stimulation, however the exact mechanism and downstream mediators by which gelsolin contributes to insulin secretion are unclear. We studied islets and β-cells from mice lacking gelsolin (-/-) and INS-1 832/13 cells following gelsolin over-expression or shRNA-mediated gelsolin knockdown. Over-expression of gelsolin enhances the insulin secretory response by 31% in INS-1 832/13 cells ($p=0.05$). Conversely, shRNA-mediated gelsolin knockdown increased F-actin levels, and blunted glucose-stimulated F-actin deplolymerization and insulin secretion (-31%, $p<0.01$). The cellular architecture of islets in pancreatic sections from gelsolin -/- mice was not different from wild-type littermates. In β-cells from the gelsolin-null mice cortical F-actin density was increased, and in intact gelsolin -/- islets the bi-phasic insulin secretory response to glucose was severely impaired (by 66%, $p<0.01$). Surprisingly, the secretory defect was not rescued by the actin depolymerizing agent latrunculin B. Downstream, the exocytotic response to membrane depolarization was impaired in gelsolin -/- β-cells (by 79% compared to wt cells, $n=20$, $p<0.001$). This resulted from a reduced β-cell voltage-dependent Ca^{2+} channel activity since Ca^{2+}-currents was detectable in 5/15 gelsolin -/- cells studied (versus 13/17 wild type cells) and glucose-stimulated islet Ca^{2+} responses were impaired in gelsolin -/- islets (by 57%, $n=4$-6, $p<0.01$). Finally, the direct application of either 200 nM or 2 mM free-Ca^{2+} to the cell interior was sufficient to rescue exocytosis in gelsolin -/- β-cells ($n=16$ and 21). Thus, we show that gelsolin is a positive regulator of glucose-stimulated insulin secretion in primary β-cells, and provide evidence that this is not due solely to it's ability to mediate actin depolymerization *per se*. Rather, the present data implicates an important role for gelsolin in the down-stream regulation of β-cell Ca^{2+} and exocytotic responses.

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Triple FoxO Knockout in B-Cells Impairs Glucose-Induced Insulin Secretion and Leads to Diabetes

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The pathogenesis of pancreatic b-cell failure in diabetes is incompletely understood.

We have previously shown that forkhead box-O transcription factor FoxO1 regulates different aspects of b-cell biology. Two additional FoxO isoforms, FoxO3a and FoxO4, show overlapping expression patterns and functional redundancy with FoxO1, and are expressed in pancreatic b-cells. To study their role in b-cell function, we generated mice lacking FoxO1, 3a, and 4 in b-cells (b-FoxO-TKO) by crossing Rat Insulin Promoter (RIP)-cre transgenic mice (Herrera strain) with mice carrying floxed alleles of all 3 FoxOs. b-FoxO-TKO mice showed normal pancreas size, islets mass and islet cell type distribution up to 8 weeks of age. At that point, they began to display fasting and fed hyperglycemia and glucose intolerance. We assessed the insulin secretory response in vivo and in isolated islets. Upon intraperitoneal glucose administration, b-FoxO-TKO mice demonstrated a substantially impaired insulin response. In primary islets isolated from b-FoxO-TKO mice, insulin release was decreased in response to any glucose concentration tested, indicating that b-cells require intact FoxO functions to regulate insulin secretion in response to glucose. To elucidate the mechanism of impaired glucose-induced insulin secretion, we analyzed expression levels of proteins required for stimulus-secretion coupling. TKO b-cells showed substantial decreases of Glut-2 and Glucokinase compared to controls. mRNA measurements indicated that TKO islets express markedly elevated levels of *lactate dehydrogenase* and *Neuropeptide Y*, while expression levels of *Kir6.2* and *Sur1*, the two subunits of the ATP-sensitive K+ channel, were reduced by ~60% compared to WT islets. The findings indicate that FoxOs are collectively required to maintain insulin secretion in response to glucose by virtue of their combined actions on the expression of components of the stimulus/secretion coupling machinery. Expression patterns in TKO islets are reminiscent of the gene expression signature of immature b-cells, suggesting that FoxOs are required for the maturation of b-cells.

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Metabolic Deceleration Protects β-Cell Function under Chronic Nutrient Overload Conditions

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We have previously suggested that hypersecretion of insulin is detrimental to the b-cell in states of increased demand eg obesity/insulin resistance. ADOPT/RECORD provided clinical evidence that drugs known for stimulating insulin secretion (IS) (Glyburides) had detrimental effects on glycaemic control when used long term. To test whether metabolic deceleration is paradoxically beneficial, we generated transgenic (tg) mice overexpressing the gluconeogenic enzyme fructose-1,6-bisphosphatase (FBPase) in islet β-cells.

We have published that β-cell specific FBPase tg mice have reduced IS compared to control mice following a chow (CH) diet as a result of reduced glycolytic flux. In this study we assessed whether under high fat (HF) conditions FBPase tg mice would be protected against glucose intolerance and reduced IS. To test this hypothesis 8 week old FBPase tg and control mice were fed either a CH or 60% HF diet for 8 weeks and then subjected to oral and intravenous glucose tolerance tests (OGTT and IVGTT). Following a HF diet FBPase tg mice showed lower fasting plasma glucose levels and when subjected to OGTT they had better glucose clearance and higher plasma insulin levels compared to controls.

Furthermore, following a HF diet FBPase tg mice showed higher plasma insulin levels during the IVGTT compared to controls. To further investigate the mechanism of HF induced protection we isolated islets from FBPase tg and control mice and incubated with or without 1mM palmitate for 48 hours. Assessment of IS confirmed the *in vivo* results that FBPase tg mice are protected against HF induced β-cell defects. Apoptosis was also assessed in islets and showed a significant increase in cell death in the control mice following HF exposure while FBPase tg mice were unchanged. Taken together these results show that FBPase tg mice display better glucose tolerance and insulin secretory function following a HF diet compared to controls, which is associated with reduced apoptosis. This data supports our hypothesis that metabolic deceleration can be beneficial and may provide a therapeutic strategy to protect the islet β-cell when exposed to a nutrient rich milieu.

For author disclosure information, see page 785.

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PI3Kα and PI3Kβ Have Distinct Roles in Rodent and Human β-Cell Function

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Phosphatidyinositol-3-OH kinases (PI3Ks) are important regulators of islet function. This is demonstrated most recently in work from Kaneko and colleagues (Cell Metab., 2010) demonstrating impaired insulin secretion and exocytosis following genetic ablation of class 1A PI3K activity in mice. This is in contrast to the known stimulatory effect of the non-selective PI3K inhibitor wortmannin, and suggests an important role for class 1A PI3K isoforms in maintaining secretory competence. In the present work we investigated the role of class 1A PI3K isoforms in human and rodent β-cell function. We find that islets and INS-1 insulinoma cells express two class 1A PI3K catalytic submits; p110α and p110β. The specific pharmacological inhibition of p110α (PIK-75, 100 nm overnight) increased glucose-stimulated insulin secretion in mouse (n=5, $p<0.01$) and human islets (n=3) consistent with the effect of wortmannin; and increases the exocytotic response measured by single-cell capacitance in human β-cells (n=14-18 $p<0.05$). While the pharmacological inhibition of the p110β (TGX-221, 100 nm overnight) had no effect on glucose-stimulated insulin secretion from mouse and human islets, the shRNA-mediated knockdown of p110β severely blunted the exocytotic response in both mouse (n=18-21, $p<0.05$) and human (n=22-21, $p<0.01$) β-cells. In intact human islets from 3 donors, shRNA-mediated knockdown of p110β decreased both first and second phase insulin secretion (n=3, $p<0.01$). Finally, knockdown of p110β in INS-1 cells resulted in a 42% reduction in insulin granule density at the plasma membrane, as shown by TIRF microscopy (n=26-28). Therefore, we now show that the inhibition of p110α likely accounts for the majority of wortmannin's stimulatory effect on insulin secretion in mouse and humans; and that knockdown of p110β expression significantly reduced the secretory response, likely by reducing insulin granule exocytosis at the plasma membrane, suggesting a role for p110β in secretory granule recruitment and/or docking that may be separate from it's catalytic activity.

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Multiple Mechanisms of Cell-Cell Communication Regulate Islet Function and Insulin Secretion

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Within the islet of Langerhans, cellular proximity is critical for the regulation of hormone secretion. β-cells within the islet secrete insulin in coordinated pulses, with a many-fold enhanced response to glucose compared to isolated β-cells. Gap junctions are important to coordinate intracellular free-calcium ($[Ca^{2+}]_i$) oscillations and regulate insulin secretion dynamics, but other coupling mechanisms can also regulate insulin secretion levels. We have determined the precise role of gap junctions compared to other means of cell-cell communication in regulating islet function. We measured the metabolic, electrical and insulin secretion response in islets that lack gap junction coupling and compared the responses to dissociated β-cells which lack cellular proximity and thus any means of cell-cell communication. A deletion of Connexin36 ($Cx36^{-/-}$) eliminates electrical coupling between β-cells and leads to significant elevations in $[Ca^{2+}]_i$ at low glucose; similar to isolated β-cells and thus revealing a heterogeneous β-cell population. Insulin secretion from these islets was minimally altered, in contrast to isolated β-cells. Introducing mosaic expression of a loss-of-function K_{ATP} channel mutation into $Cx36^{-/-}$ islets further elevated $[Ca^{2+}]_i$ at low glucose, but insulin secretion was still suppressed. This suppression could be reversed by elevated cAMP via activating PKA, or disrupting EphA-ephrinA signaling, but only in islets that lack gap junctions. We conclude that multiple cell-cell communication mechanisms are important for the normal regulation of insulin secretion. Gap junctions coordinate β-cell heterogeneity to suppress basal $[Ca^{2+}]_i$ elevations and enhance the glucose-stimulated $[Ca^{2+}]_i$ response. Other cell-cell communication mechanisms, regulated by glucose and PKA and including EphA signaling, act distal to $[Ca^{2+}]_i$ elevation to additionally suppress elevations in basal insulin secretion. These results elucidate how cell-cell communication in the islet can regulate stimulus-secretion coupling, which yields further insight for the development of pharmacological treatments as well as cell-based therapies for diabetes.

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The Role of GABA Shunt and γ-Hydroxybutyrate (GHB) in Regulation of Glucagon Secretion in Human Islets

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To examine the role of amino acid and glucose in integrating islet insulin and glucagon secretion, we used stable isotope methods to trace the flux of glucose into amino acids using islets isolated from normal and type 2 diabetic (T2D) human organ donors. The results suggest an alternative hypothesis to the proposal that insulin and/or zinc are the paracrine effectors mediating glucose-induced suppression of glucagon release.

In islets isolated from non-diabetic human controls, exposure to a sub-stimulatory level of [U-13C]glucose (5 mM) gave a parallel 40% suppression of both glucagon release and islet GABA levels, while increasing 13C enrichment of GABA. A stimulatory level of glucose (25 mM) increased insulin secretion, but gave only a small further suppression of glucagon release and islet GABA levels (to 50% of basal). Islets from T2D had impaired insulin responses to glucose, but showed two types of glucagon responses: one group with normal basal islet GABA and GAD expression showed normal suppression of glucagon; a second group with markedly reduced GABA levels and GAD expression failed to suppress glucagon secretion in response to glucose. To explore the role of the GABA shunt in glucose suppression of glucagon release, we used vigabatrin to inhibit the GABA transaminase reaction. In control human islets, vigabatrin increased GABA levels by 100% and blocked the fall in GABA levels following glucose stimulation; glucose stimulation of insulin release was not altered, but glucose failed to inhibit glucagon release. This suggested that the GABA shunt effect on glucagon release was distal to GABA and might involve Succinic semialdehyde or its metabolite, GHB, a potent inhibitory neurotransmitter. Stimulation of control human islets with [U-13C]glucose demonstrated flux into GHB and dose-dependent release of 13C-GHB into the media. Stimulation GHB receptor blocked amino-acid stimulated glucagon release; inhibition of the receptor blocked glucose-induced suppression of glucagon release.

These results suggest that GHB is a potential mediator of the paracrine mechanism by which glucose stimulation of β-cells leads to suppression of α-cell glucagon release.

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MECHANISMS OF ATHEROGENESIS IN DIABETES—ANIMAL AND CELLULAR MODELS

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AdipoR2 in Endothelial Cells and AdipoR1 in Macrophages Play Pivotal Roles in the Prevention of Atherosclerosis *In Vivo*

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The adipocyte-derived hormone adiponectin (Ad) has been proposed to play central roles as antidiabetic and antiatherogenic adipokine. However, whether Ad receptors AdipoR1 (R1) and AdipoR2 (R2) have protective roles against atherosclerosis *in vivo* are still undetermined.

Ad has been shown to be downregulated in obesity. Here we showed that in mice, ApoE deficiency and obesity induced by high-fat diet or leptin deficiency resulted in significantly decreased expression of R2 in the aorta.

Ad KO mice has been shown to exhibit severe neointimal formation. Next, we showed that these phenotypes of Ad KO mice were associated with increased proinflammatory molecules such as MCP-1 and CCR2 as well as increased molecules involved in adhesion such as ICAM-1 and also increased proliferation of vascular smooth muscle cells in injured arteries. We showed that R1KO mice exhibited more neointimal formation in response to external vascular cuff injury than wild-type mice (intima to media volume ratio (I/M); WT:25.3±1.1; R1KO:30.1±1.8%). R2KO or R1·R2 double KO mice exhibited much more neointimal formation in response to cuff injury than R1KO mice (I/M; R2KO:42.8±4.0; R1R2KO:49.6±2.6%). Interestingly, neointimal formation induced by cuff injury was increased by transplantation of bone marrow (BMT) from R1KO but not increased by BMT from R2KO mice. Importantly, the extent of neointimal formation observed in endothelial cells-specific R2KO mice was almost the same as that in conventional R2KO mice. Moreover, adenovirus-mediated supplement of R2 in cuff injury region or endothelial cells-specific R2 upregulation significantly attenuated neointimal proliferation and increased PPARγ and oxidative stress-detoxifying enzymes such as SOD1 in the aorta. Conversely, targeted disruption of R2 in endothelial cells resulted in decreased PPARγ and SOD1.

For author disclosure information, see page 785.

This study provides the direct evidence that R2 in endothelial cells and R1 in macrophages play protective roles against atherosclerosis. Moreover, this study raises the possibility that a therapeutic strategy to increase AdipoRs in injured artery could be useful in preventing vascular diseases such as restenosis after angioplasty.

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A New Mouse Model of Diabetes Related Increased Atherosclerosis in the Absence of Lipid Changes

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The role of diet in the development of obesity, diabetes and atherosclerosis is well known from both human and animal studies. Diabetes has been shown to be a major contributor to the development and severity of atherosclerosis independent of plasma lipid levels in human clinical studies. The male LDLR-/- mouse has been shown to be a model of obesity and diabetes when fed a western style high fat and cholesterol diet. While the mice develop diabetes there is also an increase in plasma lipids, most notably cholesterol, in these mice that mask any direct diabetic effects in the development of atherosclerosis. There is an additional model in which streptozotocin is given to apoE-/- mice to develop type 1 diabetes, but they also exhibit increased plasma lipids. We developed a model of mice in which obesity and diabetes results in increased atherosclerosis in the absence of plasma lipid changes. We placed young (3 months of age) male LDLR-/- mice on three commonly used chow diets, 1) continued Harlan 8904 (n=9), 2) Purina 5061 (n=6) and 3) Harlan 2920 (n=6) for 9 months. The mice on 2920 developed rapid weight gain and central obesity compared to the other 2 diets after 1 month (3.4 g vs 0.7g for 8904 and 1.1g for 5061, $p<0.001$). This change was due to a 4.6% increase in body fat in the 2920 mice as determined by NMR compared to no significant body fat changes in the other diets. This increased body weight and obesity was maintained throughout the experiment. An oral glucose tolerance test was performed after 4 months of diet and the 2920 mice had a significantly increased AUC compared to the other diets. At sacrifice the plasma cholesterol, triglycerides and free fatty acids were not different between the various diets but the mice on the 2920 diet had significantly increased (3-fold increase in atherosclerosis by the en face method. This is the first mouse model in which increased atherosclerosis is seen due to obesity and diabetes without an increase in plasma lipids being seen. Use of this model will allow the determination of the proatherogenic mechanisms without the confounding factor of plasma lipid alterations.

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Triple Knockout of Foxos Increases eNOS Expression, and Prevents Activation of Inflammatory Pathways and ROS Generation in Endothelial Cells Independently of Akt Phosphorylation

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Objective: Insulin resistance in vascular endothelial cells (ECs) promotes atherosclerotic plaque formation. The mechanism linking insulin resistance in ECs to atherosclerosis is unknown. We investigated the role of Foxo transcription factors in this process. Methods: We generated mice lacking Foxo1 alone (single Foxo1 knockout: SKO), or all three Foxos (triple Foxos-knockout: TKO) in ECs, by intercrossing *Tie2*-Cre transgenics with mice bearing "floxed" *FoxO* alleles. Thereafter, we analyzed mice in vivo and ex vivo.

Results: Surprisingly, SKO and TKO mice were born in Mendelian ratios and grew normally, without detectable defects of glucose metabolism. In ECs derived from SKO and TKO mice, LPS failed to induce inflammatory markers (iNOS, MCP-1, IL-6, and osteopontin), adhesion molecule (ICAM-1), and NF-κB activity. Strikingly, insulin-stimulated Akt and ERK1/2 phosphorylation was substantially reduced in TKO lung ECs. This finding was associated with decreased expression of IRS-1 and IGF-1 receptor. In TKO aortae, lung and aortic ECs, total eNOS and insulin-induced phospho-eNOS levels were increased. In contrast, free-cholesterol-, 7-ketocholesterol-, and hydrogen peroxide-induced reactive oxygen species were significantly decreased in TKO lung ECs, as was NADPH oxidase expression.

Conclusion: Endothelial Foxos are required to activate inflammatory responses to atherogenic stimuli. Moreover, triple Foxos deletion impairs Akt activation and NADPH oxidase expression, while increasing eNOS expression and phosphorylation.

These data indicate that Foxos are required for key responses to atherogenic stimuli, and portend a protective effect of Foxo ablation on atherosclerosis development. Studies of TKO mice crossed with Ldl receptor knockout mice to investigate the latter possibility are currently underway.

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Downregulation of Endothelial Cell VCAM-1 through FoxO1, and Anti-Atherosclerotic Action of Insulin

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Impaired insulin action in endothelial cells may contribute to increased cardiovascular risk in metabolic syndrome or type 2 diabetes. We have recently published that in apolipoprotein E mice with insulin receptor knockout targeted to endothelium, atherosclerosis was increased by up to 3-fold compared to controls, in the absence of any differences in whole-body insulin action, plasma lipids or blood pressure (Cell Metab 2010;11:379). In these mice, leukocyte-endothelial cell adhesion *in vivo* was increased by up to 4-fold, but decreased to below control levels after administration of a VCAM-1 blocking antibody. Here we studied the molecular mechanisms involved in regulation of VCAM-1 by insulin. In endothelial cell culture, insulin reduced VCAM-1 protein by 46±7% at 48 hours ($p=0.005$) and by 57±2% at 72 hours ($p=0.01$). Since FoxO transcription factors are commonly involved in downregulation of gene targets by insulin, we overexpressed FoxO1 in endothelial cells. This increased VCAM-1 by 201±44%, while 72 hours of insulin treatment was still able to reduce VCAM-1 protein by 31±9%. Expression of a triple FoxO1 mutant, which cannot be phosphorylated by insulin stimulation, increased VCAM-1 protein by 187±11%. In this condition insulin decreased VCAM-1 protein by only 14±2% ($p=0.02$). VCAM-1 mRNA half-life was 4 hours based on experiments with actinomycin D, but VCAM-1 mRNA did not decrease until 48 hours after the start of insulin stimulation, suggesting an indirect mechanism. Nitric oxide (NO) is known to decrease VCAM-1 transcription and insulin can increase NO production. Accordingly, L-NAME, an inhibitor of NO synthase, prevented 37% of the downregulation of VCAM-1. eNOS mRNA decreased by 45±8% and 81±8% after expression of wild-type or dominant-negative FoxO1, respectively. Insulin increased eNOS by 56±18% in cells overexpressing wild-type FoxO1, but had no significant effect in cells expressing the mutant. We conclude that FoxO1 mediates insulin-stimulated downregulation of VCAM-1 indirectly through upregulation of eNOS. Improving insulin sensitivity of this pathway may help prevent cardiovascular complications in people with metabolic syndrome or type 2 diabetes.

ADA-Funded Research

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Role of MicroRNA-137 in Diabetes Induced Pro-Inflammatory Phenotype of Vascular Smooth Muscle Cells

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Diabetes promotes pro-inflammatory responses in vascular smooth muscle cells (VSMC), which contribute to accelerated vascular complications. We recently demonstrated the role of epigenetic chromatin histone methylation in enhanced inflammatory responses of VSMC and metabolic memory under diabetic conditions. Evidence shows that microRNAs (miRNAs) play important roles in gene regulation and VSMC dysfunction, but much less is known in diabetic complications. We investigated the role of miRNAs in epigenetic mechanisms associated with pro-inflammatory phenotype of VSMC derived from type 2 diabetic db/db mice. Results showed that miR-137 levels were upregulated in db/db VSMC relative to non-diabetic db/+VSMC. MiRNA- target prediction software revealed Lysine specific demethylase 1 (LSD1), a histone H3K4 demethylase, as a potential miR-137 target. Luciferase activity of a reporter with LSD1-3' UTR was inhibited by miR-137 mimics and this was reversed by mutating the miR-137 binding site, confirming LSD1 to be a true target of miR-137 in VSMC. Transfection of miR-137 mimics into non-diabetic cells downregulated LSD1, and increased inflammatory gene expression and H3K4-dimethylation at their promoters. Immunoblotting and ChIP assays showed reduced LSD1 protein levels and LSD1 occupancy at inflammatory gene promoters in diabetic db/db VSMC. Knockdown of LSD1 in db/+ VSMC with siRNAs induced a diabetic phenotype of increased inflammatory genes and monocyte binding. MiR-137 mimics similarly increased monocyte-VSMC binding in db/+ VSMC. In contrast, miR-137 inhibitors blocked the enhanced monocyte binding in db/db VSMC, thus reversing its inflammatory phenotype. These results demonstrate a novel cross talk between miRNAs (such as miR-137) and epigenetic mechanisms for sustained vascular inflammation and possibly metabolic memory via downregulation of LSD1 in diabetic VSMC. MiRNAs may be novel targets to treat uncontrolled diabetic vascular complications.

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Anti-Inflammatory and Anti-Oxidant Properties of High Density Lipoproteins (HDL) Are Impaired in Type 2 Diabetes

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Despite achieving recommended targets of serum cholesterol, blood pressure, and glycaemia, type 2 diabetic (T2D) patients remain at high risk of vascular events;other factors must therefore be involved in this residual risk. Recent evidence suggests that HDL athero-protective role depends not only on their plasma concentration but also on their anti-inflammatory and anti-oxidant functions. In the present work, we sought to determine whether HDL functions are impaired in T2D and whether *ex-vivo* treatment with L-4F, an ApoA-I mimetic peptide, could improve HDL function. in 93 T2D patients and 31 healthy subjects the anti-inflammatory function of HDL was determined by measuring the ability of HDL to inhibit LDL-induced monocyte chemotactic activity in cultured human aortic endothelial cell monolayers. HDL inflammatory index(HII) was calculated as the monocyte migration induced by standard LDL(sLDL) in the presence of test HDL normalized for the value obtained with sLDL alone. The HDL anti-oxidant property was measured by a cell-free assay in which lipoprotein oxidation increases the fluorescence intensity of dichlorofluoresceine diacetate(HDL+sLDLfluorescence/sLDL fluorescence) and by HDL intrinsic oxidation. For ten T2D patients and ten controls, HII was repeated after incubation of plasma with L-4F. The mean HII for diabetic subjects was 1.42±0.29 and for controls it was 0.70±0.19($p<0.001$). HDL anti-oxidant properties were impaired in T2D patients when compared with controls (2.03±1.35 vs.1.60±0.80, $p<0.05$) and HDL intrinsic oxidation was higher in T2D patients when compared with healthy subjects (1708±739 vs.1233±601 rfu,$p<0.001$). HII correlated with spontaneous HDL fluorescence ($r=0.23$) and with serum Lp(a) concentration($r=0.23$).*Ex-vivo* treatment of plasma with L-4F, restored HDL anti-inflammatory properties in T2D(1.26±0.17 vs.0.71±0.11,$p<0.001$) and to a lesser extent in healthy subjects(0.81±0.16 vs.0.66±0.10, $p<0.05$). We conclude that HDL from T2D patients has abnormal anti-inflammatory and anti-oxidant properties. ApoA-I mimetic peptide improved HDL function and might be a novel therapy for the prevention of vascular events in T2D.

PHARMACOLOGIC TREATMENT OF DIABETES OR ITS COMPLICATIONS

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A High Dose of Aspirin Is Required for Positive Influence on Plasma Fibrin Network in Patients with Type 1 Diabetes

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The fibrin network is an important component of an arterial thrombus. Type 1 diabetes is associated with a tighter fibrin network formation, which may contribute to the increased risk of cardiovascular disease (CVD) in these patients. Treatment with low dose aspirin lowers the risk of CVD in non-diabetic patients, however, the effect seems reduced in patients with diabetes. This relative aspirin resistance may be due to a competition between glycation and acetylation of the fibrinogen molecule. In the present study, the effect of low and high dose of aspirin on fibrin network formation in relation to glycemic control was investigated in patients with type 1 diabetes. Twenty-four patients (12 women) with good glycemic control (glycated haemoglobin (HbA_{1c}; IFCC reference method) <57 mmol/mol) and 24 patients (12 women) with poor glycemic control (HbA_{1c} >68mmol/mol) were included in an open-labelled cross-over study with randomization to treatment with aspirin 75mg and 320mg, respectively. The treatment periods of 4 weeks were separated by a wash-out period of 4 weeks. Mean age and diabetes duration were 51 ±12 (mean ±SD) and 25 ±16 years, respectively. Plasma fibrin network was assessed by determination of the permeability coefficient (Ks) before and at the end of each treatment period. There were no significant differences in Ks levels at baseline between the patients with good or poor glycemic control. During treatment with aspirin 75mg, no changes in Ks levels were observed, while Ks increased from 9.8 ±3.3 to 11.0 ±3.4 $cm^2 \times 10^{-9}$ during treatment with aspirin 320mg ($p=0.004$). The increase in Ks was most pronounced in patients with poor glycemic control ($p=0.02$), whereas a non-significant increase was observed in patients with good glycemic control ($p=0.06$). Our results indicate that a high dose of aspirin is required to influence the fibrin network structure in patients with type 1 diabetes, and that the effect is more pronounced in patients with poor glycemic control. These results may partly explain why treatment with low dose aspirin is less effective for prevention of CVD in patients with type 1 diabetes.

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Metformin, Renal Function and Lactic Acidosis, a Population Based Study

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The use of metformin is limited by impaired renal function and fear of lactic acidosis. In the city of Malmö, population 300 000, all patients who had filled three prescriptions of metformin for each year 2008-2009 were identified and laboratory records were obtained. All patients had at least one P-Kreatinine (P-Kr) recorded. There is one Intensive Care Unit (ICU), at the University Hospital.

Estimated GFR expressed in ml/min/1.73 m^2 (eGFR) was calculated from P-Kr using the CDK-EPI formula.

5408 patients were identified, highest and lowest eGFR were calculated. Patients were stratified by age <60 (1),60-69 (2), 70-79 (3) and 80-89 (4). Groups 2-4 were compared to a population-based database of renal function in the elderly already published, diabetes patients excluded (controls). Average eGFR for metformin-treated vs controls in the 3 groups were 77±0.4 vs 87± 0.3, 66±0.7 vs 76±0.4 and 56±0.6 vs 66±0.6 ml/min/1.73 m^2±SEM. $P<0.001$ for all comparisons.

In the highest age group, 4, 38% of the patients have a highest eGFR of <60 and 66% have a lowest eGFR <60. Corresponding values for eGFR <45 is 12% and 34% In group 3 16% have highest and 44% lowest eGFR <60. In group 4 32% showed a >25% reduction of eGFR at any time during the two years, 8% showed a 50% reduction.

All records from the ICU regarding patients with diabetes were examined. Three cases of lactic acidosis associated with the use of metformin were identified. In one case eGFR was 41 prior to the acidosis, in one case eGFR was >90 and in one case unknown. None of the patients were over 79 years of age.

Metformin-treated patients have a higher eGFR than controls for all age-groups, probably due to selection. In the highest age group a very large group of the patients have eGFR <60, in many countries considered a contraindication for metformin treatment. Three cases of metformin-associated lactic acidosis were recorded, none from the highest age group. Treatment with metformin seems safe even at reduced eGFR, this may be due to the fact that metformin is largely eliminated by tubular secretion, not necessarily parallell to GFR. Stopping treatment when acutely ill is probably more important than not treating when eGFR is low but stable.

365-OR

Is Insulin Exposure Associated with Higher Risk of Cancer-Related Hospitalization or Death? Analysis of 5-Year Data from the ACCORD Trial

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Type 2 diabetes mellitus is associated with higher risk of cancer. Retrospective studies suggest therapy with insulin, especially glargine, may enhance this risk. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study included 10,251 participants with high cardiovascular risk who were randomized to intensive or standard glycemic therapy; insulin use was common (79% and 61%, respectively). Average follow up was 5 years with systematic tracking of ACCORD-managed treatments and medical outcomes. At baseline, mean age was 62±7 yrs and BMI 32.2±5.5. We analyzed these data to determine whether exposure to any insulin or specific kinds of insulin (updated average units/kg/day over the time of observation) was independently associated with a composite outcome (CO) of cancer-related hospitalizations or cancer death. We projected that an anticipated 300 events would provide 80% power to detect an HR of 1.38 or greater by Cox regression analysis; 304 events (101 patients with cancer-related nonfatal hospitalizations and no death, 203 deaths) were identified. All models included as covariates baseline age, gender, BMI, insulin-use, tobacco-use, alcohol-use, and history of cardiovascular events; subsequent assignment to glycemia treatment group, the blood pressure (BP) or lipid trials, or intensive BP or fibrate treatment; and on-treatment average A1c. Risk of CO was higher among current and previous smokers and in association with higher BMI. Insulin exposure-associated risks of CO, after adjustments for above mentioned factors, are summarized in the table.

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Exposure	HR	95% CI	P-Value
Total Insulin	1.19	0.79, 1.79	0.41
Basal Insulin	1.18	0.65, 2.14	0.6
Glargine	1.00	0.53, 1.86	0.99
Prandial Insulin	2.30	1.08, 4.90	0.03
Prandial insulin after correction for basal insulin exposure	2.41	1.05, 5.49	0.03

Conclusions: 1) Exposure to any insulin or to basal insulin or glargine specifically was not associated with increased risk of cancer-related outcomes. 2) Exposure to prandial insulin might be associated with increased risk. 3) Current findings are hypothesis generating. Further data collection may provide greater statistical power.

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Changes over Time in Glycemic Control, Insulin Sensitivity and Beta-Cell Function in Response to Low-Dose Metformin and Thiazolidinedione Combination Therapy in Patients with Impaired Glucose Tolerance

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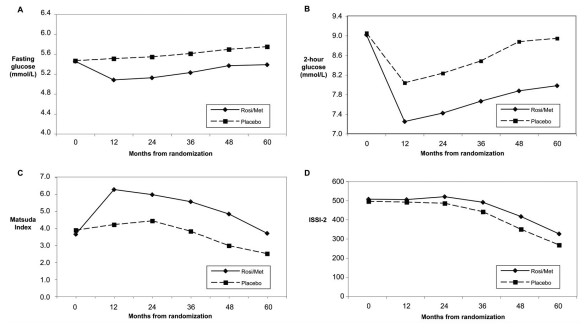
While lifestyle and anti-diabetic medications can prevent the development of type 2 diabetes (T2DM) in subjects with impaired glucose tolerance (IGT), the long-term durability of these interventions will likely depend on their capacity to modify the insulin resistance and beta-cell dysfunction that characterize the early pathophysiology of T2DM. In the Canadian Normoglycemia Outcome Evaluation (CANOE) trial, low-dose rosiglitazone/metformin (Rosi/Met) reduced the risk of incident T2DM in subjects with IGT by 66%. For insight on the disease-modifying capacity of this therapy, we evaluated the temporal changes in glycemic control, insulin sensitivity and beta-cell function during this trial. In CANOE, 207 subjects were randomized to either Rosi/Met (2/500mg bid)(n=103) or matching placebo (n=104), and followed for median 3.9 years. They underwent annual oral glucose tolerance testing, enabling temporal comparison of glycemia, insulin sensitivity (Matsuda index) and beta-cell function (Insulin Secretion-Sensitivity Index-2 (ISSI-2)) between the study arms. Fasting glucose, 2-hr glucose, and insulin sensitivity improved in the Rosi/Met arm in Year 1, but then deteriorated in the years thereafter as in the placebo arm (Fig A-C). Beta-cell function did not improve (Fig D).



Generalized estimating equation analysis confirmed that both insulin sensitivity and beta-cell function declined over time (Matsuda: beta=-0.0515,p<0.0001; ISSI-2: beta=-6.6507,p<0.0001), with no significant time-by-treatment interaction (Matsuda: p=0.57; ISSI-2: p=0.22). In summary, low-dose Rosi/Met had an early effect on insulin sensitivity that reduced incident T2DM but was not sustained. This therapy thus did not appear to have a long-term disease-modifying effect.

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Genetic Predisposition and Nongenetic Risk Factors of Thiazolidinedione-Related Edema in Subjects with Type 2 Diabetes

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We aims to study the association of thiazolidinedione (TZD)-related edema with genetic and clinical variables and develop a simple point system to predict the risk of developing TZD-related edema.

Fifty-eight (21.6%) of 268 type 2 diabetic patients who received TZD developed peripheral edema. Twenty-eight tag SNPs of the candidate genes were genotyped.

Patients with edema are older, predominantly female, and have greater weight gain. The *AQP2* rs296766 T allele was associated with TZD-related edema (allelic *P*=0.0059;OR,2.89;95% CI,1.61–5.17). The *SLC12A* rs12904216 G allele had borderline significance (allelic *P*=0.049), which disappeared after correction for multiple testing. Patients with 2 SNP-based (*AQP2* rs296766 and *SLC12A1* rs12904216)weighted genetic scores (WGS)within the top quartile had a higher risk of developing TZD-related edema (OR,5.65;95% CI,2.05–15.58). The predictive power of adding the WGS of 2 SNPs or all SNPs to predict TZD-related edema, in addition to age and sex, was significant. We also developed a simple point system to predict a patient's risk for TZD-related edema based on risk factor information.

Table 1. Basic characteristics of the study subjects

	Edema (n=58)	Non-edema (n=210)	*P* value
Male/female (male %)	14/44 (24.1%)	112/98 (53.3%)	**<0.001**
Age (years)	66.8 ± 11.7	62.6 ± 10.9	**0.011**
BW at baseline (kg)	63.7 ± 13.4	65.8 ± 11.8	0.273
SBP (mmHg)	134.8 ± 12.6	132.7 ± 15.3	0.321
DBP (mmHg)	76.1 ± 8.2	77.1 ± 9.6	0.468
Maximal BW gain after TZD (kg)	5.6 ± 4.7	2.0 ± 3.1	**<0.001**

Table 2. Cumulative effect of risk alleles on TZD-related edema

Weighted genetic score	Edema, n (%)	Non-edema, n (%)	HR (95% CI)*	*P* for trend
***AQP2* (rs296766) and *SLC12A1* (rs12904216)**				
0	9 (16.1)	75(36.8)	1.00	<0.0001
0-0.62	13 (23.2)	68(33.3)	1.49(0.58-3.83)	
0.62-2.00	20(35.7)	41(20.1)	4.43(1.77-11.10)	
2.00	14(25.0)	20(9.8)	5.65(2.05-15.58)	
All 28 SNPs				
<16.02	10(17.9)	94(46.1)	1.00	<0.0001
16.02-19.55	9(16.1)	43(21.1)	2.38(0.87-6.53)	
19.55-22.69	15(26.8)	37(18.1)	4.26(1.69-10.73)	
22.69	22(39.3)	30(14.7)	6.71(2.75-16.39)	

* adjusted for age and sex

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For author disclosure information, see page 785.

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Reversibility of Fenofibrate-Induced Renal Function Impairment in ACCORD Type 2 Diabetic Participants

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Fenofibrate therapy is commonly prescribed to manage elevated triglyceride levels in diabetic patients but can cause a persistent increase in serum creatinine (SCr) levels in some patients within 3 months of starting therapy. The reversibility of this increase in SCr after cessation of fenofibrate therapy is uncertain. We conducted an on-drug/off-drug ancillary study to the ACCORD Lipid Trial to investigate changes in renal function after the Trial close-out visit. Eligible participants were recruited into a prospective nested 3 group study based on retrospective on-trial SCr levels: Fenofibrate (F-) Cases (≥20% increase in SCr from baseline to month-4 visit, N=321); Fenofibrate (F-) Controls (≤2 % increase, N=175); and Placebo (P-) Controls (no criterion; N=565). Serum creatinine and estimated glomerular filtration rate (eGFR) were measured at trial end and 6-8 weeks after discontinuation of fenofibrate or placebo therapy.

At trial end, F-Cases had the highest SCr (±SE) (1.11±0.02 mg/dL) and lowest eGFR (±SE) (72.2±1.3 mL/min/1.73m^2) vs. F-Controls (1.01±0.02; 80.0±1.7) and P-Controls (0.98±0.01; 83.3±1.0). After 51±10 (±SD) days off drug, F-Cases had a SCr (0.97±0.02) that was still higher (p=0.0008), and eGFR (83.5±1.3) still lower (p=0.004) than F-Controls (0.90±0.02; 90.0±1.8) but not different from P-Controls (0.99±0.01; 81.6±1.0). Five-year change in renal function for F-Controls compared to a matched subgroup of P-Controls (≤ 2% increase in SCr baseline-to-month 4, N=358) revealed F-Controls had significant SCr decline (-0.03±0.02 mg/dL, p=0.03) but no change in eGFR (0.5±1.4 mL/min/1.73m^2); P-controls had significant SCr increase (0.05±0.01, p<0.0001) and eGFR decline (-6.5±1.0, p<0.0001). The differences between groups (F-Controls minus P-Controls) were also significant.

In summary, at study end after 5.2 years of treatment, F-Cases returned to the same level of renal function as P-Controls, and F-Controls had net preservation of renal function compared to a matched subgroup of P-Controls. The fenofibrate-associated on-trial increases in SCr were reversible, and the reversal was essentially complete after 51 days off drug.

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The Effects of Pioglitazone on Near-Normoglycemic Remission, β-Cell Function and Insulin Sensitivity in New-Onset Hyperglycemic Crises

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Most African American (AA) patients with new-onset T2DM presenting with diabetic ketoacidosis (DKA) or severe hyperglycemia (HG) achieve near-normoglycemic remission at ≤ 12 weeks on insulin. The optimal treatment after stopping insulin is unclear; thus, we investigated if pioglitazone could delay hyperglycemic relapse in AA patients with history of DKA or HG (BG ≥ 400 mg/dL).

Ninety newly-diagnosed AA patients were recruited after resolution of DKA (n=35;22M/13F; age: 43±11; BMI:40±12; BG:639±259 mg/dl, mean±SD) and HG (n=55; 31M/24F; age:44±10; BMI:38±9; BG:645±238); of them, 73 % with DKA and 59% with HG discontinued insulin within 12 weeks. A total of 20 DKA and 24 HG patients were randomized in a double-blinded fashion to pioglitazone 30 mg/day (n=22, PIO) or placebo (n=22, PBO) and followed for up to 36 months while in remission. β-cell function (BCF) and insulin sensitivity (IS) were assessed by IV glucagon and glucose stimulation tests and minimal model analysis (MinMod) within 1 week of diagnosis and at 1 week following insulin discontinuation. Oral glucose tolerance tests (OGTT) were done at remission, 3 months and every 6 months during the remission phase. Relapse was defined as fasting BG >130 mg/dl or random BG >180 mg/dl x2 and A1c ≥7.0%.

Both groups were comparable in age, sex, BMI, A1C, GAD antibody status and duration of insulin therapy. There were no significant differences in BCF or IS between PIO and PBO groups at diagnosis or at remission. Compared to PBO, PIO significantly reduced the number of patients with hyperglycemic relapse (68% vs 32% respectively, p=0.03). Furthermore, the PIO group compared to PBO remained in remission longer (median:809 vs 162 days, p=0.01). The PIO group compared to PBO had lower glucose and insulin levels, higher BCF and improved IS throughout repeated OGTT tests.

In conclusion, pioglitazone compared to placebo resulted in prevention of recurrence of hyperglycemia and prolonged the insulin-free period of remission in overweight AA patients with new-onset, hyperglycemic crises. This effect appears to correlate with improvement in β-cell function and insulin sensitivity.

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Colesevelam HCl Improves Fasting Plasma Glucose Clearance and Glycolytic Disposal of Oral Glucose but Has No Effect on Appearance Rate of Oral Glucose or Gluconeogenesis

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Colesevelam (COL) is a bile-acid sequestrant approved for glycemic control in subjects with type 2 diabetes (T2DM). The aim of this randomized, double-blind, placebo-controlled study was to elucidate glucose-lowering mechanisms of COL. Subjects with T2DM (A1C, 6.7-10.0%) were randomized to 3.75g/d COL (n=27) or placebo (n=28) for 12 weeks and studied at baseline and post-treatment. Fasting endogenous glucose production (EGP), gluconeogenesis (GNG), glycogenolysis, and glucose clearance were measured using [U-$^{13}C_6$]glucose and [2-$^{13}C_1$]glycerol. Appearance rate of oral glucose (Ra meal), postprandial EGP, appearance rate of total glucose (Ra total) and total glucose disposal rate (Rd total) were measured during a test meal using a dual tracer method. Glycolytic disposal of oral glucose is the amount of ^2H_2O released from 15g of [6,6-$^2H_2$]glucose administered with the test meal. Plasma glucose, insulin, total glucagon-like peptide-1 (GLP-1), total glucose-dependent insulinotropic polypeptide (GIP) and glucagon concentrations were measured during the fasting state and following the test meal. Results are treatment differences in least square mean ± SE. Compared to placebo, COL improved A1C (-0.6 ± 0.2 %, P<0.01), fasting glucose (-23 ± 11 mg/dl, P<0.05), glucose AUC (-24 ± 12 mg/dl/min, P<0.05), fasting glucose clearance (0.31 ± 0.11 ml/kg FFM/min, P<0.01) and glycolytic disposal of oral glucose (6 ± 2 %load, P<0.01). COL had no affect on fasting GNG, Ra meal, postprandial EGP, Ra total and Rd total. Fasting EGP and glycogenolysis increased with placebo (P<0.05) but were unchanged with COL (treatment effect was NS). Compared to placebo, COL increased fasting total GLP-1 (10 ± 4 pmol/l, P=0.01), total GLP-1 AUC (8 ± 3 pmol/l/min, P<0.01), GIP AUC (13 ± 3 pmol/l/min, P<0.01) and HOMA-B (18 ± 5%, P<0.01) while insulin, glucagon and HOMA-IR were unchanged. COL, a non-absorbed agent, improved tissue glucose metabolism in the fasting and postprandial states which was associated with an increased incretin response.

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TYPE 2 DIABETES GENETICS—ASSOCIATION AND FUNCTION

371-OR

Genes and Pathways Targeted by Anti-Diabetes Medications Are Enriched for Common DNA Variant Associations with Type 2 Diabetes

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Genome-wide association studies (GWAS) have uncovered ~40 common variants associated with type 2 diabetes (T2D) that collectively explain ~10% of the genetic contribution to T2D risk. Several genes in these associated regions encode direct or indirect targets of anti-diabetes medications, such as *PPARG* for thiazolidinediones (TZD), or *KCNJ11* and *ABCC8* for sulfonylureas. We therefore hypothesized that other genes in pathways targeted by anti-diabetes drugs may also be associated with T2D with modest effects that jointly have a significant impact on T2D. To address this, we created a list of 102 genes involved in pathways targeted by various classes of clinically available anti-diabetic medications including insulin, biguanides, sulfonylureas, TZD, meglitinides, alpha-glucosidase inhibitors, glucagon-like-peptide 1 mimetics, dipeptidyl peptidase 4 inhibitors, and amylin mimetics. We selected these genes via literature review of human, animal and cell culture-based studies characterizing the pathway of the drug class target and downstream effects.

To test whether the drug target gene set is enriched for genes associated with T2D or related glycemic traits, we applied our newly developed method MAGENTA (Meta-Analysis Gene-set Enrichment of variaNT Associations) to the most recent GWAS meta-analysis of T2D (DIAGRAM+) and seven MAGIC meta-analyses of glucose and insulin-related traits. We found strong enrichment for genes associated with T2D (MAGENTA p=1.2x10^{-5};

For author disclosure information, see page 785.

excess of 19 genes), primarily driven by insulin and TZD targets, and modest enrichment of associations with fasting glucose levels (p=0.01) and an index for beta-cell function, HOMA-B (p=0.05). The T2D signal was largely due to multiple genes of modest effects (p=3.5x10^{-5}, after removing known T2D loci), suggesting new T2D associations to be followed up in independent samples and pharmacogenetic studies. About half the drug target genes that passed the enrichment cutoff for T2D (18 genes) were also highly ranked with respect to fasting glucose (e.g. *ACSL1, ACACA*, *GLUT1* and *GLUT5*). These results may provide new avenues for individualized drug selection and T2D treatment design. **ADA-Funded Research**

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TCF7L2 Chromatin Occupancy and Its Role in Hepatic Glucose Production In Vitro

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Previous *in vitro* and *in vivo* studies have focused on the link between TCF7L2 and insulin secretion as an explanation for the association between TCF7L2 and T2DM. However, TCF7L2 and the Wnt/β-catenin pathway are important for metabolic zonation in the liver. This raises the interesting possibility that TCF7L2 may influence glucose homeostasis by regulating hepatic glucose production (HGP). To examine this question, we utilized the H4IIE cell as a model of HGP. Inhibition of HGP in H4IIE cells from lactate and pyruvate was highly sensitive to physiological concentrations of insulin and metformin. Silencing of TCF7L2 protein expression induced a 5-fold increase in basal HGP (P<0.0001), and this was accompanied by marked changes in the expression of several key gluconeogenic genes. FBPase, PEPCK and G6Pase mRNA were up-regulated 2.5-fold (P<0.0001), 1.4-fold (P<0.01) and 2.3-fold (P<0.0001), respectively, compared to scramble siRNA. Compared to their respective baseline values, insulin and metformin suppressed HGP equally in the scramble and TCF7L2 siRNA cells, but HGP remained elevated in TCF7L2 silenced cells due to the increased baseline HGP. Phosphorylation of Akt ser^{473} and GSK-β ser^{9} by insulin was comparable in TCF7L2 and scramble siRNA cells. Chromatin immunoprecipitation sequencing (ChIP-Seq) was used to investigate the genome-wide DNA binding activity of TCF7L2. A total of 2194 ChIP peaks were detected, and 1167 (53%) were located either within a gene or no more than 10 kb away from the TSS or 3'UTR. TCF7L2 bound genes demonstrated clustering into endocrine (247 genes), genetic (529 genes) cardiovascular (248 genes) and inflammatory categories (302 genes). Genes related to T2DM made up 65% (P=1.5 x 10^{-18}) of all genes in the endocrine category, and included PDK1, PPARγ, PPRC1, INSR, PIK3C2G, NRF1 and KCNJ11. Cellular processes represented by the dataset were consistent with a role for TCF7L2 in the Wnt/β-catenin pathway and included tissue development and survival. Our findings point to an important role for TCF7L2 in regulating HGP and indicate that TCF7L2 may modulate glucose metabolism in liver cells by influencing the expression of key metabolic genes.

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Distillation of Potential Drug Targets for Type 2 Diabetes by Overlaying TCF7L2 ChIP-Seq and Pancreatic Islet Open Chromatin Maps

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Variation in *TCF7L2* is strongly associated with type 2 diabetes (T2D). We previously mapped the genomic regions bound by TCF7L2 using ChIP-seq in the colorectal carcinoma cell line, HCT116, where it is abundantly expressed. The list of genes bound by TCF7L2 harbor a highly significant over-representation of loci uncovered in genome wide association studies (GWAS) of T2D and cardiovascular disease. Further support for our conclusions came from a subsequent vitamin D receptor ChIP-seq study that gave a similar enrichment of GWAS-discovered genes, albeit for immune and cancer related traits. As such, it is our hypothesis that a substantial portion of the reminder of genes bound by TCF7L2 on our list, not previously implicated in disease, also harbor genetic variants associated with T2D. With these facts in mind, we aimed at distilling down genes bound by TCF7L2 that encode proteins that would be considered relatively attractive drug targets. Firstly, we analyzed the overlap of loci between our TCF7L2 ChiP-seq gene list (limited to within 5kb from a transcription start site) and the published open chromatin map for pancreatic islets in order to get an indirect measure of relevant sites in this key tissue; indeed it has been reported that chromatin is more open in the presence of the key *TCF7L2* T2D-risk allele. We found that 94 genes overlapped, and interestingly, 36% of the gene products fall in to drug target classes. Attempting to distill this gene list further, we also carried out ChIP-seq in another cell line where *TCF7L2* is abundantly expressed i.e. HepG2. With this extra layer of data, 24 genes remained of which 7 encode what would be considered druggable targets i.e. *CHD2, CNGB3, AKT2, CSNK1A1, AP3M1, ATP9A* and *ITGB5.* We also carried out siRNA knock-down of *TCF7L2* expression in our cell-lines, shedding light on downstream effects. In summary, we have distilled down loci that are both bound by TCF7L2 across two cell lines and coincide with open chromatin in human pancreatic islets, of which a number encode potentially attractive drug targets that are also likely candidates to be genetically validated when sequenced in a T2D cohort.

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A Genome-Wide Association Analysis of >90,000 Individuals Identifies Sex-Specific Effects for Fasting Glycemic Traits

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Meta-analyses of genome-wide association studies (GWAS) for glycemic traits to date have described 16 loci influencing fasting glucose (FG) and two influencing fasting insulin (FI) levels. The higher prevalence of impaired fasting glucose in men and impaired glucose tolerance in women is established, but the mechanisms for these sex differences are unknown. Within MAGIC, we aimed to investigate sex-specific differences in genetic regulation of FG/FI levels.

We performed sex-stratified (2df test to model sex heterogeneity) meta-analyses of 36 GWAS with FG/FI measures in up to 32,993 (27,870 for FI) men and 42,149 (34,940 for FI) women without diabetes. SNPs at 27 loci were selected for *in silico* follow up and were available on the Metabochip, a custom iSELECT array¸ genotyped in 14 additional cohorts (up to 10,006 men and 7,457 women).

Among selected loci, a second FG signal at *G6PC2* independent of the previously-reported rs560887 showed the largest differential effect estimates (P_{2df}=1.9x10^{-13}) stronger in men (β=0.122 [SE=0.01], P=8.9x10^{-12}) than in women (β=0.059 [SE=0.017], P=5.4x10^{-4}). The signal at *IRS1*, in LD (r^2=0.74) with the reported T2D signal, showed a sex-stratified effect for FI (P_{2df}=5.4x10^{-9}) with higher estimates for men (β=0.023 [SE=0.004], P=2.3x10^{-8}) than women (β=0.009 [SE=0.004], P=0.009). Another T2D locus, *CDKN2A/2B,* demonstrated a sex-stratified effect for FG (P_{2df} =2.7x10^{-12}) with larger estimates for women (β=0.027 [SE=0.004], P=6.3x10^{-11}) than men (β=0.015 [SE=0.005], P=0.001). At *PCSK1* we detected larger effect estimates for FG in women (β=0.023 [SE=0.004], P=1.2x10^{-11}) than men (β=0.011 [SE=0.004], P=0.006; P_{2df}=2.5x10^{-12}). Meta-analysis of FI revealed larger effect estimates in women at *SLC30A10* (β=0.025 [SE=0.004], P=1.7x10^{-9}) than men (β=0.011 [SE=0.004], P=0.02; P_{2df}=8.6x10^{-10}).

Some genetic loci have greater contribution to the variability of fasting glycemic traits in men (*G6PC2, IRS1*) than in women and vice versa (*CDKN2A/2B, PCSK1, SLC30A10*) owing to differential sex-related mechanisms affecting regulation of glycemic traits. This analysis underscores the need to consider sex differentiated effects on glycemia.

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Meta-Analysis of Genome Wide Association Studies Reveals New Loci Associated with Childhood Obesity

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Obesity is a major risk factor for type 2 diabetes. A number of genetic determinants of adult obesity have already been established through large scale meta-analyses of genome wide association studies (GWAS), several of which were also confirmed in the context of childhood obesity. However, less progress has been made to establish genetic influences specific to childhood obesity though similar approaches. To identify novel genetic factors that influence early-onset obesity, we performed a meta-analysis of genome-wide genotyped datasets consisting of 5,447 cases (≥95th percentile of BMI achieved any time from age 2 to 18 years old) and

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8,185 controls (<50th percentile of BMI consistent throughout all measures during childhood) of European ancestry. Following the meta-analysis of ~2.54 million SNPs (directly genotyped or imputed), variation at seven loci showed notable evidence for association with childhood obesity ($P < 5x10^{-8}$). All these loci have been previously reported in the context of adult BMI GWAS (*FTO*, *MC4R*, *TMEM18*, *POMC*, *FAIM2*, *TNNI3K* and *SEC16B*), but their relative magnitude of association were different in the childhood obesity setting. *TMEM18* gave the strongest evidence for association while *TNNI3K* and *POMC*, which were only detected in adult studies when using hundreds of thousands of participants, were readily detected in our relatively small sample size. We elected to take forward all novel loci yielding association with $P < 5x10^{-6}$ (n = 11) in order to test for replication in multiple existing datasets. To date, we observe consistent evidence for replication at two loci, harboring the genes encoding alpha-protein kinase 1 (*ALPK1*) on 4q25 and glypican 5 (*GPC5*) on 13q31, respectively. As more data for the replication attempt is received, we anticipate additional signals will survive this effort. In summary, as a consequence of extensive North American-Australian-European collaborative meta-analyses of genome-wide genotyped datasets on children, we have uncovered at least two novel obesity loci.

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Early Growth Retardation and Insulin Resistance in JAZF1 KO Mice

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Genome-wide association studies surveying thousands of single nucleotide polymorphisms (SNPs) distributed throughout the human genome have recently detected an association of a SNP in the first intron of the JAZF1 gene and individuals with type 2 diabetes (T2D). Additionally, a separate SNP located in the first intron of JAZF1 and physically closely linked to that correlated with T2D was found to be associated with increased height, suggesting a functional connection between JAZF1 and both growth and T2D. However, little is known about the biologic bases of these associations or the function of JAZF1 in general. To investigate the possible role of JAZF1 in growth and glucose metabolism, we generated JAZF1 knockout (KO) mice and examined body composition by ^{1}H-nuclear magnetic resonance and insulin sensitivity by the hyperinsulinemic [3mU/(kg-min)]-euglycemic clamp. The KO mice were healthy and had no anatomic defects in major organs. Nevertheless, JAZF1 KO mice were found to show postnatal growth retardation, reflected by ~15% reduced body weight, ~20% shorter body length, and ~27% reduced serum IGF1 concentration (WT, 370 ± 38 ng/ml; KO, 265 ± 26 ng/ml, P<0.05) at young age (<5 month-old), compared to the age-matched wild-type control (WT) mice. After 7-8 months, the JAZF1 KO mice rapidly caught up to WT mice in size but showed excess fat accumulation (body fat composition: WT, 8.4 ± 0.4; KO, 12.1 ± 1.5%, P<0.05). Fasting plasma glucose and insulin concentrations as well insulin sensitivity were similar in young JAZF1 KO and WT mice. In contrast, lean body mass and insulin-stimulated whole body glucose uptake were both decreased by ~5% and ~11% in adult JAZF1 KO mice, respectively, compared to age-matched WT mice (7-8 month-old). Furthermore, insulin-stimulated 2 deoxy-glucose uptake in muscle was decreased by 30% (P<0.05) in adult JAZFI KO mice. Conclusion: Deletion of the JAZ1 gene in mice leads to early growth retardation, which is associated with reduced plasma IGF-1 levels, and in adulthood to decreased muscle mass, increased fat mass, and insulin resistance. These results provide potentially important mechanisms by which genetic alterations in JAZF1 result in T2D and altered stature.

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Enrichment of Diabetes and CVD EQTLs in Mexican Americans

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We previously reported association with T2D of a common SNP within VTI1A upstream of TCF7L2 (rs7095620, p= 0.00006) in the San Antonio Family Diabetes Study (SAFDS). This SNP is correlated with expression of both VTI1A and TCF7L2 in lymphocytes of SAFDS and San Antonio Family Heart Study (SAFHS) subjects. VTI1A is a component of insulin-sensitive GLUT4-containing vesicles and may regulate glucose transport and adiponectin secretion in adipocytes. To explore any effect of this variant on pathways involved in diabetes, we examined the correlation with expression profiles of primary lymphocytes from 1240 SAFHS subjects. Using the program SOLAR to account for family relations, we conducted measured genotype analysis under an additive model using the genotypes at rs7095620 on all 15,830 autosomal-encoded transcripts detected in lymphocytes. Correlation with one eQTL (CRMP1) was significant (p=3x10-6). We tested for enrichment, among the top 1% of eQTL correlations, of regions previously connected to diabetes (T2D, T1D, MODY). To do this, we first selected all genes within a 1Mb interval surrounding previously published genetic variants associated with diabetes. We then removed any gene whose transcription was not detected in lymphocytes (since our expression data pertained to lymphocytes), leaving 229 genes to compare for enrichment. Upon comparison of this list to our list of associated eQTLs, we observed significant enrichment of loci from the diabetes regions (Fisher's $p = 3 \times 10^{-6}$). Next, GO analysis with Ingenuity identified 7 eQTLs among the "enriched" set to have previous reports for diabetes mellitus (CRMP1, INSR, KCNQ1, NOTCH2, P2RX4, PLAGL1, TMEM132D), 5 with cardiovascular disorder (AGGF1, INSR, KCNQ1, TMEM132D, VTI1A) and 6 with inflammatory disease (CRMP1, INSR, KCNQ1, NOTCH2, SLC15A4, TMEM132D). We repeated the analyses with other SNPs of similar MAF on 10q, with TCF7L2 rs7903146, and with the SNPs (or their LD proxies) that defined the associated diabetes regions and did not observe enrichment (p-value range 0.06 – 1) so the pattern of associated eQTL profiles appears to be unique to subjects bearing this SNP. These regions may be of particular interest for Mexican American populations.

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Genetic Analysis of Lipidomic Profiles Influencing Diabetes Risk in Mexican Americans

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Lipids are a diverse class of molecules with essential roles in cellular structure, signaling, and energy storage. Lipid dysregulation can lead to the development of several diseases with varied consequences including Alzheimer's, atherosclerosis, and diabetes. These lipid molecules may represent intermediate phenotypes that are closer to gene action than classical lipid markers, making them extremely valuable for genetic analysis.

In the most extensive large-scale analysis of the human lipidome to date, we profiled 1,202 Mexican Americans from ~40 large pedigrees of the San Antonio Family Heart Study. Using liquid chromatography electrospray ionization-tandem mass spectrometry, we identified and quantified 356 different canonical lipid species. Quantitative genetic analysis and genome-wide association analyses using over one million SNPs were performed to examine potential genetic factors involved in the variation in lipidomic profile. Preliminary analyses showed almost all 356 measured lipid species are significantly heritable. Our results identified 210 lipid species that correlate with diabetes status (FDR ≤ 0.05); of which 128 also predict progression to diabetes in non-diabetics followed for ~10 years.

The single best predictor of progression to diabetes is dhCer (dihydro-ceramide) 18:0, the biosynthetic precursor to ceramide 18:0. dhCer is significantly heritable ($h^2 = 0.247$; $p = 1.6 \times 10^{-9}$) and is markedly increased in diabetics ($p = 2.5 \times 10^{-7}$). In non-diabetics, those that progress to diabetes within 10 years also show higher dhCer 18:0 levels at baseline than non-progressors ($p = 2.2 \times 10^{-8}$). This predictive relationship is maintained ($p = 1.2 \times 10^{-4}$) even when baseline fasting glucose and insulin levels are taken into account, indicating that this lipid component appears to be an independent predictor of diabetes risk. A genome-wide association analysis reveals two

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rare SNPs (MAF = 0.02) on chromosome 3 exhibiting genome-wide significant association with dhCer 18:0 levels ($p = 9\times10^{-8}$). These SNPs are located between the *SNORA62* and *MOBP* genes at 3p22. Our results suggest that lipidomic profiles may represent endophenotypes that may help us identify genes involved in diabetes risk.

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