LATE BREAKING ABSTRACTS
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Late Breaking Abstracts LB1
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ComplIcAtions—MacrovasculAr—Atherosclerotic CardiovasculAr disease and HuMan dIabetes

1-LB
Adolescents with Type 1 DiabeteS (T1D) Have Abnormal Global Circumferential Left VentrIcular Strain Which Correlates to GlycerCmia


Myocardial mechanics are altered in diabetes. Prior studies focused on adults with type 2 diabetes, showing that decreased longitudinal strain (LS) as a result of sub-endocardial fibrosis occurs early in the course of diabetes; abnormalities in circumferential strain (CS), suggesting midwall fiber damage, occur with more severe disease. Few studies have assessed global strain in T1D, or relationship to disease severity or functional capacity. We reported decreased maximal exercise capacity (VO₂max) and diastolic dysfunction in T1D youth, and hypothesized that myocardial strain may also be altered.

Echocardiograms and VO₂max were assessed in 34 T1D(A1c 8.4 ± 1.5%) and 20 control youth. Paraestrally short axis views at the papillary muscles were analyzed with sparkle tracking to ascertain global CS. Global values from 3 apical views were averaged to obtain global LS. Global parameters were obtained by analyzing the left ventricular (LV) myocardium as a whole rather than averaging each segment. T1D youth had significantly lower VO₂max, CS, and fractional shortening (Table). Among T1D, CS correlated with A1c (r=0.35, p=0.04). Echo parameters were otherwise unrelated to VO₂max, A1c or T1D duration.

Control T1D p-Value

| Age (yrs) | 16.4 ± 2.6 | 15.4 ± 2 | 0.13 |
| CS (cm) | 50.6 ± 8.2 | 44.1 ± 7.5 | <0.01* |
| LVIDd (cm) | 4.3 ± 0.4 | 4.4 ± 0.4 | 0.42 |
| LVIDs (cm) | 2.7 ± 0.4 | 2.9 ± 0.3 | 0.029* |
| Fractional shortening (%) | 37.8 ± 7.5 | 33.4 ± 6.1 | 0.02* |
| Longitudinal strain (%) | -19.4 ± 2.6 | -19.2 ± 2.9 | 0.85 |
| Circumferential strain (%) | -23.8 ± 4.3 | -21.7 ± 3.4 | <0.05* |

In conclusion, T1D adolescents have significant LV dysfuntion, including lower CS that correlated with A1c. Poor glycemic control may precipitate cardiac dysfunction and contribute to functional decline. The pattern of low CS, but preserved LS, suggests that damage to midwall fibers mediates cardiac dysfunction and contribute to functional decline. The pattern of low CS, but preserved LS, suggests that damage to midwall fibers mediates cardiac dysfunction and contribute to functional decline.

2-LB
Coronary Artery Calcium Score and Prediction of Cardiovascular Mortality in Diabetes. Diabetes Heart Study

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In type 2 diabetes mellitus (T2DM), it remains unclear whether coronary artery calcium (CAC) score provides additional information about cardiovascular disease (CVD) mortality beyond traditional risk factors. A total of 1,123 T2DM affected participants, ages 34-86, in the Diabetes Heart Study (DHS), a family study in which siblings concordant for T2DM, their unaffected siblings as well as unaffected family members, recruited from internal medicine and endocrinology clinics in western North Carolina, were followed for an average of 7.4 years (range 4-12 years). CAC (Agatston Score) was measured daily. All groups had similar basal PG—6.5 mmol/l and PI—600 pmol/l levels. During infusion hyperglycaemia (>25 mmol/l) and hyperinsulinemia (>2700 pmol/l) were immediately manifested in the CHG group and maintained throughout the study. The FLU group showed regular fluctuations of PG—21 mmol/l and PI—2500 pmol/l at peak level and returned back to basal levels in between pulses. Initially, the CLG group displayed an increase in PG—2700 pmol/l in T2DM and at baseline during infusion whereas PI levels were gradually increased to levels of ~2000 pmol/l during infusion. Plasma MDA was significantly increased in the FLU group at 72 and 96 h (72 h: VEH: 1.42±0.15 vs. FLU: 2.3±0.15; 96 h: VEH: 1.34±0.15 vs. FLU: 2.15±0.15; p<0.05; µmol/l) while the CHG group only showed significance at 72 h (VEH: 1.42±0.15 vs. CHG: 2.35±0.16; p<0.05; µmol/l). We show that fluctuating glucose levels lead to oxidative stress similarly to that of sustained hyperglycaemia despite a much lower total glycaemic exposure. Thus our data supports the notion that fluctuating glucose may be relatively more deleterious than sustained hyperglycaemia.

For author disclosure information, see page LB42.

3-LB
Fluctuating Hyperglycaemia Induces Relatively Higher Levels of Oxidative Stress than Sustained Hyperglycaemia in Insulin Resistant Rats

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Fluctuating hyperglycaemia has been hypothesized to promote the production of reactive oxygen species more than sustained hyperglycaemia and thus lead to increased levels of oxidative stress. In this study, we compare the effects of fluctuating with sustained high or low levels of glucose on levels of oxidative stress. Catecholized diet induced obese (DIO) insulin resistant male rats (Age: 38 weeks) were randomized into 4 groups (n=7-8) and connected to Accusamplers® for automated blood sampling and intravenous glucose infusions. Animals, fasted for 6 h received either a continuously high (CHG) or low (CLG) glucose levels and the FLU group received nine daily 30 min glucose pulse infusions for 2.5 h. In total FLU and CLG groups received equal amounts of glucose whereas the CHG group received three times this amount. Plasma glucose (PG) and insulin (Pl) was monitored throughout the study. Plasma malondialdehyde (MDA), a biomarker for oxidative stress, was measured daily. All groups had similar basal PG—6.5 mmol/l and PI—600 pmol/l levels. During infusion hyperglycaemia (>25 mmol/l) and hyperinsulinemia (>2700 pmol/l) were immediately manifested in the CHG group and maintained throughout the study. The FLU group showed regular fluctuations of PG—21 mmol/l and PI—2500 pmol/l at peak level and returned back to basal levels in between pulses. Initially, the CLG group displayed an increase in PG—2700 mmol/l but declined to basal levels during infusion whereas PI levels were gradually increased to levels of ~2000 mmol/l during infusion. Plasma MDA was significantly increased in the FLG group at 72 and 96 h (72 h: VEH: 1.42±0.15 vs. FLU: 2.3±0.15; 96 h: VEH: 1.34±0.15 vs. FLU: 2.15±0.15; p<0.05; µmol/l) while the CHG group only showed significance at 72 h (VEH: 1.42±0.15 vs. CHG: 2.35±0.16; p<0.05; µmol/l). We show that fluctuating glucose levels lead to oxidative stress similarly to that of sustained hyperglycaemia despite a much lower total glycaemic exposure. Thus our data supports the notion that fluctuating glucose may be relatively more deleterious than sustained hyperglycaemia.

4-LB
RNA-Sequencing Analysis of High Glucose Treated Monocytes Reveals Novel Transcriptome Signatures and Epigenetic Profiles

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Recent advances in powerful high throughput and next generation sequencing (Seq) technologies to evaluate genome-wide transcriptomic and epigenomic changes such as RNA-Seq, chromatin immunoprecipitation linked to microarrays (ChIP-array) have afforded unprecented research opportunities. In this study, we used these tools and a systems biology approach to gain new insights into genome-wide changes occurring in response to high glucose (HG) in monocytes. THP-1 monocytes were cultured in normal glucose (NG, 5.5 mM) or HG (25 mM) for 72 hr and RNA subjected to transcriptional profiling using RNA-Seq. Follow up bioinformatic analyses revealed 337 up-regulated genes and 326 down-regulated genes (p<0.05, fold change >2). Gene Ontology analysis revealed that pathways related to interferon signaling, cytosolic pattern recognition receptors, ERK/MAPK signaling, activated interferon regulatory factor (IRF), etc. were enriched among the up-regulated genes. To explore the role chromatin structure and epigenetic changes, we used ChIP-array to profile histone H3K4me3, H3K9Ac, H3K27me3 or Pol II marks around the promoters of HG—regulated genes under basal NG conditions. By comparing each marker’s average signal at genes subsequently regulated by HG to the average signal of all Refseq promoters, we found higher H3K9Ac around promoters of up-regulated genes, and higher H3K9Ac, H3K4me3 and Pol II occupancy in down-regulated genes, suggesting an open chromatin state (premarking with H3K9Ac) at promoter regions is prerequisite for subsequent regulation by HG. Next, by comparing HG versus NG, we found clear overall increase in Pol II Ser5P occupancy at promoters of HG up-regulated genes. In summary, results of these new
Diabetes is a major risk factor for peripheral arterial disease (PAD). Patients with type 2 diabetes are characterized by pathological derangements including dyslipidemia and inflammation, and by accelerated coronary atherosclerosis and its complications. Pharmacological therapies directed at abnormalities linked to dyslipidemia (e.g., statins) have been shown to improve the burden of excess coronary events in type 2 diabetes.

Anti-diabetic drug exenatide via GLP-1 receptor exerts glucose-dependent insulinotropic effects in pancreatic beta cells. Little is known however about the action and mechanisms of exenatide on atherogenic dyslipidemia in type 2 diabetes. In the current study, we examined whether administration of exenatide ameliorated the defects of lipoprotein metabolism in ob/ob.Ldl receptor-/- mice, a model of diabetic atherosclerosis. Exenatide-treated mice fed Western-type diet displayed decreased plasma VLDL/LDL cholesterol and TG levels as well as reduced glucose and insulin levels compared with those of pair-fed, saline-treated obese controls (p<0.05), concurrent with similar beneficial changes in hepatic lipid contents and expression of SREBP-1c and its target genes. In vivo treatment of exenatide led to a significant reduction of infiltration of CD68+ Kupffer macrophages, and of M1 macrophage markers TNF-alpha, IL-1beta, and iNOS-2 in the liver of ob/ob.Ldl receptor-/- mice (p<0.05). M2 markers including YM-1 and arginase/1 were increased, suggesting an exenatide-mediated change in macrophage polarization in vivo. When challenged ex vivo with inflammatory stimuli palmitate-BSA, modified LDL, oxysterol, or lipopolysaccharide, macrophages isolated from exenatide- vs. saline-treated obese mice had much reduced production of pro-inflammatory cytokines, e.g., TNF-alpha, with an increase in PPARdelta. Similar suppressive effects on these cytokines could be recapitulated by ex vivo treatment of macrophages with exenatide, in which canonical GLP-1 receptor signaling cascade was active. Thus we uncover a novel direct anti-inflammatory action of exenatide in macrophages, thereby likely contributing to exenatide-mediated reversal of dyslipidemia, insulin resistance and inflammation in type 2 diabetes.

Figure 1. (A) Primary antibodies to colocalize radio isotope and double mice treated with GW3965 (GW) for 12 weeks in WT and LXRα-/β-/- mice were stained on a high fat and high cholesterol diet ± STZ (streptozotocin). (B) Double knockout with BM1 part female.

A Protective Role for the Cholesterol Sensor LXR in Diabetic Nephropathy
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Diabetes is the leading cause of end stage renal disease and affects ~30% of diabetics. Diabetic nephropathy (DN) is highly correlated with glucose control but has also been linked to high serum cholesterol. The liver X receptors (LXRα and LXRβ) are cholesterol sensors. LXR expression is decreased in mouse models of diabetes and many target genes of LXR are altered in DN. Thus, we hypothesized that LXR activation would be beneficial for improving renal function and that loss of LXR would accelerate the progression of DN.

db/db and db/m mice were treated with vehicle or GW3965 (LXR agonist) in the diet for 12 weeks. In separate experiments, diabetes was induced using streptozotocin in WT and LXRα-/-/β-/β-/- mice on a high fat/high cholesterol diet for 14 weeks. Proteinuria was measured and kidneys excised for histology and molecular analyses (QPCR). GW3965 treatment significantly decreased proteinuria in db/db mice. LXRα-/-/β-/- mice had significantly elevated proteinuria compared to the control mice. Histological analysis showed an accumulation of lipids in the kidney of diabetic LXRα-/-/β-/- mice. Kidney gene expression analysis showed decreases in cholesterol efflux transporters and increases in proinflammatory growth factors, inflammatory macrophage and oxidative stress markers in the LXRα-/-/β-/- mice and the db/db mice. Inflammatory and oxidative stress markers were reduced with the LXR agonist in db/db mice. These data support a role for LXR in decreasing inflammation and increasing cholesterol efflux in the kidney and is consistent with a beneficial role for LXR activation in DN. Furthermore, diabetic LXRα-/-/β-/- mice showed elevated proteinuria, increased lipid accumulation in the kidney and detrimental changes in gene expression supporting an accelerated progression to DN.

8-LB
aV3 Integrin and Diabetic Nephropathy
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Hyperglycemia increases the αVβ3 integrin ligands thrombospondin (TS-1), osteopontin (OPn) and vitronectin. Binding of these ligands to a unique region of the β3 subunit (C-loop), distinct from the RGD site, increases the responsiveness of cells to IGF-1. An increase in the production of TS-1 and OPn has been reported in diabetic nephropathy (DN) and increased IGF-1 action is associated with capillary leakage and increased glomerular filtration rate. We hypothesized that the increase in these ligands contributes to the changes in diabetic kidneys by enhancing IGF-1 action. We used rat and pig models of STZ induced hyperglycemia to examine the ability of a specific, anti-C-loop β3 antibody to inhibit the development of DN. Hyperglycemia was confirmed with a mean glucose of >350mg/dL. In the first rat study the C-loop (or control antibody) was administered intraperitoneally every 72 hours for 8 weeks (0.3mg/kg) at the onset of hyperglycemia. Hyperglycemia
induced a 4 ± 0.2 fold increase in urinary albumin (UA) secretion after 8 weeks which was reduced by 37% with C-loop antibody treatment. In the second rat study antibody injections were commenced 4 weeks after the onset of hyperglycemia and continued for 8 weeks. The 3.8 ± 0.3 fold increase in UA with 12 weeks of hyperglycemia was normalized by C-loop treatment. There was a significant increase in the abundance of TS-1, type IV collagen, CTGF and TGF-b; treatment with the C-loop antibody normalized these changes. The hyperglycemia stimulated 2 ± 0.03 fold decrease in nephrin was prevented by the C-loop treatment. The pigs study was similar to the first rat study however antibody injections were given subcutaneously for 16 weeks. UA was significantly elevated in the diabetic pigs and this was normalized by treatment with the C-loop antibody. PAS staining of kidney sections demonstrated a significant increase in the mesangial index in kidneys from the diabetic pigs; again this was normalized by the treatment with the C-loop antibody. Taken together these studies suggest that increased activation of the J3 integrin contributes to the pathology of DN and specifically targeting the C-loop region of the J3 integrin is a potential mechanism for the treatment of this complication.

9-LB Circulating HbA1c, Lipids, TNF α, 8-isoprostanes, and Advanced Glycation Endproducts (AGEs) Are Markedly Reduced by Seques-
tration of Intestinal AGEs in Type 2 Diabetic (T2D) Patients with
Diabetic Nephropathy (DN)
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Patients with T2D and DN have increased HbA1c, AGEs, oxidative stress
(OS) and inflammation due to hyperglycemia and dietary AGEs. These factors correlate with progression of DN and cardio-metabolic disorders. Since dietary AGE-restriction is associated with improved insulin resistance and inflammation in man and animals, we hypothesized that a drug that binds and removes AGEs in the gut could be useful in T2D therapy. Sevelamer (Sev) is a non-absorbable hydrogel that binds ingested phosphates (P) and AGEs. In an intention-to-treat, open label, randomized, cross over study, we compared Sev to CaCO3, another P-binder that does not bind AGEs, in T2D with DN (n=20; 10 males, 10 females; 41% white, 45% African American 14% Asian; age 61±2 yrs) (eGFR 40±5 ml/min/1.73m2). Subjects received either S (1600 mg tid) or CaCO3 (1200 mg tid) for 8 weeks, followed by a 1 week washout, and then the alternate drug for 8 weeks. Diabetic or dietary management was not modified. Differences in the Table below are calculated as the A Sev (start-end) - A CaCO3 (start-end) 95% confidence interval:

<table>
<thead>
<tr>
<th>Variable</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.11</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.03</td>
</tr>
<tr>
<td>TNFα</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8-isoprostanes</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CML (serum)</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>MG (serum)</td>
<td>0.029</td>
</tr>
<tr>
<td>CML (intracellular)</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>MG (intracellular)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Within 2 months, HbA1c, circulating lipids, AGEs (carboxymethyl-
lysine,CML and methyl-glyoxal, MG), peripheral monocyte AGEs (iCML, iMG) and TNFα, and lipid peroxidants, 8-isoprostanes decreased by Sev, but not by CaCO3. There were no changes in fasting serum glucose, P, calcium or renal function. Conclusions: 1) Sequestration intestinal oxidants i.e. AGEs by Sevelamer, but not CaCO3, effectively improves cardio-metabolic factors. 2) Reduction of HbA1c and lipids may be due to lower OS and improved insulin action. 3) These results confirm the pathogenic role of dietary AGEs in T2D and DN and suggest the need for further studies.

10-LB Inhibition of a Novel p66ShcA Signaling Pathway in Podocytes Prevents Hyperglycemia-Induced Danger Signals and Apoptosis
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Podocytes are highly specialized, terminally differentiated, epithelial
cells. Podocyte loss and apoptosis are prognostic indicators for diabetic
glomerulosclerosis and other glomerular diseases that lead to renal failure.

Here, we demonstrate inhibition of a novel p66 signaling pathway in
podocytes, prevents hyperglycemia-induced reactive oxygen species (ROS)
and angiostatin II (ANG II) generation, while promoting expression of the
survival phenotype. p66-deficient podocyte cell lines were generated by
transfecting conditionally immortalized differentiated human podocytes
(CIDHP) with p6ShRNA or dominant negative p66 expression vector.

Cells were maintained in serum free media at 5 mM glucose (NG) or 40
mM glucose (HG) for 48h, compared with p66-deficient CIDHP, wild

type(wt)-CIDHP maintained at HG show an exponential increase in ROS
production, coupled with 1.5 fold increase in apoptosis (P <0.01). To explore
the mechanism(s) by which silencing p66 rescues podocytes from HG
stress signals, mitochondrial levels of p66, p66/cytochrome c complexes
and mitochondrial transmembrane potential (Ψm) were examined. At HG,
wt-CIDHP show increased levels of p66 and p66ShcA/cytochrome c complex;
collapse of Ψm and release of cytochrome c to the cytoplasm whereas
these parameters remained unchanged in p66-deficient CIDHP. Acute
knock down of the proly isomerase Pin 1, reversed the increased
mitochondrial levels of p66 and attenuated p66 redox function. Our data
show for the first time HG increases angiostatin II (ANG II) synthesis and
release in wt-CIDHP via p66 redox signals that activate transcription of p53
dependent genes (angiostatinogen; ANG II type 1 receptor). This redox
sensitive signaling module was suppressed in p66-deficient CIDHP at HG,
along with ANG II levels detected in the conditioned media (P<0.01). Taken
together, p66 functions as a maladaptive stress gene, that is indispensable
for HG-induced ROS and ANG II danger signals, that trigger apoptosis.

11-LB Mineralocorticoid Receptor Antagonism Improves Proximal Tubule Function Independent of Blood Pressure in a Transgenic Model of
RAAS Overexpression
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Rationale: Mineralocorticoid receptor antagonism reduces proteinuria
perhaps through improvements in cardiac stress; however, little is known
whether this occurs independent of changes in blood pressure. In addition
to glomerular origins of proteinuria, recent evidence also suggest impaired
tubular reabsorption of protein contributes to low level proteinuria in
the face of activation of the renin-angiotensin-aldosterone system (RAAS).

Accordingly, this investigation was designed to ascertain if mineralocorticoid
receptor antagonism improves glomerular and tubular contributions to
proteinuria independent of changes in blood pressure.

Experimental Design: Male TGfmRen2/27 (Ren2) and age-matched
Sprague-Dawley rats were treated with either low dose (~1 mg·kg\(^{-1}\)·day\(^{-1}\))
or a vasodilatory, conventional dose (~30 mg·kg\(^{-1}\)·day\(^{-1}\)) of spironolactone or placebo for three weeks.

Results: Ren2 rats displayed increases in systolic blood pressure
(SBP), proteinuria, and the tubular marker beta-N-acetylglucosaminidase
(beta-NAG). The Ren2 also displayed increases in the NADPH oxidase
subunit Nox2 and a marker for lipid peroxidation 3-nitrotyrosine with
increased phosphorylation of mTOR at Ser2486 and S6K at Thr389. Findings
that occurred in parallel with reductions in the podocyte specific proteins
GLEPP-1, a tyrosine phosphatase, and ezrin, a marker for adhesion, as well
as proximal tubule markers megalin, an endocytic receptor for albumin.

Low dose spironolactone had no effect on SBP but improved proteinuria
and beta-NAG comparable to the conventional dose that led to reductions
in SBP. Both doses of spironolactone led to comparable reductions in Nox2,
3-nitrotyrosine as well as p-mTOR and p-S6K and increases in GLEPP-1,
megalin, and N-cadherin.

Conclusions: These data support the notion mineralocorticoid receptor
antagonism improves glomerular and tubular functional and structural
contributions to proteinuria independent of changes in systolic blood
pressure.

12-LB Nox4 Regulates ER Stress in Podocytes: Role in Diabetic Nephropath-
y
YOUNG-HYUN YOU, SAN LY, ANGELA CHENG, ROBYN CUNARD, KUMAR
NOX4 regulates ER stress in podocytes: role in diabetic nephropathy.

For author disclosure information, see page LB42.
The Role of Ets-1 for the Upregulation of NAD(P)H Oxidase NOX4 in Diabetic Renal Tissue

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We reported markedly lower prevalence of nephropathy in diabetic patients with Gilbert syndrome, a congenital hyperbilirubinemia (JAMA 2007; 298 [12]: 1398-400), suggesting a beneficial effect of bilirubin (BIL) on diabetic (DM) nephropathy. To directly examine this, we showed that administration of BIL and biliverdin (BVD) protected against oxidative stress and renal damage in db/db mice via downregulating renal NAD(P)H oxidase NOX4 (Kidney Int 2010; 78 (9): 905-919). However, the detailed regulation mechanism for NOX4 expression has not been elucidated yet. In this study, we show the role of transcriptional factor Ets-1 on the regulation of NOX4 expression in cultured normal human mesangial cells (NHMCs) and DM renal tissues and thus show that Ets-1 may be a novel therapeutic target molecule for DM nephropathy.

First we investigated the effect of Ets-1 overexpression on NOX4 expression in the NHMCs. The levels of mRNA/protein for NOX4 were significantly increased from 48 hours after transfection with the plasmid containing Ets-1 gene. Next, we performed the transfection using siRNA directed against Ets-1. After knockdown of Ets-1 by siRNA, the expression of NOX4 mRNA/protein was significantly decreased.

Furthermore, we evaluated the mRNA/protein levels for Ets-1 and NOX4 in kidneys from db/db mice. The mRNA/protein levels for Ets-1/NOX4 were significantly increased in db/db compared with control db/+ . Administration of BVD completely normalized Ets-1/NOX4 expression, oxidative stress markers (8-hydroxy-2'-deoxyguanosine and dihydroethiduim staining), albuminuria and renal mesangial expansion in parallel. In the NHMCs, angiotensin II (AngII) stimulated Ets-1/NOX4 expression. BIL/BVD inhibited AngII-induced Ets-1/NOX4 expression in parallel, but other antioxidants NAC/CaLA did not.

In conclusion, Ets-1 plays a role in the regulation of NOX4 expression in the NHMCs. In DM kidneys, upregulation of NOX4 may be at least in part due to increased levels of Ets-1. The beneficial effect of BIL/BVD on DM nephropathy may be mediated by downregulation of Ets-1/NOX4. Ets-1 may be a novel therapeutic target molecule for DM nephropathy.

COMPICATIONS—OCULAR

Preservation of Dark Adaptation, Visual Field and Macular Microperimetry in Patients with Proliferative Diabetic Retinopathy Treated with Pegaptanib Sodium Versus Laser Photocoagulation

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To evaluate the efficacy of intravitreal injection of pegaptanib sodium in the regression of Proliferative Diabetic Retinopathy (PDR) and the impact of each therapy on retinal function.

24 eyes in 24 subjects were randomized to either intravitreal injection of 0.3mg of pegaptanib every 6 weeks for three injections followed by either quarterly pegaptanib maintenance or targeted laser therapy vs. standard PDR in eyes with PDR. The subjects were assessed at predetermined intervals over a 6 month period. Best corrected ETDRS visual acuity, optical coherent tomography, macular micropereimetry, dark adaptation and visual fields were assessed. Safety outcomes included observed and reported adverse events.

Pegaptanib treated eyes had a marked regression of retinal neovascularization in 90% of the group within 3 weeks from baseline as compared to minimal regression in about 20% of the PRP group. Functional studies including dark adaptation and micropereimetry were less altered from baseline in the pegaptanib (95%, 90%) treated group versus the laser group (15%, 80%). Visual acuity and optical coherence tomography values were more likely to improve in the pegaptanib treated group versus laser. Pegaptanib appears to be as effective in the regression of PDR as PRP. The intravitreal injection of this well tolerated drug was noted to preserve more normal dark adaptation, macular micropereimetry, visual fields and visual acuity. Intravitreal pegaptanib and targeted PRP were associated with less alteration of these functional studies as compared to standard PRP. Pegaptanib sodium appears to reduce the risk of severe vision loss secondary to PDR while preserving retinal function.

13-LB

The Benefits of Renin Angiotensin Blockade on Retinopathy in Type 1 Diabetes Vary with Glycemic Control

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Retinopathy (DR) is a major disabling microvascular complication of diabetes and may be a major risk factor for mortality in type 1 diabetes. Optimal glycemic control slows DR development and progression, and is the standard of care for type 1 diabetes. However, these glycemic goals are difficult to achieve and sustain in clinical practice. Recently, the Renin Angiotensin System Study (RASS) showed that renin angiotensin system (RAS) blockade can slow DR progression in normotensive, normoalbuminuria type 1 diabetic patients. In the present study, we examined

14-LB

Evidence of Early Involvement of A5 and C-Fibers in Proximal Sites in Type 2 Diabetes

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Somatic and autonomic neuropathies are among the most common long-term complications of type 2 diabetes (T2DM). Damage to the small Aβ and C fibers has a great impact on morbidity and quality of life in patients. There are currently no objective measures for these nerve fibers nor has the natural disease progression been defined.

We compared the nerve function of 21 healthy controls and 18 participants with T2DM using neurologic and sensory exams, nerve conduction, autonomic nervous system (ANS) tests, and contact heat evoked potential stimulation (CHEPS). Contact heat was administered at 51°C to the thenar eminence, dorsal hand, volar and dorsal aspect of forearm, distal lower limb, and lower back. Evoked potentials were recorded from the vertex (Cz) using an EEG. Latencies and amplitudes were quantified. Perception of pain was recorded using a verbal rating scale.

Patients with T2DM had mild/moderate neuropathy, with an average total neuropathy score (TNS, indicative of somatic neuropathy) of 7.3 ± 0.88, E/I ratio of 1.11 ± 0.02, Salsa vera ratio of 1.18 ± 0.04, Postural ratio of 1.14 ± 0.02, and sural conduction velocities of 37.53 ± 2.3. CHEPS summed amplitudes (IA) of controls and T2DM were significantly different in the glabrous forearm (p=0.008), hairy forearm (p=0.03), and lower back (p=0.02). Significant differences between the groups were also seen in TNS (p<0.0001) and ANS tests (E/I ratio: p=0.01; Salsa vera ratio: p=0.01; Postural ratio: p=0.01). Significant correlations were shown between TNS and IA of the dorsal hand (p=0.04) and lower back (p=0.04). Additional correlations were shown between TNS and IA of the dorsal hand (p=0.03), glabrous forearm (p=0.04), and lower back (p=0.02).

These results suggest that CHEPS is capable of detecting abnormalities in Aβ and C-fiber function in the back and forearm of patients with T2DM and neuropathy. This data correlates with measures of the ANS and mitigates against the notion that neuropathy in diabetes progresses from the dying back of long myelinated fibers. There also appears to be universal involvement in thin unmyelinated fibers and that cardiac autonomic abnormalities occur in parallel with peripheral C-fiber dysfunction.

15-LB

ACUTE AND CHRONIC COMPLICATIONS

16-LB

The Benefits of Renin Angiotensin Blockade on Retinopathy in Type 1 Diabetes Vary with Glycemic Control

For author disclosure information, see page LB42.
whether glycemic control influenced the benefit of RAS blockade on DR progression in the RASS patients who were randomized to receive 5 years of enalapril, losartan, or placebo. The primary endpoint was ≥2-steps DR progression in the concatenated 15-steps ETDRS scale, this previously has been shown to be clinically meaningful in predicting more severe DR lesions. There were 223 patients who had both baseline and follow-up fundus photographs. Of these 223 patients, 147 (65.9%) had DR at baseline (47 of 74 patients (63.5%) in placebo and 100 of 149 patients (67.1%) in the combined enalapril and losartan treatment group (p=0.67)). 57% of the patients had mild non-proliferative DR, while only 9% had moderate to severe non-proliferative DR. Patients who had ≥2-steps DR progression had higher baseline HbA1c, than those without progression (9.4% vs 8.2%, p<0.001). There was no beneficial effect of RAS blockade (p=0.52) in patients with baseline HbA1c ≤7.5%. In contrast, 30 of 112 (27%) patients on the active treatment arms with HbA1c > 7.5% had ≥2-steps DR progression compared to 26 of 56 patients (46%) in the placebo group (p=0.03).

Thus, RAS blockade significantly reduced DR progression in normotensive, normoalbuminuric type 1 diabetic patients in RASS study only if their baseline HbA1c was > 7.5%. Whether this therapy could benefit patients with HbA1c ≤ 7.5% will require longer-term studies of much larger cohorts. These findings may inform clinical decisions on appropriate therapeutic approaches to slowing DR progression.

**DIABETES EDUCATION**

**A Cluster Randomized Trial of a Mobile Phone Personalized Behavioral Intervention for Blood Glucose Control**

CHARLENE C. QUINN, MICHELLE D. SHARRELL, MICHAEL L. TERRIN, ANN L. GRUBER-BALDINI, Baltimore, MD

The objective of this study was to test whether adding mobile application coaching and patient/provider web portals to community primary care compared with standard diabetes management would reduce glycated hemoglobin levels in patients with type 2 diabetes. The cluster-randomized clinical trial, the Mobile Diabetes Intervention Study (MDIS), randomly assigned 26 primary care practices to one of three stepped treatment groups or a control group (usual care). One hundred sixty three patients were enrolled and included in this analysis. Maximum treatment was a mobile and web-based self-management patient coaching system and provider decision support. The pre-specified primary outcome was change in glycated hemoglobin levels over a one-year treatment period compared maximum treatment to usual care. Secondary outcomes were changes in patient-reported diabetes symptoms, diabetes distress, depression, and other clinical (e.g. blood pressure) and laboratory (e.g. lipid) measures. Patients received automated, real-time educational and behavioral messaging in response to individually analyzed blood glucose values, diabetes medications, and lifestyle behaviors communicated by mobile phone. Providers received quarterly reports summarizing patient’s glycemic control, diabetes medication management, lifestyle behaviors, and evidence-based treatment options. The mean declines in glycated hemoglobin were 1.9% in the maximum treatment group and 0.7% in the usual care group (P<0.001) over 12-months. Apprecriable differences were not observed between groups for patient-reported diabetes distress, depression, diabetes symptoms, or blood pressure and lipid levels (all P>0.05). In conclusion, the combination of a behavioral mobile coaching system with blood glucose data, lifestyle behaviors, and patient self-management data individually analyzed and presented with evidence-based guidelines to providers substantially reduced glycated hemoglobin levels over one year.

**WITHDRAWN**

**EXERCISE—ANIMAL**

**Physical Exercise Prevents Diet-Induced Cutaneous Xanthomatosis and Modulates Lipoprotein Profile in Low Density Lipoprotein Receptor Deficient (LDLr ko) Mice**

OMAR AL-MASSADI, ELISABETH DONELAN, SONAL SOMVANSHI, MAARIT LEHTI, CHADLER RESS, JON WEBER, CHUN-XIA YI, MATTHIAS H. TSCHOEP, SUSANNA M. HOFMANN, Cincinnati, OH

Secondary dyslipidemia due to insulin resistance and uncontrolled diabetes are commonly associated with cutaneous xanthomatosis. Herein we used LDLr ko mice, a model for diet-induced hypertriglyceridemia and –cholesterolemia to investigate whether regular physical activity may modulate lipid metabolism and inflammatory response resulting in improvement and/or prevention of the onset of xanthomatosis. Age-matched male LDLr ko mice were placed on western diet with high-fructose

For author disclosure information, see page LB42.
corn syrup sweetened beverage [sodas] and divided in to two groups, one sedentary (sed) and one undergoing exercise (exe) for 30 min at 5 m/min 5 times per week on a treadmill for 14 weeks. Exe mice did not exhibit skin lesions throughout the study compared to 50% of sed mice that developed severe xanthomatosis (P = 0.0001). Investigating whether the prevention of xanthomatosis is associated with improved dyslipidemia we measured triglyceride and cholesterol levels, and determined lipoprotein profiles by PPLC in exer and sed mice. We detected no differences in triglyceride levels (246 ± 32 vs 256 ± 40 mg/dl, N = 14) and triglyceride-rich lipoprotein (TRLP) composition [AUC 85 vs 88], suggesting that exercise may directly modulate inflammatory activity ultimately leading to decreased skin disease. To our surprise we found significant higher cholesterol levels in exer mice (1566 ± 40 vs 1303 ± 94 mg/dl, N = 14, P = 0.01) compared to sed mice that reflected an increased in VLDL and LDL particles (AUC 864 vs 637). In addition, exer mice displayed a higher body weight (bw) (47.5 ± 1.3 vs 32.5 ± 1.3 g, N = 7-14, P = 0.0001) and fat mass (41.3 ± 1.3 vs 19.3 ± 2 % of bw) compared to sed mice with xanthomatosis suggesting that exercise may prevent bw loss associated with xanthomatosis by modulating energy balance. Ongoing experiments investigating neurological circuits involved in energy homeostasis will identify underlying mechanisms. We conclude that physical activity protects against xanthomatosis by modulating energy balance independently of changes in triglyceride metabolism.

**NUTRITION—CLINICAL**

### 21-LB

The Correlation between Body Composition and Adipokineses Levels after PUFA-Omega 3 Administration in Type 2 Diabetes Patients

**ANDREEA D. DRAGOMIR, GABRIELA RADULIAN, EMILIA RUDU, CRISTIAN PAINEA, DAN M. CHETA, Bucharest, Romania**

The objective of this study was to assess the impact of 18 months administration of ω-3 PUFA supplements on adipokineses levels and body composition.

In the study were included 314 patients, 154 women and 160 men, aged 65 ± 6.8 years, with stable, diet-controlled type 2 diabetes. They were allocated to 2 groups, matched by sex, age and weight: group A – received a nutritional program consisting in diet intervention and regular physical activity; group B – received the same nutritional program + capsules of fish oil (1g eicosapentanoic acid, 1g docosahexanoic acid, 0,1g α-tocopherol acetate). Body fat mass (BFM) and body fat percent (%BF) were measured according to standard procedures. The oxidative stress was assessed using FormOX systems monitor on a blood drop. Patients were evaluated before and after the intervention.

Baseline characteristics were similar between groups. After 18 months, omega-3 supplements determined a significant improvement of adipokineses levels and decrease of oxidative stress.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A - diet</th>
<th>Group B - diet + PUFA omega 3</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>18 ± 4.3</td>
<td>13 ± 3.2</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>10.02 ± 2.9</td>
<td>13.08 ± 2.7</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Leptin/Adiponectin ratio</td>
<td>1.79 ± 1.48</td>
<td>0.99 ± 1.18</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Oxidative stress (Fort units)</td>
<td>301 ± 84</td>
<td>268 ± 57</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Body Fat Mass (kg)</td>
<td>27.84 ± 7.2</td>
<td>23.46 ± 5.7</td>
<td>p &lt; 0.002</td>
</tr>
<tr>
<td>Body Fat Percent (%)</td>
<td>28.56 ± 5.4</td>
<td>24.83 ± 6.2</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Waist-to-hip Ratio</td>
<td>1.05 ± 0.04</td>
<td>0.96 ± 0.05</td>
<td>p &lt; 0.002</td>
</tr>
</tbody>
</table>

Mean BMI, mean %BF, mean BFM and mean waist-to-hip ratio (WHR) were significantly lower in group B vs. group A. BFM was statistically correlated with leptin values (p<0.0001) and adiponectin values (p<0.001), while %BF was correlated with leptin values (p<0.0001) and leptin to adiponectin ratio.

Our study demonstrated that PUFA-omega 3 improve adipokineses levels and body composition, while decreasing the oxidative stress and delaying the damage of endothelial cells.

### 22-LB

The Role of Self-Monitored Blood Glucose Testing Frequency in the Relationship between Depression and Hemoglobin A1c (HbA1c) in Adults with Type 1 Diabetes (T1DM)

**ERIN M. RABIDEAU, KRISTOFFER S. BERLIN, MARY DE GROOT, JAY SHUBROOK, FRANK SCHWARTZ, Athens, OH, Indianapolis, IN**

Depression has been commonly associated with poorer hemoglobin A1c (HbA1c) values and increased onset of diabetes complications in adults with T1DM. Self-Monitored Blood Glucose testing Frequency (SMBGF) has been associated with improvements in HbA1c and depression in adults with type 2 diabetes. Studies have not examined the role of SMBGF in the relationship between depression and HbA1c in adults with T1DM. The purpose of the current study is to examine the indirect effect of depressive symptoms on HbA1c through its negative impact on average weekly SMBGF among adults with T1DM.

A total of 42 adults with T1DM (54.8% female) were recruited through diabetes centers in southeast Ohio. Inclusion criteria consisted of: 1) at least 18 years of age 2) T1DM for at least 1 year, and 3) most recent HbA1c less than 10% (M=7.4%, SD=0.95). Depressive symptoms were measured using the Center for Epidemiologic Studies Depression Scale (M=18.50, SD=14.89).

It was hypothesized that depressive symptoms would predict SMBGF, which in turn would predict HbA1c. This hypothesized model was tested using structural equation modeling, and provided an excellent fit to the data: χ²(1)=0.008, p=0.93, RMSEA = 0.001; and CFI = 1.0 (see Figure 1 for a description of the results). Depressive symptoms were indirectly related to HbA1c through self-monitored blood glucose testing frequency in adults with type 1 diabetes (T1DM), indicating that SMBGF may be an important mechanism through which depression impacts HbA1c. Future studies seek to identify other potential mediating factors.

### 23-LB

Can a Tool That Automates Insulin Titration Be the Key to Diabetes Management?

**RICHARD M. BERGENSTAL, ERAN BASHAN, MARGARET MCSHANE, MARY L. JOHNSON, ISRAEL HODISH, Minneapolis, MN, Ann Arbor, MI**

Most insulin users do not achieve optimal glycemic control (A1C<7%) and thereby become susceptible to complications. Numerous clinical trials have shown that frequent insulin dosage titration is a key element for successful outcome. Unfortunately, implementation of such a paradigm has been hindered by a lack of medical expertise, inadequate reimbursement, and fear of hypoglycemia. We hypothesized that the Diabetes Insulin Guidance System (DIGS™) software, which automatically adjusts patients’ insulin dosage between clinic appointments, would improve glycemic control.

We enrolled insulin treated patients with suboptimal glycemic control in a 16 week trial. Patients were divided into 3 groups: i) Type 1 diabetes treated with basal-bolus insulin, incorporating carbohydrate-counting. ii) Type 2 diabetes treated with basal-bolus therapy. iii) Type 2 diabetes treated with biphasic insulin. Following a 4-week run-in period, glucose readings reported in diaries were processed by DIGS on a weekly basis for 12 weeks and its insulin dosage recommendations, based on glucose patterns and trends, were communicated to patients. Insulin formulations were not changed during the study. Efficacy was assessed by reduction in weekly mean glucose, and safety by frequency of hypoglycemia.

Forty-six patients were recruited and 8 withdrew (6 in the run-in period). During the run-in period weekly mean glucose was stable at 174mg/dl±37). During the following 12 weeks, weekly insulin dosage recommendations
made by DIGS software resulted in progressive improvement in weekly
mean glucose to 163mg/dl(±35)p<0.03. A1C decreased from 8.4%(±0.8)
to 7.9%(±0.9)p<0.05. Concomitantly, frequency of hypoglycemia decreased by
25%. No severe hypoglycemia was reported. In only 3 out of 1,734 instances
the study-team over-rode the DIGS software recommendations.

This is one of the first reports showing that insulin dosage titrated by
software (DIGS), result in effective and safe insulin therapy. Widespread
implementation of DIGS may lead to effective titration of insulin, reduced
patients’ and care-providers’ effort, and reduced costs while improving
outcomes.

24-LB
Decision Support Tools Dramatically Improve Clinicians’ Ability To
Interpret Structured SMBG Data

HELENA W. RODBARD, OLIVER SCHNELL, JEFFREY UNGER, CHRISTEN REES,
CHRISTOPHER PARKIN, ZHONG JELSOVSKY, UNDA AMSTUTZ, NATHAN
WEGMANN, MATTHIAS SCHWEITZER, ROBIN WAGNER, Rockville, MD, Munich,
Germany, Chino, CA, Indianapolis, IN, Las Vegas, NV, Tampa, FL, Mannheim, Germany

We developed an automated decision support tool (DST) that analyzes
SMBG data from the Accu-Chek® 360º View tool, a paper form used to
document 7-point glucose profiles obtained on 3 consecutive days. The DST
presents a printed report that identifies the primary glycemic abnormality
and recommends appropriate therapeutic options. We evaluated the impact
of the DST on clinicians’ ability to identify glycemic abnormalities in SMBG
data. In this prospective, randomized, controlled, multi-center study, 288
clinicians (39.6% family practice physicians, 37.9% internal medicine
physicians and 22.6% nurse practitioners) were randomized to four study
groups: structured SMBG alone (Group A, n=72); structured SMBG with DST
(Group B, n=72); Structured SMBG with educational DVD (Group C, n=72);
and structured SMBG with the DST and educational DVD combined (Group
D, n=72). Clinicians were asked to analyze 30 clinical cases and identify the
primary glycemic abnormality. An expert clinician panel reviewed the cases
and established correct answers. 223 clinicians completed all 30 patient
cases with no major protocol deviations. Significantly more Group D clinicians
(87%) correctly identified the primary glycemic abnormalities than Group A
clinicians (51%, p<0.0001), Group B clinicians (77%, p=0.0454) and Group
C clinicians (72%, p<0.0001) (Figure 1). Recent studies have demonstrated
improved clinical efficacy of structured SMBG when combined with clinician
education. Automated decision support technology can be equally effective
as clinician education, but combing both approaches significantly improves
clinicians’ ability to accurately interpret structured SMBG data.

25-LB
Feasibility and Accuracy of a Cell-Based Continuous Glucose Mon-
itoring System in Adults with T1D

TADEJ BATTELINO, NATASA BRATINA, TAMIR GIL, ITAMAR WEISMAN, ORLY
GRINBERG, YARON PEREG, BOAZ HYMAN, TEHLA HYMAN, Ljubljana, Slovenia,
Lod, Israel

Continuous glucose monitoring (CGM) improves metabolic control in
people with diabetes. A reliable long-term sensor would improve the
acceptance of CGM technology. GluSense sensor detects an optical signal,
emitted from cells expressing a fluorescent glucose binding protein after
FRET reaction.

The GluSense sensor was inserted in the subcutaneous tissue of the
forearm for 15 days to 12 subjects (6 males) with T1D (age 18-46, BMI 20.4-
31 kg/m², HbA1c 5.8%-8.2%). Visits were at days 1, 4, 8, and 15, for mixed
meal tolerance test (MMTT), after which blood glucose was lowered to 35
mg/dl for 10 min by an insulin bolus. Blood samples were collected every 10
min, and plasma glucose concentration measured by Olympus AU400. Paired
points of GluSense CGM and the reference measurements were analyzed by
curve-fit (retrospective calibration, Figure 1) and real-time methods.

Seven out of 12 sensors performed throughout the 15 days. Error grid
analysis for curve-fit method demonstrated 95.9% (n=483) values in zones A
and B and 4.1% (n=13) in zones C and D. Similarly, real-time method showed
95.4% (n=267) values in zones A and B and 4.6% (n=13) in zones C and D.
No values in zone E were detected. The overall MARD using the curve-fit
and the real-time analysis were 13.1% and 14.9%, respectively. Sensor
accuracy was similar during periods of hypo-, hyper- and euglycemia, and
during days 1 or 15. The GluSense CGM was safe and well tolerated with
no skin reactions.

This first clinical study examining the use of an optical cell-based CGM
demonstrated acceptable safety and accuracy with a potential for routine
clinical use.

26-LB
Short-Term Real-Time Continuous Glucose Monitoring (RT-CGM)
Improves Short- and Long-Term Glycemic Control in Patients with
Diabetes Mellitus Type 2

ROBERT A. VIGERSKY, NICOLE EHRHARDT, MARY CHELLAPPAPA, SUSAN WALKER,
STEPHANIE J. FONDA, Washington, DC

RT-CGM has been primarily studied in patients with Type 1 diabetes
mellitus (DM) or in those with Type 2 DM on prandial insulin. We investigated
the effect of episodic, RT-CGM on short- and long-term glycemic control in
patients with Type 2 DM not on prandial insulin.

This was an investigator-initiated prospective, 3-month intervention/9
month follow-up trial of 100 patients with DM Type 2. Patients were
randomized to either pre-meal/bedtime SMBG or to a DexCom-SEVEN RT-
CGM in 4 cycles (2 weeks/1 week off) over 3 months. Patients received
diabetes care by their regular provider; no therapeutic intervention was
made by the study team.

The groups were well-matched in baseline metrics and therapy (oral
only–51%; oral+basal insulin–33%; oral+exenatide–9%; diet+exercise–7%).
Mean changes in A1C by group are shown in the Figure. Usage of the RT-
CGM for ≥48 (n=34) vs. <48 days (n=16) resulted in greater A1C (mean ± SD)
responses at 12 weeks (1.23% ± 1.09 vs. 0.59% ± 1.08, respectively) and
52 weeks (1.03% ± 1.50 vs. 0.25% ± 1.30, respectively). There were fewer
medication additions and/or dose increases in the CGM group at 12 weeks (26 vs. 32) and 52 weeks (53 vs. 69) and fewer patients placed on basal insulin (6 vs. 12). Weight and BP did not differ at 3 or 12 months between groups.

Three months of RT-CGM improved glycemic control significantly more than SMBG - a difference that persisted for one year. The improvement was greater with more regular use of the technology and achieved with a lower pharmacotherapy burden. We speculate that increased awareness of glycemic changes with RT-CGM promotes salutary lifestyle changes and improves medication adherence.

The opinions expressed reflect the personal views of the authors and not the official views of the US Army or the Dept. of Defense.

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**CLINICAL THERAPEUTICS/NEW TECHNOLOGY—INSULIN DELIVERY SYSTEMS**

**27-LB**

Addition of Human Hyaluronidase to Rapid Analog Insulin Reduces the Absolute Variability of Early Insulin Absorption across Infusion Set Life

LINDA MORROW, DOUGLAS B. MUCHMORE, MARCUS HOMPESCH, DANIEL E. VAUGHN, Chula Vista, CA, San Diego, CA

Pharmacokinetics(PK) and glucodynamics of an insulin aspart formulation (Aspart-PH20) with human hyaluronidase (rHuPH20) were compared to the commercial insulin aspart formulation (NovoLog®) approximately ½ and 2½ days following initiation of continuous subcutaneous infusion (CSII). Data are available for 13 (9 male, 4 female; mean age 39.7 (±9.7); mean BMI 26.2 (± 3.4)) of planned 18 T1DM patients who regularly use insulin pumps. Euglycemic clamp experiments followed a 0.15 U/kg bolus; usual basal rate was continued during clamps and PK results are thus baseline-subtracted. Euglycemic clamp experiments followed a 0.15 U/kg bolus; usual basal rate was continued during clamps and PK results are thus baseline-subtracted. For commercial aspart, insulin absorption was dramatically accelerated after 2½ days relative to ½ day CSII (Panel A). Aspart exposure also increased after 2 ½ days CSII compared to ½ day (Panel B), with insulin exposure in the 1st h increasing 67% from 22±6 to 37±15% of total AUC (p=.009) and exposure beyond 2 hr of 33%, p=.08). The absolute shift in early exposure over infusion set life as a unique challenge to CSII therapy. Euglycemic clamp experiments followed a 0.15 U/kg bolus; usual basal rate was continued during clamps and PK results are thus baseline-subtracted. For the 1st h exposure was 22±6 to 37±15% of total AUC (p=.009) and exposure beyond 2 hr of 33%, p=.08). The absolute shift in early exposure over infusion set life as a unique challenge to CSII therapy.

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### Administration of Rapid-Acting Insulin Analogs by Needle-Free Jet Injection Technology Advances the Onset of Glucose-Lowering Action and Reduces the Duration of Hyperinsulinemia

BASTIAAN E. DE GALAN, ELSEMIK E. ENGWERDA, EVERTINE J. ABBINK, CEES J. TACK, Nijmegen, The Netherlands

Background: Insulin jet injectors use a high-velocity jet to deliver insulin into the subcutaneous region without penetrating the skin with a needle. This study evaluated whether this injection method advances absorption of insulin into the circulation and subsequent glucose-lowering action of insulin.

Methods: Euglycemic glucose clamp tests were performed in 18 healthy volunteers (M/F 5/13; age 27±3 years, BMI 23.6±2.8 kg/m²) after subcutaneous administration of 0.2 U/kg aspart insulin, either by jet injection or by conventional pen injection, using a double-blind, double-dummy cross-over study design.

Results: Compared to the insulin pen, administration of insulin by the jet injector resulted in both a more rapid absorption of insulin and a faster onset of glucose lowering action (table 1). In addition, the durations of hyperinsulinemia and of glucose lowering action were shorter when insulin was injected with the jet injector rather than with the insulin pen. The efficacy of the jet injector was not modulated by age, sex or BMI.

Conclusion: Jet injectors enhance the absorption and glucose-lowering action of rapid-acting insulin analogs. Administration of rapid-acting insulin analogs by jet injection may provide better postprandial insulin substitution than analogs administered by conventional pens.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Jet injector</th>
<th>Conventional insulin pen</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-INSGLUC (min)</td>
<td>25±1</td>
<td>44±2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T-INSMAX (min)</td>
<td>31±3</td>
<td>64±6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T-INSGLUC (min)</td>
<td>101±14</td>
<td>181±26</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T-INSMAX (min)</td>
<td>111±5</td>
<td>147±5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-INSGLUC (mU/L)</td>
<td>108±13</td>
<td>93±7</td>
<td>0.017</td>
</tr>
<tr>
<td>C-INSGLUC (U/min/mL)</td>
<td>14.6±1.6</td>
<td>15.2±1.4</td>
<td>0.53</td>
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</table>

<table>
<thead>
<tr>
<th>Pharmacodynamic parameters</th>
<th>Jet injector</th>
<th>Conventional insulin pen</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>T-GLR (min)</td>
<td>40±2</td>
<td>64±3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T-GLR (min)</td>
<td>51±3</td>
<td>105±11</td>
<td>0.001</td>
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<tr>
<td>T-GLR (min)</td>
<td>123±7</td>
<td>166±6</td>
<td>&lt;0.0001</td>
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<tr>
<td>T-GLR (min)</td>
<td>191±11</td>
<td>237±7</td>
<td>0.0005</td>
</tr>
<tr>
<td>GIR (mg/kg/min)</td>
<td>6.49±0.58</td>
<td>6.09±0.56</td>
<td>0.50</td>
</tr>
<tr>
<td>GIR (g)</td>
<td>70.0±6.9</td>
<td>83.3±8.8</td>
<td>0.19</td>
</tr>
</tbody>
</table>

T-INSGLUC: time to maximal insulin concentration; AUC: area under the curve; T-GLR: time to maximal glucose infusion rate; C-INSGLUC: maximal insulin concentration.
Angiotensin-(1-7) Increases Bone Marrow-Derived Endothelial Progenitor Cells and Reduces Diabetes-Induced Oxidative Stress In Vivo

NICHOLAS M. MORDWININK, CHRISTOPHER J. MEEMS, THERESA ESPINOZA, NORMA RODA, STAN G. LOUIE, KATHLEEN E. RODGERS, Los Angeles, CA

Diabetics have a reduced number of endothelial progenitor cells (EPC), in part due to oxidative stress as a result of eNOS uncoupling. The heptapeptide, angiotensin-(1-7) [Ang-(1-7)], has been shown to increase the proliferation of EPC in vitro. The goal of this study is to evaluate the in vivo effects of Ang-(1-7) treatment on EPC numbers, endothelial nitric oxide synthase (eNOS) uncoupling, and oxidative stress in diabetic bone marrow, as well as its pathways.

BKS.Cg-Depstk+/−/Lepdm/J mice and their heterozygous controls were administered Ang-(1-7) alone or combined with A-779, L-NAME, or icatibant subcutaneously for 14 days. The mice were then euthanized and bone marrow collected to measure EPCs (culture and flow cytometry), nitrite levels, and mRNA and protein expression of NOS isoforms, eNOS phosphorylation, superoxide dismutase (SOD) isoforms, and p22-phox.

EPC counts and nitrite levels in the bone marrow were significantly decreased in diabetic mice, where treatment with Ang-(1-7) significantly increased these measures in diabetic mice (p<0.01). This effect was blocked by the co-administration of A-779, L-NAME, or icatibant. In addition, Ang-(1-7) treatment reversed the paradoxical increase in eNOS and neuronal nitric oxide synthase (nNOS) expression seen in diabetic mice. Ang-(1-7) also reversed diabetes-induced oxidative stress, by significantly decreasing p22-phox expression and increasing SOD3 expression, as well as significantly decreased nitrosylation in diabetic bone marrow (p<0.05).

Our findings demonstrate that Ang-(1-7) treatment increased bone marrow-derived EPC counts in diabetic mice. Ang-(1-7) administration also decreased diabetes-induced oxidative stress in the bone marrow and had significant effects on cellular components involved in eNOS uncoupling. Pharmacological treatment with Ang-(1-7) along with other first-line therapies may prove useful to treat diabetes-induced oxidative stress and prevent its associated long-term complications.

Cardiovascular Risk with Linagliptin in Patients with Type 2 Diabetes: A Pre-Specified, Prospective, and Adjunctive Meta-Analysis from a Large Phase III Program

ODD-ERIK JOHANSEN, DIETMAR NEUBACHER, MAXIMILIAN VON EYNATTEN, SANJAY PATEL, HANS-JUERGEN WOERLE, Askar, Norway, Biberach, Germany, Ingehelm, Germany, Bracknell, United Kingdom

The cardiovascular (CV) benefit of glucose lowering, particularly if too intensive, in type 2 diabetes mellitus (T2DM) is currently debated. Some modalities have even been reported, unexpectedly, to be associated with worse CV outcomes. To investigate the CV profile of the novel DPP-4 inhibitor linagliptin, a pre-specified meta-analysis of all CV events was conducted. CV events were prospectively adjudicated by a blinded, pre-specified, prospective, and independently adjudicated CV meta-analysis of all CV endpoints (TABLE). This is the first pre-specified, prospective, and independently adjudicated CV meta-analysis of a DPP-4 inhibitor in a large Phase III program. Although a meta-analysis, with distinct limitations, the data support a potential reduction of CV events with linagliptin. This hypothesis will be tested prospectively in CAROLINA, an ongoing outcomes trial.

30-LB

CNX-011-67, a Novel Orally Available GPR40 Agonist, Enhances Glucose Stimulated Insulin Secretion and Significantly Reduces Fasting and Non-Fasting Hyperglycemia—Studies in In Vitro and in a Preclinical Model of Type 2 Diabetes


While the role of GPR-40 (highly expressed in pancreatic beta cells) has been studied extensively in the amelioration of beta cell dysfunction, its potential as a therapeutic target has not been fully explored. We report the development and application of a highly selective potent and stable GPR40 agonist, CNX-011-67, which exhibits unique properties to improve beta cell function with potential for further development as an oral antidiabetic agent for treating type 2 diabetes mellitus (T2D). In cultured rat islets subjected to severe glucolipotoxic conditions (11mM glucose + 0.5mM palmitate for 72 hrs) chronic treatment with CNX-011-67 (1µM) as a GPR40 agonist (n=3) restored normal expression levels of GCK, PDX1 and PC mRNA, improved intracellular ATP content leading to enhanced glucose stimulated insulin secretion (GSIS) and intracellular insulin content. Treatment with CNX-011-67 also reduced expression of IL-1β, TNFα and CHOP and significantly decreased beta cell apoptosis (decrease of 25%+/-3%, n=3). Acute treatment of human T2DM islets with CNX-011-67 also enhanced GSIS by >30%. Chronic treatment of male prediabetic ZDF rats (n=8) with CNX-011-67 for 7 wks significantly enhanced insulin secretion in response to oral glucose load and delayed onset of fasting glycemia (204±32 (untreated) vs 133±12 (treated) mg/dl) by 3 weeks, reduced non-fasting glucose excursions (403±31 (untreated) vs 305±41 (treated) mg/dl), reduced fasting free fatty acid ([11.1±0.2 (untreated) vs 10.3±0.5 (treated)] mmol/l), and triglyceride levels ([289±14 (untreated) vs 184±19 (treated)] mg/dl). Treatment with CNX-011-67 reduced HbA1c ([5.5±0.3 (untreated) vs 5.18±0.21 (treated)]) significantly reduced plasma fructosamine levels ([236.7 ± 19.1 (untreated) vs 111.25 ± 25.98 (treated)] and HOMA-IR ([64.28 ± 6.2 (untreated) vs 41.82± 2.59 (treated)]. These data suggest that long-term oral therapy with CNX-011-67 could be of clinical value to improve islet function by multiple mechanisms and provide good glycemic control.

31-LB

Effects of Canagliflozin (CANA), a Sodium Glucose Co-Transporter 2 Inhibitor, on Vulvovaginal Candida Colonization and Symptomatic Vulvovaginal Candidiasis in Patients with Type 2 Diabetes Mellitus (T2DM)

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Women with T2DM are at increased risk for vulvovaginal Candida colonization and symptomatic vulvovaginal candidiasis (VVC), potentially related to increased urinary glucose excretion. In a 12-week, double-blind, placebo (PBO)-controlled study, subjects with T2DM with inadequate glycemic control on metformin were randomized to the addition of CANA 50, 100, 200, 300 mg qd, or 300 mg bid; sitagliptin (SITA) 100 mg qd or PBO for 12 weeks. Women with a history of VVC within 3 months were excluded. At baseline (BL), Week 12, and during the trial, if symptoms consistent with VVC were reported, a self-administered vaginal swab for Candida culture was to be collected. The study included 215 women: mean age 53 y, A1C 7.6%, BMI 32 kg/m2. In the overall study (N=451), CANA improved glycemic control (A1C reduced by 0.45%–0.73% relative to PBO). At BL, vaginal cultures for any Candida species were positive in 23 (11.6%–7.1%)

For author disclosure information, see page LB42.
Efficacy and Safety of Lixisenatide Once-Daily vs Exenatide Twice-Daily in Type 2 DM Inadequately Controlled on Metformin (GetGoal-X)

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Exenatide yields better cell improvement and weight loss in individuals with diabetes but has not been well-studied in individuals with prediabetes. We recruited healthy volunteers with BMI 25-37 kg/m² with prediabetes as defined by FPG ≥100 mg/dl or ≥2 hr glucose 141-199 mg/dL on screening OGTT. Insulin-mediated glucose uptake was quantified with the modified insulin suppression test with steady-state plasma glucose concentrations (SSPG). Beta cell function was calculated using the insulinogenic index(ΔA/ΔG). Subjects were randomized, double-blind, to exenatide(EX), 10 mcg BID, or placebo(P) for 30wks. All subjects took a hypocaloric diet with biweekly oversight by study dietitians for 18wk. During the last 12wks, no dietary counseling was provided. We hypothesized that individuals assigned to EX would experience greater increase in insulin secretion, weight loss, and improved insulin sensitivity than the P group. 68 subjects were randomized and 10 dropped out. At baseline, subjects were NS different with regard to mean age, BMI(33 kg/m²), sex, SSPG, or insulinogenic index. EX and P subjects had lost 8.8 and 7.8% of initial body weight at 18wks and 8.9 and 8.1%, at 30wks respectively (NS betw groups). Despite reduction in insulin resistance, beta cell function increased in the EX group(ΔA/ΔG 1.4 to 1.6). In the P group, beta cell function decreased(2.3 to 2.0) (p=0.13). Insulin resistance decreased significantly in both groups, and was correlated with weight loss (r=0.46, p<0.01). FBG decreased but was NS in both groups. 2hr glucose decreased significantly in both groups, reverting from IGT to NGT on average (NS betw groups). Change in FBG was predicted by 2hr weight loss, whereas change in 2hr glucose was predicted by insulinogenic index. In conclusion, obese prediabetic subjects assigned to EX plus hypocaloric diet lose weight, improve insulin sensitivity, and revert from IGT to NGT. Insulin resistance and FBG decrease in proportion to weight loss, whereas 2 hr glucose decreases in proportion to insulinogenic index. Despite reduction in insulin resistance, beta cell function increased with EX, but not P. Otherwise, intensive dietary management alone appears to yield comparable results to EX plus diet.

Flexible Once-Daily Dosing of Insulin Degludec Does Not Compromise Glycemic Control or Safety Compared to Insulin Glargine Given Once Daily at the Same Time Each Day in People with Type 2 Diabetes

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Current basal insulin preparations should be injected at a consistent time to ensure optimal biologic action. However, patients have difficulty adhering to strict dosing schedules for many reasons. Insulin degludec (IDeg), an ultra-long-acting basal insulin with a flat and stable action profile, may enable more flexible dosing intervals as an alternative to the recommended strict dose timing of current insulins.

The primary objective of this 26-week, open-label, randomized trial in people with type 2 diabetes (mean: A1C 8.4%; FPG 161mg/dl, duration of diabetes 10.6 yr) was to evaluate non-inferiority of IDeg dosed once-daily in a flexible regimen (IDeg Flex, a compulsory, rotating morning and evening schedule, creating 8-40 h dosing intervals; n=229) compared to insulin glargine (IGlar, n=230). Insulin was added to existing OAD therapy (if any) and titrated to FPG <90mg/dl.

For both groups, 88% of participants completed the trial. At 26 weeks, IDeg Flex and IGlar reduced A1C by 1.28 and 1.26%, respectively (estimated treatment difference [ETD] IDeg Flex–IGlar: 0.04% [-0.12, 0.20]; non-inferiority was confirmed as the upper 95% CI limit was <0.4). Mean FPG at Week 26 was significantly lower for IDeg Flex than IGlar (104 vs. 112mg/dl, ETD: -7.8mg/dl [-14.8 to -0.8], p=0.04); mean daily insulin doses were similar between groups. Rates of confirmed hypoglycemia (FPG <56mg/dl or severe) were similar for IDeg Flex and IGlar (ICD 9.2 vs. 9.4 episodes/patient-yr; estimated rate ratio (ERR) IDeg Flex/IGlar: 1.03 [0.75, 1.40], p=NS), as were rates of nocturnal confirmed hypoglycemia (0.6 vs. 0.8 episodes/patient-yr; ERR: 0.77 [0.44, 1.35], p=NS) and rates of adverse events. Severe hypoglycemia was rare (2 episodes/group).

In conclusion, by using extreme dosing intervals of 8–40 h, this trial shows that IDeg can be dosed flexibly at any time of day so that injection times
36-LB

IL-1β Antibody (Canakinumab) Improves Insulin Secretion Rates in Subjects with Impaired Glucose Tolerance (IGT) and Type 2 Diabetes (T2DM)

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Canakinumab (C) is a human monoclonal anti-human IL-1β antibody of the IgG1k isotype that binds to human IL-1β and blocks the interaction of the cytokine with its receptors, thereby neutralizing the effect of IL-1β. IL-1β-mediated inflammation has been implicated in suppression of insulin (I) secretion and worsening of β-cell function in patients with diabetes. This trial evaluated C effects on meal challenge–derived I secretion rate (ISR) relative to glucose.

Subjects received a single dose of C 150 mg s.c. at wk 0 and underwent a standard meal challenge at wk 0 and 4. Subjects were randomized either to C 150 mg s.c. or placebo (P) in 2:1 ratio except for IGT subjects who were randomized 1:1.

Randomized set: 246 subjects (C=154 [T2DM, n=126, IGT, n=28], P=92 [T2DM, n=65; IGT, n=27]; mean age 57.4 y, 54% male). Mean baseline HbA1c was 7.1% in the T2DM group and 6.1% in the IGT group. Mean duration of T2DM=9.4 y.

<table>
<thead>
<tr>
<th>Groups</th>
<th>C-P</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0 to 0.5 h)</td>
<td>(0 to 1 h)</td>
</tr>
<tr>
<td>Metformin (M)</td>
<td>-1.40</td>
<td>0.42</td>
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<tr>
<td>M+ sulfonylurea (SU)</td>
<td>-0.18</td>
<td>-0.11</td>
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<td>M+ Sul + thiazolidinedione</td>
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</tr>
<tr>
<td>IGT</td>
<td>3.921</td>
<td>2.54</td>
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*Primary endpoint.

Modest increase in ISR relative to glucose (0-0.5 h) reflecting first-phase I secretion was seen in I-treated T2DM and IGT groups. Other groups did not show consistent effects on ISR relative to glucose. There were no deaths or serious adverse events (AEs). The most frequently reported AEs (at least n=2) were tremor (n=6, 2.5%), hyperglycemia (n=5, 2.0%), dizziness (n=4, 1.8%), hypoglycemia (n=4, 1.6%) and nasopharyngitis (n=3, 1.2%). The safety assessment of C was consistent with the known safety experience to date.

1. M–treated T2DM and IGT groups trended toward improving ISR relative to glucose (0-0.5 h; numerically) when treated with C. This supports the hypothesis that blocking IL-1β in pancreatic islets has the potential to reduce suppression of I secretion by IL-1β–mediated inflammation, thereby improving β-cell function. C as a single injection at 4-wk follow-up was safe and well tolerated.

Insulin Degludec Has a Two-Fold Longer Half-Life and a More Consistent Pharmacokinetic Profile Than Insulin Glargine

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Insulin degludec (IDeg) is an ultra-long-acting basal insulin which forms soluble multi-hexamers upon subcutaneous injection resulting in a depot from which IDeg is continuously and slowly absorbed into the circulation leading to a flat and stable glucose-lowering effect. We describe the pharmacokinetic (PK) properties of IDeg in comparison to those of insulin glargine (IGlar) under steady-state (SS) conditions in people with type 1 diabetes.

In this randomized, double-blind, two-period, crossover trial, 66 people with type 1 diabetes (55 males/11 females, mean age 37 yrs. BMI 24.9 kg/m², A1C 8.1%) received one of three fixed doses (0.4, 0.6 or 0.8 U/kg) of IDeg and IGlar once daily for 8 days with 7–21 days wash-out between treatments. A euglycemic glucose clamp was performed on treatment Day 8 and PK samples were taken throughout each treatment period and for 120 h after the last dose.

IDeg showed stable PK concentrations under steady-state conditions that showed minimal fluctuations and that increased proportionally with increasing dose. The serum exposure to IDeg was equally distributed between the first and the second 12 hours post-dosing (indicated by a ratio between AUC_{0-12h} & AUC_{12-24h} of 0.5) whereas IGlar showed a higher exposure during the first 12 hours (AUC_{0-12h} / AUC_{12-24h} = 0.6). Likewise, the cumulated AUC below and above the average glucose infusion rate (AUC_{<GR average} & AUC_{>GR average}) was considerably lower for all doses of IDeg (0.25, 0.37, 0.38 U/kg/min) than with IGlar (0.39, 0.54 and 0.73 mg/kg/min). IDeg was detectable in the serum for at least 120 h following the final dose, whereas, for most subjects, IGlar fell below the lower limit of quantification after 36–48 h post-dosing. Mean terminal half-life was twice as long for IDeg than IGlar (25.4 vs. 12.5 h). Both insulin preparations were well tolerated and no safety concerns were identified.

38-LB

JD-5037, a Non-Brain-Penetrant CB₆ Receptor Inverse Agonist Improves Glycemic Control in Mouse Models of Insulin Resistance

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Overactivity of the endocannabinoid system has been associated with insulin resistance, a prominent feature of the metabolic syndrome and type-2 diabetes. Cannabinoid 1 receptor (CB₁,R) antagonists improve insulin sensitivity in obese humans and rodents, but their value as anti-obesity/anti-diabetic agents is limited by side effects mediated at CB₁,R in the CNS. CB₁,R are also present in peripheral tissues including liver, skeletal muscle, pancreatic beta cells and fat, where their activation contributes to obesity-related metabolic/hormonal abnormalities. Here, we describe a novel, peripherally restricted and orally bioavailable CB₁,R inverse agonist, JD-5037, and its effects in genetically obese (ob/ob) and high fat diet-induced obese (DIO) mice. JD-5037, an analog of the brain-penetrant CB₁,R inverse agonist AM6545, has high CB₁,R affinity (Kᵢ 0.3 nM) and 700-fold CB₁,R/CB₆ selectivity, with minimal brain penetration as evidenced by 11 brain/plasma ratio <2% after acute or ~7% after 28-day oral dosing at the maximally effective dose of 3 mg/kg. 2) lack of brain CB₁,R occupancy, verified by CB₁,R positron emission tomography as well as ex vivo ligand binding, and 3) no behavioral effects, as tested for catalepsy, ambulatory activity and anxiety. Oral JD-5037 treatment for 28 days at 3 mg/kg/day normalizes the hyperinsulinemia and hyperglycemia as well as improves glucose tolerance and insulin sensitivity in DIO mice. A shorter (7-day) treatment has similar effects in both DIO and ob/ob mice, even though weight and food intake are unaffected in ob/ob but reduced in DIO mice, indicating that the glycemic effects are weight- and food intake independent. At doses equieffective for reducing hepatic steatosis, JD-5037 causes significantly greater insulin sensitization than does the peripherally restricted CB₁,R neutral antagonist, AM6545, indicating the importance of CB₁,R inverse agonism in improving glycemic control. We conclude that peripherally restricted CB₁,R inverse agonists have therapeutic value in the management of obesity-related insulin resistance/type-2 diabetes.

Linagliptin Has Similar Efficacy to Glimiride but Improved Cardiovascular Safety over 2 Years in Patients with Type 2 Diabetes Inadequately Controlled on Metformin

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Type 2 diabetes mellitus (T2DM) often requires combination therapy to maintain glycemic control. Adding a sulfonylurea to metformin therapy improves glycemic control, but can cause hypoglycemia and weight gain. This 2-yr double-blind trial investigated the long-term efficacy and safety of adding linagliptin or glimepiride to ongoing metformin to treat T2DM. T2DM patients on stable metformin (≥500mg/d) for ≥10 weeks were randomized to linagliptin 5mg/d (N=764) or glimepiride 1-4mg/d (N=755) over 2 years. Efficacy analyses were based on HbA₁c changes from baseline in the full analysis set (FAS) and per-protocol (PP) population. Safety evaluations included pre-specified, prospective, and adjudicated capture of cardiovascular (CV) events (CV death, non-fatal myocardial infarction or stroke, unstable angina with hospitalization). Baseline characteristics were well balanced in the 2 groups (HbA₁c, 7.7% for both). In the PP population, adjusted mean (±S.E) HbA₁c changes from baseline were -0.4% (±0.04%)

For author disclosure information, see page LB42.
for linagliptin 5mg/d vs -0.5% (±0.04%) for glimepiride (mean dose 2mg/d). Mean between-group difference was 0.17% (95% CI, 0.08–0.27%; p=0.0001 for noninferiority). Similar results were observed in the FAS population. Fewer patients experienced investigator-defined, drug-related hypoglycemia with linagliptin than glimepiride (7.5% vs 36.1%; p<0.0001). Body weight was decreased with linagliptin and increased with glimepiride (-1.4 kg vs +1.3 kg; adjusted mean difference, -2.7 kg; p=0.0001). CV events occurred in 15 (1.7%) linagliptin patients vs 26 (3.4%) glimepiride patients revealing a significant 50% reduction in relative risk for the combined CV endpoint (RR, 0.50; 95% CI, 0.26–0.96; p=0.04). In conclusion, when added to metformin monotherapy, linagliptin provides similar HbA1c reductions to glimepiride but with less hypoglycemia, relative weight loss, and significantly fewer adjudicated CV events. A long-term outcomes study (CAROLINA; NCT01249424) is ongoing to confirm the promising CV safety data seen with linagliptin to date. [ClinicalTrials.gov, NCT00622284]

40-LB
Long-Term Efficacy and Safety of Add-On Dapagliflozin vs Add-On Glipizide in Patients with T2DM Inadequately Controlled with Metformin: 2-Year Results
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Dapagliflozin (DAPA), a selective SGLT2 inhibitor, reduces hyperglycemia in an insulin-independent manner by increasing urinary glucose excretion. In a randomized, double-blind trial of DAPA (up to 10 mg/d; n=406) vs glipizide (GLIP, up to 20 mg/d; n=408) as add-on therapies to metformin (MET, mean dose 2000 mg/d) in patients with T2DM (A1C: 7.0-120 mm Hg) (D169C000004, mean baseline HbA1c 7.72%), DAPA was non-inferior to GLIP in reduction of HbA1c (primary endpoint, both -0.52%), produced weight loss, and reduced hyperglycemia. We now report efficacy and safety after 2 years of treatment, in which patients continued to receive DAPA (n=315) or GLIP (n=309) added to MET. At the end of year 2, change from baseline in HbA1c with DAPA was -0.32% (95% CI -0.42, -0.21) vs -0.14% (-0.25, -0.03) with GLIP. DAPA produced sustained reductions in body weight: -3.70 kg (-4.16, -3.24) vs +1.36 (0.88, 1.84) for GLIP, with low risk of hypoglycemia in both arms over 2 years. On active questioning, proportions of subjects reporting signs, symptoms, and events suggestive of UTI mostly occurred in DAPA (4.2% vs GLIP 45.8%) and proportion reporting signs, symptoms, and events suggestive of genital infections was 14.8% for DAPA (8.0% in men, 23.3% in women) and 2.9% (0.4% in men, 5.9% in women) for GLIP over 2 years. The majority of events occurred in year 1, were mild to moderate in intensity, and responded to standard care. One discontinuation in each arm due to UTI and 3 discontinuations in the DAPA arm due to genital infections occurred during year 1; no discontinuations due to UTI or genital infections occurred in year 2. There was no clinically relevant change in renal function due to UTI and 3 discontinuations in the DAPA arm due to genital infections. Proportional hazards regression models were used to assess two-way interactions between glycemia and BP treatment arm assignment for each microvascular complication.

Over a mean follow-up of 4.7 years, the primary microvascular outcome occurred in 527 of 4733 participants, including 11.4% in the intensive BP arm and 10.9% in the standard BP arm (HR=1.08, 95% CI 0.91-1.28). Whereas intensive glycemic control reduced the incidence of microalbuminuria and a few other microvascular outcomes, intensive BP control only reduced development of microalbuminuria (HR=0.84, 95% CI 0.72-0.97). The observed reductions in microvascular outcomes by intensive glycemic control were not affected by the BP treatment arm (no interaction).

Intensive BP control improved only 1 of 10 pre-specified microvascular outcomes. None of the pre-specified outcomes were further significantly reduced in participants intensively treated for both glycemia and BP compared to those treated with either regimen alone, signifying the lack of an additional beneficial effect from combined intensive treatment. [ClinicalTrials.gov. Gov Number, NCT00000620]

42-LB
Multi-Hexamer Formation Is the Underlying Mechanism behind the Ultra-Long-Glucose-Lowering Effect of Insulin Degludec
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Insulin degludec (IDeg) is a new-generation basal insulin that has been shown in clinical studies to have a flat and stable action profile and a lower risk of hypoglycemia compared to other basal insulins. A continuous and sustained release of IDeg multi-monomers into the circulation from a soluble multi-hexamers that form after subcutaneous (sc) injection is considered to be the mechanism that underlies the distinct characteristics of IDeg.

Here we use transmission electron microscopy (TEM) to verify the formation of IDeg multi-hexamers, and investigate the pharmacodynamic consequences of IDeg multi-hexamer formation in people with type 1 diabetes. For TEM, samples of IDeg (5 zinc ions per insulin hexamer) were examined with a FEI Morgagni 268 electron microscope. Under conditions mimicking the sc interstitial fluid, elongated structures were seen with a uniform width (6.3±0.9 nm) consistent with the expected width of insulin hexamers. No such structures were visible under conditions corresponding to the pharmaceutical IDeg formulation, indicating that multi-hexamers only form after sc injection. Addition of EDTA to chelate zinc ions disrupted the multi-hexamic structures, showing that multi-hexamer formation is reversible, and suggesting that a gradual release of zinc ions from sc multi-hexamers leads to the release of IDeg monomers for absorption.

The pharmacodynamic effect of IDeg was assessed in 42-h euglycemic glucose clamps (clamp blood glucose [BG] level: 100 mg/dl) conducted after 8 days of once-daily dosing of IDeg (0.4, 0.6 or 0.8 U/kg). For all doses, the glucose-lowering effect of IDeg was >42 h. End of action (BG >150 mg/dl) did not occur within the 42-h clamp period for any subjects dosed with 0.6 or 0.8 U/kg IDeg, and for only 3 of 21 subjects on 0.4 U/kg IDeg. Moreover, mean BG profiles measured over the 42-h clamp remained almost horizontal for the 0.6 and 0.8 U/kg dose groups showing that BG was controlled throughout.

In conclusion, formation of soluble IDeg multi-hexamers at the sc injection site gives rise to an ultra-long glucose-lowering effect beyond 42 h at clinically relevant doses in people with type 1 diabetes.
was collected at baseline and Week 12. Repeat urinalysis and culture were obtained during the trial if symptoms consistent with UTI were reported. Urine cultures were considered positive if ≥10^5 CFU/mL bacteria or ≥10^6 CFU/mL Candida were isolated. Eighteen women (9.3%) and 6 men (3.4%) had positive bacterial growth at baseline. Escherichia coli was the most common. No baseline cultures were positive for fungal growth. At Week 12, CANA improved glycemic control (A1C decreased by 0.45%-0.73% relative to PBO, lowering HbA1c 1.3%-2.3% relative to PBO), and increased urinary glucose excretion (UGE) (35.4-61.6 mg/mg creatinine). Conversion from negative baseline urine bacterial culture to positive culture during the trial was not discernibly different in the pooled CANA group vs pooled PBO/SITA group (4.8% vs 3.7%, respectively). The most common pathogenic bacterial cultured were E. coli and Klebsiella pneumoniae UTI adverse events (AEs) (both symptomatic and positive postbaseline urine culture reported as a UTI) occurred in 21 (4.7%) subjects: 16 (5.0%) in the pooled CANA group and 5 (3.8%) in the pooled PBO/SITA group. All UTI AEs were considered mild or moderate, and none led to discontinuation. In this 12-week study, CANA increased UGE, but did not increase bacteriuria or UTI AEs.

**44-LB**

Ranolazine Treatment Delays the Development of Diabetes in ZDF Rats through β-Cell Preservation

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Ranolazine (RAN) is an anti-anginal drug has been shown to reduce HbA1c in patients with Type II diabetes mellitus (T2DM); however the mechanism(s) for this effect has not been fully characterized. Studies were performed in Zucker Diabetic Fatty (ZDF) rats (a model of T2DM) to characterize the effect of RAN on glycemia and diabetes disease progression. Pre-diabetic male ZDF rats were treated for 9 or 14 weeks with RAN (160 mg/kg/d) in chow to achieve clinically relevant drug levels (12.0±0.3 µM). Compared to vehicle, RAN treatment improved several diabetes parameters including reductions in HbA1c (Fig A, % decrease wk 8, 27±1.5%, p<0.001; wk 14, 20±4%, p=0.004), non-fasting plasma glucose (Fig B, wk 8, 29±5%, p<0.001; wk 13, 43±11%, p<0.001), fasting plasma glucose (wk 8, 36±2%, p=0.002; wk 13, 28±7%, p<0.001), and water consumption (wk 8, 53±4%, p<0.001; wk 13, 39±1%, p=0.002). There was no effect of RAN on body weight. Fasting insulin peaked at week 4 for vehicle and at week 10 for RAN treated animals indicating that RAN delays the deterioration of β-cell function. In RAN compared to vehicle treated animals, total pancreas islet area (% increase wk 9, 124±4%, p=0.003; wk 14, 88±28%, p=0.35), islet insulin staining (wk 9, 13.6±0.4%, p<0.001; wk 14, 6.0±0.8%, p<0.001) and the ratio of islet insulin to glucagon staining (wk 9, 150±11%, p<0.001; wk 14, 82±18%, p=0.002) were increased. Muscle glycogen content was 49±9% (p=0.003, mean of wks 9 and 14) higher in the RAN group compared to vehicle. These data, in a well established animal model of diabetes, confirm the clinical finding that RAN reduces HbA1c and that this effect is associated with improvement in plasma glucose and the diabetic phenotype. The mechanism(s) for these anti-diabetic effects include preservation of insulin secreting β-cells.

**45-LB**

Risk of Pancreatitis from Exenatide in the Privately-Insured Population

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Concern has arisen about the safety of exenatide. We performed a case-control study of exenatide use and acute pancreatitis within employer-provided health plans. We identified 390,701 privately-insured patients with Type 2 diabetes from 33 large employers from July 2005 to December 2009. An exenatide prescription was filled by 14,448 beneficiaries. Patients with a history of pancreatitis were excluded from the analysis, as were statin-taking users. We compared employer-level rates of exenatide use to hospitalization rates for acute pancreatitis within 6-month windows. The figure shows unadjusted rates (weighted by plan size), with utilization measured by the proportion of patients with Type 2 diabetes with at least one fill of an exenatide prescription; we also analyzed average months supplied.

Regressions controlled for age, gender, risk factors (gallstones or alcohol abuse), comorbid conditions, time since diabetes diagnosis, and secular trends in pancreatitis incidence. There was a negative but statistically insignificant relationship between exenatide months supplied and pancreatitis hospitalization: a one standard deviation increase in utilization (1.02 months) was associated with a 0.009 percentage point decrease (95% confidence interval: -0.030 to 0.012) in the hospitalization rate (mean: 0.135%).

To address potential confounding, we constructed a natural experiment based on the out of pocket cost of 30-day exenatide prescription, which serves to quasi-randomize beneficiaries into exenatide use. Using this instrumental variable, we find a statistically insignificant relationship, with a standardized increase in months supply associated with a 0.005 percentage point increase (95% confidence interval: -0.040 to 0.050) in hospitalization. These findings indicate that exenatide use does not increase the risk of acute pancreatitis.

**46-LB**

Safety and Efficacy of Once-Monthly Exenatide over 20 Weeks in Patients with Type 2 Diabetes

LEIGH MACCONIEL, JARET MALLOY, WEN YING HUANG, BRENA CIRINCIONE, LARRY SHEN, LISA PORTER, San Diego, CA

Exenatide, a GLP-1 receptor agonist, improves glycemic control and body weight with twice daily or once weekly subcutaneous (SC) injections in patients with type 2 diabetes (T2DM). A new formulation, exenatide suspension, uses the extended-release microspheres of exenatide once weekly (ExQW) with a triglyceride-based diluent that enables delivery of higher doses with less frequency. The safety and efficacy of 3 monthly doses of exenatide suspension (ExQM: 5, 8, or 11mg SC or ExQW (2mg) as a reference arm, were assessed in a randomized, open-label, controlled study in 121 patients (36%F, 50±10y, WT 97±19kg, A1C 8.5±1.2%, FPG 185±45mg/dL, diabetes duration 6±5y, meanSD) with T2DM treated with diet/exercise, metformin (MET), pioglitazone (PIO), or MET+PIO. Across 20wks, patients received 5 monthly ExQM injections or 20 weekly ExQW injections, with high patient retention (94%). Sustained plasma levels were achieved all ExQM doses. Greater peak to trough variability was observed with ExQW than ExQW, however mean trough concentrations remained within the therapeutic range with all ExQW doses. The 2 highest ExQW doses achieved levels similar to ExQW. As with ExQW, ExQM approached undetectable levels 8wks after last injection. A1C, FPG, and WT were substantially improved with all doses of ExQM and were comparable to ExQW (Table). Evaluable and ITT results were comparable. No unique safety findings were observed with ExQM relative to ExQW. The most frequent AEs were: ExQM, headache (17-27%) and nausea (17-23%); ExQW, headache (30%) and diarrhea (27%) (ITT). No major or minor hypoglycemia was observed. One AE (vomiting/ExQM) led to withdrawal. There was no evidence of prolonged AE duration with ExQM or ExQW. ExQM was well-
tollerated with robust improvements in glycemic control in patients with T2DM, supporting further development of the suspension formulation.

<table>
<thead>
<tr>
<th>N Baseline</th>
<th>ATC (%)</th>
<th>ATC -7% (Wk20)</th>
<th>FPFG (mg/dL)</th>
<th>WT (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>8.1±0.2</td>
<td>-1.5±0.2</td>
<td>48%</td>
<td>-3±0.9</td>
</tr>
<tr>
<td>26</td>
<td>8.1±0.2</td>
<td>-1.5±0.2</td>
<td>50%</td>
<td>-2±0.8</td>
</tr>
<tr>
<td>28</td>
<td>8.1±0.2</td>
<td>-1.5±0.2</td>
<td>57%</td>
<td>-3±10</td>
</tr>
<tr>
<td>27</td>
<td>8.1±0.2</td>
<td>-1.5±0.2</td>
<td>70%</td>
<td>-4±9.8</td>
</tr>
</tbody>
</table>

MeansSE Δ from baseline to Wk20, Evaluable Population.

47-LB

Safety, Tolerability and Efficacy of Subcutaneous (SC) LY2189102 (LY), a Neutralizing IL-1β Antibody, in Patients (pts) with Type 2 Diabetes (T2DM)

JOANNE SLOAN-LANCASTER, EYAS ABU-RADDAD, JOHN POLZER, JEFFREY W. MILLER, JOEL C. SCHERER, JOLENE K. BERG, WILLIAM H. LANDSCHULZ, Indianapolis, IN, San Antonio, TX

Determine the efficacy, safety, and tolerability of LY2189102, a humanized neutralizing IL-1b antibody, in T2DM pts.

A randomized, double-blind, placebo (pbo) controlled, parallel design study which enrolled 106 pts with T2DM on diet and exercise, with or without anti-diabetic medication (excluding thiazolidinediones [T2D] and insulin). Pts equally randomized to LY (0.6, 18, 180 mg) or pbo received weekly SC doses for 12 weeks (wks; 13 doses) and 12 wks follow-up. The primary objective was the change from baseline (CBF) in HbA1c after 12 wks of dosing for the cohort set (n=11 doses). Sample size based on 1-sided 75% confidence interval (CI) in CBF between LY and pbo being ≥-0.68% HbA1c. All LY dose groups significantly (based on 75% 1-sided CI) reduced HbA1c at 12 wks compared to pbo (Table 1) with no obvious dose-response relationship; numerical reduction remained evident at 24 wks compared to pbo. A significant (p<0.05) early reduction in C-reactive protein (CRP) occurred with other inflammatory molecules also reduced. LY was well tolerated with 4 treatment emergent (TE) serious adverse events (SAEs) in 2 pts (0.6mg, 18mg) considered not drug-related. A similar percentage of Pts had ≥1 TEAE across all treatment groups.

Table 1:

<table>
<thead>
<tr>
<th>Placebo</th>
<th>0.6mg LY</th>
<th>18mg LY</th>
<th>180mg LY</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=23</td>
<td>n=21</td>
<td>n=16</td>
<td>n=19</td>
</tr>
</tbody>
</table>

Baseline

<table>
<thead>
<tr>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.93 (0.815)</td>
<td>7.45 (0.835)</td>
<td>7.95 (0.702)</td>
</tr>
<tr>
<td>8.27 (0.930)</td>
<td>8.19 (0.813)</td>
<td>8.21 (0.796)</td>
</tr>
</tbody>
</table>

End-of-Dosing CBF

<table>
<thead>
<tr>
<th>LS Mean (SE)</th>
<th>LS Mean Difference*</th>
<th>75% 1-sided CI*</th>
<th>pvalue*</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.13 (0.131)</td>
<td>-0.274</td>
<td>-0.378</td>
<td>-0.245</td>
</tr>
<tr>
<td>-0.06 (0.16)</td>
<td>-0.01</td>
<td>-0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>0.095</td>
<td>0.045</td>
<td>0.16</td>
<td></td>
</tr>
</tbody>
</table>

*From ANCOVA model: treatment, site, baseline HbA1c as covariates.

Overall, LY was well tolerated with no drug-related serious adverse events. A similar percentage of pts had ≥1 TEAE across all treatment groups.

49-LB

Ultra-Long-Acting Insulin Degludec Has a Flat and Stable Glucose-Lowering Effect

LESZEK NOSEK, TIM HEISE, SUSANNE G. BOTTCHER, HANNE HASTRUP, HANNE HAAHR, Neuss, Germany, Aalborg, Denmark, Søborg, Denmark

Insulin degludec (IDeg) is a new-generation, ultra-long-acting basal insulin that forms soluble multi-hexamers upon subcutaneous injection, resulting in a depot from which IDeg is continuously and slowly absorbed into the circulation.

In this double-blind, two-period, crossover trial, we investigated the dose-response relationship of three doses of IDeg (0.4, 0.6 and 0.8 U/kg) at steady state (SS) in people with type 2 diabetes. Participants (insulin-treated people with type 2 diabetes without concomitant oral anti-diabetic agents, n=49; mean age: 58.7 years, BMI: 29.6 kg/m²; A1C: 7.8%, duration of diabetes: 14.1 years) were given IDeg once-daily for 6 days, with a washout period of 13–21 days between treatments. Following dosing on Day 6, subjects underwent a euglycemic glucose clamp (Biostator; clamp blood glucose level: 90 mg/dL). Pharmacokinetik samples were taken up to 120 h after the last injection of IDeg.

For all dose levels, mean 24-h glucose infusion rate (GIR) profiles were flat and stable (Figure 1). Total glucose-lowering effect (AUC_GIR, 0–120 h) increased linearly with increasing dose. Over 24 h, the glucose-lowering effect of IDeg was evenly distributed between the first and second 12 h for all 3 dose levels (AUC_GIR, 12–24h; AUC_GIR, 24–36h = 0.5). The blood glucose levels of all participants stayed very close to the clamp level until the end of the experiment (mean blood glucose levels in the last 10 min of a 24-h dosing interval were 90–92 mg/dL for all IDeg doses). The terminal half-life estimated across the three dose levels after the last dose was 25.1 hours. IDeg was well tolerated and no safety concerns were identified.

Figure 1: Mean 24-h glucose infusion rate profiles at steady state.

For author disclosure information, see page LB42.
ZGN-433 Is Well-Tolerated, Reduces Body Weight Rapidly and Improves Cardiovascular Risk Markers in Obese Subjects: The ZAF-001 Proof of Concept Trial

THOMASE HUGHES, JAMESE VATH, JOANNE MARJASON, JOSEPH PROIETTO, Cambridge, MA, Brisbane, Australia, Heidelberg, Australia

ZGN-433 (ZGN) is a selective methionine aminopeptidase 2 (MetAP2) inhibitor. MetAP2 inhibitors have been found to reduce food intake, increase ketogenesis, and reduce expression of hepatic fatty acid synthetic genes while reducing expression of pro-inflammatory genes in liver and adipose tissue. ZAF-001 was a 4-week, randomized, double-blind, placebo (PBO)-controlled trial that evaluated the safety, tolerability, and efficacy of intravenously administered ZGN. Subjects received ZGN at 0.1, 0.3, or 0.9 mg/m² by twice weekly infusion or placebo, without diet or exercise counseling.

The trial enrolled 31 surgically sterile or post-menopausal women: mean age 52.3±0.9 yr, weight 102±2 kg, and BMI 37.8±0.6 kg/m². Twenty-six subjects completed the trial (PP population). After 26 days of treatment, subjects on 0.9 mg/m² ZGN lost 3.6 kg from baseline (0.98 kg per week, P<0.001, n=8) vs. 1.2 kg for PBO (0.31 kg per week, p=0.15, n=6). LDL cholesterol decreased 22% in the 0.9 mg/m² ZGN group vs a 2% increase in PBO (p=0.02); C-reactive protein decreased 64% in the 0.9 mg/m² ZGN group vs 12% for PBO (p<0.001). Blood pressure did not change with treatment. The most frequent AE's were headaches, cutaneous related to cannulation for the infusion site, and nausea and diarrhea (all were equivalent for PBO and ZGN treatment). There were no clinically significant hematologic or serum chemistry values for any of the subjects in any of the treatment groups. Consistent with results in obese mice, treatment with 0.9 mg/m² ZGN increased β-hydroxybutyrate by 188% (p<0.05), increased plasma adiponectin concentrations by 59% (p<0.005) and increased the ratio of adiponectin/leptin by 24% (p=0.001). Insulin and glucose were not affected by treatment in this normoglycemic study population.

In conclusion, ZGN treatment was associated with rapid weight loss and improvements in LDL cholesterol and C-reactive protein as compared to placebo, with no evidence of major tolerability or safety issues. MetAP2 inhibition is a promising novel strategy for treating obesity.

HEALTH CARE DELIVERY—ECONOMICS

51-LB

Achieving Treatment Targets in Multi-Condition Collaborative Care for Diabetes, Coronary Heart Disease and Depression

ELIZABETH E. LIN, MICHAEL VON KORFF, PAUL CIECHANOWSKI, DO PETERSON, EVETTE J. LUDMAN, CAROLYN RUTTER, MALIA OLIVER, BESSE A. YOUNG, JOCHEN GENSCHEN, MARY MCGREGOR, DAVID MCCulloCH, WAYNE J. KATON, Seattle, WA, Jena, Germany

Multi-condition collaborative care for complex chronic disease patients demonstrated improved outcomes in diabetes, coronary heart disease (CHD), and depression.

The objective of this study was to evaluate factors resulting in better control of blood glucose, blood pressure, low-density lipoprotein, and depression. We conducted a randomized controlled trial in 14 primary care clinics among 214 patients with poorly controlled diabetes (glycated hemoglobin [HbA1c]<8.5%) and/or CHD (blood pressure> 140/90mmHg and/or low-density lipoprotein> 130 mg/dl) with co-existing depression (Patient Health Questionnaire-9> 10). The intervention consisted of an integrated care management for multiple conditions employing a treat-to-target strategy for diabetes, CHD and depression, delivered by a nurse care manager working closely with primary care physicians and consultants. Outcomes included: pharmacotherapy initiation, treatment adjustments, medication adherence, and disease self-monitoring.

Comparing collaborative care management to usual care at 12-months, glucose self-monitoring was higher (relative rate [RR]=1.28; p=0.006), and blood pressure self-monitoring was three-fold higher (RR=3.20; p<0.001). Treatment initiation and adjustment rates were more than six-fold greater for anti-depressant medicines [RR = 6.20; p<0.001], three-fold greater for insulin (RR=2.97; p<0.001), and nearly two-fold greater for anti-hypertensive medicines (RR=1.86, p<0.001). Patient outcomes improved even though no differences in medication adherence for any of the targeted medication classes were observed. In summary, benefits of a multi-condition collaborative care management intervention for glycemic, blood pressure, lipid, and depression control were likely achieved by improved self-monitoring of disease control parameters, and by more frequent and timely initiation and adjustment of medications by primary care physicians. Benefits were achieved without changes in medication adherence. We conclude that pro-active changes in health care team management of chronic illness were critical to achieving improved clinical outcomes for diabetes and co-morbidities.

52-LB

Clinical and Economic Outcomes of Appropriate Oral Antidiabetic Drug (OAD) Treatment among Type 2 Diabetes Mellitus (T2DM) Patients with Chronic Kidney Disease (CKD)

SHIH-YIN CHEN, BIRGIT KOVACS, MICHAEL STOKES, STEPHEN SANDER, KIMBERLY SIU, FREETH RAO, LUKE BOULANGER, Lexington, MA, Ridgefield, CT, Dorval, QC, Canada

CKD is prevalent among T2DM patients. Clinical guidelines recommend OAD treatment adjustment in CKD, but published data on actual treatment practice is limited. This study assessed outcomes associated with appropriate OAD treatment (AT) using electronic health record data provided by an integrated health system in the United States. Patients were selected if they had at least 1 prescribed OAD, 1 diagnosis of T2DM, and stage 3-5 CKD based on diabetes or lab results (GFR<60). Patients with any one of the following were excluded from the analysis (first identified CKD): Patients were considered appropriately treated (IT) if they were prescribed OADs that are recommended to be avoided or require dose adjustment but were not dose adjusted according to guidelines developed by the National Kidney Foundation. Glycemic control (HbA1c<7), hypoglycemic events, hospital admissions, and costs of encounters were assessed 12 months following the index date. Regression analyses were conducted to assess the association between AT and outcomes adjusting for patient demographic and clinical characteristics.

<table>
<thead>
<tr>
<th>Age (mean)</th>
<th>Male (%)</th>
<th>Glycemic Control (%)</th>
<th>Hypoglycemic Events (%)</th>
<th>Hypoglycemia Related Costs (mean)</th>
<th>Hospital Admissions (%)</th>
<th>Annual Encounter Costs (mean)</th>
<th>Diabetes Related Costs (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>69.2</td>
<td>37.6</td>
<td>46.1</td>
<td>10.2</td>
<td>$239</td>
<td>23.9</td>
<td>$9,885</td>
<td>$6,694</td>
</tr>
</tbody>
</table>

Cost Effectiveness of a Human Fibroblast-Derived Dermal Substitute for the Treatment of Diabetic Foot Ulcers in Medicare and Commercially Insured Populations

YIDUO ZHANG, PAUL HOGAN, Falls Church, VA

A Markov model was developed to compare cost-effectiveness of a human fibroblast-derived dermal substitute (HDFs, Dermagraft) to conventional care (CC) in the treatment of diabetic foot ulcers (DFUs).

The model simulated health status over 52 weeks of a cohort of 10,000 patients with a DFU treated either with HDFs or CC. Weekly health state transition probabilities were directly derived from results of a published U.S. clinical trial (N=245). Health states were verified by medical review and included healed, unhealed newly infected, cellulitis, osteomyelitis and three types of amputations (toe, foot [includes TMA], below-knee). Due to similar costs, bone resections were collapsed with toe amputation. Transition to bone resection/amputation occurred in 4.6% of HDFs and 11.4% of CC patients. Medicare costs were estimated from 100% of the 2009 Medicare claims data covering 480,447 DFU patients. Costs for a commercially insured population came from a 2009 proprietary claims database covering 34,888 DFU patients. Medical claims data from initial DFU diagnosis date were cumulated over 1 year for each patient. Actual payments based on the

For author disclosure information, see page LB42.

53-LB

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The proportion of healed ulcers was 76% (HFDS) vs. 51% (CC), median time to heal was 19-20 weeks (HFDS) vs. 51-52 weeks (CC). Patients receiving HFDS had fewer infections and amputations. The average expected cost to Medicare per treated patient over 52 weeks was $23,080 (HFDS) vs. $28,505 (CC). The average estimated cost per healed ulcer was $30,344 (HFDS) vs. $58,156 (CC). Cost neutrality for HFDS was achieved at 6 months for Medicare payers and 8 months for private insurers. When using commercial reimbursement rates, HFDS provided similar but smaller cost effective ratios. HFDS treatment pays for itself in as early as 6 months from the payer's perspective. Additional costs for HFDS were offset by medical savings from accelerated wound healing and reduced DFU complications and amputations.

No Change in Preventable Hospitalizations for Diabetes over Seven Years in a Midwestern State

ERIC S. ARMRECHT, LESLIE PORTH, THOMAS E. BURROUGHS, ST. LOUIS, MO, JEFFERSON CITY, MO

Population-level preventable hospitalization (PH) rates for diabetes may indicate suboptimal outpatient care delivery and provide guidance to hospitals and public health agencies for setting community health priorities. To assess changes in PH over time, we used Prevention Quality Indicators (PQI), developed and validated by the Agency for Healthcare Research and Quality. We assessed trends for 12 PQI measures (three diabetes, six other chronic and three acute) across seven years (2002-2009) using all hospitalizations for Missouri residents. Between 2002 and 2009, the risk-adjusted rate of PH fell 16%, from 2.187 per 100,000 to 1.843 per 100,000. Approximately 10.5% of hospitalizations were classified as preventable. Of all PH statewide in 2008, 61% were attributable to three conditions—congestive heart failure (24%), bacterial pneumonia (22%) and chronic obstructive pulmonary disease (15%). At 200.4 per 100,000 Missourians, diabetes was the fifth leading cause of PH, accounting for 10.3% of all PH statewide. The overall seven-year trend for diabetes, per a linear regression model, was flat (-5.2%, p=0.30) but varied for its PQI-defined sub-types: short-term complications (+18.2%, p<0.001), long-term complications (-1.3%, p=0.02), and uncontrolled (-14.1%, p=0.16). By comparison two common co-morbidities associated with diabetes had distinctly different trends. PH rates fell for congestive heart failure fell (-18.9%, p=0.001) and rose for hypertension (+15.7%, p=0.04). Although total PHs have fallen between 2002 and 2009, the progress does not appear to be attributable to changes in overall diabetes-related hospitalizations, as measured by the established PQI method. Reductions attributable to long-term complication rates were offset by increases in short-term complication rates. Observed gains in the quality of diabetes care across Missouri, measured by surveillance studies, may not translate into population-level PQI indicators for diabetes. Further study of the PQI indicator for diabetes is warranted, especially since community health planning efforts may use PQI results for setting priorities and evaluating progress.

Telemedical Management of Diabetic Patients by Computer Versus Traditional Care: A Controlled Clinical Trial

STEVEN B. LEICHTER, ROYCE ANN ADKINS, KELLY L. BOWMAN, COLUMBUS, GA

The application of telemedicine to diabetes care is evolving, but previous models often require extensive telephone support or new technology, which increases the costs of care. We carried out a non-inferiority study of 100 patients (Pts) with known diabetes, comparing a control group (CG) managed by 4 traditional office visits per year to a study group (SG), in which half their visits were carried out by communication by computer, using a modified version of a widely available software program, associated with blood sugar monitoring devices (Roche Diagnostics, Indianapolis, IN). This program allowed SG to transmit their home capillary glucose measurements, as well as home blood pressures, food diaries and body weights. Patients in SG were given a home blood pressure monitor and a body weight scale. The patients were assigned to each group to achieve similar percents treated with insulin alone, oral agents alone, insulin plus oral agents or a combination of insulin oral agents, but no other criteria for group assignment were considered. Baseline characteristic for the groups were similar for BMI, blood pressure, age, Hgb A1c, HDL/LDL cholesterol ratios, serum triglycerides, and percent Black and Caucasian ethnicity (p > 0.1613 for each variable). After 1 year, CG had similar Hgb A1c levels (7.25 ± 0.25%) vs SG (7.84 ± 0.29%), (p = 0.1418), and the change in Hgb A1c from baseline in each group was the same (+0.4%). BP, BMI, serum triglycerides and HDL/LDL-cholesterol ratios were similar (p > 0.1469 for each variable). The study demonstrated no inferiority in the 1 year status of the endpoints studied in SG vs CG. The staff time to serve the patients in the SG was the same or less than the CG, but the loss of time from work, and costs of travel to office visits were much less for SG. This study demonstrates that telemedical applications of low-cost computer software can expand access to care for pts and save costs for pts, employers, and providers.

Markers for Oxidative Stress in Obese Children

JAMES R. EBERT, MIRYOUNG LEE, STEFAN A. CZERWINSKI, DAYTON, OH

Increased oxidative stress (OS) has been related to inflammatory processes leading to endothelial dysfunction. Adult studies have shown that OS markers such as plasma oxidized LDL (ox-LDL) and 8-iso-prostaglandin F2α (8-iso-PGF2α), may also be important contributors to the inflammatory processes present in metabolic syndrome and insulin resistance. We examined the relationships of the OS markers and cardiometabolic risk factors in the context of childhood obesity. We hypothesized that there would be a significant positive relationship between OS markers and risk factors in a group of obese children. OS marker data were obtained on 223 children who had BMI's that were above the 90th% for age. Patients with known diabetes, inflammatory diseases, or taking glucose lowering or lipid lowering medications were excluded. Height, weight, and blood pressure were measured on each subject. Fasting blood glucose, insulin, lipid profile, ox-LDL, and 8-iso-PGF2α were obtained. To calculate the total area under the curve (e.g., AUC0-30min), 211 children also completed a 5 hour OGTT with insulin levels. The mean age of subjects was 12.6 years ranging from 6.1 to 18.5. The median BMI was 33.9 kg/m2 and ranged from 20.2 to 75.7. Approximately 40% were dyslipidemic, and 14% had impaired glucose tolerance. There was no significant correlation between OS markers and demographics. Anthropometric measurements (e.g., BMI z-score) were not significantly related to ox-LDL or 8-iso-PGF2α. Levels of ox-LDL were positively correlated with total cholesterol, LDL-C, and triglycerides (p-values <0.0001). While there was no correlation between ox-LDL and insulin resistance indices and AUC measures, 8-iso-PGF2α values were significantly related to HOME-IR and other parameters. 8-iso-PGF2α positively related to HOME-IR (r= 0.143, p-value <0.05) and AUC0-30min (F= 0.148, p-value <0.05) and negatively correlated to glucose-to-insulin ratio (r= -0.148, p-value <0.05). These correlations were independent of age, sex, and BMI z-score. In this sample of significantly obese children, ox-LDL correlated with dyslipidemia and 8-iso-PGF2α, correlated with indicators of insulin resistance.

EBV Infection May Influence Therapeutic Potential of ccCD3 Therapies

LIMEI WANG, YOSHIKAI OKUBO, DOUGLAS E. BURGER, DENISE L. FAUSTMAN, BOSTON, MA

The recently released results of two Phase III trials testing humanized ccCD3 monoclonal antibodies (MoAbs) in new onset type 1 diabetes (T1D) may be better understood by the discovery of an association between ccCD3 efficacy and Epstein-Barr virus (EBV) infections. Latent EBV infections are reactivated by ccCD3 MoAbs at higher doses or by immunosuppression. In Phase II testing, a six-day course of ccCD3 (total dose ~64 mgs) transiently reactivated EBV in the majority of adults with T1D and showed efficacy. However, in Phase III testing at a lower total dose, few patients experienced transient EBV reactivation, and efficacy was not shown. Likewise, administration of the ccCD3 MoAb at lower doses in a different trial, resulted in EBV reactivation in a subset of T1D patients. The select patients with EBV reactivation demonstrated the release of insulin autoreactive T cells. We have been able to serially follow a patient with long-term T1D during an acute EBV infection and study T cells and pancreas responses at weekly intervals for 20 weeks. The data reveal that acute EBV infection in a longterm T1D patient results in:

- Massive release of dead insulin autoreactive T cells into the circulation within 7 days
Insulin Resistance in Type 1 Diabetes (T1D) Is Not Associated with Ectopic Fat
KRISTEN J. NADEAU, AMY WEST, DAVID BRUMBAUGH, ANN SCHERZINGER, MARK S. BROWN, JANE E.B. REUSCH, Aurora, CO

Insulin resistance (IR) is associated with a metabolic syndrome phenotype and ectopic lipid deposition. We have reported that T1D youth are significantly IR relative to nondiabetic youth of similar pubertal stage and BMI, unrelated to glyceremia. Despite their IR, T1D youth paradoxically lacked obesity and dyslipidemia, leaving the mechanism of IR in T1D unclear.

To further investigate the phenotype of T1D in youth and potential underlying mechanisms, we used abdominal MRI and soleus MRS to assess hepatic, visceral, subcutaneous (SQ) and extra- and intramyocellular (EMCL, IMCL) lipid, and hyperinsulinemic euglycemic clamps to assess IR in T1D youth versus youth with normal weight, obesity and type 2 diabetes (T2D), of similar age and pubertal stage.

Results in the table show significantly higher BMI, triglycerides, IR, SQ, visceral, hepatic, IMCL and Dextrafat, and lower HDL and adiponectin in T2D youth versus controls, with intermediate results in obese youth. In contrast, despite significant IR, T1D youth were similar to control youth in all of these parameters. No T1D youth had hepatic fat fraction $>$5%, compared to 70% of T2D youth.

<table>
<thead>
<tr>
<th>T1D</th>
<th>Lean Control</th>
<th>Obese Control</th>
<th>T2D</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>18</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>15.6 ±1.8</td>
<td>14.8 ±2.3</td>
<td>13.5 ±2.4</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>21.4 ±3.4</td>
<td>20.5 ±3.7</td>
<td>30.0 ±3.3</td>
</tr>
<tr>
<td>A[α]%</td>
<td>8.1 ±1.44</td>
<td>5.3 ±3.4</td>
<td>5.2 ±0.1</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>79 ±43</td>
<td>77 ±23</td>
<td>300 ±109</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>48 ±5.7</td>
<td>54 ±12.4</td>
<td>42 ±7</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>107 ±37</td>
<td>9.2 ±3.5</td>
<td>5.7 ±29</td>
</tr>
<tr>
<td>Insulin Sensitivity (mg/kg/mnin)</td>
<td>10.8 ±3.7</td>
<td>19.6 ±3.6</td>
<td>5.4 ±3.3</td>
</tr>
<tr>
<td>Hepatic Fat(%)</td>
<td>0.9 ±0.1</td>
<td>0.3 ±0.18</td>
<td>2.4 ±2.1</td>
</tr>
<tr>
<td>Visceral Fat(cm2)</td>
<td>19 ±19.7</td>
<td>20 ±8.5</td>
<td>45 ±19.8</td>
</tr>
<tr>
<td>SQ Fat(cm2)</td>
<td>107 ±279</td>
<td>103 ±271</td>
<td>230 ±109</td>
</tr>
<tr>
<td>IMCL(AU)</td>
<td>468 ±230</td>
<td>403 ±266</td>
<td>827 ±292</td>
</tr>
<tr>
<td>EMCL(AU)</td>
<td>465 ±199</td>
<td>505 ±243</td>
<td>1334 ±496</td>
</tr>
<tr>
<td>Dextrafat</td>
<td>72 ±7.6</td>
<td>23 ±7.9</td>
<td>40 ±4.4</td>
</tr>
</tbody>
</table>

*p<0.001 vs T1D, @p<0.05 vs T1D, $p<0.001 vs lean, $p<0.05 vs lean, #p<0.05 vs obese.

In conclusion, T1D youth have a unique phenotype of IR without other typical correlates of IR, including normal fat distribution. Ongoing studies will now address other potential contributors to the unique mechanisms of IR in T1D, to allow targets for future treatment of IR in T1D.

Maternal Efficacy and Safety Outcomes in a Randomized Trial Comparing Insulin Detemir with NPH Insulin in 310 Pregnant Women with Type 1 Diabetes
ELISABETH R. MATHIESEN, PETER DAMM, MOSHE HOD, DAVID R. MCCANCE, MARINA IVANISEVIC, SANTIAGO DURAN-GARCIA, LOIS JOVANOVIĆ, Copenhagen, Denmark, Petah Tiqa, Israel, Belfast, United Kingdom, Zagreb, Croatia, Sevilla, Spain, Santa Barbara, CA

The aim of this prospective, randomized, controlled, parallel-group, open-label trial was to compare the efficacy and safety of insulin detemir (IDet) vs. NPH (both with prandial insulin aspart) in pregnant women with type 1 diabetes (t1DM). t1DM women (HbA1c $\leq 8\%$ at pregnancy confirmation) were randomized to IDet (n=152) or NPH (n=158) up to 12 months before pregnancy or during pregnancy at 8-12 weeks gestation. The primary objective was to confirm efficacy of IDet by showing that IDet was non-inferior to NPH with respect to HbA1c at 36 gestational weeks (GWs) (primary endpoint). Non-inferiority was shown if the upper limit of the 95% CI for the treatment difference of IDet vs. NPH was below the pre-specified non-inferiority margin of 0.4% for both the Full Analysis Set (FAS) and Per Protocol Set (PP).

The data were analyzed using linear regression. 79 and 83 women in the IDet and NPH groups, respectively, were pregnant at randomization while 73 and 75 women, respectively, became pregnant following randomization. Mean±SD baseline demographics were: age 30.1±4.4 yrs; BMI 24.8±4.1 kg/m2; HbA1c 7.01±0.79%; fasting plasma glucose (FPG) 5.94±3.25 mmol/l and diabetes duration 12.3±8.0 yrs. For FAS, the estimated HbA1c at GW36 was 6.27% for IDet and 6.33% for NPH. IDet was declared non-inferior to NPH.

For author disclosure information, see page LB42.
(FAS: -0.06%, 95% CI: -0.21; 0.08, PP: -0.151%, 95% CI: -0.34; 0.04). FPG was significantly lower with IDet vs. NPH (table). Hypoglycemia rates were similar between groups. In summary, lower FPG, but comparable HbA1c in late pregnancy were obtained using insulin detemir in comparison to NPH insulin in women with type 1 diabetes.

<table>
<thead>
<tr>
<th>IDet</th>
<th>NPH</th>
</tr>
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<tbody>
<tr>
<td>n=152</td>
<td>n=158</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Estimated mean FPG (mmol/L)</th>
<th>Treatment difference, 95% CI, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At GW24</td>
<td>5.38</td>
</tr>
<tr>
<td>At GW36</td>
<td>4.76</td>
</tr>
</tbody>
</table>

Hypoglycemia rates (episodes/yr) Estimated mean rate ratio, 95% CI, p value

| Overall major | 1.1 | 1.2 | 0.82 [0.39, 1.75] | p=0.615 |
| Overall minor | 104.4 | 101.0 | 1.10 [0.88, 1.37] | p=0.393 |
| Nocturnal major | 0.3 | 0.2 | 1.15 [0.40, 3.33] | p=0.785 |
| Nocturnal minor | 15.6 | 17.4 | 0.96 [0.72, 1.27] | p=0.733 |

Association of Skin Intrinsic Fluorescence with Type 1 Diabetes Complications in DCCT/EDIC Study

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The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) is evaluating the relationship of skin intrinsic fluorescence (SIF), glycemic exposure and diabetes complications. SIF has been shown to correlate with advanced glycation endproducts (AGEs) in dermal collagen. We have shown in DCCT/EDIC that dermal AGEs measured in skin biopsies are independently associated with microvascular complications. Our hypothesis is that SIF will correlate significantly with complications independent of A1c.

SIF was measured noninvasively on the volar forearm of 1,080 participants and quantified using SCOUT devices in 28 clinical centers during the 23rd/24th year of DCCT/EDIC. Glycemic exposure was calculated by summing DCCT/EDIC eligibility A1c x duration of diabetes at baseline, DCCT mean A1c x years of follow-up in DCCT and EDIC mean A1c x years of follow-up in EDIC. The participants were 54% male, mean age 52±6.9 years, and type 1 diabetes duration 20±4.9 years. Proliferative diabetic retinopathy (PDR) was present in 19% of participants, 39% had cardiac autonomic neuropathy (CAN), 30% had confirmed clinical neuropathy (CCN), 14% had sustained albumin excretion rate AER>30, and 29% had coronary calcium CC>0.

For author disclosure information, see page LB42.
diabetic counterparts (Figure, bottom). The BMI-mortality relationship is substantially different in adults with vs without diabetes. Future studies should account for diabetes status in investigations of the BMI-mortality relationship.

We conducted a prospective study of 3615 French men and women, aged 30–65 years, with normal baseline fasting glycaemia (FG), who participated in the 9-year follow-up D.E.S.I.R study (Data from an Epidemiological Study on the Syndrome of Insulin Resistance), Paris, France, Villejuif, France, La Riche, France.

Recent data indicate an independent association between plasma copeptin, a surrogate for vasopressin, and risk of diabetes mellitus. Despite the known influence of water intake on vasopressin secretion, no study has investigated a possible association between usual daily water intake and incidence of hyperglycemia.

We conducted a prospective study of 3615 French men and women, aged 30–65 years, with normal baseline fasting glycaemia (FG), who participated in the 9-year follow-up D.E.S.I.R study (Data from an Epidemiological Study on the Syndrome of Insulin Resistance), Paris, France, Villejuif, France, La Riche, France.

65-LB

Low Water Intake and Risk for New-Onset Hyperglycemia


Recent data indicate an independent association between plasma copeptin, a surrogate for vasopressin, and risk of diabetes mellitus. Despite the known influence of water intake on vasopressin secretion, no study has investigated a possible association between usual daily water intake and incidence of hyperglycemia.

Positive associations were observed between daily water intake and risk of developing hyperglycemia. Further studies are needed to establish whether interventions to increase water intake may protect against hyperglycemia. Further studies are needed to establish whether interventions to increase water intake may protect against hyperglycemia.

Odds Ratios (ORs) and 95% Confidence Intervals (95% CIs) for the incidence of hyperglycemia (impaired FG or diabetes, i.e., FG ≥ 6.1 mmol/l) or treatment for diabetes were calculated according to water intake classes.

During follow-up, 565 incident cases of hyperglycemia were identified. After adjustment for confounding factors (sex, baseline age, body mass index, FG, physical activity, smoking status, triglycerides, HOMA-IR, total cholesterol, gamma-GT and familial history of diabetes) ORs (95% CI) for hyperglycemia associated with the volume of daily water intake (<0.5 L, 0.5 to <1.0 L, more than 1.0 L) were 1.00, 0.64 (0.49–0.83), and 0.73 (0.55–0.97), P=0.003. The ORs were similar when stratified by various characteristics, including gender and alcohol consumption.

Self-reported water intake was inversely associated with the risk of developing hyperglycemia. Further studies are needed to establish whether vasopressin levels mediated this association and whether interventions to increase water intake may protect against hyperglycemia.

Model 1: Adjusted for confounding factors (see text).

Model 2: Further adjusted on mean daily volumes of beer-cider, sweet drinks, and wine.

66-LB

Micro- and Macrovascular Outcomes in Primary Care Patients with Type 2 Diabetes Treated with Insulin Glulisine or Human Regular Insulin: A Retrospective German Database Analysis

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Aims Analog insulin glulisine has a higher efficacy in reducing post-prandial glucose excursions and in restoring normal postprandial microcirculation than regular human insulins. Besides glycemic control, insulin glulisine has also favorable effects in maintaining normal endothelial function. Therefore, the aim was to compare the incidence of macro- and microvascular outcomes in type 2 diabetic patients treated with insulin glulisine or regular human insulin.

Methods Computerized data from 952 glulisine (age: 61 ± 11 yrs) and 11,157 regular insulin (65 ± 11 yrs) users in general practices throughout Germany (Disease Analyzer, 11/2004 to 3/2010) were analysed. Hazard ratios (HR, Cox regression) for 3.5-year risk of macro- or microvascular outcomes were adjusted for age, sex, diabetes duration, health insurance, residency, diabetologist care, hypertension, hyperlipidemia, depression, and co-medication (basal insulin, oral antidiabetics). Furthermore, adjustment was carried out for baseline microvascular complications when analyzing macrovascular outcomes and vice versa.

Results Overall, risk for both macro- and microvascular outcomes was 20% lower for patients using insulin glulisine (p=0.05). There was a decreased risk for coronary heart disease (HR; 95% CI: 0.78, 0.62–0.98), and a trend for lower events of myocardial infarction (0.66; 0.43–1.02). Also for microvascular complications, the adjusted hazard ratios for retinopathy, nephropathy and neuropathy were below 1.0, indicating a lower risk for the insulin glulisine group, however, which was statistically significant for neuropathy only (0.74; 0.58–0.93).

Conclusions The prescription of the rapid-acting insulin analog glulisine was associated with a reduced incidence of macro- and microvascular outcomes in type 2 diabetes under real-life conditions in a retrospective database analysis. It is important to confirm this finding in a randomized controlled trial.
and from The Health Improvement Network (THIN) primary care database in the U.K.,N=160,348 and N=2,548,349 respectively. To account for differences in age and sex distribution between patients with and without diabetes, between the two countries and over years, all mortality rates were standardised to the age and sex distribution of the 2006 Canadian census population.

The age and sex-adjusted annual mortality rates in patients with diabetes deceased by 2006 from 19.0 to 11.6 per 1,000 patients in Canada from 1996 to 2009, and there was a corresponding 54% decrease in adjusted rates in the U.K. from 30.1 to 13.7 per 1,000 patients. In contrast, adjusted annual mortality rates in the non-diabetes populations declined by 24% (10.5 to 8.0 per 1,000 individuals) and 44% (14.9 to 8.4) in Canada and U.K. respectively. Consequently, the adjusted mortality risk ratios for diabetes versus no diabetes decreased from 1.9 to 1.5 in Canada and from 2.0 to 1.6 in the U.K. The excess mortality associated with diabetes was similar in men and women in both Canada and the U.K.

In conclusion, mortality rates have declined in both patients with and without diabetes, but those with diabetes have experienced a greater decline in death rates over the last decade irrespective of sex. The gap in mortality attributed to diabetes decreased by almost 20% in both Canada and the U.K., which may be due to better diabetes care in developed nations in the twenty-first century.

GENETICS—TYPE 2 DIABETES

69-LB

Fine-Scale Genetic Mapping of Type 2 Diabetes Loci Using the Metabochip

TERESA FERREIRA, ANUBHA MAHAAJAN, STEVEN WILSHIRE, INGA PROKOPENKO, CECILIA M. LINDGREN, PANOS DELOUKAS, PETER DONNELLY, TIM FRAYLING, ANDREW HATTERSLEY, COLIN N.A. PALMER, ANDREW D. MORRIS, WELLCOME TRUST CASE CONTROL CONSORTIUM, MARK I. MCCARTHY, ANDREW P. MORRIS, Oxford, United Kingdom, Hinton, United Kingdom, Exeter, United Kingdom, Dundee, United Kingdom

There are now over 40 established loci influencing susceptibility to type 2 diabetes (T2D). These loci often extend over hundreds of kilobases and contain many genes with plausible functional impact on the disease. The aim of this study was to use imputation to fine-map known T2D loci to search for secondary signals of association and refine the location of the underlying causal variant(s).

We considered 5,938 T2D cases and 9,356 controls of European descent from the UK T2D Genetics Consortium, WTCCC and Warren 2 repository. The samples were genotyped using the "Metabochip", a custom iSELECT array containing ~195,000 SNPs, designed to support fine-scale mapping of established loci for T2D and other cardiometabolic traits and follow-up of putative novel associations. We performed imputation across 30 known T2D loci using IMPUTE2 and the European reference panel from the 1000 Genomes Project (August 2010 release). In each cohort, we tested for association of each variant (MAF>1%) assuming a linear trend in allelic effects, and performed conditional analyses by including the allele dosages of leading SNPs as covariates.

There are 3 independent (p<0.05) signals of association conditional on P<0.010 at the KCNQ1 locus: rs163184 (p=4.9x10^-8, OR=1.14[1.09-1.20]), rs2237839 (p=7.3x10^-8, OR=1.31[1.17-1.48]) and rs78131 (p=5.3x10^-5, OR=1.10[1.05-1.15]). The first 2 of these tag previously observed signals of association in European and East Asian populations. There are 2 signals of association at the CDKN2A/B locus: rs12555274 (p=4.4x10^-5, OR=1.17[1.11-1.24]) and rs10181166 (p=5.5x10^-5, OR=1.20[1.12-1.28]). These 2 variants tag 3 clades of haplotypes which have been previously demonstrated to be associated with T2D.

Fine-mapping of known T2D loci using the Metabochip highlights evidence of multiple causal variants at KCNQ1 and CDKN2A/B. Further refinement and replication of signals in these regions are currently underway within the DIAGRAM Consortium. Our results highlight the importance of fine-mapping to fully reveal the genetic architecture of T2D associations within known loci, showing great promise for furthering our understanding of the biological mechanisms underpinning susceptibility to the disease.

70-LB

Genome-Wide Association Analysis of Rare Variants with Type 2 Diabetes

REEDIK MAGI, JUHUI CHEN, KYLE GAULTON, JENNIFER ASIMIT, ELEFTHERIA ZEGGINI, MARK I. MCCARTHY, INGA PROKOPENKO, ANDREW P. MORRIS, Oxford, United Kingdom, Cambridge, United Kingdom

Genome-wide association studies (GWAS) of common variants have been successful in identifying novel type 2 diabetes (T2D) susceptibility loci. However, the joint effects of these variants account for no more than 15% of T2D heritability. The aim of this study was to assess the evidence for association of T2D with rare genetic variation, defined here to have minor allele frequency (MAF) less than 1%, which is poorly captured by GWAS genotyping products.

We performed imputation in 1,926 T2D cases and 2,942 controls of European descent, genotyped using the Affymetrix GeneChip 500K Mapping Array Set by the Wellcome Trust Case Control Consortium. Imputation was undertaken using IMPUTE2 and the European reference panel from the 1000 Genomes Project (August 2010 release). We tested for association with accumulations of minor alleles at rare variants in disease genes, incorporating 50kb up- and down-stream to allow for additional functional elements and regulatory regions. The analysis was performed using GRANVIL, which models disease status as a function of the proportion of rare variants at which an individual carries at least one minor allele in a logistic regression framework.

The strongest signal of association of rare variants with T2D was observed for BMP2 (p=1.0x10^-4, genome-wide significant correcting for 30,000 genes). Common variants within this gene have been shown to be associated with height and body mass index. Strong evidence of association (p<10^-4) was also observed for IGFB (p=2.4x10^-4) and CKLR (p=6.4x10^-4). IGFB belongs to a family of signalling molecules that play crucial roles in cellular energy metabolism and in growth and development.

The analysis has demonstrated strong evidence of association of T2D with rare variation in three genes. These signals of association warrant follow-up for author disclosure information, see page LB42.
71-LB
Haploinsufficiency of Sel1L Predisposes Mice to High-Fat Diet-Induced Hyperglycemia
RAJNI SINGH, ADAM B. FRANCISCO, GIAOMING LONG, XI YAN, XINGEN LEI, Lhacra, NY
Suppressor-enhancer-in-12t1-1-like (Sel1L) encodes a cytoplasmic factor present at high abundance in the mature pancreas. As a type I endoplasmic reticulum (ER) membrane protein, Sel1L has previously been implicated in ER-associated degradation (ERAD), a protein quality control process that is crucial for maintaining ER homeostasis. We have recently reported the first mouse genetic mutation for Sel1L, a hypomorphic allele generated through gene trapping. While homozygous mice (Sel1L-/−) are embryonically lethal, heterozygous mice (Sel1L+/−) are viable and show a normal glucose metabolic profile when fed with a normal diet. We hypothesize that haploinsufficiency of Sel1L may impair the ability of mature beta cells to functionally adapt to increased insulin demand under the condition of insulin resistance. In the present study, we tested this hypothesis in a diet-induced obesity model. Male Sel1L−/− mice and their wild-type littermates (10-weeks of age) were fed with a high-fat diet (HFD) for 24 weeks. Sel1L−/− mice showed progressively higher fasting blood glucose levels than their wild-type control mice after 8 weeks of HFD feeding. At the end of the HFD-feeding period, Sel1L−/− mice were glucose intolerant and had an impaired glucose-stimulated insulin secretion as compared to wild-type mice, as revealed by glucose-tolerance test (GTT) and glucose-stimulated insulin secretion (GSIS) analysis. Pancreatic morphometry indicated that Sel1L−/− mice had a markedly reduced β-cell mass. No observable increase of β-cell apoptosis but a significant decrease in β-cell proliferation was detected in the pancreas of Sel1L−/− mice. Pancreatic islets isolated from Sel1L−/− mice showed elevated expression of Bbig, Herp, Xbp-1 and Chop mRNA, indicating that the unfolded protein response (UPR) pathway was activated. Finally, cultured mouse and rat isletcell lines ectopically expressing a deletion mutant Sel1L exhibited impaired GSIS and reduced cell proliferation. Taken together, our in vivo and in vitro data strongly suggest that Sel1L has a critical functional role in the mature pancreas, most likely through maintaining normal β-cell proliferation and function.

72-LB
Haplotype Analysis of Established Type 2 Diabetes Loci Using the “Metabochip”
ASHISH KUMAR, ANDREW P. MORRIS, PANOS DELOUKAS, PETER DONNELLY, TIM FRAYLING, ANDREW HATTERSLEY, C.N.A. PALMER, A.D. MORRIS, WELCOME TRUST CASE CONTROL CONSORTIUM, MARK I. MCCAVERY, Oxford, United Kingdom, Exeter, United Kingdom, Dundee, United Kingdom
The number of established loci influencing susceptibility to type 2 diabetes (T2D) is now in excess of 40. Most of these loci have been identified through single-SNP analysis of genome-wide association studies, but together they explain less than 15% of the disease heritability. We have thus undertaken haplotype-based analysis within established T2D loci to identify multi-SNP signals of association that might better explain the contribution of the loci to disease susceptibility. We have considered 5,938 T2D cases and 9,356 controls of European descent from two cohorts: the UK T2D Genetics Consortium and the WTCCC and Warren 2 repository. The samples were genotyped using the “Metabochip”, a custom SELECT array containing ~195,000 SNPs, designed to support large-scale follow-up of putative novel associations for T2D and other cardio-metabolic traits, as well as fine-scale mapping of established loci. Within each cohort, we performed step-wise selection in each fine-mapping region of the Metabochip by adding SNPs in turn to the haplotype so as to maximise the signal of association. Meta-analysis of haplotype association p-values across the two cohorts was performed via Fisher’s method.

Our results highlighted haplotype association with stronger signals of association than any single SNP in two loci: KCNQ1 and CDKN2A/B. In KCNQ1, the strongest signal of association was obtained for a three SNP haplotype (rs6135184, rs2237896 and rs10400376; p=1.98x10−14), compared with the strongest single-SNP association (rs6135184, p=1.08x10−9). Moreover, these SNPs are not in linkage disequilibrium (r2<0.04), and thus represent independent effects on T2D. In CDKN2A/B, the strongest signal of association was obtained for a 2 SNP haplotypes (rs7018475; rs10811660; p=5.19x10−13), compared with the strongest single-SNP association (rs7018475; p= 6.01x10−9). These SNPs define three clades of haplotypes that have been previously shown to be associated with T2D. These results clearly outline the potential for haplotype-based analysis to identify secondary association signals within established T2D susceptibility loci, and elucidate the complex underlying genetic architecture of the disease.

73-LB
Metabolite Quantitative Trait Loci (mQTL) and Their Role in Type 2 Diabetes and Insulin Sensitivity
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Using metabonomic approaches, recent studies have identified several associations between small molecules and type 2 diabetes and insulin resistance. These metabolites may represent useful biomarkers, but their causal role in diabetes disease processes is less certain.

We aimed to identify common genetic variants associated with circulating metabolites relevant to insulin sensitivity and type 2 diabetes. Using 1004 non-diabetic individuals from the RISC study we performed a genome wide association study on 14 metabolites measured by mass spectrometry. We next assessed the association of these variants with diabetes related traits using the RISC data. We identified eight signal associations with four separate insulin sensitivity related metabolites. A previously reported variant in the FADS2 gene cluster (p = 2.90E-09) was associated with adrenate levels. Two variants were associated with betaine levels, one in the SLC6A12 gene (p< 8.1E-09) and one in the BHMT (betaine-homocysteine methyltransferase) (p = 1.40E-06). Two variants were associated with glycine levels, one, previously reported, in the CPS1 gene (p = 5.32E-30), and one in the ALDH1LI (aldehyde dehydrogenase) gene (p= 7E-5). Three variants were associated with serine levels, one in the PHGDH (3-phosphoglycerate dehydrogenase) gene (p = 1.52E-09) and two independently (r 2 = 0.01) in the PSPH (phosphoserine phosphatase) gene (p = 2.00E-05 and p = 4.00E-04).

The allele in the ALDH1LI gene associated with raised glycine levels was associated with increased sensitivity (P = 0.002, +0.14μmol.min⁻¹kg⁻¹FFM⁻¹, 95%CI (-0.05, 0.22) an effect consistent with a causal effect of glycine levels on insulin sensitivity, although further studies are needed to confirm this association.

74-LB
Rare Non-Functional Mutant Melatonin MT2 Receptors Contribute to Increased Type 2 Diabetes Risk
AMÉLIE BONNEFOND, NATHALIE CLÉ MENT, KATHERINE FAWCETT, LOIC YENGO, EMMANUEL VAUILANT, JEAN-LUC GUILLAUME, ROBERT SLADEK, MARK MCCARTHY, INÈS BARROSO, RALF JOCKERS, PHILIPPE FROGUEL, Lille, France, Paris, France, Cambridge, United Kingdom, Montreal, QC, Canada, Oxford, United Kingdom
Genome-wide association studies (GWAS) revealed that common non-coding variants in MTHR1 (encoding melatonin MT1 receptor) increase type 2 diabetes (T2D) risk. Although the strongest association signal was highly significant (P<10⁻10), its contribution to T2D risk was only modest (OR=1.15). This mirrors the effects seen for all common T2D-associated variants discovered by GWAS, which explain less than 10% of T2D heritability. It has been suggested that the ‘dark matter’ might be partially explained by the presence of numerous rare mutations with stronger effect.

To define the genetic architecture of T2D risk at the MTHR1 locus, we performed large-scale exon resequencing in 7,832 Europeans including 2,186 T2D patients, followed by a four-tier functional investigation of all identified MT2 mutations (cell surface expression, 2(125I)-iodomelatonin binding, melatonin-dependent G protein and ERK1/2 activation). The total loss-of-function MT2 variants were further genotyped in 11,854 European subjects. The effect of common variants on T2D risk was assessed by a logistic regression adjusted for age, sex and BMI. Rarer variants (minor allele frequency [MAF]<1%) were analysed by pooling them via the KBAC method embedded in a logistic regression adjusted for age, sex and BMI.

We identified 40 non-synonymous variants, including 36 very rare variants (MAF<0.1%) that associated with T2D (OR=3.31; 95%CI 2.00E-05 and P=4.00E-04). The four variants with MAF<0.1% did not contribute to T2D risk. The functional investigation of all 40 MT2 mutants revealed that 14 were non-functional and rare (MAF<1%), including four very rare mutations which completely ablate melatonin binding and downstream signaling. Altogether, the very rare
variants with partial or total loss-of-function contributed to T2D (OR=5.67, 95% CI: 3.00-12.5), whereas the very rare neutral variants did not associate with T2D. The four total loss-of-function MT variants strongly contributed to T2D (OR=8.153; 95% CI: 10.38, 6.37x10^-10). This post-GWAS study demonstrates a firm functional link between MTNRI-B and T2D risk along with the relative importance of rare and common genetic variations in establishing this risk.

75-LB TCF Transcription Activity Is Decreased by Expression of a Tissue-Specific TCF7L2 Splice Variant Influenced by GWAS Risk Alleles

HEATHER R. MANNING, PETER A. ANTINOZZI, Winston-Salem, NC

Common genetic variants of TCF7L2, a transcription factor of Wnt/β-catenin signaling, have been identified as the strongest genetic risk factors for Type 2 diabetes (T2D). The causal role of TCF7L2 risk alleles on its function is not firmly established; however, recent correlation of risk genotypes to TCF7L2 tissue-specific alternative splicing may provide insights to the mechanism. In particular, risk alleles (rs12255372 and rs7903146) influence the abundance of TCF7L2 splice variants including exon 13. To evaluate the role of exon 13 on β-catenin dependent-TCF transcriptional activity, we established a cell-based assay using the highly specific 8XTOPFlash luciferase-based Wort reporter system. In the normal, inactive state of the Wnt pathway, β-catenin is phosphorylated by glycogen synthase kinase 3β (GSK3β) and targeted for degradation by the proteasome. Thus, β-catenin protein levels largely drive TCF transcriptional activity. In our assay, expression of human β-catenin increased Wnt signaling activity 2.6-fold ± 0.20 over basal levels. To evaluate the role of TCF7L2 variants, co-expression of β-catenin with a specific TCF7L2 variant containing exon 13 (NM_001146284; variant 4) was evaluated. Variant 4 expression reduced β-catenin-mediated transcriptional activity to basal levels. This observation demonstrates the presence of exon 13 has a negative impact on Wnt/β-catenin signaling. Although this observation is novel it is not unprecedented. Alternatively spliced TCF7L2 variants have been shown to both activate and inhibit β-catenin transcriptional activity. Potential functional roles of exon 13 include altered association with transcriptional co-repressors and direct effects on β-catenin activity and degradation. Evidence to support the latter scenario includes co-expression of variant 4 with a degradation-resistant isoform of β-catenin, which restored activity to 135% ± 26% of the level observed with β-catenin alone. In conclusion, TCF7L2 risk alleles for T2D may influence the abundance of tissue-specific TCF7L2 splice variants, which may ultimately have direct effects on Wnt/β-catenin signaling.

76-LB The Expression of the Killer Cell Lectin-Like Receptor Subfamily D, Member 1 (KLRD1) Is Down Regulated in Insulin Resistant and Type 2 Diabetic Human and Cynomolgus Monkeys

MICHAEL BENZINOU, JIAN WANG, JIE CIE, SHIJUN YU, XIAOLI WANG, YUPENG FANG, CHEN WANG, XINGYU WANG, YOKIN WANG, TAICANG, China, Beijing, China

Insulin resistance (IR) is a precursor to type 2 diabetes mellitus (DM). To identify new molecular markers of IR, we conducted a large-scale, genome-wide gene expression profiling of 12 extreme IR and insulin-sensitive (IS) Chinese individuals selected from a general population cohort (99th percentile). The comparison of patients with excessive phenotype and a consequent increased heritability identified the Killer cell lectin-like receptor subfamily D, member 1 (KLRD1), an antigen preferentially expressed on natural killer cells. KLRD1 expression levels were found to be significantly lower in IR patients when compared to IS patients. To validate this association of KLRD1 with IR and DM, we also tested its expression by quantitative RT-PCR in cynomolgus monkeys with elevated homeostasis model assessment of insulin resistance (HOMA-IR) index, glucose intolerance by oral and intravenous glucose tolerance test and reduced tissue-specific TCF7L2 splice variants, which may ultimately have direct effects on Wnt/β-catenin signaling.

77-LB CD4 T Cells from Human T1D Patients Respond to a Peptide from Chromogranin A

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The response of human CD4 T cells to a peptide from a newly described autoantigen, chromogranin A (Chga), was tested in T cells from patients with type 1 diabetes (T1D) or controls. The peptide WE14 from Chga is a naturally occurring proteolytic cleavage product in islet beta-cells and was reported to be weakly stimulatory for diabetogenic T cell clones derived from the NOD mouse (Stadinski, Delong et al., Nat Immunol, 2010). Treatment of WE14 with the enzyme transglutaminase (Tgase) yielded a highly antigenic version of this peptide. Human PBMC responses to WE14 in both forms, untreated or Tgase converted, were measured by production of IFNγ in response to test antigen, detected by an indirect ELISPOT assay. T1D patients (N=23) were recent onset patients within 6 months of diagnosis who were typed for HLA-DQB. Controls (N=17) were either 1st degree relatives with no antibodies or from the HLA-matched general population cohort of DAISY/TEDDY.

Results indicated that WE-14 alone or with Tgase modification can be recognized by human T1D T cells and that treatment with Tgase may increase recognition at lower concentrations of peptide.

For author disclosure information, see page LB42.
normal mean +3SD. PAA were detected in 7-20%, sera from recent onset (7/100) and long-standing (10/50) T1D patients as well as patients with other autoimmune diseases or pancreatic cancer. The specificitiy of PAA in T1D and pancreatic cancer in selected samples was confirmed by Pdx1 competition assays. Therefore, we have developed a sensitive, specific, and non-radioactive assay for detecting PAA in human sera, providing a useful tool for evaluating the clinical significance of PAA.

79-LB
Loss of Anergy in Insulin-Specific B Cells from New-Onset Type 1 Diabetics
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Although T cell-derived immune effectors mediate islet destruction in type 1 diabetes (T1D), it has become increasingly clear that B cells also play an important role in disease development, and further that the offending cells are islet antigen, e.g. insulin, specific. This may indicate that normal B cell tolerance mechanisms fail in T1D.

In this study we explored the status of insulin-specific B cells in the peripheral blood of normal (N=24), new onset (N=12), and >1 year diabetic individuals (N=12), aided by magnetic particle-based enrichment of antigen binding cells. Insulin-specific B cells could be found in similar numbers in all subjects, indicating that at least some of these cells escape receptor editing and clonal deletion in all subjects, and diabetes is probably not associated with failure of these silencing mechanisms. Insulin-specific B cells were found predominantly in CD27+CD45RA+ B cell populations, occurring in compartments thought to be naive as well as compartments thought to be anergic (Breg). Importantly, insulin-specific B cells transiently disappeared from the anergic population in early onset diabetics, returning to normal levels in patients who were diabetic for >1 year. We observed a correlated increase in insulin-specific B cells among IgG1+ IgM+ naive B cells during this time period. We hypothesize that B cells recognizing insulin with high avidity are normally silenced by anergy, but that this anergy is transiently lost during the prediabetic and new onset period. B cells that have lost anergy may promote or instigate disease development by antigen presentation to diabetogenic T cells.

In the course of our studies we also noted that, irrespective of their specificity, B cells in T1D individuals express reduced levels of IgM. In the mouse, this phenotype is associated with mutations that impair signaling pathways that negatively regulate antigen receptor signaling. Our findings therefore suggest that T1D may be associated with mutations that impair maintenance of anergy in B cells, and thereby contribute to disease development.

80-LB
The Efficacy of Thymic Negative Selection Increases with Age in NOD Mice
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Type 1 Diabetes (T1D) is characterized by the T cell-mediated destruction of the pancreatic β cells. Defects in central and peripheral tolerance are believed to contribute to the development and expansion of β cell-specific T cells. During central tolerance, autoreactive T cells are typically deleted upon recognition of self-antigen in the thymus through a process referred to as negative selection. Inefficient negative selection has been reported in NOD mice, which is expected to lead to an increased frequency of β cell-specific T cells in the periphery. The current study investigates the events during thymic negative selection that promote the development of diabetogenic T cells in NOD mice. Specifically, we assessed the efficiency of thymic negative selection as a function of age. Interestingly, intravenous injection of peptide into NOD.BDC and NOD.CA.T1 cell receptor transgenic mice resulted in inefficient induction of thymocyte apoptosis in newborn versus 4 week-old animals. The observed differences in the efficacy of negative selection were not the result of inefficient trafficking of antigen into the newborn thymus or inherent differences in the capacity of thymocytes from different aged thymi to undergo apoptosis following antigen encounter. Consistent with the latter, an increased frequency of β cell-specific mature thymocytes, as determined by tetramer staining, was detected in the thymus of newborn versus 4 week-old NOD mice. In an initial attempt to define the mechanism influencing the efficacy of negative selection, thymic dendritic cells (DC) were examined from different aged NOD mice. Independent of age, thymic DC exhibited minor differences in the expression of co-stimulatory and MHC molecules. However, thymic DC isolated from 4 week-old NOD mice exhibited an increased stimulatory capacity compared to their newborn counterparts. Together, our findings indicate that the efficacy of thymic negative selection increases with age due in part to changes in the stimulatory capacity of thymic DC. Furthermore, our data suggest that the peripheral repertoire of autoreactive T cells in NOD mice is largely established early in life.

81-LB
Encapsulated hESC Derived Islets as a Transplantation Therapy for Diabetes without Immunosuppression
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The major obstacles to widespread application of islet transplantation for the treatment of diabetes are the scarcity of human islets and the need for chronic immunosuppression. Previously we determined that a durable encapsulation device protected murine islets from both allograft rejection and autoimmune disease in the NOD model of Type I diabetes. Moreover, in a preclinical trial it was established that the device is also immunoprotective in primates. In order to address the shortage of islets, we investigated the use of human embryonic stem cells (hESC) derived tissue, a virtually unlimited source of cells. Previously Novocell/ViaCyte reported a method for inducing pancreatic epithelium formation from hESC in vitro. In the present study encapsulated hESC derived pancreatic epithelium was transplanted subcutaneously into mice (n=27). Within 7-9 weeks mice exhibited circulating human insulin (measured as C-peptide). From weeks 7 to 16 both the level of glucose stimulated C-peptide and the stimulation index (maximal/ fasted C-peptide) rose significantly. In vivo bioluminescent imaging (BLI) of luciferase expressing cells revealed that cell mass within the device remained constant during the period of dramatic increases in glucose responsive insulin secretion. Together the data provide evidence of robust cell maturation in the device. The level of circulating human insulin achieved from 20UL of packed cells, was sufficient to influence glucose homeostasis in multiple cohorts of animals treated with the drug allocan to destroy their endogenous b-cells (n=21). In animals exhibiting 4000 pmol C-peptide, diabetes reversal was complete for 23 days, the longest period tested. The data establish encapsulation of hESC derived pancreatic epithelium as a promising strategy for providing a widely available, non-immunosuppressive, and minimally invasive transplantation therapy for diabetes.

82-LB
Intra-Renal Transplantation of Bioactive Renal Cells Preserves Renal Functions and Extends Survival in the ZSF1 Model of Progressive Nephropathy
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There are ~200,000 diabetic patients in the US with End-Stage Renal Disease. Dialysis and pharmacological intervention insufficiently support kidney function longterm, culminating in kidney transplantation or death. New treatment paradigms that slow or reverse progression of chronic kidney disease (CKD) are needed to relieve patient and healthcare burdens. Recent work in our laboratory demonstrated that a selected population of bioactive renal cells (BRCs), established from autologous diseased kidney tissue, regenerated in situ functional kidney mass, stabilized filtration function, and prolonged survival following intra-renal delivery in a 5/6 nephrectomy model of terminal CKD. In the present study, the in vivo function of these BRCs was evaluated in the ZSF1 rodent model of progressive nephropathy secondary to a metabolic syndrome of diabetes, obesity, dyslipidemia, and hypertension. Injection of syngeneic BRCs into the ZSF1 renal parenchyma elicited a regenerative response that significantly improved renal functions, including filtration (eGFR, BUN, sCr), protein handling (albumin), electrolyte balance (K, Na, Phos) and the ability to concentrate urine (osmolality) just as occurred in the mass reduction model of CKD. Multivariable linear regression analysis showed that each of the renal functions affected by treatment significantly predicted ZSF1 survival beyond the one year timeline for follow up. The characteristic hypertension in the ZSF1 model was normalized by the cell treatment; these data were further supported by statistically significant modulation of physiological regulators of blood pressure, including the pituitary and adrenal pressor hormones, ACTH and cortisol. Also consistent with previous results, implantation of the BRCs in the ZSF1 model resulted in significant reduction of circulating plasmogen activator inhibitor (PAI),
a master regulator of tissue fibrosis. These results collectively support the utility of the intra-renal delivery of an adult autologous and regenerative renal cell population for slowing progression and improving survival in patients with CKD secondary to metabolic syndrome.

**INSULIN SIGNALING/INSULIN ACTION**

**INSULIN ACTION—GLUCOSE TRANSPORT**

83-LB

**Manipulation of BACE1 Activity/Expression in C2C12 Myotubes Alters Glucose Uptake and Utilization**

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There is accumulating evidence that insulin resistance and neurodegeneration are intimately linked. A key enzyme in the progression of neurodegeneration is the aspartic protease BACE1 (beta site APP cleaving enzyme 1). BACE1 activity is the rate-limiting step for the generation of toxic amyloid peptides normally associated with neurodegeneration. Previous work from our laboratory has demonstrated that BACE1 knockout mice display improved insulin glucose disposal and insulin sensitivity as measured by glucose and insulin tolerance tests. Additionally, these animals are resistant to diet induced obesity and insulin resistance suggesting that BACE1 activity regulates whole body glucose and energy homeostasis. We hypothesized that manipulation of BACE1 in the skeletal muscle cell line, C2C12, would lead to alterations in glucose metabolism. We utilised C2C12 myotubes treated overnight with a cell permeable BACE1 inhibitor (250nM (BACE1 inhibitor IV, Calbiochem (BACE1i))) or C2C12 myotubes stably expressing either empty vector (EV) or BACE1 and carried out a range of measures such as glucose oxidation and uptake. We found that overnight inhibition of BACE1 with the BACE1 inhibitor significantly (p<0.05) enhanced 2-Dextroseglucose (2DG) uptake [vehicle=9.98±0.54 (pmol/min/mg), BACE1i=12.62±0.67 (pmol/min/mg)] and insulin stimulated 2DG uptake [vehicle =13.48±0.74 (pmol/min/mg), BACE1i=16.82±0.91 (pmol/min/mg)]. Additionally the BACE1 inhibitor significantly (p<0.05) increased glucose oxidation [vehicle=6.47±0.69 (pmol/min/mg), BACE1i=8.58±0.09 (pmol/min/mg)]. Alternatively BACE1 over expression significantly (p<0.05) increased insulin stimulated glucose uptake [EV=18.31±2.38 (pmol/min/mg), BACE1=14.54±1.55 (pmol/min/mg)] and significantly (p<0.05) reduced glucose oxidation [EV=14.13±1.72 (pmol/min/mg), BACE1=11.07±1.34 (pmol/min/mg)]. These data demonstrate that BACE1 is a novel regulator of skeletal muscle substrate uptake and metabolism. They also indicate that this secretase acts in a cell autonomous manner suggesting that targeting skeletal muscle BACE1 may be a novel means to alleviate skeletal muscle insulin resistance.

84-LB

**The First Luminal Loop Is Responsible for the Insulin Responsiveness of the Glucose Transporter Isoform 4**

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Glucose transporter isoform 4, or Glut4, is expressed in insulin-sensitive tissues and is responsible for the post-prandial blood glucose clearance. The mechanistic dissection of Glut4 regulation is crucial for our understanding of the molecular nature of insulin resistance and diabetes mellitus. Unlike most other glucose transporters, Glut4 is compartmentalized inside the cell primarily in small insulin-responsive-vesicles, or IRVs, that are translocated to the cell surface upon insulin stimulation. From the plasma membrane, Glut4 is internalized and is rapidly delivered to either a sub-domain of the trans-Golgi network (TGN) or recycling endosomes that represent the donor compartment for the formation of the IRVs. Such complicated pattern of intracellular trafficking is apparently defined by unique signals in the Glut4 molecule. Previous studies have suggested that sequences in the cytoplasmic tails of Glut4 are important for its faithful intracellular traffic. In this study, we explored the targeting role of the first luminal loop of Glut4. For that, we exchanged the first luminal loops of Glut4 and cellulinyrin, a 4-transmembrane protein which is absent from the IRVs. Using immunofluorescence microscopy and biochemical fractionation, we found that the first luminal loop of Glut4 is sufficient to confer insulin responsiveness to cellulinyrin. Mechanistically, the first luminal loop of Glut4 targets the reporter protein to the IRVs by interacting with the sorting receptor sortilin. We propose a model according to which targeting of Glut4 into the IRVs requires at least two distinct steps: targeting into the donor compartment (TGN and/or recycling endosomes) provided by sequences in the cytoplasmic tails and targeting from the donor compartment into the IRVs that depends on the first luminal loop of the transporter.

85-LB

**Increased Inflammatory Gene Expression in Skeletal Muscle Cells from Insulin Resistant Type 2 Diabetic Patients**

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Skeletal muscle is the key site of peripheral insulin resistance in type 2 diabetes. Insulin-stimulated glucose uptake has previously been shown to be decreased in differentiated skeletal muscle cells derived from type 2 diabetic patients. This study was designed to examine changes in gene expression with differentiation between the diabetic and control cultures. Skeletal muscle cell cultures were established from insulin-resistant type 2 diabetic patients and matched controls. Gene expression analysis was performed using Affymetrix chips to compare gene expression in the diabetic and control cultures. Genes and pathways upregulated with differentiation in the diabetic cultures were identified using GeneSpring and Gene Set Enrichment Analysis (GSEA).

Many of the top genes upregulated in the diabetic cultures were genes involved in the immune or inflammatory response. These include CXCL2 (p=0.02, compared with control), CCLS (p=0.03) and ILB (p<0.05). The pathways predominantly upregulated in the diabetic cultures were derived from treatment with interferons. Quantitative PCR confirmed that expression of chemokines including MCP-1, ILB and IL8 showed the same pattern of increase with differentiation in the diabetic cultures. Treatment of control muscle cells with either interferon alpha or interferon gamma also increased expression of these genes. Interferon-induced upregulation of inflammatory gene expression was accompanied by decreased insulin-stimulated glucose uptake and Akt phosphorylation. While basal glucose uptake was comparable across treatment groups, treatment with interferon-gamma resulted in a reduction in insulin-stimulated glucose uptake from 489.6±190.1pmol/min/mg in untreated cells to 124.3±32.8pmol/min/mg (p<0.05). This was accompanied by a 37% reduction in insulin-stimulated phosphorylation of Akt after interferon treatment (p<0.02).

Taken together, these data show that pro-inflammatory gene expression is increased in diabetic skeletal muscle and that this is likely to contribute directly to the decreased peripheral insulin action observed in the diabetic state.

**INSULIN ACTION—METABOLISM**

86-LB

**A Metabolomic Profile in Three Ethnic Groups Is Associated with Insulin Resistance and Conversion to Diabetes in the IRAS Study**

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We hypothesized that metabolomic profiling of acylcarnitines and amino acids would identify specific analytes associated with dynamically measured insulin sensitivity and conversion to type 2 diabetes (T2D) in a multiethnic sample from the Insulin Resistance Atherosclerosis Study. Metabolites including 69 amino acids and acylcarnitines were profiled by mass spectrometry from baseline. Subjects also had an intravenous glucose tolerance test measuring insulin sensitivity (SI) and acute insulin response (AIR). T2D was determined by 2-hour OGTT. Samples were from European Americans, African Americans and Hispanics to represent 1) extremes of the SI distribution and 2) subjects converting to T2D between baseline and 5-year follow-up. Logistic regression analysis identified metabolites that differentiated subjects by high and low SI and by conversion to T2D between baseline and 5-year follow-up. Analysis from 74 high SI (mean 5.6) compared to 73 low SI (mean 0.22) subjects adjusted for age, gender, BMI, and ethnicity, revealed the insulin resistant subjects had significantly decreased Gin/P (p=0.0005), and increased Val/P (p=0.0002), Leu/Ile/P (p=0.0015), Phe(0.0004), and Glu/Gln (p=0.0023). The associations were strongest in European Americans. When comparing amino acid profiles between subjects that converted to T2D (76 converters; 94 non-converters), a similar pattern was observed.
ETV5 Inactivation Protects from Obesity, but Provokes Glucose Intolerance

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ETV5 is a transcription factor involved in the development of the kidney, spermatogenesis and pancreas. Recently, genome-wide association studies have revealed an association of ETV5 with human obesity. To begin to identify the role of this gene in obesity, we exposed mice lacking the ETV5 gene to a high-fat diet (HFD). ETV5 knock-out (KO) mice were smaller, leaner and had a reduced body weight (BW) compared to their wild-type (WT) controls at 8 weeks of age on a low-fat chow diet. When these mice were exposed to a HFD for 8 weeks, the KO mice maintained their same BW than before exposure to HFD (19.6 ± 0.6 vs 19.9 ± 0.8 g), whereas the BW of the WT mice was significantly increased (23.7 ± 0.8 vs 32.2 ± 1.3 g). Fat mass was increased four-fold after exposure to HFD in the WT mice (2.6 ± 0.3 vs 12 ± 0.7 g), and 1.5-fold in the KO mice (1.8 ± 0.7 vs 2.9 ± 0.3 g) and no differences were observed in the lean mass. When corrected for body weight, there were no differences of food intake between WT and KO mice. Moreover, there were also no changes of energy expenditure per metabolic mass in either WT or KO mice. Interestingly, an intraportal glucose tolerance test revealed a glucose intolerant phenotype in the KO mice fed the HFD, and the insulin levels of the KO mice were significantly decreased. Further, there were no apparent differences of insulin sensitivity between WT and KO mice. The KO mice had more and smaller adipocytes compared to the WT mice. Taken together, these experiments demonstrate that ETV5 KO mice are resistant to obesity, possibly due to an impaired insulin production and secretion. Further studies of the implication of the transcription factor ETV5 in the development of the pancreas, and hence, possible repercussions on insulin metabolism, will have to be performed.
INSULIN SIGNALING/INSULIN ACTION

91-LB
Exposure to Common Food Additive Carrageenan Leads to Glucose Intolerance, Insulin Resistance, and Inhibition of Insulin/IGF-1 Signaling
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This study was performed to determine the impact of the common food additive carrageenan on glucose tolerance, insulin sensitivity, and insulin signaling. Groups of male C57BL/6J mice were tested for the effect of carrageenan (10 µg/ml) in their drinking water by glucose and insulin tolerance tests. HepG2 and human colonic epithelial cells (NCM460, InCell) human subcutaneous preadipocytes, and human multipotent adipose-tissue. Through cGMP and cGMP-dependent protein kinase (PKG), NPs hemodynamic balance. NP receptors have been found in human adipose

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Insulin resistance (IR) is accompanied by chronic low grade systemic inflammation and deregulation of total body energy homeostasis. We induced inflammation in human adipose and liver tissue in vivo in order to mimic inflammation in vivo, with the aim to identify tissue-specific processes and biomarkers implicated in IR. Human adipose and liver tissues were cultured with or without LPS and DHA Microarray Technology was applied for their transcriptome analysis. The transcriptome data were validated with proteomics analysis of the inflamed adipose tissue secretome using CILAR technology. LPS significantly affected 667 and 484 genes in adipose and liver tissues respectively. During inflammation adipose tissue, compared to liver tissue, had more significantly upregulated genes, GO terms, and functional clusters related to inflammation and angiogenesis. The secretome pathway of LPS significantly affected 667 and 236 genes in adipose and liver tissue respectively. The secretomes of both tissues shared 66 genes. The adipose tissue specific biomarkers were represented by fractalkine, tumor necrosis factor, pentraxin-related protein or interstitial collagenase (matrix metallopeptidase 1) and the liver specific biomarkers were for example chemokine (C-X-C motif) ligand 9, chemokine (C-X-C motif) ligand 3, or follistatin-like 3 (secreted glycoprotein). The transcriptome data of the inflamed adipose tissue secretome showed excellent correlation with the proteomics data. The higher number of altered proinflammatory genes, GO processes, and genes encoding for secretome during inflammation in adipose tissue compared to liver suggests that adipose tissue is the major organ in the development of systemic IR. Our study led to the identification of differential pathways and biomarkers suggesting tissue specific changes, which could be applied for tissue specific detection and treatment of IR.

92-LB
JNK1 Enhances Lipotoxicity through Inhibition of SIRT1
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A decrease in SIRT1 expression is associated with the development of hepatic steatosis, which leads to insulin resistance through lipotoxicity. However, the intracellular signaling pathway for SIRT1 inhibition is not known. In this study, we investigated SIRT1 protein degradation in response to hyperinsulinemia or hyperglycemia in dietary obese mice. We found that in response to insulin or glucose, SIRT1 was phosphorylated by JNK1 (c-Jun N-terminal kinase 1) at Ser46 and Ser47 in human SIRT1, one of the four potential JNK1 targeting residues. The phosphorylation led to SIRT1 ubiquitination and degradation in proteasomes. The protein degradation inhibited SIRT1 function and contributed to the development of hepatic steatosis in dietary obese mice. The phosphorylation was observed in the wild-type mouse embryonic cells, but not in the JNK1 null cells. Mutation of Ser46 to alanine prevented the SIRT1 degradation by blocking phosphorylation and ubiquitination in response to JNK1. JNK1 was compared with JNK3 in the regulation of SIRT1 degradation. JNK1 was required for the degradation as shown in the liver of JNK1-KO mice. JNK 2 exhibited an opposite activity in the regulation of SIRT1 degradation as shown in the JNK1 (1+2) KO mice. The data suggest that SIRT1 activity is inhibited by JNK1 in cells. This mechanism is responsible for SIRT1 degradation in response to insulin or glucose. This signaling pathway is involved in the pathogenesis of hepatic lipotoxicity in dietary obesity, and represents a new signaling pathway for JNK1 in the pathogenesis of insulin resistance.

PHYSIOLOGY—ADIPOCYTE BIOLOGY

93-LB
Cardiac Natriuretic Peptide: New Activator of p38 MAPK-Dependent Adaptive Thermogenic Program and Respiration in Human Adipocytes
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Cardiac natriuretic peptides (NPs) are key hormones in fluid and haemodynamic balance. NP receptors have been found in human adipose tissue. Through cGMP and cGMP-dependent protein kinase (PKG), NPs can stimulate lipolysis in human adipocytes with a potency similar to catecholamines via β-adrenergic receptors (βARs). Using differentiated human subcutaneous preadipocytes, and human multipotent adipose-derived stem (hMADS) cells, we asked whether NPs could also promote a “brown” adipocyte phenotype as can be elicited by βARs. Atrial NPs (ANP) indeed significantly increased the expression of UCP1, PGC1α, cytochrome c and the broader network of genes for mitochondrial biogenesis and function. ANP also increased the activity of transfected reporter genes for the Ucp1 enhancer and the Pgc-1α promoter. We previously showed that βAR agonists via cAMP/PKA drive expression of UCP1 and PGC-1α through a signaling cascade to p38 MAPK (p38). ANP was also able to activate p38 and its downstream targets MK-2 and the transcription factors ATF-2 and PGC-1α and subsequent biochemical machinery of brown fat thermogenesis. Like βAR agonists, ANP recruited ATF-2 and PGC-1α to the human Ucp1 enhancer in a PKG and p38 dependent manner. Human adipocytes also responded to ANP by increasing respiratory uncoupling. Finally β-agonists and ANP acted in a synergistic or additive manner to increase expression of UCP1, PGC-1α, cytochrome c, SIRT1, among others. Our results support the concept of ANP as a cardiometabolic hormone and reveal a novel pathway parallel to βARs that can coordinate brown adipocyte gene expression and function. Given the recognition of brown adipocytes in adult humans, the ability of NPs to work in concert with catecholamines and βARs presents new investigative opportunities toward increasing energy expenditure, combating obesity and its negative cardiovascular consequences.

94-LB
Organ-Specific Candidate Biomarkers of Inflammation Found by Comparative Analyses of the Human Hepatic and Adipose Tissue Transcriptome and Secretome during LPS Treatment
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Insulin resistance (IR) is accompanied by chronic low grade systemic inflammation and deregulation of total body energy homeostasis. We induced inflammation in human adipose and liver tissue in vitro in order to mimic inflammation in vivo, with the aim to identify tissue-specific processes and biomarkers implicated in IR. Human adipose and liver tissues were cultured with or without LPS and DNA Microarray Technology was applied for their transcriptome analysis. Gene Ontology (GO), gene functional analysis, and prediction of genes encoding for the secretome were performed using DAVID, STRING, and SecretomeP as bioinformatics tools. The transcriptome data were validated by proteomics analysis of the inflamed adipose tissue secretome using CILAR technology. LPS significantly affected 667 and 484 genes in adipose and liver tissues respectively. During inflammation adipose tissue, compared to liver tissue, had more significantly upregulated genes, GO terms, and functional clusters related to inflammation and angiogenesis. The secretome pathway of LPS significantly affected 667 and 236 genes in adipose and liver tissue respectively. The secretomes of both tissues shared 66 genes. The adipose tissue specific biomarkers were represented by fractalkine, tumor necrosis factor, pentraxin-related protein or interstitial collagenase (matrix metallopeptidase 1) and the liver specific biomarkers were for example chemokine (C-X-C motif) ligand 9, chemokine (C-X-C motif) ligand 3, or follistatin-like 3 (secreted glycoprotein). The transcriptome data of the inflamed adipose tissue secretome showed excellent correlation with the proteomics data. The higher number of altered proinflammatory genes, GO processes, and genes encoding for secretome during inflammation in adipose tissue compared to liver suggests that adipose tissue is the major organ in the development of systemic IR. Our study led to the identification of differential pathways and biomarkers suggesting tissue specific changes, which could be applied for tissue specific detection and treatment of IR.
insulin signaling/insulin action

96-LB
Prolonged Exposure to S-Adenosylhomocysteine (SAH) Perturbs Adipocyte Biology

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Dietary micronutrients (MC) affect target tissue functionality through the regulation of methyl donor availability (via 1-carbon metabolism) and its resultant effect on gene expression. How specific MC might influence adipocyte function in particular remains poorly understood. We have investigated the effects of prolonged exposure to SAH, a pivotal metabolite in the 1-carbon cycle, on adipogenic differentiation and function. 3T3-L1 preadipocytes were proliferated and differentiated in -10uM and 100uM SAH. Mature adipocytes were assessed for basal and insulin-stimulated (100M; 20min) glucose uptake (GU), basal and isoproterenol (ISO)-stimulated (10uM) lipolysis and lipid accumulation. Total glucose transporters (GLUT)-1 and -4 that mainly mediate basal and insulin-stimulated GU respectively, were assessed by Western blot. Total RNA was extracted to assess adipogenic gene expression using semi-quantitative RT-PCR. Prolonged SAH exposure impaired basal GU by 30% (p<0.05) at 10uM and 45% (p<0.05) at 100uM SAH. Insulin-stimulated GU was reduced by 50% at both 10uM (p<0.05) and 100uM (p<0.01) SAH. However, SAH did not alter total GLUT1 and -4 levels. SAH exposure did not alter total lipid accumulation but impaired basal lipolysis by 70% (p<0.05) at 10uM SAH. ISO-stimulated lipolysis was impaired by 50% (p<0.05) at 10uM SAH respectively. Whilst gene expression of the key adipogenic marker peroxisome proliferator activated receptor-α was unchanged by SAH, CAAT enhancer binding protein α and retinol x receptor α were dramatically reduced by 90% (p<0.05) and 50% (p<0.01) respectively at 100uM SAH. In summary, prolonged SAH exposure impairs key aspects of adipocyte functionality, lipolysis and GU (in cells that apparently differentiates) and significantly changes adipogenic gene expression. Our data emphasizes that dietary MC can specifically alter adipocyte biology and may influence adiposity and the onset of obesity under conditions of MC imbalance.

97-LB
Role of miRNAs in Pathophysiology of Obesity

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Obesity is a result of increased energy intake and/or decreased energy expenditure. This energy imbalance varies in different individuals; certain people gain weight more rapidly than others when subjected to similar high calorie diet. However, the etiology and mechanistic insight into these variations are poorly understood. Studying this phenotype in humans is complicated due to variation in diet, physical activity, etc. Therefore, we developed diet induced obese (DIO) and diet resistance (DR) mice to investigate the phenomenon of variable weight gain in response to a high fat diet. C57BL/6J mice were fed high fat diet and bred for multiple generations to derive pure DIO and DR colonies. The DIO and DR mice had similar body weight at birth, but responded differently to HFD (i.e. DIO gained more weight than DR), despite ingesting similar caloric intake. We next inquired the mechanistic reasons for this phenotypic difference. Interestingly, we observed that the body temperature was significantly increased in DR mice, compared to the DIO mice. Consistent with this, we observed increased BAT, muscle and mitochondria specific genes and decreased WAT specific genes in the white adipose tissue (WAT) of DR mice. Considering these mice are genetically similar, we hypothesized that epigenetic modification, such as micro RNA (miRNAs), may play a role in the phenotype diversity. miRNAs are small oligonucleotides that regulate gene expression at the transcriptional and translation levels. We thus profiled expression levels of miRNAs in the WAT of DIO and DR mice. Interestingly, we observed that 8 miRNAs were upregulated and 11 downregulated, while few miRNAs were switched on or off depending on whether the WAT was derived from DIO or DR mice. These data suggest that miRNAs are differentially expressed in the context of adiposity. Further work is in progress to understand the molecular targets of these miRNAs and to definitively ascertain whether change in miRNA expression is an early and causative event in the pathogenesis of obesity.

98-LB
The eif2x2-CHOP Pathway Has an Essential Role in Suppressing Adipocyte Differentiation

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Obesity is characterized by excess body fat with consequent adverse effects on health. Recent studies suggest that the UPR sensors play a role in adipocyte differentiation; however, it is not known how each individual UPR sensor affects adipogenesis. Here, we showed that ER stress during adipogenesis attenuated the differentiation potential of preadipocytes. The attenuation of adipogenesis was augmented in preadipocytes undergoing a greater level of UPR due to deletion of a chaperone protein, p58. In addition, preadipocytes that have a mutation at Ser 51 to Ala of eIF2α showed enhanced adipocyte differentiation. Furthermore, high fat-fed mice that harbor the same mutation showed increased obesity over time. In contrast, induction of eIF2x2 phosphorylation attenuated adipogenesis in 3T3-L1 cells. The forced expression of CHOP in 3T3-L1 cells significantly inhibited adipogenesis, evidenced by less fat accumulation and reduced expression levels of C/EBPα, PPARγ, and PGC-1α. CHOP-/- mice showed greater body fat mass compared to wildtype mice on regular or high fat diet. These results demonstrate that the eif2x2-CHOP pathway of the UPR plays an important role in suppressing adipogenesis. This pathway may be a potential therapeutic target to decrease fat mass in obesity.

99-LB
The Multi-Level Action of Fatty Acids on Adiponectin Production by Fat Cells

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Current epidemicics of diabetes mellitus is largely caused by widespread obesity.

Although obesity can provoke insulin resistance and diabetes via multiple mechanisms, the best-established connection between obesity and insulin resistance is the elevated and/or dysregulated levels of circulating free fatty acids and, in particular, palmitate that cause and aggravate insulin resistance, type II diabetes, cardiovascular disease and other hazardous metabolic conditions. We investigated the effect of the major dietary fatty acid, palmitate, on the insulin-sensitizing adipokine adiponectin produced by cultured adipocytes. We found that palmitate rapidly inhibits release of adiponectin from adipocytes in a time- and concentration-dependent fashion. Adiponectin transcription is also suppressed by palmitate. Transcription of adiponectin is controlled primarily by PPARγ and C/EBPs. Using mouse embryonic fibroblasts from C/EBPa-null mice, we found that the latter transcription factor is not likely to mediate the inhibitory effect of palmitate. However, substitution of PPARγ for the unphosphorylatable mutant Ser273Ala blocks the effect of palmitate on adiponectin transcription. This suggests that palmitate inhibits adiponectin gene expression by stimulating phosphorylation of PPARγ on Ser273. The acyl-CoA synthetase inhibitor, Triacsin C, blocks the negative effect of palmitate on adiponectin transcription. We suggest therefore, that the latter effect is not explained by activation of the cell surface receptors, but requires intracellular metabolism of palmitate via the acyl-CoA synthetase-mediated pathway. In addition, we found that the lysosomal inhibitor, chloroquine, rapidly suppressed the effect of palmitate on adiponectin release from adipocytes. Thus, palmitate not only decreases adiponectin expression and the level of transcription but also stimulates lysosomal degradation of newly synthesized adiponectin in adipocytes.
FGF21 Suppresses Hepatic Glucose Production through the Activation of Atypical Protein Kinase C Isomers

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FGF21 growth factor (FGF21) has been identified as a potent and robust metabolic regulator. Administration of recombinant FGF21 protein to rodents and rhesus monkeys exerts strong anti-diabetic effects. Previous work demonstrated that FGF21 inhibits glucose output by the rat H4IIe hepatoma cell line. We performed pharmacological studies to investigate the mechanisms by which FGF21 regulates glucose production. H4IIe cells were cultured in glucose-free medium then treated with 16 μM vehicle, insulin or FGF21. Total RNA was collected for gene expression analysis by RT-qPCR and cell medium was collected to measure glucose levels. We found that both insulin and FGF21 suppressed gene expression of Glic-6-Pase and PEPCK. Accordingly, glucose production was inhibited.

Treatment of cells with the PI-3 kinase (PI3K) inhibitor LY294002 reversed the effects of both insulin and FGF21. Interestingly, a specific Akt2 inhibitor only inhibited insulin’s impact on glucose production, but not that of FGF21. In further studies, we found that FGF21 did not alter AMPK or acetyl-CoA carboxylase phosphorylation, indicating that the polypeptide did not change AMPK activity. In contrast, FGF21 induced PKCy/β phosphorylation in a PI3K-dependent manner. Furthermore, the non-isouform selective PKC inhibitor, Ro31-8220, blocked FGF21 inhibition of glucose production, while Go6976, the inhibitor of classical and novel PKC isoforms, had no effect on FGF21 inhibitory activity.

These results suggest that FGF21 suppresses H4IIe cell glucose production by activating atypical PKCs in a PI3K-dependent manner. To determine if FGF21 affects atypical PKC activity in vivo, we treated diabetic db/db mice with FGF21 and harvested livers for PKC phosphorylation measurement by immunoblot. We found that hepatic PKCy/α phosphorylation was significantly upregulated by FGF21. We conclude that FGF21 may inhibit hepatic glucose production by activating atypical PKC.

ZBTB20 Regulates B Cell Function through Transcriptional Repression of Fructose-1,6-Biphosphatase 1

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The glucoregulatory function is critical for β cell function. Fructose-1,6-biphosphatase 1 (FBP1), a key enzyme involved in hepatic gluconeogenesis and representing a potential target for diabetic treatment, is expressed by β cells, and regulates their glucoregulatory metabolism and insulin secretion, but its transcriptional regulatory mechanism is poorly defined. Here we show that the zinc finger protein ZBTB20 is a transcriptional repressor of FBP1, and plays an important role in the regulation of glucose-stimulated insulin secretion (GSSS) in β cells. Abundantly expressed in β cells, ZBTB20 was found to bind to FBP1 promoter by chromatin immunoprecipitation, and inhibit its transcriptional activity in vitro. Reducing ZBTB20 levels in β cell lines by RNA interfering or β cell-specific disruption of ZBTB20 by gene targeting resulted in increased FBP1 expression and markedly impaired GSSS, the latter was largely restored by inhibition of FBP1 activity. β cell-specific ZBTB20 knockout mice exhibited hyperglycemia, hypoinsulinemia, and glucose intolerance. Taken together, these data suggest a critical role of ZBTB20 in β cell function and a potential target for diabetes treatment.
Raising Circulating HDL Levels in Mice Is Associated with Enhanced Glucose Utilization and Lipolysis

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Abnormal glucose metabolism is a central feature of disorders associated with increased rates of cardiovascular disease (CVD). One of the strongest predictors of CVD in type 2 diabetes (T2D) is a low level of HDL. Herein we used a genetic mouse model with markedly increased HDL levels (apoAI tg; 174 ± 41 mg/dl) and one with severely reduced HDL levels (apoAI ko; 23 ± 10 mg/dl) compared to wt mice (wt; 70 ± 9 mg/dl) to test the hypothesis that circulating HDL levels may modulate glucose utilization and lipid utilization. Fasting glucose levels are reduced in apoAI tg mice and increased in apoAI ko mice compared to wt mice (respectively, 142 ± 5 vs 196 ± 12 ± 16 ± 1 % fat of BW, N = 13, P < 0.05) and fasting NEFA levels were increased in apoAI tg mice compared to wt and apoAI ko mice (0.56 ± 0.02 vs 0.44 ± 0.04 vs 0.38 ± 0.02, N = 13, P < 0.05) suggesting that lipolysis in adipose tissue of apoAI tg mice is enhanced compared to wt mice. To understand whether FGF21, a novel inhibitor of lipolysis, may mediate the increased NEFA release observed in apoAI tg mice we determined FGF21 expression in liver. We found that FGF21 expression levels were significantly reduced in apoAI tg mice compared to apoAI ko and wt mice (0.6 ± 0.15 vs 2 ± 0.6 vs 1.4 ± 0.2 mM×240 min, N = 5, P = 0.03) suggesting that FGF21 mediated inhibition of lipolysis is reduced in apoAI tg mice. Since HDL levels are low in T2D, our findings point to a novel role of HDL-raising therapies in modulating glucose and lipid homeostasis and thus address key aspects of T2D.

INTEGRATED PHYSIOLOGY—OTHER HORMONES

Ghrelin Administration Impairs Postprandial Glucose Tolerance at Physiologic Levels in Healthy Humans

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Administration of intravenous ghrelin inhibits the acute insulin response to glucose and worsens IV glucose tolerance in healthy subjects. We hypothesize that ghrelin exerts similar effects on glucose homeostasis to glucose and worsens IV glucose tolerance in healthy subjects. We administered ghrelin and saline to healthy volunteers and measured glucose and lipid homeostasis and thus address key aspects of T2D.

Preserved Postprandial GLP-1 Responses in Cholecystectomized Subjects: No Evidence of a Physiological Role of Gallbladder Emptying in Postprandial GLP-1 Release

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Besides their well-established roles in dietary lipid absorption and cholesterol homeostasis, bile acids are increasingly being recognized for their function as metabolic regulators. Preclinical studies suggest that gallbladder emptying - via bile acid-induced activation of the G protein-coupled receptor TGR5 in intestinal L cells - plays a significant role in the secretion of the incretin hormone glucagon-like peptide-1 (GLP-1). We hypothesized that human gallbladder emptying potentiates postprandial release of GLP-1 and aimed to evaluate whether cholecystectomized patients exhibit impaired postprandial GLP-1 secretion.

Ten cholecystectomized subjects (age; 49±4 years (mean±SEM); BMI; 25.0±4 kg/m²; HbA1c; 5.9±0.1%) and 10 healthy age-, gender- and BMI-matched control subjects (age; 48±4 years; BMI; 24±0.5 kg/m²; HbA1c; 5.7±0.3%) were studied. None had any family history of diabetes and all had normal oral glucose tolerance according to 75 g-oral glucose tolerance test. Subjects received a liquid meal (with acetalaminophen for evaluation of gastric emptying) during which blood samples were drawn and duodenal aspirate (for evaluation of intraduodenal bile acid concentrations) was collected through a duodenal tube placed fluoroscopically. Similar fasting plasma glucose levels were observed in the two groups (5.4±0.1 (mean±SEM) vs. 5.2±0.1 mM, P=0.2) whereas postprandial plasma glucose excursions were exaggerated in the cholecystectomized group compared to control subjects (1,431±31 vs. 1,313±36 mM×240 min, P=0.023). Cholecystectomized subjects exhibited preserved postprandial GLP-1 responses compared to the control subjects (3,585±313 vs. 3,990±313 mM×240 min, P=0.37).

In conclusion, cholecystectomized subjects exhibit preserved postprandial GLP-1 responses suggesting that the physiologically role of gallbladder emptying for postprandial GLP-1 release indicated by preclinical studies is of less importance in humans. Thus, the physiological relevance of potentiation of GLP-1 release via bile acid-induced activation of TGR5 in small intestinal L cells is still questionable in humans.

OBESITY—ANIMAL

ALB-127158(a): An MCH, Receptor Antagonist That Exhibits Weight Loss and Improvements in Insulin Sensitivity in Diet-Induced Obese Mice

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Melanin-concentrating hormone (MCH) is a cyclic neuropeptide involved in appetite regulation and energy homeostasis. Antagonists of the MCH receptor have been shown to be promising new mediators of weight loss in animal models. ALB-127158(a), a selective, high affinity antagonist of the human MCH1 receptor, demonstrated significant reductions in food intake and body weight in a 28-day feeding study in hyperinsulinemic male diet-induced obese (DIO) C57BL/6J mice. At twice daily oral doses of 5 and 15 mg/kg and a once daily oral dose of 30 mg/kg, ALB-127158(a) reduced food intake (12.4%, 18.9%, 20.2%), body weight (12.3%, 17.3%, 18.1%), and fasting plasma insulin levels (25.7%, 30.3%, 29.0%), respectively relative to control animals. An oral glucose tolerance test (OGTT) performed after 28 days of dosing also demonstrated improvements in glucose tolerance and insulin sensitivity. Reductions in plasma insulin were observed 15 minutes (-17%, 38.6%, 49.4%), 30 minutes (19.1%, 23.2%, 37.2%) and 60 minutes (22.3%, 29.6%, 31.2%) after challenge and reductions in plasma glucose were observed 15 minutes (8.1%, 9.8%, 23.4%), 30 minutes (18.4%, 9.3%, 14.4%) and 60 minutes (22.7%, 13.8%, 17.1%) after challenge, respectively relative to control animals. These data indicate that ALB-127158(a) produces significant weight loss and body weight in a mouse model of obesity with concomitant improvements in insulin sensitivity. ALB-127158(a) was recently shown to be well tolerated in a Phase I clinical study. The preclinical efficacy and clinical data support the continued development of ALB-127158(a) as a potential once daily treatment for obesity and related disorders. p<0.05, p<0.01, p<0.001.
Anti-Diabetic Effects of Panax notoginseng Extract Containing Dammarane-Type Triterpenes in KKAy Mice

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The root of Panax notoginseng has been traditionally used as an herbal treatment for diabetes, and a group of steroidal saponins is considered to comprise the active ingredients. Since saponins are converted into sapogenins (non-sugar portion of saponins) during the digestion process, we produced dammarane-type triterpenes (DT) from Panax notoginseng extract in which all sugars were fully removed from saponins. To evaluate the anti-diabetic effects of DT, genetically obese diabetic mice, KKAy mice, (4 weeks old, male) were fed a high-fat diet without or with 0.12% DT for 10 days. The increase in blood glucose due to a high-fat diet was significantly inhibited in the DT group compared with that in the control group. The result of glucose tolerance test in the DT group was also significantly improved compared with that in the control group. There were no significant differences in plasma insulin levels between the two groups. To determine whether the improved whole body glucose tolerance was associated with improved insulin sensitivity in skeletal muscle, glucose transport in response to maximal insulin (50mU/ml) was measured in isolated sartorius muscles.

The DT group demonstrated significantly higher insulin-stimulated glucose transport compared with that in the control group. Furthermore, the addition of DT in cell culture media for L6 myotubes significantly increased glucose transport compared with that in the control group. Furthermore, the addition of DT in cell culture media for L6 myotubes significantly increased glucose transport, indicating that DT directly affected the skeletal muscle. In conclusion, we found an anti-diabetic effect of DT, which is derived from Panax notoginseng. DT seems to directly affect the skeletal muscles and elicit anti-diabetic effects.

Effects of Acipimox on Development of Non-Alcoholic Fatty Liver Disease in High Fat-Rats

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In obesity, hypertrophic Adipocytes Have Higher Lipolysis Rate, Which Results In Overproduction Of Free Fatty Acid (FFA) And Insulin Resistance. These Are Associated With The Pathogenesis Of Non-Alcoholic Fatty Liver Disease (NAFLD). Acipimox (APX), A Lipolysis Inhibitor, Has Been Reported To Improve Insulin Resistance By Lowering FFA. We, Thus, Investigated The Effects Of APX On Development Of NAFLD In High Fat-Rats (N=18). At 8 Weeks Of Age, Male Sprague-Dawley Rats Were Randomly Assigned To One Of Three Groups: Standard Diet, High-Fat Diet (HFD), HFD With APX. After 4 Weeks, We Performed An Insulin Tolerance Test And Measured Serum Adipokines. Junchaooxylin and eosisin, (B) Oil red O (x 200) (C) TNNF-α stain. The bars represent 100 μm.

Cdc2-Like Kinase 2 (Clk2) Is a Key Modulator of Leptin and Insulin Signaling/Action in the Hypothalamus

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Cdc2-like kinase 2 (Clk2) is an insulin-regulated suppressor of hepatic gluconeogenesis and is downregulated in the liver from db/db mice. In vitro studies have shown that insulin is able to induce Clk2 threonine and serine phosphorylation (pThr/Ser), and these phosphorylations seem to be important for Clk2 signaling/activity. However, the role of insulin and leptin modulating Clk2 in the hypothalamus and in the regulation of energy balance were not yet investigate. Thus, the aim of the present study was to investigate in vivo firstly the effect of insulin and leptin on Clk2 regulation and on energy homeostasis and secondly the effect of nutritional status and obesity on the modulation of this kinase in the hypothalamus. By western blot and immunofluorescence we detected Clk2 expression in all hypothalamic nuclei. Fasting decreased and refeeding increased Clk2 pThr/Ser in the hypothalamus from control mice. Intracerebroventricular (ICV) injection of insulin or leptin increases hypothalamic Clk2 pThr/Ser in time and dose dependent manner. ICV P3K (LY294002) or Akt (Akt VIII) inhibitors blocked insulin-induced Clk2 pThr/Ser suggesting that the P3K/Akt pathway mediates the effects of insulin on Clk2 pThr/Ser. Furthermore, the inhibition of Clk2 for 5 days with ICV oligonucleotide antisense (ASO) or with TG003 (pharmacological inhibitor) increased body weight and adiposity due to increased food intake (FI). The inhibition of Clk2 by ICV ASO or TG003 decreased by 30-40% the anorexigenic effects of leptin and insulin and reduced POMC, CART, CRH levels in the hypothalamus. Further, in db/db and diet induced obesity mice hypothalamic insulin-induced Clk2 pThr/Ser in the hypothalamus was downregulated. In summary, our data provide evidence, in vivo that hypothalamic Clk2 pThr/Ser is regulated by nutritional status and by insulin and leptin. This regulation is blunted in obese mice. Moreover these findings suggest that hypothalamic Clk2 is required for the appropriate expression of hypothalamic neuropeptides to modulate FI. Thus, the potential for hypothalamic Clk2 as a site for new therapeutic approach of obesity and energy balance deserves further exploration.

Myocardial Cross-Talk between the Angiotsin II Type 2 Receptor (AT2R) and mTOR-S6K1-RPS6 Signaling in Obese Hypertensive Zucker Rats

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The cardio-protective protein, angiotensin II (Ang II) type 2 receptor (AT2R), is often compensatorily elevated with cardiac disease, however, the underlying mechanisms are unclear. This investigation was undertaken to determine whether the mammalian target for rapamycin (mTOR)–Ribosomal S6 kinase (S6K1)-signaling pathway, which is activated by excess nutrients, insulin (INS) or Angiotensin II (Ang II), contributes to elevation of AT2R protein in cardiomyocytes as a protective feedback mechanism under conditions of over-nutrition and hypertension. Blood pressure measurements by telemetry, and cardiac functional analysis by cine-magnetic resonance imaging and pressure-volume loop analysis established elevated blood pressures, increased septal wall thickness and diastolic dysfunction of left ventricle (LV) (p<0.05) in 9-12 week old male Zucker obese (ZO) rats compared to age matched ZZ controls. Immunoblotting revealed elevated AT2R protein and activation of mTOR complex 1 (mTORC1)-signaling in ZO myocardium.
Early improvement in glucose homeostasis after RYGB can be explained simply by caloric restriction. However, RYGB subjects exhibit a distinctive change in the dynamic response of insulin and incretin to meal challenge, which might explain the accentuated and early improvement in glucose homeostasis observed with surgical intervention.

114-LB

Effects of Gastric Bypass Surgery and High-Protein-Low-Calorie Diet on Postprandial Plasma Glucose, Insulin and Gut Hormone Levels in Obese Diabetic Patients


Both high-protein-low-calorie diets (HPLC) and roux-en-y-gastric bypass surgery (RYGB) improve glucose homeostasis in obese patients with type 2 diabetes (T2DM) within weeks. Caloric restriction and altered secretion of incretins may be responsible for these effects. This study aimed to quantify postprandial glucose, insulin and gut hormone secretion before and after a HPLC or RYGB in obese subjects with T2DM.

We studied 2 groups of females at baseline and 3 weeks after RYGB (n=15, age 51.3±7.3y) or after the start of a HPLC (n=12, age 50.8±2.7y). We performed a meal tolerance test and measured glucose, insulin and gut hormones for 3 hours. BMI declined similarly in both groups (RYGB: 43.5±1.1 vs. 40.4±1.1kg/m2; HPLC 40.2±1.9 vs. 37.9±1.7kg/m2). Fasting plasma levels of glucose, insulin and HbA1c also decreased similarly. Area under the response curve (AUC) of glucose decreased comparably after RYGB (1889±1479mmol/L/3h, p<0.05) and after HPLC (1774±1346mmol/L/3h, p<0.05), whereas AUC of insulin increased after RYGB (5663±6780 mmol/L/3h, p=0.2) and significantly decreased after HPLC (5827±4084 mmol/L/3h, p<0.05). Moreover, the peak insulin response was lower and delayed after HPLC (98±123 min, p=0.05), whereas postprandial insulin peaked earlier after RYGB (102±42 min, p<0.05).

RYGB increased AUC GLP-1 fivefold (488±2373 mmol/L/3h, p<0.05), but levels did not change after HPLC (362±833 mmol/L/3h, p=ns). Total GIP levels however, increased significantly after HPLC (30232±41636 mmol/L/3h, p<0.05), whereas RYGB did not alter GIP secretion (31015±34615 mmol/L/3h, p=ns).

These data suggest that RYGB and HPLC ameliorate glucose metabolism in obese women with type 2 diabetes through distinct mechanisms. RYGB enhances insulin release, likely via stimulation of incretin hormone secretion before and after a meal.

Young rats injected daily with UGP281 at doses of 5 µg/kg and 20 µg/kg exhibited an immediate acute dosedependent reduction in food intake of 55% and 84%, respectively and a sustained weight loss of 12% and 20%, respectively in a 20 day chronic dosing study, young rats injected daily with UGP281 at doses of 5 µg/kg and 20 µg/kg exhibited an immediate acute dosedependent reduction in food intake of 55% and 84%, respectively. A placebo-controlled study in Beagle dogs with enteric-coated capsules containing UGP281 demonstrated a sustained weight loss reduction of 8% compared to placebo for 12 weeks.

For author disclosure information, see page LB42.
ISLET BIOLOGY/INSULIN SECRETION

116-LB
Islet Amyloid Polypeptide Degradation by Pitrilysin
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Type 2 diabetes mellitus (T2DM) is characterized by a progressive loss of pancreatic beta-cell function, a decrease in beta-cell mass due to increased beta-cell apoptosis, and accumulation of islet amyloid that is derived from islet amyloid polypeptide (IAPP; amylin). The in vivo steady concentration of hIAPP is regulated by its rate of production versus its rate of clearance. A shift in this balance either by up-regulation of synthesis and secretion or down-regulation of degradation could contribute to IAPP accumulation and aggregation. Although there are numerous studies regarding the production of IAPP, its clearance through enzymatic degradation is poorly understood. Insulin-degrading enzyme (IDE) was the first enzyme to be reported to degrade IAPP and IDE inhibition in insulinoma cells did indeed impair IAPP degradation and accelerated amyloid formation. Here we report that a related peptidase pitrilysin, another member of invercizinc family, is able to hydrolyze IAPP in vitro. Human IAPP (hIAPP) was incubated with recombinant pitrilysin and cleavage products were separated by HPLC and identified by mass spectrometry. The overall degradation of hIAPP by pitrilysin resulted in the production of several 9-15 amino acid fragments with the most abundant product being the COOH-terminal peptide 25-37-amide. The catalytic constant (Kcat) and K_m value for pitrilysin with hIAPP were 13.5±0.1 min⁻¹ and 0.38±0.01 µM, respectively. Compared with IDE, the catalytic efficacy of pitrilysin towards hIAPP is about 40% of that of IDE (Kcat/K_m = 35.5 and 94.5 min⁻¹µM⁻¹ for pitrilysin and IDE, respectively). Exogenously applied pitrilysin was able to reduce the level of synthetic hIAPP or endogenously secreted hIAPP induced cytotoxicity/apoptosis in insulinoma cells (INS-1, 832/13 cells), showing that the degradation products of hIAPP degradation by pitrilysin are not toxic. Thus pitrilysin might be involved in IAPP clearance and up-regulation of pitrilysin could be a therapeutic approach to prevent or treatment of T2DM.

Pancreas-Specific Deletion of 12-Lipoxygenase Protects Against Islet Dysfunction in Mouse Models of Cytokine-Mediated Diabetes
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12-lipoxygenase (12-LO) catalyzes the oxygenation of cellular polyunsaturated fatty acids to form lipid inflammatory mediators. Increased expression or activity of 12-LO increases the production of HETE products, which may accelerate inflammation, antagonize the action of PPAR-γ, and activate signaling pathways promoting oxidative stress. Whole-body 12-LO knockout mice are protected against low-dose STZ-induced diabetes and high fat diet-induced islet dysfunction. When introgressed onto the NOD background, 12-LO knockout mice are protected against the development of insulitis and type 1 diabetes. Because of the broad expression of 12-LO in immune cells, fat, and islets, the underlying protection against hyperglycemia in these animal models is difficult to attribute to any single cell type. To clarify the role of 12-LO in the pancreas, we bred 12-LOloxP/LoxP mice to mice harboring the Cre transgene driven by the 4.7 kb Pdx1 promoter to generate mice with a pancreas-wide deletion of 12-LO (pLOKO). Isolated islets from pLOKO mice showed reduced insulin secretion and improved glucose-stimulated insulin secretion compared to islets from WT littermates. In conclusion, our results suggest for the first time that an intrinsic role of 12-LO in the pancreas may contribute to islet dysfunction in models of cytokine-mediated diabetes, and that the targeting of 12-LO activity may represent a novel and viable approach for the treatment of diabetes.

Divergent Regulation of Pancreatic Endocrine Cell Differentiation from Endocrine Progenitor Cell Line Tec-3p Cells
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Differentiation of pancreatic endocrine progenitors is controlled by local factors and physical interactions with the microenvironment, leading to transcriptional activation of endocrine markers. Neurogenin (ngn3), which is transiently expressed in pancreatic endocrine progenitors, is an essential transcription factor in the differentiation of the endocrine lineage. However, the mechanisms by which the endocrine progenitors differentiate into specific cell types, such as alpha and beta cells, are incompletely understood. We have established a novel cell line, named Tec-3p, from diabetic neoplasia driven by Simian Virus 40 large T antigen. Tec-3p cells are spherical in shape and non-adherent and are unique as they express endocrine lineage markers, ngn3, beta2, nkk2.2 and nks8.1 but not insulin, which suggests that Tec-3p cells resemble immature endocrine progenitors. Here we show that the treatment of Tec-3p cells with several factors, such as retinoic acid and ligands for the class B GPCR family, can induce glucagon and insulin gene expression, respectively. When treated with retinoic acid under low glucose conditions, gene expression of ngn3 is downregulated and the cells acquire adherence properties. The adherence phenotype of these cells, which we refer to as Tec-3, is irreversible even after withdrawal of retinoic acid. Immunohistochemical analysis demonstrates that Tec-3 cells express glucagon but not insulin. In contrast, treatment of Tec-3p cells with the GPR1 analogue exendin-4 induces insulin I gene expression under high-glucose conditions. Taken together, adherence to extracellular matrix components such as collagen appears to play an important role in facilitating endocrine lineage gene expression. These results suggest that the coordinated interaction with local factors and microenvironment controls the differentiation of early endocrine progenitors to alpha and beta cells, and establishes Tec-3p cells as useful model to study these processes in vitro and screen for agents with regenerative potential in the diabetes.

Identification of Novel Small Molecule Regulators of Human Pancreatic Endocrine Cell Specification
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Expression of Neurogenin3 (NEUROG3) is an obligate step in the production of pancreatic endocrine cells from multipotent embryonic progenitors. Understanding the mechanisms that control NEUROG3 expression will greatly improve the efficiency of endocrine cell production from human embryonic stem cells (hESC) as well as to expand our knowledge of the underlying biology regulating endocrine cell development and regeneration. In an effort to identify novel regulators of endocrine cell genesis, Pfizer Regenerative Medicine and ViaCyte (formerly Novocell), Inc. partnered to screen hESC-derived pancreatic epithelial progenitors (PE) for small molecules that induce NEUROG3 expression and subsequent endocrine cell commitment. Using a high-throughput multiplex qRT-PCR screening method, multiple unique small molecules/mechanisms were identified that induce de novo NEUROG3 and NKX2.2 expression in uncultured pancreatic progenitors. Of these molecules, a multimodal kinase inhibitor "Compound A" was selected for further analysis to validate our experimental approach. Addition of Compound A to PE significantly increases the number of NKX2.2-positive/ChromograninA-positive endocrine cells in vitro, and enhances the magnitude of in vivo graft function relative to controls after transplantation of Compound A-treated PE cells into mice. Efforts are ongoing to further characterize this molecule, as well as to characterize the other molecular pathways identified in this screen to develop small molecule regulators of beta cell differentiation and regeneration.
DNA methylation creates epigenetic marks critical for determining chromatin accessibility and controlling gene expression. DMR at Cpg island shores can discriminate between different somatic cell types including reprogrammed cells. To find DMRs that distinguish pancreatic β-cells and liver cells, we screened methylation status across the rat Pdx1 and insulin I promoters in Cpg islands and shores using DNAs isolated from rat insulinoma cells (INS1), rat liver stem cells (WB), and rat liver by treating DNA with bisulfate followed by PCR/sequencing. Three major Cpg islands in the Pdx1 promoter were mostly unmethylated in all cell types. However, DMRs were identified in the promoters of Pdx1 (-1282 to -599 nt) and insulin-I (-357 to -110 nt) exhibiting opposite methylation patterns. The Cpg sites at DMR within Pdx1 are methylated (95%) in INS1 cells and unmethylated (98%) in liver cells. In contrast, the Cpg sites at DMR of insulin are unmethylated (98%) in INS1 and methylated (97%) in liver. We tested the hypothesis that DMRs within Pdx1 and insulin I promoters might serve as epigenetic marks for monitoring the process of liver cell reprogramming by step-by-step reprogramming WB cells into pancreatic precursor cells: 1) first introducing Pdx1 into WB cells via lentivirus for a month, 2) followed by transfecting cells with either Ngn3 or NeuroD mRNA for an additional month under first low- then high-glucose culture conditions. Methylation status analysis revealed an increasing frequency of methylated Cpg sites in Pdx1-DMR as hepatic cells were reprogrammed toward beta cells (liver-4%, WB-11%, WB-Pdx1-44%, WB-Pdx1/Ngn3 or NeuroD-62%, INS1-95%); and decreasing methylation in the insulin I promoter-DMR (liver -97%, WB-80%, WB-Pdx1-83%, WB-Pdx1/Ngn3 or NeuroD- 58%, INS1- 2%). Among each Cpg site within DMRs, there are sequential changes during cell reprogramming. In conclusion, DMRs of Pdx1 and insulin I promoters are useful epigenetic markers for monitoring pancreatic lineage commitment during directed liver-to-endocrine pancreas transdifferentiation.

Reprogramming Hepatic Cells into Pancreatic Precursors by Delivery of mRNAs of Pdx1-VP16 and Ngn3

Generating autologous liver-derived insulin-producing cells (iPcs) for diabetes therapy requires the development of efficient viral-and DNA-free reprogramming strategies. Previously, we found that reprogramming hepatic cells into functional IPCs can be achieved by transgene expression of pancreatic transcription factors (PTFs) delivered by viral vectors. However, the timing of the reprogramming process is poorly understood due to uncontrollable expression of PTF genes. Further, virus-mediated random insertional mutation poses safety concerns that prevent clinical translation. Recent progress in delivering modified mRNA opens new avenues for somatic cell reprogramming, allowing sustained, high-level expression of active proteins. To test the feasibility of reprogramming hepatic cells into IPCs by mRNA, we constructed expression plasmids containing T7 promoter-driven PTF genes (Pdx1-VP16 and Ngn3) and then generated modified PTF mRNA-containing a 120-nucleotide polyA tail by in vitro transcription. We first transfected cells with PTF mRNA (0.25-5 µg) and then determined their kinetics and half-life. Then we transfected human hepatic (HuH7) cells daily for 3d with dual-color-dual-lineage reporters (aFP-GFP/MyoRFP) in the presence of innate immunity suppressor B18R to determine the reprogramming efficiencies as assessed by visualization of RIP-RFP and inactivation of aFP-GFP and by evaluating pancreatic and hepatic gene expression by realtime PCR. We found that 1µg is an ideal dose for reprogramming 106 cells and the PTF-mRNAs peaked around 18-24hrs and were undetectable after 48hrs. Combined Pdx1-VP16/Ngn3 mRNA showed powerful reprogramming efficiency (up to 90%) as assessed by emergence of RIP-RFP+ cells as early as 18hrs after first transfection. We also saw activation of several pancreatic endocrine genes (Pdx1, NeuroD, Pax4, Islet-1, insulin) and downregulation of hepatic genes (aFP, TTR, Alb). In conclusion, RNA-based technology is more efficient than lentivirus-based transgene expression in hepatic cell reprogramming.

Reprogramming of Hepatic Stem-Like WB Cells into Endoderm Progenitor Cells

Induced pluripotent stem cells (iPSCs) hold great promise for cell therapy. However, the low efficiency of lineage-specific differentiation and teratoma formation adversely impacts its clinical application. We hypothesized that somatic cells reprogrammed into endoderm progenitor cells (EPCs) might proliferate comparably to iPSCs but having a higher capacity for differentiating into pancreatic endocrine progenitor cells (PEPCs). We aimed at reprogramming rat liver stem (WB) cells into EPCs by transducing with variable combinations of reprogramming factors (RFs) Oct4, Sox2, Klf4 and Myc delivered by retroviral vectors. EPCs were selected based on unique colony morphology (small and round cells with a high N/C ratio). They grew faster than the parental WB cells (MTT assay), expressed pluripotency markers (Oct4 and Sox2) and EPC markers (Sox17, Foxa2) by RT-PCR, stained negatively for alkaline phosphatase (AP), and failed to form teratomas in vivo. Expression arrays revealed that EPCs had a dramatically different gene expression profile than parental WB cells with upregulation of stem cell markers (Opa5, Ednb, Fgf4, Gabrb3, Gal, Kit, Lefty2 and Serp2). Importantly, EPCs were more susceptible to directed differentiation into PEPCs by retrovirus-borne Oct4, Ngn3, and Sox2 than were WB cells, as characterized by expression of endogenous Pdx1, Ngn3, Nkx6.1, Ngn3, Klf4, and Myc. We hypothesized that somatic cells reprogramming into EPCs may be a safer, more effective strategy to generate pancreatic beta cells for cell replacement therapy of type 1 diabetes.

The Cdk4-E2F1 Pathway Regulates Pancreatic Progenitors and Endocrine Precursor Development

Importantly, EPCs were more susceptible to directed differentiation into PEPCs by retrovirus-borne Oct4, Ngn3, and Sox2 than were WB cells, as characterized by expression of endogenous Pdx1, Ngn3, Nkx6.1, Ngn3, Klf4, and Myc. We hypothesized that somatic cells reprogramming into EPCs may be a safer, more effective strategy to generate pancreatic beta cells for cell replacement therapy of type 1 diabetes.
tcf19 is a putative transcription factor containing a forkhead-associated (FHA) domain. It is largely uncharacterized, but is expressed during cell division, beginning at G1/S phase. In a mouse model of obesity-associated diabetes, we identified tcf19 within a group of cell cycle genes whose expression correlates with adaptive islet proliferation. In confirmation of these microarray data, we observed 3.3-fold upregulation of tcf19 mRNA in islets from obese non-diabetic C57Bl/6J (B6) mice vs. lean (n=5, p<0.01). This obesity-driven upregulation was not observed in islets from the diabetic BTBR strain. A possible explanation for this expression difference is a non-coding single nucleotide polymorphism we identified between the B6 and BTBR strains within the 5’UTR of exon 1 of tcf19. To examine the tissue distribution of tcf19 expression, we measured tcf19 mRNA levels across a panel of 13 lean B6 tissues and in obese B6 islet. Tcf19 is most highly expressed in mouse islet. Specifically, we observe tcf19 expression in mouse β-cell nuclei by immunostaining. In addition, in BioGPS human expression profiling, Tcf19 is most highly expressed in pancreas. This is an unusual pattern of expression for a cell cycle regulator since numerous tissues have higher basal proliferation rates and suggests that tcf19 may play a more specific role in cell cycle regulation in the islet.

We then directly examined the role of tcf19 in beta cell growth. After siRNA-mediated knockdown of tcf19 in INS-1 cells, we found a significant reduction in the number of viable cells (n=5, p<0.05). We directly measured proliferation by 3H-thymidine incorporation and found a 40% reduction with tcf19 knockdown within a group of cell cycle genes whose expression correlates with adaptive islet proliferation. In confirmation of these microarray data, we observed 3.3-fold upregulation of tcf19 mRNA in islets from obese non-diabetic C57Bl/6J (B6) mice vs. lean (n=5, p<0.01). This obesity-driven upregulation was not observed in islets from the diabetic BTBR strain. A possible explanation for this expression difference is a non-coding single nucleotide polymorphism we identified between the B6 and BTBR strains within the 5’UTR of exon 1 of tcf19. To examine the tissue distribution of tcf19 expression, we measured tcf19 mRNA levels across a panel of 13 lean B6 tissues and in obese B6 islet. Tcf19 is most highly expressed in mouse islet. Specifically, we observe tcf19 expression in mouse β-cell nuclei by immunostaining. In addition, in BioGPS human expression profiling, Tcf19 is most highly expressed in pancreas. This is an unusual pattern of expression for a cell cycle regulator since numerous tissues have higher basal proliferation rates and suggests that tcf19 may play a more specific role in cell cycle regulation in the islet.

A VGF-Derived Peptide Attenuates Development of Type 2 Diabetes Via Specific Effects on Islet β-Cell Mass and Function

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Islet β-cell mass is a major underlying determinant in the onset of type 2 diabetes (T2D). The progressive decline in islet mass coupled with increasing insulin resistance ultimately results in an inappropriate insulin response to meet metabolic demand. We previously reported that overexpression of the homeodomain transcription factor Nkx6.1 can both enhance islet secretory function and increase islet cell replication. In the current study, we have identified a downstream target of Nkx6.1 as the prohormone VGF (non-acyclic; unrelated to VEGF), which has been previously linked to hypothalamic regulation of energy expenditure. Here we show that acute addition of a VGF peptide potentiates GSIS by 30-35% in isolated rat islets. In Wistar rats, a glucose challenge following pre-administration of the VGF peptide resulted in a significant improvement in glycemic control that correlated with a preservation of islet β-cell mass and a reduction in β-cell death. In addition, we show that the VGF peptide is comparable to the GLP-1 mimetic, exendin-4, in its ability to enhance stimulus-coupled Akt signaling and prevent islet cell apoptosis in vitro. These studies identify a VGF peptide as a potential novel therapy for the treatment of T2D.

Human Pancreatic Islets from Diabetic Subjects Recover Function after Treatment with a Glucokinase Activator

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The health of the pancreatic beta cell (β cell) is compromised in type 2 diabetes (T2D) and persistent deterioration of β cell function accelerates the progression of this disease. In subjects with T2D, the β cells lose first phase insulin release and experience a shift in the threshold for glucose stimulated insulin secretion to higher glucose concentrations than seen in normal subjects. This in conjunction with relative insulin deficiency and decreased insulin sensitivity leads to hyperglycemia. The same pattern of defects is seen with ex vivo stimulation of pancreatic islets isolated from T2D subjects (diabetic islets). Here we tested the hypothesis that glucose phosphorylation is impaired in diabetic islets and this contributes to the loss of glucose sensitivity. To address this, diabetic islets were treated ex vivo with an activator of glucokinase, the enzyme responsible for the conversion of glucose to glucose-6-phosphate, and dynamic measurements of insulin secretion were used as an assessment of β cell function. Here we show that diabetic islets stimulated with glucose in the presence of a glucokinase activator (GKa) recover first phase insulin release and secrete more insulin than those stimulated with glucose alone. Significantly, treatment of diabetic islets with a GKa was able to restore sensitivity to lower glucose concentrations and, in fact, they secreted insulin at glucose concentrations comparable to islets from normal subjects. Interestingly, preliminary results also indicate that pretreatment of diabetic islets with a GKa for 48 hrs resulted in a sustained improvement in glucose stimulated insulin secretion even after the activator was removed from the system. These findings support the use of a GKa for the treatment of T2D by suggesting that a GKa will restore β cell sensitivity to glucose and potentially recover first phase insulin secretion. In summary, a decreased glucose phosphorylation rate is suggested to play a role in the insensitivity of the diabetic β cell to glucose and this can be reversed with a glucokinase activator.

Metabolomic Analysis of Pancreatic β-Cell Insulin Release in Response to Glucose

MEI HUANG, JAMIE W. JOSEPH, Kitchener, ON, Canada

Defining the key metabolic pathways that are important for fuel-regulated insulin secretion is critical to providing a complete picture of how nutrients regulate insulin secretion. We have performed a detailed metabolomics study of the clonal β-cell line 832/13 cells and rat islets using a gas chromatography-mass spectrometer (GC-MS) to investigate potential coupling factors that link metabolic pathways to insulin secretion. Mid-polar and polar metabolites, extracted from 832/13 β-cell line and rat islet, were derivatized followed by identification and quantification. 454 out of 527 mass spectra were identified by our metabolomic platform belonging to more than 30 metabolic pathways. These identified metabolites allowed us to perform a systematic analysis of key pathways involved in glucose-stimulated insulin secretion (GSIS). Of these metabolites, 33 were consistently identified in all experiments as biomarker for GSIS by partial least-squares discriminant analysis. 16 out of 33 are from common metabolic pathways including glycolytic, polyol, pentose phosphate pathway, and the TCA cycle suggesting these pathways play an important role in GSIS. Lipogenesis was strongly associated with GSIS since several precursors, glycerol, glycerol-3-phosphate, glycerol 2-phosphate, malonic acid, acetyl-CoA, and palmitate were shown to contribute to the clustering of high glucose sample groups. Alanine and serine are up-regulated by glucose whereas aspartate and proline were down-regulated by glucose suggesting they might act as important coupling factors regulating insulin release. In summary, a coordinated signalling cascade elicited by glucose metabolism in pancreatic β-cells is revealed by our GC-MS based metabolomics platform. This integrating machinery might provide a new conceptual framework for future research and/or drug discovery.
in the endocrine pancreas, and demonstrated that PTP1B directly regulates substrate of PTP1B. Together, our study identified a novel role for PTP1B was disrupted by pervanadate treatment, suggesting that EphA5 is a phosphorylation. We further demonstrate that EphA5 could form a complex of EphA5, and reconstitution of PTP1B into KD cells restored EphA5 tyrosyl phosphorylation. In MIN6 cells, PTP1B knockdown (KD) led to attenuated GSIS while PTP1B reconstitution restored GSIS in knockdown cells. Notably, basal and glucose-stimulated EphA5 tyrosyl phosphorylation was enhanced in PTP1B deficient islets, suggesting that PTP1B deficiency enhanced EphA5 forward signaling to attenuate GSIS in pancreas-specific PTP1B KO mice. In line with this observation PTP1B KD cells also exhibited enhanced tyrosyl phosphorylation of EphA5, and reconstitution of PTP1B into KD cells restored EphA5 tyrosyl phosphorylation. We further demonstrate that EphA5 could form a complex with PTP1B "substrate-trapping" PTP1B D181A mutant in MIN6 cells, which was disrupted by pervanadate treatment, suggesting that EphA5 is a substrate of PTP1B. Together, our study identified a novel role for PTP1B in the endocrine pancreas, and demonstrated that PTP1B directly regulates insulin secretion in β cells, at least in large part, through EphA5.

Characterization of Melatonin Receptor Mutant Mice

CECILIA NAGORNÝ, HEDVIG BENNET, MALIN FEX, NILS WIERUP, HINDRIK MULDER, Malmö, Sweden

Several independent studies have now confirmed our initial finding that the melatonin receptor 1B (MTNR1B or MT1) is associated with an increase in fasting plasma glucose over time, impaired early insulin secretion as well as Type 2 Diabetes Mellitus (T2DM). Both melatonin receptors are expressed in human islets, and given the increased expression of MTNR1B in islets from individuals at risk of T2DM and a presumed insulinostatic effect of the hormone, the pharmacological effects are likely started directly on β-cells and α-cells.

In vitro findings show a direct inhibitory effect of melatonin on insulin secretion from cloned β-cells. It remains unsolved which receptor mediates this effect. To further elucidate this, we were kindly provided with murine null mutants for MTNR1A (MT1), 1B (MT2), and both receptors (MT1/2) from David Weaver (UMASS). We observed an increased weight in MT2 null mice compared to wild type (WT) (WT: 33.84 ± 0.77g; MT2: 39.17 ± 1.23g (p = 0.001)). Immunohistochemical analyses showed distinct locations of the two receptors in islets. MT2 receptors were located in β-cells, while MT1 receptors were expressed in α-cells. An oral glucose tolerance test (OGTT) revealed improved glucose tolerance in MT2 null mice compared to WT (Area under the curve (AUC): WT 1322 ± 80 arbitrary units (AU); MT2 1046 ± 332 AU (p=0.017)). MT2 mice were less insulin-sensitive as demonstrated by a lesser drop in glucose levels during an insulin tolerance test (ITT) (AUC: WT 284 ±14 AU, MT2 339 ± 45 AU (p = 0.035)). In vitro batch incubations of isolated islets from MT2 null mice showed enhanced insulin secretion upon stimulation with 15mM glucose (WT 0.31 ± 0.12 ng/islet/hr, MT2 0.45 ± 0.10 ng/islet/hr).

Our data thus show differences in whole body metabolic regulation in MT2 mice, since they are significantly heavier than WT, MT1, and MT2/2. The immunohistochemical localization of the MT2 receptor to the islet β-cells suggests that our in vitro findings, where melatonin has a direct inhibitory effect on insulin secretion, are mainly mediated via the MT2 receptor. Also, our batch incubations and OGTT in the MT2 mice support a role for melatonin signaling in CA-mediated insulin secretion, thus implicating the intrinsic pathway.

Role of Oxidative Stress-Mediated ATF3 in the Progression of Type 2 Diabetes in Ethanol-Fed OLETF Rats: GCK and SIRT1 Down-regulation

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As like in our previous report (JBC,2010,285:37251-62), alcohol drinking is well known as an independent risk factor for the progression of type 2 diabetes through metabolic alteration; however, there is still a great deal of controversy concerning the relationship between alcohol drinking and development of diabetes. Here, we investigated the effects of alcohol consumption and exact molecular mechanisms involved in the development of type 2 diabetes. To this, we have used 10-week OLETF rats, a spontaneous type 2 diabetes model, and non-diabetic LEOt rats, which were fed for 6 weeks with ethanol-containing liquid diet. 40% of ethanol-fed OLETF rats develop to type 2 diabetes with hyperglycemia and hyperlipidemia, but not in pair-fed rats. Islet cell mass was enlarged in ethanol-fed rats, but the ability of insulin synthesis was significantly decreased, correlated with GCK downregulation and induction of activating transcriptional factor 3(ATF3), a stress-inducible transcription factor. Especially, C-terminal domain of ATF3 directly inhibits the transcriptional activity of GCK by increasing ATF3 acetylation through downregulation of SIRT1, a protein deacetylase, in chronic ethanol-treated cells, which were attenuated by ATF3 siRNA and D/NATF3. Furthermore, ATF3-mediated GCK downregulation and pancreatic β-cell dysfunction in ethanol-treated cells were strongly prevented by wild-type SIRT1 and its activator resveratrol. ATF3-mediated SIRT1 downregulation was also associated with the increase of oxidative stress by reducing the nuclear factor E2-related factor2(Nrf2)-mediated antioxidant H.O-1 and Ppx-1, results in the increase of GCK tyrosine nitration. The susceptibility of nitrated GCK into ubiquitination was decreased by CA-NR12 and CA-SIRT1. Consequently, we demonstrated that alcohol drinking may accelerate the development of type 2 diabetes by oxidative stress-induced ATF3. Furthermore, ATF3-mediated the downregulation of SIRT1 and Nr1-2/HO-1 might play as novel and efficient modulators of alteration of glucose metabolism and insulin synthesis via GCK downregulation in pancreatic β-cells.

For author disclosure information, see page LB42.
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Reiss, Chandler ............................................... Disclosed no conflict of interest
Reusch, Jane E R .............................................. Disclosed no conflict of interest
Revers, Marian J ............................................... Disclosed no conflict of interest
Reynolds, Rebecca M ....................................... Disclosed no conflict of interest
Rissane, Aila .................................................. Advisory Panel: Boehringer Ingelheim Pharmaceuticals, Inc., Novartis Pharmaceuticals Corporation, Novo Nordisk A/S
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