COMPLICATIONS—HYPOGLYCEMIA

1-LB
Peripheral GABA Infusion in Diabetic Rats Enhances the Glucagon Counterregulation and Protects Against Insulin Hypoglycemia
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Glucagon counterregulation (GCR) is often defective in type 1 diabetes (T1DM). Our animal, clinical, and modeling studies suggest that hyperglucagonemia contributes to the GCR impairment and alpha-cell inhibitors (ACI) may be used to improve GCR. This study further supports this hypothesis by showing that constant peripheral GABA infusion enhances GCR and protects against hypoglycemia. Blood glucose (BG) and glucagon hypoglycemia responses were tested in 2 groups of conscious STZ-treated male Wistar rats. BG was lowered to ~150 mg/dL after which a constant (50U/min) jugular iv infusion of saline (N=7) or 4mg/mL of GABA (N=9) started (t=0 min). At t=0 min, a 12U/kg iv insulin bolus was given. Blood sampling was done every 10 min for BG and glucagon from t=10 to 80 min. GCR was estimated via the product R=\{mean of 2 lowest BG values from t=10 to 40 min\} x {mean glucagon from t=-10 to 80 min}. GABA treated group was also better protected against hypoglycemia assessed by the two lowest BG values after the insulin bolus: 68±6.5 mg/dL vs. 57±9.5 mg/dL; p=0.02. These results are predicted by our mathematical GCR model in which reduction of basal glucagon by ACI enhances the defective GCR. They differ from prior animal work where ACI were given intrapancreatically and which reduction of basal glucagon by ACI enhances the defective GCR. They were 72% higher in the GABA group: 3159±900.5 vs. 2273±690.4, p=0.04. The GABA treated group was also better protected against hypoglycemia assessed by the two lowest BG values after the insulin bolus: 68±6.5 mg/dL vs. 57±9.5 mg/dL; p=0.02. These results are predicted by our mathematical GCR model in which reduction of basal glucagon by ACI enhances the defective GCR. They differ from prior animal work where ACI were given intrapancreatically and switched off at hypoglycemia. Thus, for the first time GCR enhancement is achieved by peripheral ACI infusion without a switch-off or other manipulation. Such treatment could lead to novel strategies for glycemia control in T1DM with enhanced protection against hypoglycemia.

Supported by: NIH (R01DK082805)

2-LB
Lower Risk of Hypoglycemia in Elderly Type 2 Diabetes Patients when Linagliptin is Added to Basal Insulin: An Exploratory Analysis
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Elderly T2DM patients (pts) with long-standing disease often require insulin (INS) therapy, yet hypoglycemia is a major concern. It has recently been shown that linagliptin (LINA) added to stable basal INS in elderly T2DM pts reduced HbA1c by -0.77% vs. placebo (PBO), notably with less hypoglycemia. Here we further explore hypoglycemia risk in these pts (n=247; mean±SD age 74±4 yrs, HbA1c 8.2±0.8%) on basal INS (baseline [BL] dose 36±25 U/day) from two phase 3 studies of 24 and ≥52 weeks. Odds ratios (OR) for overall and confirmed hypoglycemia (blood glucose ≤70 mg/dL) were assessed (INS doses did not change notably). Overall (~37%) and confirmed (~34%) hypoglycemia risk was lower with LINA than PBO (OR 0.63 [95% CI 0.37-1.10] [Fig] and 0.66 [0.36-1.21], respectively). Significantly less (~59%) confirmed hypoglycemia was found in LINA pts with mild-moderate BL hyperglycemia (HbA1c 7.5-9.0%, OR 0.41 [0.21-0.84]; p=0.014). Similar directional trend in hypoglycemia risk with LINA vs. PBO was also observed in pts with BL HbA1c <7.5% (overall OR 0.77) and subgroups for glargine, detemir or NPH (overall OR 0.74, 0.59, 0.49, respectively). Despite significantly reduced HbA1c and no relevant on-trial INS dose reductions, adding LINA to basal INS appears to decrease hypoglycemia risk. This trend is in stark contrast to other oral agents when combined with INS. The biological underpinnings of this phenomenon are unclear but deserve further study.

Supported by: Norwegian Diabetes Association

3-LB
The Effects of Diabetes Duration on Hypoglycemia Symptom Intensity and Prevalence of Impaired Awareness of Hypoglycemia
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Diabetes duration influences hypoglycemia symptom profile and prevalence of impaired awareness of hypoglycemia (IAH); viz. a diminished ability to perceive onset of hypoglycemia. By questionnaire, hypoglycemia symptoms and prevalence of IAH were assessed in an outpatient population with type 1 diabetes. Symptom presence and intensity were measured by the Edinburgh Hypoglycaemia Scale, using a Likert scale of 1 to 7. Hypoglycemia awareness was assessed by the method of Gold et al., based on the question “do you know when your hypos are commencing?”, using a scale from 1 to 7 (1 = always aware, 7 = never aware; ≥ 4 = IAH, < 4 = normal awareness [NAH]). The response rate was 70% (445/636). IAH was present in 17% (CI: 14-21%). With progressive diabetes duration, the prevalence of IAH increased (from 3% for duration 2-9 years to 8% for duration ≤30 years, p for trend < 0.001), the mean intensity of autonomic (A) symptoms declined (p for trend < 0.001) (Fig.1), the intensity of trembling and hunger decreased (p < 0.001 and p = 0.004, respectively), while the mean intensity of neuroglycopenic (NG) symptoms did not change (p = 0.55). The mean (SD) ratio of NG/A symptoms was higher in IAH than in NAH subjects (1.16 (0.43) vs. 1.01 (0.33), p = 0.001). In conclusion, with progressive diabetes duration, the prevalence of IAH rises and the intensity of autonomic symptoms, particularly trembling, declines. Neuroglycopenic symptoms predominated in those with IAH.

Supported by: Boehringer Ingelheim Pharmaceuticals, Inc.
COMPLICATIONS—MACROVASCULAR—ATHEROSCLEROTIC CARDIOVASCULAR DISEASE AND HUMAN DIABETES

4-LB

Limiting Amylin Aggregation Protects the Heart in Diabetes
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Recent data revealed that the islet amyloid polypeptide (IAPP; amylin), an amyloidogenic protein making up the pancreatic amyloid in type-2 diabetes mellitus (T2DM), also accumulates in failing hearts from obese or T2DM patients. Cardiac deposition of amylin accelerates diabetic heart failure in a T2DM rat model transgenic for human amylin (the HIP rat). In this study, we assessed the attachment/incorporation of oligomerized amylin to cardiac cells in humans and tested the ability of pro-fibroinolytic eicosanoids to reduce amylin deposition and its deleterious cardiac effects in HIP rats. Oligomerized amylin was identified within coronary arteries, cardiac myocytes and atherosclerotic lesions in failing hearts from diabetic humans, but not in control hearts. Intriguingly, significant amylin deposition was found in cardiac cells from patients that developed T2DM post-transplantation, we treated animals in pre-diabetic that amylin builds up in the heart and may affect myocardial structure and function even in pre-diabetes. To elevate the blood level of eicosanoids and block cardiac amylin deposition in HIP rats, we treated animals in pre-diabetic state with an inhibitor of soluble epoxide hydrolase, the enzyme that degrades endogenous eicosanoids. Treatment doubled the blood concentration of pro-fibroinolytic eicosanoids, which drastically limited the attachment/incorporation of oligomerized amylin to cardiac myocytes. Animals in the treated group displayed reduced cardiac hypertrophy and left-ventricular dilatation. We show that possible cardioprotective effects include limiting amylin-induced cardiac oxidative stress and myocyte Ca<sup>2+</sup> cycling alteration. The present studies point to cardiac amylin accumulation as a novel therapeutic target in diabetic heart disease and elevating the blood level of pro-fibroinolytic molecules as a pharmacological strategy to reduce amylin deposition and amylin-mediated cardiotoxicity.

Supported by: NSF-CBET 1133339

5-LB

Blood Pressure and Vascular Function in Sprague-Dawley Rats With Insulin-Treated Type I Diabetes Mellitus
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The purpose of this study was to determine if insulin-treated type I diabetes mellitus (T1DM) altered conscious resting blood pressure (BP) through a NO mechanism in Sprague-Dawley rats. Rats were divided into 2 groups, control (C) and T1DM (daily streptozotocin injections of 20 mg/kg for 5 days); followed by a subcutaneous insulin pellet implant: 1 IU/12 h; fed state blood glucose ~16 mmol/L). Resting conscious BP and the BP response to L-NG-Monomethylarginine (L-NMMA; 30 mg/kg) infusion was measured at week 1 and week 10 following insulin therapy. At week 11, rats were anaesthetized, the right jugular vein was cannulated for acetylcholine (ACh; 25 ug/kg) and prazosin (PRZ: 85 ug/kg) infusions and the right carotid artery was cannulated for nitroglycerin (~16 µg). In C group, the levels of 24h urinary volume, 24h UMA, BUN, TG, LKW/BWT and kidney nitrogen (BUN), Triglyceride (TG), 24h urinary microalbumin (UMA) and kidney hypertrophy index (LK/W/BWT) were determined at week 12. In T1DM group, the levels of 24h urinary volume, 24h UMA, BUN, TG, LKW/BWT and kidney nitrogen (BUN), Triglyceride (TG), 24h urinary microalbumin (UMA) and kidney hypertrophy index (LK/W/BWT) were determined at week 12. All the indicators related to renal function such as blood urea nitrogen (BUN), Triglyceride (TG), 24h urinary microalbumin (UMA) and kidney hypertrophy index (LK/W/BWT) were determined in rats sacrificed after 8 weeks of experiment. The 24 hour urine of all the rats were collected one day before they died. All the nephridial tissues underwent hematoxylin and eosin stain to observe pathological morphology of nephridial tissues. The protein levels of TGF-β1 and c-Ski were determined by immunohistochemistry. Compared with NC group, the levels of 24h urinary volume, 24h UMA, BUN, TG, LKW/BWT and TGF-β1 were significantly higher (P<0.01), while c-Ski was significantly lower in the DM group(P<0.01). After treatment with Pioglitazone, all the related biochemical data and TGF-β1 were determined decreased (P<0.05), while c-Ski was higher (P<0.01). In conclusion, Pioglitazone could significantly up-regulate the protein level of c-Ski and inhibit TGF-β1 in kidney tissue of diabetic rats, which may play an important role in ameliorating the process of diabetic nephropathy.

COMPLICATIONS—NEPHROPATHY—BASIC AND EXPERIMENTAL SCIENCE

7-LB

The Regulation of PPAR-γ on TGF-β1 and c-Ski in Kidney Tissue of Diabetic Rats
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TGF-β1 is the critical cytokine of glomerulosclerosis and renal interstitial fibrosis. As the repressor of TGF-β1/Smad pathway, c-Ski could inhibit the Smad compound activating the transcription of its downstream target gene. In this study, the diabetic models were induced with streptozotocin, and some of them were treated with Pioglitazone. We observed the expressions of c-Ski and TGF-β1 in renal tissue of diabetic rats, to investigate their relationship with diabetic nephropathy and the effect of PPAR-γ on TGF-β1 and c-Ski. All the SD rats were randomized into normal control group (NC), diabetes group (DM) and treatment group (PT). The body mass weight was measured every week, and the level of blood glucose was measured every two weeks. All the indicators related to renal function such as blood urea nitrogen (BUN), Triglyceride (TG), 24h urinary microalbumin (UMA) and kidney hypertrophy index (LK/W/BWT) were determined in rats sacrificed after 8 weeks of experiment. The 24 hour urine of all the rats were collected one day before they died. All the nephridial tissues underwent hematoxylin and eosin stain to observe pathological morphology of nephridial tissues. The protein levels of TGF-β1 and c-Ski were determined by immunohistochemistry. Compared with NC group, the levels of 24h urinary volume, 24h UMA, BUN, TG, LKW/BWT and TGF-β1 were significantly higher (P<0.01), while c-Ski was significantly lower in the DM group(P<0.01). After treatment with Pioglitazone, all the related biochemical data and TGF-β1 were determined decreased (P<0.05), while c-Ski was higher (P<0.01). In conclusion, Pioglitazone could significantly up-regulate the protein level of c-Ski and inhibit TGF-β1 in kidney tissue of diabetic rats, which may play an important role in ameliorating the process of diabetic nephropathy.
COMPLICATIONS—Nephropathy—Clinical and Translational Research

8-LB
Blood Pressure and Pulse Pressure Effects on Renal Outcomes in the Veterans Affairs Diabetes Trial (VADT)
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The Veterans Affairs Diabetes Trial (VADT) was a prospective, randomized study of 7191 veterans with T2DM. The primary goal was to determine whether intensive glucose control prevented major cardiovascular disease events. Our current objective was to determine whether on study systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP) affected renal outcomes measured as albumin creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) evaluated by time-varying covariates survival analyses and hazard ratios (HR) for worsening of renal outcomes.

Compared with SBP ≥140 mmHg, the risk of ACR worsening increased significantly for SBP 130-139 mmHg (HR=1.879; 95% CI=1.276-2.769; P=0.001), and for SBP ≥140 mmHg (HR=2.506; CI=1.663-3.776; P<0.0001). A1c as a time-varying covariate also increased risk of ACR worsening (HR=1.194; CI=1.089-1.309; P=0.0002). Compared with a PP range of 40-49 mmHg, PP>40 significantly lowered risk of worsening ACR (HR=0.364; CI=0.193-0.685; P=0.022), and PP<40 significantly increased risk of worsening ACR (HR=2.362; CI=1.579-3.592; P<0.0001). Analyses of categorical BP ranges associated with eGFR worsening showed a significant interaction between patients with SBP>140 mmHg and A1c. Compared with the SBP 105-129 mmHg group, patients with SBP≥140 mmHg were 15% more likely to experience eGFR worsening (HR=1.149; 95% CI=1.003-1.317; P=0.045) for each 1% A1c increase. We conclude that SBP ≥140 mmHg, higher A1c and PP<40 were associated with worsening ACR. FF<40 showed a lower risk for worsening ACR. The results suggest that treatment of SBP to <130 mmHg may lessen ACR worsening. In light of the interaction effect between SBP>140 mmHg and A1c, our results suggest that the effect of glycemic control on reducing progression of renal disease may be even greater in hypertensive patients.

Supported by: U.S. Dept. of Veterans Affairs

COMPLICATIONS—Neuropathy

9-LB
Effect of Aerobic Exercise on Quality of Life of Individuals With Diabetic Peripheral Neuropathy: A Single Blind, Randomized Controlled Trial
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The objective of the study was to evaluate the effect of moderate intensity (Heart Rate Reserve 40-60%) exercise on Neuropathy Quality of life (NQOL) in type 2 diabetes. The study was a parallel-group, randomized clinical trial performed in a tertiary setting. People with type 2 diabetes with peripheral neuropathy: A single Blind, randomized controlled trial (VADT) was a prospective, randomized study of 7191 veterans with T2DM. The primary goal was to determine whether intensive glucose control prevented major cardiovascular disease events. Our current objective was to determine whether on study systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP) affected renal outcomes measured as albumin creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) evaluated by time-varying covariates survival analyses and hazard ratios (HR) for worsening of renal outcomes.

Compared with SBP 105-129 mmHg, the risk of ACR worsening increased significantly for SBP 130-139 mmHg (HR=1.879; 95% CI=1.276-2.769; P=0.001), and for SBP ≥140 mmHg (HR=2.506; CI=1.663-3.776; P<0.0001). A1c as a time-varying covariate also increased risk of ACR worsening (HR=1.194; CI=1.089-1.309; P=0.0002). Compared with a PP range of 40-49 mmHg, PP>40 significantly lowered risk of worsening ACR (HR=0.364; CI=0.193-0.685; P=0.022), and PP<40 significantly increased risk of worsening ACR (HR=2.362; CI=1.579-3.592; P<0.0001). Analyses of categorical BP ranges associated with eGFR worsening showed a significant interaction between patients with SBP>140 mmHg and A1c. Compared with the SBP 105-129 mmHg group, patients with SBP≥140 mmHg were 15% more likely to experience eGFR worsening (HR=1.149; 95% CI=1.003-1.317; P=0.045) for each 1% A1c increase. We conclude that SBP ≥140 mmHg, higher A1c and PP<40 were associated with worsening ACR. FF<40 showed a lower risk for worsening ACR. The results suggest that treatment of SBP to <130 mmHg may lessen ACR worsening. In light of the interaction effect between SBP>140 mmHg and A1c, our results suggest that the effect of glycemic control on reducing progression of renal disease may be even greater in hypertensive patients.

Supported by: U.S. Dept. of Veterans Affairs

10-LB
Small Particle Diet Reduces Upper Gastrointestinal (GI) Symptoms in Patients With Diabetic Gastroparesis: A Randomized Controlled Trial
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Gastroparesis is a complication to diabetes mellitus (DM). The prevalence is suggested to be 30-65%. Dietary advice is considered to be of importance to reduce GI symptoms in patients with diabetic gastroparesis (GP), but no randomized controlled trials exist.

Our aim was to compare GI symptoms in insulin treated DM subjects with GP eating a food with small particle size ("intervention diet"), compared with the recommended diet for DM ("control diet"). We randomized 56 subjects with insulin treated DM and GP (mean 53.3 ± 11.6 years age; 35 females), determined with scintigraphy, to the intervention diet or to the control diet for 20 weeks. The patients met a dietitian at 7 occasions during the study. GI symptom severity was assessed with a validated questionnaire, (PAGI-SYM), BMI and Hba1c were followed. A significantly greater reduction of nausea/vomiting, postprandial fullness, bloating, and regurgitation/heartburn were seen in patients who received the intervention diet compared with the control diet, but not for abdominal pain (see table). No differences in BMI and HbA1c were seen between the groups. The author’s conclusion: A dietary treatment with small particle size significantly improves the key symptoms of gastroparesis in patients with diabetes mellitus.

For author disclosure information, see page LB66.
11-LB

Intermittent Fasting (IF) at Night Protects from Diabetic Microvascular Complications in db/db Mice

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Previously, we identified that diabetic bone marrow (BM) neuropathy precedes diabetic retinopathy (DR) (Busik et al. 2003) and circadian dysregulation of vascular progenitors (VP) profoundly contributes to retinal vascular dysfunction. This study explored whether correction of diurnal dysfunction and restoration of VP function is a strategy for prevention microvascular complications.

db/db and age match control (db/m) mice were maintained under a 12h:12h light:dark cycle for six months starting at 4 month of age with either normal feeding or with IF, initiated at night time every other day with food (normal chow) introduced 5–30min before lights were off on the first day and removed 5–30min before the lights were off on the second day. At study end, mice were euthanized every 4 hrs for 48hrs. Glycerated hemoglobin (GlyH), VP enumeration in BM and blood (by flow cytometry), VP migration (by QCM Chemosatxis Asssay) and circadian clock gene expression, BMAA and PER2, (by RT-PCR), NF200 staining in BM for neuropathy assessment and enumeration of acellular capillaries was performed.

In db/db mice under ad libitum feeding marked dysfunction was observed: i) loss of diurnal oscillation of BMAL and PER2 mRNA expression in BM VP; ii) increased BM VP numbers iii) reduced VP migration; and iv) DR and DN development. Without changing levels of GlyH, IF initiated at night time increased the survival rate in db/db mice, corrected diurnal disruption of VP release from BM and diurnal oscillation of BMAL and PER2 gene expression, restored VP migration to normal nondiabetic levels. If of db/db mice prevented development of diabetic neuropathy as assessed by NF200 staining in BM and DR as assessed by the number of acellular capillaries.

Our results suggest that by simply changing the timing of food consumption, circadian dysregulation can be corrected and diabetic microvascular complications prevented without improving GlyH levels.

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12-LB

Histone Methyltransferase EZH2 Regulates Glucose Induced VEGF Production through H3K27 Methylation in Retinal Endothelial Cells

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Glucose induced augmented vascular endothelial growth factor (VEGF) production is a key event in diabetic retinopathy. We have previously demonstrated that downregulation of miR-200b causes overexpression of VEGF, mediating structural and functional changes in the retina in diabetes (Diabetes 60:1314,2011). However, regulation of miR-200b is not known. Histone methyltransferase, enhancer of the zeste homolog (EZH2), has been demonstrated to repress miRNAs in neoplastic process. We hypothesized that, in diabetes, EZH2 represses miR-200b through its H3K27 methylation mark.

Human endothelial cells of dermal origin, isolated from both type 1 and type 2 diabetic and non-diabetic individuals, and retinal microvascular endothelial cells were treated in high glucose (25mM) or normal glucose (5mM) for 24 hours. Expression of EZH2, VEGF and various miRNA were measured by qPCR. Loss-of-function experiments were also performed using a chemical inhibitor for EZH2, 3-Daezanepinacolin A (DN2ep). When treated with high glucose, all cell types showed significantly increased VEGF expression. Retinal endothelial cells showed increased expression of EZH2 with decreased expression of miR-200b. Dermal endothelial cells isolated from diabetic patients showed increased EZH2 and decreased miR-200b expression as well. Furthermore, inhibition of EZH2 in retinal endothelial cells produced increased miR-200b expression with parallel decreased VEGF, demonstrating a causal link.

This research has demonstrated a repressive relationship between EZH2 and miR-200b. These data further provide evidence of a novel mechanism of miRNA regulation through another epigenetic pathway, i.e., histone methylation. Understanding such pathways will potentially yield new treatment strategies.

Supported by: CDA, CIHR

13-LB

Relationship of Visceral and Subcutaneous Adiposity With the Severity of DM Retinopathy in People With Type 2 Diabetes Mellitus

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Our study was performed to determine whether Visceral adiposity (VAT) or subcutaneous adiposity (SAT) was associated with the severity of DM retinopathy in people with type 2 DM. Nine hundred and twenty-nine people with type 2 DM and who had undergone abdominal computed tomography assessment of the VAT and SAT areas were included, the severity of DM retinopathy graded to 9 categories/no retinopathy, microaneurysms only, mild NPOD, mild NPOD, severe NPOD, very severe NPOD, PDR without HRC, PDR with HRC, advanced PDR). VAT was positively associated with the severity of DM retinopathy after adjustment for the clinical variables (P-coefficient =0.085, P = 0.034), while SAT was not significantly associated with the severity of DM retinopathy. When stratifying the individuals by BMI groups, VAT was positively associated with severity of DM retinopathy in the overweight and obese subjects after adjustment for the clinical variables, while there was no significant association between the VAT and the severity of DM retinopathy in the normal weight subjects. SAT was not significantly associated with severity of DM retinopathy in the normal weight, overweight and obese subjects. Our data suggest that VAT may be an additional prognostic factor for the severity of DM retinopathy especially in the overweight or obese subjects with type 2 DM.

14-LB

The Association of HbA1c (Long-Term Hyperglycemia) With the Risk of Pulmonary Embolism (PE)

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Background: Diabetes mellitus is a well-known pro-thrombotic condition. Hyperglycemia is associated with arterial thrombosis, and also has shown increased risk of venous thrombosis. The objective of this study is to evaluate the association of long-term hyperglycemia with the risk of pulmonary embolism.

Methods: We conducted a retrospective, case-control study that reviewed patients who were admitted to Albert Einstein Medical Center from 2005 to 2011. The case group was 140 patients with confirmed pulmonary embolism by diagnostic study (positive CT chest w/ contrast or high probability with V/Q scan) during the period. Controls were selected age-, sex-matched, in a 1:2 ratio from individuals who had negative diagnostic studies for PE during the same period. Those who had Hba1c values measured during the admission were included. Patients who were on anticoagulation at the time of admission for any reason were excluded. Logistic regression was used for statistical analysis.

Results: The mean age of the study population was 64. 60% of the patients were women, and 71% were African American. The prevalence of diabetes was not different in two groups (85.0% vs. 72.3%; p=0.125). However, Hba1c was statistically significantly higher in the case group than in the control group (7.53% vs. 7.09%; p=0.012). The unadjusted Odds ratio was PE with respect to Hba1c was 1.16 (CI 95%, 1.03-1.30). Multi-logistic regression adjusted for demographics and known risk factors for PE such as immobilization, recent surgery, malignancy and history of venous thromboembolism also yielded odds ratio of 1.28 (CI 95%, 1.09-1.50). When stratified by status of diabetes, the adjusted odds ratio was 1.06 (CI 95%, 0.76-1.49) in non-diabetes and 1.38(CI 95%, 1.17-1.62) in diabetes.

Conclusion: The study suggests that long-term hyperglycemia, particularly those in diabetes, is associated with a significant risk of pulmonary embolism.

15-LB

Hyaluronic Acid in Type 2 Diabetic Patients

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Background: Our previous studies have demonstrated that diabetes-induced oxidative stress can alter the homeostasis of retinal nerve growth factor (NGF) resulting in accumulation of its precursor, proNGF at the expense of NGF. This imbalance coincided with retinal damage in experimental diabetes. Here we test the hypothesis that alteration of NGF and proNGF levels observed in retina and ocular fluids will be mirrored in experimental and clinical diabetes. Methods: Blood and vitreous samples were collected from patients undergoing vitrectomy at Georgia Reagents University under approved IRB. Samples included patients with diabetic retinopathy and non-diabetic (controls). Western Blot analysis was performed on serum samples collected
from diabetic and non-diabetic patients as well as samples (retina and serum) collected from C57Bl/6 mice that were kept diabetic for 5- weeks using STZ-model. Results: Diabetes significantly increased proNGF levels to 2.25 fold of the control levels in both retina and plasma of the same STZ-mice (n=4-5). NGF expression was markedly attenuated in diabetic mice to 50% and 60% in retina and plasma of the same animals, respectively. In human samples, vitreous and sera from diabetic patients showed 3-fold and 1.4-fold increase, respectively compared to non-diabetic (n=4-6). Vitreous and sera from diabetic patients showed significant 40% and 50% reduction in NGF levels, respectively when compared to non-diabetics. Conclusion: Our results showed that diabetes-induced expression of proNGF and impaired NGF expression was comparable between ocular fluid and serum. NGF plays an important role in improved wound healing, inflammatory responses, and preserving retinal function. Further characterization of the imbalance of proNGF to NGF ratio may facilitate its utility as an earlier biomarker for diabetic complication including diabetic retinopathy.

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16-LB
Plasma Biomarkers For Diabetic Retinopathy Discovered Using SOMAScan™
ALEX STEWART, BRITTA SINGER, ROBERT E. MEHLER, ANTONIO CIARDELLA, TIM BAUER, STEPHEN WILLIAMS, Boulder, CO, Denver, CO

A blood test that provides a timely and accurate diagnosis of diabetic retinopathy (DR), especially in the 50% of diabetic subjects not adherent to eye exams, could be used to drive preventive interventions. A prospective case-control study enrolled 88 diabetic subjects. Blood samples were collected just before an eye examination by an ophthalmologist. Thirty-five (35) subjects had DR and 53 had no evidence of DR. Of the subjects with DR, 5 had Grade 1, 12 Grade 2, 9 Grade 3, 5 Grade 4, and 4 Grade 5. The 88 plasma samples were run on SOMAScan™, SomaLogic’s SOMAmer-based proteomic assay that identifies and quantifies over a thousand proteins across approximately eight logs of concentration in small sample volumes. Sixteen proteins were significantly related to the presence of retinopathy, and were used to construct random forests classifiers to distinguish patients with DR from those with no DR. Peak performance was achieved by a 7-protein classifier which displayed sensitivity of 0.74, specificity of 0.89, and AUC of 0.92. For several proteins the concentration related to the stage of DR. The biologic classifications of the 7 proteins included inflammatory, neuron-derived, and cardiovascular. We are planning validation studies.

17-LB
Protecting Retinal Pigment Epithelium and the Outer Blood-Retinal Barrier: Role of X-Box Binding Protein 1
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Normal function of the retinal pigment epithelium (RPE) is essential for maintaining integrity of the outer blood-retinal barrier (BRB). Oxidative injury of the RPE resulting in disturbance in RPE tight junctions is implicated in diabetic retinopathy. The transcription factor NF-E2-related factor 2 (Nrf2) is a central regulator of cellular antioxidant responses. Enhancing Nrf2 activity protects RPE cells from oxidative injury, and recent studies suggest an important role of Nrf2 in regulation of epidermal barrier function. In the present study, we examined the role of X-box binding protein 1 (XBP1), an ER stress-inducible transcription factor, in regulation of Nrf2 and tight junctions in the RPE. Our results show that in RPE-specific XBP1 knockout (KO) mice, Nrf2 level in the RPE was significantly lower compared to wildtype (WT) mice. Expressions of Nrf2 target genes were also decreased in XBP1-deficient RPE. Confocal microscopy of RPE flatmount shows disturbed tight junctions between RPE cells in XBP1-deficient mice. In line with the in vivo findings, primary RPE cells isolated from XBP1 KO mice expressed less Nrf2 than those from WT mice. In cultured human RPE cells, overexpressing XBP1 increased Nrf2 level, while knockdown of XBP1 by siRNA or inhibiting XBP1 splicing resulted in a decrease in Nrf2 level. Conclusion: Our results suggest XBP1 is required for Nrf2 expression in RPE cells and that deficiency of XBP1 in RPE results in decreased anti-oxidant response contributes to oxidative injury and tight junction damage of the RPE.

Supported by: NIH (EY019949)

18-LB
Safe and Selective Small Molecule RxRα Agonist Modulates Glucose and Lipid Metabolism in ob/ob Mice
M.R. JAGANNATH, B.P. SOMESH, M.V. VENKATARANGANNA, O. ANUP, S. MANGUJKUMAR, D. ANILKUMAR, YOGANAND MOOLEMATH, R. MADHUSUDAN, ASHOK R. KUMAR, JAIDEEP SINGH, V. SUNIL, Bangalore, India

Retinoid X Receptor α (RXRα) regulates intracellular receptor signaling pathways involved in, among others, glucose and lipid metabolism and has potential to impact multiple risk factors associated with the metabolic syndrome. In the present study we investigated the effect of a potent and selective RXRα agonist CNX-013-B2 (30 mg/kg, p.o., BID for 4 weeks) in obese-hyperglycemic ob/ob mice. Treatment with CNX-013-B2 did not increase either food intake or body weight. In comparison with control ob/ob animals treatment with CNX-013-B2 resulted in a 22% reduction in fed glucose (196.30±3.16 Vs. 153±3.83 mg/dl), 16% in fasting serum triglycerides (141.8±58 Vs. 119±3.3 mg/dl), 20% free fatty acids, 14% fasting glycerol, 14% cholesterol and 26% LDL (low-density lipoprotein). In an oral glucose tolerance test a 19% decrease in glucose AUC was observed in the agonist treated animals indicating improvement in insulin sensitivity. After 4 weeks there was no significant change in weight of different depots of fat, kidney and pancreas. A non-significant increase in liver weight was observed in treated animals. In muscle expression of PDK4, SREBP1c, UCP3 and ABCA1 was significantly increased suggesting enhanced glucose and fat metabolism. In liver increased expression of SREBP1c, FASN and SCD1 suggested enhanced de novo lipogenesis. However the increase in liver triglyceride accumulation was non-significant (7%). Expression levels of Cyp7a1, bile acid transporters like MDR3, MRP4 and NTCP and cholesterol transport genes like ABCG5 suggest a robust modulation of cholesterol metabolism in treated animals. Gene expression profile in inguinal fat (PPARγ, UCP2, SREBP1c) indicates increased insulin sensitivity. Treatment of C57BL/6j/DIO mice on HFD with 100mg/kg for 5 weeks with CNX_013_B2 did not reduce serum levels of T3, T4 and TSH which indicates minimal impact on the HPT axis. CNX-013-B2 is a highly active and selective RXR agonist with good potential to provide glycemic and lipid control.

Supported by: NIH (EY019949)

DIABETIC DYSLLIPIDEMIA

For author disclosure information, see page LB66.
Acute and Chronic Complications

Debio 0930, a Novel Direct AMPK Activator, Improves Glycemic Control and Lipid Profile in Metabolic Disease Models

Patrick Muzzin, Maria Von Hoftey, Maryse Barber, Robert Lysek, Andreas Macalister, Lusanne, Switzerland

AMP-activated protein kinase (AMPK), a key cellular energy sensor, is a promising target for the treatment of metabolic disorders. This study describes the in vivo metabolic effects of a novel direct AMPK activator, Debio 0930, which is under preclinical development for type 2 diabetes.

Debio 0930, a small molecule, activated at least two recombinant human AMPK heterotrimers containing the B1 subunit in a submicromolar range (5-12 fold stimulation). In human HepG2 hepatocytes, Debio 0930 promoted AMPK activation without any changes in AMP/ATP ratio, supporting a direct mechanism of action. Debio 0930 was also found to have attractive DMPK properties with a favorable in vitro safety profile.

In vivo efficacy of the compound was examined in diet-induced obese (DIO) mice and dyslipidemic hamsters. Following 4-week oral repeat dosage in the DIO mice, Debio 0930 at 60 mg/kg BID reduced fasting plasma glucose and hepatic glucose production, and ameliorated insulin resistance (HOMA-IR). In addition, the treatment demonstrated marked improvement in liver lipid content (TG, Chol, FFA). In dyslipidemic hamsters, oral administration of Debio 0930 at 60 mg/kg for two weeks lowered fasting blood glucose and enhanced the HDL/LDL ratio. Plasma lipoprotein analysis demonstrated that Debio 0930 caused a significant reduction in VLDL and LDL and a substantial rise in HDL compared to vehicle treated animals. Food intake was not affected by Debio 0930 in either study.

In conclusion, Debio 0930 is a novel direct AMPK activator that improves both glycemic control and lipid profile, and potentially could be a new oral agent for the treatment of type 2 diabetes and dyslipidemia.

Foot care—lower extremities

Heat Shock Protein 70 (HSP70) Gene Polymorphism: A Risk for Diabetic Foot Amputation

Anil Kumar, S.K. Gupta, Ashok Kumar, S.K. Singh, Varanasi, India

Heat shock protein (HSP) has been identified playing role in repair of damaged tissue. It might have role in impaired diabetic wound healing also. Present study was designed to assess HSP gene polymorphism and it association with severity and prognosis of diabetic foot ulcer.

Venous blood was taken from 50 patients with diabetic foot. DNA was extracted and HSP A1B/ HSPA1L genes were amplified by PCR using specific primers. Following enzyme (restriction endonucleases PstI and NcoI ) digestion of the amplified fragments, RFLP analysis was done by gel electrophoresis.

HPS A1 B polymorphism was identified as AG in 82%, GG in 18% and GA in none of the patients with diabetic foot ulcer. HPS A1 L gene polymorphism was identified as TT in 80% , CT in 18 % and CC in 2 % of the patients. AG polymorphic variant HPS A1 L gene polymorphism was associated with severity of diabetic foot ulcer.

Table. NMR Fractions During Glycemic Stages (Values expressed as mean ± SD)

<table>
<thead>
<tr>
<th>NMR fraction</th>
<th>Euglycemia stage</th>
<th>Hyperglycemia stage</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMR protein, ng/mL</td>
<td>466.1 ± 39.4</td>
<td>436.1±31.9</td>
<td>0.0003</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>147.6 ± 24.5</td>
<td>135.7 ± 22.4</td>
<td>0.0004</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>77.6 ± 27.8</td>
<td>55.1 ± 20.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol (LDL-C), mg/dL</td>
<td>81.8 ± 19.1</td>
<td>79.4 ± 18.7</td>
<td>0.224</td>
</tr>
<tr>
<td>HDL cholesterol (HDL-C), mg/dL</td>
<td>49.9 ± 7.6</td>
<td>45.9 ± 7.6</td>
<td>0.0014</td>
</tr>
<tr>
<td>Total LDL particles (LDL-p), nm/L</td>
<td>902.4 ± 359</td>
<td>860.7 ± 334.0</td>
<td>0.192</td>
</tr>
<tr>
<td>LDLp size (nm)</td>
<td>213 ± 109</td>
<td>213 ± 106</td>
<td>0.681</td>
</tr>
<tr>
<td>Total small LDL particles (LDL-p), nm/L</td>
<td>801.0 ±245.1</td>
<td>931 ±350.2</td>
<td>0.772</td>
</tr>
<tr>
<td>Mean HDLp size (nm)</td>
<td>9.1 ± 0.48</td>
<td>9.1 ± 0.51</td>
<td>0.104</td>
</tr>
<tr>
<td>Total small HDL particles (HDL-p), µmol/L</td>
<td>30.4 ± 4.79</td>
<td>28.7 ± 4.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Large HDL pth(1,3)D, µmol/L</td>
<td>7.85 ± 2.67</td>
<td>7.17 ± 2.53</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Supported by: NIH (UL1TR000135)

For author disclosure information, see page LB66.
DIABETES EDUCATION

24-LB

Safe Use of Non Insulin Therapies e-Learning—Module Evaluation

JUNE R. JAMES, HEATHER STEPHENS, LOUISE RICHARDS, GRACE SWEENEY, HELEN WILKINSON, ANNA MORTON, Leicester, United Kingdom, Newcastle upon Tyne, United Kingdom, London, United Kingdom

A "safe use of non-insulin therapies" e-learning module aimed at reducing medication error by hospital and community staff launched in August 2012, was evaluated by learners in March 2013.

The aims were to review the impact of module completion on individuals’ confidence and practice. 2747 (31.3%) of 8813 had completed training by 1/3/2013. Those completing Sept – Nov 2012 were asked to complete an on-line survey comprising of 11 questions previously pilot tested. This tool combined both quantitative and qualitative data collection.

Data was collected from 191 (17.4%) staff, 65.4%, n=125 (nurses), 14.1% n=27 (doctors), 20.4%, n=39 others.

69.6%, n=133 participated for CPD, for 34% n=65, it was mandated, 16.2% n=31 were advised to complete by a manager. Other reasons (12.6% n=24) included; assessing for others, personal interest, compulsory element within another course.

Increased confidence with non-insulin therapies reported as a result of course completion were in:

• Managing (127/188, 67.5%)
• Administering (110/189,61.1%)
• Prescribing (59/150,39.3%)

86 (45%) reported changes in working practice, 44% (n=84) no changes, 11% (n=21) unsure. Changes were reported in management (37.7%, n=72), administration (20.9%, n=40) and prescribing (12.6%, n=24) of non-insulin therapies.

Self-reported changes of individual working practice showed 3 themes:

• Increased confidence and improved knowledge
• Improved assessment in choosing the correct therapy
• Improved advice to patients on lifestyle and medication

93.7%, n=164 of 175 responders would recommend the course.

Conclusion: This module enabled the delivery of a standardised training to staff and increased confidence in managing (68%), administering (60%), and prescribing (40%) non-insulin therapies. There is evidence to suggest that the module led to changes in individuals working practice. Poor response rate limits generalisability; but provides some indication of the early impact of this intervention.

NUTRITION—CLINICAL

25-LB

HbA1c Reduction With a Low Carbohydrate Diet and Skills that Promote Behavior Change in Type 2 Diabetes Mellitus

LAURA SASLOW, SARAH KIM, JENNIFER J. DAUBENMIER, JUDITH T. MOSKOWITZ, STEPHEN D. PHINNEY, VERONICA GOLDMAN, RACHEL M. COX, PATRICIA MORAN, ELIZABETH MURPHY, FREDERICK M. HECHT, San Francisco, CA

We tested if a low carbohydrate (CHO) diet would improve glucose control in overweight adults with type 2 diabetes or prediabetes. We randomized participants with an HbA1c > 6.0% to a low fat, carbohydrate restricted diet (LFCR), restriction of 500 calories below estimated need for weight maintenance, 45-50% of energy from CHO, 25-35% of energy from fat; n = 18) or a diet low in carbohydrate (LC), a mean weight loss was twice as high in the LC group, even though they did not aim to calorie restrict but the LFCR group did. Despite its relatively high fat content, blood lipids did not worsen on the LC diet. As this was a small pilot with a short follow-up, further testing is warranted. However, our results suggest that a lower carbohydrate diet coupled with skills that promote behavior change may improve glucose control in type 2 diabetes.

Supported by: Boxers Fund

26-LB

Development and Validation of a Carbohydrate and Insulin Dosing Knowledge Quiz in Asian Patients With Diabetes Mellitus on Prandial Insulin

ANGELA KOH, ANURADHA NEGI, MEE LI YAP, PEI LING KOH, CHEE FANG SUM, Singapore, Singapore

The ability to recognize and estimate carbohydrate(carb) in food is vital in diabetes mellitus (DM), particularly for prandial insulin users, to ensure matching of insulin dose to carbohydrate intake. Tools developed in the west to gauge one’s carb counting ability have limited utility in Asian subjects, since the Asian diet is different from a Western one with more varied/different carb choices.

We aimed to develop and validate a carb and insulin dosing knowledge quiz for Asian patients with DM. Items for the carb section were chosen from commonly eaten food in Singapore based on food records from patients from a diabetes centre in a single tertiary hospital. We tested: carb recognition in food, single food carb estimation, meal carb estimation, food label reading, insulin dosing for carb, blood glucose and for a meal. We compared the quiz against dietitians’ and physicians’ rating of the patient’s carb and insulin dosing knowledge respectively.

55 patients with DM on prandial insulin were recruited, with mean age of 42.6±1.8 years, and insulin use duration of 8.4±1.2 years. 54.5% were Chinese, 18.2% Malay and 27.3% Indian in ethnic origin. 60.4% of the subjects had type 1 DM, and 69.1% were on multiple daily dose of insulin(MDI) or insulin pump. Mean score for the quiz was 64.7±2.3%. The total quiz score, carb domains and only insulin dosing domains only were significantly correlated with the respective healthcare provider ratings. Internal validity for the quiz was good, with Cronbach alpha of 0.875 and Guttmann split half coefficient of 0.923. Quiz scores were significantly higher in type 1 DM subjects vs. type 2 (72.4±2.3% vs.54.5±3.8%, p<0.001) and subjects with more complex insulin regimens (MDI/CSSL vs. bid: 70.0±2.6% vs. 53.2±3.4%, p<0.001).

Our preliminary analysis suggests that this quiz may be a useful screening tool to assess carb and insulin dosing knowledge in Asian patients with diabetes.

Supported by: Alexandra Health

For author disclosure information, see page LB66.
Type 2 Diabetes: Effectiveness of Weight Loss Interventions on A1C, Lipids, Blood Pressure
MARION J. FRANZ, JACKIE L. BOUCHER, Minneapolis, MN

A systematic review of weight loss interventions (WLI) in overweight/obese adults with type 2 diabetes was conducted to determine their baseline to 1-year effectiveness. Study inclusion criteria: randomized clinical trial >1 year, completion rate of ≥70%, published between Jan 2000 to Feb 2013. Ten studies met study criteria; 7 compared WLI and 3 compared WLI to usual care/control. WLI in 17 arms: meal replacements (2), individualized food plans (2), group behavioral (2), low-fat (3), high monounsaturated fat (MUFA) (1), high carbohydrate (CHO) (2), low CHO (2), high protein (1), Mediterranean-style diet (MED) (1), intensive lifestyle intervention (ILI) (1). Weight losses: 14 WLI reported losses of 2.4 to 4.8 kg; the largest: MED, 6.2 kg and ILI, 8.4 kg; the smallest: low-CHO, 1.9 kg. Figure 1 illustrates average weight losses per subject from WLI. Six WLI improved 1-year A1C; however, 11 WLI reported NS A1C changes. Four trials compared WLI with differing macronutrient percentages; weight changes did not differ between groups (1.9 to 4.0 kg) and all reported NS changes in A1C. NS changes in lipids were reported from the majority of the 17 WLI; of 17 WLI reported ↑ in HDL-C, 3 of 17 ↓ in TG, 1 of 14 ↓ in TC, and 1 of 16 ↓ in LDL-C. Five WLI reported positive blood pressure changes and 3 NS changes. The ILI and the MED consistently reported improvements in A1C, lipids, and blood pressure. All other WLI interventions reported minimal, if any, benefits on these outcomes.

In addition to previously reported robust face and content validity, these psychometric analyses demonstrate the HypoA-Q has satisfactory structure, internal consistency reliability, and validity (convergent, divergent, and known groups). The HypoA-Q is likely to enable improved recognition of IAH and evaluation of medical fitness for activities including driving. Its responsiveness now needs to be examined in clinical trials.

Supported by: Diabetes UK

Evaluating the Effect of a Stage Matched Intervention or a Framing Effects Intervention on LDL in Patients With Diabetes: Primary Results of the TACTICS Trial
MARIA ANTONIA RODRIGUEZ, JENNIFER FRIEDBERG, SANGMIN JUNG, IRIS LIN, JOHN CHOI, BINHUAN WANG, YIXIN FANG, JUDITH WYLIE-ROSSET, SUNDAR NATARAJAN, New York, NY, Bronx, NY

Low-density lipoprotein cholesterol (LDL) is an established modifiable risk factor for cardiovascular disease in diabetes and most patients are prescribed treatment. Despite treatment, a substantial proportion have uncontrolled LDL (LDL ≥ 100 mg/dL), probably because of patient nonadherence. Tailored interventions hold promise to improve adherence. We evaluated if a stage-matched intervention (SMI) based on the Transtheoretical Model or a framing effects intervention (FEI) based on Prospect Theory will improve adherence and lower LDL.

Veterans with diabetes and uncontrolled LDL were randomized to SMI, FEI, or Attention Placebo (AP). LDL was assessed at baseline and 6 months. All patients received monthly phone counseling for 6 months. SMI and FEI were tailored to diet, exercise, and medications. We evaluated the effect on LDL, both as a continuous and as a dichotomous outcome, by initial unadjusted analyses and then while controlling for baseline LDL and BMI in regression models that accounted for physician clustering.

We randomized 247 veterans with Type 2 diabetes and hyperlipidemia. The findings are summarized below.

The TACTICS (Targeting Adherence to Cholesterol-lowering Treatment to Improve Control Study) Randomized Clinical Trial: Primary Results

Framing Effects Intervention (FEI) - Stage Matched Intervention (SMI) - Attention Placebo (AP)

<table>
<thead>
<tr>
<th>Baseline (n=207; FEI 64, SMI 82, AP 61) comparisons of median LDL level (mg/dL) and LDL control % (LDL &lt; 100 mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL level (mg/dL)</td>
</tr>
<tr>
<td>LDL control % (LDL &lt; 100 mg/dL)</td>
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</tbody>
</table>

The effect on LDL at 6 months (n=222; FEI 75, SMI 73, AP 74) evaluated using: a) median LDL level (mg/dL), b) LDL control % (LDL < 100 mg/dL), c) Linear regression and d) Logistic regression

| LDL level (mg/dL) | 105 mg/dL | 112 mg/dL | 111 mg/dL | 0.12 (Wilcoxon) |
|---------------------------------------------------------------|
| LDL control % (LDL < 100 mg/dL) | 42.17% | 28.75% | 25.00% | 0.05 (Chi-Sq test) |

The FEI lowered LDL and improved LDL control. This FEI may be a potent facilitator for reaching lipid lowering goals in patients with Type 2 diabetes and hyperlipidemia.

The HypoA-Q lowered LDL and improved LDL control. This FEI may be a potent facilitator for reaching lipid lowering goals in patients with Type 2 diabetes and hyperlipidemia.
Results from the Sunshine Study among Women With Type 2 Diabetes and Comorbid Depression: Mental Health Professionals, Research Explained in Simple Terms, Use of Humor, an Improvement in Their Mood Immediately After Reading or Sharing About Finding Supportive Personal Stories from Others. 72% reported experiencing frequently cited reasons for using diabetes-related social media included respondents (81%) were patients; 62% were 18-30 years of age. The 3 most used categories were caregivers (i.e., parent/spouse). Descriptive statistics and thematic analyses to members from 4 national online diabetes communities. The majority of caregivers in better understanding social support needs of this current study was to address gaps in the current literature base and assist health professionals in better understanding social support needs of this population. A 14-item survey developed by the researchers was administered to 4 national online diabetes communities. Participants included 154 patients with a diagnosis of T1, T2, or gestational diabetes or caregivers (i.e., parent/spouse). Descriptive statistics and thematic analyses were used to determine patterns of social media use, perceived outcomes, and suggestions for improving diabetes online communities. The majority of respondents (81%) were patients; 62% were 18-30 years of age. The 3 most frequently cited reasons for using diabetes-related social media included 1) having one's voice heard, 2) finding information related to coping, and 3) finding supportive personal stories from others. 72% reported experiencing an improvement in their mood immediately after reading or sharing about diabetes online. Recommendations to inform future social media content for the diabetes community included: interdisciplinary input from medical and mental health professionals, research explained in simple terms, use of humor, and local community connections.

Supported by: The Patterson Foundation

Supported by: NIDDK/NIH

Vitamin D Supplementation Decreases Severity of Pain Symptoms among Women With Type 2 Diabetes and Comorbid Depression: Results from the Sunshine Study

TODD DOYLE, SUE PENCKOFER, PATRICIA MUMBY, MARY BYRN, MARY ANN EMANUELE, DIANE WALLIS, Maywood, IL, Chicago, IL

Type 2 diabetes (T2DM) is associated with complications such as depression and pain. Few studies have examined how co-morbid pain impacts depression treatment in patients (Ps) with T2DM. No studies have examined Vitamin D₂ (25-OH-D) supplementation on this association. We aimed to 1) determine if pain was affected by Vitamin D₂ supplementation for treatment of Major Depressive Disorder (MDD) in T2DM & 2) if differences in baseline (BASE) pain were associated with changes in pain symptom severity over time. The Sunshine Study was a single-arm repeated measures trial designed to test the efficacy of weekly Vitamin D₂ supplementation (50,000 IU) for 6 months on MDD in women with T2DM. The Diabetes Symptom Checklist subscales of neuropathic pain and sensory pain were used to measure pain symptom severity at BASE & 3- & 6-month follow-ups (3MFU & 6MFU). Ps (N=46) had a mean age of 54.8 years (SD=10.5), HbA1c=6.8% (SD=0.82%), and T2DM duration=7.8 years (SD=7.1). 61% of Ps reported neuropathic pain and 74% sensory pain at BASE. Repeated measures ANOVA showed clinical improvements in neuropathic pain (F[2, 135]=2.25, p<.11, BASE M=3.2, 3MFU M=1.8, 6MFU M=2.1) and sensory pain (F[2, 135]=1.76, p=.18, BASE M=7.3, 3MFU M=5.0, 6MFU M=5.9). There was a significant change in neuropathic pain (F[2, 134]=3.45, p<.001) and sensory pain (F[2, 134]=28.1, p<.001) according to BASE pain severity. Ps with higher neuropathic pain at BASE (M=5.2) showed significantly (p<.05) decreased pain severity at 3MFU (M=2.5) compared to Ps with lower neuropathic pain. Ps with higher neuropathic pain at BASE (M=10.6) showed significantly (p<.05) decreased pain severity at 6MFU (M=6.5) compared to Ps with lower sensory pain. Ps with elevated neuropathic and sensory pain at BASE showed improved changes in pain severity at 3MFU & 6MFU following Vitamin D₂ supplementation for MDD in T2DM. Vitamin D₂ supplementation for the treatment of pain and MDD in T2DM is promising.

Supported by: NIDDK/NIH

Social Media Use by Individuals With Diabetes

EMILY SHAFFER-HUDKINS, NICOLE JOHNSON, STEPHANIE MELTON, Tampa, FL

Patients with diabetes are often looking online for information and support related to their chronic health condition. An analysis of the 10 most popular social media websites tailored to individuals living with a chronic disease found the sites to have an average of 6,700 members and up to 100 new posts daily (Weitzman et al., 2011). The current study focuses on social media use of patients with diabetes, given the high level of self-management required and correlations with mental health and social support needs. In recent examinations of online networking by patients with Type 1 (T1) and Type 2 (T2) diabetes, researchers found the most common topics to include sharing personal clinical information, requesting disease-specific guidance, and receiving emotional support (Armstrong, et al., 2011). The purpose of the current study was to address gaps in the current literature base and assist health professionals in better understanding social support needs of this population. A 14-item survey developed by the researchers was administered to members from 4 national online diabetes communities. Participants included 154 patients with a diagnosis of T1, T2, or gestational diabetes or caregivers (i.e., parent/spouse). Descriptive statistics and thematic analyses were used to determine patterns of social media use, perceived outcomes, and suggestions for improving diabetes online communities. The majority of respondents (81%) were patients; 62% were 18-30 years of age. The 3 most frequently cited reasons for using diabetes-related social media included 1) having one’s voice heard, 2) finding information related to coping, and 3) finding supportive personal stories from others. 72% reported experiencing an improvement in their mood immediately after reading or sharing about diabetes online. Recommendations to inform future social media content for the diabetes community included: interdisciplinary input from medical and mental health professionals, research explained in simple terms, use of humor, and local community connections.

Supported by: The Patterson Foundation

Glucose Metabolism Is Associated With Acute and Chronic Stress in Depressed Patients

LI LI, RICHARD C. SHELTON, Birmingham, AL

Early life stress (ELS) is recognized as a risk factor not only for psychiatric disorders such as depression, but also for metabolic diseases including prediabetes. Prediabetes is a state of abnormal glucose homeostasis characterized by the presence of impaired fasting glucose, impaired glucose tolerance, or both. Individuals with prediabetes are at increased risk for type 2 diabetes and cardiovascular diseases. The estimated cost of ELS-related illnesses including depression and prediabetes in the U.S is over $200 billion a year. Although numerous studies indicate a reciprocal relationship between depression and diabetes, little is known about the association between chronic and acute stress with prediabetes in depressed patients. Our aim is to elucidate the relationship between acute psychosocial stress, ELS, i.e. sexual and physical abuse, and glucose homeostasis in depressed patients. A history of ELS is assessed using the Childhood Trauma Questionnaire. Depressed patients and matched controls are exposed to an acute psychosocial stress, Trier Social Stress Test (TSST), to assess acute stress paradigm. Subjects provide blood samples for oral glucose tolerance tests (OGTT). Fasting glucose levels are positively correlated with the severity of depression and the severity of sexual and physical abuse in depressed patients. Compared to controls, depressed patients show impaired glucose tolerance during OGTT. Depressed patients with higher fasting glucose levels have stronger acute stress response represented by higher anxiety, fatigue and irritation scores during the TSST. Pro-inflammatory cytokines such as interleukin-6 and interleukin-8 are elevated by the TSST in depressed patients compared with controls. These results suggest a key relationship between glucose homeostasis, ELS and acute stress in depressed patients. Our findings emphasize the importance to identify and intervene stresses in depressed patients to improve their health outcomes and to reduce the cost.

Supported by: DHHS/HRSA (H58MC12788)

For author disclosure information, see page LB66.
We Are in this Together: Partner Perspectives of Living With a Loved One With Diabetes

SHELLEY YIP, MATTHEW SIGNAL, GREG SMITH, GRANT BEBAN, MICHAEL CLERK, LINDA CALDWELL, PAUL BROWN, HELEN GORDON, JANET CROWLEY, JENNY HUMPHREYS, JENNIFER BOURNE, LINDSEY BESS, STEPHANIE MELTON, NICOLE JOHNSON, Tampa, FL

Partners or spouses play a significant role in providing diabetes support and care for loved ones with Type 1 Diabetes. The demands of diabetes care placed on partners are not significantly addressed or acknowledged in the medical community. The purpose of this qualitative study is to assess partner perceptions of how diabetes affects their personal relationship and the challenges they face in caring for their loved one, as well as to identify unmet needs for intervention.

In-depth interviews were conducted with 19 partners of individuals with Type 1 Diabetes. The sample represents a diverse group of couples in relationships from one to twenty-six years. Interview transcripts and notes were analyzed for themes using a grounded theory approach with qualitative software.

The challenges partners face revolve around the daily demands of diabetes care and the emotional weight from worrying about their loved one. They experience chronic stress, fear and grief over the risk diabetes poses for their loved one. They report experiencing grief over the loss of time with their spouse due to expected shortened lifespan, they face diabetes complications, and they worry over having to provide emergency care for their significant other. However, only 26% report receiving any form of diabetes education, while most (90%) have administered life-saving care for their loved one. The role of the partner is mediated by the perceived effectiveness of diabetes management. When depended upon, the partner becomes the primary caregiver, even so, they struggle to not “nag” or overstep their loved one’s independence.

This study highlights the emotional strain partners face in providing diabetes support. Partners need more psycho-social support services, care giving training and formal diabetes education. The outcomes of this research support the notion that incorporating spouses into diabetes care plans can have positive impact on diabetes management and quality of life for families impacted by diabetes.

Supported by: The Patterson Foundation

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Clinical Trials of a Closed-Loop Artificial Pancreas With Large Unannounced Meals

FRASER CAMERON, GÜNTER NIEMEYER, DARRELL M. WILSON, KARI BENASI, PAULA CLINTON, B. WAYNE BEQUETTE, BRUCE A. BUCKINGHAM, Troy, NY; Glendale, CA; Stanford, CA; Stanford, CA

Closed-loop control of blood glucose (BG) levels in people with type 1 diabetes can reduce patient burden and the incidence of complications, particularly if meals do not need to be announced. We tested a multiple model probabilistic predictive controller (MMPPC) on four preliminary patients, revised it and tested six primary patients. Each admission lasted for 32 hours with five unannounced meals containing 1 g/kg of carbohydrate (CHO). The closed-loop therapy used an Abbott Navigator CGM and Insulet insulin pump with the MMPPC implemented through the UCSB artificial pancreas system. Therapy began at 9 AM with unannounced meals at 9 AM, 1 PM, 5:30 PM, and 9 AM and 1 PM the next day. The patients had a mean (±SD) HbA1C of 7.3±0.6%, age of 28±5 years, total daily dose of 43±13 U, and weight of 74±13 kg. The controller was initialized only with the patient’s total daily dose and daily basal pattern.

The MMPPC algorithm explicitly estimates and predicts BG uncertainty stemming from past and potential future meals, endogenous glucose production, and insulin sensitivity. Insulin boluses are calculated to lower predicted BG levels until there is a roughly 3% risk of BG levels below 80 mg/dL. At night, the MMPPC targets a BG level of 100 mg/dL with attenuated control providing smooth corrections.

On a 24-hour basis, the primary patients had mean reference/CGM values of 161/142 mg/dL, with 63/78% of time spent between 70 and 180 mg/dL. Two preventable system failures led to a manual bolus (per subject settings) and an underestimation of active insulin which subsequently required CHO intervention for hypoglycemia. One other CHO treatment was given for a nocturnal glucose of 66 mg/dL with a rate of change of -0.25 mg/dL per min. For the 30 unannounced meals the mean pre-meal, post-meal maximum, and 3-hour post-meal values were 139/132, 223/208, and 168/156 mg/dL respectively.

The MMPPC was tested in-clinic against repeated, large, unannounced meals and maintained good control overnight and during meals.

Supported by: JDRF
Continuous Glucose Monitoring via Telemetry in Rat

TAMER COSKUN, LIBBEY O’TARRELL, ROBERT BICKWAY, PAUL HAENFER, RICHARD G. PETERSON, CHARLES V. JACKSON, Indianapolis, IN, St. Paul, MN

The current standard for routine glucose measurements in preclinical research is often glucometers and test strips. These pose significant limitations in terms of accuracy, animal stress, and frequency of sampling. Until now, continuous monitoring options for preclinical research have been very limited. The present study evaluates the use of a novel prototype device (Data Sciences International) for acute and chronic glucose measurements in rats. The device is 1.4cc and provides temperature, activity, and direct continuous blood glucose readings for 4 weeks or longer. The devices were evaluated in 4 diabetic and 4 normal Zucker fa/fa (ZDF) rats and in 10 Zucker diabetic/Sprague Dawley (ZDS) rats. Each animal was surgically instrumented with glucose sensors in the abdominal aorta and the telemetry device placed in the intraperitoneal (ip) cavity. Continuous glucose readings were recorded for 5-7 weeks with periodic fasting GTTs (glucose, 2-3 g/kg, po or ip). Daily and GTT reference values were recorded with a StatStrip Xpress glucometer (Nova Biomedical). The glucose sensors provided high resolution data and demonstrated the ability to accurately assess chronic diurnal patterns matching with the feeding pattern of rats from 3 days up to 7 weeks after surgery. These devices hold great potential for comparing physiologic processes associated with glucose regulation in normal and disease condition rats; monitoring diabetes progression and developing preventive treatments for type II diabetes.

A Study of Islet Auto-Antibodies and B-Cell Functional of Ketosis-Proone Diabetes

BAO MING-JING, Chengdu, China

To observe the clinical characteristics, islet auto-antibodies, β-cell function in Ketosis-prone diabetes (KPD), and to investigate the effect of insulin intensive therapy or oral antidiabetic drug (OHA) on the β-cell in the patients with KPD.

According to auto-antibodies (A) and β-cell function (I), a total of 162 patients with KPD were divided into four groups, including A+I+ (group 1, n=25), A-β- (group 2, n=38), A+β- (group 3, n=41), A-β+ (group 4, n=58). Islet auto-antibodies, including glutamic acid decarboxylase antibody (GAD-Ab), insulin cell antibody (ICA), insulin autoantibody (IAA) and protein tyrosine phosphatase antibody (IA-2Ab) were measured. The clinical characteristics, biochemical parameters and FPG, HbA1c, FCP and 2h CP were compared between each groups. Group 1, 2 treated with insulin intensive therapy and group 4 treated with Metformin (Glucophage). The treatment targets were FPG <6.0mmol/L, 2hPG <8.0mmol/L, HbA1c<7%. After 6 months, Total 131 patients, in each group were 21, 29, 34 and 47 cases finished this clinical test. At the same time, β-cell function and auto-antibodies were detected and analyzed.

Patients in group 2 demonstrated the youngest age at onset, lowest level of FCP and 2hCP. Compared with group 1, 2 and 3, patients in group 4 owed older age at onset, higher BMI, more obesity, dyslipidemia and hypertension, higher FCP and 2h CP. The phenotype of patients in group 1 and 2 were intermediate between group 2 and 4. After 6 months observation the β-cell function showed that the 2h CP of group 1 and 3 improved compared with basic value. In group 2, the 2h CP progressively deteriorated. So in group 4, FCP and 2h CP had no changed.

There are significantly different in β-cell function, auto-antibodies, clinical characteristics and biochemical parameters in KPD who may need different therapeutic strategies. Insulin intensive therapy may protect the β-cell in patients with KPD.

Glucose Variability and Physical Activity Among People With Type 1 Diabetes

HANJONG PARK, LAURIE QUINN, ALI CINAR, CHANG PARK, KAMURAN TURKOY, ELIF SEYMA BAYRAK, ELIZABETH LITTLEJOHN, SARAH SCHWARZ, DEIRDRE FISHER, Chicago, IL

Glucose variability (GV) and physical inactivity appear to contribute to the development of diabetes complications in T1DM. A cross-sectional pilot study was performed to examine relationships among demographics, GV, and physical activity (PA) in 15 adults (10F/SM) using insulin pump therapy (ages:31±13.3 years, duration of diabetes:20±13.0 years; A1C:7±0.8%, BMI:25±5.0 kg/m2)

For author disclosure information, see page LB66.
Liraglutide formulations may be directly effective in reducing the duration and AUC of hyperglycemia, being overweight/obese and experience longer duration of hypoglycemia. PA was associated with longer duration of hyperglycemia (rs = -.604, p = .017) which competed with decreasing the risk of developing diabetes complications.

The patients of Group 2 (Mean±1 day) reached target glycemic control in less time than that of Group 1 (Mean±2 days) (p < 0.044). The daily insulin doses of patients were not significantly different between 2 groups during the 2 weeks of maintaining normoglycemia (day2: 0.86±0.18 units/kg vs. 0.89±0.15 units/kg, p = 0.942; day7: 0.53±0.21 units/kg vs. 0.57±0.19 units/kg, p = 0.942; day13: 0.45±0.16 units/kg vs. 0.47±0.19 units/kg, p = 0.289). And the HbA1c of all patients had a considerable change after the treatment, while the level of Group 1 decreased 1.77% ± 0.16% and of Group 2 decreased 1.47% ± 0.17% (p = 0.21). The AIR improved significantly compared to baseline, however, the improvement of Group 2 [AIR=39.54±5.92 (μU·min·mL⁻¹)] was much greater than that of Group 1 [AIR=19.70±5.92 (μU·min·mL⁻¹); p = 0.013]. The combination of Liraglutide and CSII may have better effect in improving β cell function than CSII only.

The AUTONOMY Study: Initiating and Adjusting Lispro Therapy in Patients With Type 2 Diabetes Mellitus Not Adequately Controlled on Basal Insulin Therapy and Oral Agents

STEVEN V. EDELMAN, JENNAL JOHNSON, RONG LIU, LEONARD C. GLASS, San Diego, CA, Indianapolis, IN

Evidence on optimal methods of prandial insulin initiation and adjustment, especially in the primary care setting, is limited. AUTONOMY evaluated 2 approaches to introduce lispro therapy in patients with T2DM ≥18 to ≤85 yo, on basal insulin glargine (GLA), NPH, NPL, or detemir (≥20 U/day) plus oral antihyperglycemic agents for ≤3 months, with a screening HbA1c >7.0% to ≤12.0%, and who required prandial therapy. T2DM studies (A and B) followed a randomized, double-blind, three-period crossover study, the pharmacokinetics and local injection site toleration measured with a 100 mm visual analog scale (VAS) of BIOM-238 and BIOM-250 were compared to HU in 12 subjects with type 1 diabetes. Mean times to half maximal insulin concentrations were 13.7 ± 1.9, 14.6 ± 1.9, and 24.8 ± 2.9 min for BIOM-238, BIOM-250, and HU, respectively (p < 0.001 for BIOM-238 and p = 0.001 for BIOM-250 vs. HU). Time to maximal insulin concentrations and areas under the curves for the first 30 and 45 minutes for BIOM-238 and BIOM-250 all indicated significantly increased early lispro absorption compared to HU. The 2 algorithms showed an equivalent clinically significant drop in HbA1c compared to HU at 24 weeks (HbA1c 7.3 ± 1.7% for BIOM-238 and 8.2 ± 4.5% for HU). The mean VAS score for BIOM-238 was significantly higher than that associated with HU (2.4 ± 7.0 mm for BIOM-238 and 0.0 ± 0.29 mm for HU). Safety results were comparable between treatments. In conclusion, this study demonstrates that Na2EDTA/citrate formulations of insulin lispro result in more rapid absorption and more rapid declines from peak concentrations compared to HU. Furthermore, the presence of magnesium sulfate in BIOM-250 significantly mitigates local injection site discomfort without altering the ultra-rapid pharmacokinetic profile.

Effect of Liraglutide Combined With Short-Term Continuous Insulin Infusion on β Cell Function in Newly Diagnosed Type 2 Diabetic Patients

WEEJIAN KE, JUAN LIU, LIEHUA LIU, HAI LI, DONGHONG FANG, YANBING LI, Guangzhou, China

To investigate the effect on of Liraglutide combined with short-term continuous insulin infusion (CSII) in newly diagnosed and diagnosed type 2 diabetic patients with fasting plasma glucose of 7.0-16.7 mmol/L were enrolled and randomly assigned to therapy with CSII (Group 1, n=19) or Liraglutide at a dose of 0.6 mg injected subcutaneously per day combined with CSII (Group 2, n=20). The treatment was stopped after normoglycemia maintained for 2 weeks. Intravenous glucose tolerance tests (IVGTTs) were performed to investigate acute insulin response (AIR) and blood glucose, HbA1c, insulin were measured before and after.

45-LB

46-LB

The AUTONOMY Study: Initiating and Adjusting Lispro Therapy in Patients With Type 2 Diabetes Mellitus Not Adequately Controlled on Basal Insulin Therapy and Oral Agents

STEVEN V. EDELMAN, JENNAL JOHNSON, RONG LIU, LEONARD C. GLASS, San Diego, CA, Indianapolis, IN

Evidence on optimal methods of prandial insulin initiation and adjustment, especially in the primary care setting, is limited. AUTONOMY evaluated 2 approaches to introduce lispro therapy in patients with T2DM ≥18 to ≤85 yo, on basal insulin glargine (GLA), NPH, NPL, or detemir (≥20 U/day) plus oral antihyperglycemic agents for ≤3 months, with a screening HbA1c >7.0% to ≤12.0%, and who required prandial therapy. T2DM studies (A and B) followed a randomized, double-blind, three-period crossover study, the pharmacokinetics and local injection site toleration measured with a 100 mm visual analog scale (VAS) of BIOM-238 and BIOM-250 were compared to HU in 12 subjects with type 1 diabetes. Mean times to half maximal insulin concentrations were 13.7 ± 1.9, 14.6 ± 1.9, and 24.8 ± 2.9 min for BIOM-238, BIOM-250, and HU, respectively (p < 0.001 for BIOM-238 and p = 0.001 for BIOM-250 vs. HU). Time to maximal insulin concentrations and areas under the curves for the first 30 and 45 minutes for BIOM-238 and BIOM-250 all indicated significantly increased early lispro absorption compared to HU. The 2 algorithms showed an equivalent clinically significant drop in HbA1c compared to HU at 24 weeks (HbA1c 7.3 ± 1.7% for BIOM-238 and 8.2 ± 4.5% for HU). The mean VAS score for BIOM-238 was significantly higher than that associated with HU (2.4 ± 7.0 mm for BIOM-238 and 0.0 ± 0.29 mm for HU). Safety results were comparable between treatments. In conclusion, this study demonstrates that Na2EDTA/citrate formulations of insulin lispro result in more rapid absorption and more rapid declines from peak concentrations compared to HU. Furthermore, the presence of magnesium sulfate in BIOM-250 significantly mitigates local injection site discomfort without altering the ultra-rapid pharmacokinetic profile.
Glycemic Control and Treatment Satisfaction in Type 2 Diabetes: Basal Plus Compared With Biphasic Insulin in the LANSCAPE Trial

JITEN VORA, NEALE COHEN, MARC EVANS, ANDREW HOCKEY, JANE SPEIGHT, CAROLINE WHATELY-SMITH, LONDON, Kingdom, Melbourne, Australia, Cardiff, United Kingdom, Guildford, United Kingdom, Homchuch, United Kingdom, Hunton Bridge, United Kingdom

Biphasic insulin is a frequent intensification step when basal insulin alone provides inadequate glycemic control. Basal plus main-meal fast-acting insulin may be equally effective and more acceptable. The LANSCAPE study tested whether “Basal Plus” insulin glargine (Lantus) once daily and insulin glulisine aspart protamine 30/70 (NovoMix30) with respect to glycemic control (primary objective) and provided superior treatment satisfaction.

LANSCAPE was an international, controlled trial of adults with type 2 diabetes receiving basal insulin. Participants’ mean (SD) age was 61.6 (8.5) years, diabetes duration 12.9 (6.4) years and A1C 8.62 (0.94)%. During an 12- week run-in, oral agents except metformin were stopped and insulin glargine optimized. After run-in, 335 participants with fasting glucose <126mg/dl were randomized to “Basal Plus” (n=170) or “Biphasic” (n=165) regimen. Active insulin titration followed standardized algorithms; 89% of patients (91.8% and 86.1% respectively) completed treatment. At 24 weeks A1C fell by 1.02% in the Basal Plus, 1.22% in the Biphasic arms; mean difference 0.21% (SE 0.09, upper 95% CI 0.38%), implying non-inferiority of Basal Plus relative to the Biphasic regimen (pre-defined margin 0.4%). More subjects reached target A1C 7% with Basal Plus than Biphasic (76.5% vs. 66.1%, p=0.049). There was no difference in overall glycemia rates (15 vs. 18 events/patient-year respectively p=0.2), but slightly more nocturnal events with Basal Plus (5.7 vs. 3.6 events/patient-year p=0.02). Significant advantages favoring Basal Plus were seen in DTSQc and ITSQ, and provided superior treatment satisfaction.

In type 2 diabetes Basal Plus provides comparable glycemic control to a Biphasic regimen, better patient reported outcomes, and may present a more acceptable option for insulin initiation/intensification.

Supported by: Sanofi
bed tertiary care hospital. The SCIP criteria for glycemic control were the percentage of glucose values in the target range on post-op day two. We also measured avoidance of hospital acquired (never pay) glycemic conditions such as hypoglycemic coma or diabetic ketoacidosis. Data was provided by CMS, the CDC and the National Health Care Survey.

Results: Immediate and sustained improvement in SCIP was noted starting from 88% in 2008 to 99% in 2011, compared to the national average of 95%. This data represents a doubling in cost savings and places our hospital in the top 10% of all U.S. hospitals for SCIP. In addition, we noted immediate and sustained reductions in hospital-acquired conditions improved from 0.083/1000 in 2008 to 0.032 in 2011 compared to the national average of 0.058/1000. Conclusions: The Endotool™ intravenous insulin system used in our hospital surgical ICUs resulted in a dramatic improvement in SCIP and a reduction in hospital acquired (never pay) glycemic conditions with a highly favorable economic impact.

**CLINICAL THERAPEUTICS/NEW TECHNOLOGY—NON-INSULIN INJECTABLES**

**51-LB**

Antisense Suppression of Serum ApoC-III Improves Hypertriglyceridemia and Insulin Sensitivity in Multiple Species

RICHARD G. LEE, MARK J. GRAHAM, WUXIA FU, VERONICA J. ALEXANDER, WALTER SINGLETON, RICHARD GARY, JENNIFER BURKEY, SANJAY BHANOT, ROSANNE M. CROOKE, Carlsbad, CA

Chronic elevation in fasting serum triglycerides (TG) is a hallmark of metabolic syndrome and type 2 diabetes (T2D). A key regulatory factor in TG homeostasis is apolipoprotein C-III (apoC-III), an apolipoprotein present on VLDL, LDL and HDL particles. We have previously demonstrated in rodent models, monkeys, and normal human volunteers that antisense oligonucleotide (ASO) mediated suppression of apoC-III concomitantly reduced serum TG, with no target-related safety issues observed. In BB rats, a model of human type 1 diabetes, apoC-III ASO treatment produced a significant delay in pancreatic beta cell death, demonstrating that apoC-III may represent an important factor in disease progression. In Western diet fed human apoC-III transgenic mice, treatment with the human apoC-III ASO for eight weeks produced concordant and significant reductions in hepatic human apoC-III mRNA (~87% reduction vs. control ASO), plasma human apoC-III protein (79 ± 6 mg/dL with apoC-III ASO vs. 173 ± 1 mg/dl with control ASO) and fed plasma TG (227 ± 16 mg/dL with apoC-III ASO vs. 899 ± 59 mg/dL with control ASO). Fed plasma insulin levels were also reduced (1.8 ± 0.1 ng/ml with apoC-III ASO vs. 5.8 ± 1 ng/ml with control ASO) without a change in plasma glucose concentrations, indicating an improvement in insulin sensitivity. A Phase 2 double-blind placebo controlled study is underway in dyslipidemic T2D patients to determine whether apoC-III ASO treatment will improve insulin sensitivity as assessed by hyperinsulinemic-euglycemic clamps conducted pre and post 3 month ASO administration, as ASO treatment will improve insulin sensitivity as assessed by hyperinsulinemic-euglycemic clamps conducted pre and post 3 month ASO administration.

For A1C (baseline 8.1 ± 0.8%), Albi treatment (txt) difference was superior to Pbo (−81%; P < 0.0001), Sita (−35%; P = 0.0001) and SU (−27%; P = 0.003). Albi had superior txt difference (P < 0.05) in FPG (mg/dL) vs. comparators: Pbo −27.7; Sita −15.5; SU −10.1. Weight (baseline 90.7 kg±19) (kg) Δ was similar for Albi (P = NS) compared to Pbo (txt difference: −2) and to Sita (−4). Weight loss was greater with Albi than SU (txt difference: −2.4; P < 0.0001).

Gastrointestinal AEs through Wk 104 with Pbo/Sita/SU/Albi were: nausea, 11%/7%/8%/10%; diarrhea, 11%/9%/9%/13%; vomiting, 1%/4%/4%/0%. Injection site reactions occurred in: Pbo 5%, Sita 6%, SU 8%, Albi 17%. The incidence of documented (≥70 mg/dL) symptomatic hypoglycemia events (prior to the addition of hyperglycemia rescue medication) was Pbo 4%, Sita 2%, SU 18%, Albi 3%; no severe events.

Albi treatment for T2D as add on to met was durable and superior to Sita and SU in A1C and PFD reduction, superior in weight loss to SU, and well tolerated at Wk 104.

**52-LB**

**HARMONY: 3; 104 Week (Wk) Efficacy of Albiglutide (Albi) Compared to Sitagliptin (Sita) and Glimepiride (SU) in Patients (pts) With Type 2 Diabetes Mellitus (T2DM) on Metformin (Met)**

BO AHREN, MURRAY STEWART, DEBORAH CIRKEL, FRED YANG, CAROLINE PERRY, SUSAN JOHNSON, Lund, Sweden, Upper Merion, PA, Stavenage, United Kingdom, Research Triangle Park, NC

This 3-year, randomized, double-blind, placebo- and active-controlled, phase IIb III study examined Albi 30 mg added to Met vs. Pbo + Met, Sita + Met, or SU + Met in pts with T2DM (A1C 7-10%) on Met. Pts meeting predefined hyperglycemia criteria qualified for blinded dose titration (SU 2 to 4 mg, albiglutide 30 to 50 mg). Primary endpoint was the mean difference in A1C change (A) from baseline at Wk 104.

For A1C (baseline 8.1 ± 0.8%), Albi treatment (txt) difference was superior to Pbo (−81%; P < 0.0001), Sita (−35%; P = 0.0001) and SU (−27%; P = 0.003). Albi had superior txt difference (P < 0.05) in FPG (mg/dL) vs. comparators: Pbo −27.7; Sita −15.5; SU −10.1. Weight (baseline 90.7 kg±19) (kg) Δ was similar for Albi (P = NS) compared to Pbo (txt difference: −2) and to Sita (−4). Weight loss was greater with Albi than SU (txt difference: −2.4; P < 0.0001).

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Albi treatment for T2D as add on to met was durable and superior to Sita and SU in A1C and PFD reduction, superior in weight loss to SU, and well tolerated at Wk 104.

**53-LB**

Superagonistic Activation of GLP-1 Receptor by CA Exendin-4 (Exendin-4 Analog) With Fast Dissociation Rate Constant

IN YOUNG CHOI, SUNG HEE PARK, SANS YOON HWANG, KYU HANG LEE, YOUNG HOON KIM, SE CHANG KWON, Hwasung-si, Republic of Korea

Exendin-4 is a glucagon-like peptide-1 (GLP-1) receptor agonist used for the treatment of type 2 diabetes. We have developed a novel Exendin-4 analog, Exendin-4 superagonistic activation of GLP-1 receptor by CA exendin-4 to give prolonged efficacy outcome.

**54-LB**

For author disclosure information, see page LB66.
HARMONY 2 Wk 52 Results: Albiglutide Monotherapy in Drug Naive Patients With Type 2 Diabetes Mellitus

MICHAEL NAUCK, MURRAY STEWART, CHRISTOPHER PERKINS, ANGELA JONES-LEONE, FRED YANG, CAROLINE PERRY, RICKEY REINHARDT, MARC RENDELL, Harz, Germany, Upper Manor, PA, Winston-Salem, NC, Omaha, NE

This 3-year, double-blind, placebo (Pbo)-controlled study examined efficacy/safety of GLP-1 receptor agonist albiglutide at 30mg (Albi30) and 50mg (Albi50) vs. placebo (Pbo) in patients with A1C (7-10%) on diet and exercise. Both Albi groups began Albi30 QW (Albi50 started at Wk 12) and were allowed to continue if requiring hyperglycemic rescue (R). Primary objective was A1C change from baseline at Wk 52 with statistical testing performed for Albi50 vs. Pbo then Albi30 vs. Pbo.

Baseline characteristics were similar between groups: mean A1C 8.1%; mean age 53 years; BMI 34 kg/m^2; duration of diabetes 4 years. Wk 52 A1C difference (Albi – Pbo) was −0.84% (95% CI −1.11, −0.58) for Albi30 and −1.0 4% (95% CI −1.31, −0.77) for Albi50, both P<0.001. Fasting plasma glucose decreased rapidly and the improvement mirrored A1C cut to 52 wks:−34 mg/dL (95% CI −46, −22) for Albi30 and −43 mg/dL (95% CI −55, −31) for Albi50, both P<0.001. Weight (kg) decreased in all groups: Pbo: −0.7, Albi30: −0.4, Albi50: −0.9. GI adverse events (% participants including R) for Pbo/Albi30/ Albi50 were: nausea 8/10/9; diarrhea 12/10/13; vomiting 1/3/3. Injection site reactions (% participants including R) were higher for Albi30 (18) and Albi50 (22) vs. Pbo (10). Incidence of pre-R documented (≥70 mg/dL) symptomatic hypoglycemia events (%) was Albi30 (1) and Albi50 (0) vs. Pbo (2); no severe events reported.

Albi monotherapy resulted in robust, durable A1C reduction through Wk 52 and was well tolerated.

Supported by: GlaxoSmithKline
observed which could explain the increase in the Treg populations in vivo. The increase in Tregs is also of interest in the treatment of diabetic nephropathy where Treg dysfunction appears to correlate with disease. In summary DM-199 could represent a novel medicament for the treatment of both Type 1 and 2 diabetes, to be elucidated in upcoming clinical trials.

57-LB

HARMONY 1 Week 52 Results: Albiglutide vs. Placebo in Patients With Type 2 Diabetes Mellitus Not Controlled On Pioglitazone ± Metformin

JANE REUSCH, MURRAY STEWART, CHRISTOPHER FERGUS, PAUL DRUDONNEAU, JUNE YE, CAROLINE PERRY, RICKEY RENHARDT, BRUCE BODE, Denver, CO, Upper Merion, PA, Winston-Salem, NC, Research Triangle Park, NC, Atlanta, GA

This 3-year, randomized, double-blind, placebo (Pbo)-controlled study evaluated efficacy and safety of once-weekly GLP-1 receptor agonist albiglutide 30 mg (Albi) + Pio ± Met vs. Pbo + Pio + Met in patients inadequately controlled (A1C >7%) on Pio ± Met. Patients were allowed to continue if requiring hyperglycemic rescue (R). Primary objective was A1C change from baseline at week 52.

Baseline demographics were similar between groups; mean A1C 8.1%; mean age 55 years; BMI 34 kg/m², duration of diabetes 8 years. Week 52 change from baseline A1C was −0.05% for Pbo and −0.81% for Albi, treatment difference (TD) −0.76% (95% CI −0.96%, −0.56%).

A1C (%) −0.55 (0.06) −0.80 (0.00) *0.33 (0.08)*

PFS (mg/dL) −12.4 (2.9) −31.4 (2.9) *11.5 (4.4)*

Weight (kg) −0.4 (0.2) +4.4 (0.2) *−0.4 (0.4)*

Adverse events (% participants)

Table: Efficacy (Adjusted Mean Difference From Baseline [SE]) and Adverse Event (%) Findings at 52 Weeks

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59-LB

HM11260C, a New Generation Long Acting GLP-1R Agonist With a Unique Pharmacokinetic Profile Improves Glucose Control and GI Tolerability: A Phase Iia Clinical Trial in Type 2 Diabetes Mellitus

JAHDON KANG, SODIMIN CHOI, IN YOUNG CHOI, SE CHANS KWON, MICHAEL TRAUTMANN, MARCUS HOMPESCH, JEEWOUNG SON, Seoul, Republic of Korea, Hwaseong-si, Republic of Korea, Chula Vista, CA.

A very long T1/2 (~180 hrs) and no burst absorption (Tmax:: ~144 hrs) of HM11260C was confirmed in previous single ascending dose study in T2DM. The aim of this double-blinded, randomized, placebo controlled phase IIa study is to investigate safety, tolerability, PK and PD of HM11260C when treated repeatedly with weekly or monthly regimens in T2DM. HM11260C was administered subcutaneously over an 8-week for weekly regimens or a 8-week for monthly regimens.

Data through Day 57 from 48 patients in W1 (1 mg/wk), W2 (2 mg/wk), W3 (4 mg/wk), M1 (8 mg/mo) and M2 (12 mg/mo) are reported. Key demographics were (active vs. placebo; mean [SD]): age 53.7 [0.0] vs. 52.9 [8.7] years; HbA1c, 8.4 [0.1] vs. 8.1 [0.9] %. At Day 57, patients treated with weekly regimens or monthly regimens experienced clinical significant improvements from baseline HbA1c, fasting plasma glucose, body weight compared with placebo. Most common AEs were nausea, vomiting and diarrhea. Weekly regimen showed fewer GI AEs and most events were reported after first injection. No treatment effect was observed on vital signs, laboratory and ECG. HM11260C demonstrated meaningful improvements in blood glucose control and good tolerability after repeated treatment in all weekly and monthly cohorts. Further development of HM11260C is warranted to explore its full potential as mono and combination therapy in patients with T2DM.

Support by: Korea Drug Development Fund R&D Project (KDDF-201204-03)

58-LB

52-Week Efficacy of Albiglutide vs. Placebo and vs. Pioglitazone in Triple Therapy (Background Metformin and Giapreza) in People With Type 2 Diabetes: HARMONY 5 Study

PHILIP HOME, MURRAY STEWART, FRED YANG, CAROLINE PERRY, MOLLY C. CARR, Newcastle upon Tyne, United Kingdom, Upper Merion, PA.

A randomized, double-blind, multicenter study evaluated the efficacy and safety of the GLP-1 receptor agonist albiglutide once weekly (QW) vs. placebo and vs. pioglitazone all on background metformin and Giapreza in people with A1C >7.0-10.0% on dual therapy. The primary objective was A1C change from baseline at week 52. Uptitration of albiglutide 30 mg to 50 mg QW and pioglitazone 30 mg to 45 mg QD was allowed if needed. Patients were allowed to continue if requiring hyperglycemic rescue (R). Primary objective was A1C change from baseline at week 52.

Baseline demographics were similar between groups; mean A1C 8.1%; mean age 55 years; BMI 34 kg/m², duration of diabetes 8 years. Week 52 change from baseline A1C was −0.05% for Pbo and −0.81% for Albi, treatment difference (TD) −0.76% (95% CI −0.96%, −0.56%).

A1C (%) −0.55 (0.06) −0.80 (0.00) *0.33 (0.08)*

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<th>PFS (mg/dL)</th>
<th>Weight (kg)</th>
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<td>Albiglutide (n=273)</td>
<td>−0.80 (0.00)</td>
<td>−31.4 (2.9)</td>
<td>+4.4 (0.2)</td>
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<tr>
<td>Placebo (n=115)</td>
<td>−0.80 (0.00)</td>
<td>−31.4 (2.9)</td>
<td>+4.4 (0.2)</td>
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</table>

Supported by: GlaxoSmithKline

For author disclosure information, see page LB66.
Durability of Dapagliflozin vs. Glipizide as Add-On Therapies in T2DM Inadequately Controlled on Metformin: 4-Year Data

STEFANO DEL PRATO, MICHAEL A. NAUCK, SANTIAGO DURÁN-GARCÍA, KATJA ROHWEDEER, ANETT THEUERKAUF, ANNA MARIA LANGKILDE, SHAMIK J. PARIKHI, Pisa, Italy; Bad Lauterberg, Germany; Seville, Spain, Wedel, Germany; Cologne, Germany, Mölndal, Sweden, Wilmington, DE

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 (≥10 mg/d, n=408) vs. glipizide (GLIP; ≥20 mg/d, n=408) as add-on to metformin (median 2000 mg/d) in T2DM (NCT0066907, baseline HbA1c 7.72%), DAPA was non-inferior to GLIP in HbA1c change at 52 weeks (primary endpoint, both −0.52%; produced weight loss and reduced hypoglycemia [hypo]). Here we report 4-year data from this study, the longest duration of therapy studied for any SGLT2 inhibitor to date. 161 DAPA vs. 141 GLIP patients completed Year 4. Effect of therapy on HbA1c attenuated over time in both groups, but DAPA showed more persistent benefits vs. GLIP up to Year 4 (change from baseline of −0.10 vs. +0.20%; treatment difference −0.30% [95% CI −0.51, −0.09], Sustained and stable weight loss was observed with DAPA vs. weight gain with GLIP (−3.95 vs. +1.12 kg), difference −5.07 kg [95% CI −6.21, −3.93]. Mean systolic BP was reduced with DAPA but not with GLIP, difference −3.7 mmHg [95% CI −5.9, −1.4]. Rate of patients with hypo was −10-fold less with DAPA (5.4%) vs. GLIP (51.5%); most patients with hypo first presented in Year 1. All major hypoglycemia were with GLIP. There were no discontinuations due to hypo with DAPA. Overall rates of AEs and SAEs were similar between groups. Discontinuation due to AEs was 13.3% for DAPA vs. 11.3% for GLIP. Proportion of patients with UI+T = 13.5% for DAPA vs. 9.3% for GLIP (upper UI+T: 1 DAPA vs. 3 GLIP patients). Genital infections (GenI) occurred in 14.3% vs. 2% of patients. Most patients with UI+/GenI first presented in Year 1. The majority of events were of mild/moderate intensity and resolved with standard treatment. UI+/GenI were more common in women. In summary, DAPA demonstrated sustained metabolic benefits including stable weight loss and low rates of hypo compared with GLIP over 4 years. Therapy was well-tolerated, with no new safety signals identified.

Supported by: Bristol-Myers Squibb/AstraZeneca

Imeglimin: A New Antidiabetic Agent That Provides Added Benefit to DPP-4 Inhibitor Therapy

PASCALE FOUQUIER, CLIFFORD J. BAILEY, VALDIS PIRAGS, MICHAELA DIAMANT, GUNTRAM SCHERNTAINER, HAROLD E. LEBOVITZ, SILVIO E. INZUCCHI, Lyon, France; Birmingham, United Kingdom; Amsterdam, Netherlands, Vienna, Austria; New York, NY; New Haven, CT

This 12-week study assessed the efficacy and tolerability of imeglimin as add-on therapy to sitagliptin in type 2 diabetes patients inadequately controlled with sitagliptin monotherapy. This was a multi-center, randomized, double-blind, placebo-controlled, parallel-group study of imeglimin (1,500 mg BID) or placebo added to sitagliptin (100 mg QD) in 170 patients with type 2 diabetes (mean age, 56.8 years; 52.9% male; BMI, 32.2 kg/m²) who were inadequately controlled with sitagliptin alone (A1C ≥ 7.5%) during a 12-week run-in period. The primary efficacy endpoint was change in A1C from baseline to placebo; secondary endpoints included corresponding changes in fasting plasma glucose (FPG), % A1C responders, and certain non-glycemic parameters. Imeglimin-sitagliptin reduced A1C (LS mean) from baseline (8.5%) by 0.80% compared with an increase of 0.12% with placebo (P < 0.001), for a placebo-adjusted decrease of 0.72% with imeglimin. The corresponding changes in FPG were a decrease of 0.80 mmol/L with imeglinim vs. a decrease of 0.19 mmol/L with placebo (P = 0.014). 54.3% of subjects achieved a decrease in A1C ≥ 0.5% with imeglimin vs. 21.6% with placebo (P < 0.001), and 19.8% of subjects receiving imeglimin achieved an A1C ≤ 7% compared with placebo (1.1%). (P = 0.004). Sitagliptin-imeglimin was generally well tolerated with a comparable safety profile to the sitagliptin-placebo group and no related treatment-emergent adverse events. Imeglimin demonstrated incremental efficacy as an add-on therapy to sitagliptin, with comparable tolerability to sitagliptin-placebo, highlighting the potential for imeglimin to complement the efficacy of oral anti-hyperglycemic treatments.
A Euglycemic Clamp Pilot Study Assessing the Effects of the Glucagon Receptor Antagonist LY2409201 on 24-h Insulin Requirement in Patients With T1DM

CHRISTOF M. KAUDA, PARAG GARHYAN, YING DING, RONAN P. KELLY, THOMAS A. HARDY, CHRISTOPH KAPITZA, Neeuw-Sur-Seine, France, Indianapolis, IN, Singapore, Singapore, Neuss, Germany.

Recent research shows that blocking glucagon action prevents lethal metabolic effects seen in mice with type 1 diabetes mellitus (T1DM). LY2409201 (LY), a potent, selective human glucagon receptor antagonist, is being investigated as a treatment for T2DM. To test its effects in T1DM, we assessed whether single oral doses of LY could result in clinically meaningful reductions of the 24-hour insulin requirement. Twenty T1DM patients with a mean age of 43.0 years (SD, 10.3 years), a mean diabetes duration of 19.0 years (SD, 18.8 years), and a mean baseline hemoglobin A1c of 7.8% (SD, 0.7%) had euglycemia (100 mg/dL) maintained overnight by a glucose-controlled insulin infusion system (Biostator) after usual insulin regimens were discontinued. On Day 1, euglycemia was maintained using variable intravenous insulin infusion and standardized meals. These regimens were readministered on Day 2, but patients, randomized 1:2:2, also received a placebo or a 100- or 300-mg single dose of LY before breakfast. The placebo-corrected 24-hour insulin dose needed to maintain euglycemia was reduced by a mean of 17.0% (95% confidence interval [CI], -33.7% to -0.4%; P=0.046) and a mean of 19.6% (95% CI, -35.0% to -4.3%; P=019) in 100- and 300-mg dose groups, respectively. Group mean glucose values were well matched and maintained near euglycemia throughout the clamp procedure. Although LY led to an expected 2- to 3-fold dose-dependent increase in plasma glucagon levels, no significant changes from placebo values were observed for other pharmacodynamic parameters, including levels of glucagon-like peptide-1 (total and active), C-peptide, lipids, and β-hydroxybutyrate. No clinically significant differences between LY and placebo groups were observed in hypoglycemia or adverse event frequency after and during the clamp procedure. Results suggest glucagon antagonism can reduce insulin requirements in T1DM.

Supported by: Eli Lilly and Company

Canagliflozin (CANA) Demonstrates Durable Glycemic Improvements Over 104 Weeks versus Glimpiride (GLIM) in Subjects With Type 2 Diabetes Mellitus (T2DM) on Metformin (MET)


This 3-arm, multicenter, randomized, double-blind, active-controlled study evaluated the durability of the efficacy and safety of albiglutide (ALO) compared to glipizide in combination with metformin (MET) in type 2 diabetic patients inadequately controlled on stable-dose MET. The duration of the study was 104 weeks. The treatment arms were: ALO 12.5 mg OD + MET (ALO 12.5) (n=880), ALO 25 mg OD + MET (ALO 25) (n=885), and glipizide 5 mg titrated to a maximum of 20 mg + MET (GLIP) (n=874). The primary efficacy endpoint was least square mean change from baseline in HbA1c (A1c) at 104 weeks. The majority of patients were white (62.3%); 50.3% were women. Mean age was 55.4 years; body mass index, 31 kg/m²; diabetes duration, 5.5 years; and baseline A1c, 7.6%. Reductions in A1c at Week 104 were -0.68%, -0.72%, and -0.59% for ALO 12.5, ALO 25, and GLIP, respectively. More patients achieving an A1c ≤7% at Week 104 with ALO 25 (48.5%) vs. GLIP (42.8%) (P=0.004); the proportion for ALO 12.5 was 45.6% (not significant vs. GLIP). Changes in fasting plasma glucose at Week 104 were -0.9 mg/dL for ALO 12.5, -3.2 mg/dL for ALO 25, and 5.4 mg/dL for GLIP (P<0.001 for both ALO vs. GLIP). Mean weight changes at Week 104 were -0.68, -0.89, and 0.95 kg for ALO 12.5, ALO 25, and GLIP, respectively (P<0.001 for all ALO decreases vs. GLIP). More GLIP patients (23.2%) experienced a hypoglycemic event than ALO 12.5 (2.5%) or ALO 25 (1.4%) patients; severe hypoglycemia occurred in six GLIP, one ALO 12.5, and no ALO 25 patients. Pancreatitis occurred in one ALO 25 patient and three GLIP patients. Numbers of patients experiencing a adverse event or an event leading to treatment discontinuation were similar across the 3 groups. Eleven deaths occurred: 3 in the ALO 12.5 group, 3 in the ALO 25 group, and 5 in the GLIP group. In summary, albiglutide efficacy was sustained through 104 weeks. The safety profile was similar among the treatment arms, although considerably lower incidences of hypoglycemia were observed in the ALO dose groups.

The selective FFAR1/GPR40 agonist fasiglifam (TAK-875), an antidiabetic drug in phase 3 development, potentiates insulin secretion in a glucose-dependent manner by activating FFAR1 expressed in pancreatic β cells. Fasiglifam significantly improved glycemic control in diabetic patients with a low risk of hypoglycemia in phase 2 studies. However, precise mechanism of the involvement of endogenous free fatty acids (FFAs) on the efficacy of fasiglifam in vivo is not fully understood. Here, we show that fasiglifam acts as a positive allosteric modulator with partial agonist activity for FFAR1. In a Ca²⁺-influx assay using CHO-HFFR1 cells, the EC₅₀ of γ-linolenic acid (γ-LA), an FFAR1 endogenous ligand, was shifted from 5.39 μM to 1.07 μM in the presence of fasiglifam (1 μM), indicating positive cooperativity between fasiglifam and FFAR1. In mouse insulinaoma MIN6 cells and mouse islets, the combination of fasiglifam (10 μM) and γ-LA (100 μM) dramatically potentiated glucose-induced insulin secretion (Fast, 1.8 fold; γ-LA, 3.5 fold; Fasgy-LA, 12.2 fold increase vs. control in mouse islets), while the insulinoytic activities of these agonists were completely abolished in FFAR1/-/- mouse islets. Furthermore, reduction of plasma FFA levels with the lipolysis inhibitor acipimox (30 mg/kg) caused significant suppression of the insulinogetic effect of fasiglifam (10 mg/kg) in N-STZ-1.5 rats, suggesting that plasma FFAs affect insulin release by fasiglifam in vivo. Point mutations of FFAR1 differentially affected the Ca²⁺-influx activating activity of fasiglifam and γ-LA, while these mutations utilize distinct binding sites. Our results indicate that...
Regardless of the Degree of Glycemic Control, Linagliptin (LINA) has Lower Hypoglycemia Risk than All Doses of Glimepiride (GLIM) at All Times of Treatment

BAPTIST GALLWITZ, JULIO ROSENSTOCK, SANJAY PATEL, MAXIMILIAN VON EYNATTEN, UWE HENIKE, LUDWIG MEHLBURGER, KLAUS A. DUGI, HANS-JUERGEN WOERLE, Tübingen, Germany; Dallas, TX; Bracknell, United Kingdom; Ingelheim, Germany.

Sulfonylurea (SU)-induced hypoglycemia is a common problem in type 2 diabetes. In a 2-y, randomized, double-blind study of the DPP-4 inhibitor LINA 5 mg/d (n=764) vs. the SU GLIM 1.4 mg/d (n=755) in pts uncontrolled by metformin, LINA provided noninferior reductions in HbA1c to GLIM with a lower hypoglycemia risk and no weight gain. This exploratory analysis evaluated the risk for investigator-reported hypoglycemia with GLIM based over time, overdose, and, by HbA1c reduction vs. LINA. Pts randomized to GLIM started at 1 mg. Pts not achieving FPG ≤110 mg/dL at 4 wks and not at hypoglycemia risk were uptitrated stepwise to 4 mg. The % pts with hypoglycemia at the maximum GLIM dose was: 1 mg, 45.0%; 2 mg, 50.8%; 3 mg, 36.1%; 4 mg, 27.7%. During the study, the % pts with hypoglycemia was higher with GLIM vs. LINA (36.1 vs. 7.5%; p<0.0001); the % pts with hypoglycemia was higher with GLIM vs. LINA (36.1 vs. 7.5%; p<0.0001); the % pts with hypoglycemia was not increased with greater reductions in HbA1c in either group. In all 4-wk intervals, the % pts with hypoglycemia was lower with LINA vs. GLIM (Figure). In summary, LINA was associated with a lower risk for hypoglycemia than GLIM at all times, all dose levels, and regardless of change in HbA1c.

Empagliflozin Improves Glycemic Parameters and Cardiovascular Risk Factors in Patients With Type 2 Diabetes (T2DM): Pooled Data from Four Pivotal Phase III Trials

THOMAS HACH, JOHN E. GERICH, AFSHIN SALSAI, GABRIEL KIM, STEFAN HANTEL, HANS J. WOERLE, ULI C. BROEDL, INGELHEIM, Germany; ROCHester, NY; Biberach, Germany.

We analyzed pooled data from 2477 patients with T2DM (mean [SD] age 55.6 [10.2] years, HbA1c 7.99 [0.85], BMI 28.7 [5.5]) from four randomized, placebo-controlled Phase III trials that investigated empagliflozin (EMPA) 10 mg or 25 mg given for 24 weeks as monotherapy, add-on to metformin (MET), add-on to MEt + SU, or add-on to pioglitazone + MET. Effects on HbA1c, fasting plasma glucose (FPG), weight, systolic and diastolic blood pressure (SBP and DBP) were evaluated in the full analysis set (placebo [PBO]: n=825; EMPA 10 mg: n=831; EMPA 25 mg: n=821). Effects on lipids and uric acid were evaluated in all treated patients (PBO: n=825; EMPA 10 mg: n=830; EMPA 25 mg: n=822). Effects on SBP and DBP were also evaluated in patients with uncontrolled BP (SBP >130 mmHg or DBP >80 mmHg) at baseline (PBO: n=501, EMPA 10 mg: n=517, EMPA 25 mg: n=506).

EMPA significantly reduced HbA1c, FPG, weight, SBP, DBP and uric acid at week 24 vs. PBO. Reductions in SBP and DBP were more pronounced in patients with uncontrolled BP at baseline. Small increases in HDL- and LDL-cholesterol and small decreases in triglyceride levels were observed compared to EMPA vs. PBO.

In conclusion, in a pooled analysis of data from four Phase III trials, 24 weeks’ treatment with EMPA 10 mg or 25 mg provided clinically meaningful improvements in glycemic parameters, weight, and BP, with positive effects on uric acid and small effects on lipids.

**CLINICAL DIABETES/THERAPEUTICS**

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<th>Empagliflozin 10 mg</th>
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<tr>
<td><strong>HbA1c (%)</strong></td>
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<td></td>
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</tr>
<tr>
<td>Baseline† (SE)</td>
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<td>7.99 (0.03)</td>
<td>7.96 (0.03)</td>
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<td>Change from baseline at week 24 (SE)</td>
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<td>-0.70 (0.03)</td>
<td>-0.76 (0.03)</td>
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<tr>
<td>FG (mg/dL)</td>
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<td></td>
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<tr>
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<td>153.7 (1.3)</td>
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<td>-20.5 (1.6)</td>
<td>-23.2 (1.6)</td>
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<tr>
<td>Difference vs. placebo (95% CI)</td>
<td>-27.9</td>
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<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
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<td>28.7 (5.5)</td>
<td>28.7 (5.5)</td>
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<td>-4.3 (0.4)</td>
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<td>-3.8</td>
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<tr>
<td>SBP (mmHg)</td>
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<td>-1.5 (-1.9, -0.5)</td>
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<td>DBP (mmHg)</td>
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<td>Total cholesterol (mmol/L)†</td>
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<tr>
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<td>LDL cholesterol (mmol/L)†</td>
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<td>-0.11 (0.04)</td>
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<td>Uric acid (mmol/L)†</td>
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<tr>
<td>Baseline</td>
<td>287.6 (13.8)</td>
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Supported by: Boehringer Ingelheim Pharmaceuticals, Inc.

*ADA-Funded Research

For author disclosure information, see page LB66.
70-LB
Exploring the Potential of Dapagliflozin in Type 1 Diabetes: Phase 2a Pilot Study
ROBERT R. HENRY, JULIO ROSENSTOCK, ALEXANDROS-GEORGIOS CHALAMANDARIS, SREENEERANJ KASICHAYANULA, ALLYSON BOGLE, STEVEN C. GRIFFEN, San Diego, CA, Dallas, TX, Braine-l’Alleud, Belgium, Princeton, NJ
Insulin therapy for type 1 diabetes (T1D) is associated with weight gain and is often insufficient in maintaining glycemic control. Dapagliflozin (DAPA), an insulin-independent sodium glucose cotransporter 2 inhibitor that increases urinary glucose excretion, has shown antihyperglycemic efficacy in type 2 diabetes. This 2-week randomized, double-blind, placebo-controlled, Phase 2a study evaluated DAPA added to insulin in patients with suboptimally controlled T1D. Adults on stable insulin with HbA1c 7-10% (baseline mean 8.5%) were randomized to receive DAPA (1, 2.5, 5, or 10 mg) or placebo once daily for 14 days (Days -3 to 7 as inpatients). DAPA PK/PD, including continuous glucose monitoring (CGM) and 24-hour urine glucose, was assessed at Day 7. Seventy patients were randomized to treatment; 62 (88.6%) completed the trial. As expected, there was a dose-dependent increase in urine glucose with DAPA. CGM data suggested a potential for reduced glucogenic levels and diminished glucogenic variability with DAPA. Marked reductions in total daily insulin dosing at Day 7 were reported for DAPA 5 mg (-19%) and 10 mg (-18%). Hypoglycemia was common in all treatment groups; 1 event (DAPA 5 mg) was major and led to discontinuation. The incidence of AEs was 38.5% to 61.5%; there was 1 non-treatment-related serious AE (DAPA 5 mg) and no deaths. DAPA was generally well tolerated in this T1D population. Further studies to determine the potential benefit of DAPA as treatment of T1D are warranted.

72-LB
Novel Mechanism of Luseogliflozin, SGLT2 Inhibitor, Induced Beneficial Serum Urinary Acid-Lowering Effect
YUKIHIRO CHINO, HIROSHI ARAKAWA, YOSHISHIGE SAMUKAWA, SOICHI SAKAI, JUN-ICHI YAMAGUCHI, TAKEO NAKANISHI, IKUMI TAMAI, Kanazawa, Japan, Tokyo, Japan
Sodium glucose co-transporter 2 (SGLT2) inhibitors have known as lowering serum uric acid (UA) levels likely a class effect. This is the first report to elucidate a possible mechanism of the serum UA-lowering effect by SGLT2 inhibitors. Initially, we analyzed the laboratory data of healthy subjects and patients with type 2 diabetes mellitus dosed with Luseogliflozin. It was found that serum UA levels decreased to 0.5-1.8 mg/dL (0.5-25 mg single) and 0.4-0.8 mg/dL (0.5-10 mg daily for 12 weeks), respectively, from the baseline level (4.8-6.4 mg/dL). In healthy subjects, a negative correlation was observed between serum UA and urinary UA levels, and the renal UA clearance increased after dose of Luseogliflozin. These results suggest that the decrease of serum UA is due to the increased renal UA clearance. On single dosing in healthy subjects, UA excretion reached a plateau at a low dose of 1 mg. The increase of urinary UA excretion could be a treatment of urinary glucose (Glc) excretion, but not to the pharmacokinetics of Luseogliflozin. From these findings, we focused on Glc which concentrated in urine and Glc transporter 9 isoform 2 (GLUT9-iso2, SLC2A9) which expresses in the apical membrane of proximal tubule epithelial cells and transports both of UA and Glc. We examined the effect of Glc on the efflux of 14C-UA by Xenopus oocytes expressing GLUT9-iso2. As a result, higher Glc concentration of over renal threshold of Glc clearly stimulated 14C-UA efflux, suggesting that GLUT9-iso2 plays an important role on facilitation of UA secretion by exchangning urinary Glc with intracellular UA after dosing of Luseogliflozin. Therefore, GLUT9-iso2 could be a main player of serum UA lowering by SGLT2 inhibitors. In addition, this mechanism may also explain the relevance in appearance of urinary Glc and declining trend of serum UA in diabetes.

73-LB
Canagliflozin (CAN) is Effective and Generally Well Tolerated in Subjects With Type 2 Diabetes Mellitus (T2DM) and Stage 3 Chronic Kidney Disease (CKD)
VINCENT WOO, MELANIE DAVIES, DICK DE ZEEUW, GEORGE BAKIRS, VLADKO PERKOVIC, CRISTINA GASSMANN-MAYER, UJJWALA VIJAPURKAR, KEITH USISKIN, GARY MEININGER, Winnipeg, MB, Canada, Leicester, United Kingdom, Groningen, Netherlands, Chicago, IL, Sydney, Australia, Titusville, NJ, Rantin, NJ.
The efficacy/safety of the SGLT2 inhibitor, CANA, was assessed by a pooled analysis in subjects with T2DM from 4 randomized, placebo (PBO)-controlled studies (3 wk, 1 study; wk 26, 3 studies) with an eGFR ≥30 and <60 mL/min/1.73 m2 (N = 1,085) and in subgroups with eGFR ≥60 mL/min/1.73 m2 (N ≥40). Across populations, with CANA 100 and 300 mg reduced A1C, body weight, and systolic BP versus PBO across populations (Table); A1C and body weight changes were larger in subjects with eGFR ≥60 mL/min/1.73 m2. For the pooled CANA group, overall AE rates were higher than PBO across populations (eGFR ≥30 to <60: 74.7% vs. 70.4%; ≥60: 71.0% vs. 66.8%; ≥45: 81.5% vs. 78.4%); serious AE rates were higher with PBO than CANA and AE-related discontinuation rates were low across populations. Rates of osmotic diuresis-related AEs (e.g., polyuria, polydipsia) were higher with CANA than PBO in subjects with eGFR ≥30 and <60 (4.0% vs. 3.7%) and <45 mL/min/1.73 m2 (N ≥40). CANA 100 and 300 mg reduced A1C, body weight, and systolic BP versus PBO across populations (Table); A1C and body weight changes were larger in subjects with eGFR ≥60 mL/min/1.73 m2. For the pooled CANA group, overall AE rates were higher than PBO across populations (eGFR ≥30 to <60: 74.7% vs. 70.4%; ≥60: 71.0% vs. 66.8%; ≥45: 81.5% vs. 78.4%); serious AE rates were higher with PBO than CANA and AE-related discontinuation rates were low across populations. Rates of osmotic diuresis-related AEs (e.g., polyuria, polydipsia) were higher with CANA than PBO in subjects with eGFR ≥30 and <60 (4.0% vs. 3.7%) and <45 mL/min/1.73 m2 (4.8% vs. 2.6%). Rates of AEs related to intrarenal vasoconstriction (e.g., postural dizziness, orthostatic hypotension) were higher with CANA than PBO across populations (eGFR ≥30 to <60: 6.8% vs. 2.6%; ≥45: 5.9% vs. 3.4%; <45: 8.9% vs. 1.7%). Rates of renal-related AEs that were serious or led to discontinuation were low and similar across groups. In summary, in subjects with T2DM and Stage 3 CKD, CANA reduced A1C with a greater effect in subjects with higher eGFR, and was generally well tolerated.

For author disclosure information, see page LB66.
**Table. Summary of Efficacy Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Placebo</th>
<th>Empagliflozin</th>
<th>PBO</th>
<th>Difference vs PBO</th>
<th>PBO</th>
<th>Empagliflozin</th>
<th>250 mg</th>
<th>300 mg</th>
<th>Placebo</th>
<th>Empagliflozin</th>
<th>PBO</th>
<th>Empagliflozin</th>
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<tbody>
<tr>
<td>AUC 0-48</td>
<td>220 ± 33</td>
<td>269 ± 46</td>
<td>239 ± 33</td>
<td>289 ± 40</td>
<td>-60 ± 44</td>
<td>60 ± 45</td>
<td>70 ± 47</td>
<td>100 ± 50</td>
<td>110 ± 52</td>
<td>30 ± 40</td>
<td>40 ± 50</td>
<td>70 ± 53</td>
<td>100 ± 55</td>
</tr>
</tbody>
</table>

**Number of episodes per patient, n (%)**

<table>
<thead>
<tr>
<th>Level</th>
<th>n</th>
<th>Male, n/N (%)</th>
<th>Female, n/N (%)</th>
<th>Male, n/N (%)</th>
<th>Female, n/N (%)</th>
<th>Male, n/N (%)</th>
<th>Female, n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>57</td>
<td>14/37 (25.4)</td>
<td>43/118 (36.2)</td>
<td>16/28 (57.1)</td>
<td>10/24 (41.7)</td>
<td>11/20 (55.0)</td>
<td>7/14 (50.0)</td>
</tr>
<tr>
<td>Moderate</td>
<td>9 (1.1)</td>
<td>2/24 (8.3)</td>
<td>7/20 (35.0)</td>
<td>2/6 (33.3)</td>
<td>0/6 (0.0)</td>
<td>1/4 (25.0)</td>
<td>0/4 (0.0)</td>
</tr>
<tr>
<td>Severe</td>
<td>2 (0.2)</td>
<td>1/10 (10.0)</td>
<td>1/10 (10.0)</td>
<td>1/5 (20.0)</td>
<td>0/5 (0.0)</td>
<td>0/2 (0.0)</td>
<td>0/0 (0.0)</td>
</tr>
</tbody>
</table>

**Intensity of worst episode, n (%)**

<table>
<thead>
<tr>
<th>Level</th>
<th>n</th>
<th>Male, n/N (%)</th>
<th>Female, n/N (%)</th>
<th>Male, n/N (%)</th>
<th>Female, n/N (%)</th>
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<td>0/6 (0.0)</td>
<td>1/4 (25.0)</td>
<td>0/4 (0.0)</td>
</tr>
<tr>
<td>Severe</td>
<td>2 (0.2)</td>
<td>1/10 (10.0)</td>
<td>1/10 (10.0)</td>
<td>1/5 (20.0)</td>
<td>0/5 (0.0)</td>
<td>0/2 (0.0)</td>
<td>0/0 (0.0)</td>
</tr>
</tbody>
</table>

**Patients with events consistent with UTI leading to treatment discontinuation, n (%)**

<table>
<thead>
<tr>
<th>Level</th>
<th>n</th>
<th>Male, n/N (%)</th>
<th>Female, n/N (%)</th>
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<th>Female, n/N (%)</th>
<th>Male, n/N (%)</th>
<th>Female, n/N (%)</th>
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<td>0/2 (0.0)</td>
<td>0/0 (0.0)</td>
</tr>
</tbody>
</table>

Support: Boehringer Ingelheim Pharmaceuticals, Inc.
DR may provide a method to treat renalally impaired type 2 diabetic patients with metformin without increasing the risk of Met associated lactic acidosis.

**76-LB**

**Efficacy and Safety of Canagliflozin (CANA) in Older Subjects With Type 2 Diabetes Mellitus (T2DM)**

BRUCE W. BODE, ALAN SINCLAIR, STEWART HARRIS, UJJWALA VIJAPURKAR, CRISTIANA GASSMANN-MAYER, ALBERT FUNG, WAYNE SHAW, KEITH USISKIN, MEHUL DESAI, GARY MEININGER, Atlanta, GA, Luton, United Kingdom, London, ON, Canada, Rantou, NJ, Nauselle, NJ.

The efficacy and safety of CANA, an SGLT2 inhibitor, were evaluated using pooled data in subjects with T2DM from 4 randomized, placebo (PBO)-controlled, 26-week studies (N = 2,313) and analyzed by age: <65 y (n = 1,868, male, 49.1%; mean age, 52.8 y; A1C, 8.0%; body weight, 90.1 kg; eGFR, 90.8 mL/min/1.73 m2) or ≥65 y (n = 445; male, 51.5%; mean age, 69.3 y; A1C, 7.3%; body weight, 85.1 kg; eGFR, 76.9 mL/min/1.73 m2; CANA 100 and 300 mg reduced A1C, body weight, and systolic BP relative to PBO in subjects <65 and ≥65 y (Table); similar lipid changes were seen in both age groups. Overall adverse event (AE) rates were similar with CANA 100 and 300 mg and PBO in subjects <65 y (serious AEs: 2.5%, 2.5%, 3.3%; AE-related discontinuations: 3.3%, 3.2%, 2.8%), and higher with CANA 100 mg than CANA 300 mg or PBO in subjects ≥65 y (serious AEs: 6.9%, 3.4%, 3.6%; AE-related discontinuations: 8.8%, 5.4%, 4.4%). As in subjects <65 y, those ≥65 y who received CANA had higher rates of PBO than genital mycotic infections in women and men and osseous diuresis-related AEs; rates of AEs related to reduced intravascular volume were low in both age groups. UTI and renal-related AEs were similar in age groups in subjects ≥65 y. In summary, both CANA doses provided reductions in A1C and body weight and were generally well tolerated in older subjects with T2DM.

<table>
<thead>
<tr>
<th>Effect</th>
<th>PBO</th>
<th>CANA 100 mg</th>
<th>CANA 300 mg</th>
<th>PBO (n=1,868)</th>
<th>CANA 100 mg (n=1,623)</th>
<th>CANA 300 mg (n=1,623)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in A1C</td>
<td>-0.6 (0.9)</td>
<td>-0.9 (0.9)</td>
<td>-1.0 (0.9)</td>
<td>-0.9 (0.9)</td>
<td>-1.0 (0.9)</td>
<td></td>
</tr>
<tr>
<td>Change in body weight (kg)</td>
<td>-0.5 (2.2)</td>
<td>-1.1 (2.2)</td>
<td>-1.0 (2.2)</td>
<td>-0.8 (2.3)</td>
<td>-0.9 (2.3)</td>
<td></td>
</tr>
<tr>
<td>Change in systolic BP (mmHg)</td>
<td>-2.5 (4.0)</td>
<td>-3.4 (4.0)</td>
<td>-3.6 (4.0)</td>
<td>-2.5 (3.9)</td>
<td>-3.5 (3.9)</td>
<td></td>
</tr>
<tr>
<td>Change in total cholesterol (mg/dL)</td>
<td>-1.5 (40.0)</td>
<td>-6.0 (39.0)</td>
<td>-6.4 (39.0)</td>
<td>-1.4 (39.0)</td>
<td>-6.2 (39.0)</td>
<td></td>
</tr>
<tr>
<td>Change in LDL cholesterol (mg/dL)</td>
<td>-1.5 (4.0)</td>
<td>-6.0 (4.0)</td>
<td>-6.4 (4.0)</td>
<td>-1.4 (4.0)</td>
<td>-6.2 (4.0)</td>
<td></td>
</tr>
<tr>
<td>Change in HDL cholesterol (mg/dL)</td>
<td>0.4 (4.0)</td>
<td>0.7 (4.0)</td>
<td>0.7 (4.0)</td>
<td>0.4 (4.0)</td>
<td>0.7 (4.0)</td>
<td></td>
</tr>
<tr>
<td>Change in triglycerides (mg/dL)</td>
<td>1.2 (4.0)</td>
<td>3.7 (4.0)</td>
<td>4.0 (4.0)</td>
<td>1.2 (4.0)</td>
<td>3.7 (4.0)</td>
<td></td>
</tr>
</tbody>
</table>

Results: 108 patients, with the mean age of 51 years, HbA1c 7.7%, BMI 26.6 kg/m² were enrolled. 54 patients were assigned to metformin group, the other in acarbose group. Baseline characteristics of both groups were even. After 24-week treatment, glucose control improved significantly in both metformin group and acarbose group (HbA1c=1.24% and 1.28%.; fasting plasma glucose: 2.09 mmol/L and 1.53 mmol/L; 0.5h postprandial glucose: 2.27 mmol/L and 2.87 mmol/L; 2h postprandial glucose: 3.18 mmol/L and 2.35 mmol/L respectively). The early-phase insulin secretion index ΔINS/ΔG30 was improved only in acarbose group; Body weight decreased (metformin: -2.5 kg vs. acarbose: -2.6 kg); Decrease in fasting and 0.5h postprandial glucose.

**Conclusion:** In newly diagnosed type 2 diabetic patients metformin and acarbose have similar effect on improving glucose control and decreasing body weight as monotherapy. It seemed that acarbose may improve islet β cell function better than metformin, representing by the greater decrease of fasting and 0.5h postprandial glucose. Although the improvement of A30/A530 can decrease postprandial glucagon, further studies are needed to explore the related pathophysiology mechanism.

**77-LB**

**Effect of Metformin and Acarbose in Islet a Cell Function in Overweight and/or Obese Patients With Newly Diagnosed Type 2 Diabetes**

WEIPING SUN, GUOHUA LI, LIZHEN LIAO, YING WANG, Xiangtan, China.

Aims: To explore the effect of metformin and acarbose on islet β cell function in overweight and/or obese patients with newly diagnosed type 2 diabetes.

Materials and methods: Drug naïve patients with newly diagnosed type 2 diabetes, whose HbA1c between 6.3% and 9%, BMI greater than 24 kg/m² were enrolled. Patients were randomly assigned to metformin (1.5g/d) and acarbose (100mg tid) group for a predictive follow-up period of 24 weeks. Plasma glucose, insulin and glucagon at 0, 0.5h, and 2 h after the meal and HbA1c were measured at baseline and 24 weeks. Results: 108 patients, with the mean age of 51 years, HbA1c 7.7%, BMI 26.6 kg/m² were enrolled. 54 patients were assigned to metformin group, the other in acarbose group. Baseline characteristics of both groups were even. After 24-week treatment, glucose control improved significantly in both metformin group and acarbose group (HbA1c=1.24% and 1.28%; fasting plasma glucose: 2.09 mmol/L and 1.53 mmol/L; 0.5h postprandial glucose: 2.27 mmol/L and 2.87 mmol/L; 2h postprandial glucose: 3.18 mmol/L and 2.35 mmol/L respectively). The early-phase insulin secretion index ΔINS/ΔG30 was improved only in acarbose group; Body weight decreased (metformin: -2.5 kg vs. acarbose: -2.6 kg); Decrease in fasting and 0.5h postprandial glucose.

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**78-LB**

**Resveratrol Synergy in Pre-Diabetes**

JANIE LIPPS, SCOTT HAGAN, MICHELLE CLARK, DIANNE DAVIS, LIBBY STONE, LIBBY SURVANT, WANDA SNEAD, KEVIN NISWENDER, Nashville.

Impaired glucose tolerance (IGT) and fasting hyperglycemia (FH) are risk factors for diabetes. Caloric restriction sensitizes to insulin, in part via SIRT1. Resveratrol (Res) is a SIRT1 activator shown to increase insulin sensitivity. The utility of resveratrol in metabolic disease has been limited by the large doses required (>1-6 g/d) and oral bioavailability. Leucine (Leu) and metabolites such as β-Hydroxy β-Methylbutyrate (HMB) also stimulate SIRT1 activity. Resveratrol in combination with either leucine or HMB left-shifts in vitro SIRT1 activity dose-response curves and reduces the EC50 from micromolar to nanomolar concentrations. In rodent resveratrol-leucine (ResLeu) and resveratrol-HMB (ResHMB) improved metabolic function.

Humans with FH (100-125 mg/dL) or IGT (75 g OGTT, 2 hour between 140-199 mg/dL) were randomized to receive resveratrol (50 mg/leucine (1.11 g) (ResLeu), resveratrol (50 mg)/HMB (500 mg)(ResHMB, n=11) or placebo (n=12) twice daily (bid) in a blinded fashion. Primary outcomes were derived from change in glucodynamic responses on 75 g OGTT from baseline to 28 days of treatment: change glucose area under the curve (AUC), insulin AUC, and disposition index (0-120 insulin AUC/0-120 glucose AUC * 1/fasting insulin). Fasting plasma IRISIN levels were also assessed by commercial ELISA assay.

Relative to placebo, ResHMB significantly improved the change in glucose AUC (2384 placebo vs. -273 HMBRes relative units; p=0.001). ResLeu significantly lowered the change in insulin AUC relative to placebo (2871 placebo vs. -3803 LeuRes; p=0.02) while ResHMB trended to do so (1037; p=0.09). Finally, relative to placebo, both LeuRes (p=0.03) and HMBRes (p=0.01) generated larger changes in disposition index. Finally, while neither placebo nor HMBRes changed fasting insulin levels, LeuRes increased circulating insulin by nearly 50% from 340±60 to 502±82 mg/dL (p=0.02). Thus, capitalizing on synergistic properties of HMB and Leu with resveratrol may be an attractive nutraceutical strategy to improve metabolism.

**Supported by:** Janssen Research & Development, LLC.
15% (p<0.0001) (1.25% in overall study). LDL changes were concordant in a limited subset with available LDL samples. Mean BP was unchanged, with a lower rate of hypertensive adverse events (AEs) (8%) than that reported historically for ESAs. Overall, FG-4592 was well tolerated, with no drug-related SAEs. In diabetic subjects with CKD, FG-4592 corrected/maintained Hb without IV Fe and with a favorable cardiovascular risk profile. This suggests a distinct pharmacological and clinical profile that may provide a safer and more convenient therapy for treatment of CKD anemia. Phase 3 trials are in progress.

**80-LB**

**Reversal of Suppressed Estrogen Receptor α and Anti-Oxidant Levels: Possible Benefits for Cardiovascular Disease (CVD) in Diabetic Kidney Disease (DKD)**

GARY E. STRIKER, ELENA M. YUBERO-SERRANO, SHOBHA SWAMY, SHARON J. ELIOTT, WIZJING CAI, XUE CHEN, ELIZABETH MICKEE, ANITA KALAJ, FRIEDERIKE KRUCKELMANN, LAUREN TIRIN, KAMALA MANTHA, ELIOT J. RAYFIELD, JAIME URIBARRI, NIKOLAS HARBORD, RONALD TAMLER, GRISHMA PARIKH, AGUSTIN BUSTA, LEONID PORETSKY, MARK WOODWARD, HELEN Vlassara, New York, NY, Miami, FL, Baltimore, MD

The expression/actions of estrogen receptor (ER) are reduced in post-menopausal females, high oxidative stress (OS) and inflammation (Infl) and may underlie CVD risk in DKD. Advanced glycation endproducts (AGEs) cause of increased OS/Infl in DKD. Sevelamer carbonate (SevCarb), blocks AGE absorption from food, reduces OS and Infl, restores cellular anti-OS defenses and improves glucose and lipids in DKD. We asked if SevCarb increases ER expression in T2D. Subjects with HbA1c >6.5, eGFR 25-80ml/min/1.73m², and type 2 diabetes (T2D) were randomized to SevCarb (4800mg/day) or CaCO₃ (1950mg/day) for 6 months in an intention-to-treat trial. At 3 months there was a robust increase of ERf levels and NrF2 and AGER1 (markers of anti-oxidant and anti-AGE defenses). Full-length RAGE, a pro-oxidant receptor was reduced and there was a strong trend for reduction of TNFR1, a marker of risk for CVD and progression of DKD.

Sevelamer Carbonate (n = 56) Calcium Carbonate (n = 50)

| Mononuclear Cell | Mean | SD | p value | Mean | SD | p value | p value *
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ER α</td>
<td>1.503</td>
<td>3.515</td>
<td>0.003 -0.358</td>
<td>0.941</td>
<td>0.011</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>NrF2</td>
<td>0.497</td>
<td>1.662</td>
<td>0.025 -0.131</td>
<td>0.981</td>
<td>0.350</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>AGER1</td>
<td>0.284</td>
<td>1.001</td>
<td>0.035 -0.045</td>
<td>0.446</td>
<td>0.532</td>
<td>0.028</td>
<td>0.028</td>
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<tr>
<td>TNFR1</td>
<td>-0.359</td>
<td>1.841</td>
<td>0.144 0.331</td>
<td>1.491</td>
<td>0.168</td>
<td>0.052</td>
<td>0.052</td>
</tr>
<tr>
<td>RAGE</td>
<td>-0.100</td>
<td>0.705</td>
<td>0.086 0.419</td>
<td>1.413</td>
<td>0.059</td>
<td>0.011</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*Statistical significance between deltas of both treatments (3 months minus baseline).

In conclusion, ERα restoration after sevelamer carbonate treatment may underlie and reduce OS and inflammation and other anti-oxidant defenses in DKD. It also decreased markers of progression in DKD and other risk factors for CVD. A longer and larger clinical trial is necessary to determine if these changes affect clinical outcomes.

**82-LB**

**Foot-In-Wallet Disease: Tripped Up by "Cost Saving" Reductions**

GRANT H. SKREPNEK, JOSEPH L. MILLS, DAVID ARMSTRONG, Tucson, AZ

The purpose of this study was to assess changes in inpatient-related outcomes associated with diabetic foot infections (DFIs) among adult beneficiaries of Arizona Medicaid (Arizona Health Care Cost Containment System, AHCCCS) following the 2009 announcement of reimbursement coverage cancellation to podiatric physicians that was implemented in 2010 and intended to reduce costs (Arizona 49th Legislature, 7th Special Session; SB1003, HB 2003). Inpatient discharge records from the Agency for Healthcare Research and Quality Healthcare Cost and Utilization Project were used in this retrospective cohort study spanning 2006-2010. Inclusion criteria involved cases of all-listed diagnoses of inpatient DFIs among AHCCCS beneficiaries ≥18 years of age. An autoregressive integrated moving average (ARIMA) interrupted time-series was used to estimate post-announcement changes in inpatient admissions, charges, length of stay, and severe aggregate outcomes (SOAs) involving mortality, amputation, sepsis, or surgical complications. Across the 5-year time period, 8,485 inpatient cases of DFIs among adult AHCCCS beneficiaries were observed, averaging 64.1 ± (20.4) cases each month. Per case, the average length of stay was 7.0 (±5.7) days and mean charges were $54,046 ± (64,368), amounting to a total of $228 million (USD 2012). SOAs occurred in 31.1% of cases. Following the announcement of changes in AHCCCS podiatric service coverage, results of the interrupted time-series analysis indicated 56.3% more admissions, 42.1% longer lengths of stay, 52.1% higher inpatient charges, and 88.1% more SOAs (p<0.001). This evaluation of the cancellation of podiatric services within AHCCCS suggests a marked worsening of patient care in terms of increased inpatient admissions, lengths of stay, charges, and severe clinical outcomes. Restricting access to preventive care among people with diabetes may manifest in serious unintended consequences, particularly among the poor and underserved.

**83-LB**

**The Cost of Diabetes: Escalating Trends and Cost Drivers**

NINA RAN, JESSICA DONG, MICHAEL DOUGAN, RICHARD KAHN, MARISSA H. LYNN, LISA ROTENSTEIN, MARK YARCOHA, KELLY CLOSE, San Francisco, CA, Boston, MA, Chapel Hill, NC, Philadelphia, PA

Diabetes-related costs place a significant burden on the US healthcare system, accounting for 13% of total healthcare spending in 2012. The purpose of this study was to analyze trends in costs and to determine which components of diabetes care have made the greatest impact.

We analyzed published reports that used data derived from Federal databases; one report with 2012 data was published only last month. The cost of diagnosed diabetes in 2012 ($245 billion) continues to rise inexorably from previous years: 2007: $174 billion; 2002–$132 billion; 1997–$98 billion; and 1992–$92 billion. Costs have grown at a faster rate from a higher base, rising 41% (27% inflation adjusted) from 2007 to 2012 compared to 32% (15%) from 2002-2007, 35% (20%) from 1997-2002, and 7% (7%) from 1992-1997. Accounting for inflation and the number of diagnosed cases of diabetes, annual total costs per capita has steadily decreased from $20,700 in 1992 to $11,000 in 2012. Per capita institutional healthcare expenditure (i.e., inpatient hospital days, nursing/residential care, hospice care) attributed to diabetes has shown a parallel 54% decline from $8,890 in 1992 to $4,070 in 2012. This has been partially offset by a steady increase in per capita costs for outpatient care from $1,391 in 1992 to $3,820 in 2012; while a large absolute increase, the per capita outpatient cost remains far less than that of inpatient care. These data suggest a considerable reduction in the cost of expensive diabetes complications that has been offset by improvements in outpatient services. The cost of the latter consists mainly of medicines and supplies, as well as provider-patient encounters. Thus, the greater and appropriate use of

**HEALTH CARE DELIVERY—ECONOMICS**

**81-LB**

**Cost-Effectiveness of an Internet-Delivered Lifestyle Intervention in a High Cardiovascular Risk Population in Southwestern Pennsylvania**

SHIHCHEN KUD, JANICE C. ZGIBOR, KENNETH J. SMITH, KATHLEEN M. MCFIGUE, RACHEL HEIS, TINA BHARGAVA, CINDY L. BRYCE, Pittsburgh, PA, Kent, OH

While Internet-delivered lifestyle interventions are effective for weight control, their cost-effectiveness for diabetes prevention and risk reduction in primary care settings is unclear. A Markov state-transition model was developed to estimate the cost-effectiveness of using an Online adaptation of the Diabetes Prevention Program lifestyle intervention (ODPP) compared to usual care to reduce metabolic risk in an overweight/obese cohort (mean age 53) over a 10-year time horizon. Intervention costs and weight change outcomes were obtained from a prospective ODPP pilot study, other costs, disease progression data, and utilities were drawn from published reports. In the model, diabetes risk was a function of weight change with/without the program. Compared to usual care, the base case incremental cost-effectiveness ratio (ICER) of the ODPP in our pilot study cohort (30% diabetic) was $14,351 and $23,331 per quality-adjusted life-year (QALY) gained from a health system and societal perspective, respectively. In a hypothetical cohort without diabetes, the ICER was $7,777 and $18,263 per QALY gained, respectively. When excluding website-related costs (licensing, maintenance, and technical support), the ODPP was cost-saving (health system) or cost $14,143 per QALY gained (societal). Results were robust in sensitivity analyses, but enrolling cohorts with lower annual risk of developing diabetes (<1%), enrolling fewer participants (<16), or increasing the hourly cost (-30%) in 2012, per-participant time required (>1.44 hours) for ODPP technical support could increase the ODPP ICER to be >$20,000 per QALY gained. In probabilistic sensitivity analyses, the ODPP was cost-effective in 20-58% of model iterations using an acceptability threshold of $20,000, 73-92% at $50,000, and 95-99% at $100,000/QALY. The ODPP delivered in primary care settings for weight management appears to be a economically reasonable intervention.

**Supported by: USAMRAA (W81XWH-04-2-0038)**
84-LB
Resource Utilization and its Impact on the Inpatient Diabetes Management in the Non-Critical Care Units

VIVEK BANSAL, NOORMUHAMMAD ABBASAKODOR, EUNICE Y. CHUANG, TARANEER K. PAWAR, OSAMA HAMDY, Boston, MA

With the growing number of admitted patients with diabetes and the amplified hospitalization cost, it became quite important to explore the best model of resource utilization for inpatient diabetes management. In this double-blind study, we evaluated two models of care at an academic center one offered by primary service team (PST) vs. another by specialized diabetes team (SDT). A total of 766 admissions to non-critical care units were evaluated; 392 met eligibility criteria (type 2 DM for >3 months and non-pregnant). From them, 262 were matched in 1:1 ratio based on the mean of the first four blood glucose values (4BG) with equal proportions from surgical unit (45%). Baseline demographics for PST vs. SDT included: mean age 68.9 ± 11.0 vs. 59.0 ± 14.9 years (p=0.01), female gender 41.2% vs. 45.0% (p=0.5), mean 4BG values 202.2 ± 52.5 g vs. 203.1 ± 60.6 mg/dL (p=0.5), and mean of most recent A1C 7.3 ± 1.3 vs. 8.7 ± 2.1% (p=0.001). Overall 30-day readmission percentage was 22.1% with relative percentages of 21.4% in PST vs. 22.9% in SDT (p=0.089), and rates of 30-day readmissions were 1.3 ± 0.5 vs. 1.1 ± 0.3 (PST vs. SDT, p=0.107). Length of stay (LOS) was found to be significantly shorter for the PST group (4.8 ± 1.9 vs. 5.6 ± 2.3 days, p=0.038). Surgical subgroup analysis showed shorter LOS for PST (4.8 ± 1.9 vs. 5.6 ± 2.2 days, p=0.04), but the 30-day readmission was 20% with a frequency of 1.5 in PST and 1.1 in SDT (1.5 ± 0.7 vs. 1.1 ± 0.3 times, p=0.026). No differences were seen in the medicine subgroup for all outcomes. In interpreting these results, we should appreciate that the care for sicker patients with higher A1C was also reflected in the observed wide variability of BG levels during hospitalization. In conclusion: utilizing PST for inpatient diabetes management in non-critical care unit is better resource utilization and is advisable. Reserving SDT to only sicker patients with higher A1C and for patients admitted to surgical non-critical care units may significantly impact the 30-day readmission rate.

85-LB
Improved Real-World Glycemic Outcomes With Liraglutide versus Other Incretin-Based Therapies in Type 2 Diabetes

MITCHELL DEKOVEN, WUN CHAN LE, JONATHAN R. BOUCHARD, MARJAN EDWY, JAKOB LANGER, Alexandria, VA, Princeton, NJ

Outcomes on A1C in clinical practice were retrospectively compared among patients ≥18 years with type 2 diabetes (T2D) who initiated liraglutide (LIRA), exenatide (EXEN) or sitagliptin (SITA), including sitagliptin/metformin, using the IMS integrated data warehouse. Patients were required to be GLP-1 and DPP-4-naive during a 6-month pre-index period, with ≥1 prescription for LIRA, EXEN or SITA between January 2010 and December 2011 (index period). Only patients who were persistent on their index treatment (LIRA, EXEN or SITA) regimens for 180 days post-index were included in the analysis. Patients who were pregnant or used insulin in the pre- or post-index periods were excluded. Changes in A1C from baseline (45 days pre-index through 7 days post-index) to follow-up (180 days post-index ± 45), and the proportion of patients reaching A1C<7%, were examined using multivariable regression methods to adjust for confounding factors such as age, gender, region, comorbidities, baseline A1C and background antiabetic treatment. At follow-up, changes in A1C (%-point) and the proportion of patients achieving A1C<7% were significantly greater with LIRA compared with EXEN and SITA (Table). These real-world data suggest that initiating LIRA was associated with significantly greater reductions in A1C and improved glycemic goal attainment than EXEN and SITA among patients with T2D.

<table>
<thead>
<tr>
<th>LIRA</th>
<th>EXEN</th>
<th>SITA</th>
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<tr>
<td>Sample size</td>
<td>234</td>
<td>182</td>
</tr>
<tr>
<td>Baseline A1C</td>
<td>7.8%</td>
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<td>Change in A1C from baseline</td>
<td>-0.8%</td>
<td>-0.75%</td>
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<tr>
<td>Pct. achieving A1C&lt;7%</td>
<td>64%</td>
<td>54%</td>
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Supported by: Novo Nordisk, Inc.

86-LB
Optimal Glycemic Control Improves Clinical Outcomes in Patients With Type 2 Diabetes

BORIS DRAZNIN, YUNJIAO WANG, STACEY A. SEGGELKE, MATTHEW HAWKINS, JOANNA GIBBS, NEDA RASOULI, CECILIA L. WANG, Aurora, CO, Denver, CO

While the importance of glycemic control is well established for patients with diabetes hospitalized for surgical problems, it has not been supported by clinical studies for patients with diabetes hospitalized on the medical floors. We conducted a retrospective study of 378 patients with Type 2 diabetes admitted for cardiac or infectious disease (ID) diagnosis between Sep 1, 2011 and August 1, 2012. Exclusion criteria included Type 1 diabetes, admission to the intensive care unit (ICU), hospital stay shorter than 3 days, and daily glucocorticoid dose greater than 20 mg. The primary composite outcome included death during hospitalization, ICU transfer, initiation of enteral or parenteral nutrition, line infection, deep vein thrombosis, pulmonary embolism, rise in plasma creatinine by 1 or over 2 mg/dL, new infection, an infection lasting for more than 20 days, and re-admission within 30 days and between 1 and 10 months after discharge. Patients were stratified by mean blood glucose (BG) level: Group 1 had mean BG of less than 180 mg/dL (n=286, mean BG 142±23 mg/dL) while Group 2 had mean BG levels greater than 180 mg/dL (n=92, mean BG 216±34 mg/dL, p<0.0001). Group 2 had a 45% higher occurrence of the primary outcome (p<0.0004). The rate of unfavorable events was greater in cardiac and ID patients with worse glycemic control (Group 2). Consultation by the inpatient Glucose Management Team (GMT) in Group 2 patients resulted in a lower rate of composite outcome (p=0.03), less variability in blood glucose levels (p=0.05), and an increase in the proportion of BG levels in the acceptable glycemic range of 100-200 mg/dL (65% vs. 12% in GMT-treated patients vs. 36%±15% in non-GMT-treated patients, p<0.002). These data demonstrate that poor glycemic control is associated with worse outcomes in hospitalized medical patients with Type 2 diabetes. The involvement of a specialized GMT improves outcomes in these patients by reducing glycemic variability and increasing the proportion of BG values within an acceptable range.

87-LB
Validity of the 13C-Glucose Breath Test as a Screening Tool to Identify Metabolic Syndrome in Mexican Pediatric Population

ALEJANDRA SALAS-FERNÁNDEZ, JORGE MALDONADO-HERNÁNDEZ, AZUCENA MARTÍNEZ-BASILA, GABRIEL MARTÍNEZ RAZO, Distrito Federal, Mexico

Metabolic syndrome (MS) is an important risk factor in the pediatric population for the early onset of type 2 diabetes mellitus and cardiovascular disease. New non-invasive tools are required to identify MS in this population to prevent chronic diseases in the future; the 13C-breath test shows different advantages: simplicity, portability, sample stability and ease administration. The aim of this cross-sectional study was to determine the validity of the 13c-glucose breath test to identify MS in Mexican pediatric population. Children between 10 and 16 years old were recruited and divided in two groups: the control group (n=31) included subjects without any component of MS and the MS group (n=33) consisted of subjects with MS according to the modified criteria proposed by the International Diabetes Federation in pediatric population. A blood sample was taken to determine glucose, triglycerides and HDL-cholesterol concentrations. The waist circumference and blood pressure were determined. After the ingestion of 1.75 g/Kg of glucose and 1.5 mg of 13C-glucose breath test to identify MS in Mexican pediatric population was performed. From them, 262 were matched in 1:1 ratio based on the mean of the first four blood glucose values (4BG) with equal proportions from surgical unit (45%). Baseline demographics for PST vs. SDT included: mean age 68.9 ± 11.0 vs. 59.0 ± 14.9 years (p=0.01), female gender 41.2% vs. 45.0% (p=0.5), mean 4BG values 202.2 ± 52.5 g vs. 203.1 ± 60.6 mg/dL (p=0.5), and mean of most recent A1C 7.3 ± 1.3 vs. 8.7 ± 2.1% (p=0.001). Overall 30-day readmission percentage was 22.1% with relative percentages of 21.4% in PST vs. 22.9% in SDT (p=0.089), and rates of 30-day readmissions were 1.3 ± 0.5 vs. 1.1 ± 0.3 (PST vs. SDT, p=0.107). Length of stay (LOS) was found to be significantly shorter for the PST group (4.8 ± 1.9 vs. 5.6 ± 2.3 days, p=0.038). Surgical subgroup analysis showed shorter LOS for PST (4.8 ± 1.9 vs. 5.6 ± 2.2 days, p=0.04), but the 30-day readmission was 20% with a frequency of 1.5 in PST and 1.1 in SDT (1.5 ± 0.7 vs. 1.1 ± 0.3 times, p=0.026). No differences were seen in the medicine subgroup for all outcomes. In interpreting these results, we should appreciate that the care for sicker patients with higher A1C was also reflected in the observed wide variability of BG levels during hospitalization. In conclusion: utilizing PST for inpatient diabetes management in non-critical care unit is better resource utilization and is advisable. Reserving SDT to only sicker patients with higher A1C and for patients admitted to surgical non-critical care units may significantly impact the 30-day readmission rate.

LIRA | EXEN | SITA
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Supported by: CONACYT

For author disclosure information, see page LB66.
A Community-Based Intervention for Diabetes Risk Reduction in Inner-City Obese Adolescents
MARY SAVOYE, SONIA CAPRIO, JAMES DZIRUA, ANNE CAMP, FANGYONG LI, MELISSA SHAW, GRACE KIM, WILLIAM V. TAMBORLANE, New Haven, CT

Childhood obesity has been accompanied by an increasing prevalence of type 2 diabetes (T2D), particularly in minority children. 20-30% of obese youth have "pre-diabetes" a precursor to diabetes marked by insulin resistance, β-cell dysfunction and impaired glucose tolerance (IGT). The Diabetes Prevention Program demonstrated T2D could be prevented/delayed by lifestyle modification in adults with pre-diabetes, but efficacy of similar interventions in youth has not been established. Therefore, we evaluated the effects of the Bright Bodies (BB) Healthy Lifestyle Program on 2-hr OGTT glucose in comparison to children receiving standard care of with a parallel-group randomized controlled trial comparing BB with standard clinical care (CC) in obese adolescents (10-16 y) with elevated OGTT 2-hr blood glucose (130-199 mg/dl) from an ethnically-diverse population.

OGTTs, including anthropometric and metabolic syndrome assessments, were conducted at baseline and 6 mos. Children attended BB twice weekly for exercise and nutrition/behavior modification and CC group received clinical care from their pediatrician. Primary outcome was change in 2-hr OGTT glucose and % conversion from elevated 2-hr blood glucose to non-elevated (<130 mg/dl) 2-hr blood glucose. Changes in outcomes were compared between groups using a mixed model with covariate adjustment for baseline outcome and multiple imputation for missing data. Least squares means and 95% CIs were estimated for changes in outcomes.

Reductions in 2-hr glucose were more favorable in BB compared to CC (27.2 vs. -10.1 mg/dl; df=17.1, 95% CI, p= 0.005). Moreover, greater conversion to <130 mg/dl 2-hr glucose occurred in BB than CC (p=0.03). Other insulin sensitivity indices were significantly improved, as well as the prevalence of metabolic syndrome in the BB group (p=0.004).

Compared to standard of care, the Bright Bodies Program is a more effective means of reducing the risk of T2D in obese adolescents with elevated 2-hr blood glucose. Supported by: NCRR, NIH.

CLINICAL DIABETES/THERAPEUTICS

88-LB

89-LB

Even If Not Macroscopic, Children of Diabetic Mothers Tend to be Overweight at Age 17
ZVI LARON, ALON FARFEL, RODA RABINOWITZ, TAMAR LARON-KENET, MOSHE HOD, RONY CHEN, GADI KAMPINO, DORIT TZUR, ESTELLA DERAZNE, ZVI LARON, ALON FARFEL, RONA RABINOVITZ, TAMAR LARON-KENET, MOSHE MELISSA SHAW, GRACE KIM, WILLIAM V. TAMBORLANE, NEW HAVEN, CT

We have shown previously that newborns born long and/or overweight tend to be tall and obese at age 17, however data on adult height and weight of children born to diabetic mothers is scant. We studied all full term neonates of diabetic mothers born between 1987 and 1993 in the Rabin Medical Center in Israel. The control group consists of neonates born to healthy mothers during the same period. The birth length and weight and height, weight and BMI at age 17 as measured at the recruitment centers of IDF, were compared between groups. There were 447 children (235 males) from mothers with pre-gestational diabetes (GDM), 97 children (51 males) from mothers with gestational diabetes (PGDM) and 544 children (265 males) in the control group. At age 17 we were able to track 674 adolescents (61.95% of the original groups). The main findings are shown in the Table. There was no significant difference in birth weight or length between the groups. Our study shows that improved outcomes of metabolic syndrome in the BB group (p=0.004).

Compared to standard of care, the Bright Bodies Program is a more effective means of reducing the risk of T2D in obese adolescents with elevated 2-hr blood glucose. Supported by: NCRR, NIH.

90-LB

Metabolites as Novel Biomarkers for Childhood Obesity-Related Traits in Mexican American Children
VIDYA S. FAROOK, LAVANYA REDDIVARI, GEETHA CHITTOOR, SOBHA PUPPALA, RECTOR ARYA, SHARON P. FOWLER, BIRUNDA MOHAN, KELLY J. HUNT, JOANNE E. CURRAN, ANTHONY G. EDMONZIE, DONNA M. LEHMAN, CHRISTOPHER P. JENKINS, JANIE L. LYNCH, RAHAL A. DEFRONZO, JOHN BLANGERO, DANIEL E. HALE, RAVINDRANATH DUGGIRALA, JAIRAM VANAMALA, SAN ANTONIO, TX

Childhood obesity has become a major public health issue and has spurred continued efforts to understand the mechanisms influencing it. Although newer approaches have identified several metabolites associated with obesity, there is a paucity of such studies in ethnic minorities including Mexican American (MA) children. We therefore, attempted to identify systemic metabolites, reflective of metabolic processes, associated with obesity by performing global serum metabolite screening in 14 obese, 13 overweight and 15 normal MA children (6-17 years), using the UPLC system with Micromass Q-ToF Micro mass spectrometer. Among the ~850 metabolites detected, we identified 14 metabolites with significant (P<0.01) differences between normal and obese weight children by ANOVA. Higher levels of bradykinin, phosphocholine and phosphotidylethanolamine and lower levels of L-thyronine, naringenin, indole-3- propionic acid, 2-methylbutyrylcarnitine, 3-hydroxyquinine, 1, 2-dihydroxy-23,24,25,26,27-pentanovitomin D3, lysophosphatidylcholine (18:1), calicoflor B, diglyceride, malvidin3-(6-acetyl glucoside) and ileoelic acid were found in obese children. After adjustment for multiple testing significance threshold = P<5x10-3, L-thyronine (18:2 fold), bradykinin (4.2 fold), and naringenin (1.3 fold) remained significant. We examined associations between these metabolites and 6 cardio-metabolic traits: waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), HOMA-IR, triglyceride (TG), and HDL-cholesterol (HDL-C) using SOLAR. Interestingly, except for 2-methylbutyrylcarnitine, all the metabolites were significantly (P<0.05) associated with one or more of the obesity-related traits. For example, L-thyronine was negatively correlated with WC, SBP, DBP, HOMA-IR, TG and positively correlated with HDL-C. To our knowledge, this is the first study, albeit pilot to identify these novel biomarkers of childhood obesity.

PEDIATRICS—TYPE 1 DIABETES

91-LB

Performance of a New CGM System in Youths With Diabetess: Comparisons With SMBG and YSI
LORI M. LAFFEL, BRUCE BUCKINGHAM, DAVID PRICE, KATHERINE NAKAMURA, TIMOTHY S. BALEY, MARK DANIELS, DAVID R. LILJENQUIST, PETER CHASE, BOSTON, MA, STANFORD, CA, SAN DIEGO, CA, ESCONDIDO, CA, CHINO, CA, IDAHO FALLS, ID, DENVER, CO

We studied the performance of the new Dexcom G4 PLATINUM (DG4P) CGM system in 176 youth (age 2 - 17, mean 11.5±3.9 yrs, 54% male) using an in-clinic session on sensor days 1, 4, 7 and in youth ≥ 6 yrs, the session lasted up to 6 hrs in which “arterialized” (via a heating pad over an arm) venous YSI samples q15 mins and SMBG samples q30 mins were collected. In youth <6 yrs, there was only SMBG samples q30 mins collected for up to 4 hrs. Compared to SMBG, the DG4P MARD was 15% (n= 16318), 14% on the abdomen, and 16% on the buttocks. Accuracy was similar when supplemental topical adhesives to secure the sensor or during different time of the day. The MARD was 17% in ages 2-5, 16% in ages 6-12, and 15% in ages 13-17. The MARD of the sensor decreased from 15% on day 1 to 12% on day 7. In a comparison of SMBG to YSI, MARD was 13% (n= 1298), higher than expected. As a result, the DG4P MARD using YSI reference was 17% (n=2922). After adjustment for the bias of venous and capillary glucose values in simulated post-hoc analyses, the MARD of CGM to YSI reduced to 15%, similar to the SMBG reference. The precision analysis showed a CV of 7% overall, 6% on the abdomen, and 7% on the buttocks. 95% of sensors lasted 7 days and 95% of possible data displayed on the receiver. There were no serious or unanticipated device events, sensor fractures, or infections. This is largest pediatric CGM performance study to date, and included young children 2-5. DG4P performance compared favorably to the CGM system currently approved for pediatric use. There were minor differences at wear sites and across age groups. Performance of SMBG and CGM in comparison to YSI was likely impacted by arterialization challenges in youth. After adjustment, the DG4P performance to YSI was similar to SMBG.

For author disclosure information, see page LB66.

ADA-Funded Research

For full effects, see page LB66.

supported by: NIH, ADA-Funded Research.
Iowa City children With type 1 diabetes (T1D) are used to assess insulin secretory reserve levels, impaired glucose tolerance in the proinsulin molecule. The measurement of C-peptide in serum and urine is designed as a two-step reaction utilizing a pair of antibodies. One antibody is conjugated with an ECL signal (MSD Sulfo tag™) molecule (capture) is immobilized on the carbon surface of the MSD 96-well plate while the other antibody is conjugated with a biotin hapten that is captured from the sample by a pair of biotinylated antibodies immobilized on a magnetically stirred carbon coated graphite/stainless steel electrode. A complex emits light upon application of electrochemical stimulation initiated at the electrode surfaces of the microplate.

The wide dynamic range ECL technology allowed an assay range of 0.004 ng/mL to 8 ng/mL. The lower limit of quantitation (inter-assay CV 20.4% and bias 2.3%) was 0.004 ng/mL. Inter-assay CV for serum samples at 1.5, 2.7 and 4.8 ng/mL was 5.0%, 3.2% and 6.9% respectively. Dilutional linearity of high C-peptide serum samples provided mean recoveries of 101% and 110%. Recovery in serum was within ±15% in the presence of high levels of bilirubin, Intralipid or hemoglobin and there was little or no effect on C-peptide in the presence of spiked normal physiological levels of proinsulin or insulin. C-peptide in serum and urine were stable at ambient temperature for one day, refrigerated or frozen temperature for up to 7 days, and up to six freeze/thaw cycles.

The C-peptide serum and urine ECL method combines novel technology with commercial diagnostic C-peptide assays. The C-peptide serum and urine ECL method combines novel technology with commercial diagnostic C-peptide assays. The C-peptide serum and urine ECL method combines novel technology with commercial diagnostic C-peptide assays.

Identification of type 1 diabetes (T1DM) can impair linear growth in children due to its effect on the insulin like growth factor 1 (IGF-1) system.

Studies have shown that Type I diabetes (T1DM) can impair linear growth in children due to its effect on the insulin like growth factor 1 (IGF-1) system.

We conducted a survey of adolescents in the pediatric diabetes clinic to assess their knowledge of impaired growth as a complication of uncontrolled T1DM and to determine which knowledge would motivate them for better diabetes control. Twenty-seven patients with T1DM were included (18 males, 9 females). The average A1C for all patients was 9.2% (S = 1.1) and average height difference was 2.1 inches (S = 2.3). Only three patients showed a gain in height from the predicted height and all had an A1C less than 18. The data was analyzed by linear regression of height difference on A1C. Height difference was compared to the subject’s average hemoglobin A1C (A1C) values from age of onset to 18. The data was analyzed by linear regression of height difference on A1C. Height difference was compared to the subject’s average hemoglobin A1C (A1C) values from age of onset to 18. The data was analyzed by linear regression of height difference on A1C. Height difference was compared to the subject’s average hemoglobin A1C (A1C) values from age of onset to 18. The data was analyzed by linear regression of height difference on A1C. Height difference was compared to the subject’s average hemoglobin A1C (A1C) values from age of onset to 18.

The scatter plot of A1C versus height difference shows a striking trend. For every 1% increase in the A1C, children are predicted to lose about one-half inch from predicted height (slope B = 0.51, S = 0.4, CI = -0.31-1.34).

For author disclosure information, see page LB66.
Circulating Markers of Endothelial Dysfunction and Glutathione Peroxidase Activity in Normal Pregnancy

XINHUA CHEN, THERESA O. SCHOLL, Stratford, NJ

Endothelial dysfunction is positively related to insulin resistance and cardiovascular disease. Oxidative stress increases and antioxidant decreases expression of endothelial adhesion molecules. We examined the relationship between markers of endothelial dysfunction and glutathione peroxidase activity, an indicator of antioxidant status, in normal pregnancy.

Pregnant women (N=230) were randomly selected from a prospective cohort of normotensive, non-diabetic gravidas (African-American 35%, Hispanic 46%, Caucasian 19%) age 21.7±0.2 (yr), pregravid BMI (kg/m²) 25.2±0.3. Serum levels of soluble intercellular and vascular cell adhesion molecules (sICAM-1, sVCAM-1 and E-selectin) and glutathione peroxidase (GPx) were measured at entry to care (week 16) and the 3rd trimester (week 30). Data were analyzed using multiple regression analysis for controlling for age, BMI, smoking, parity and ethnicity. At entry, significant negative associations were observed between GPx activity and endothelial dysfunction markers including sICAM-1 (-1.896 ng/ml/mg Hb GPx, p=0.008), sVCAM-1 (-2.668 ng/ml/mg Hb GPx, p=0.026) and sE-selectin (-3.348 ng/ml/mg Hb GPx, p=0.008). These associations persisted at the 3rd trimester for sICAM-1 (-2.911 ng/ml/mg Hb GPx, p=0.027) and sE-selectin (-4.460 ng/ml/mg Hb GPx, p=0.007) but not sVCAM-1 (-0.970 ng/ml/mg Hb GPx, p=0.05).

In conclusion, inverse associations between maternal circulating soluble adhesion molecules and GPx activity suggest a link between endothelial dysfunction and antioxidant defenses. Increasing antioxidant status may modulate circulating levels of soluble adhesion molecules and prevent endothelial damage, thus reducing susceptibility to the pregnancy complications like preeclampsia or gestational diabetes mellitus.

Supported by: NIH

ACE C1237T Gene Polymorphism in Indian Women With Gestational Diabetes Mellitus

PAPUL AGARWAL, KRISHNA DALAL, NUTAN AGARWAL, NHIBRITI DAS, SUMIT SHARMA, New Delhi, India, Linköping, Sweden

Polymorphisms in the Angiotensin Converting Enzyme (ACE) gene in patients with Type 2 diabetes have been reported to have contradicting results in different populations. A report studying ACE Insertion/Deletion (ACE I/D) in the intron16 of ACE gene with respect to Gestational Diabetes Mellitus (GDM) in Czechoslovakian women found no association. Villard et al reported several polymorphisms in the ACE gene, among which was a polymorphism ACE 6 (ACE C1237T) in exon 9. Two previous studies involving (Keaveny et al in 1998 and Zhu et al 2000) ACE-6 polymorphism and hypertension focused in haplotyping only. The current study is the first to evaluate the role of ACE 6 polymorphism in women with GDM. Our study determined the occurrence of genotype and allele frequencies of ACE 6 polymorphism in a genetically homogeneous population. We enrolled 215 Indian women, comprising of 115 healthy pregnant women (control group) and 100 pregnant patients with clinical diagnoses of GDM (study group). The ACE 6 alleles were visualized by assays based on polymerase chain reaction and restriction endonuclease analysis. The ACE C1237T polymorphism showed a strong association with GDM (2.5±0.0, p=0.0190). Further analysis revealed that the ACE T/T 1237 genotype was positively associated (95% CI=1.135-0.9281, OR=0.34) with GDM. This is the first study reporting association of the ACE 6 polymorphism with GDM, probably indicating that ACE 6 gene can be considered as one of the genetic marker for GDM.

Supported by: AIIMS (to K.D.)
vacular density, capillary diameter and pericyte coverage. In old islets, however, blood vessels expressed the inflammatory markers ICAM-1 and VCAM-1 and the density of macrophages increased. These results suggest that old islets are able to adapt to increased insulin demand, but this is associated with local inflammation. To isolate islets from the systemic influences of the aging organism we transplanted old and young islets into the eye of young diabetic animals. Young islets readily engrafted and reversed diabetes. By contrast, old islets showed poor engraftment, as evidenced by defective revascularization with disproportionately large blood vessels, and a 30d delay to return to normoglycemia. Three months after transplant, recipients of old islets were normoglycemic but were glucose intolerant and had significantly lower plasma insulin. However, glucose metabolism in these animals gradually became better concomitantly with an increased incidence of blood vessels with smaller diameter in old islet grafts. Our results suggest that old islets transfer their inflamed vascular phenotype that delays engraftment and revascularization but, as the islet recipient continues to gain weight, old (and young) islet grafts grow, and increasingly display a normal microvasculature to become fully functional.

Supported by: DOIST (Republic of Korea)

Epidemiology—Cardiovascular Disease

101-LB

Pain Qualities and STEMI: The Croatian Experience: Can Type 2 Diabetics Benefit from Silent Myocardial Ischemia Screening? 
Marina Gradišer, Edgar Dolavš, Jasna Cmičnjak, Branko Ostrički, Goran Topše, Korana Ćurić, Miljenka Igreč, Davor Miločić, Maša Katč, Cakovec, Croatia, Zagreb, Croatia

Coronary artery disease (CAD) is one of the leading causes of morbidity and mortality in patients with type 2 diabetes mellitus (DM). The aim of our study was to determine whether diabetic STEMI patients arrive in the Emergency room (ER) later than nondiabetics, compare the differences in pain quality and quantity between these groups, and measure differences in the outcome of the index hospitalization. We expected impaired pain perception and atypical symptoms to cause diabetic patients to seek out medical help later than nondiabetics, and subsequently have worse outcome. A total of 266 patients with first STEMI were included in our study during 2 years, 62 were diabetic and 204 were nondiabetics. Pain intensity and qualities at admission were measured using a modified McGill short form questionnaire. Other data was collected from hospital electronic records. Diabetic patients did not arrive and significantly later than nondiabetics; 95% arrived within 120 minutes of symptom onset. Most (66%) diabetic patients described their pain as “slight” or “none”, while most (78%) non-diabetics graded their pain as “moderate” or “severe”. The quality of pain tended to be more distinct in non-diabetics, while diabetic patients reported mainly shortness of breath. Diabetic patients were more likely to suffer an in-hospital fatal outcome (6.1% vs. 3.4%), and were less suitable for single vessel PCI (58% vs. 82%). Earlier arrival times and cautious evaluation of diabetic patients alone are not enough to significantly improve overall survival; a multidisciplinary approach is necessary before neuropathy and irreversible cardiovascular damages set in.

Epidemiology—Clinical—Diagnosis and Screening

102-LB

The Finnish Diabetes Risk Score in Detecting Undiagnosed Diabetes and Pre-Diabetes in U.S. Adults by Gender and Race/Ethnicity
Lu Zhang, ZhenZhen Zhang, Bang Hu, Yurong Zhang, New Orleans, LA, East Lansing, MI, Baton Rouge, LA, Xi’an, China

This study aimed to evaluate the Finnish Diabetes Risk Score (FINDRISC) in detecting the undiagnosed diabetic and pre-diabetes in U.S. adults and to examine whether there was a gender and racial/ethnic difference. This cross-sectional analysis included 20,833 adults (≥ 20 years of age) who participated in the National Health and Nutrition Examination Survey (NHANES) 1999-2010, consisting of 49.8% women, 53.0% non-Hispanic White, 18.1% non-Hispanic Black and 24.9% Hispanics. The overall prevalence (weighted) of undiagnosed diabetes (fasting glucose ≥ 126 mg/dL, HbA1C ≥ 6.5%), or glucose ≥ 200 mg/dL on a 2-h oral glucose tolerance test (OGTT) and pre-diabetes (fasting glucose between 100 and 125 mg/dL, HbA1C between 5.7 and 6.4%, or glucose between 140 and 199 mg/dL on a 2-h OGTT) was 4.1% and 35.5%, respectively. FINDRISC (range: 0-26) was positively associated with the prevalence of diabetes (OR=1.49, p<0.001) and pre-diabetes (OR=1.15, p<0.001). The area under the receiver operating characteristic curve (AUC) for detecting undiagnosed diabetes was 0.75 for total population, 0.74 for men and 0.78 for women (p=0.04), 0.76 for White, 0.76 for Black and 0.72 for Hispanics (p=0.03 for White vs. Hispanics). The AUC for detecting pre-diabetes was 0.67 for total population, 0.66 for men and 0.70 for women (p<0.001; 0.68 for White, 0.67 for Black and 0.65 for Hispanics (p<0.001 for White vs. Hispanics). The optimal cutoff point for detecting undiagnosed diabetes was 9 (sensitivity=0.83) for men and 10 (sensitivity=0.88) for women. The optimal cutoff point for detecting pre-diabetes is 8 (sensitivity=0.68) for men and 9 (sensitivity=0.76) for women. In summary, findings from this study suggest that the FINDRISC can be used as a simple and non-invasive screening tool to identify individuals at high risk for diabetes in the U.S. adult population. In this evaluation, the FINDRISC performed better in women than in men, non-Hispanic White than in Hispanics.

103-LB

Association of White Blood Cells Types With Incident Type 2 Diabetes: The Insulin Resistance Atherosclerosis Study
Carlos Lorenzo, Anthony J. Hanley, Steven M. Haffner, San Antonio, TX, Toronto, ON, Canada

The relation between white blood cell (WBC) type and development of diabetes has received scant attention. Neutrophil count may reflect inflammation. Therefore, we hypothesized significant diabetic risk associated to this WBC type. We examined this issue in 866 participants who were non-diabetic at baseline. Incident diabetes was ascertained after a 5.2-year follow-up using the 2003 ADA diagnostic criteria. Insulin sensitivity index (SI) was directly measured. All three WBC types were related to metabolic traits with neutrophil and lymphocyte counts being more strongly associated with inflammation and adiposity, respectively.

Spearman’s correlation coefficients relating WBC types to metabolic variables

<table>
<thead>
<tr>
<th></th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-h glucose</td>
<td>0.16 *</td>
<td>0.14 *</td>
<td>0.05</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.13 *</td>
<td>0.22 *</td>
<td>0.08 †</td>
</tr>
<tr>
<td>Insulin sensitivity index</td>
<td>-0.21 *</td>
<td>-0.24 *</td>
<td>-0.10 †</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.28 *</td>
<td>0.17 *</td>
<td>0.12 *</td>
</tr>
</tbody>
</table>

*p <0.001; † p <0.01; ‡ p <0.05

Lymphocyte count predicted incident diabetes, whereas neutrophil and monocyte counts did not. SI explained much of the relationship between lymphocyte count and incidence of diabetes.

OR and 95% CI of incident diabetes by tertiles of WBC type counts

<table>
<thead>
<tr>
<th></th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjustment model 1st tertile 2nd tertile 3rd tertile p for trend</td>
<td>Referent 1.27 (0.80, 2.02) 1.48 (0.94, 2.33) 0.999</td>
<td>Referent 1.30 (0.82, 2.04) 1.23 (0.78, 1.95) 0.373</td>
<td>Referent 1.45 (0.90, 2.33) 1.83 (1.06, 3.19) 0.010</td>
</tr>
</tbody>
</table>

In conclusion, lymphocyte count carries prognostic information in terms of risk of developing diabetes.

Supported by: NHLBI; NCRR, GCRC

104-LB

An Evaluation of the Ipswich Touch Test for Peripheral Neuropathy Screening in a Developing Country: A Comparative Study
Zulfiqarali G. Abbas, Janet Lutale, Lennox K. Archibald, Dar es Salaam, United Republic of Tanzania, Gainesville, FL

Diabetic foot ulcers (DFU) are associated with substantial morbidity and mortality in persons with diabetes in Dar es Salaam, Tanzania. Peripheral neuropathy (PN) is the major risk factor for DFU. The Ipswich Touch Test (IpTT) was touted as a sensitive, user-friendly screening method for PN in settings with limited resources. Thus, we carried out this study to determine the utility of the IpTT for PN screening in Dar es Salaam when compared with three standard methods: (i) monofilament (MF), (ii) monofilament (MF), (iii) and (iv) quantitative sensory testing (QST).

For author disclosure information, see page LB66.
vibration perception threshold (VPT); and (iii) hot/cold perception threshold (HCPT). We studied consecutive persons attending a large diabetes clinic in Dar es Salaam. The IpTT involved touching the tips of the 1st, 3rd, and 5th toes and dorsum of the hallux with tip of index finger for 1-2 s. Pressure sensation on these toes was assessed with 10-g MF applied for 1-2 s. VPT was measured bilaterally in the wrists, knees, ankles, and halluces by biothesiometry. HCPT was ascertained at 3rd, 5th, and 6th toes, heel, and plantar surfaces with a semitometer. Among 171 individuals screened, 57/9 (80.6%) were ethnic Africans, 8% Asian Indians, and 6% Arabs. Median age was 52 range: 17-90 years; median duration of diabetes (DOD) was 5 range: 1-40 years. Overall PN prevalence by HCPT, VPT, MF, IpTT was 89%, 50%, 38%, and 10%, respectively. Using HCPT as the gold standard, the overall sensitivity of VPT, MF, IpTT was 53%, 42%, and 12%, respectively. The sensitivity of VPT for each DOD quartile was 37%, 41%, 68%, the corresponding sensitivity of MF was 40%, 36%, and 47%, respectively. In conclusion, the IpTT was not useful as a screening test for PN because of relatively low sensitivity. HCPT demonstrated a high prevalence 37%, 41%, 69%; the corresponding sensitivity of MF was 40%, 36%, and 47%, respectively.

**T2DM diagnosis status**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total N</th>
<th>% Readmitted (n)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CHF diagnosis</td>
<td>21,074</td>
<td>11.5 (2,422)</td>
<td>0.514 (0.463-0.570)</td>
</tr>
<tr>
<td>Diagnosed diabetes</td>
<td>10,541</td>
<td>10.4 (1,099)</td>
<td>1.201 (1.015-1.421)</td>
</tr>
<tr>
<td>Undiagnosed diabetes</td>
<td>3,029</td>
<td>19.2 (583)</td>
<td>0.611 (0.421-0.815)</td>
</tr>
</tbody>
</table>

**Pre-period CHF diagnosis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Not readmitted, mean (SD)</th>
<th>Readmitted, mean (SD)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>28,596</td>
<td>10.3 (2,948)</td>
<td>NA</td>
</tr>
<tr>
<td>No</td>
<td>3,029</td>
<td>19.2 (583)</td>
<td>8.059 (1.023-0.647)</td>
</tr>
</tbody>
</table>

**Post-period non-inpatient visits**

| Index length of stay, days | 4.17 (5.04) | 5.60 (6.03) | 1.039 (1.028-1.049) |

**Post-premier emergency room visits**

| OR vs. no CHF | 0.61 (1.72) | 0.96 (2.74) | 1.058 (1.040-1.076) |

**Post-premier emergency room visits**

| OR vs. undiagnosed | 0.61 (1.72) | 0.96 (2.74) | 1.058 (1.040-1.076) |

**OR vs. no CHF**

| OR vs. undiagnosed | 0.61 (1.72) | 0.96 (2.74) | 1.058 (1.040-1.076) |

**OR vs. no CHF**

| OR vs. undiagnosed | 0.61 (1.72) | 0.96 (2.74) | 1.058 (1.040-1.076) |

**OR vs. no CHF**

| OR vs. undiagnosed | 0.61 (1.72) | 0.96 (2.74) | 1.058 (1.040-1.076) |

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| OR vs. undiagnosed | 0.61 (1.72) | 0.96 (2.74) | 1.058 (1.040-1.076) |

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| OR vs. undiagnosed | 0.61 (1.72) | 0.96 (2.74) | 1.058 (1.040-1.076) |

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**OR vs. undiagnosed**

| OR vs. undiagnosed | 0.61 (1.72) | 0.96 (2.74) | 1.058 (1.040-1.076) |
Alcohol Consumption, Plasma Fetuin-A and Risk of Type 2 Diabetes in Women
SYLVIA H. LEY, QI SUN, MONIK C. JIMENEZ, KATHRYN M. REXRODE, JOANN E. MANSON, MAJKEN K. JENSEN, ERIC B. RIMM, FRANK B. HU, BOSTON, MA

Benefits of moderate alcohol consumption on type 2 diabetes have been well-documented and postulated to involve a mechanism of improved insulin sensitivity. Fetuin-A, a liver-derived protein that inhibits insulin signaling, has emerged as a biomarker associated with type 2 diabetes risk. Therefore, alcohol intake may influence circulating fetuin-A concentrations and subsequently diabetes risk through altering insulin signal. We hypothesized that moderate alcohol consumption would be associated with lower plasma fetuin-A and that fetuin-A would partly explain the association between alcohol consumption and type 2 diabetes in mid-aged and older women. Multiple linear regression was conducted among the Nurses’ Health Study female participants with measures of plasma fetuin-A and alcohol consumption (n=1381). The proportion of alcohol consumption and type 2 diabetes association explained by fetuin-A was assessed within 470 matched incident diabetes case-control pairs from 2000 to 2006. Higher total alcohol intake was associated with lower plasma fetuin-A (p-trend=0.008). Least-squares means±SE 476.6±5.7 ug/mL for abstainers, 469.6±5.1 ug/mL for 0.1-4.9 g/d consumers, 496.4±6.8 ug/mL for 5.0-14.9 g/d, and 449.3±2.1 ug/mL for ≥15 g/d. The association between alcohol consumption and diabetes explained by fetuin-A and fasting insulin were 18.3 % (95% CI 0.1-36.4) and 65.2 % (14.7-115.6) (both p-contribution<0.05), while liver enzymes were not a significant contributor of this association. Further, fasting insulin explained 61.7 % (25.7-97.8) of the association between fetuin-A and diabetes (p-contribution=0.008). In conclusion, moderate total alcohol consumption is associated with lower plasma fetuin-A concentrations in women. Fetuin-A and insulin explain a significant proportion of the association between alcohol consumption and type 2 diabetes in this population. Further, studies are needed to determine whether there are biological mechanisms underlying this association.

Supported by: NIH; CHRP

Prospective Study of Fast-Food Consumption and the Risk of Gestational Diabetes: The SUN Cohort
FRANCISCO JAVIER BASTERRA-GORTARI, LIGIA J. DOMINGUEZ, MIGUEL ANGEL MARTINEZ-GONZALEZ, ALFREDO GEA, CARMEN DE LA FUENTE, LLUIS FORGA, MAIRA BES-RASTRILLO, Pamplona, Spain, Palermo, Italy

Little is known about the influence of fast-food consumption on incident gestational diabetes mellitus (GDM). Therefore, our objective was to evaluate the association between fast-food consumption and GDM in a cohort of university graduates. The prospective dynamic SUN cohort included data of 2903 women free of diabetes or previous GDM who reported at least one pregnancy between 1999 and 2010. Fast-food consumption was assessed through a validated semi-quantitative food frequency questionnaire. Fast-food was defined as the consumption of hamburgers, sausages, and pizza. Three categories of fast-food were established: low (0-3 servings/month), intermediate (>3 servings/month-2 servings/week) and high (>2 servings/week). Non-conditional logistic regression models were used to adjust for potential confounders.

We identified 169 incident cases of GDM during follow-up. After adjusting for age, baseline body mass index, smoking, physical activity, alcohol intake, fiber intake, Mediterranean dietary pattern, soft drinks consumption, family history of diabetes, cardiovascular disease and hypertension at baseline, and parity, regular fast-food consumption was significantly positively associated with incident GDM. Women in the intermediate category of consumption had an adjusted OR of 1.35 (95% CI 0.84-2.17) and those in the highest category had an adjusted OR of 1.72 (95% CI: 1.08-2.91) compared with the low consumption group; p for linear trend 0.018. Our results suggest that pre-pregnancy higher consumption of fast-food (defined as the consumption of hamburgers, sausages, and pizza) was a risk factor for GDM.

Supported by: Spanish Government (PI10), (022930), Navarre Government (45/2011)

The Risk of Fractures after Initiating Oral Anti-Diabetic Drugs: Results from the National Claim Registry
HYUNG JIN CHOI, CHANMI PARK, YOUNG-KYUN LEE, YONG-CHAN HA, SUNMEE JANG, CHAN SDO SHIH, Cheongui Su, Chungcheongbuk-Do, Republic of Korea, Seoul, Republic of Korea, Seogungnam, Republic of Korea, Gimhae-si, Republic of Korea

Thiazolidinediones (TZD) increase fracture risk. However, the effect of other oral anti-diabetic drugs (OADs) on fracture risk is not well known. We examined the risk of fractures after initiating OADs using the nationwide database of medical and pharmacy claims in South Korea. Among 2,886,555 subjects with antidiabetes prescriptions, 207,558 subjects aged 50 years and older, who initiated OADs from January 2008 to June 2011, were analyzed. Based on medication possession ratio data, subjects were classified as: non-user, metformin alone, sulfonylurea (SU) alone, alpha-glucosidase inhibitor alone, metformin+SU combination, metformin+TZD combination, metformin+DPP4 inhibitor combination and SU+TZD combination. The outcome measure was the first occurrence for a vertebral fracture or a non-vertebral fracture. The incidence of fracture was analyzed controlling for age, gender, comorbidity score, diagnosis of osteoporosis, osteoporosis treatment, and osteoporosis related diseases. Total of 5,996 fractures were observed among 207,558 subjects during the observation period. Fracture rate per 10,000 person-years varied significantly across type of OADs, with metformin+DPP4 inhibitor combination group having the lowest rate [124.9, 95% confidence interval (CI) 106.0-147.1] and SU+TZD combination group having the highest rate (289.6, 95% CI 222.1-327.4). Metformin+DPP4 inhibitor combination group had significantly reduced fracture risk compared with non-users (hazard ratio (HR)=0.83, 95% CI 0.70-0.98, P=0.025). In models adjusting for all confounding factors, metformin+DPP4 inhibitor combination group showed a trend of lower non-vertebral fracture risk compared with metformin+SU combination group (HR=0.82, 95% CI 0.65-1.03, P=0.080). TZD was significantly associated with increased risk of fracture (HR=1.59, 95% CI 1.38-1.82, P<0.001). These findings suggest that DPP4 inhibitor may have a protective effect on bone metabolism.

Supported by: Spanish Government (PI10), (022930), Navarre Government (45/2011)

Diepaptidyl Peptide 4 Inhibitors and Comparative Pancreatic Cancer Risk
MUGDA GOKHALE, TIL STURMER, CHRIS GRAY, VIRGINIA PATE, ALISON MARQUIS, JOHN B. BUSE, Chapel Hill, NC

A recent study analyzing human pancreata described potentially detrimental effects of sitagliptin, a dipeptidyl peptidase 4 inhibitor (DPP4i), on human pancreas with implications for incident pancreatic cancer (PC). This adds to concerns raised by an analysis of the FDA Adverse Events Reporting System which reported increased PC rates with incretin-based drugs. Both studies are limited by many shortcomings. We compared PC risk after initiation of DPP4i versus sulfonylureas (SU) and thiazolidinediones (TZD) using a 20% sample of the 2006-10 Medicare claims. To address concerns about potential outcome detection bias, we compared the cumulative incidence of diagnostic work-up in the two cohorts before and after initiation (index date). This was a new user active comparator cohort study consisting of patients ≥65 years requiring a second prescription of the same drug within 180 days of initiation with follow-up starting at the second fill date. Using an as-treated approach, we used propensity score adjusted Cox models to estimate hazard ratios (HR) and 95% confidence intervals (CI). Diagnostic work-up pre and post index was compared using risk ratios (RR). There were 19294 DPP4i initiators with mean age 74. Over a 9 month median follow-up, 29 DPP4i initiators had a PC diagnosis. The hazard of PC with DPP4i was lower relative to SU (HR 0.5, CI 0.3 - 1.0) and similar to TZD (HR 1.1, CI 0.7 - 1.8). Excluding the first 9 months after drug initiation to reduce the potential for reverse causality did not alter results. In the 6 months post index, the cumulative incidence of diagnostic work-up among sitagliptin initiators (79.4%) was similar to TZD (74.0%) (RR 1.07, CI 1.06 - 1.08) and SU (74.6%) (RR 1.06, CI 1.05 - 1.07). The probability of diagnostic workup pre index was similar for all groups (~80%). Though limited by sample size and real world duration of treatment, contrary to previous evidence, our data suggest no increased pancreatic cancer risk with DPP4i relative to SU or TZD and that diagnostic work-up is not affected by DPP4i use.

Supported by: Spanish Government (PI10), (022930), Navarre Government (45/2011)
Combination Therapy With Metformin Plus Sulfonylureas versus Metformin Plus DPP-4 Inhibitors and Risk of All-Cause Mortality

CRAIG J. CURRIE, SARA JENKINS-JONES, JAYANTI MUKHERJEE, CHRISTOPHER L.L. MORGAN, Cardiff, United Kingdom, Wallingford, CT

Aims: The aim of this study was to evaluate the risk of all-cause mortality for patients exposed to dual therapy with metformin and sulfonylurea (SUs) vs. metformin and DPP-4 inhibitors (DPP-4i).

Materials and methods: Retrospective data were extracted CPRD: a data resource comprising approximately 10% of patients treated in primary care in the UK. Patients with type 2 diabetes initiated with treatment comprising metformin with either a SU or a DPP-4i between 2007 and 2012 were included, regardless where these regimens were used in the natural history of the disease. Time to all-cause mortality was compared using Cox proportional hazards models. In addition to the main comparative analysis adjusting for key covariates within the model, two additional sensitivity analyses were performed. Firstly, a matched-cohort study using the following matching criteria at baseline: age (±2 years), gender, diabetes duration (±1 year), BMI (±3 Kg/m²), serum creatinine (±10 µmol/L) and HbA1c (±1%). Secondly, patients were also matched by propensity score predicted by the same candidate variables.

Results: In the main analysis, 27,261 patients were prescribed metformin in combination with a SUs, and 5,215 were prescribed metformin in combination with a DPP-4i. 3,454 patients were included in each arm of the direct matched cohorts and 4,703 in each arm in the propensity matched analysis. With respect to all-cause mortality, in the main analysis the adjusted hazard ratio (aHR) was increased using SUs (aHR=1.285, 95%CI 9.900–1.779). The aHR was significantly increased for metformin-SUs compared with metformin+DPP-4i for those matched directly (aHR=2.314, 1.348–3.973) and those matched on propensity score (aHR=1.691, 1.135–2.519).

Conclusion: There was a consistent reduction in mortality for patients prescribed metformin in combination with DPP-4i versus metformin in combination with SUs. These data should be considered when initiating dual therapy with metformin.

Supported by: Bristol-Myers Squibb

42-Months Intervention on Glucose Control and End Events in Type 2 Diabetes Patients With Different Level of Education in Beijing Communities

GUANG-RAN YANG, MINGXIA YUAN, GANG WANG, HANJING XUEPING DU, YULING LI, YU JI, XIAONING GU, YUE LI, MINGXIA YUAN, SAN ANTONIO, TX

To investigate the effects of educational level on glucose control and end events after 42-months intervention in type 2 diabetes patients in Beijing communities.

Using multi-stage sampling, 2,866 type 2 diabetes patients from 15 Beijing urban communities were investigated. After 42-months intervention, end events including the incidence of macrovascular complications (such as malignant tumors and aggravation of renal disease) were recorded. Educational attainment was categorized into three levels: low (elementary school or illiteracy), medium (middle school) and high (college or academic degree). (1) At attainment was categorized into three levels: low (elementary school or illiteracy), medium (middle school) and high (college or academic degree). (2) At baseline, the numbers of patients reaching good glucose control (HbA1c < 7.0 %) were 49.09%, 54.82% and 62.59%, respectively (p all <0.05). (3) After adjustment for confounding factors, educational level was independently associated with the numbers of patients reaching good glucose control (medium OR=0.782, High OR=0.589, p all <0.05). It showed that the educational level was found to be associated with glucose control after 42-months intervention. Educational attainment seems to be related with the incidence of macrovascular complications, malignant tumors and aggravation of renal disease in type 2 diabetes in Beijing.

Supported by: Capital Medical Development Foundation of China
non-veteran civilians (OR 1.4 95% CI 1.4-1.5). For females, veterans had 10% less risk for diabetes than civilians but with no statistical significance (OR 0.9 95% CI: 0.7-1).

Conclusion: Overall the prevalence of diabetes was higher in male veterans than non-veteran civilians. This association remained significant after adjusting for socio-demographic and health behavior factors.

**GENETICS—TYPE 2 DIABETES**

116-LB

**SNPs in ADAMTS7, the 9p21 Region and UBE2E Interact With Type 2 Diabetes Status to Modify the Risk of Coronary Artery Disease**

NIGEL W. RAYNER, NATALIE VAN ZUYDAM, BENJAMIN F. VOIGHT, CLAES LADENVALL, RONA J. STRAWBRIDGE, SARA M. WILLEMS, ERIK P.A. VAN IPEREN, JAKA HARTIALA, EFTHYMIA VLACHOPULOU, EVELIN MIHALCOV, LYDIA KWEE, CHRIS NELSON, UIMING DU, ANUJ GOEL, MARC KRUSZ, JITENDER KUMAR, STAVROULA KANONI, CARDIOGRAMPLUSC4D, SUMMIT, Oxford, United Kingdom, Dundee, United Kingdom, Philadelphia, PA, Malmö, Sweden, Stockholm, Sweden, Rotterdam, Netherlands, Amsterdam, Netherlands, Los Angeles, CA, Helsinki, Finland, Tartu, Estonia, Durham, NC, Leicester, United Kingdom, Heidelberg, Germany, Upsala, Sweden, Cambridge, United Kingdom.

Patients with type 2 diabetes (T2D) are 3-4 times more likely to suffer from coronary artery disease (CAD). Despite this no overlapping loci between CAD and T2D have been identified to date. In this study we aimed to: identify loci that modify the risk of CAD in patients with T2D, assess whether known CAD loci have a different effect on CAD risk in patients with T2D and evaluate the influence of known T2D loci on CAD risk in non-diabetic individuals compared to patients with T2D.

Summary statistics for 2,295,146 SNPs from 16,942 patients with T2D (6,022 CAD cases and 10,920 CAD free controls) and 2,727 non-diabetic individuals (10,892 CAD cases and 17,835 CAD free controls) were combined in a fixed effects meta-analysis and stratified by T2D status. The meta-analysis of SNP effects on CAD in patients with T2D identified associations in ADAMTS7 represented by two independent SNPs previously reported for CAD. Rs11072811 (OR 1.8, p=1.7E-07) and rs11634042 (OR=1.2, EAF=0.58, p=5.7E-08) were reported for CAD. Rs11072811 (Odds Ratio (OR)=1.17, effect allele frequency (EAF) = 0.53, p=3.9E-11) and rs11634042 (OR=1.15, EAF =0.58, p=5.7E-08): significant for interaction (p=4.2E-02).

Rs1556516, a proxy for the known CAD SNP rs1333049 in the well-established 9p21 locus, had a smaller OR in non-diabetic individuals (OR=1.08, p=1.2E-02) when compared to its effect in patients with T2D, and rs11072811 had a smaller effect on CAD risk in non-diabetic individuals (OR=1.08, p=1.2E-02) when compared to its effect in patients with T2D, and this interaction with T2D status was nominally significant (p=3.5E-02).

For author disclosure information, see page LB66.ADA-Funded Research

118-LB

**TCF7L2 Overexpression and Type 2 Diabetes: Dissecting the Function of Tcf7l2 as a Regulator of Glucose Metabolism**

KATHLEEN A. BAILEY, SOO-YOUNG PARK, JERRY KILIMNIK, MANAMI HARA, PIOTR WITKOWSKI, GRAEME I. BELL, MARCELO NOBREGA, CHICAGO, IL.

Genome-wide association studies to identify variants associated with type 2 diabetes (T2D) consistently identify a region of non-coding variation within transcription factor 7-like 2 (TCF7L2). To test if variation in this region could cause a change in TCF7L2 expression leading to T2D risk, we recombined additional copies of Tcf7l2 into the mouse leading to global overexpression. Intrapertoneal glucose tolerance test (IPGTT) on these overexpression mice identified glucose intolerance, highlighting a role for Tcf7l2 in glucose homeostasis.

Global overexpression of Tcf7l2 in the mouse leads to glucose intolerance but does not explain the tissue-specific mechanism by which overexpression leads to hyperglycemia. To isolate the effect of Tcf7l2 overexpression in each tissue, we restore wildtype expression in a single tissue using a Cre-loxP system and look for rescue of the glucose intolerance phenotype.

We first restored normal Tcf7l2 expression in beta-cells while maintaining overexpression elsewhere. Using IPGTT, we discovered normal expression in beta-cells led to more severe hyperglycemia compared to global overexpression. Perfusion on isolated islets indicated that beta-cells with Tcf7l2 overexpression secrete more insulin than beta-cells with wildtype expression. Immunohistochemistry found that global overexpression mice have a larger beta-cell area than mice with normal expression in beta-cells.

We compared mice with global overexpression of Tcf7l2 and mice with normal expression in beta-cells but overexpression elsewhere. When we restore normal Tcf7l2 expression in beta-cells, we decrease beta-cell area, we reduce insulin secretion, and we increase hyperglycemia. These data suggest that Tcf7l2 overexpression in beta-cells protects against T2D rather than causing glucose intolerance. It also points to a mechanism in the periphery as responsible for the glucose intolerance seen in T2D patients harboring the TCF7L2 risk variants.

119-LB

**Pleiotropic Effects on Lipid Levels and Obesity Identified in Multi-Trait Meta-Analysis of Genome-Wide Association Studies (GWAS) of Type 2 Diabetes (T2D) Related Traits**

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Serum lipid levels, fat storage and obesity are related to T2D risk through shared biochemical pathways and can be influenced by common genetic factors. Analysis of the genetic effects on multiple phenotypes simultaneously allows dissection of variable patterns of multi-trait associations.

Within the ENGAGE consortium, we assessed multi-trait genetic effects on four blood lipids (high-/low-density lipoprotein and total cholesterol, triglycerides [IDL/IDL/TG] and body mass index (BMI). The 1000 Genomes reference panel (06/2011) was used for imputation in up to 41,752 individuals from 18 European GWAS. Each study carried out multi-trait analysis by fitting a multiple logistic regression on SNP genotypes allowing for joint effects of four lipid traits and BMI. Single-trait meta-analyses, conditional on remaining traits, were used to verify the independence of trait-specific genetic effects.

For author disclosure information, see page LB66.
120-LB
Variation in Glucose Homeostasis Traits due to P2X7 Polymorphisms in Mice and Humans

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ATP, a key molecule in energy metabolism, also acts as an extracellular signal via two families of purinergic receptors, P2X and P2Y. Both receptor types are expressed in pancreatic β-cells and ATP is contained in insulin secretory granules. We hypothesized that purinergic signaling might influence glucose regulation.

We generated a mouse model of purinergic signaling dysfunction by crossing 129SvJ mice with C57Bl/6 mice that have a naturally hypomorphic P2x7 variant (P451L). There were no significant differences in weight, fasting glucose, or fasting insulin for mice with the two different P2x7 alleles at baseline. We categorized baseline obesity to influence T2D risk and to identify genetic loci where such interaction occurs. Small studies have reported certain gene

Four independent SNPs met significance for the joint effect: rs3919258 at the TNF-1B locus, rs2671490 in NFKBIL1, rs1115764 in SEZBL2, and rs1019856 in TGFBR2. For rs1019856 in TGFBR2, the SNP main effect (p = 9.8 x 10 -4) seemed to drive this joint effect, but SNP-smoking interaction (p = 3.3 x 10 -5 to 5.8 x 10 -4) seemed to drive the joint effect of the other three. Variants in SEZBL2 and TGFBR2 have been previously reported to be associated with fasting insulin and blood pressure, respectively. These results merit replication in larger studies and may elucidate new causal pathways in smoking and T2D risk.

122-LB
Genome-Wide Association With Fasting Glucose (FG) in 20,000 African Americans Suggests New Loci and Allelic Heterogeneity at Known Loci: The African American Glucose and Insulin Genetic Epidemiology (AAGILE) Consortium

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Hyperglycemia disproportionately affecting African Americans (AA) may have a genetic basis. We used meta-analyses (m-a) of genome-wide (g-w) association studies (GWAS) of FG in AA to test whether FG loci identified in Europeans (EU) also are associated in AA, and to find new AA FG loci.

We performed FG GWAS in 16 cohorts of 20,209 non-diabetic AA (mean age 56 yr) using additive genetic models to test associations of FG with 3.3M single nucleotide polymorphisms (SNPs) and combined results in METAL using inverse-variance weighted m-a. To leverage possible AA-EU heterogeneity at each SNP, we combined AA METAL results with MAGIC published results (Manning 2012, PMID 22581228). The first two results files using MANTRA, a Bayesian method accounting for allelic heterogeneity among population clusters that returns a Bayes Factor, were shown to have significant p < 2x10^{-5} for replication.

Four known FG loci, 1 Index (MTNR1B) and 1 Best SNP (GCK) were significantly associated with low FG in AA and plus EU. For 23 known FG loci, 1 Index and 1 Best SNP (GCK) were significantly associated with low FG in AA and plus EU.

The Collaborative Cross is a next-generation genetic resource, developed using the “gene mine” to identify novel diabetes-susceptibility Loci. For 23 known FG loci, 1 Index and 1 Best SNP (GCK) were significantly associated with low FG in AA and plus EU. For 23 known FG loci, 1 Index and 1 Best SNP (GCK) were significantly associated with low FG in AA and plus EU.

For author disclosure information, see page LB66.
IMMUNOLOGY/TRANSPANTATION

Resolution of Autoimmune Diabetes Mellitus Precipitated by Interferon Therapy for Chronic Hepatitis C
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Interferon (IFN) is a commonly used agent for chronic hepatitis C and is a rare cause of latent autoimmune diabetes in adults (LADA). LADA is characterized by circulating autoantibodies against pancreatic beta cell antigens (islet-cell antibodies [ICA] or glutamic acid decarboxylase antibody [GAD-Ab]). The infrequency with which LADA is encountered and the variability of onset can present a diagnostic challenge. In addition, the disease course is not well-described in patients taking interferon. We present a unique case of interferon-associated LADA which resolved after interferon cessation.

A 68-year-old male was evaluated for acute onset of hyperglycemia of 473mg/dl, 7 months after initiating ribavirin and interferon-alpha (IFN-α) for hepatitis C. Anion gap was absent, serum and urine ketones were negative. Hemoglobin A1C (HBA1C) was 7.2% at diagnosis of diabetes.

After a trial of oral medications including Metformin, the patient was referred to endocrinology for persistently elevated blood glucose. GAD-Ab was elevated at 17,818U/ml (normal<.5). Insulin was initiated and the patient’s A1c improved. IFN was discontinued after an extended 72-week course. GAD-Ab was 10,595, 3041, and 2024U/ml at 1, 4, and 11 months post therapy respectively. The patient was weaned off insulin 4 months after completion of IFN with a HBA1C of 5.2%. Two years later, he remains euglycemic. IFN-α has antiviral, anti-proliferative, and immunomodulatory effects and its use has been associated with autoimmune diseases such as multiple sclerosis, systemic lupus erythematosus, thyroid disease, and Type 1 diabetes (T1D). Subjects with IFN-associated diabetes have higher levels of C-peptide and GAD-Ab than T1D. The onset of diabetes ranges from 2-13 months from initiation of IFN to 4 months after therapy completion. Once diagnosed, insulin is often necessary to achieve glycemic control.

This is the only reported case of resolution of hyperglycemia following the cessation of IFN therapy.

126-LB

NCoR KD Reprograms Macrophage Lipid Metabolism Increasing ω3 Fatty Acid Synthesis and Insulin Sensitivity
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Macrophage-mediated inflammation is an major contributor to obesity-associated insulin resistance. The co-repressor NCoR inhibits inflammatory pathway activation in macrophages, and one would predict that removal of this co-repressor should lead to activation of inflammatory responses. Surprisingly, we find that macrophage-specific deletion of NCoR leads to an anti-inflammatory phenotype causing robust systemic insulin sensitization in obese mice. We traced this mechanism of the paradoxical effect to the ability of NCoR to co-repress the nuclear receptor LXR. NCoR deletion led to LXR derepression with activation of its downstream transcriptional targets, including lipogenic pathway genes. These lipogenic genes promote the biosynthesis of omega-3 fatty acids which produce strong, local, anti-inflammatory insulin sensitizing effects. Thus, macrophage NCoR deletion leads to reprogramming of macrophage lipid metabolism, turning these cells into local factories for the production of omega-3 fatty acids. Therapeutic methods to harness this mechanism could lead to a new approach to insulin sensitizing therapies.

127-LB

128-LB

Augmentation of Leptin Receptor Signaling by Loss of Socsc3 Induces Development of Gastric Tumors in Mice
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It is well known that leptin derived from adipose tissue acts on its receptor (ObR) in the hypothalamus to inhibit food intake and energy expenditure. Leptin and ObR are also expressed in the gastrointestinal tract; however, the physiological significance of leptin signaling in the gut remains uncertain. Suppressor of cytokine signaling 3 (SOC3) is a key negative feedback regulator of ObR-mediated signaling in the hypothalamus. We now show that gastrointestinal epithelial cell-specific SOC3 conditional knockout (T3b-SOC3 CKO) mice developed gastric tumors by enhancing leptin production and the ObRβ/signal transducer and activator of transcription 3 (STAT3) signaling

IMMUNOLOGY

Loss of a Novel Immune Regulatory Pathway in Type 1 Diabetes
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In type 1 diabetes (T1D), recruitment of T cells to the pancreatic islets contributes to the destruction of insulin secreting beta cells. Very little is known about the mechanisms by which T cell migration is regulated during inflammation, and it is thus difficult to target this aspect of pathology for the development of therapies. We tested the hypothesis that adiponectin, an anti-inflammatory adipose tissue derived cytokine, regulates T cell migration. In vitro, videomicroscopy was used to assess the migration of lymphocytes isolated from healthy donors or patients with T1D across TNF-α/IFN-γ activated endothelial cells (EC). In vivo, lymphocyte migration was assessed in a model of zymosan driven peritoneal inflammation. Adiponectin receptors expression was measured by flow cytometry.

We observed that migration of human lymphocytes was dose-dependently blocked by adiponectin (EC50=37nM). This effect was lost when B cells were absent, but could be regained by the addition of supernatants from adiponectin stimulated B cells. Mass spectrometry analysis identified the adiponectin-induced B cell-derived peptide, subsequently named PEPTIM (PEPptide Inhibitor of Trans-Endothelial Migration). Interestingly, PEPTIM could also effectively inhibit T cell migration in vitro (EC50=19pM). In vivo, lymphocyte recruitment was ameliorated by treatment with PEPTIM in vivo.

Lymphocytes isolated from patients with T1D expressed lower levels of adiponectin receptors, and were released from the inhibitory effects of adiponectin on transmigration. The addition of PEPTIM to T1D lymphocytes re-established the block on transmigration. We hypothesise that modulating the PEPTIM pathway has therapeutic potential for T1D.

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WITHDRAWN

125-LB

For author disclosure information, see page LB66.
pathway. All T2b-SOCS3 cKO mice developed tumors in the stomach but not in the bowels by 2 months of age, even though the SOCS3 deletion occurred in both the epithelium of stomach and bowels. The tumors developed in the absence of the inflammatory response and all cKO mice died within 6 months. These tumors displayed pathology and molecular alterations, such as an increase in MUC2 (Mucin 2) and TFF3 (trefoil factor 3), resembling human intestinal-type gastric tumors. Administration of anti-leptin antibody to T2b-SOCS3 cKO mice reduced hyperplasia of gastric mucosa, which is the step of the initiation of gastric tumor. These data suggest that SOCS3 is an anti-
gastric tumor gene that suppresses leptin overexpression and ObRb/STAT3 hyperactivation, supporting the hypothesis that the leptin/ObRb/STAT3 axis accelerates tumorigenesis and that it may represent a new therapeutic target for the treatment of gastric cancer.

Supported by: JSPS

129-LB
Elevated Frequencies of Th22 Cells in Peripheral Blood from Obesity and Type 2 Diabetes Patients Correlate With Insulin Resistance and Islets β-Cell Function Loss
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The chronic low-grade inflammation has long been recognized as the central link between obesity-associated insulin resistance and type 2 diabetes. In light of the role of Th22 cells in the pathogenesis of chronic inflammation, we first identified increased frequencies of Th22 cells in peripheral blood from patients with obesity and type 2 diabetes. Consistently, we detected elevated IL-22 levels in plasma and increased gene expressions of Th22 specific transcription factor in peripheral blood mononuclear cells from patients. Moreover, the remarkable positive correlation of Th22 frequency with both homeostatic model assessment-insulin resistance index and residual islets β-cell function indicates that the expansion and hyperactivity of Th22 cells might have a critical role in the development of obesity-associated insulin resistance and disease progression to type 2 diabetes.

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130-LB
Hyperglycemia Inhibits Complement-Mediated Immunological Control of S. aureus in a Rat Model of Peritonitis
KENJI M. CUNNION, CLIFFORD MAUERILO, PAMELA HAIR, REUBEN ROHN, Norfolk, VA

Previous work in our laboratory demonstrated that elevated glucose levels (> 10 mM) dramatically inhibited complement-mediated immune effectors critical for control of S. aureus infection. In vitro, high-glucose levels inhibited opsonization of S. aureus with C3b/iC3b as well as generation of the anaphylatoxin C5a, which is the step of the initiation of gastric tumor. These data suggest that SOCS3 is an anti-
gastric tumor gene that suppresses leptin overexpression and ObRb/STAT3 hyperactivation, supporting the hypothesis that the leptin/ObRb/STAT3 axis accelerates tumorigenesis and that it may represent a new therapeutic target for the treatment of gastric cancer.

Supported by: JSPS

131-LB
Hyperglycemia and Associated Findings in Autologous HCT Recipients
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Independent of history of diabetes, patients with cancer are at an increased risk for hyperglycemic events due to the malignancy, treatments, nutritional alterations, physical inactivity, and stress. Hyperglycemia promotes proinflammatory cytokine, chemokine, and prostaglandin expression. This inflammatory response further impairs an already compromised immune system, leading to increased risk for microorganismal invasion and related adverse outcomes. To better understand associations between hyperglycemia and its contributors, immune status, and presence of microorganisms in patients with cancer, we prospectively investigated patients with hematological malignancies who received autologous hematopoietic cell transplantation (HCT). Daily morning fasting blood glucose (BG) and leukocytes, documented microorganisms, and patient demographics in 45 autologous HCT recipients were collected. In this initial study phase, we used descriptive statistics and Pearson correlations to evaluate associations between patient factors, leukocytes, and presence of microorganisms. A total of 1,024 BG and WBC/ANC values among 27 female and 18 male adult/older adult patients were analyzed. The mean age was 56 years among an ethnically diverse patient population. The mean Body Mass Index (BMI) was 28.1. Microorganism growth occurred in 27 patients, with multiple microorganisms. Coagulase-negative staphylococci (N = 14) and Clostridium difficile (N = 10) were the most prevalent. Pearson correlations of interest included BG and leukocytes (r = -142, p < .0001), mean BG and presence of microorganism (r = .078, p < .014), age and BMI (r = .138, p < .0001), and BMI and presence of microorganism (r = .229, p < .0001). In summary, associations were found between BG, age, leukocytes, BMI, and presence of microorganisms among autologous HCT recipients. Further investigation and intervention studies are warranted.

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132-LB
Positivity for Islet Cell Autoantibodies in Subjects With Monogenic Diabetes Is Associated With Later Diabetes Onset and Higher Hba1c Level
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Islet cell autoantibodies (iAbs) are associated with the autoimmune insulinitis, and belong to the main diagnostic criteria of type one diabetes mellitus (T1DM). Nevertheless, growing evidence suggests the iAbs presence also in other diabetes types, surprisingly, in MODY (Maturity Onset Diabetes of the Young). The aim of the study was to characterize the cohort of Czech MODY patients positive for iAbs.

Autoantibodies against glutamic acid decarboxylase 65 (GADA) and protein tyrosine phosphatase IA-2 (IA2A) were analyzed in a cohort of 31 Czech MODY subjects, all confirmed by genetic testing. Selected clinical data were correlated to the iAbs status and kinetics.

Almost one quarter of the MODY subjects examined (7/31; 22.6%) was positive for iAbs. GADA were more prevalent (7/7) over IA2A (1/7). The iAbs incidence did not correlate with the HLA risk of T1DM. The iAbs-positive subjects manifested diabetes significantly later than the iAbs-negative ones, but displayed worse diabetes control (significantly higher Hba1c level). Secretion of IA2A decreased with any improvement of diabetes compensation. Only one of the examined subjects did not correspond to the above and displayed combined MODY and T1DM signs.

The data suggest transient but highly prevalent iAbs expression in Czech MODY subjects. The iAbs were found in subjects with rather delayed diabetes for author disclosure information, see page LB66.
manifestation, and in times of insufficient diabetes control. Since improving of diabetes compensation was associated with decrease of IABs levels, their presence may reflect the kinetics of beta-cell destruction induced by other than autoimmune causes.

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133-LB
Amelioration of Type 1 Diabetes in NOD Mice by Allogeneic Newborn Blood Transfer Is Associated With Restoration of Self-Tolerance
SUNDARRAJAN JAYARAMAN, MARK HOLTERMAN, BELLUR PRABHAKAR, Chicago, IL, Peoria, IL
We have previously shown that a single injection of allogeneic newborn blood, functionally equivalent to umbilical cord blood, into un-preconditioned pre-diabetic NOD mice prevented the onset of type 1 diabetes, which was accompanied by transient chimerism and alteration of gene expression in T-cells (Jayaraman et al. 2010, J. Immunol. 184:3038-150). We now show that allogeneic newborn blood transfer results in the elimination of diabetes-causing potential of T lymphocytes. Global unresponsiveness, predominant under diabetic condition was substantially reduced whereas alloantigen driven T-cell proliferation was enhanced in mice cured of diabetes. Protection against diabetes did not accompany altered frequency of CD4+ cells expressing CD25/CD62L, CD44 or Foxp3. Both the ability of CD4+CD25/Foxp3+ regulatory cells to suppress and the sensitivity of CD4+CD25 T effector cells to T regulatory cell mediated suppression were similar in diabetic and cured mice. Gene expression analysis of splenocytes derived from cured mice using qRT-PCR revealed the repression of pro-inflammatory genes such as Cela2b and enhancement of Mif, respectively implicated in diabetes manifestation and protection by our recent transcriptome analysis (Jayaraman et al. 2012, PloS One, 8: e56574). Importantly, activation induced T-cell death, crucial for maintaining peripheral tolerance, was substantially enhanced in CD4+ T-cells derived from cured mice. Taken together, these data indicate that allogeneic newborn blood transfer affords protection against type 1 diabetes by restoring self-tolerance and modifying gene expression.

134-LB
MIF Contributes to the Inflammatory Process in Type 1 Diabetes by Mediating Macrophages and Dendritic Cells Maturation
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Type 1 diabetes (T1D) is characterized by a cellular infiltrate in pancreatic islets where β cells are destroyed. The recognition of antigens and auto antigens takes place by macrophages (Mo) and dendritic cells (DCs). Previously we have showed that Macrophage migration inhibitory factor (MIF) induces the expression of co-stimulatory molecules on Mo and DCs on infection diseases. However, the role of MIF on Mo and DCs has not been explored in T1D. Here, we determined the expression of co-stimulatory molecules on Mo and DCs from WT and MIF-/mice with experimental T1D induced by STZ. Cells extracted from pancreas and spleen were treated in vitro with antibodies anti- CD80, CD86, CD40, MHC-II, TLRs and CD4+CD25+Foxp3+ cells. MIF-/mice did not increase high glucose levels compared with WT mice after STZ. Pro-inflammatory cytokines presence in serum was diminished in MIF-/mice but, the anti-inflammatory cytokines was higher than WT T1Z mice during all the experiment. These suggest the absence of MIF prevents an exacerbated inflammatory response, which correlates with blood glucose levels. Moreover, MIF-/ STZ mice had less expression of CD80, CD86, MHCII, TLRs and CD4+CD25+Foxp3+ cells. MIF-/mice did not increase high glucose levels compared with WT mice after STZ. In addition, CD4+CD25+ spleen cells from healthy MIF-/mice increased FOXP3 transcription factor expression regarding WT mice. Eight weeks after of T1D induction FOXP3 expression was higher in MIF-/mice than WT mice. Our results suggest MIF favors the expression of co-stimulatory molecules in the Mo and DCs in T1D.
Additionally we propose that MIF down-regulate the proliferation of regulatory T cells and MIF targeted therapy, combined with existing can help to decrease T1D course.

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135-LB
Beta-Cell-Targeted PDL1-CTLA4Ig Over-Expression Protects Allogeneic Islets from Acute Rejection
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Islet transplantation has the potential to cure type 1 diabetes mellitus (T1D) and a subset of type 2 diabetes. Major barrier for wide-spread use of islet transplant is the requirement of long term immunosuppressive treatment. Programmed death 1 (PD1) and its ligand, PDL 1, supply inhibitory signals during T cell activation. CTLA4Ig (Cytotoxic T lymphocyte Associated antigen 4 IgG) has been used as an inhibitor of T cell co-stimulation. In this study, we determined the influence of beta cell-targeted over-expression of PDL1 and CTLA4Ig on T1D development and allo-islet rejection. We employed adeno-associated virus 9 (AAV9) vectors with a mouse insulin 2 promoter to achieve beta cell-specific expression of an artificial PDL1-CTLA4Ig poly-protein. Beta cell-targeted overexpression of PDL1-CTLA4Ig protected non-obese diabetic mice (NOD) from developing hyperglycemia. Immuno-histology revealed the suppression of autoimmune-mediated insulitis in PDL1-CTLA4Ig expressing islets. We then analyzed the effects of PDL1-CTLA4Ig expression on rejection of allo-islets in NOD-matched recipient mice. Streptozoxin (STZ)-induced diabetic DBA2 mice received allo-islets isolated from BALB/c mice with or without pretreatment of the PDL1-CTLA4Ig-expressing vector. As a positive control, we also transplanted allogeneic-encapsulated allo-islets into diabetic DBA mice. Although untreated islets were rejected within 10 days, mice transplanted with the PDL1-CTLA4Ig-expressing islets remained normoglycemic for at least 40 days. Encapsulation of islets delayed immune-rejection for 3 weeks after transplantation. The present study demonstrated the utility of the beta cell-targeted AAV9 vector system and the potent immune-suppressive effects of beta cell-targeted PDL1-CTLA4Ig overexpression against autoimmunity and acute graft rejection. Beta cell-targeted PDL1-CTLA4Ig expression can provide an alternative strategy for immunosuppression-free islet transplantation.

Supported by: Mayo Foundation

136-LB
The Potential Contribution of Beta-Cell Purinergic Signaling and Ectonucleotidases in the Pathophysiology of Diabetes: Preliminary Rodent Study
CARMEN FOTINO, R. DAMARIS MOLANO, OLIVER UMLAND, ANDREA VERGANI, FABIO GRASSI, RODOLFO ALEJANDRO, CAMILLO RICORDI, PAOLO FIORINA, ANTONELLO PILEGGI, Miami, FL, Boston, MA, Bellinzona, Switzerland
Extracellular ATP is regulated by purinergic signaling and ectonucleotidases (ENTPDases), and may amplify β-cells inflammation during autoimmunity and islet rejection. We evaluated P2X7R and ENTPDases (CD39 and CD73) expression in mouse islets and pancreas.
Viable β-cells (R2D6+) in islets exposed to 24h stress were 45.5% in control, 65.7% in IFN-γ, 66.6% in cytokines, and 61.3% in high glucose. P2X7R, E-NTPDases and MHC-I expression is shown in Table 1.
In dissociated pancreases of C57BL/6, prediabetic NOD (pNOD) and NOD.SCID β-cells were 80%, 29.9% and 30.2%. R2D6+P2X7R+ cells were 71.5%, 26.2%, and 74%. R2D6+CD39+ cells were 8.5%, 46%, and 26%. R2D6+CD73+ cells were 8.2%, 5.7% and 26.6%. CD3+ cells were 10.9%, 15.3% and 0%. CD3+P2X7R+ cells were 11.7% in pNOD and 9.1% in C57BL/6. CD3+CD39+ cells were 23.6% in pNOD and 3.3% in C57BL/6, and CD3+CD73+ similar (55.4% and 66%, respectively). P2X7R expression by CD31+ endothelial cells was similar in C57BL/6 and NOD.SCID (62.1% and 62.4%), and 30.5% in pNOD; CD3+ was 66%, respectively. P2X7R expression by CD3+P2X7R+ cells was similar (66%, 71.5% and 74%, respectively). CD3+P2X7R+ cells were 11.7% in pNOD and 9.1% in C57BL/6. CD3+CD39+ cells were 23.6% in pNOD and 3.3% in C57BL/6, and CD3+CD73+ similar (55.4% and 66%, respectively). P2X7R expression by CD31+ endothelial cells was similar in C57BL/6 and NOD.SCID (62.1% and 62.4%), and 30.5% in pNOD; CD3+ was similar in all strains (40.1%, 42.5%, and 51.8%, respectively). CD3+CD73+ cells were 13.8% in NOD.SCID, 4.8% in pNOD and 5.3% in C57BL/6.
P2X7R and E-NTPDases expression increase in β-cells after stress. In pNOD P2X7R and CD39 are low and high in β-cells, both increase in C3+ cells. ATP/ P2X and E-NTPDases may contribute to islet immnunotun. Their modulation could be an alternative strategy for immunosuppression-free islet transplantation.

Supported by: DRIF

For author disclosure information, see page LB66.
INSULIN ACTION/MOLECULAR METABOLISM

INSULIN ACTION—ADIPOCYTE BIOLOGY

137-LB
AKT2 Is Not Required for Insulin Regulation of Lipolysis In Vivo
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Regulation of lipolysis is under tight hormonal control mainly by catecholamines and insulin. The general consensus is that insulin antagonizes catecholamine-activated lipolysis through Akt phosphorylation and activation of PDE3B. Recently, this hypothesis was challenged as in vitro studies demonstrate that insulin suppresses lipolysis by an Akt-independent pathway. To address this question in vivo, we studied lipolysis in mice deficient for AKT2, the major isoform expressed in adipocytes. We found that AKT2 whole-body knock-out (KO) mice have mildly reduced adiposity and comparable levels of adipokines when compared to wild type (WT) mice. In the fed state and following an oral glucose challenge, AKT2 KO mice are glucose intolerant and display hyperinsulinemia; however, exhibit normal insulin-mediated suppression of lipolysis. Furthermore, insulin significantly inhibits lipolysis in both genotypes during insulin tolerance test (ITT) and hyperinsulinemic-euglycemic clamp (attached figure) in the presence of equivalent insulin levels. Insulin inhibits catecholamine-induced lipolysis in primary differentiated brown fat adipocytes (BFA) of AKT2 KO to a similar extent as in BFA from WT mice. These results suggest that while AKT2 is an important component of insulin signaling in glucose metabolism, it is not required for insulin regulation of lipolysis in vivo. The existence of multiple pathways by which insulin regulates metabolism and adipose tissue may lead to more targeted therapeutics.

A

GLUCOSE LEVELS

B

GIR (glucose infusion rate)

C

GLYCEROL

D

NEFA

Hypothalamic Euglycemic Clamp. WT n=6 and AKT2 KO n=6.
NEFA = Non-Estivated Fatty Acids
* = p<0.05; ** = p<0.005

INSULIN ACTION—CELLULAR AND MOLECULAR METABOLISM

138-LB
Amyloid β42 Administration Impairs Energy Metabolism In Vivo and In Vitro
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Amyloid β42 (Aβ42) is a protein implicated in Alzheimer’s Disease (AD), in part through causing impaired neuronal metabolism. Obese and diabetic patients have increased circulating Aβ42, yet it is unknown whether circulating Aβ42 contributes to altered metabolism in these conditions. The aim of this study was to determine whether Aβ42 alters metabolism of insulin sensitive cells in vitro and whole body metabolism in vivo. Monomeric Aβ42 (m42) increased glucose production in FAO hepatocytes, while aggregated Aβ42 (a42) had no effect. Similarly, m42 impaired glucose uptake in 3T3-L1 adipocytes, while a42 had no effect. We next investigated the effect of m42 or scrambled Aβ42 (control) administration to mice (1μg / day; I.P injection) over 2 weeks. Administration of m42 had no effect on bodyweight or food intake compared with control mice. However, administration of m42 reduced oxygen consumption and total carbohydrate oxidation compared with control animals (p<0.05). This data shows that monomeric Aβ42 impaired glucose metabolism while aggregated Aβ42 had no effect. This data suggests that not only is Aβ42 involved in the pathology of AD, but it may also be involved in the dysregulation of metabolism in obesity and type 2 diabetes, where circulating Aβ42 levels are elevated.

Supported by: NHMRC

140-LB
LRP6 Mutation Alters Expressions of Insulin and IGF Receptor and Causes Insulin Resistance in Humans
RAJIV SINGH, RENATA BELFORT DE AGUIAR, SARITA NAIK, SHEIDA MANI, KAMAL OSTAASHAFIR, DETLEF WENCKER, MASOOD SOTOODEH, REZA MALEKZADEH, ROBERT S. SHERWIN, ARYA MANI, New Haven, CT, Isfahan, Islamic Republic of Iran, Hartford, CT, Tehran, Islamic Republic of Iran

We have identified a large kindred in whom a non-conservative mutation (R611C) in the Wnt co-receptor LRP6 underlying the development of autosomal dominant early onset CAD, type 2 diabetes and metabolic syndrome. Healthy non-diabetic LRP6 mutation carriers exhibited insulin resistance compared to noncarrier relatives during oral glucose tolerance test. The skeletal muscle biopsies and skin fibroblasts showed diminished Wnt/TCF7L2-dependent transcription of insulin receptor and decline in insulin signaling activity. Further investigations showed that LRP6 mutation increases phosphorylation of multiple serine residues of the IRS-1 in LRP6R611C fibroblasts, which was accounted for by enhanced activation of mTORC1. Increased mTORC1 activity correlated with higher IGF expression and subsequent activation of ERK1/ERK2 in LRP6R611C compared with wildtype fibroblasts. Further investigations revealed posttranscriptional regulation of IGFR by LRP6. In IGFR-1 treated LRP6 knockdown cells IGFR was stabilized by somatostatin. These findings identify the Wnt/LRP6/TCF7L2 axis as a regulator of glucose metabolism and a potential therapeutic target for insulin resistance.

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141-LB
Mechanism of Angiotensin II-Infused Insulin Signaling Pathway
EUN KYUNG KOH, HYUN-JU JANG, HAE-SUK KIM, JEONG-A KIM, CHARLOTTESVILLE, VA

It is well recognized that hypertensive individuals have elevated level of angiotensin II (ANG II). Elevated level of ANG II is known to be related to insulin resistance in the metabolic and cardiovascular tissue. Mammalian target of rapamycin (mTOR) is thought to be important in insulin resistance, cardiac and
vascular smooth muscle cell hypertrophy. In the previous study from our lab, we reported that activation of mTOR/p70S6K by ANG II impairs insulin-stimulated vasodilation through phosphorylation of IRS-1 at Ser 636/639. In the present study, we investigated upstream/downstream molecules that are involved in ANG II-activated mTOR/p70S6K pathway. We treated ANG II (100 nM) on HEK 293 stably transfected with AT1R (HEK 293 AT1a) cells for various time points (5 min, 10 min, 20 min, 30 min, 40 min, 60 min and 120 min, respectively). We observed that ANG II stimulated phosphorylation of both mTOR and p70S6K as early as 5 min and maximally stimulated between 30 min to 60 min. ANG II-stimulated phosphorylation of p70S6K was decreased when the cells were pre-treated with wortmannin (PI 3-kinase inhibitor, 100 nM), Diphynylethyleniodium (NADPH oxidase inhibitor, 10 µM), N-acetyl cysteine (antioxidant, 10 mM), and losartan (AT1R blocker, 10 µM), but not PD98059 (MEK inhibitor, 20 µM), and PD123319 (AT2R blocker, 10 µM). This suggests that NADPH oxidase is involved in ANG II-stimulated mTOR activity. Next, we examined whether PKC and receptor endocytosis, or SGK (serum glucocorticoid kinase) are involved in ANG II-stimulated mTOR activity. ANG II-stimulated phosphorylation of p70S6K was decreased when cells were treated with rottlerin (PKCδ inhibitor, 10 µM), dynasore (endocytosis inhibitor, 80 µM) and GSK650394 (SGK inhibitor, 10 µM). Interestingly, ANG II stimulated phosphorylation of PKCδ which was inhibited by SGK inhibitor, GSK 650394. From these results, we conclude that ANGII-stimulated SGK/PKC/ NADPH oxidase may play a role in activation of mTOR which contributes to inhibition of insulin signaling.

**INTEGRATED PHYSIOLOGY—INSULIN SECRETION IN VIVO**

142-LB

**WITHDRAWN**

**143-LB**

**Arginine (Arg) Stimulation Provides Repeatable Measures of Beta Cell Function (BCF) that Can Distinguish Across Spectrum of Glucose Tolerance (GT)**

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The ability to reproducibly measure BCF in longitudinal clinical trials is desirable. Useful BCF methods should distinguish among normal GT (NGT), prediabetes (PDM), and type 2 DM (T2DM). Acute insulin responses to Arg under both basal (AIRg) and glucose-potentiated (AIRgMAX) conditions measure BCF, but within-subject variability across spectrum of GT has not been assessed. The objectives of this study are to assess: 1. Response to Arg in men and women with NGT, PDM, and T2DM; and 2. Repeatability (R) of the methodology.

During 2 separate visits, subjects received Arg (5 gm IV) after overnight fast. AIRg was determined in first 5 min post Arg followed by a 60 min infusion of glucose and repeat injection of Arg (AIRgMAX). AIRgMAX - AIRg = Insulin Secretory Reserve (ISR). All AIR parameters are adjusted for basal insulin (AIRg/basal insulin) in µU/mL. Table includes intraclass correlation coefficient (ICC), a measure of R. ICC > 0.8 = high R.

**144-LB**

**Modeled Response to a Standard Meal Is Useful Method to Characterize Beta Cell Function (BCF) and Insulin Sensitivity (IS) Across Spectrum of Glucose Tolerance (GT): Corroboration With FSIGT**

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It is important to easily measure BCF and IS in longitudinal clinical trials and show similar responses with methodology like the FSIGT. Mixed meal tolerance tests (MMTT) are appealing due to simplicity and relevance to enteric physiology, yet complex due to variable absorption. The goal of this study is to measure BCF and IS to a standard, commercially available 450 kcal meal across the spectrum from normal GT (NGT) to prediabetes, both within and across GT. In conclusion, Arg testing has high repeatability within each GT population and distinguishes among NGT, PDM, and T2DM, suggesting that it may be useful to assess changes in BCF over time.

Supported by: FNIH Biomarkers Consortium Beta Cell Project

For author disclosure information, see page LB66.
For MMITT and FSIGT, SI, f(t)/f(O) and DI, values decline from NGT to T2DM (all P<0.001). Correlation analysis for each MMITT/FSIGT parameter pair across ALL 3 GROUPS: SI = [r=0.69; f(t)/f(O)=r=0.73; and DI (r=0.74), suggesting that values from the tests tracked similarly across GT states. In conclusion, modeled results from the MMITT correspond to FSIGT-derived parameters across range of responses of GT spectrum.

Supported by: FNIH Biomarkers Consortium Beta Cell Project

A Arginine (Arg) is Preferred to Glucagon (Glgn) in Stimulation Testing for Beta Cell Function (BCF)
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Arg and Glgn have been used as stimuli to quantify acute insulin secretory responses (AIR). To date no study has compared the responses to Arg and Glgn nor the repeatability (R) of the tests within the same subjects. The objects of this study were to determine: 1. the tolerability of both procedures; and 2. the repeatability of the BCF measures obtained with Arg and Glgn.

Obese (BMI 31.5±2.8 (kg/m²) subjects (n=23 (12M/11W)) with normal glucose tolerance were studied twice with Arg or Glgn in a randomized crossover. On separate days during each of 2 visits, AIRs to Arg (5 gm IV over 30 sec) and Glgn (1 mg IV over 30 sec) were measured at basal glucose (AIRarg and AIRglgn) and after 60 min infusion (900 mg/min) of glucose (glucose-over. On separate days during each of 2 visits, AIRs to Arg (5 gm IV over 30 sec) and Glgn (1 mg IV over 30 sec) were measured at basal glucose (AIRarg and AIRglgn) and after 60 min infusion (900 mg/min) of glucose (glucose-potentiated AIRs (AIRargMAX and AIRglgnMAX)). Table summarizes results. Insulin Secretory Reserve (ISR)= AIRargMAX/AIRarg. R assessed using intraclass correlation coefficient (ICC), values of ICC > 0.8 = high R.

All subjects had significant responses to Arg and Glgn. Arg yielded better R than Glgn. AIR to Arg and Glgn correlated with one another across tests (AIRarg/AIRglgn r= 0.64; AIRargMAX/AIRglgnMAX r=0.64). Most common adverse events with Arg were mild transient flushing (33%) and oral paresthesias (46%). For Glgn, nausea was common (43%) and was moderate in severity in 13%. Due to better repeatability and tolerability with Arg, we recommend Arg over Glgn as a stimulus for testing BCF.

Supported by: FNIH Biomarkers Consortium Beta Cell Project

B A Broad Range, Highly Sensitive, Small Sample Volume Chemiluminescent ELISA for Measuring Insulin in Mouse and Rat Models
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Mouse and rat models are common preclinical models in diabetes research. Quantification of insulin in serum or plasma over a wide range of disease states or pre and post drug treatment is vital in furthering diabetes research. Many commercial rodent insulin ELISAs require extra sample dilution steps to ensure concentrations fall within a limited standard curve range. This results in increased sample preparation time as well as potential re-dilution and re-testing of samples. The aim of this study was to develop a broad range, highly sensitive, small volume (<10 µL) mouse/rat insulin ELISA to address these issues. The sandwich assay uses a black 96 well plate. Standards (0.1 -200 µg/mL) and samples (0.1 -200 ng/mL) were added to the plate followed by conjugate, incubation, plate wash, chemiluminescent substrate, and reading after 1 min. Sample concentrations were calculated from the relative light units which are directly proportional to the amount of insulin in the sample. Analytical and functional sensitivity were 0.08 ng/mL and 0.10 ng/mL, respectively. Sample linearity for mouse and rat samples (n=3 ea) across 3 dilutions ranged from 99-107% with r² values of 0.997. Samples were spiked with 3 levels of insulin and recovered at averages of 95, 91, and 97% at the low, mid, and high spikes, respectively. Serum and plasma concentrations from normal, fasted or fed diabetic and non-diabetic mice and rats ranged from 0.24 ng/mL to 129.7 ng/mL (n=76). CVs of the sample duplicates were 0.14-12.1% (median 1.7%). This study demonstrates the accuracy and linearity over the 2000-fold dynamic range of this chemiluminescent ELISA using a 5 µL sample size. The assay eliminates the need for sample dilution and provides the flexibility to run both mouse and rat samples on the same plate. Other advantages include a short 2-hour run time, the use of a standard chemiluminescent plate reader and potential time and cost savings for high volume screening labs.

Supported by: NIH

ADA-Funded Research

Identification of Monounsaturated Fatty Acids (MUFA) as Endogenous FAAH Inhibitors
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High-fat diet induced obesity (DIO) is associated with increased hepatic expression of lipogenic genes, including sterol-CoA desaturase-1 (SCD1), and mice deficient in SCD1 are resistant to DIO. DIO has also been linked to increased activity of the endocannabinoid/CB1 receptor system, including increased hepatic levels of the endocannabinoid anandamide (AEA), due to the reduced activity of the AEA-degrading enzyme, fatty acid amide hydrolase (FAAH). We have shown endocannabinoids contribute to diet-induced insulin resistance in mice via hepatic CB1-mediated inhibition of insulin signaling and clearance. Here we show that hepatic levels of AEA and FAAH activity remain unaffected by high-fat diet (HFD) in SCD1−/− mice, and that the monounsaturated fatty acid (MUFA) products of SCD1, palmitoleic acid and oleic acid, inhibit FAAH activity in vitro with a higher potency as compared with saturated fatty acids (16:0 and 18:0) and polyunsaturated fatty acid (20:4n6 and 22:6n3). HFD markedly increases hepatic SCD1 activity in wild type mice as well as in CB1−/− mice with transgenic re-expression of CB1 in hepatocytes (htCB1−/−), but not in global CB1 knock-out (CB1−/−) mice. Treatment of HFD-fed mice with the SCD1 inhibitor A939572 prevented the HFD-induced reduction of hepatic FAAH activity, normalized endogenous AEA level, and improved insulin sensitivity. SCD1−/− mice on HFD remain insulin sensitive, but develop glucose intolerance and insulin resistance in response to chronic treatment with the FAAH inhibitor UR8597. We conclude that the MUFA products of SCD1 act as endogenous FAAH inhibitors. This may account for the HFD-induced increase in hepatic AEA, which then activates hepatic CB1 receptors to induce hepatic insulin resistance.

Supported by: NIH

Ethnic Differences in Circulating Lipoprotein Profiles May Be Protective Against NASH in African Americans
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Nonalcoholic fatty liver disease (NAFLD) is a disorder of excessive hepatic fat (simple steatosis, SS) associated with lipoprotein derangements in a dose dependent fashion, independent of obesity and insulin resistance. Most reported studies, however, were performed in Class I-II (BMI: 25-35 kg/m2) obese, but little is known about such derangements in Class III (BMI: >40) obesity.

We assessed lipid profiles in 31 Class III Caucasian and African American subjects scheduled for bariatric surgery. Using NMR spectroscopy, we determined triglycerides (TG), HDL cholesterol, ApoB100, VLDL, LDL, and HDL concentrations and respective lipoprotein particle sizes and subclass concentrations. Subjects were segregated into 3 age-, BMI- and gender-matched cohorts based on NAFLD Activity Scoring (NAS) of liver biopsies obtained at surgery; this led to classifying subjects as normal (n=11), SS (n=11), or NASH (n=9). African Americans (n=9) did not display NASH. Insulin clamps performed in 4 subjects of each group revealed significant decreases (p<0.05) in hepatic insulin sensitivity in NASH compared to SS. Plasma lipid profiles revealed that NASH subjects had increased TG (65%), large VLDL concentration (53%) and size (16%) and lipoprotein insulin resistance scores (LP-IR, 60%). Compared to Caucasians, African Americans with SS had decreased levels of total and small, medium and large VLDL, LDL, TG, LP-IR, and ApoB100, and had increased HDL size (p<0.05). The improved lipid profile in African Americans may contribute to protection from NASH, suggestive that altered circulating lipoprotein production or uptake may be indicative of NAFLD progression in Class III obese insulin resistant Caucasians.
Hepatic Fat Is a Determinant of Hepatic Insulin Sensitivity in Pre-Diabetes

ISABEL ERAZZURIZ CRUZAT, CHIARA DALLA MAN, SIMMI DUBE, CLAUDIO COBELLI, ANANDA BASU, JOHN PORT, RITA BASU, Rochester, MN; Padova, Italy.

Hepatic fat has been implicated as a marker of insulin resistance. However, the relationship between hepatic fat and hepatic insulin sensitivity (ability of insulin to suppress hepatic glucose production) has not been assessed in prediabetes. To do so, we studied individuals with prediabetes (n=15, 7M/8F, age 58±14 yrs, FPG 6.1±0.5 mM, 2-hour glucose 9.3±1.9mM, BMI 31±3 kg/m², LBM 48±8 kg) with or without abdominal obesity. Total hepatic fatty acid content was measured using single-breath-hold liver magnetic resonance spectroscopy, with spectra processed using LCModel. The methyl peak (Lip09) was used to quantify total fatty acid (FA) concentration. Hepatic insulin sensitivity (SI) was determined by both model independent (IAUC endogenous glucose/IAUC insulin) method and our validated oral minimal model to measure hepatic insulin sensitivity. Am J Physiol Endocrinol Metab 2008;295:E1152-1159

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INTEGRATED PHYSIOLOGY—MACRONUTRIENT METABOLISM AND FOOD INTAKE

Hypothalamic Circadian Control of the Homeostatic Refeeding Response and Insulin Resistance in Mice

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The pacemaker clock within the central nervous system programs 24 hr cycles of behavior and peripheral tissue metabolism across the sleep-wake/fasting-feeding period. However, it is not clear how circadian signals regulate mechanisms of energy and glucose homeostasis. Here we demonstrate that the homeostatic refeeding response to fasting is regulated by circadian timing, as acute 12 hr food consumption following a 24 hr fast is greater when feeding resumes in the night time compared to the day time, despite equivalent leptin levels. In addition, we find that exogenous leptin suppresses the refeeding response only during the daytime, in parallel with increased hypothalamic pSTAT3 signaling, suggesting leptin sensitivity in the hypothalamus is regulated in a circadian manner. We further discover that Bmal1 expression in the hypothalamus is necessary for the circadian control of the acute refeeding response to fasting in mice, and that genetic ablation of the hypothalamic clock leads to increased peripheral insulin resistance during hyperinsulinemic euglycemic clamp. Surprisingly, restriction of feeding to the night reversed insulin resistance in circadian mutant mice. These results provide genetic evidence for the regulation of energy and glucose homeostasis by the hypothalamic clock and have therapeutic implications for circadian intervention to treat metabolic disorders.

Supported by: ADA-Funded Research

149-LB
Dietary Iron Regulates the Circadian Rhythm of Hepatic Glucoseogenesis Through Heme Synthesis
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The circadian rhythm of the liver is important in the maintenance of glucose homeostasis, and disruption of this rhythm is associated with type 2 diabetes risk. Feeding is one factor that sets the circadian clock in peripheral tissues, but relatively little is known about the role of specific dietary components in that regard. We have assessed the effects of dietary iron on the circadian rhythm of the liver. Dietary iron affects circadian glucose metabolism through heme-mediated regulation of the interaction of Rev-Erbx with its cosuppressor NCOX. Loss of regulated heme synthesis was achieved by aminolevulinic acid (ALA) treatment of mice or cultured cells, to bypass the rate-limiting enzyme in hepatic heme synthesis, ALAS1. ALA treatment abolishes differences in hepatic glucose production and in the expression of gluconegenic enzymes seen with variation of dietary iron. The differences among diets are also lost with inhibition of heme synthesis by treatment with bisnorchondrohydrazine. Heme levels respond to dietary iron through modulation of the level of Peroxisome Proliferator-Activated Receptor Coactivator 1α (PGC-1α), a transcriptional activator of ALAS1. Treatment of mice with the antioxidant n-acetylcysteine diminished the PGC-1α variation observed among the iron diets, suggesting that iron may be acting through reactive oxygen species signaling to regulate PGC-1α. Together, these studies show that dietary iron alters the circadian rhythm of metabolism through control of intracellular heme synthesis.

Supported by: U.S. Dept. of Veterans Affairs; NIH

152-LB
Glycine Sensing in the Dorsal Vagal Complex Lowers Food Intake
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Glycine is in the dorsal vagal complex (DVC) activates N-methyl-D-aspartate receptors to lower hepatic glucose production and triglyceride-rich lipoprotein secretion in rats. Given that leptin and GLP-1 signalling in the DVC lowers appetite, we tested whether DVC glycine sensing regulates food intake. Male SD rats were implanted with stereotoxic cannulae into the DVC. After a 22-h fast, glycine (10 µmol/L, n=6) or saline (n=5) was injected into the DVC, and food intake was measured every hour for 6 h and at 20 h post-refeeding. DVC glycine administration (10 µmol/L) increased DVC tissue glycine levels 1.5-fold (P<0.02). This was recapitulated by peripheral glycine administration (50 nmol/kg, iv), which elevated circulating glycine levels 2-fold (741±67 vs. 1291±277 µM/L, P<0.04) and similarly increased DVC glycine levels 1.5-fold (P<0.02). Acute DVC glycine lowered cumulative food intake compared with saline controls at 5 h (-30%; 17.3±2.2 vs. 24.7±1.6, P<0.03), 6 h (-35%; 19.7±3.5 vs. 30.2±2.3, P<0.05), and 20 h (-12%; 38.7±1.6 vs. 44.2±1.8 g, P<0.04) post-refeeding.

To evaluate the therapeutic potential of glycine delivery to lower food intake in diet-induced obesity, we tested the effects of 1% (w/v) glycine in drinking water in high fat diet-fed rats. This elevated plasma glycine levels 2.5-fold (786±29 vs. 1906±153 µM/L, P<0.05; but not 3.0-fold (P>0.05). Using donor marrow expressing RFP-coupled MCP-1 receptors (CCR2-RFP), we confirmed that post-ablative microglial were ablated by peripheral DT injection. As for slices, post-ablative microglia were ablated by peripheral DT injection. As for slices, post-ablative microglia were ablated by peripheral DT injection. We lethally irradiated head-shielded CD11b-DTR mice, transplanted them with marrow lacking DTR. After recovery, microglia ablation in slices was transient, and new microglia restored tissue content within 10 days. We also lethally irradiated head-shielded CD11b-DTR mice, transplanted them with marrow lacking DTR. After recovery, microglia ablation in slices was transient, and new microglia restored tissue content within 10 days. We also lethally irradiated head-shielded CD11b-DTR mice, transplanted them with marrow lacking DTR. After recovery, microglia ablation in slices was transient, and new microglia restored tissue content within 10 days. We also lethally irradiated head-shielded CD11b-DTR mice, transplanted them with marrow lacking DTR. After recovery, microglia ablation in slices was transient, and new microglia restored tissue content within 10 days. We also lethally irradiated head-shielded CD11b-DTR mice, transplanted them with marrow lacking DTR. After recovery, microglia ablation in slices was transient, and new microglia restored tissue content within 10 days.

In summary, we found that benzodiazepine receptor agonists suppress daily food intake, and acute treatment of the DVC with glycine lowers food intake in diet-induced obesity. These findings suggest that glycine or a glycine analog may have nutraceutical benefits to lower food intake in obesity by triggering the CNS.

Supported by: CHR

153-LB
Microglia Modulate a Hypothalamic Inflammatory Response to Saturated Fatty Acids in Mice
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High-fat diets promote the accumulation and inflammation of inflammatory (M1) activation of macrophages in peripheral tissues and of microglia, CNS analogs of macrophages, in the hypothalamus. Saturated fatty acids (SFAs) trigger macrophage activation, but what triggers microglial activation is unknown. We found that feeding mice a 6-week diet rich in SFAs altered levels of several lipid classes, including the SFA palmitic acid, specifically in the hypothalamus in association with microglial activation and accumulation. Moreover, treating primary murine microglia or hypothalamic slice cultures with long-chain SFAs, but not unsaturated or short-chain species, increased M1 gene mRNA levels and stimulated inflammatory cytokine secretion. We also found that hypothalamic cultures of microglia by treatment with clodronate liposomes or by using diphtheria toxin (DT) to treat slices from mice expressing diphtheria toxin receptor under control of the CD11b promoter (CD11b-DTR mice). Microglial ablation greatly reduced SFA-induced cytokine secretion, pointing to the importance of hypothalamic microglia for the inflammatory response to SFAs. Microglial ablation in slices was transient, and new microglia restored tissue content within 10 days. We also lethally irradiated head-shielded CD11b-DTR mice, and transplanted them with marrow lacking DTR. After recovery, microglia were ablated by peripheral DT injection. As for slices, post-ablative microglial proliferation occurred in vivo. Using donor marrow expressing RFP-coupled MCP-1 receptors (CCR2-RFP), we confirmed that post-ablative microglial proliferation does not involve peripheral monocytes. This proliferation increased basal hypothalamic microglial content vs. DTR-mutant controls. Our results indicate that SFAs stimulate microglia-dependent hypothalamic

For author disclosure information, see page LB66.
The effects of high fat feeding on cardiovascular health and hypothalamic function have been studied extensively. Diets high in saturated fat have been shown to lead to an immediate decrease in weight gain, as seen in the study where rats fed a lard-based high-saturated fat diet (HF) had a weight gain of 5% at W2, 8% at W6, and 16% at W24 (n=4 for W24 cardiac data; p<0.05). No additional changes were observed in left ventricular mass, dimensions, or blood pressure at W2, -26% at W6, -16% at W24. This study provides new insight into the development of cardiometabolic dysfunction in as little as 2 weeks.

Cardiovascular disease is a major complication of Type 2 Diabetes Mellitus (T2DM). Increased dietary fat consumption is a risk factor for each of these diseases and we propose that it is a common mechanism during the development of both. Thus, it is critical to identify areas of intervention during the early stages of cardiometabolic disease progression. We hypothesize that high fat feeding will lead to an immediate and observable decrease in cardiometabolic function in as little as 2 weeks.

Intravenous glucose tolerance tests and cardiac magnetic resonance imaging (cMRI) were conducted in eight male dogs at baseline (W0), and after 2, 6, and 24 weeks of fat feeding (W2, W6, W24). Normal Chow consisted of 40% fat. Fat feeding led to a significant decrease in body weight and age, resulting in a reduction in food intake and increased activity. MG+ spines had reduced proteoglycan content of the nucleus pulposus (Fig. 1) and higher vertebral cortical thickness and area than MG^- mice (p<0.05). AGE/MG+ mice exhibited greater MG accumulation in vertebral endplates, higher bone mineral density (p<0.01) and lower connectivity density in superior endplates (p<0.01) than MG^- mice.

These results support our hypothesis that acute high fat feeding impairs cardiometabolic function, and provides new insight into the development of cardiovascular disease in T2DM.
The common food additive carrageenan produced glucose intolerance, insulin resistance, and impaired insulin signaling in C57BL/6 mice treated with high molecular weight carrageenan in the water supply (10 µg/ml) for 18 days. To analyze further the impact of carrageenan, hyperinsulinemic, euglycemic clamp studies were performed. Studies were performed in 24 mice (twelve treated and twelve controls) that were fasted for 5 h. Mice had indwelling jugular vein and carotid artery lines, and insulin was infused at 4mU/kg/min and the glucose infusion rate was adjusted to achieve glucose homeostasis with glucose measured at 10 minute intervals. The carrageenan-treated animals demonstrated: significantly higher arterial glucose levels due to a reduced glucose clearance and lower endogenous glucose production rates at baseline. The time course of glucose infusion rate to achieve a steady state glucose concentration during the clamp was slowed with carrageenan-treatment. Other studies demonstrated that in mice given carrageenan in their water, controls and treated animals had similar glucose tolerance at 3 days, but by six days, carrageenan-treated animals had significantly higher glucose levels at all time points. The chemokine IL-8 (or KC, the mouse homolog) was increased following carrageenan exposure, and experiments in human hepatocytes showed that IL-8 treatment inhibited production of phospho(Ser473)-AKT and PI3K activity following insulin, suggesting that some of the effects that follow carrageenan may be mediated by IL-8. Future studies will further clarify the mechanisms by which oral carrageenan exposure induces systemic inflammation with extracolonic effects, including impaired insulin signaling, and may contribute to development or progression of clinical diabetes.

**159-LB**

The FGF21 Response to Fructose in Humans: Defining a Fructose Tolerance Test

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Recent evidence implicates high fructose consumption as a risk factor for obesity, diabetes and metabolic syndrome (MetS). Currently, there is no method to quickly assess an individual’s biological response to fructose. Fibroblast growth factor 21 (FGF21) is a metabolic hormone involved in lipid and glucose metabolism. In humans, FGF21 levels positively correlate with features of MetS, however its regulation is poorly understood. Dietary manipulations such as fasting and ketogenic diet increase FGF21 in rodents but have little or no effect in humans. In rodents, FGF21 levels increase markedly with fructose consumption. We therefore hypothesized that fructose ingestion might regulate FGF21 levels in humans. We administered a 75g oral fructose load to 10 lean subjects. Serum FGF21 rose within 90 min and peaked at 120 min with a fourfold increase over baseline [P < 0.0002]. In contrast, a 75 gram oral glucose load had no effect on FGF21 at this time point. Next we evaluated the FGF21 response following oral fructose in 7 subjects with MetS. The FGF21 excursion was higher in MetS compared to lean controls [FGF21 AUC: Control 53 ± 4.4 ng/ml vs. MetS 141.7 ± 115, P < 0.007]. We also compared the FGF21 excursion following ingestion of a combined fructose + glucose load (37.5g each). While there was an 8-fold variation in the FGF21 excursion across subjects, for any given individual there was a strong correlation between the response following fructose and the response following the mixture (R2=0.75, P<0.001). The variability across but reproducibility within individuals indicates that genetic or chronic environmental factors, such as long-term dietary composition, may govern an individual’s FGF21 response to fructose. To our knowledge, FGF21 is the only known measurable circulating biomarker that specifically assesses an individual’s acute metabolic response to fructose ingestion. This bioassay will form the foundation for a new paradigm for investigating fructose-associated metabolic disease.

Supported by: JPB Foundation

**161-LB**

Elf2α Phosphorylation in Skeletal Muscle Increases Fgf21 Expression as a Myokine and Prevents Diet-Induced Obesity by Increasing Energy Expenditure

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Endoplasmic reticulum (ER) stress has emerged as an important cause of diabetes. Unfolded protein response is an adaptive process in response to ER stress that is activated by three transducers, IRE1, ATF6, and PERK. We previously reported that the expression of gluconeogenic and lipogenic genes in liver (Cell Metab 2008) requires PERK-mediated elf2 phosphorylation, but the role of PERK signaling pathway in skeletal muscle remains unclear. In this study, we generated skeletal muscle-specific transgenic (TG) mice that overexpress Fx2E-PERK, which promotes elf2 phosphorylation by the artificial ligand AP20187 uncoupled from ER stress in a dose-dependent manner. These TG mice are a powerful tool to dissect the role of PERK signaling; we found that elf2α phosphosylated TG mice were resistant to high-fat diet-induced obesity because of increased energy expenditure. In skeletal muscle, the fiber type composition and metabolic gene expression of TG mice were similar to those of wild-type mice. However, mRNA expression of thermogenic genes in brown adipose tissue was higher in TG mice. Microarray analysis of mRNA expression in the skeletal muscle of TG mice revealed higher expression of the metabolic hormone Fgf21, which is known to have anti-diabetic, anti-hyperlipidemic, and anti-obesity effects. Consistent with this, plasma FGF21 concentration was markedly increased in TG mice. Promoter analysis identified that elf2α regulates Fgf21 in a downstream transcription factor, ATF4, dependent manner. Our results show that elf2α phosphorylation in skeletal muscle prevents obesity by increasing energy expenditure of brown adipose tissue but not skeletal muscle. We conclude that this phenotype is mediated via FGF21 from skeletal muscle, suggesting that FGF21 is an ER stress/PERK/elf2α-induced myokine and that phosphorylation of elf2α is a potential therapeutic target for diabetes and obesity.

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**158-LB**

Insulin Resistance Following Exposure to the Common Food Additive Carrageenan Demonstrated by Hyperinsulinemic, Euglycemic Clamp Studies

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Mitochondrial dysfunction is proposed to be both a cause and consequence of insulin resistance and recent data suggest that mitochondrial morphology and function using intravitral microscopy. Subsarcolemmal (SS) and intramyofibrillar (IMF) mitochondria were imaged in muscle fibers using a mitochondrial-specific GFP tag or by average NADH-positive organelle area (pixel area). Mitochondria oxidation was measured by the level of NADH autofluorescence (AF; grey value/µm²). Mice were studied in the fed state or after a 24 hr fast that induced marked glucose intolerance (p<0.003). Fasting caused mitochondrial fragmentation as indicated by a 62% (SS) and 43% (IMF) decrease in NADH area (p<0.01). Fasting also caused a 70% (SS) and 84% (IMF) decrease in mitochondrial oxidation (p<0.02). The AMPK activator AICAR (1gr/kg i.v) rapidly (30 min) normalized both the fasting-induced fragmentation and decreased oxidation. To determine the effects of AMPK activity independent of nutritional state, skeletal muscle-specific transgenic (TG) mice with increased or decreased AMPK activity were studied in the fed state and compared to their respective littermate controls. TG mice with increased AMPK activity had mitochondrial elongation [SS:492%, IMF:324% increase in NADH area; (p<0.003)] and increased oxidation [SS:237%, IMF:212% increase in NADH AF; (p<0.03)]. TG with decreased AMPK activity had mitochondrial fragmentation [SS:40%, IMF:85% decrease in NADH area (p<0.003)] and decreased oxidation [SS:51%, IMF:57%, decrease in NADH AF (p<0.03)]. Fasting did not further alter mitochondria morphology and oxidation in TG mice. In conclusion, fasting-induced insulin resistance causes rapid mitochondrial dysfunction that is reversed by AMPK activation. AMPK is an important regulator of mitochondrial morphology.

Supported by: JPB Foundation

**160-LB**

Fasting-Induced Mitochondrial Dysfunction Is Reversed by AMPK Activation in Mouse Skeletal Muscle

HANS P. LAURITZEN, LAURIE J. GOODYEAR, Boston, MA

Mitochondrial dysfunction is critical in organelle function. We determined the effects of fasting-induced insulin resistance and AMPK activity on mitochondrial morphology and function using intravitral microscopy. Subsarcolemmal (SS) and intramyofibrillar (IMF) mitochondria were imaged in muscle fibers using a mitochondrial-specific GFP tag or by average NADH-positive organelle area (pixel area). Mitochondria oxidation was measured by the level of NADH autofluorescence (AF; grey value/µm²). Mice were studied in the fed state or after a 24 hr fast that induced marked glucose intolerance (p<0.003). Fasting caused mitochondrial fragmentation as indicated by a 62% (SS) and 43% (IMF) decrease in NADH area (p<0.01). Fasting also caused a 70% (SS) and 84% (IMF) decrease in mitochondrial oxidation (p<0.02). The AMPK activator AICAR (1gr/kg i.v) rapidly (30 min) normalized both the fasting-induced fragmentation and decreased oxidation. To determine the effects of AMPK activity independent of nutritional state, skeletal muscle-specific transgenic (TG) mice with increased or decreased AMPK activity were studied in the fed state and compared to their respective littermate controls. TG mice with increased AMPK activity had mitochondrial elongation [SS:492%, IMF:324% increase in NADH area; (p<0.003)] and increased oxidation [SS:237%, IMF:212% increase in NADH AF; (p<0.03)]. TG with decreased AMPK activity had mitochondrial fragmentation [SS:40%, IMF:85% decrease in NADH area (p<0.003)] and decreased oxidation [SS:51%, IMF:57%, decrease in NADH AF (p<0.03)]. Fasting did not further alter mitochondria morphology and oxidation in TG mice. In conclusion, fasting-induced insulin resistance causes rapid mitochondrial dysfunction that is reversed by AMPK activation. AMPK is an important regulator of mitochondrial morphology.

Supported by: JPB Foundation
162-LB
High-Fat Diet-Induced Impairment of Skeletal Muscle Insulin Action Is Not Prevented by SIRT1 Overexpression
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SIRT1 has been implicated in the regulation of skeletal muscle metabolism in response to changes in nutrient availability, though its role in the modulation of skeletal muscle insulin action remains to be completely defined. Previous studies have demonstrated that SIRT1 expression decreases under insulin-resistant conditions, such as those induced by a high-fat/hypercaloric diet (HFD). Thus, we sought to determine whether augmenting skeletal muscle SIRT1 levels via constitutive activation in a mouse model would prevent HFD-induced skeletal muscle insulin resistance. To address this, mice with muscle-specific overexpression of SIRT1 (mOX) and their wildtype (WT) littermates were fed low-fat control diet (CON; 10% calories from fat) or a HFD (60% of calories from fat) for 12 weeks beginning at 10 weeks of age. Magnetic resonance imaging and indirect calorimetry were used to measure body composition and energy expenditure (EE), respectively. Insulin-stimulated glucose uptake was measured using a 2-deoxyglucose uptake assay at a physiological insulin concentration of 0.36 nmol/L (80 µU/mL) in isolated soleus and extensor digitorum longus (EDL) muscles. SIRT1 protein abundance was ~50-300-fold higher in soleus and EDL muscles from mOX vs. WT mice. As expected, body weight and percent body fat were increased by 30% and 300%, respectively, in HFD vs. CON animals, while there was no effect of genotype on these parameters. In addition, EE was not affected by diet or genotype, though HFD increased the contribution of fat to total EE. Importantly, 12 weeks of HFD decreased insulin-stimulated glucose uptake in skeletal muscle by 50%, and this impairment was not prevented in mOX mice. These impairments in insulin action were paralleled by decreased insulin-mediated activation of Akt and GSK3β. Taken together, the present results demonstrate that upregulation of SIRT1 activity in skeletal muscle does not prevent HFD-induced impairments in skeletal muscle insulin action.

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163-LB
BAF60c Drives Glycolytic Muscle Formation and Improves Glucose Homeostasis through Deptor-Mediated Akt Activation
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A shift from oxidative to glycolytic metabolism has been associated with skeletal muscle insulin resistance in type 2 diabetes. However, whether this metabolic switch is deleterious or adaptive remains controversial, in part due to limited understanding of the regulatory network that directs the metabolic and contractile specification of fast-twitch glycolytic muscle. Here we show that BAF60c, a transcriptional cofactor enriched in fast-twitch muscle, promotes a switch from oxidative to glycolytic myofiber type through Deptor-mediated Akt activation. Muscle-specific transgenic expression of BAF60c activates a program of molecular, metabolic, and contractile changes characteristic of glycolytic muscle. In addition, BAF60c is required for maintaining glycolytic capacity in adult skeletal muscle in vivo. BAF60c expression is significantly decreased in skeletal muscle from obese mice. Unexpectedly, transgenic activation of the glycolytic muscle program by BAF60c protects mice from diet-induced insulin resistance and glucose intolerance. Further mechanistic studies revealed that Deptor is induced by the BAF60c/Six4 transcriptional complex and mediates activation of Akt and glycolytic metabolism by BAF60c in a cell-autonomous manner. This work defines a fundamental mechanism underlying the specification of fast glycolytic muscle and illustrates that the oxidative to glycolytic metabolic shift in skeletal muscle is potentially adaptive and beneficial in the diabetic state.

Supported by: NIH, AHA

164-LB
Effect of Age and Exercise on FNDC5 and PGC1alpha Gene Expression in Human Skeletal Muscle
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Older subjects are at increased risk of developing insulin resistance and type 2 diabetes. Peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1α) is a master regulator of energy metabolism and some evidence indicates that PGC1α is down regulated with aging. In mouse muscle, overexpression of PGC1α enhances the mRNA level of fibronectin type III domain containing 5 (FNDC5), a gene that encodes for irisin, a protein that is secreted into the circulation. The goals of this study were to: 1) examine whether aging affects the gene expression of muscle FNDC5 and PGC1α and; 2) examine whether exercise up regulates the expression of these genes in aging. Insulin sensitivity (M) was measured with a hyperinsulinemic (40 mU/mL) euglycemic clamp and vastus lateralis muscle biopsies were performed in 24 young, non diabetic (age=22±3 y, BMI=24.1±0.5 kg/m², V02max=25.4±2 ml/kg/min, M/I=16.8±1.7 mg/kg FFM/min/m²), young (age=23±2 y, BMI=24.4±0.4, V02max=17.0±1.9, M/I=14.1±2.2 y, respectively). In 17 older and 10 younger, these measurements were done before and after 16 week aerobic exercise program. At baseline, both PGC1 and FNDC5 mRNA levels were significantly lower in older (50% and 24% of younger, respectively; P<0.05). PGC1α mRNA directly correlated with FNDC5 (r=0.61, P<0.0001). Both PGC1 and FNDC5 mRNA negatively correlated with age (r=-0.51, P< 0.0001; r=-0.27, P< 0.02, respectively). Exercise program increased PGC1α and FNDC5 expression in both older (40% and 30%, respectively; P<0.05) and younger (50% in both; P<0.05), accompanied by an increase in M/I (20% in older and 22% in younger) and VO2, (11% in older and 18% in younger). Conclusions: 1) Aging is associated with decreased PGC1 and FNDC5 gene expression, and these molecular changes could be involved in the metabolic alterations that occur during aging. 2) Exercise enhances the gene expression of PGC1 and FNDC5, an effect that possibly contributes to the beneficial metabolic effects of exercise in the elderly.

Supported by: NIA, NIDDK

165-LB
Deletion of Tribbles 3 (TRB3) Protects Mice from High Fat Diet-Induced Insulin Resistance and Hepatosteatosis
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TRB3 is known to regulate metabolism in multiple tissues and we have recently shown that TRB3 is important for Endoplasmic Reticulum (ER) stressor-induced insulin resistance in skeletal muscle. However, the role of TRB3 in nutritional stress-induced insulin resistance remains unclear. To determine the role of TRB3 in high fat diet-induced obesity and insulin resistance, we studied global TRB3 knockout (TRB3KO) mice fed a high fat diet (HFD; 60% fat by kcal) for eight weeks. TRB3KO mRNA expression in skeletal muscle was significantly increased by HFD in wild type mice by 5.2-fold (P<0.05). TRB3KO mice exhibited lower body weight (11%) and fasting blood glucose (205±9 vs. 151±14 mg/dl), and improved glucose tolerance (35% decrease in AUC) compared to wild type, with no difference in food intake. Serum leptin, insulin, and total cholesterol were lower and adiponectin concentrations higher in TRB3KO mice, indicating improved whole body glucose and lipid metabolism. Skeletal muscle from TRB3KO mice had normal levels of GLUT1, GLUT4, glycogen, triglycerides, and mRNA expression of ER stress markers. However, muscles from TRB3KO mice had increased insulin-stimulated glucose uptake and phosphorylation of IRS1 (Y912), Akt (T308), FoxO1 (S256), and FoxO3a (S253), demonstrating improved insulin sensitivity. Liver triglycerides were decreased by 57% and tissue glycogen by 49% lower. However, TRB3KO mice are protected from HFD-induced steatosis. There were no differences in mRNA expression of genes involved in ER stress and gluconeogenesis, but decreased expression of lipogenic genes (SREBP1, FAS, ACC, and SCD1) in livers from TRB3KO mice. In conclusion, TRB3KO mice are protected from high fat diet-induced muscle insulin resistance and hepatic steatosis. TRB3 is a potential target for type 2 diabetes treatment.

INTEGRATED PHYSIOLOGY—OTHER HORMONES

166-LB
Testosterone Restores Insulin Sensitivity in Males With Hypogonadotropic Hypogonadism (HH) Through Its Novel Anti-Inflammatory Actions and the Suppression of Free Fatty Acids (FFA). Tumor Necrosis Factor (TNF) α, Suppressor of Cytokine Signaling (SOCS)-3 and IkB Kinase (IKK) β Independently of Weight Loss
SANDEEP DHINSA, HUSAM GHANIM, KELLY GREEN, SANAA ABUAYSEH, NITESH KUHADIYA, SARTAJ SANDHU, MANAV BATRA, ANTOINE MAKDISSI, AJAY CHAUDHURI, PARESH DANDONA, NITESH KUHADIYA, SARTAJ SANDHU, MANAV BATRA, ANTOINE MAKDISSI, AJAY CHAUDHURI, PARESH DANDONA, Buffalo, NY
We have recently demonstrated that HH occurs in one third of men with type 2 diabetes (T2D) and these patients have a significantly greater degree (by 30%) of insulin resistance. We have now asked the question whether the replacement of testosterone (T) in such patients reverses insulin resistance and whether the factors known to be inflammatory and to interfere with insulin signal transduction are suppressed by T. 20 men with T2D and HH were randomized to receive intramuscular T (250 mg) or placebo (1ml saline) every 2 weeks for 6 months (n=10 in each group). Insulin sensitivity was calculated from the glucose infusion rate(GIR) during the last 30 min of a 4 hour For author disclosure information, see page LB66.
Type 2 diabetes is associated with chronic inflammation that affects organs such as the heart. Monocyte chemotactic protein-1 (MCP-1) is a pro-inflammatory factor that contributes to the development of diabetic cardiomyopathy by facilitating hyperglycemia-induced cardiomyocyte endoplasmic reticulum (ER) stress and apoptosis. Activation of the glucagon-like peptide-1 receptor (GLP1R) by Ex4 prevents hyperglycemia-induced cardiomyocyte ER stress and apoptosis. The present study tests the hypothesis that Ex4 is also cardioprotective in a mouse model of inflammatory cardiomyopathy. Cardiac specific overexpression of MCP1 (MHC-MCP1) increases monocyte infiltration, ER stress, cardiac fibrosis and left ventricular dysfunction. Three month-old MHC-MCP1 mice were implanted with osmotic minipumps delivering either Ex4 (24 nmol/kg/day) or PBS for 12 weeks and were compared to wild-type mice receiving PBS (WT). Compared to WT, MHC-MCP1 mice receiving PBS exhibited decreased fractional shortening (FS; 48.5±1.2 vs. 32.3±1.0%; p<0.05) and ejection fraction (EF; 84.3±1.2 vs. 67.3±1.2%; p<0.05). MHC-MCP1 mice receiving Ex4 displayed FS and EF values comparable to WT (42.6±1.2% and 82.6±1.3%; p>0.05 vs. MHC-MCP1 PBS). Despite the improved cardiac function, MHC-MCP1 mice infused with Ex4 did not exhibit a decrease in monocyte infiltration or cardiac fibrosis. However, compared to MHC-MCP1 mice receiving PBS, MHC-MCP1 mice receiving Ex4 displayed reduced apoptosis as indicated by lower TUNEL staining (3.3±0.3 vs. 0.9±0.1%, p<0.05) and caspase 3 cleavage (2.9±0.6 vs. 0.6±0.5 cleaved,uncleaved, p<0.05). Ex4 also attenuated expression of the ER stress markers GRP78, PDI and CHOP. In sum, Ex4 improves cardiac function and reduces ER stress and cardiomyocyte apoptosis.
apoptosis in MHC-MCP1 mice. This demonstrates a novel anti-inflammatory role for Ex4 and proposes that the GLP1R is a viable target for the treatment of cardiomyopathies associated with inflammatory diseases such as type 2 diabetes.

**171-LB**

**Synergistic Glucose-Lowering Effects of SGLT1- and ASBT-Inhibitor Combinations in ZDF Rats**

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The profound, rapid, and weight-independent antidiabetic effects of bariatric surgery affirm the promise of modulating gastrointestinal function for therapeutic purposes. We have previously demonstrated separate glucose-lowering effects of inhibitors of the sodium-glucose cotransporter 1 (SGLT1) and the apical sodium-dependent bile acid transporter (ASBT) in human and/or animal studies.

A potential synergy between SGLT1 inhibition and ASBT inhibition was tested in male Zucker-fatty diabetic rats using a novel dose-ratio scanning method. First, equi-effective (ED50) glucose-lowering doses of an SGLT1 inhibitor (KGA2727; 0.5 mg/kg bid) and an ASBT inhibitor (GSK2299027; 0.14 mg/kg bid) were determined by dose-response analysis of each agent. At high doses, both KGA2727 and GSK2299027 significantly decreased blood glucose. In subsequent studies, a maximally effective dose of an ASBT inhibitor (Z64W94) reduced plasma [glucose] to 196 ± 33 mg/dL versus 341 ± 24 mg/dL in vehicle controls. A series of 9 combination treatments in ZDF rats (n=16 rats each), the subtraction of one agent was made up by the addition of the other, were conducted at the same time. With a 50%-60% or 50%-50% of ED50 mixture (KGA2727, 0.25 mg/kg; GSK2299027, 0.07 mg/kg), maximal effect was attained in ceum weight, fecal water content, or fecal bile acid excretion.

In summary, we demonstrate synergy of the glucose-lowering, but not the adverse effects of distinct gastrointestinal mechanisms (ASBT and SGLT1 inhibition), each capable of being invoked by agents restricted to the gut lumen.

**172-LB**

**Low Circulating Levels of Insulin-Like Growth Factor-I (IGF-I) in Healthy Adult Males Are Associated With Reduced Beta-Cell Function, Increased Intramyocellular Lipid Content and Enhanced Fat Utilisation**

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Low IGF-I levels are linked to increased risk of T2D in epidemiological studies. We explored fasting metabolism associated with variations in IGF-I levels in healthy adults. IGF-I levels were measured in 300 healthy, non-obese male volunteers (age: 34.8±8.9 years; BMI 23.4±0.9 kg/m^2; WHR 0.90±0.02) and eight age & BMI matched Caucasian subjects (age: 29.4±4 years; BMI 23.1±0.9 kg/m^2; WHR 0.90±0.02) were studied. All were confirmed to have normal oral glucose tolerance following a 75 gm. glucose load.

All subjects underwent a 4-h paired oral glucose tolerance test (50 gm.) and isoglycaemic i.v. glucose (20%) infusion study involving sampling for insulin, c-peptide, glucagon, GLP-1 & GIP on 2 separate days. Blood glucose was measured using an YSI STAT analyser. Gastrointestinally mediated glucose disposal was calculated using the formula 100x[(glucose IGII-glucose OGTT)/glucose OGTT] with glucose OGTT being 50 gm, for all subjects.

There were no significant differences in the gastrointestinally mediated glucose disposal (IGID%) (South Asians 63.9±4.9; Caucasians 58.9±5.3). IGID is one of the ways of describing the incretin effect. Given our findings, other aspects of incretins need to be studied to confirm potential ‘racial’ differences in incretin biology.

**174-LB**

**Adipochip 2.0: Simultaneous Monitoring of Fatty Acid and Glycerol Secretion from Adipocytes using Microfluidic Enzyme Assays**

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Research investigating the mechanisms and functions of adipose tissue has been spurred in recent years by the growing concern over medical and economical impacts of the increasing population of individuals living with obesity-related disorders. Lipolysis is an important metabolic function of adipocytes that results in the release of fatty acids and glycerol from triacylglycerol (an energy storage molecule). The physiological energy needs vary throughout the day, resulting in differing amounts of the released fatty acids that are recycled back into triacylglycerol or used by peripheral tissues. Dysfunctional lipolysis and fatty acid recycling are observed in obesity-related disorders like type 2 diabetes. Our goal is to develop a method of simultaneously monitoring fatty acid and glycerol concentrations secreted from the same group of adipocytes to learn more about fatty acid recycling and adipocyte function. Microfluidics is an ideal platform for perfusing cells and analyzing secreted products compared to conventional methods because of its inherent ability to reduce cell and reagent requirements, improve temporal resolution, and allow automation.

A novel multi-layer PDMS chip has been developed that integrates murine 3T3-L1 adipocyte perfusion, reaction of secreted fatty acid or glycerol with a fluorescence enzyme assay and detection on one device. The on-line limit of detection (LOD) of the glycerol assay is 1 µM, and the LOD of the fatty acid assay is 5 µM. Adipocytes are cultured on glass coverslips and are transferred to a reversibly-sealed cell chamber on the chip, where they are perfused for at least an hour. Secreted fatty acid and glycerol concentrations from adipocytes are monitored while under basal conditions and during lipolysis stimulation by the application of isoproterenol.
OBESITY—ANIMAL

175-LB

The Ubiquitin Ligase Siah2 Regulates Inflammatory Gene Expression in Obesity
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Obesity is a major risk factor for developing insulin resistance and type 2 diabetes and the chronic low-grade inflammation associated with obesity is an important link in the relationship between obesity and type 2 diabetes. Adipogenesis depends on the peroxisome proliferator-activated receptor gamma (PPARγ), a protein that functions as the "master switch" in regulating lipid and carbohydrate metabolism in adipocytes. The insulin-sensitizing thiazolidinediones are PPARγ ligands that have potent anti-inflammatory effects, but are associated with adverse effects that limit their use. In our studies to understand how PPARγ activity is regulated by posttranslational modification of PPARγ by ubiquitin, we identified the mammalian homolog of Drosophila seven-in-absentia, Siah2, as a regulator of ligand-mediated changes in PPARγ activity and protein levels in adipocytes. Our current studies in Siah2-/mice indicate Siah2 plays a role in the relationship between obesity and inflammation of adipose tissue. Siah2-/mice become obese on a high-fat diet and although the adipocytes are uniformly large, there are significantly fewer "crown-like" structures in the adipose tissue compared to wild-type mice. This correlates with increased levels of PPARγ protein in adipose tissue and improved insulin sensitivity as determined by glucose and insulin tolerance testing and lipolysis assay of isolated adipocytes, suggesting Siah2 is an important determinant of insulin sensitivity in obesity. Microarray analysis of the adipose tissue from high-fat fed wild-type and Siah2-/- mice shows significant changes in PPARγ activity and protein levels in adipocytes. Our current studies to understand how PPARγ activity is regulated by posttranslational modification of PPARγ by ubiquitin, we identified the mammalian homolog of Drosophila seven-in-absentia, Siah2, as a regulator of ligand-mediated changes in PPARγ activity and protein levels in adipocytes. Our current studies in Siah2-/mice indicate Siah2 plays a role in the relationship between obesity and inflammation of adipose tissue. Siah2-/mice become obese on a high-fat diet and although the adipocytes are uniformly large, there are significantly fewer "crown-like" structures in the adipose tissue compared to wild-type mice. This correlates with increased levels of PPARγ protein in adipose tissue and improved insulin sensitivity as determined by glucose and insulin tolerance testing and lipolysis assay of isolated adipocytes, suggesting Siah2 is an important determinant of insulin sensitivity in obesity. Microarray analysis of the adipose tissue from high-fat fed wild-type and Siah2-/- mice shows significant changes in PPARγ activity and protein levels in adipocytes. Genes encoding adipocyte-secreted pro-inflammatory proteins such as serpine-1 (PAI-1) and serum amyloid A3 (Saa-3) are significantly down-regulated in the adipose tissue from high-fat fed wild-type and Siah2-/- mice compared with wild-type mice. This correlates with increased levels of PPARγ protein in adipose tissue and improved insulin sensitivity as determined by glucose and insulin tolerance testing and lipolysis assay of isolated adipocytes, suggesting Siah2 is an important determinant of insulin sensitivity in obesity. Microarray analysis of the adipose tissue from high-fat fed wild-type and Siah2-/- mice shows significant changes in PPARγ activity and protein levels in adipocytes. Genes encoding adipocyte-secreted pro-inflammatory proteins such as serpine-1 (PAI-1) and serum amyloid A3 (Saa-3) are significantly down-regulated.

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176-LB

Deletion of ABHD6 in Mice Protects from Diet-Induced Obesity, Hyperglycemia and Insulin Resistance, and Enhances Locomotor Activity
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Lipogenesis and lipolysis, two essential components of glycerolipid/fatty acid cycling, play an important role in regulating insulin secretion and sensitivity. In the pancreatic β-cells, which have very low levels of monoacylglycerol (MAG) lipase, MAG hydrolysis is conducted mostly by membrane bound ε/δ-domain hydrolase-6 (ABHD6). We have recently found that MAG levels increase in β-cells upon suppression of ABHD6 activity both in vitro and in vivo, associated with enhanced glucose stimulated insulin secretion, suggesting that MAG is a metabolic coupling factor in glucose induced insulin secretion.

ABHD6 KO male mice on a chow diet grow normally and did not show difference in food intake, body weight gain, glucose tolerance and insulin sensitivity over a span of 26 weeks age. Female mice on chow diet showed reduced body weight gain and improved glucose tolerance and insulin sensitivity. However, when fed with high fat diet for 8 weeks, both male and female ABHD6-KO mice showed reduced food intake in food body weight, body weight gain, insulinemia and glycemia, improved glucose tolerance and insulin sensitivity, as compared to corresponding wild type mice. Metabolic studies indicated that ABHD6-KO mice show increased glucose homeostasis, with elevated respiratory exchange ratio and enhanced locomotor activity. Overall the phenotype in female mice was more pronounced. In conclusion, ABHD6-KO mice on a high fat diet show a unique phenotype with enhanced glucose homeostasis, associated with reduced appetite, body weight gain, as well as increased thermogenesis and physical activity. Collectively, these results identify ABHD6 as a novel target for metabolic syndrome, obesity and diabetes.

Supported by: CIHR

177-LB

Probiotics Improve Glucose Tolerance and Insulin Sensitivity in DIO Mice via Intestinal Permeability and Microbiota Modulations
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Obesity is the main risk factor to the development of insulin resistance and type 2 diabetes. The common basis among these events is an inflammatory process characterized by the activation of toll-like receptor 4 (TLR4) by its main ligand lipopolysaccharide, LPS. Its concentration is higher in obese people and it is believed that changes in composition of the gut microbiota and epithelial functions may play a role in the inflammation associated with obesity. The aim of the study was to evaluate the effects of probiotic on the insulin sensitivity, TLR4 signaling, intestinal permeability and microbiota composition in diet-induced obese mice. Male adult Swiss mice composed randomly 2 groups: chow diet (CTC) and high-fat diet by 5 consecutive weeks (DIO). During these 5 weeks, some mice of the DIO and CTC groups received daily a pool of probiotics. Glucose tolerance, insulin signaling (IR, IRS-1, Akt), TLR4 pathway (TLR4, IKK, JNK, iNOS), gut microbiota and intestinal tight junctions proteins were evaluated. The DIO animals that received probiotic presented an expressive improvement in their glucose tolerance test, fasting glucose and in parallel a significant increase in the phosphorylation levels of insulin induced IR, IRS1 and Akt in muscle, liver and adipose tissue. There was a relevant reduction in the TLR4-Mydd88 interaction, IKKβ and JNK phosphorylation and Inos expression in DIO mice treated with probiotic. This treatment also improved the expression of ideal tight-junctions proteins (ZO-1, Occludin), decreased LPS portal levels and the concentrations of bacteria of the phylum Firmicutes (associated with obesity) in feces. In conclusion, our results show that probiotics, through their effects on intestinal permeability and microbiota composition, can improve insulin sensitivity and signaling of DIO mice, reducing their inflammation and suggesting potential beneficial effects in the treatment of insulin resistance and type 2 diabetes.

178-LB

ACAM (Adipocyte Adhesion Molecule)/CLMP Inhibits Adipocyte Hypertrophy in Obesity
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We identified adipocyte adhesion molecule (ACAM) / CLMP which belongs to cortical thymocyte marker in Xenopus (CTX) gene family. ACAM is predominantly expressed in white adipose tissues and up-regulated in obese rodents and human subjects with obesity. Two immunoglobulin-like domains exist in extracellular segment and they are involved in the adhesion process and homophilic aggregation of the cells (Biotechnol. J 2005). To explore the functional role of ACAM in obesity and type 2 diabetes, we generated ACAM transgenic (Tg) mice under aP2 promoter. Under high fat high sucrose diet, the increase in body weight was significantly ameliorated in Tg mice compared with wild type (WT) mice. The fat pad weight and adipocyte size in 1g mice were reduced. In glucose tolerance and insulin sensitivity tests, plasma glucose levels were significantly lower in Tg mice. The oxygen consumption rate was significantly higher in Tg mice and lipid droplets in brown adipose tissues were prominently depleted. Thus, we investigated mRNA expression of UCP-1 and PGC1α and they were significantly up-regulated in ACAM Tg mice compared with WT mice. To further give insights into the mechanism for the reduction of lipid accumulation in adipose tissues, we investigated the role of ACAM in differentiation of 3T3-L1 cells. ACAM is differentially expressed during the maturation of 3T3-L1 adipocytes; it has two expression peaks at 6 hrs and 10 days after hormonal induction. We identified that KLF4 and CEBP/β up-regulate the transcriptional activity of ACAM revealed by luciferase assay using pGL3 vector and the knockdown of ACAM mRNA inhibits mitotic clonal expansion and lipid accumulation. Finally, we identified myosin II-A and g-actin as interacting proteins forming protein complexes with ACAM by tandem-affinity purification method. Taken together, the adhesion process and cytoskeletal organization mediated by ACAM may have an inhibitory role in adipocyte hypertrophy and lipid accumulation in obesity.

Supported by: NIH (R56DK089020)
**179-LB**

**Pro-Inflammatory Effects of Central Leptin on Adipose Tissue Are Abolished by Adrenergic Denervation**

KEHAO ZHANG, JULIE CHEN, WEILE LI, CECELIA DAVIS, YANG ZHANG, XINYUAN DONG, BING LIU, GARY SCHWARTZ, ROGER GUTIERREZ-JUAREZ, MEREDITH HAWKINS, Bronx, NY

Obesity is characterized by infiltration of adipose tissue by macrophages and other inflammatory cells. The fat-derived cytokine leptin, whose levels are increased in obesity, activates immune cells. We previously showed that central leptin administration rapidly induced adipose inflammation (~2-fold increase in cytokine expression) and activation of adipose tissue macrophages (ATM) in normal rats. These effects persisted with high fat feeding, despite resistance to leptin’s effects on energy balance, suggesting that leptin could fuel adipose inflammation in obesity (Endocrine Reviews 2011 32:3). To confirm that central leptin caused adipose inflammation via neural inputs to fat pads, we examined the impact of perirenal denervation on leptin’s ability to induce adipose inflammation. Selective, unilateral disruption of adrenergic signaling to perirenal fat pads was performed in n=16 normal male Sprague Dawley rats (age=10 weeks, weight ~350 g) using multiple injections of 6-OH-dopamine (bmg/ml) with contralateral perirenal saline injections. The effects of intracerebroventricular leptin 0.25 µg/h (n=8) vs. vehicle (n=8) infusion on adipose tissue inflammation were compared in the denervated vs. sham-injected fat pads. Denervation of perirenal fat pads diminished the leptin-induced adipose tissue inflammation, with decreased expression of TNF-α, IL-6 and iNOS by 38% (p=0.036), 45% (p=0.035) and 32% (p=0.045), respectively, and with corresponding 39% (p=0.039), 37% (p=0.045) and 28% (p=0.057) reductions in the expression of these cytokines by ATMs. Thus, central leptin-administration rapidly induces inflammation and ATM activation in perirenal adipose tissue of rats, and selective adrenergic denervation abolished the stimulatory effects of leptin on adipose inflammation. Increased leptin production could contribute to activation of adipose macrophages with weight gain, thereby exacerbating the metabolic and inflammatory consequences of obesity.

**180-LB**

**Simulating Rapid Gastric Emptying With Duodenal Nutrient Infusions Reduces Decreased Food Intake and Reduced Weight Gain**

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Decreased caloric intake contributes to weight loss occurring soon after some bariatric surgical procedures. We asked whether rapid gastric emptying following vertical sleeve gastrectomy (VSG) and gastric bypass bariatric procedures contributes to early satiation and subsequent weight loss. To simulate rapid gastric emptying, 8 male Long-Evans rats received a duodenal catheter (DC) and another 8 rats received a similar catheter but placed in the gastric compartment (GC). Each time rats consumed Ensure Plus from an available sipper tube, a 50% diluted Ensure Plus was infused via the catheter into either the duodenum or stomach. The start and end point of infusion coincided with the start and end point of the ad-lib meal. Over 3 weeks of these infusions, DC rats exhibited early satiation in the form of decrease in meal size resulting in significant decrease in daily total caloric intake (oral plus infusion calories) and significantly reduced total body weight (~10%) as compared to the GC rats receiving the same infusions into the stomach. NMR body composition analysis demonstrated that the weight loss in DC rats was due to decrease in adiposity and not in lean mass. This study suggests that simulating rapid gastric emptying is sufficient to produce reduced meal size and cause weight loss. These data suggest that rapid gastric emptying seen after some bariatric surgical procedures may be important to the observed reduction in food intake and body weight.

Supported by: NIH/NIDDK, Ethicon Endo-Surgery, Inc.

**181-LB**

**Aryl Hydrocarbon Receptor Deficiency Attenuates Diet-Induced Obesity and Insulin Resistance in Mice**

CAN-XIN XU, CHUN WANG, CASSIE JAEGER, STACEY KRAGER, KATHLEEN BOTTUM, SHELEY TISCHKAU, Springfield, IL

The aryl hydrocarbon receptor (AhR), together with its partner AhR nuclear translocator (ARNT), has been explored extensively for its role in xenobiotic metabolism. AhR is implicated in xenobiotic-induced insulin resistance and type 2 diabetes. Our previous study has shown that AhR knockout mice display decreased peroxisome proliferator activated receptor alpha (PPARα) expression, improved glucose tolerance and insulin sensitivity on normal chow diet. Since “Western diet” is implicated in the development of obesity and diabetes, we sought to further define the role of AhR in energy homeostasis by exploring the effects of a high-fat diet on metabolism in AhRKo mice. 6-week old wild-type, AhR heterozygous and knockout male mice were exposed to a normal chow diet (NCD, 10% fat diet) or a high-fat diet (HFD, 60% fat diet) for 14 weeks. AhR deficiency inhibited HFD-induced obesity, hepatic steatosis and insulin resistance, which resulted from increased energy expenditure, increased PPARγ, adiponectin and leptin expression, decreased inflammation and enhancement insulin receptor substrate 1 and 2 (IRS1 and IRS2) expression and insulin-induced Akt phosphorylation in adipose tissue, liver and muscle. Mechanistically, the metabolic benefits of AhR deficiency were related to the inhibition of c-Jun N-terminal kinase (JNK) activation and nuclear factor-κB (NF-κB) pathway. These findings demonstrate an important role for the AhR in obesity and insulin resistance, and the AhR signaling pathway may become a potential therapeutic target. In future, AhR antagonists may be developed to prevent and treat obesity and type 2 diabetes.

Supported by: NIH (ES017774)

**182-LB**

**Dietary Methionine Restriction Induces Weight-Loss, Promotes Insulin Sensitivity and Decreases Adipose Tissue Macrophage Accumulation in Diet-Induced Obese Mice**

GENE P. ABLES, MARK PEFFERS, HEIDI SEYMOUR, INES AUGIE, CARMEN PERRONE, DAVID ORENTREICH, NORMAN ORENTREICH, Cold Spring, NY

Methionine restriction (MR) in rodent models extends lifespan and induces favorable metabolic changes on glucose metabolism with a concomitant reduction in adipose tissue mass. Since the recruitment of adipose tissue macrophages is implicated in obesity and insulin resistance, we hypothesized that MR diet reduces the accumulation of adipose tissue macrophages which consequently attenuates obesity and insulin resistance.

Diet-induced obese (DIO) C57BL/6J mice were fed isocaloric high-fat control (HFD - CF /0.86% methionine) or methionine restricted (HFD - MR/0.12% methionine) diets containing 60% fat for 12 weeks. The HFD - MR mice had decreased body weight despite increased food consumption and energy intake compared to HFD - CF mice. Plasma amino acid analysis by ultra-performance liquid chromatography (UPLC) assay showed that HFD - MR mice had decreased concentrations of methionine, cysteine, taurine and lysine, while glycine, proline, serine, tyrosine and threonine were increased compared to the HFD - CF. The decreased methionine in the diet lowered fasting blood glucose, plasma insulin, leptin, PAI1 and resistin concentrations suggesting increased insulin sensitivity in the HFD - MR mice, which was confirmed in glucose and insulin tolerance tests. Perigonadal (PGAT), subcutaneous (SCAT) and brown adipose tissue (BAT) mass were significantly reduced in the HFD - MR mice due to increased lipolysis as shown by an increase in free fatty acid (FFA) levels following an overnight fast. Finally, immunohistochemistry staining of the perigonadal adipose tissue for the macrophage marker, F4/80, showed decreased macrophage content in HFD - MR mice fat depots. Taken together, these data suggest that dietary MR in obese mice could induce weight loss and attenuate insulin resistance due to reduced accumulation of adipose tissue macrophages.

Supported by: Orentreich Foundation for the Advancement of Science

**183-LB**

**Interferon Regulatory Factor 4 Regulates Obesity-Induced Inflammation Through Regulation of Adipose Tissue Macrophage Polarization**

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Interferon regulatory factors (IRFs) play functionally diverse roles in the transcriptional regulation of the immune system. We have previously shown that several IRFs are regulators of adipogenesis, and that IRF4 is a critical transcriptional regulator of adipocyte lipid handling. However, the functional role of IRF4 in adipose tissue macrophages (ATMs) remains unclear, despite high expression there. Here we show that IRF4 expression is regulated in both primary macrophages and ATMs of high-fat-diet-induced obese mice. IRF4/-/- macrophages produce higher levels of proinflammatory cytokines, including IL-1β and TNFα, in response to fatty acids. In co-culture experiments, IRF4 deletion in macrophages leads to reduced insulin signaling and glucose uptake in 3T3-L1 adipocytes. To determine the macrophage-specific function of IRF4 in the context of obesity, we generated myeloid cell-specific IRF4 knockout (MI4KO) mice. MI4KO mice develop significant insulin resistance on high fat diet despite no difference in adiposity. This phenotype is associated with increased expression of inflammatory genes and decreased insulin signaling in adipose tissue, skeletal muscle, and liver. Furthermore, IRF4/-/- ATMs express markers suggestive of enhanced M1 polarization. These findings indicate that IRF4 is a negative regulator of inflammation in diet-induced obesity, in part through regulation of macrophage polarization.

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For author disclosure information, see page LB66.
184-LB
Imbalance between Neutrophil Elastase and its Inhibitor α1-Antitrypsin in Obesity Alters Insulin Sensitivity, Inflammation, and Energy Expenditure

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Background: Neutrophil elastase (NE), an enzyme produced by neutrophils, is known to cause tissue damage and inflammation. However, the role of ELI in the development of obesity and related complications remain unclear. Using a quantitative serum proteomic approach, we identified α1-antitrypsin (A1AT), a natural inhibitor of neutrophil elastase (NE), was significantly reduced in obese mice. Further validation studies demonstrated that both human obese subjects and obese mice have increased activity of neutrophil elastase (NE) and decreased serum levels of A1AT. NE null (Ela2−/−) mice were resistant to high fat diet (HFD)-induced bodyweight gain, insulin resistance, fatty liver, neutrophil and macrophage infiltration, inflammation and fibrosis in white adipose tissues. Overexpression of human A1AT also alleviated HFD-induced phenotypes in mice. NE small molecule inhibitor GW316161A reversed insulin resistance and bodyweight gain in long term HFD-fed mice, suggesting a potential therapeutic effect. Compared with wild-type mice, Ela2−/− mice augmented circulating high molecular weight (HMW) adiponectin levels, phosphorylation of AMP-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC), and fatty acid oxidation (FAO) in the liver and brown adipose tissue (BAT). These data suggest a novel link of the A1AT-NE system to the AMPK signaling, FAO, and energy expenditure axis. Hence, the imbalance between A1AT and NE contributes to the development of obesity and related inflammation, insulin resistance and liver steatosis.

Exchange Protein Directly Activated by cAMP 1 Plays an Important Role in β3-Adrenergic Induction of UCP1 in WAT and Thermogenesis via Regulating Lipolysis

YINXIAN CHEN, ANDREW C.P. TAI, ALAN K.L. KAI, SIDNEY TAM, KAREN S.L. LAM, STEPHEN S.M. CHUNG, AIMIN XU, SOKKJA K. CHUNG, Hong Kong, China, Zhuhai, China

Background: The exchange protein directly activated by cAMP 1 (Epac1) and thermogenesis has drawn great interest to counteract obesity. Previously, the exchange protein directly activated by cAMP 1 (Epac1)−/−-deficient mice showed less induction of beige cells with a significantly less UCP1 expression in white adipose tissue (WAT) and lower circulating free fatty acid (FFA) after chronic CL316,243. To test whether reduced FFA is due to compromised lipolysis, glycerol release from WAT explants was examined ex vivo. To further study the role of Epac1 in β3-adrenergic induction of UCP1 and its function, energy expenditure and thermogenesis were determined. By indirect calorimetry, the Epac1−/−-deficient mice showed slightly lower oxygen consumption from 9-16 hours after CL (1mg/kg) administration compared to that of wt mice. By using rectal thermometer, continuously lower rectal temperature within 30 min after the ninth dose of CL administration was observed in the Epac1−/− deficient mice relative to that of wt mice. These results suggest that in the absence of Epac1, increase of energy expenditure and thermogenesis induced by β3-adrenergic activation are compromised, which could be due to lower FFA and UCP1 induced by CL in the Epac1−/− deficient mice. To test whether the reduced FFA is due to compromised lipolysis, glycerol release from WAT explants was examined ex vivo. Interestingly, Epac1−/− deficient WAT explants showed impaired CL-stimulated glycerol release, indicating that absence of Epac1 diminishes β3-adrenergic receptor mediated lipolysis in WAT. In addition, Western blot showed that phosphorylation of hormone sensitive lipase at Ser660 by protein kinase A (PKA) was not different in Epac1−/− deficient WAT explants with incubated CL (10μM) for 10 min, compared to that of wt mice. Taken together, Epac1 plays an important role in β3-adrenergic induction of UCP1 in WAT and thermogenesis via mediating lipolysis independent of PKA.

OBESITY—HUMAN

185-LB
Propathine carboxypeptidase Expression Is Altered in Experimental Animal Models of Obesity

ZIA SHARIAT-MODAR, TAHMINEH TABRIZIAN, ALEXANDRE A. DA SILVA, JUSSARA M. DA SILVA, JOHN E. HALL, Jackson, MS

Background: Propathine carboxypeptidase (PRCP) plays an essential role in the regulation of the melanocortin system. PRCP deficiency is associated with obesity, insulin resistance, prediabetes, and type 2 diabetes. However, the role of PRCP in the development of obesity and related complications remain unclear. Using a quantitative serum proteomic approach, we identified α1-antitrypsin (A1AT), a natural inhibitor of neutrophil elastase (NE), was significantly reduced in obese mice. Further validation studies demonstrated that both human obese subjects and obese mice have increased activity of neutrophil elastase (NE) and decreased serum levels of A1AT. NE null (Ela2−/−) mice were resistant to high fat diet (HFD)-induced bodyweight gain, insulin resistance, fatty liver, neutrophil and macrophage infiltration, inflammation and fibrosis in white adipose tissues. Overexpression of human A1AT also alleviated HFD-induced phenotypes in mice. NE small molecule inhibitor GW316161A reversed insulin resistance and bodyweight gain in long term HFD-fed mice, suggesting a potential therapeutic effect. Compared with wild-type mice, Ela2−/− mice augmented circulating high molecular weight (HMW) adiponectin levels, phosphorylation of AMP-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC), and fatty acid oxidation (FAO) in the liver and brown adipose tissue (BAT). These data suggest a novel link of the A1AT-NE system to the AMPK signaling, FAO, and energy expenditure axis. Hence, the imbalance between A1AT and NE contributes to the development of obesity and related inflammation, insulin resistance and liver steatosis.

Beloranib, a Novel Methionine Aminopeptidase 2 (MetAP2) Inhibitor, Appeared Safe and Showed Dose Responsive Weight Loss Over 12 Weeks in Interim Analysis of Ongoing Phase 2 Trial

DENNIS D. KIM, JAYNÉS E. VATH, ALICE CHEN, JOHANNE MARJASON, JOE PRICKETT, THOMAS E. HUGHES, Cambridge, MA, Herston, Australia, Heidelberg, Australia

Beloranib is a MetAP2 inhibitor that increases fatty acid oxidation and reduces hunger. Previous proof of concept studies over 4 weeks showed ~4% weight loss with 1-3 mg subcutaneous (SC) beloranib. This is a double-blind, placebo-controlled study to investigate the safety/ tolerability, PK/PD, and metabolic effects of SC beloranib in obese women. Beloranib is a MetAP2 inhibitor that increases fatty acid oxidation and reduces hunger. Previous proof of concept studies over 4 weeks showed ~4% weight loss with 1-3 mg subcutaneous (SC) beloranib. This is a double-blind, placebo-controlled study to investigate the safety/tolerance, PK/PD, and metabolic effects of SC beloranib. Obese men and women were randomized to 0.6 (n=37), 1.2 (n=36), or 2.4 mg (n=34) of SC beloranib.
189-LB Rescue of Intracellularly Retained Human Melanocortin-4 Receptor Mutants
HUI HUANG, Y-A XIONG TAO, Auburn, AL
The melanocortin-4 receptor (MC4R) is a critical regulator of energy homeostasis. Mutations of MC4R gene have been identified as the most common cause of monogenic obesity. Most of the inactivating mutants are defective in intracellular forward trafficking. Some of these mutants can activate G proteins once expressed on the cell surface. In this study, we investigated whether small molecule MC4R ligands could act as pharmacological chaperones, promoting the proper folding of intracellularly retained MC4R mutants in Neuro2A and NIE115 cells. Three MC4R ligands including 2 antagonists (Lipsen 5i and ML0253764) and 1 agonist (THIQ) were studied. Totally 14 human MC4R mutations were studied, including 10 (N62S, I69R, P78L, C84R, G98R, Y157S, W174C, P260Q, P261S, and C271Y) that are retained intracellularly, and 4 (A88-92, D90N, I102S, and N274S) that are expressed normally on the cell surface. Cells transiently transfected with the empty vector, WT or mutant receptors were treated with the small molecules for 24 h, and then the maximal cAMP production stimulated by 10-6 M NDP-MSH was measured. The results were similar in the two cell lines studied. With 10-8 M Lipsen 5i treatment, 7 mutants (N62S, I69R, P78L, C84R, W174C, P260Q, and C271Y) restored function in cAMP production. With 10-5 M THIQ treatment, 6 mutants (N62S, P78L, C84R, W174C, P260Q, and C271Y) restored function in cAMP production in Neuro2A cells and 7 (including I69R) in NIE115 cells. With 10-5 M ML0253764 treatment, 4 mutants (N62S, C84R, W174C, and C271Y) restored function in cAMP production in Neuro2A cells and 3 (excluding C271Y) in NIE115 cells. None of these small molecules had effect on the 4 control mutants. In summary, we identified 3 small molecule ligands that could act as pharmacological chaperones, rescuing intracellularly retained MC4R mutants in neuronal cells. These results will be useful in research towards personalized medicine for obese patients carrying MC4R mutations.

190-LB Exercise Following Gastric Bypass Surgery Maintains Higher Fatty Acid Oxidation
TRACEY WOODLIEF, PAUL M. COEN, NICOLE L. HEBLING, GABRIEL S. DUBIS, JOSEPH A. HOUMARD, BRETH R. GODDASTER, Pittsburgh, PA, Greenville, NC
Severe obesity has been associated with impaired skeletal muscle fatty acid oxidation. Although gastric bypass surgery (GPS) is an effective and increasingly common treatment option for severe obesity, the effects of regular exercise following GPS on preference for fatty acid oxidation are not clear. The purpose of the study was to determine the effects of GPS, both with and without regular exercise, on fatty acid metabolism. Subjects were recruited 1-3 months post-GPS and completed 6-months of either moderate structured exercise (EN, n=26) or GPS only control (CON, n=45). Percutaneous biopsies of the vastus lateralis were obtained, before and after the 6-month interventions. 14C-palmitate oxidation was measured in muscle homogenates. Resting metabolic rate (RMR) and respiratory quotient (RQ) were determined by indirect calorimetry. Aerobic capacity (VO2 max) was determined by a graded exercise test. Cardiorespiratory capacity (VO2 max) increased in the EN group (+160 VS. -25 m/min, p<0.026). In this subset of completers, the EN group also lost significantly more weight (-23.9 VS. -18.7 Kg, p<0.05). Resting RQ increased in the CON group (Pre = 0.74, Post = 0.77, p = 0.03), while not changing in the EN group (Pre = 0.74, Post = 0.74, p = 0.87), suggesting a shift in substrate preference to carbohydrate in the CON group and a maintenance of higher fatty acid oxidation in the EN group. Palmitate oxidation in muscle homogenate decreased to a greater extent in the CON group compared to the EN group (-3.40 VS. 0.10 nmol/g tissue/min, p=0.03), again suggesting a shift towards CHO oxidation in the CON group and a maintenance/enhanced fatty acid oxidation in the EN group. Exercise intervention during surgically induced greater weight loss maintains higher fatty acid oxidation at the level of both whole body and skeletal muscle.

191-LB Differential Effects of Inverse Agonists on cAMP and ERK1/2 Signaling Pathways in Six Naturally Occurring Constitutively Active Mutant Human Melanocortin-4 Receptors
XI-U LEI MO, Y-A XIONG TAO, Auburn, AL
The melanocortin-4 receptor (MC4R) is a G protein-coupled receptor that plays an essential role in regulating energy homeostasis. Defects in MC4R are the most common monogenic form of obesity, with more than 150 distinct mutations identified in human. In addition to the conventional Gs-stimulated adenyl cyclase pathway, it has been recently demonstrated that activation of mitogen-activated protein kinases, extracellular signal-regulated kinases 1 and 2 (ERK1/2), is involved in MC4R-mediated energy balance. Herein, we investigated the potential of four MC4R ligands (including the agonist-related peptide (AgRP), MCL0020, Lipsen 5i and ML0253764), which are inverse agonists at the Gs-cAMP signaling pathway, to regulate the activity of phospholipid-ERK1/2 (pERK1/2) in wild type (WT) and six naturally occurring constitutively active mutant (CAM) MC4Rs. We show that these four inverse agonists acted as agonists for the ERK1/2 signaling cascade in WT and CAM MC4Rs. Three mutants (P230L, L250Q and F280L) had significantly increased pERK1/2 level upon stimulation with all four inverse agonists, with maximal induction ranging from 1.6 to 4.2 fold. WT and one mutant MC4R (D148N) had significantly increased pERK1/2 level upon stimulation with AgRP, MCL0020 or ML0253764, but not Lipsen 5i. The pERK1/2 levels of 2 mutants (H76R and S127L) were significantly increased only upon stimulation with AgRP or MCL0020. In summary, our studies demonstrated for the first time that the conventionally identified MC4R inverse agonists exert divergent efficacy on cAMP and ERK1/2 signaling pathways. These results suggested that there are multiple activation states of MC4R with ligand-specific and/or mutant-specific conformations capable of differentially coupling the MC4R to distinct signaling pathways, adding a new layer of complexity to the MC4R signaling.

192-LB Improvement in Insulin Sensitivity and Beta Cell Function in Severely Obese Adolescents Following Gastric Bypass Surgery
TOM INGE, STEPHEN BENEDT, TODD JENKINS, RONALD PRIGEON, DEBORAH ELDER, LAWRENCE M. DOLAN, DAVID A. D’ALESSIO, Cincinnati, OH, Baltimore, MD
The metabolic changes associated with Roux en Y gastric bypass (RYGB) have not been previously examined in detail in adolescents. We studied 15 non-diabetic adolescents who underwent serial intravenous glucose tolerance tests before and after RYGB. Insulin sensitivity (SI) was determined using Bergman’s minimal model. Insulin secretion was measured as the acute insulin response to glucose (AIRg) and the disposition index (DI) was computed as AIRg/DI.

Mean age was 17 yr; 66% female, 78% Caucasian. Mean BMI decreased 39% by 1 yr (p<0.01). Mean fasting glucose fell by 11% by 2 weeks (p<0.01), while fasting insulin normalized by 3 mo. SI increased nearly 3-fold by 1 year (p<0.01). The acute insulin response to glucose (AIRg) decreased by 59% by 1 year. Taking into account changes in SI, insulin secretion (as DI) doubled by 1 year (p<0.01). Indexed to normal, lean adults, DI increased from the 11th to the 32nd percentile over 1 year (p<0.02).

These data demonstrate that severely obese adolescents have severe insulin resistance, with high secretory demands to maintain normoglycemia during glucose challenge. When surgery is used late in the development of adolescent severe obesity (class III), a significant reduction in BMI is achieved, but subjects remain severely obese. Postoperatively, SI slowly normalizes, leading to a significant compensatory improvement in beta cell function, despite persistence of class II obesity.
### INTEGRATED PHYSIOLOGY/OBESITY

#### 193-LB

**The Metabolically Healthy But Obese Phenotype Is Associated With Lower Plasma Levels of Persistent Organic Pollutants**

MARIE-SOLEIL GAUTHIER, REMIRABASA-LHORET, DENIS PRUD’HOMME, DAWEI GENG, BERT VAN BAVEL, JEROME RUZZIN, MARIE-SOLEIL GAUTHIER, REMI RABASA-LHORET, DENIS PRUD’HOMME, DAWEI GENG, BERT VAN BAVEL, JEROME RUZZIN, MARIE-SOLEIL GAUTHIER, REMI RABASA-LHORET, DENIS PRUD’HOMME, DAWEI GENG, BERT VAN BAVEL, JEROME RUZZIN, MARIE-SOLEIL GAUTHIER, REMI RABASA-LHORET, DENIS PRUD’HOMME, DAWEI GENG, BERT VAN BAVEL, JEROME RUZZIN, MARIE-SOLEIL GAUTHIER, REMI RABASA-LHORET, DENIS PRUD’HOMME, DAWEI GENG, BERT VAN BAVEL, JEROME RUZZIN, MARIE-SOLEIL GAUTHIER, REMI RABASA-LHORET, DENIS PRUD’HOMME, DAWEI GENG, BERT VAN BAVEL, JEROME RUZZIN, MARIE-SOLEIL GAUTHIER, REMI RABASA-LHORET, DENIS PRUD’HOMME, DAWEI GENG, BERT VAN BAVEL, JEROME RUZZIN, MARIE-SOLEIL GAUTHIER, REMI RABASA-LHORET, DENIS PRUD’HOMME.

**Supported by:** NIH (R03DK068228)

*Obesity is a major risk factor for the development of insulin resistance (IR) and type 2 diabetes (T2D). However, a subset of obese individuals does not develop IR and remain Metabolically Healthy but Obese (MHO). They represent a distinctive human model to delineate key factors that either contribute to or prevent the development of metabolic abnormalities without the confounding effects of differences in body fat mass. In the recent years, exposure to environmental Persistent Organic Pollutants (POPs) has been shown to cause IR in rodents and to be associated with increased incidence of T2D in humans. POPs, such as dioxins, polychlorinated biphenyls (PCBs), and organochlorine pesticides are highly toxic, lipophylic and resistant to degradation. They thus accumulate in the environment, food chain and human body. We examined the plasma levels of various classes of POPs in a cohort of well phenotyped non-diabetic obese patients stratified into MHO (n=38) or Metabolically Abnormal Obese (MAO, n=40) according to the results of a euglycemic-hyperinsulinemic clamp. Despite similar age, BMI, and fat mass, MAO patients had 50% lower insulin sensitivity, decreased serum levels of HDL-C, and increased levels of ApoB, triglycerides and hsCRP as compared to MHO subjects (p<0.05). The plasma levels of all but 5 POPs out of a total of 18 POPs measured were significantly higher in MAO than in MHO subjects (p<0.05). More specifically, the levels of octachlorodibenzodioxin and all measured dioxin-like PCBs (congeners 105, 118, 156, 157 and 189) were higher in MAO than in MHO subjects (1.4-2.9 fold; p<0.05). MAO patients also had higher levels of transnonachlor and several nondioxin-like PCBs (congeners 74, 99, 138, 153, 170, and 194)(1.4-2.0 fold; p<0.05). Overall, this data shows a close link between higher POPs exposure and the presence of metabolic abnormalities in obese patients. It also provides potential mechanistic explanations for the metabolically protected MHO phenotype.*

#### 194-LB

**Obesity Associated Increase in Fasting Insulin Is Related to Decreased Hepatic Glucokinase Activity in Women**

DARKO STEFANOVSKI, RUCHI MATHUR, RICHARD N. BERGMAN, GENG, BERT VAN BAVEL, JEROME RUZZIN, MARIE-SOLEIL GAUTHIER, REMI RABASA-LHORET, DENIS PRUD’HOMME.

*Obesity is the increased expression of glucose regulatory protein (GCKR) brought forth by the rise in fasting insulin.*


*For author disclosure information, see page LB66.*

#### 195-LB

**Reduced Coenzyme Q10 Content and Redox Status Modification in Obesity and Adipocyte Hypertrophy**

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*The development of obesity-related metabolic complications is closely related to adipose tissue dysfunction. This phenomenon is characterized by altered lipid metabolism and adipokine secretion as well as chronic inflammation. Occurrence of oxidative stress in white adipose tissues may contribute to this phenotype. Coenzyme Q10 (CoQ10) is a lipid molecule playing a central role in the regulation of reactive oxygen species and cellular oxidative status.*

**Effects of Long Term Administration of Liraglutide on Insulin Uptake, Body Weight and Hypoglycemia in Type I Diabetes**

ANTONIOS LEPOURAS, ANASTASIA LINARDI, NIKOLAOS ALEXANDROPOULOS, Athens, Greece.

*This study was designed to investigate the effect of administration of liraglutide to patients with type 1 diabetes. More specifically, insulin uptake, weight and lipid profile were investigated.*

11 volunteers (8 females, 3 males) received liraglutide with their diabetes treatment for up to 31 months. Mean age was 45.09 yrs and disease duration was 22.73 yrs. Mean BMI was 30.96 Kg/m², mean total cholesterol 195.56 mg/dL and mean glucose was 19.83 and 54.55 IU respectively. Liraglutide was generally well tolerated by all patients. Average patient TRIM-D total score was 72.5 indicative of well accepted treatment for diabetes. All patients reported rare or no episodes of hypoglycemia while on liraglutide treatment compared to daily or frequent episodes prior to treatment.

**Average Change Average % Change**

| Weight | -7.70 | -9.40% |
| BMI | -3.37 | -11.64% |
| Total Cholesterol | -21.00 | -11.09% |
| LDL | -26.50 | -22.23% |
| Triglycerides | 6.29 | 6.57% |
| Slow insulin | 0.40 | 1.33% |
| Fast Insulin | -10.00 | -54.95% |

*Administration of liraglutide resulted in significant weight reduction both in terms of absolute weight and in terms of BMI. The lipid profile of patients also improved with reductions in total cholesterol and LDL, while HDL and triglycerides do not appear to be decreased. Insulin uptake was significantly reduced.*

**For author disclosure information, see page LB66.**
197-LB
Two-Year Outcomes of a Randomized Controlled Trial of Behavioral Treatment for Depression and Obesity in Women

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The co-occurrence of obesity and depression is problematic given that each contributes to risk for type 2 diabetes. Patients often prioritize weight loss over depression, but data show that weight loss outcomes are poor in people with depression. The purpose of this study was to compare two approaches to treating co-occurring obesity and depression, one that addresses depression prior to weight, and another that addresses weight only. Two-year outcomes are reported. Obese women with major depressive disorder (N=161, mean age=45.9, SD=10.8) were randomized to one of two 6-month interventions. In one condition, 10 weeks were devoted to behavior therapy for depression and then a 16 week lifestyle intervention (BA) was initiated at week 8. In the other condition all 24 weeks were devoted to a lifestyle intervention (LI). We hypothesized that devoting treatment time to depression will improve both weight and depression outcomes. Main outcome measures included weight and depression remission. Results. Intention-to-treat analyses revealed both conditions lost significant weight, but no differences were observed between conditions at 6 months (BA= -3.0%, SE = -0.65%, LI = -3.7%, SE = 0.63%; p = 0.48), 1-year (BA= -2.6%, SE = 0.77%, LI= -3.1%, SE=0.74%, p=0.72), or 2-years (BA= -0.8%, SE = 1.10%; LI= -2.5%, SE=1.00%; p = 0.26). The BA condition evidenced significantly higher depression remission rates relative to the LI condition at 6 months (BA = 60.7%, LI =39.6%, p=0.01), 1-year (BA =66.4%, LI =74.4%; p =0.03), and 2-years (BA =73.8%, LI =56.9%, p<0.03). Conclusion. Devoting treatment time to depression does not compromise or enhance weight loss but improves depression significantly. Although weight rebounded at 2 years, depression remission rates continued to rise. Behavior therapy for depression appears to have long lasting effects on mood and may be a helpful first step in treatment for women with depression and obesity.

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198-LB
Effect of 1, 25-Dihydroxyvitamin D3 on VDR Gene Expression and Apoptogenesis in Human Adipose Tissue

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Vitamin D has been associated to obesity. Vitamin D exerts its action by means of the vitamin D receptor (VDR) which forms a heterodimer with the retinoid X receptor (RXR), acting as transcription factor of a number of genes. 1,25-Dihydroxyvitamin D3 (1,25(OH)2D3, the active form of vitamin D) up-regulates VDR gene expression and inhibits adipogenesis in 3T3-L1 preadipocytes. By contrast, 1,25(OH)2D3 promotes the differentiation of human and mouse preadipocytes. Moreover, it has been reported a higher adipose tissue VDR gene expression in obese patients than in lean subjects, which suggests a differential vitamin D dynamics in adipose tissue according to the obesity degree. Thus, the aim of this study was to analyze the effect of 1,25(OH)2D3 on the gene expression of VDR, RXRα, acting as transcription factor of a number of target genes such as Heme Oxygenase 1, NADPH: Quinone Oxidoreductase 1, Glutathione S-transferase, as revealed by qRT-PCR, which was further confirmed using luciferase reporter driven by ARENQO1 and GST1 promoters. 1,25(OH)2D3 promoted the differentiation of human and mouse preadipocytes. Moreover, it has been reported a higher adipose tissue VDR gene expression in obese patients than in lean subjects, which suggests a differential vitamin D dynamics in adipose tissue according to the obesity degree. Thus, the aim of this study was to analyze the effect of 1,25(OH)2D3 on the gene expression of VDR, RXRα and adipogenic markers in human adipose tissue explants from morbidly obese (ATMO) and lean (ATL) donors were cultured and undergone to a range of 1,25(OH)2D3 concentrations (10-6 M, 10-7 M, 10-8 M). Gene expression of VDR, RXRα and adipogenic markers (PPARγ, C/EBPα, SREBP1 and aP2) was measured.

VDR gene expression was significantly higher in ATMO than in ATL. 1,25(OH)2D3 significantly increased VDR gene expression in ATMO, but had no effect in VDR gene expression in ATL. No significant effect of 1,25(OH)2D3 in ATMO or ATL was seen on the gene expression of RXRα or the adipogenic markers analyzed, although a trend towards lower mRNA levels of C/EBPα and aP2 after stimulation with 1,25(OH)2D3 (10-6M) was observed in both ATMO and ATL.

There is a different VDR gene expression response to 1,25(OH)2D3 in visceral adipose tissue according to the obesity degree. By contrast, 1,25(OH)2D3 has a similar effect on visceral adipose tissue from lean and morbidly obese subjects with respect to the gene expression of adipogenic markers.

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199-LB
WITHDRAWN

ISLET BIOLOGY/INSULIN SECRETION

200-LB
Reportper Protein Complementation System Identifies Pterostilbene as Nrf2 Activator and Protects Pancreatic β-Cells Against Apoptosis

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Nrf2 (Nuclear factor erythroid 2 Related factor 2, a “master regulator” of cell survival, modulates the expression of phase II metabolic enzymes and antioxidant genes for maintaining cellular homeostasis. Under normal conditions, Nrf2 is associated with its inhibitor Keap1 (Kelch-like ECH associated protein 1) which, upon activation, translocates into the nucleus and triggers antioxidant response through Antioxidant Responsive Element (ARE). Thus, Nrf2 activation through ligands is a promising approach for combating oxidative stress-mediated disorders, including diabetes. We described a cell-based luciferase enzyme fragment complementation (EFC) assay to identify potent Nrf2 activators, based on specific interaction of Nrf2 and Keap1. In order to study the mechanism of Nrf2 activation by molecular imaging, CLuc-Nrf2 and NLuc-Keap1 constructs that showed maximum level of complement dissociation signal were used to develop Hek293T cell stably co-expressing the fusion proteins. Among the several Nrf2 activators screened, pterostilbene (PTS), a naturally available stilbene compound, showed effective Nrf2 activation, as observed by luminoimetric screening and validation in a high throughput-screening platform. Follow-up studies were focused on PTS to reveal its mechanistic role in hyperglycemia. PTS reduced hyperglycemia-induced ROS formation, and also prevented mitochondrial and nuclear DNA damage in INS-1E cells. PTS increased the expression of Nrf2 downstream target genes such as Heme Oxygenase 1, NADPH: Quinone Oxidoreductase 1, Glutathione S-transferase, as revealed by qRT-PCR, which was further confirmed using luciferase reporter driven by ARENQO1 and GST1 promoters. These findings may improve the understanding of mechanisms mediating anti-apoptotic effects of PTS on pancreatic β-cells, and will also form the basis for its potential use as a therapeutic agent in diabetes management.

Supported by: SRM University, Stanford University

201-LB
The Transcriptome of Metabolically Stressed Human Islets

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Pancreatic β cell dysfunction and death are central in the pathogenesis of type 2 diabetes (T2D). Saturated free fatty acids cause metabolic stress and contribute to β cell failure. Here we profiled the transcriptome of human islets exposed to palmitate to map mechanisms of β cell demise. Using RNA-sequencing we identified transcripts of 5 human islet preparations, basally or following 48 h palmitate exposure (0.5 mM, 1% BSA). Organ donors were aged 55±9 years. Islet β cell purity by insulin immunostaining was 50±5%. Samples were sequenced on Illumina Genome Analyzer II and data analyzed using GEM mapper and Flux Capacitor. Transcript expression was considered changed by Benjamini-Hochberg-corrected Fisher tests (p<0.05) and if modified in the same direction in 4/5 samples. Genes were annotated manually or using Ingenuity Pathway Analysis (IPA) or DAVID. Human islets expressed 30,026 transcripts corresponding to 19,882 genes. Palmitate induced 428 genes and downregulated 897 genes, including genes regulating the endoplasmic reticulum (ER) stress response, ubiquitin and proteasome function, autophagy and apoptosis. Transcripts related to innate immunity were upregulated and several HLA transcripts downregulated. Several transcription factors controlling β cell phenotype were inhibited, including PDX1 and GATA6, which is now functionally studied. 52/63 of the T2D for author disclosure information, see page LB66.
candidate genes were expressed with an RPKM ≥1, and palmitate modified expression of 11 of these. Palmitate caused a shift in alternative splicing in 574 transcripts. IPA confirmed that top changed functions related to cell death and cell development. DAVID analysis of transcription factor binding sites in palmitate-modified genes pointed to a role for KLF1 and ATRF, mediating the ER stress response. In conclusion, we used RNA-sequencing to map the human islet transcriptome and identified novel mechanisms of palmitate-induced β-cell dysfunction and death. The data point to crosstalk between metabolic stress and T2D candidate genes at the β-cell level.

Supported by: FF7 BETABAT

202-LB

Protective Effect of Nicotinamide on High Glucose/Palmitate-Induced Glucotoxicity to INS-1 Beta Cells Is Attributed to Its Inhibitory Activity on Sirtuins

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This study was initiated to determine whether the protective effect of nicotinamide (NAM) on high glucose/palmitate (HG/PA)-induced INS-1 beta cell death was due to its role as an anti-oxidant, nicotinamide dinucleotide (NAD+) precursor, or inhibitor of NAD+-consuming enzymes such as poly (ADP-ribose) polymerase (PARP) or sirtuins. All anti-oxidants tested were not protective against HG/PA-induced INS-1 cell death. Direct supplementation of NAD+ or indirect supplementation through NAD+ salvage or de novo pathway did not protect the death. Knockdown of the NAD+ salvage pathway enzymes such as nicotinamide phosphoribosyl transferase or nicotinamide mononucleotide adenyltransferase did not augment death. On the other hand, pharmacological inhibition or knockdown of PARP did not affect death. However, sirtinol as an inhibitor of NAD-dependent deacetylase or knockdown of Sirt3 or Sirt4 significantly reduced the HG/PA-induced death. These data suggest that protective effect of NAM on beta cell glucotoxicity is attributed to its inhibitory activity on sirtuins.

ISLET BIOLOGY—BETA CELL—DEVELOPMENT AND POSTNATAL GROWTH

203-LB

Intrapatient Variations in Type 1 Diabetes-Specific Ips Cell Differentiation Into Insulin-Producing Cells

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Nuclear reprogramming of adult somatic tissue enables embryo-independent generation of patient-specific induced pluripotent stem (iPS) cells. Exploiting this emergent regenerative platform for individualized medicine applications requires the establishment of bioequivalence criteria across derived pluripotent lines and lineage-specific derivatives. Here, from individual patients with type 1 diabetes (T1D) multiple human IPS clones were produced and prospectively screened using a battery of developmental markers to assess respective differentiation propensity and proficiency in yielding functional insulin (INS)-producing progeny. Global gene expression profiles, pluripotency and prospectively screened using a battery of developmental markers to assess respective differentiation propensity and proficiency in yielding functional insulin (INS)-producing progeny. Global gene expression profiles, pluripotency and pluripotency expression patterns, and the capacity to differentiate into SOX17- and FOXA2-positive definitive endoderm-like cells were comparable among individual IPS clones. However, notable intrapatient variation was evident upon further guided differentiation into HNF4α- and HNF1β-expressing primitive gut tube, and INS- and glucagon-expressing islet-like cells. Differential dynamics of pluripotency-associated genes and pancreatic lineage-specifying genes underlined clonal variance. Successful generation of glucose-responsive INS-producing cells required silencing of stemness programs as well as the induction of stage-specific pancreatic transcription factors. Thus, comprehensive fingerprinting of individual clones is mandatory to secure homogenous pools amenable for diagnostic and therapeutic applications of IPS cells from patients with T1D. We will also present the proprieties of IPS cells for pancreatic differentiation and teratoma formation in immuno-compromised mice.

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204-LB

mTOR Signaling Contributes to Developmental Programming of Pancreatic Beta-Cell

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Nutrients and growth factors converge on mTOR, which is involved in the regulation of growth and development of many organs including the pancreas. We hypothesize that mTOR plays a role on β-cell during fetal development. To develop a mouse model of maternal low-protein, pregnant C57BL/6 mice were fed control or low-protein diet throughout (LP0.5) pregnancy. We examined islet morphology in newborns and metabolic studies on LP0.5 offspring during adulthood. Rescue experiments to assess the involvement of mTOR was studied in LP0.5 offspring with transient gain of mTOR complex 1 function. A significant decrease in insulin levels and β-cell fraction in LP0.5 newborns was observed. These changes were associated with reduced phosphorylation of Ribosomal protein S6 in islets of LP0.5 offspring, suggesting that LP treatment had a negative impact on mTOR signaling and that this pathway could be involved in this process. Adult LP0.5 showed glucose intolerance despite enhanced insulin sensitivity, which points to a primary defect at the β-cell level. Indeed, glucose-induced insulin secretion from LP0.5 islets was blunted. KCl-induced insulin secretion was reduced, implying a defect that was distal to Ca2+ influx in LP0.5 islets. Reduced Insulin2 expression and insulin total content were associated with down-regulation of Pdx-1 message and total protein in LP0.5 islets. A significant reduction in mTOR protein, specifically in LP0.5 islets, was observed. The normalization of the β-cell mass defect by gain of mTORC1 function was explained at least in part by enhanced proliferation. β-cell over-expression of RhoB transiently during the last week of development was sufficient to rescue the impairment in glucose tolerance in adult LP0.5 mice. These data suggest that nutrient environment during fetal life programs glucose homeostasis by inducing permanent changes on mTOR expression and signaling. In addition, these experiments underscore a novel role of mTOR signaling in β-cell development and fetal programming.

Supported by: NIH

205-LB

Function of CISH and SOCS2 on Beta-Cell Proliferation During Pregnancy

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Prolactin / placental lactogen (PL/PRL) regulate beta-cell proliferation during pregnancy. After PL/PRL binds to the prolactin-receptor (PRL-R), the receptor dimerizes, JAK2 kinase is activated and phosphorylates the PRL-R. STAT5 is recruited to phosphorylated PRL-R and is phosphorylated in turn by JAK2. Phosphorylated STATS then dimerizes and translocates into the nucleus to regulate gene expression as a transcription factor. A group of known targets of STAT5 is up-regulated during pregnancy, including Ph-r, Glut-2, and Cyclin D2, resulting in increased beta-cell proliferation and insulin secretion. Cish and Socs2 are also up-regulated in mouse islets during pregnancy, forming a negative feedback loop to inhibit JAK2/ STAT5 signaling, thus potentially limiting proliferation. SOCS2 and CISH are “ Suppressors of the Cytokine Signaling” proteins, a family of eight members with similar structure. Different gene ablation models and transgenic mice for multiple Socs genes have been described, and show various phenotypes depending on which cytokine signal they regulate.

It is unclear whether CISH and SOCS2 limit beta-cell proliferation during pregnancy. Since lactogen signaling is critical for beta-cell proliferation and beta-cell function during pregnancy, and Cish, and to a lesser extent Socs2, are induced during pregnancy, we hypothesized that these two SOCS proteins negatively regulate beta-cell proliferation and beta-cell function. Here, we derived a novel mouse model with conditional ablation of the Cish gene in beta-cells to test the hypothesis that removing this negative feedback inhibitor could be exploited to stimulate beta-cell replication. Our findings reveal that: first, Cish deficiency in beta-cells is not sufficient to increase beta-cell DNA replication during pregnancy, and does not alter glucose tolerance before, during, or after pregnancy; second, Cish deficiency does not alter Stat5 signaling. And third, Socs2 might be compensating for Cish deficiency during pregnancy.

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Adult pancreatic duct cells exposed to Liraglutide—A pharmaceutical strategy for the treatment of diabetes. 

We aimed to investigate whether liraglutide, a human long-acting GLP-1 analogue, could enhance differentiation of human adult pancreatic duct cells into insulin-producing cells in vitro.

CAF19.3+ duct cells were purified by magnetic cell sorting from the exocrine fraction of pancreas from 12 human cadaveric organ donors (4 male, age 56±3; BMI 27.8±1.62). Sorted cells were cultured in suspension for 30 days in differentiation medium with or without liraglutide (300nM), and/or EGF (20ng/ml). Gene expression was determined by real-time RT-PCR. Replication (BrdU) and protein expression were analyzed by immunofluorescence. Insulin and C-peptide were determined by ELISA.

After 2-3 days in culture, the sorted cell population clustered into pancreato-spheres (92±4.0% duct; 0.19±0.07% C-peptide+ cells). Gene expression of krt19 was reduced on days 7, 30 and compared with post-sorting day (p<0.05). Duct cell replication remained low along the 30 days of culture with or without liraglutide. EGF alone and in combination with liraglutide resulted in similar stimulation of duct cell replication (p<0.05). Gene expression of ins, stz, ggl and pdx-1 was similarly increased in liraglutide-treated and non-treated cultures at day 30 compared with post-sorting day and day 3 (p<0.01). Insulin and C-peptide content (corrected per DNA) was significantly increased on day 30 compared with post-sorting day (p<0.05), but remained very low compared with human islets (1%). It was similar in liraglutide-treated and non-treated cultures. Insulin secretion did not increase in response to glucose stimulation.

These results support the hypothesis that adult human pancreatic duct cells can differentiate into insulin-producing cells. The GLP-1 analogue liraglutide did not improve human adult duct cell differentiation into insulin-positive cells, and did not exert a mitogenic effect in human adult duct cells.

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206-LB

Replication and Differentiation Into Insulin-Producing Cells of Human Adult Pancreatic Duct Cells Exposed to Liraglutide In Vitro

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A Key Role for Pyruvate Kinase Regulating Insulin Secretion Independent of Enzymatic Activity

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Mitochondria are intimately involved in the signal coupling glucose metabolism to insulin secretion. Putative mitochondrial metabolic signals include ATP produced via oxidative metabolism as well as several cataplerotic metabolic fluxes and cycles. The glycolytic enzyme pyruvate kinase (PK) is poised to play an essential role either by regulating the concentration of PEP and/or the availability of pyruvate. Pancreatic beta-cells contain both the PK-L and the PK-M2 isoforms that are both subject to regulation by allosteric, transcriptional and post-translational modifications. To determine if metabolic control is exerted at the PK step, we overexpressed the constitutively active human muscle isofrom (PK-HM1) in INS-1 B32/13 cells. In dominant PK-HM1 cells, HM1 message increased to 80% of endogenous PK-M2 message. PK-M2 message declined 31% (P=0.038) while PK-L increased 45% (P=0.0005) and together with PK-HM1 the total PK activity increased 7-fold. Glucose-stimulated and uncoupled respiration tended higher as did the ATP concentration. Proximal mitochondrial metabolites were the same, though malate and fumarate were reduced. In contrast, both proximal and distal TCA cycle flux (measured using mass isotopologue multi-ordinate mass spectroscopy) was identical to controls. Despite having the same total insulin content, ATP content and higher O2 consumption, glucose-stimulated insulin secretion was reduced >55% in PK-HM1 dominant cells (control 316±10 vs. PK-HM1 141±7 ng/mL/hr/mg protein, P=3x10^-3). While the cells had indistinguishable tolbutamide-stimulated insulin release, pyruvate-stimulated insulin secretion was surprisingly reduced as well (control 87±7 vs. PK-HM1 58±6, P=0.011) implicating PK in metabolic steps distal to pyruvate metabolism. These results suggest that PK-M2 exerts a positive regulatory influence that can be metabolically separated from total PK activity and is in keeping with an important role for PEP cycling in insulin release.

209-LB

Clock-Controlled Output Gene Dbp Is a Regulator of Arnt/Hif-18 Gene Expression in Pancreatic Islet B-Cells

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Aryl hydrocarbon receptor nuclear translocator (ARNT) / hypoxia-inducible factor-18 (HIF-1-ß) has emerged as a potential determinant of pancreatic ß-cell dysfunction and type 2 diabetes in humans. An 82% reduction in Arnt expression was observed in islets from type 2 diabetic donors as compared to non-diabetic donors. However, few regulators of Arnt expression have been identified. Meanwhile, disruption of the clock components CLOCK and BMAL1 is known to result in hypoinsulinemia and diabetes, but the molecular details remain unclear. In this study, we identified a novel molecular connection between Arnt and two clock-controlled output genes, albumin D-element binding protein (Dbp) and E4 binding protein 4 (E4bp4).

By conducting gene expression studies using the islets of WTfs1/2+/A mice that develop severe diabetes due to ß-cell apoptosis, we demonstrated clock-related gene expressions to be altered in the diabetic mice. Dbp mRNA decreased by 50%, E4bp4 mRNA increased by 50%, and Arnt mRNA decreased by 30% at Zeitgeber Time (ZT) 12. Mouse pancreatic islets exhibited oscillations of clock gene expressions. E4bp4, a D-box negative regulator, oscillated anti-phase to DBP, a D-box positive regulator. We also found low-amplitude circadian expression of Arnt mRNA, which peaked at ZT4. Over-expression of DBP raised both mRNA and protein levels of ARNT in HEK293 and MIN6 cell lines. Arnt promoter-driven luciferase reporter assay in MIN6 cells revealed that DBP increased Arnt promoter activity by 10-fold and that E4BP4 competitively inhibited its activation. In addition, on CHIP assay, DBP and E4BP4 directly bound to D-box elements within the Arnt promoter in MIN6 cells. These results suggest that in mouse pancreatic islets mRNA expression of Arnt fluctuates significantly in a circadian manner and that the down-regulation of Dbp and up-regulation E4bp4 contribute to direct suppression of Arnt expression in diabetes.

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210-LB

Critical Role of Actin Dynamics Regulated by N-WASP and Cofilin in the Biphasic Response of Glucose-Induced Insulin Secretion

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Actin dynamics is involved in insulin secretion, but molecular mechanisms of the regulation of actin dynamics in pancreatic β-cells and its role in has insulin secretion are not known. Here, we examined the role of actin dynamics regulated by neuron Wiskott-Aldrich syndrome protein (N-WASP) and cofilin in glucose-induced insulin secretion (GII). N-WASP, which promotes actin polymerization through activation of actin nucleation factor Arp2/3 complex, was activated in insulin-secreting clonal pancreatic β-cells (MIN6-KB) by glucose stimulation. Introduction of a dominant-negative mutant of N-WASP (DN-N-WASP), which lacks G-actin and Arp2/3 complex-binding region WA, into MIN6-KB β-cells or knockdown of N-WASP suppressed GII. We performed perfusion experiment using DN-N-WASP-introduced or N-WASP-knockdown MIN6-KB β-cells and found that the second phase of GII was specifically reduced. We also found that cofilin, which severs F-actin in its dephosphorylated (active) form, is converted to the phosphorylated (inactive) form in MIN6-KB β-cells by glucose stimulation, thereby promoting F-actin remodeling. In addition, perfusion experiment using MIN6-KB β-cells showed that a dominant-negative mutant of cofilin, which inhibits activation of endogenous cofilin, or knockdown of cofilin reduced the second phase of GII, indicating that activity of cofilin is critical for the second phase. In contrast, the first phase of GII arises mostly in G-actin-dependent process, in which cofilin activity predominates over N-WASP activity. Taken together, these results indicate that actin dynamics, which is regulated by the balance of N-WASP and cofilin activities, is critical in determining the biphasic response of GII.

211-LB

Enabling Structure Base Drug Discovery Using Stabilised Receptors—Identification of Novel GPR39 Agonists that Stimulate GLP-1 and Insulin Secretion In Vivo

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Heptares creates new medicines targeting clinically important GPCRs (G protein-coupled receptors) through application of a powerful structure-based drug discovery (SBDD) capability. By using stabilised receptors (Staar®), Heptares apply advanced computational and structural analyses together with fragment-based drug discovery to drive the identification of novel hits and leads for relevant GPCR targets. The G-protein-coupled receptor 39 (GPR39) has recently been implicated in metabolic regulation and pancreatic islet function. In vitro GPR39 responds to Zn2+ coupling via Gαxs and Gαq/g signaling cascades to increase cAMP and IP3/Ca2+ respectively. Using this approach we have identified novel GPR39 agonists that have been used to examine the potential of GPR39 activation for the treatment of metabolic diseases. Zn2+ and the identified GPR39 agonists G4G01 and G4G99 stimulated the accumulation of cAMP and IP3 in GPR39 transiently transfected HEK293 cells with EC50 values of 0.84µM/4µM, 2.4µM and EC50 0.6µM/1.2µM, 3.2µM (Zn2+/G4G01/G4G99). The secretion of insulin and GLP-1 in response to GPR39 agonism was assessed using the pancreatic β-cell line, N1T1, and primary mouse intestinal epithelial cells (mIECs). Zn2+ and G4G01/G4G99 dose-dependently stimulated glucose-dependent insulin secretion from N1T1 cells (EC50 0.26µM/0.94µM/0.45µM). Furthermore these effects were significantly diminished using GPR39 siRNA supporting a specific GPR39-mediated response. Consistent with their insulin secretory activity Zn2+ and compounds G4G01/G4G99 also stimulated GLP-1 secretion from primary mIECs with EC50 values of 1.6µM, 1.2µM and 3.4µM respectively. In conclusion GPR39 agonists stimulate insulin and GLP-1 secretion in vitro from native/primary cell systems and suggest that GPR39 agonists may represent efficacious agents for the treatment of metabolic disease.

212-LB

GLP-1 Is Not Able to Preserve the Beta Cell Function in Mice With Mitochondrial Diabetes

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Mitochondrial diabetes is an unremarkable form of diabetes characterized with progressive loss of beta cell function by the mutation of mitochondrial DNA. Beta cell failure in mitochondrial diabetes is related with imbalance between apoptosis and proliferation of beta cells. GLP-1 appears to inhibit the apoptosis and stimulate the proliferation of pancreatic beta cells. However, it has not been evaluated whether GLP-1 may restore beta cell function or islet mass in the mitochondrial diabetes.

In this study, we have developed the new animal model of beta cell specific mitochondrial dysfunction by breining the Crif1(+/−) mice with RIP2-cre mice. CRIF1 is a protein required for the intramitochondrial production of mtDNA-encoding OXPHOS subunits; therefore, CRIF1 deficiency results in specific failure of OXPHOS capacity. CRIF1 deficiency in pancreatic beta cells resulted in functional defect of insulin secretion without decrease of islet beta cell area in 4 week-old mice. 11 week-old mice with beta cell specific CRIF1-deficiency showed diabetic phenotypes with marked defect of insulin secretion with 70% decreased islet area. GLP-1 receptor agonist (Exenatide, 10 nM/kg) was given to the beta cell specific CRIF1 knockout mouse (4 week-old) for 4 weeks and measured the insulin secretion and islet area. The administration of GLP-1 receptor agonist in wild type mice enhanced insulin secretion and increased islet area (35%) compared to vehicle group. However, GLP-1 receptor agonist in beta cell specific CRIF1 knockout mice did not improve the insulin secretion and not preserve the islet area. Consequently, GLP-1 receptor agonist did not improve the diabetic phenotypes in mice with beta cell specific CRIF1 deficiency. Based on these findings, we concluded that short-term treatment of GLP-1 receptor agonist was not effective to reverse the diabetic phenotypes by preserving islet mass and insulin secretion in mite model of mitochondrial diabetes with beta cell specific CRIF1 deficiency.

213-LB

Sulfonlyureas Act as an Enhancer of Epac2 Activation in CAMP-Induced Insulin Secretion

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CAMP is a key signal in β-cells that amplifies insulin secretion. Incretins such as glucagon-like peptide 1 (GLP-1) stimulate insulin secretion through cAMP signaling in β-cells. We have shown that Epac2, which belongs to a new class of CAMP-binding proteins, plays a critical role in incretin-induced insulin secretion. In addition, we have found that Epac2 is also a target of sulfonlourylic (SUs). Here we have identified and characterized SU binding site in Epac2. We first predicted the amino acid residues of Epac2 that interact with SUs by molecular docking simulation, and the predicted amino acids were mutated individually to alanine in vitro. Analyses of these mutants by FRET (fluorescence resonance energy transfer), SU binding, and Rap1 activity revealed that SU-binding site is located in the first cAMP-binding domain A (cNBD-A) and that binding of SUs to Epac2 depends on SU structures as well as the state of CAMP binding to Epac2. We also found that SU and cAMP synergistically activate Epac2 and Rap1. Modeling of CAMP binding and SU binding in cNBD-A indicates that the two binding sites are not identical, but clearly overlap, suggesting that cAMP and SU cannot bind simultaneously in cNBD-A. We next examined the effect of combination of SU and incretin or cAMP analog on insulin secretion from perfused pancreas. Potentiation by cAMP analog or GLP-1 of glibenclamide-induced insulin secretion was markedly enhanced in wild-type mice, whereas the potentiation was significantly reduced in Epac2 deficient mice. Our data indicate that cAMP and SU cooperatively activate Epac2 through binding to cNBD-B and cNBD-A, respectively, to stimulate insulin secretion. We propose that SUs act as an enhancer of activation of Epac2 by CAMP.

214-LB

Deletion of 4E-BP2 Induces Beta Cell Proliferation and Mass and Confers Resistance to Streptozotocin Induced Diabetes

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The mechanistic target of rapamycin (mTOR) signaling pathway integrates growth factors and nutrient signals and is essential for cell growth and proliferation. The mTOR complex 1(mTORC1) is sensitive to rapamycin and regulates protein translation and ribosomal biogenesis by modulation of ribosomal S6 kinase (S6K) and eukaryote initiation factor 4E binding proteins (4E-BP1 and 2). 4E-BPs repress translation by disrupting eIF4F formation, thereby preventing ribosome recruitment to the mRNA. To test the role of 4E-BPs in beta cells, we studied 4E-BP1 and 4E-BP2 deficient mice (4ebp1-/- and 4ebp2-/-). Mice deficient for 4E-BP1 or 4E-BP2 showed improved glucose tolerance test at 2 months and 1 year. However, analysis of pancreas

For author disclosure information, see page LB66.
morphology from these mice showed that beta cell mass and proliferation was enhanced only in 4ebp2-/- mice. The increase in proliferation in 4ebp2-/- mice was associated with higher levels of p-ERK and decrease p27 levels. Moreover, islets from 4ebp2-/- mice were resistant to apoptosis induced by “in vitro” treatment with of pro-inflammatory cytokines (IL1 beta, TNF alpha and interferon gamma). Finally, 4ebp2-/- mice were resistant to diabetes induced by low-dose streptozotocin and this protective effect resulted from lower levels of apoptosis and enhanced proliferation. These experiments demonstrate that the 4E-BPs relates proliferative and survival signals induced by activation of mTORC1.

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215-LB
Dynamic Changes in Oct4 Expression in Parallel With Human Islet Dedifferentiation/Redifferentiation In Vitro
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It has recently been proposed that beta cell dedifferentiation with loss of insulin expression and increased expression of mesenchymal markers may play an important role in the pathogenesis of type 2 diabetes. Animal data suggest that this is associated with upregulation of pluripotency genes as a marker of plasticity and potential for beta cell reprogramming. We have characterised expression of end-differentiated islet phenotypic markers, mesenchymal markers and the classical pluripotency marker Oct4 in intact islets, islet survivor cells (ISCs) established in proliferative 2D culture and reaggregated pseudo-islets (PIs). Dynamic changes in Oct4 expression have been further studied by live-cell imaging following infection with a lentiviral Oct4-eGFP reporter construct in control to compare CMV-eGFP construct.

Initial adherence induced proliferation originating as 2D outgrowths from 3D islet clusters. This was accompanied by decreased expression of epithelial endocrine markers including insulin, PDX1, PC1/3, Glut2, glucagon, somatostatin and pancreatic polypeptide and increased expression of CK19, vimentin and PAX4. Redifferentiation of 3D PIs at Passage 4 led to increased C-peptide storage and secretion. In parallel, increased Oct4 gene expression during ISC formation, maintenance in proliferative culture and downregulation on PI formation was demonstrated. Lentiviral Oct4-eGFP infection confirmed low frequency of Oct4 expression in intact islets with increased numbers on establishment in 2D culture. Live cell imaging demonstrated symmetrical cell division of Oct4 expressing cells with maintained expression in all progeny. PI formation was associated with decreased number of cells expressing Oct4-eGFP in comparison to control.

Upregulation of Oct4 expression has been demonstrated in human islet cells undergoing dedifferentiation with reversal following redifferentiation in live cell reporter gene imaging studies in vitro.

216-LB
Pancreatic Primary Cell Aggregates Are Functionally Superior to Age-Matched Islets
THOMAS H. HRAHA, KELLY M.T. SHEKIRO, ABIGAIL B. BERNARD, KRISTI S. ANSETH, RICHARD K.P. BENNINGER, Aurora, CO, Boulder, CO
Barriers to the effectiveness of islet transplantation include the limited number of donor islets and post-transplant graft viability. Recently, it has been shown that smaller islets have higher viability, functionality and lead to better transplant results. Therefore, a method for controlling the size of transplant islets may lead to better functional outcomes. To accomplish this, primary pancreatic cells were dissociated and re-aggregated into “pseudo-islets” using novel hydrogel microwell arrays created through photo-lithography to physically direct cell re-aggregation and create pseudo-islets of defined size. After 7 days in culture, pseudo-islets were created with a mean diameter of 45 ± 4µm and compared to age-matched islets with a mean diameter of 195 ± 23µm. Pseudo-islet function was assessed using real-time quantitative fluorescence microscopy. Compared to age-matched control islets, pseudo-islets showed significantly more coordinated [Ca2+]i dynamics at high glucose and lower [Ca2+]i activity at low glucose. This correlated with elevated glucose-stimulated insulin secretion. Two-photon microscopy showed a significant glucose-stimulated NADPH elevation in the pseudo-islets, but not in the age-matched control islets. In both of these measurements, the pseudo-islet response was similar to that of freshly isolated islets.

These data suggest that re-aggregation produces functional islet-like clusters based on highly-sensitive measurement techniques. Therefore, by increasing the functional capacity of every donor β-cell, re-aggregating large islets into smaller pseudo-islets will allow for the therapeutic delivery of β-cells with greater viability and function. In addition, this method also lends itself to study the molecular pathogenesis of diabetes and as a platform for aggregating stem cells.

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217-LB
Post-Transcriptional Regulation of SERCA2b by IRS2 in the Pancreatic Beta Cell
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Cytosolic and endoplasmic reticulum (ER) Ca2+ levels in the β cell are closely regulated by the sarco-endoplasmic reticulum Ca2+ ATPase (SERCA) pump. We have previously shown markedly diminished β cell SERCA2b mRNA and protein expression in human and rodent models of Type 2 diabetes mellitus (T2D). To study further the transcriptional and translational regulation of β cell SERCA2b, we first sought to define the protein and mRNA half-life (1/2) in basal and diabetic conditions. SERCA2b protein and mRNA expression were decreased in INS-1 832/13 cells treated with 5ng/ml IL-1β-25 mM glucose (IL-1β-HG) to mimic the pro-inflammatory and hyperglycemic milieu of T2D. Cycloheximide was used to block protein translation and actinomycin D was used to block transcription in time-course experiments. At baseline, SERCA2b protein 1/2 was ~24hr, while the mRNA 1/2 was ~8hr. IL-1β-HG reduced the protein 1/2 to 18hr, but the mRNA 1/2 was stable. IL-1β-HG led to induction of iNOS and cleaved caspase-3 and reduced SERCA2b protein 1/2 and expression. However, concurrent treatment with the iNOS inhibitor L-NMMA prevented these changes, suggesting that SERCA2b loss was NO-dependent. Given that iNOS can inhibit IRS/PI3-kinase/Akt signaling, we further investigated the relationship between this pathway and SERCA2b expression and stability. INS-1 cells were treated with IGF-1 or infected with adenoviruses to overexpress IRS-2 or IRS-1. Under basal conditions, IGF-1 stimulation and IRS-2, but not IRS-1, increased SERCA2b protein levels. Interestingly, in IL-1β-HG-treated INS-1 cells, IGF-1 and IRS-2 overexpression prolonged the protein 1/2 and restored SERCA2b levels. Together, our data suggest β cell SERCA2b protein 1/2 is decreased in an NO-dependent manner in T2D. We further demonstrate a novel connection between IRS-2 signaling and regulation of ER Ca2+, demonstrating that IRS-2 activation regulates SERCA2b expression under basal conditions and acts to stabilize the protein under diabetic conditions.

Supported by: K12DK080225; R03DK089147; R01DK093954

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ABSTRACT AUTHOR INDEX

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