Late Breaking Abstracts

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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 ~150mg/dL after which a constant (50uL/min) jugular iv infusion of saline (N=7) or 4mg/mL of GABA (N=9) started (t=-10 min). At t=0 min, a 12U/kg iv insulin bolus was given. Blood sampling was done every 10min 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= 50 to 80 min}. R measures the BG level-specific GCR with higher values indicating a better response. This design was chosen over a glucose clamp to avoid over-insulinization at the time of GCR. Even though the initial (t< 20 min) glucagon fold change relative to the basal was lower with GABA vs. saline (mean±SD) (0.9±0.28 vs. 1.4±0.54, p=0.04), the later (after t=50 min) BG level-specific GCR was 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 md/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 (RO1DK082805)

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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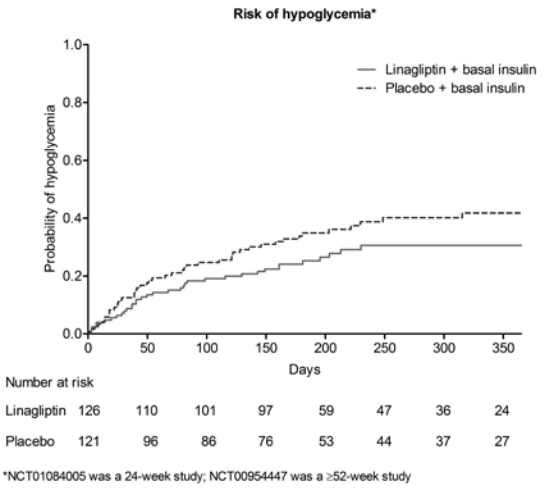
Elderly T2DM patients (pts) with long-standing disease often require insulin (INS) therapy, yet hypoglycemia is a major concern. It has recently been shown that linagliptin (LINA) added to stable basal INS in elderly T2DM pts reduced HbA1c by -0.77% vs. placebo (PBO), notably with less hypoglycemia. 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 vs. 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 deserving further study.



*NCT01084005 was a 24-week study; NCT00954447 was a ≥52-week study

Supported by: Boehringer Ingelheim Pharmaceuticals, Inc.

3-LB

The Effects of Diabetes Duration on Hypoglycemia Symptom Intensity and Prevalence of Impaired Awareness of Hypoglycemia

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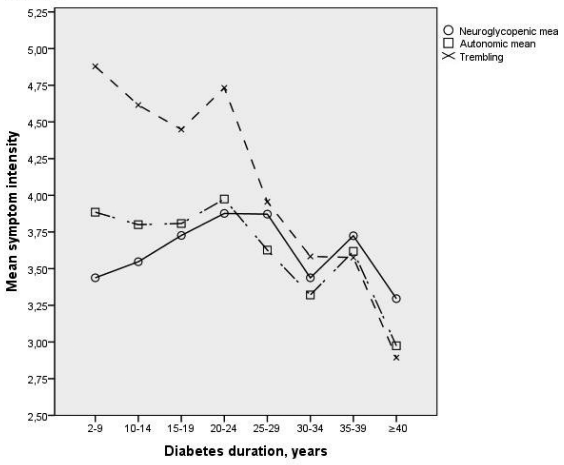
Diabetes duration influences hypoglycemia symptom profile and prevalence of impaired awareness of hypoglycemia (IAH); viz. a diminished ability to perceive onset of hypoglycemia. By questionnaire, hypoglycemia symptoms and prevalence of IAH were assessed in an outpatient population with type 1 diabetes. 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 28 % 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.

Figure 1



Supported by: Norwegian Diabetes Association

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-fibrinolytic eicosanoic acids 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, suggesting 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-fibrinolytic 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 dilation. We show that possible cardioprotective effects include limiting amylin-induced cardiac oxidative stress and myocyte Ca^{2+} 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-fibrinolytic 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 TIDM (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 continuous BP measurement. Following the experiment, femoral arteries (FA) were harvested for tissues analyses. At week 1, conscious BP (C=109±12 vs. T1DM=109±6 mmHg) and the increase in BP (~20%) following L-NMMA was the same (P≤0.72) between groups. At week 10, rats with T1DM had higher resting BP (T1DM=147±5 vs. C=113±7; P<0.01). The BP response to L-NMMA was preserved in C (P<0.01) but not T1DM (P=0.86) rats. Under anaesthetia, BP reductions (~45 mmHg) to systemic ACh infusions were similar (P=0.78) between groups. However, in rats with T1DM, reductions in BP following PRZ infusion were greater (T1DM=49±22 vs. C=17±18 mmHg; P=0.02) and basal NPY concentrations were greater (T1DM=718±118 vs. C=517±91; P<0.01). FA endothelial NO synthase content was similar (T1DM=0.95±0.04 vs. C=0.92±0.06 eNOS/β-actin; P=0.21) between groups. However, in rats with T1DM the cross sectional area of FA smooth muscle was greater (T1DM1=142±55 vs. C=96±6 μm2; P=0.05). These data suggest a role for impaired NO function, sympathetic hyperactivity and/or smooth muscle hypertrophy in the etiology of T1DM related hypertension.

Supported by: CIHR

6-LB

High Sensitivity Cardiac Troponin I as a Diagnostic Tool for the Presence of Coronary Artery Disease in Stable Diabetic Patients

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Introduction: The emergence of several high sensitivity troponin (hsTroponin) assays has shown that cardiac troponins can be chronically elevated in response to cardiovascular comorbidities. In the absence of unstable coronary artery disease, pathophysiological mechanisms as increased demand ischemia or disturbances of cell membrane integrity are possible causes for troponin leakage. We hypothesized that with a high sensitivity assay, troponin levels would be higher in diabetic patients with coronary artery disease (CAD), in comparison to diabetic patients without coronary artery disease.

Objective: To evaluate hsTroponin levels in ambulatory diabetic patients with multivessel CAD and diabetic patients with angiographically normal coronary arteries.

Methods: hsTroponin levels were determinated in 85 diabetic patients: 51 women (22 with CAD and 29 with normal coronary arteries) and 44 men (34 with CAD and 10 with normal coronary arteries). Patients were paired by age and body mass index. Both groups of patients had preserved left ventricular function measured by ventriculography or echocardiography.

Results: hsTroponin levels were significantly higher (p=0.0005) in diabetic women with CAD (average = 12.95±5.55 pg/mL) in comparison to diabetic women with normal coronary arteries (average = 8.02±2.62 pg/mL). hs Troponin levels were also significantly higher (p=0.0086) in diabetic men with CAD (average=10.60±4.39 pg/mL) in comparison to diabetic men with normal coronary arteries (average=6.94±1.67). At a troponin cutoff of 11 pg/mL 90% of diabetic men and 75% of diabetic women had CAD.

Conclusion: In this study hs Troponin I was a strong marker of CAD in diabetic patients with multivessel CAD in comparison with diabetic patients with angiographically normal coronary arteries. hsTroponin may be a useful tool to stratify the risk of CAD among diabetic patients with normal ventricular function. The discriminating power was higher among men.

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-β/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 (LKW/BWT) were detected 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 decreased (P<0.05), while c-Ski was higher (P<0.01). In conclusion , Pioglitazone could significantly up-regulate 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 1791 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=0.153-0.865; P=0.022), and PP>60 significantly increased risk (HR=2.382; CI=1.579-3.593; 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; CI=1.003-1.317, P=0.045) for each 1% A1c increase. We conclude that SBP ≥130 mmHg, higher A1c and PP>60 were associated with worsening ACR. PP<40 showed a lower risk for worsening ACR. The results suggest that treatment of SBP to below 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 clinical neuropathy were included if they had a minimum score of 7 on the Michigan Diabetic Neuropathy Score (MDNS). Following which the patients were randomly assigned to intervention or standard care for eight weeks. RANOVA was used for data analysis and $p < 0.05$ was considered significant. After randomization there were 47 participants in the control group and 40 participants in the exercise group. The results showed a significant difference in mean scores of two groups for MDNS and NQOL post intervention.The two groups had a significant difference for pain scores with F = 6.7 and p =0.01, sensory symptom scores had F =4.60 and p = 0.04, restricted activities of daily living had F =4.97 and p =0.03, disruptions in social relationships score had a F =5.43 and p = 0.02, specific impact on quality of life score had F = 9.28 and p = 0.00 and overall quality of life score had a F =28.72 and p = 0.00 and total score for NQOL had F = 31.10 and p = 0.00. Degrees of freedom for all the components were 1,62. In conclusion moderate intensity aerobic exercises helps to improve the NQOL of individuals with peripheral neuropathy in type 2 diabetes.

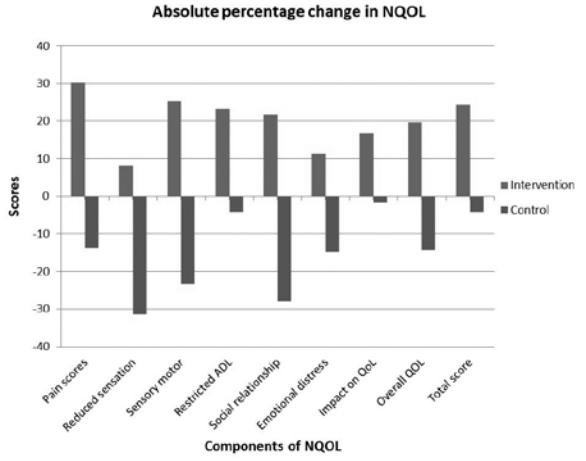

Figure 1 : Depicts the absolute percentage change in NQOL[†] (-) or (+) sign indicates deterioration or improvement in quality of life respectively of the patients in two groups.

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.

Table 1. Gastrointestinal symptoms assessed by questionnaire; Patient assessment of upper gastrointestinal symptom severity index (PAGI-SYM) at baseline and at 20 weeks study period, grouped in intervention and control group.

	Intervention diet, n = 28			Diabetes diet, n = 28		P-value ID diet	Diff Change Mean, 95 CI
	Baseline Mean ± SD Median (range)	Finished study mean ± SD Median (range)	p-value	Baseline Mean ± SD Median (range)	Change Baseline/Finished study, p-value		
PAGI-SYM Nausea+vomiting	1.08±1.1 1.00 (0.00-4.00)	0.18±0.5 0.00 (0.00-1.67)	0.002	1.49±1.5 1.00 (0.00-5.00)	ns	0.011	-0.564 (-1.014—-0.114)
Peripheral fullness	2.4±1.3 2.4 (0.00-5.00)	1.44±1.2 1.10 (0.00-4.75)	0.001	2.5±1.3 2.62 (0.25-4.75)	ns	0.021	-0.609 (-1.137—-0.081)
Bloating	2.61±1.7 2.5 (0.00-5.00)	1.75±1.5 1.75 (0.00-5.00)	0.003	3.95±1.6 3.25 (0.00-5.00)	ns	0.006	-0.865 (-1.475—-0.255)
Upper abdominal pain	1.86±1.6 2.00 (0.00-5.50)	1.34±1.3 1.10 (0.00-5.00)	*	2.11±1.6 2.00 (0.00-5.00)	ns	0.244	-0.342 (-1.010-0.285)
Lower abdominal pain	1.64±1.7 1.00 (0.00-5.00)	1.14±1.4 0.75 (0.00-5.00)	0.049	1.57±1.4 1.25 (0.00-4.00)	ns	0.123	-0.503 (-1.147-0.141)
Regurgitation/heartburn	1.43±2.2 1.42 (0.00-4.86)	0.82±1.9 0.43 (0.00-3.57)	0.007	1.21±0.9 1.21 (0.00-3.29)	ns	0.021	-0.598 (-1.845—0.072)

ns=no significant; * p > 0.05

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. 2009) and circadian dysregulation of vascular progenitors (VP) profoundly contribute towards 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. Glycated hemoglobin (GlyH), VP enumeration in BM and blood (by flow cytometry), VP migration (by QCM Chemotaxis Assay) and circadian clock gene expression, BMAL 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) increase in 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 assesses 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.

Supported by: R01DK090730, R01EY007739

COMPLICATIONS—OCULAR

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-Deazaneplanocin A (DZNep).

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 SAT and VAT areas were included. the severity of DM retinopathy graded to 9 categories(no retinopathy, microaneurysms only, mild NPDR, mod NPDR, severe NPDR, very severe NPDR, PDR without HRC, PDR with HRC, advanced PDR). VAT was positively associated with the severity of DM retinopathy after adjustment for the clinical variables (β-coefficient =0.085, P = 0.034), while SAT was not significantly associated with the severity of DM retinopathy. When stratifying the individuals by the body mass index 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 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 (65.0% 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 of 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 in those with diabetes, is associated with a significant risk of pulmonary embolism.

15-LB

Imbalance in NGF/proNGF Ratio as Biomarker of Diabetic Retinopathy

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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 vitreoctomy 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.

Supported by: JDRF; RO1EY042208; University of Georgia

16-LB

Plasma Biomarkers For Diabetic Retinopathy Discovered Using SOMAscan™

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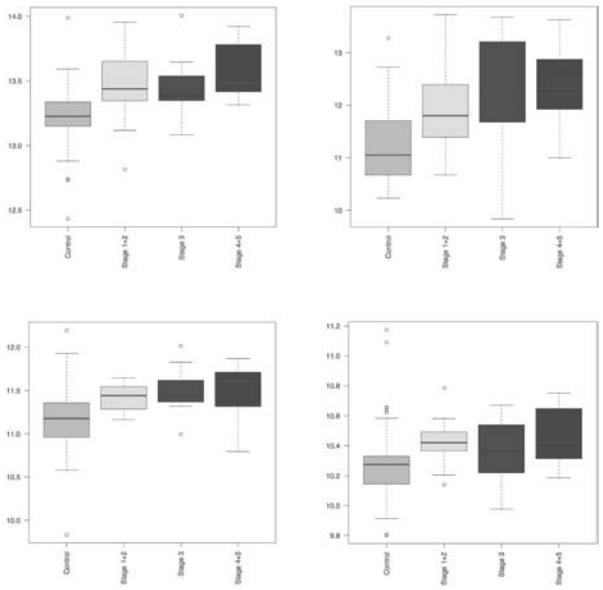
A blood test that provides a timely and accurate diagnosis of diabetic retinopathy (DR), especially in the 50% of diabetic subjects not adherent to eye exams, could be used to drive preventive interventions. A prospective case-control study enrolled 88 diabetic subjects. Blood samples were collected 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 factor2 (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 and disruption in tight junction formation. Moreover, induction of XBP1 splicing by tunicamycin and thapsigargin increased Nrf2 expression, while quinotrierixin, a XBP1 splicing inhibitor, abolished tunicamycin- and thapsigargin-induced Nrf2 upregulation. Taken together, our results indicate that XBP1 is required for Nrf2 expression in RPE cells and that deficiency of XBP1 resulting in decreased anti-oxidant response contributes to oxidative injury and tight junction damage of the RPE.

Supported by: NIH (EY019949)

DIABETIC DYSLIPIDEMIA

18-LB

Safe and Selective Small Molecule RxRα Agonist Modulates Glucose and Lipid Metabolism in *ob/ob* Mice

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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 (141.85±8 Vs. 119±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 C57BL6/j 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.

19-LB

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

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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 ß1 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 dosing 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.

20-LB

Acute Changes in Glucose Metabolism are Linked to Lipoprotein Alterations, and may Impact Endothelial Health

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We examined the effect of acute hyperglycemia on lipoprotein particle concentrations utilizing nuclear magnetic resonance (NMR) spectroscopy in healthy subjects. Baseline endothelial function was evaluated by reactive hyperemia index (RHI) using Endo-PAT. We studied 9 healthy subjects (8 females, age 46 ± 6 yrs, fasting blood sugar (BS) 5± 0.3 mmol/l, HbA1C 5 ± 0.3 %) following overnight fast during 2-step pancreatic clamp [somatostatin, glucagon, insulin (0.75 mU/KgTBW/min)] and GLP-1 (1.2 pmol/kg/min). Glucose infusions maintained euglycemia (BS 5 ± 0.5 mmol/l), followed by hyperglycemia (BS 13 ± 0.6 mmol/l) for 2 hrs. Endothelial dysfunction was RHI <2.0. NMR profile included total NMR protein,total cholesterol, triglycerides, HDL-C, LDL-C, LDL-p, sLDL-p, HDL-p, and lgHDL-p concentrations and was evaluated at baseline, euglycemia and hyperglycemia. RHI (mean 2.1 ± 0.6) was significantly correlated to baseline HDL-p (r = 0.7, p= 0.03), and maintained significance after adjusting for age (p=0.04). Significant decrease in NMR fractions during hyperglycemia was observed.

Conclusions: Acute hyperglycemia resulted in a significant decrease in total and large HDL-p concentrations measured by NMR. HDL-p had a positive correlation with baseline endothelial function, as determined by RHI. These observations suggest that acute changes in glucose metabolism are linked to lipoprotein alterations, and impact endothelial health.

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

NMR fraction	Euglycemia stage	Hyperglycemia stage	P value
NMR protein, ng/mL	466.1 ± 39.4	436.1±31.9	0.0003
Total cholesterol , mg/dL	147.6 ± 24.5	135.7 ± 22.4	0.0004
Triglycerides , mg/dL	77.6 ± 27.8	55.1 ± 20.3	<0.0001
LDL cholesterol (LDL-C),mg/dL	81.8± 19.1	79.4 ± 18.7	0.224
HDL cholesterol (HDL-C),mg/dL	49.9 ± 7.6	45.9 ± 7.6	0.0014
Total LDL particles (LDL-p),nmol/L	902.4 ± 359	860.7 ± 334.0	0.192
LDLp size (nm)	21.3 ±1.09	21.3±1.06	0.681
Total small LDL particles (sLDL-p), nmol/L	601.0 ±345.1	593.1 ± 350.2	0.772
Mean HDLp size (nm)	9.1 ± 0.48	9.1 ±0.51	0.104
Total total HDL particles (HDL-p), µmol/L	30.4 ± 4.79	28.7 ± 4.8	0.002
Large HDL p(lgHDL-), µmol/L	7.86 ± 2.67	7.17 ± 2.53	0.032

Supported by: NIH (UL1TR000135)

21-LB

Existence of a Triglyceride-Dependent Glycemic Regulatory Pathway in Patients With Type 2 Diabetes

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Gastric electrical stimulation by the Tantalus-Diamond device has been shown to reduce A1C, weight and blood pressure in patients with type 2 diabetes (T2DM) inadequately controlled with oral antidiabetic agents (J Diabetes Sci Technol 3:964-970, 2009). A proposed mechanism for these actions is a vagus nerve-mediated CNS pathway. A retrospective analysis of 40 patients treated with the Tantalus device for at least 12 months has shown that the improvement in glycemic control is inversely correlated with the fasting plasma triglyceride (TG) level (Diabetic Med. online Jan. 13, 2013). Studies in rodents have described a gut-brain-liver axis involving upper intestinal lipids to regulate glucose production (Nature 452:1012-1016, 2008). In a prospective study, 21 patients with T2DM inadequately controlled on oral agents who had been implanted with the Tantalus and had completed a 12 month randomized crossover control versus electrical stimulation study were enrolled into an additional 6 month study in which all had TG measurements and received electrical stimulation. The goal was to assess the effect of the TG levels on the A1C levels achieved at the end of the 6 months electrical stimulation. The mean A1C after 6 months in 9 patients with TG ≤ 1.7 mmol/L was 7.7 ± 0.43 % which was a 0.92 ± 0.31 % decrease from the baseline values preceding implantation of the Tantalus. This contrasted to a mean of 8.33 ± 0.45 % in 12 patients with TG >1.7 mmol/L which was a mean increase from baseline of 0.21 ± 0.36 % (treatment difference 1.23 %, $p < 0.05$). The Pearson correlation coefficient for the lnTG versus the change in A1C from baseline was 0.535 ($p = 0.012$). These data confirm the previous retrospective data and are consistent with the rodent data suggesting the existence of a triglyceride sensitive glucose regulatory pathway in humans.

Supported by: MetaCure

FOOT CARE—LOWER EXTREMITIES

22-LB

WITHDRAWN

23-LB

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

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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 amplicons, RFLP analysis was done by gel electrophoresis.

HPS A1 B polymorphism was identified as AG in 82%, GG in 18% and GG 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 A1L and TT polymorphic variant HPS A1B was associated with severe wound grade (Wagner's 3, 4 and 5) and risk of amputation. Other polymorphic variants of HPSA1L/HPSA1B genes did not show such association. HPS A1B and HPSA1B genes (encoding HPS 70 protein) polymorphism was associated with severity of diabetic foot ulcer. AG and TT Polymorphic variant of HPS A1B and HPSA1L genes increased the risk of amputation of diabetic foot. The study thus suggested role of quantitative proteomic analysis of HPS 70 as a prognostic marker of diabetic foot

DIABETES EDUCATION

24-LB

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

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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 (110/180,61.1%)
- 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

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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 a HbA1c > 6.0% to a low fat, calorie 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 CHO (LC; < 50 gm CHO/day not including fiber; n = 16). Both groups met for 13 sessions over 3 months. We excluded participants receiving insulin; 74% were taking oral diabetes medications. All were also taught psychological skills aimed at helping promote behavior change and maintenance. The primary outcome was HbA1c. During the intervention period, the mean HbA1c decreased -0.02% in the LFCR diet group and -0.59% in the LC group (p = 0.04 comparing groups). None in the LFCR group achieved an HbA1c < 5.7 compared to 13% (n=2) in the LC group. Eleven percent of participants in the LFCR group reduced or discontinued 1 or more oral diabetes medications whereas 47% in the LC group did so. 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.

	LFCR diet		LC diet		Difference (LC – LFCR)	95% Confidence Interval
	0 mo	3 mo change	0 mo	3 mo change		
HbA1c (%)	6.91	-.02	6.61	-.59**	-.57	[.02 to 1.11]
Weight (kg)	99.71	-2.60*	100.07	-5.52**	-2.92	[-.46 to 6.30]
BMI	37.38	-.94*	36.22	-1.91**	-.97	[-.22 to 2.18]
Systolic BP (mm Hg)	129.53	4.68	130.73	5.27	.59	[-9.97 to 8.79]
Diastolic BP (mm Hg)	79.94	.29	76.33	2.53	2.24	[-8.91 to 4.43]
LDL (mmol/l)	98.53	-3.41	89.20	-2.07	1.34	[-15.00 to 12.31]
HDL (mmol/l)	46.89	-.89	50.07	.93	1.82	[-6.17 to 2.52]
Triglycerides	172.22	-3.94	123.60	-22.33†	-18.39	[-20.54 to 57.32]
Fasting Glucose (mg/dL)	140.65	-1.18	124.40	-11.07	-9.89	[-16.40 to 36.18]
Fasting Insulin (μIU/mL)	10.11	1.00	12.20	-2.87	-3.87	[-.51 to 8.24]
C-Reactive Protein (mg/dL)	4.41	-.65*	7.26	-1.50*	-.85	[-.38 to 2.08]
Insulin Resistance (HOMA2; Homeostasis Model Assessment)	1.48	.09	1.67	-.41	-.50	[-.11 to 1.09]
CES-D Overall Depression	12.00	-1.17	10.56	-1.36	-.19	[-4.94 to 5.32]
Total Diabetes Distress	2.34	-.20	1.78	-.48**	-.28	[-.20 to .77]

Note: † = $p < .10$, * = $p < .05$, and ** = $p < .01$ for within-group paired-samples t-tests on scores collected at baseline and 3 months later. Means exclude participants without follow-up data: two moved away (one of those has partial 3-month data). Center for Epidemiologic Studies Depression Scale (CES-D; higher is more depressed; Radloff, 1977), Diabetes Distress Scale (higher is more distressed; Polonski, et al., 2005).

Supported by: Bowes Fund

26-LB

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

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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 only and 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 Guttman 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/CSII 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

27-LB

Type 2 Diabetes: Effectiveness of Weight Loss Interventions on A1C, Lipids, Blood Pressure

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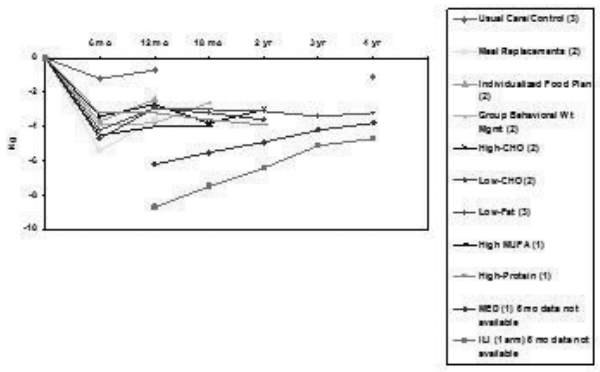
A systematic review of weight loss interventions (WLI) in overweight/obese adults with type 2 diabetes was conducted to determine their baseline to 1-yr effectiveness. Study inclusion criteria: randomized clinical trial ≥1-year, completion rate of ≥70%, published between Jan 2000 to Feb 2013. Ten studies 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-yr 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; 9 of 17 WLI reported ↑ in HDL-C, 3 of 17 ↓ in TG, 1 of 14 ↓TC, and 1 of 16 ↓ 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.

Figure 1. Average Weight Loss/Maintenance in Persons with Type 2 Diabetes (10 studies)



PSYCHOSOCIAL, BEHAVIORAL MEDICINE

28-LB

Psychometric Validation of a Novel Measure of Impaired Awareness of Hypoglycemia: The Hypoglycemia Awareness Questionnaire (HypoA-Q)

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Impaired awareness of hypoglycemia (IAH) affects 20-25% of adults with established type 1 diabetes and is associated with a six-fold increase in severe hypoglycemia. Our aim was to conduct preliminary psychometric validation of the 18-item HypoA-Q.

Questionnaires (HypoA-Q, Gold score, Clarke measure, Problem Areas in Diabetes (PAID)) were completed by 120 adults with type 1 diabetes with/without clinically diagnosed IAH (mean age: 44.4±15.8 years; duration of diabetes: 22±13.4 years; HbA1c 8.2±1.3%; 44% women) attending routine specialist clinic appointments. HbA1c was collected from clinic records.

Principal components analysis revealed the HypoA-Q to include three brief scales reflecting 'impaired awareness', 'symptom frequency', and 'symptom level' (3-5 items each; Cronbach's alpha=0.79-0.89), plus individual items measuring recall of mild/severe hypoglycemic events, nocturnal hypoglycemia/awareness and healthcare resource use. Satisfactory convergent validity was demonstrated, e.g. between 'impaired awareness' and other measures of IAH (Gold: rs=0.75; Clarke: rs=0.76). 'Impaired awareness' discriminated significantly between those on the clinical register of IAH (n=60) and those not on the register (n=60; U=640.00, p<0.001), correctly classifying 72% of participants. Divergent validity was supported, e.g. with low correlations between 'impaired awareness' and HbA1c (rs=-0.05), age (rs=0.16), duration of diabetes (rs=0.22), and diabetes-related distress (PAID; rs=0.25).

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

29-LB

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

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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)	p-value (statistical test or model)
Baseline (n=247; FEI 84, SMI 82, AP 81) comparisons of a) median LDL level (mg/dL) and b) LDL control % (LDL < 100 mg/dL)			
a) 105 mg/dL	112 mg/dL	111 mg/dL	0.12(Wilcoxon)
b) 42.17 %	28.75%	25.00%	0.05 (Chi-Sq test)
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			
a) 92 mg/dL	103 mg/dL	106 mg/dL	FEI vs. AP= 0.005; SMI vs. AP = 0.46 (Wilcoxon)
b) 62.67%	46.58%	40.54%	FEI vs. AP = 0.01; SMI vs. AP = 0.46 (Chi-Sq test)
c) -10.80 mg/dL (95% CI: -20.5 - -1.1)	-4.35 mg/dL (95% CI: -15.0 - 6.3)	Referent (0)	FEI vs. AP= 0.03; SMI vs. AP = 0.42 (Linear model adjusted by baseline data)
d) Odds Ratio: 3.8 (95% CI: 1.6 - 9.0)	Odds Ratio: 2.1 (95% CI: 1.0 - 4.5)	Referent (Odds Ratio = 1)	FEI vs. AP= 0.003; SMI vs. AP = 0.06 (Logistic model adjusted by baseline data)

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.

30-LB

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

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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 D_2 (25-OH-D) supplementation on this association. We aimed to 1) determine if pain was affected by Vitamin D_2 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 D_2 supplementation (50,000 IUs) 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 54.6 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 ($F[2, 134]=34.5$, $p<.001$) 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 sensory 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 D_2 supplementation for MDD in T2DM. Vitamin D_2 supplementation for the treatment of pain and MDD in T2DM is promising.

Supported by: NIDDK/NIH

31-LB

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

32-LB

After Baby Fitness Challenge (ABfC): Impacting Postpartum Weight in Poor Rural Postpartum Women

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The purpose of the study was to improve postpartum weight loss in poor rural women. The intervention was integrated into a Healthy Start nurse managed program improving healthcare for women and infants in East Central Georgia. The intervention consisted of bi-weekly nutrition classes/supervised exercise @ the local YMCA. The intervention facilitator provided social/problem solving support via phone, email, Facebook, attending nutrition/PA sessions. Assessed @ baseline (B), 6 and 12m were height/weight, wellbeing (I only, SF-12), and nurse/interventionist contact. 104 women (18-43yrs; X BMI = 35.3 SD= 7.8; 89% AA) enrolled from non-contiguous counties (62 **I**ntervention, 42 **C**ontrol). 48 women retained (I=19, C=29) @ 12m. T-tests within and between group characteristics @ B, 6 &12m calculated. Predictors significantly correlated with BMI tested in linear regression models. Although absolute mean BMI was greater for I @ B, BMI did not differ significantly between groups @ B, 12m, or BMI change 0-12. BMI significantly increased in C ($p < .002$) from B to 12m, but was stable for I mothers. For C, more case manager contact was associated with greater 12m BMI ($p <.01$). For I, higher 12m BMI was associated with lower B physical wellbeing ($p< .01$) and lower 6m ($p< .04$) mental wellbeing. Predicting BMI change 0-12m for I, interventionist support ($p <.02$), number of Y sessions attended ($p < .08$) and positive change in mental well-being 0-6m ($p <.01$) explained 72% of variance in BMI change 0-12. Women enrolled in a nurse management Healthy Start program were shown to stabilize post-partum weight from 6-18m when provided with a community based intervention specifically focused on facilitating better nutrition and increased PA. Attendance at supervised exercise sessions and interventionist support both independently contributed to weight stabilization. Retaining women in this high risk population remains a challenge. However this intervention model shows promise for impacting obesity in poor rural women.

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33-LB

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

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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 social 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.

34-LB

We Are in this Together: Partner Perspectives of Living With a Loved One With Diabetes

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 fear 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 deemed necessary spouses take on a more active role in diabetes care, 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

CLINICAL THERAPEUTICS/NEW TECHNOLOGY—GLUCOSE MONITORING AND SENSING

35-LB

Improved Glycemia Early After Bariatric Surgery Is Largely Explained by Caloric Restriction

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We assessed the acute impact of laparoscopic Roux-en-Y gastric bypass (GBP) or sleeve gastrectomy (SG) or matched diet alone, on glucose excursion in obese patients with type 2 diabetes and examined if this was mediated by changes in insulin resistance, early insulin response (EIR) or gut hormone levels. Six-day subcutaneous continuous glucose monitoring (CGM) recordings were obtained from patients beginning 3 days before GBP (n=11), SG (n=10) or matched diet (n=10) and analysed for glycemic changes. Glucagon like peptide -1 (GLP-1), insulin, and glucose was measured during 75g oral glucose tolerance testing at the start and end of each CGM. Post-operative hyperglycemia occurred after both GBP and SG in the first 6 hours, with a more rapid decline in glycemia after GBP ($p<0.001$). Beyond 24 hours post-operatively, glycemic variability as assessed by continuous overlapping net glycemia action (CONGA), reduced after GBP (median [interquartile range] 1.6 [1.2-2.4] to 1.0 [0.7-1.3] $p<0.05$ and after SG 1.4 [0.9-1.8] to 0.7 [0.7-1.0] $p<0.05$), similar to those on matched diet (2.2 [1.7-2.5] to 1.3 [0.8-2.8] $p<0.05$. Higher logGLP-1 increment post oral glucose, occurred after GBP (mean ± SE, 0.80 ± 0.12 vs. 0.37 ± 0.09, $p<0.05$), but not after SG (0.43 ± 0.14 vs. 0.38 ± 0.11), or after matched diet (0.18 ± 0.09 vs. 0.15 ± 0.07). Log EIR or insulin resistance estimated by HOMA-IR did not change 3 days after any of the interventions although oral glucose lowering therapies were stopped. In the subgroup with >30% of baseline glucose >7mmol/L, mean glycemia and glycemic variability improved 24 hours after both surgeries to a greater extent than matched diet. Significantly higher GLP-1 increment and lower HOMA-IR was seen after GBP in this subgroup. The acute impact of GBP and SG on improving glycemia after the initial 24 hour period of post-operative hyperglycemia, is similar to equivalent caloric restriction, despite higher GLP-1 increment after GBP.

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36-LB

^{13}C-Glucose Breath Test Is a Valid and Highly Reliable Method to Assess Insulin Resistance in Non Diabetic Mexican Adults

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Insulin Resistance (IR) constitutes a central abnormality in the pathogenesis of type 2 diabetes mellitus; many attempts are being made to diagnose IR in at risk individuals. Recently, the ^{13}C-glucose breath test (^{13}C-GBT), a noninvasive technique, has been used to assess glucose metabolism in obese and diabetic subjects. The aim of this study was to determine the repeatability and validity of the ^{13}C-GBT as a surrogate measurement of IR in non diabetic individuals.

To assess repeatability, 86 healthy volunteers were recruited; on the likelihood that they would represent a spectrum of insulin sensitivities study subjects, had a wide range of body mass indexes. All participants underwent standard oral glucose tolerance tests plus 1.5 mg/Kg of U-^{13}C-glucose (^{13}C-GBT); breath and blood samples were collected at baseline and at 10, 20, 30, 60, 90, 120, 150, and 180 min following the glucose load. The same procedure was repeated within one week. To determine validity, 25 healthy volunteers were recruited; they underwent the ^{13}C-GBT, as previously described; and a week later a hyperinsulinemic clamp (40 mU/m^2*min) was performed.

Reliability was determined using Bland & Altman coefficients of variation (CV); ^{13}C-GBT proved to be a highly reliable method (CV 7.16%), and it has a better reliability than other IR surrogates (HOMA 26.3%, fasting insulin 24.3%, Matsuda DeFronzo Index 24.9%, 2h OGTT insulin 43.9%). To validate the test, we computed correlation coefficients between the ^{13}C-GBT and M and M/I values derived from the clamp (0.65 and 0.66, *P<0.0001*). Using M/I value as IR gold standard, a cutoff point of 4.435% for the ^{13}C-GBT (expressed as % of oxidized dose at 180min) showed a sensitivity of 79% and a specificity of 83% (ROC curve AUC; 0.912, CI_{95} 0.797-1, *P=0.003*).

Our results demonstrate that the ^{13}C-GBT is reliable and valid, and may represent a non invasive alternative to determine IR in non diabetic individuals within a wide spectrum of insulin sensitivities.

Supported by: CONACYT

37-LB

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

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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

38-LB

Continuous Glucose Monitoring via Telemetry in Rat

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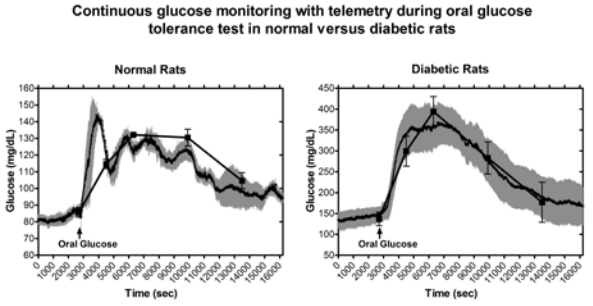
The current standard for routine glucose measurements in preclinical research is often glucometers and test strips. These pose significant limitations in terms of accuracy, animal stress, and frequency of sampling. Until now, continuous monitoring options for preclinical research have been very limited. 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 (ZDSD) 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.

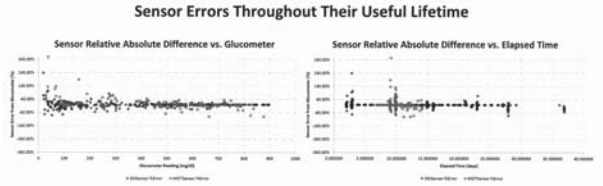


39-LB

Comparison of Continuous Glucose Monitoring Systems in Type 1 Rat Model

PAUL HAEFNER, KIMBERLY HOLLIDAY-WHITE, MARK CLEMENTS, *St. Paul, MN, Kansas City, MO*

Presently, few continuous monitoring glucose options are available for preclinical research. This study compares the performance of an existing interstitial system (Medtronic MiniMed) with a fully implantable direct blood monitoring prototype (Data Sciences International). Prototype sensors were surgically implanted in the abdominal aorta of 10 weight matched Sprague Dawley rats at 8 weeks of age. One week later, interstitial sensors were placed in the dorsal skin near the scapulae and the rats dosed with streptozotocin (65 mg/kg). Daily glucose measurements and approximately weekly GTT's (dextrose, 1-2.6 g/kg, ip; insulin, 0.5-1.0 unit/kg, im) were measured with a StatStrip glucometer (Nova Biomedical). Sensors were calibrated via an interpolated one point calibration method utilizing daily readings. A relative absolute difference was calculated for each sensor, on both GTT's and daily readings. Diabetes was induced in 8 of 10 animals. Two of 10 interstitial sensors and 2 of 10 blood monitoring sensors (improperly implanted) failed to perform correctly. Both sensor types exhibited larger errors at lower glucose. Interstitial sensors functioned an average of 5.6 days with an average delay of 8.8 minutes. Blood monitoring sensors functioned an average of 24.9 days with an average delay of -0.8 minutes. These prototype devices provide a promising alternative for chronic preclinical continuous glucose monitoring in a free roaming type 1 rat model.



40-LB

A Study of Islet Auto-Antibodies and B-Cell Functional of Ketosis-Prone 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 (β), a total of 162 patients with KPD were divided into four groups, including A+β+ (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, 2h PG, HbA1c, FCP and 2h CP were compared between each groups. Group 1, 2, 3 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 group1, 2 and 3, patients in group 4 owned older age at onset, higher BMI, more obese, dyslipidemia and hypertension, higher FCP and 2h CP. The phenotype of patients in group 1 and 3 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.

41-LB

Glucose Fluctuations in Patients During Acute Coronary Syndrome Attack Might Be Associated With Cardiovascular Adverse Events and the Change of CRP Level

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This study aimed to describe the glucose fluctuations pattern of patients during acute coronary syndrome (ACS) attack using continuous glucose monitor system (CGMS), and further observe the association between blood glucose fluctuations and cardiovascular adverse events and prognosis related biological markers. Patients suffered ACS confirmed with chest pain and coronary angiography in cardiovascular intensive care unit were enrolled from October 2011 to April 2012. A 72h retrospective CGMS was performed within 24 hours after admission. The main outcome is cardiovascular adverse events during admission, the secondary outcome is prognosis related biological markers such as C Reactive protein (CRP). A total of 38 patients (8 females, 30 males) were enrolled with age 64 ± 12 years old. Time of blood glucose level higher than 10.0 mM or lower than 3.9 mM within 24 hours was 11.1h (47.05%) and 0.267h (1.23%) respectively. The mean amplitude of glycemic excursions (MAGE) was 6.49 ±2.48 mM. Patients were grouped by tertiles according to MAGE distribution.There was no significant difference for clinical parameters between three groups at baseline. But a significant change of CRP (the difference between its level on 72h after admission and at admission) during the stay in cardiovascular intensive care unit was observed. With the increase in MAGE, the change in CRP level has an significant increase trend in three group. The incidence rate of adverse cardiovascular events were 0/13, 1/13, 3/12 respectively that showed an non-significant increasing trend. Patients suffered ACS appear to have an unsatisfactory glycemic control and blood glucose fluctuations. The blood glucose fluctuation might be related to the change of inflammatory markers CRP and adverse cardiovascular events.

42-LB

Glucose Variability and Physical Activity Among People With Type 1 Diabetes

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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/5M) 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.

(mean±SD). PA measures for 48 hours were calculated using an accelerometer. CGMS data for 48 hours were used to calculate GV measures (e.g., mean and SD, %CV, mean of daily differences (MODD), continuous overall net glycemic action (CONGAn), duration and area under curve (AUC) of hypo-, hyper-, and euglycemia). Higher BMI was positively associated with higher CONGA1 and CONGA2 (rs = .557, p = .031; rs = .546, p = .035, respectively). People with higher %CV (intra-day glucose variability) and higher MODD (inter-day glucose variability) had longer duration and larger AUC of hypoglycemia (rs = .623-.887, p values < .05). Higher %CV was associated with shorter duration of hyperglycemia (rs = -. 650, p = .009). Duration of euglycemia negatively related to the mean glucose value (rs = -.564, p = .028) and CONGA over a 3-hour interval (rs = -.529, p = .043). Duration and AUC of hyperglycemia were negatively associated with step counts (rs = -.546, p = .035) and moderate and vigorous PA (rs = -.518, p = .040). Longer duration and larger AUC of euglycemia were consistently associated with more step counts (rs =.707, p =.003; rs = 643, p =.010, respectively) and longer light, moderate, and vigorous PA (rs= .671, p = .006; rs = .671, p = .006, respectively). Less sedentary time tended to lengthen duration of euglycemia (rs = -.604, p = .017) which competed with duration of hyperglycemia. People with higher GV may have increased risk of being overweight/obese and experience longer duration of hypoglycemia. PA may be directly effective in reducing the duration and AUC of hyperglycemia, decreasing the risk of developing diabetes complications.

Supported by: NIH/NIDDK (R01DK085611-01), (P60DK020595-32S3)

CLINICAL THERAPEUTICS/NEW TECHNOLOGY—INSULINS

43-LB

New Insulin Glargine Formulation: Glucose Control and Hypoglycemia in People With Type 2 Diabetes Using Basal and Mealtime Insulin (EDITION I)

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Insulin glargine 100 U/mL (Gla-100) is widely used in people with type 2 diabetes (T2DM). A new insulin glargine formulation, 300 U/mL (Gla-300), has a longer and a flatter pharmacokinetic profile than Gla-100. The phase 3 EDITION I study compared the efficacy and safety of Gla-300 vs. Gla-100 in people with T2DM using basal plus mealtime insulin. In a multicenter, open-label study 807 people [mean age 60 (SD 8.6) yr, duration of T2DM 15.8 (7.5) yr, BMI 36.6 (6.4) kg/m^2, A1C 8.15 (0.78) %, total insulin dose 1.2 (0.47) U/kg, basal insulin dose 0.67 (0.25) U/kg] were randomized (1:1) to once daily evening, Gla-300 (n=404) or Gla-100 (n=403) while continuing mealtime insulin. The dose was titrated to achieve fasting plasma glucose 80-100 mg/dL. Primary endpoint was change in A1C from baseline to month 6, and first secondary endpoint was percent of people with ≥1 severe or confirmed (≤70 mg/dL) nocturnal hypoglycemic event from month 3 to month 6. Gla-300 was non-inferior to Gla-100 for change in A1C [least squares mean change -0.83% (0.06) in both groups; difference -0.00% (95% CI -0.11 to 0.11)]. Fewer people using Gla-300 had severe or confirmed nocturnal hypoglycemia during month 3-month 6 (first secondary endpoint: 36.1% vs. 45.5%; RR 0.79 [CI 0.67 to 0.94]; p=0.0070). Occurrence of any hypoglycaemic event (% of people with at least one event) during study period was numerically lower in the Gla-300 group than in the Gla-100 group. No between-treatment differences in adverse events were seen. In conclusion, in people with T2DM, Gla-300 was as effective as Gla-100 in controlling glycemia and was associated with a 21% reduction in severe or confirmed nocturnal hypoglycemia from month 3 to month 6. Gla-300 was well tolerated.

Supported by: Sanofi

44-LB

Lispro Formulations BIOD-238 and BIOD-250 Associated With Faster Absorption and Declines From Peak Concentrations Compared to Humalog®

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Formulations of human insulin containing citrate and sodium-EDTA (Na_2EDTA) have been shown to be more rapidly absorbed than the commercially available formulation of insulin lispro (Humalog®, HU). BIOD-238 is a Na_2EDTA/citrate formulation of lispro. BIOD-250 is a similar lispro formulation with the addition of magnesium sulfate, intended to mitigate Na_2EDTA-mediated local injection site discomfort. In this single-center, randomized, double-blind three-period crossover study, the pharmacokinetics and local injection site toleration [measured with a 100 mm visual analog scale (VAS)] of BIOD-238 and BIOD-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 BIOD-238, BIOD-250, and HU, respectively (p<0.001 for BIOD-238 and p=0.001 for BIOD-250 vs. HU). Time to maximal insulin concentrations and areas under the curves for the first 30 and 45 minutes for BIOD-238 and BIOD-250 all indicated significantly increased early lispro absorption compared to HU. The times to half maximal concentration after the peak were 123.8 ± 10.5, 132.3 ± 18.7 and 166.5 ± 10.6 min for BIOD-238, BIOD-250 and HU, respectively (p=0.009 for BIOD-238 and p=0.016 for BIOD-250 vs. HU). The mean VAS score was numerically lower, but not significantly different for BIOD-250 compared to HU (2.7 ± 1.6 mm for BIOD-250 and 8.2 ± 4.5 mm for HU). The mean VAS score for BIOD-238 was significantly higher than that associated with HU (24.2 ± 7.0 mm, p=0.029 vs. HU). Safety results were comparable between treatments. In conclusion, this study demonstrates that Na_2EDTA/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 BIOD-250 significantly mitigates local injection site discomfort without altering the ultra-rapid pharmacokinetic profile.

45-LB

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

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To investigate the effect on of Liraglutide combined with short-term continuous insulin infusion (CSII) on β cell function, newly diagnosed and drug naïve 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 normoglycaemia 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.

The patients of Group 2 (Meadian=1 day) reached target glycaemic control in less time than of Group 1 (Meadian=2 days)(p=0.044). The daily insulin doses of patients were not significantly different between 2 groups during the 2 weeks of maintaining normoglyceamia (day2: 0.68±0.18 units/kg vs. 0.69±0.15 units/kg, p=0.642; day7: 0.55±0.21 units/kg vs. 0.57±0.19 units/kg, p=0.842; day13: 0.40±0.16 units/kg vs. 0.47±0.19 units/kg, p=0.299). 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 Group2 decreased 1.475%±0.17% (p=0.21). The AIR improved significantly compared to baseline, however, the improvement of Group 2 [ΔAIR=295.56±57.82 (uU•min/ml)] was much greater than Group 1 [ΔAIR=79.96±18.63 (uU•min/ml)](p=0.0013). The combination of Liraglutide and CSII may have better effect in improving β cell function than CSII only.

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

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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. The 2 studies (A and B) were conducted under 1 protocol as a 15-country, multicenter, randomized, open-label, parallel trial in 1106 subjects. Subjects were randomized to 1 of 2 bolus insulin treatment algorithms for 24 weeks following a 6-week GLA optimization period (for those not already optimized on GLA). Both algorithms added lispro by 1, 2, or 3 injections, as needed, and subjects self-adjusted their prandial insulin with study diaries, either every 3 days (Q3D), based on a current ADA/EASD guidelines, or by 1 unit of insulin every day (Q1D). At Week 24 in each study, the 2 algorithms showed an equivalent clinically significant drop in HbA1c and low rates of hypoglycemia. AUTONOMY is the first to demonstrate that prandial insulin therapy in T2DM on GLA can be effectively and safely initiated in the primary care setting and that self-titration of lispro may be done by either of 2 simple algorithms without the complexity of carbohydrate counting and correction factor, regardless of patient age.

	Study A (N=528)			Study B (N=578)		
Treatment	**Q1D (n=267)**	**Q3D (n=261)**	**Q3D vs. Q1D p-value**	**Q1D (n=288)**	**Q3D (n=290)**	**Q3D vs. Q1D p-value**
Baseline HbA1c % (Mean ± SD)	8.33 ± 0.92	8.39 ± 0.99	0.453	8.28 ± 0.99	8.40 ± 0.98	0.162
HbA1c % Change from baseline (LSM ± SE)[a]	-1.00 ± 0.08	-0.96 ± 0.08	0.706; 95% CI (-0.15,0.22)	-0.98 ± 0.07	-0.92 ± 0.07	0.515; 95% CI (-0.12,0.24)
Proportion achieving target HbA1c ≤7.0%						
Overall—n (%)	133 (49.81)	111 (42.53)	0.128	142 (49.31)	123 (42.41)	0.162
Geriatric (≥65yo)—n (%)	38 (58.46)	40 (57.97)	0.701	38 (67.86)	30 (46.15)	0.015
Weight (kg) change from baseline (LSM ± SE)	2.15 ± 0.27	2.96± 0.28	0.014	2.47 ± 0.24	1.97 ± 0.24	0.108
FBG (mg/dL) change from baseline (LSM ± SE)	1.36 ± 4.02	6.58 ± 4.14	0.238	-6.47 ± 3.79	8.03 ± 3.73	0.002
Hypoglycemia						
Overall						
Incidence—n (%)	231 (86.2)	218 (83.2)	0.435	238 (82.4)	231 (79.1)	0.351
Rate per 30 days (LSM ± SE)	3.15 ± 0.23	3.33 ± 0.25	0.586	3.18 ± 0.26	3.33 ± 0.27	0.689
Nocturnal						
Incidence—n (%)	169 (63.1)	167 (63.7)	0.870	156 (54.0)	149 (51.0)	0.470
Rate per 30 days (LSM ± SE)	0.71 ± 0.07	0.79 ± 0.08	0.404	0.59 ± 0.07	0.68 ± 0.07	0.358
Severe						
Incidence—n (%)	5 (1.9)	2 (0.8)	0.258	7 (2.4)	8 (2.7)	0.856
Rate per 30 days (LSM ± SE)	0.00 ± 0.00	0.00 ± 0.00	0.756	0.00 ± 0.00	0.00 ± 0.00	0.934

[a] Primary object analyzed through the classification method using mixed model, repeated measure (MMRM) approach (Qu 2011).

47-LB

Glycemic Control and Treatment Satisfaction in Type 2 Diabetes: Basal Plus Compared With Biphasic Insulin in the LANSCAPE Trial

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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 (Apidra) at main meal was non-inferior to twice daily "Biphasic" insulin aspart/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) yrs, diabetes duration 12.9 (6.4) yrs and A1C 8.62 (0.94) %. During an 8-12 week run-in, oral agents except metformin were stopped and insulin glargine optimized. After run in, 335 participants with fasting glucose at target (<126mg/dl) but suboptimal A1C (>7%) were randomized to a "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 1.00% in the Basal Plus, 1.22% in the Biphasic arms; mean difference 0.21% (SE 0.09, upper 97.5% CL 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 hypoglycemia 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 treatment satisfaction measures.

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

CLINICAL THERAPEUTICS/NEW TECHNOLOGY—INSULIN DELIVERY SYSTEMS

48-LB

Reduction in Hypoglycemia and No Increase in A1C With Threshold-Based Sensor-Augmented Pump (SAP) Insulin Suspension: ASPIRE In-Home

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ASPIRE In-Home was a 3-month RCT comparing SAP with Threshold Suspend (SAP+TS), which stops insulin at a specified sensor glucose (SG) threshold for up to 2 h, to SAP alone (SAP).

The primary safety outcome was the between-group difference for ΔA1C. The primary efficacy outcome was the between-group difference for AUC of nocturnal hypoglycemia (NH) events, measured by Enlite CGM sensors. Hypoglycemia events were defined as >20 consecutive min of SG values ≤65 mg/dL without subject intervention; NH events were defined as those starting between 10:00PM and 8:00AM. Subjects with T1D, ages 16-70 who had ≥2 NH episodes in a 2-wk run-in period were randomized to SAP+TS (121) or to SAP (126).

Baseline A1C values were similar and did not change significantly in either group, meeting the safety outcome. There was a 38% reduction in mean AUC of NH events and a 32% reduction in rate of NH events in SAP+TS vs. SAP, meeting the efficacy outcome. Overall, day and night combined, there was a 31% reduction in mean AUC of hypoglycemia events and a 30% reduction in rate of hypoglycemia events in SAP+TS vs. SAP (Table). The percentage of SG values <70 mg/dL was significantly less in SAP+TS vs. SAP (p<0.001).

No reported DKA occurred during the study. Four severe hypoglycemia events occurred (4 subjects), all in the SAP group. ASPIRE In-Home demonstrated that using SAP therapy with the TS feature safely reduced nocturnal and overall hypoglycemia, without change in A1C.

A1C and Hypoglycemia Outcomes

	A1C (%)		Hypoglycemia Events and Mean Event AUC			
			Nocturnal		Overall	
	Baseline	End of Study	Events per patient-week	AUC per event (mg/dL × min)	Events per patient-week	AUC per event (mg/dL × min)
SAP + TS*	7.26±0.71	7.24±0.67	1.5±1.0	980±1200	3.3±2.0	798±965
SAP	7.21±0.77	7.14±0.77	2.2±1.3	1568±1995	4.7±2.7	1164±1590
CI or P value	95% CI: [-0.05, 0.15]	p < 0.001	p < 0.001	p < 0.001	p < 0.001	

*TS setting of 70 mg/dL

49-LB

Glucose Lowering Effects of Pre-Meal Pramlintide During Closed-Loop Insulin Delivery Is Associated With Lower Plasma Glucagon Levels and Reduced Insulin Requirements

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Exaggerated prandial glycemic excursions in type 1 diabetes (T1D) persist during closed-loop control (CLC), due in great part to delays in the absorption of subcutaneously infused insulin analogs. We previously demonstrated that pre-meal 30 mcg pramlintide injections in T1D subjects mitigated but did not normalize glycemic excursions during CLC. In this study, we sought to determine whether the improved meal excursions during CLC could be achieved with therapeutic 60 mcg pre-meal doses of pramlintide and the mechanism underlying such improvements in post-meal hyperglycemia. Ten subjects (4 males, age 16.9-23.2 y, A1c 7.2±0.6%) underwent two 24-hour CLC assessments: CL alone (Control) and then CLC plus pramlintide (Pram), separated by 3 weeks, during which time pramlintide was introduced and increased gradually to the full 60 mcg per meal dose. Identical meals were provided for both study days, and there were no meal announcement strategies. Pramlintide reduced overall mean prandial glycemic excursions during CLC from 93±10 to 59±20 mg/dL (p<0.001), a value that was significantly (p<0.020) lower than previously observed with the 30 mcg pre-meal dose. Mealtime insulin delivery also fell with pramlintide from 11.4±3.2 units to 9.4±2.9 units/meal (p<0.005). As shown in the Figure, the peak peak-post-meal glucose during CLC with and without pramlintide correlated with peak post-meal plasma glucagon concentrations. These data indicate that the reduction in prandial glycemic excursions during CLC by pramlintide is related to suppression of meal-stimulated glucagon responses which, in turn, contributes to substantially lower meal related insulin requirements.

Supported by: NIDDK (R01DK085618); JDRF (22-2009-799); NIH (UL1RR024139)

50-LB

Successful Utilization of a Computer-Guided Glucose Management System for a Surgical Care Improvement Project at a Tertiary Care Hospital

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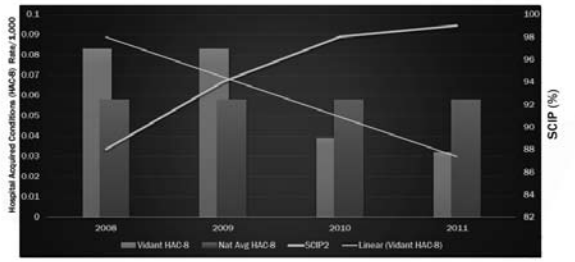
Background: Endotool™ is a computer-guided glucose management system for administration of intravenous insulin in hospital intensive care units. The Surgical Care Improvement Project (SCIP) is a national quality based effort to improve surgical outcomes in U.S. hospitals.

Methods: In 2008, the Endotool system was initiated in the surgical and cardiothoracic intensive care units of Vidant Medical Center, an 850

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

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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. 889 ± 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 well as potential reductions in glucose, insulin and hemoglobin A1c levels. Initial data suggest that therapeutic targeting of apoC-III may represent an attractive strategy for reducing serum TG and improving insulin sensitivity in T2D individuals.

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)

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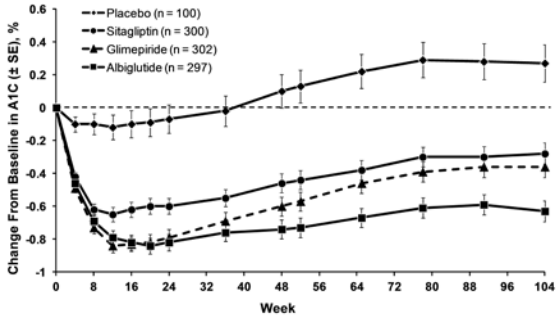
This 3-year, randomized, double-blind, placebo (Pbo)- and active-controlled, Phase III study examined Albi 30 mg added to Met vs. Pbo + Met, Sita + Met, or SU + Met in pts with T2DM (A1C 7-10%) on Met. Pts meeting predefined hyperglycemia criteria qualified for blinded dose titration (SU 2 to 4 mg, albiglutide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

For A1C (baseline 8.1±.8%), Albi treatment (txt) difference was superior to Pbo (–.91%; $P < .0001$), Sita (–.35%; $P = .0001$) and SU (–.27%; $P = .003$). Albi had superior txt difference ($P < .05$) 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; $P < .0001$).

Gastrointestinal AEs through Wk 104 with Pbo/Sita/SU/Albi were: nausea, 11%/7%/6%/10%; diarrhea, 11%/9%/9%/13%; vomiting, 1%/4%/4%/6%. 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 T2DM as add on to met was durable and superior to Sita and SU in A1C and FPG reduction, superior in weight loss to SU, and well tolerated at Wk 104.

Figure. Model-Adjusted[1] Change From Baseline in A1C Through Week 104 (Intent-to-Treat; Last Observation Carried Forward[2])



[1] ANCOVA model adjusted for baseline A1C, region, history of prior MI, and age category. A prespecified statistical testing procedure was performed for Albi vs. PBO followed by Albi vs active control for noninferiority and subsequent superiority
[2] Last observation prior to study discontinuation or hyperglycemia rescue

Supported by: GlaxoSmithKline

53-LB

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

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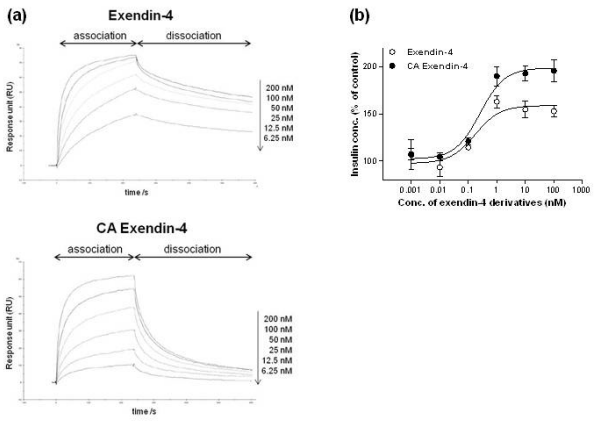
Exendin-4 is a glucagon-like peptide-1 (GLP-1) receptor agonist used for the treatment of type 2 diabetes. We have developed a novel Exendin-4 analog, CA Exendin-4, which showed potent insulinotropic and glucose lowering activity by N-terminal modification. Surface Plasmon resonance (SPR) analysis revealed CA Exendin-4 dissociated 5.5-fold faster than native Exendin-4 from human GLP-1 receptor (GLP-1R). CA Exendin-4 showed greater *in vitro* cyclic AMP accumulation and insulin release in a rat insulinoma cell line and was more effective in glycemic control in *db/db* mice. We supposed that the fast dissociation of CA Exendin-4 might elicit less internalization of GLP-1R so that it enhanced GLP-1 signaling. In the receptor internalization assay, significantly less internalization of GLP-1R was confirmed in the CA Exendin-4 treated cells. We have conjugated human Fc fragment to CA Exendin-4 and Exendin-4, named langlenatide and HM11260A respectively, to give prolonged half-life. Their binding properties to GLP-1R were conserved similarly with CA Exendin-4 and Exendin-4 even after conjugation. The glucose lowering efficacy of langlenatide was compared with that of HM11260A in *db/db* mice, and the result showed much more potent efficacies were achieved in langlenatide treated group. These results suggest that the rapid dissociation kinetics on GLP-1 receptor triggers superagonistic activation and resulted in potent efficacy outcome.



Correlation of fast dissociation kinetics of CA Exendin-4 and insulinotropic activity. (A) The receptor affinity of CA Exendin-4 was measured by a SPR assay using immobilized extracellular domain of human GLP-1 receptor which is fused to the GST (glutathione S transferase). The hGLP-1R/GST was expressed in the transformed CHO cell and purified by GST affinity chromatography. The sensorgram of CA Exendin-4 showed a rapid dissociation rate compared with Exedin-4. (B) Insulinotropic activity was measured by murine RINm5F cells. The magnitude of maximal insulinotropic activity was 2-fold higher on CA Exendin-4 ($P<0.05$).

54-LB

HARMONY 4: 52-Wk Efficacy of Albiglutide (Albi) vs. Insulin Glargine (Glar) in Patients (pts) With T2DM

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This 3-year, randomized, open-label, parallel-group, multicenter, Phase III study assessed efficacy + safety of Albi, a once-weekly (QW) GLP-1 receptor agonist, at 30 mg QW vs. Glar in T2DM pts uncontrolled (A1C 7-10%) on a regimen of metformin (met) ± sulfonylurea (SU). Pts were treated to a target A1C ≤7.0% and FPG ≤100 mg/dL. If needed, pts could uptitrate Albi to 50 mg QW and Glar per prespecified criteria. The primary objective was to evaluate the A1C change from baseline at wk 52 in Albi vs. Glar. Pts were allowed to continue if hyperglycemic rescue was required.

Baseline demographics were similar between groups; mean age 56 y, BMI 33 kg/m^2, A1C 8.3%, duration of diabetes 8.8 y, 82% receiving met + SU. A1C decreased in both groups. The wk 52 treatment difference was 0.11% (95% CI –0.04%, 0.27%). The upper bound of the CI was below the noninferior margin of 0.3%, indicating noninferiority of Albi to Glar. Change in FPG significantly favored Glar, whereas change in weight significantly favored Albi. AEs through wk 52 for Albi/Glar were nausea 9.9%/3.7%; diarrhea 7.5%/4.1%; vomiting 3.8%/3.7%. Documented (≤70 mg/dL) symptomatic hypoglycemia and severe hypoglycemic events (prior to the addition of hyperglycemia rescue meds) were less with Albi vs. Glar (18%/0.4% vs. 27%/0.4%). Injection site reactions occurred in 13.9% of Albi and 8.7% of Glar pts.Albi treatment resulted in A1C improvement at wk 52 that was noninferior to Glar with modest weight loss. Both drugs were well tolerated.

Table. Week 52 Model-Adjusted Change From Baseline Least Square Mean For Key Efficacy Parameters

	Albiglutide (n = 496)	Glargine (n = 239)
A1C, LS Mean[a] (SE), %	−0.67 (0.04)	−0.79 (0.06)
P value (noninferiority)	.0086	
P value (superiority)	.1463	
FPG, LS mean (SE), mg/dL	−15.7 (2.30)	−37.1 (3.31)
P value	< .0001	
Weight, LS mean (SE), kg	−1.05 (0.17)	+1.56 (0.25)
P value	< .0001	

[a]Last observation prior to study discontinuation or hyperglycemia rescue carried forward. Treatment comparison based on ANCOVA model was adjusted for baseline A1C, region, history of prior MI, age category, and background antidiabetic therapy.

Supported by: GlaxoSmithKline

55-LB

HARMONY 2 Wk 52 Results: Albiglutide Monotherapy in Drug Naïve Patients With Type 2 Diabetes Mellitus

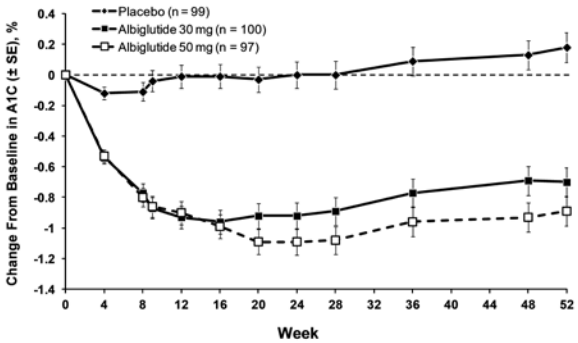
MICHAEL NAUCK, MURRAY STEWART, CHRISTOPHER PERKINS, ANGELA JONES-LEONE, FRED YANG, CAROLINE PERRY, RICKEY REINHARDT, MARC RENDELL, *Harz, Germany, Upper Merion, PA, Winston-Salem, NC, Omaha, NE*

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[1] 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 $P<.0001$. Fasting plasma glucose decreased rapidly and the improvement mirrored A1C out to 52 wks:–34 mg/dL (95% CI –46, –22) for Albi30 and –43 mg/dL (95% CI –55, –31) for Albi50, both $P<.0001$. 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.

Figure. Model-Adjusted[1] Change From Baseline in A1C Through Week 52 (Intent-to-Treat; Last Observation Carried Forward[2])



[1] Treatment comparison based on ANCOVA model adjusted for baseline A1C, region, history of prior MI, and age category

[2] Last observation prior to study discontinuation or hyperglycemia rescue

Supported by: GlaxoSmithKline

56-LB

Human Tissue Kallikrein (DM-199 Compound) Induces Diabetes-Protective Immunomodulation in NOD Mouse T1D Model

ALEXEI Y. SAVINOV, LILIA MANEVA-RADICHEVA, CHRISTINA AMATYA, CAMILLE PARKER, JACOB ELLEFSON, ILIAN RADICHEV, JACAN SIMON, PAUL BURN, MATT CHARLES, MARK WILLIAMS, *Sioux Falls, SD, Minneapolis, MN*

Recombinant human tissue kallikrein-1 (DM-199) is currently under development for the treatment of both Type 1 and Type 2 diabetes mellitus. We previously reported significant dose- and frequency-of-treatment- dependent effects including attenuation of spontaneous T1D development in female NOD mice and herein the mechanistic studies are presented.

The T1D-protective DM-199 action depended on the reduced rates of islet infiltrates formation, which lead to significantly improved preservation of β cell mass and function. Specifically after 18 weeks of treatment there was an increased retention of naive CD8+ T cells in the spleen, and a commensurate decrease in numbers of activated CD8+ cells in pancreatic lymph nodes (PLN) (up to 27% reduction; p=0.002) and islet infiltrates (up to 2-fold reduction in CD4/C8 T cell ratios; p=0.008). Treatment with DM-199 yielded a significant increase of percentage of T regulatory cells (Tregs: CD4+, CD25+, Foxp3+) in both the PLN (2.5%, p=0.014) and pancreata (~4%, p=0.0032) suggesting a change in CTL trafficking and an increase in the Treg populations to reduce insulitis.

The Tregs appeared to be induced by an increase in active TGFβ (up to 63%, p<0.05) with no effect on total TGFβ. An increase in the expression of Indoleamine 2,3-dioxygenase in dendritic cells (30%, p<0.001) was also

For author disclosure information, see page LB66.

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

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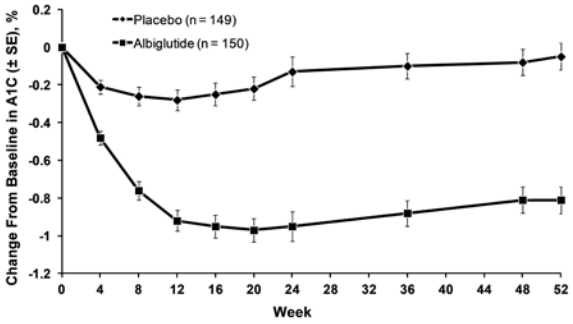
This 3-year, randomized, double-blind, placebo (Pbo)-controlled study evaluated efficacy and safety of once-weekly GLP-1 receptor agonist albiglutide 30mg (Albi) + Pio ± Met vs. Pbo + Pio ± Met in patients inadequately controlled (A1C 7-10%) on Pio ± Met. Patients were allowed to continue if requiring 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/m^2, duration of diabetes 8 years. Week 52 change from baseline A1C was –0.05% for Pbo and –0.81% for Albi, treatment difference (TD): –0.75% (95% CI –0.95%, –0.56%),

$P < .0001$. Fasting plasma glucose declined rapidly and the improvement mirrored A1C out to 52 weeks (+6.4 mg/dL for Pbo, –23.1 mg/dL for Albi, TD: –29.5 mg/dL, $P < .0001$. There was a nonsignificant difference in weight with Albi vs. Pbo (+0.45 kg for Pbo, +0.28 kg for Albi, TD: –0.18 kg). Adverse events (% participants including R) of nausea and vomiting were comparable between Albi/Pbo (10.7%/11.3% and 4.0%/4.0%, respectively) while diarrhea was higher with Albi (11.3% vs. 8.6%) and injection site reactions were higher for Albi (11.3%) vs. Pbo (7.9%). Incidence of pre-R documented symptomatic (≤70 mg/dL) and severe hypoglycemia was 1%/0% Pbo vs. 3%/1% Albi.

Albi combination therapy resulted in a robust and sustained glycemic improvement with good tolerability in patients with type 2 diabetes mellitus.

Figure. Model-Adjusted[1] Change From Baseline in A1C Through Week 52 (Intent-to-Treat; Last Observation Carried Forward[2]) in Pts Not Controlled on Pio ± Met



[1] ANCOVA model adjusted for baseline A1C, region, history of prior MI, age category, and background antidiabetic therapy
[2] Last observation prior to study discontinuation or hyperglycemia rescue

Supported by: GlaxoSmithKline

58-LB

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

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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 glimepiride 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 hyperglycemic rescue was required. Stepwise statistical analysis was first vs. placebo, then noninferiority (margin 0.30%) testing vs. pioglitazone.

Baseline mean age was 55.2 years, BMI 32.2 kg/m^2, A1C 8.2%, duration of diabetes 8.9 years. Week 52 A1C difference (albiglutide - placebo) was –0.87 (95% CI –1.07, –0.68) % (P <0.0001), and vs. pioglitazone 0.25 (0.10, 0.40) % (noninferiority P value 0.27, not shown noninferior). Changes in FPG mirrored those of A1C (Table). Albiglutide and pioglitazone differed in weight change direction (Table). Adverse events included more gastrointestinal and injection site reaction reports with albiglutide vs. placebo and vs. pioglitazone, and more hypoglycemic events with pioglitazone and albiglutide (Table).

Albiglutide in triple therapy gives effective glucose lowering and was generally well tolerated and associated with less weight gain than pioglitazone.

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

	Albiglutide (n=269)	Pioglitazone (n=273)	Placebo (n=115)
A1C (%)	−0.55 (0.06)	−0.80 (0.06)[a]	+0.33 (0.08)[b]
FPG (mg/dL)	−12.4 (2.9)	−31.4 (2.9)[b]	+11.5 (4.4)[b]
Weight (kg)	−0.4 (0.2)	+4.4 (0.2)[b]	−0.4 (0.4)[c]
Adverse events (% participants)			
Diarrhea/nausea/vomiting	8.9/9.6/2.6	5.4/4.3/1.8	2.6/3.5/0.9
Injection site reactions	12.9	3.2	3.5
Documented symptomatic/ severe hypoglycemia[d]	13.7/0.4	25.3/1.1	7.0/0.0

[a]Last observation prior to study discontinuation or hyperglycemia rescue carried forward. Treatment comparison based on ANCOVA model was adjusted for baseline A1C, region, history of prior MI, and age category; P (noninferiority) not significant (albiglutide not noninferior to pioglitazone)
[b]P <.0001 vs albiglutide; [c]Not significant; [d]Events prior to addition of hyperglycemia rescue meds.

Supported by: GlaxoSmithKline

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

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A very long $T_{1/2}$(~ 180 hrs) and no burst absorption (T_{max}: ~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 9-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, 53.3 [7.0] vs. 52.9 [8.7] years; HbA1c, 8.4 [1.0] 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.

	Weekly Regimens (Day57)				Monthly Regimens (Day57)		
	Weekly Placebo	W1 Cohort	W2 Cohort	W3 Cohort	Monthly Placebo	M1 Cohort	M2 Cohort
ΔHbA1c (%, LS mean [p-value])	-0.09	-0.92 (p=.021)	-1.02 (p=.008)	-1.24 (p=.002)	-0.22	-1.32 (p=.006)	-1.05 (p=.018)
ΔFPG (mg/dL, LS mean [p-value])	1.63	-36.16 (p=.036)	-26.43 (p=.133)	-65.73 (p<.001)	-1.18	-23.92 (p=.322)	-8.05 (p=.742)
ΔBody weight (kg, LS mean [p-value])	0.27	-1.16 (p=.275)	-1.29 (p=.206)	-2.42 (p=.030)	-0.79	-0.18 (p=.638)	0.29 (p=.376)
Nausea # Patient (%) [#AEs]	3 (33%) [3]	0	1 (11%) [1]	2 (22%) [2]	2 (29%) [5]	5 (56%) [10]	4 (44%) [7]
Vomiting # Patient (%) [#AEs]	1 (11%) [1]	0	1 (11%) [1]	1 (11%) [1]	2 (29%) [4]	2 (22%) [4]	1 (11%) [3]
Diarrhea # Patient (%) [#AEs]	1 (11%) [1]	2 (22%) [2]	0	1 (11%) [1]	1 (14%) [1]	2 (22%) [2]	1 (11%) [1]

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

60-LB

GSK2374697, a Long-Acting GLP-1 Mimetic: First Use of an AlbudAb™ in Humans—Pharmacokinetics, Pharmacodynamics, Safety, and Tolerability in Healthy Volunteers

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GSK2374697, a GLP-1 mimetic, is a recombinant fusion protein consisting of exendin-4 fused to an AlbudAb™, a small (~14 kDa) human antibody light chain variable domain that reversibly binds to albumin. We assessed the pharmacokinetics, pharmacodynamics, and safety/tolerability of GSK2374697 in normal and obese healthy volunteers, in the first evaluation of an AlbudAb in humans. In this double-blind (sponsor unblinded), randomized, placebo-controlled study, 82 subjects (18 placebo, 64 GSK2374697, 19-65 years, BMI 19.2-33.9 kg/m^2 and obese BMI 30.6-38.4 kg/m^2 received escalating doses of GSK2374697 or placebo (subcutaneous injections into the abdomen) in sequential cohorts (C) as follows: Single doses on Day 1 C1: 0.1mg, C2: 0.2mg, C3: 0.5mg, C4: 1.0mg, C5: 2.0mg, C6: 4.0mg, Divided doses on Days 1, 4, and 7: C7: 4.0mg as 1mg + 1mg + 2mg, C8: 6.0mg as 2mg + 2mg + 2mg, C9: same as C8 in subjects with higher BMI.

The median (range) plasma half-life of GSK2374697 was 6.3 d (4-22), median (range) Tmax was 57 h (12-108), and plasma exposure increased with dose. Significant reductions in average plasma glucose (doses ≥2mg) and insulin (doses ≥1mg) were observed following a mixed meal challenge 6/12 days after single/divided doses ($p<0.05$, compared to placebo, ANCOVA). GSK2374697 single doses (≥2mg) significantly reduced gastric emptying (measured using acetaminophen PK).

The most common drug-related adverse events were decreased appetite, nausea, vomiting, constipation, injection site reactions, diarrhea and dizziness. The incidence and severity of nausea and vomiting was reduced considerably when doses were divided compared to single dose administration.

In conclusion, early PK/PD of GSK2374697 supports at least once-weekly dosing with an acceptable safety/tolerability profile.

CLINICAL THERAPEUTICS/NEW TECHNOLOGY—ORAL AGENTS

61-LB

Pitavastatin for the Delay or Prevention of Diabetes Development in Individuals With Impaired Glucose Tolerance

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Although statin therapy is known to reduce cardiovascular risk, trial data and meta-analyses suggest that statins may also increase the risk of development of diabetes. However, in those trials, data were analyzed retrospectively, and the diagnostic criteria of diabetes differed. Thus the effect of statins on the incidence of diabetes has not been clearly defined. To clarify this issue, we performed the Japan Prevention Trial of Diabetes by Pitavastatin in Patients with Impaired Glucose Tolerance (J-PREDICT) study, which was a prospective randomized, controlled trial evaluating the effect of pitavastatin on the development of diabetes.

Of 8,472 patients who underwent screening, 1,269 individuals with impaired glucose tolerance (IGT) were randomized to either the pitavastatin group (lifestyle modification and pitavastatin [1-2 mg/day]) or the control group (lifestyle modification only). Every six months, a 75-g oral glucose tolerance test was performed with other standard laboratory tests. The primary outcome was cumulative incidence of diabetes, as determined by a 2-h plasma glucose ≥200 mg/dl or a fasting plasma glucose ≥126 mg/dl measured at least once.

The diabetes incidence rates for the pitavastatin and control groups were 163 and 186 cases per 1,000 person-years, respectively; the hazard ratio for progression from IGT to diabetes in the pitavastatin group was 0.82 (95% CI: 0.68-0.99; $P = 0.041$). Even in any subgroups, pitavastatin did not accelerate the incidence, unlike the effects of statins in previous reports. Pitavastatin in combination with lifestyle modification was associated with a lower incidence of diabetes than was lifestyle modification alone in Japanese patients with IGT. Statins are now used with the understanding that a slightly increased risk of diabetes is outweighed by cardiovascular benefits of the drugs. However, based on our results, it may be necessary to reconsider whether all statins really increase the risk of developing diabetes.

Supported by: Waksman Foundation of Japan, Inc.; Kowa Pharmaceuticals Co., Ltd.

62-LB

Durability of Dapagliflozin vs. Glipizide as Add-On Therapies in T2DM Inadequately Controlled on Metformin: 4-Year Data

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Dapagliflozin (DAPA), a selective SGLT2 inhibitor, reduces hyperglycemia in an insulin-independent manner by increasing urinary glucose excretion. In a randomized, double-blind trial of DAPA (≤10 mg/d, n=406) vs. glipizide (GLIP; ≤20 mg/d, n=408) as add-on to metformin (median 2000 mg/d) in T2DM (NCT00660907, 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 hypos (n=3) 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 UTI was 13.5% for DAPA vs. 9.3% for GLIP (upper UTI: 1 DAPA vs. 3 GLIP patients). Genital infections (GenI) occurred in 14.3 vs. 2.9% of patients. Most patients with UTI/GenI first presented in Year 1. The majority of events were of mild/moderate intensity and resolved with standard treatment. UTI/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

63-LB

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

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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/m^2) 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 vs. 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 0.60% 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 imeglimin 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.

64-LB

A Euglycemic Clamp Pilot Study Assessing the Effects of the Glucagon Receptor Antagonist LY2409021 on 24-h Insulin Requirement in Patients With T1DM

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Recent research shows that blocking glucagon action prevents lethal metabolic effects seen in mice with type 1 diabetes mellitus (T1DM). LY2409021 (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, 13.8 years), and a mean baseline hemoglobin A_{1c} of 7.6% (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-mg 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*=.046) and a mean of 19.6% (95% CI,-35.0% to -4.3%; *P*=.019) in 100-mg 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 during and after the clamp procedure. Results suggest glucagon antagonism can reduce insulin requirements in T1DM.

Supported by: Eli Lilly and Company

65-LB

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

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The 104-wk efficacy and safety of CANA (longest follow-up to date), an SGLT2 inhibitor, were assessed in this randomized, double-blind study. Subjects with T2DM on MET (N = 1450; mean age, 56 y; A1C, 7.8%; FPG, 166 mg/dL; BMI, 31 kg/m^2; eGFR, 90 mL/min/1.73 m^2) received CANA 100 or 300 mg or GLIM (up to 6 or 8 mg/d) during a 52-wk core period followed by a 52-wk extension (n = 1050). At Wk 104, both CANA doses reduced A1C, FPG, body weight, systolic BP, and triglycerides vs. GLIM, with HDL-C and LDL-C increases that were stable from Wk 26 to Wk 104. Fewer subjects had hypoglycemia events with CANA 100 and 300 mg than GLIM (7%, 8%, 41%). The coefficient of durability (rate of A1C rise from Wk 26 to Wk 104) was lower with CANA (0.16% both doses) than GLIM (0.37%).

Table. Summary of Efficacy Endpoints at Wk 104 (mITT, LOCF)

Parameter*	CANA 100 mg (n = 483)	CANA 300 mg (n = 485)	GLIM (n = 482)
A1C change, %	–0.65 (0.04)	–0.74 (0.04)	–0.55 (0.04)
Difference vs GLIM	–0.09 (–0.20, 0.01)	–0.18 (–0.29, –0.08)	
Coefficient of durability, %	0.16 (0.03)	0.16 (0.03)	0.37 (0.03)
Difference vs GLIM	–0.21 (–0.29, –0.12)	–0.21 (–0.30, –0.13)	
% of subjects reaching A1C <7.0%†	42.5 (2.3)	50.2 (2.3)	43.9 (2.3)
Difference vs GLIM	–1.4 (–7.9, 5.1)	6.3 (–0.2, 12.9)	
FPG change, mg/dL	–19.3 (1.7)	–22.5 (1.7)	–10.6 (1.7)
Difference vs GLIM	–8.8 (–13.0, –4.5)	–12.0 (–16.2, –7.7)	
Body weight % change	–4.1 (0.2)	–4.2 (0.2)	0.9 (0.2)
Difference vs GLIM	–5.1 (–5.6, –4.5)	–5.2 (–5.7, –4.6)	
Systolic BP change, mmHg	–2.0 (0.6)	–3.1 (0.6)	1.7 (0.6)
Difference vs GLIM	–3.7 (–5.2, –2.3)	–4.8 (–6.2, –3.4)	
Triglycerides % change	4.5 (2.7)	7.9 (2.6)	13.9 (2.6)
Difference vs GLIM	–9.4 (–16.0, –2.9)	–5.9 (–12.5, 0.6)	
HDL-C % change	9.4 (0.9)	10.1 (0.9)	0.8 (0.9)
Difference vs GLIM	8.6 (6.4, 10.7)	9.3 (7.1, 11.5)	
LDL-C % change	11.1 (2.1)	14.2 (2.1)	6.3 (2.1)
Difference vs GLIM	4.9 (–0.4, 10.1)	8.0 (2.7, 13.2)	
LDL-C/HDL-C % change	4.3 (2.3)	5.3 (2.3)	7.7 (2.3)
Difference vs GLIM	–3.4 (–9.1, 2.3)	–2.4 (–8.1, 3.4)	
Non–HDL-C % change	6.3 (1.3)	10.3 (1.3)	6.1 (1.3)
Difference vs GLIM	0.3 (–3.0, 3.6)	4.2 (0.9, 7.5)	

mITT, modified intent to treat; LOCF, last observation carried forward; LS, least squares; SE, standard error; ANCOVA, analysis of covariance; CI, confidence interval. *LS mean (SE) change from baseline using ANCOVA and GLIM-subtracted LS mean (95% CI) for all parameters except for % of subjects reaching A1C <7.0%. †% (SE) and GLIM-subtracted % (95% CI) of subjects reaching A1C <7.0%.

Overall AE rates were 73%, 78%, and 78% with CANA 100 and 300 mg and GLIM, and serious AE rates were 10%, 10%, and 14%; discontinuations due to AEs were low across groups. Genital mycotic infection rates were higher with CANA (pooled) than GLIM (women, 15% vs. 3%; men, 9% vs. 2%). Higher rates of osmotic diuresis-related AEs (6%, 7%, 2%) and UTI (11%, 9%, 7%) were seen with CANA 100 and 300 mg vs. GLIM. Rates of these AEs were generally lower in the 52-wk extension vs. core period. A larger decrease in eGFR was seen with GLIM (6%) than CANA (~1-3%) at Wk 104. In summary, CANA showed durable glycemic improvements vs. GLIM and was generally well tolerated in subjects with T2DM on MET over 104 wks.

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66-LB

Durability of the Efficacy and Safety of Alogliptin Compared to Glipizide Over 2 Years When Used in Combination With Metformin

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This 3-arm, multicenter, randomized, double-blind, active-controlled study evaluated the durability of the efficacy and safety of alogliptin (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 QD + MET (ALO 12.5) (n=880), ALO 25 mg QD + 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/m^2; 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 achieved 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 both ALO decreases vs. GLIP). More GLIP patients (23.2%) experienced ≥1 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 ≥1 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, alogliptin 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.

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A Novel Antidiabetic Drug Fasiglifam/TAK-875 Acts as an Ago-Allosteric Modulator for FFAR1

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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 Ca^{2+} influx assay using CHO-hFFAR1 cells, the EC_{50} 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 FFA. In mouse insulinoma MIN6 cells and mouse islets, the combination of fasiglifam (10 μM) and γ-LA (100 μM) dramatically potentiated glucose-induced insulin secretion (Fas; 1.8 fold, γ-LA; 3.5 fold, Fas+γ-LA; 12.2 fold increase vs. control in mouse islets), while the insulinotropic 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 insulinotropic 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 Ca^{2+} influx activities of fasiglifam and γ-LA, further supporting that these agonists utilize distinct binding sites. Our results indicate that

For author disclosure information, see page LB66.

fasiglifam is an ago-allosteric modulator for FFAR1 that exerts its potent pharmacological effects by acting cooperatively with FFAs, offering greater efficacy in the presence of endogenous ligands. These findings contribute to our understanding of fasiglifam as an attractive antidiabetic drug with a novel mechanism of action.

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Regardless of the Degree of Glycemic Control, Linagliptin (LINA) has Lower Hypoglycemia Risk than All Doses of Glimepiride (GLIM) at All Time Points Over a 2-Year Trial

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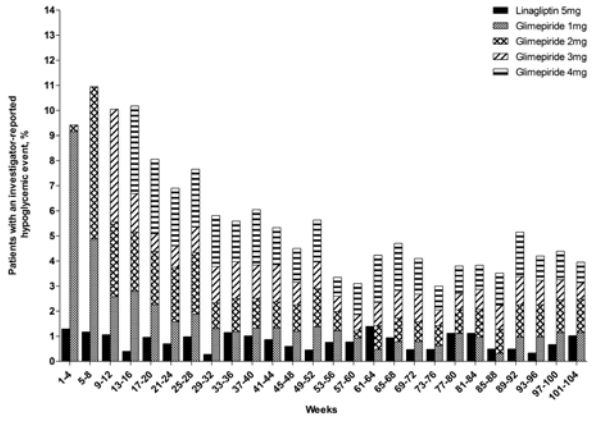
Sulfonylurea (SU)-induced hypoglycemia is a common problem in type 2 diabetes. In a 2-yr, randomized, double-blind study of the DPP-4 inhibitor LINA 5 mg/d (n=764) vs. the SU GLIM 1-4 mg/d (n=755) in pts uncontrolled by metformin, LINA provided noninferior reductions in HbA1c to GLIM with a lower hypoglycemia risk and no weight gain. This exploratory analysis evaluated the risk for investigator-reported hypoglycemia with GLIM based on dose, over time, 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); after excluding events during dose escalation (wks 0-16), this difference remained significant (wks 16-104; 25.8 vs. 5.9%; p<0.0001). At wks 4, 8, 12, 16, and 104, the % pts with hypoglycemia was higher with GLIM vs. LINA in each quartile of HbA1c change from baseline (all 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.



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

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We analyzed pooled data from 2477 patients with T2DM (mean [SD] age 55.6 [10.2] years, HbA_{1c} 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 HbA_{1c}, 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 HbA_{1c}, 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 with 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.

	Placebo	Empagliflozin 10 mg	Empagliflozin 25 mg
HbA_{1c} (%)†			
Baseline‡ (SE)	8.02 (0.03)	7.98 (0.03)	7.96 (0.03)
Change from baseline at week 24 (SE)	-0.08 (0.03)	-0.70 (0.03)	-0.76 (0.03)
Difference vs. placebo (95% CI)		-0.62 (-0.69, -0.55)***	-0.68 (-0.75, -0.61)***
FPG (mg/dL)†			
Baseline (SE)	153.7 (1.3)	152.6 (1.2)	152.6 (1.2)
Change from baseline at week 24 (SE)	7.4 (1.0)	–20.5 (1.0)	–23.2 (1.0)
Difference vs. placebo (95% CI)		-27.9 (-30.7, -25.1)***	-30.6 (-33.4, -27.8)***
Body weight (kg)†			
Baseline (SE)	78.03 (0.66)	78.77 (0.65)	79.10 (0.66)
Change from baseline at week 24 (SE)	-0.24 (0.09)	-2.05 (0.09)	-2.25 (0.09)
Difference vs. placebo (95% CI)		-1.81 (-2.05, -1.57)***	-2.01 (-2.25, -1.76)***
SBP (mmHg)†			
Baseline (SE)	128.6 (0.5)	129.6 (0.5)	129.0 (0.5)
Change from baseline at week 24 (SE)	-0.5 (0.4)	-3.9 (0.4)	-4.3 (0.4)
Difference vs. placebo (95% CI)		-3.4 (-4.4, -2.3)***	-3.8 (-4.9, -2.8)***
DBP (mmHg)†			
Baseline (SE)	78.0 (0.3)	78.7 (0.3)	78.3 (0.3)
Change from baseline at week 24 (SE)	-0.6 (0.2)	-1.8 (0.2)	-2.0 (0.2)
Difference vs. placebo (95% CI)		-1.2 (-1.9, -0.5)***	-1.5 (-2.1, -0.8)***
SBP (mmHg) in patients with uncontrolled BP at baseline ¶			
Baseline (SE)	136.3 (0.6)	136.9 (0.6)	137.4 (0.5)
Change from baseline at week 24 (SE)	-2.5 (0.5)	-7.0 (0.5)***	-7.7 (0.5)***
DBP (mmHg) in patients with uncontrolled BP at baseline ¶			
Baseline (SE)	82.4 (0.3)	83.1 (0.3)	82.6 (0.3)
Change from baseline at week 24 (SE)	-1.9 (0.3)	-3.5 (0.3)***	-3.7 (0.3)***
Total cholesterol (mmol/L)§			
Baseline (SE)	4.70 (0.04)	4.67 (0.04)	4.70 (0.04)
Change from baseline at week 24 (SE)	0.04 (0.02)	0.11 (0.02)	0.16 (0.02)***
HDL cholesterol (mmol/L)§			
Baseline (SE)	1.26 (0.01)	1.26 (0.01)	1.27 (0.01)
Change from baseline at week 24 (SE)	0.00 (0.01)	0.07 (0.01)***	0.07 (0.01)***
LDL cholesterol (mmol/L)§			
Baseline (SE)	2.62 (0.03)	2.57 (0.03)	2.57 (0.03)
Change from baseline at week 24 (SE)	0.02 (0.02)	0.08 (0.02)	0.10 (0.02)**
Triglycerides (mmol/L)§			
Baseline (SE)	1.86 (0.04)	1.95 (0.05)	1.96 (0.07)
Change from baseline at week 24 (SE)	0.03 (0.04)	-0.11 (0.04)*	-0.02 (0.04)
Uric acid (µmol/L)§			
Baseline (SE)	321.44 (2.98)	321.81 (2.89)	322.35 (2.96)
Change from baseline at week 24 (SE)	1.03 (1.83)	-28.95 (1.82)***	-29.55 (1.83)***

Adjusted means based on ANCOVA with last observation carried forward imputation; values on rescue medication were excluded from analysis of HbA1c, FPG, weight and BP but included in analysis of lipid parameters and uric acid.

†Full analysis set (all randomized and treated patients who had a baseline HbA_{1c} value).

‡Inclusion criteria: HbA1c ≥7.0% to ≤10.0%.

¶Patients with uncontrolled BP (SBP ≥130 mmHg or DBP ≥80 mmHg) at baseline.

§Treated set (all patients treated with at ≥1 dose of randomized study medication).

*p<0.05 vs. placebo; **p<0.01 vs. placebo; ***p<0.001 vs. placebo.

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ADA-Funded Research

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Exploring the Potential of Dapagliflozin in Type 1 Diabetes: Phase 2a Pilot Study

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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. Adult patients 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 glycemic levels and diminished glycemic variability with DAPA. Marked reductions in total daily insulin dosing at Day 7 were reported for DAPA 5 mg (-19%) and 10 mg (-16%). Hypoglycemia was common in all treatment groups; 1 event (DAPA 10 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.

		Placebo + insulin (n=10 to13)	DAPA 1 mg + insulin (n=10 to 13)	DAPA 2.5 mg + insulin (n=11 to 15)	DAPA 5 mg + insulin (n=13 to 14)	DAPA 10 mg + insulin (n=9 to 15)
Hypoglycemia (%)	No. pts (%) with ≥1 event	8 (61.5)	12 (92.3)	9 (60.0)	11 (78.6)	10 (66.7)
24 h urine glucose excretion	Baseline, g/24h ± SD	30.7 ± 52.0	6.6 ± 6.5	12.1 ± 12.3	11.2 ± 10.9	11.9 ± 14.3
	Day 7, g/24h ± SD	9.0 ± 7.5	48.5 ± 20.8	60.8 ± 38.2	83.6 ± 47.9	99.7 ± 58
	Mean change from baseline, g/24h (95% CI)	-21.6 (-54.4, 11.1)	41.9 (27.6, 56.1)	48.5 (28.1, 69.0)	72.4 (47.0, 97.9)	88.8 (55.2, 122.5)
24 h glucose/ CGM daily average	Baseline, mg/dL ± SD	174 ± 43	162 ± 30	174 ± 42	174 ± 33	174 ± 44
	Day 7, mg/dL ± SD	154 ± 41	146 ± 20	164 ± 37	144 ± 51	139 ± 35
	Mean change from baseline, mg/dL (95% CI)	-20.4 (-65.4, 24.7)	-15.7 (-38.6, 7.2)	-13.9 (-47.0, 19.1)	-29.5 (-47.2, -11.9)	-41.3 (-66.8, -15.7)

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Metabolic Response to Sodium-Glucose Transporter 2 (SGLT2) Inhibition With Empagliflozin in Patients With Type 2 Diabetes (T2D)

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SGLT2 inhibitors lower glycemia by enhancing urinary glucose excretion. The physiologic response to pharmacologically-induced glycosuria has not been investigated. We studied 66 T2D patients (62±7 years, BMI=31.6±4.5 kg/m2, HbA1c=7.2±0.2%, µ±SD) at baseline, after a single dose (25 mg, StudyI), and following 4 weeks of treatment with empagliflozin (25 mg/d, StudyII). For each study, patients received a mixed meal coupled with double-tracer glucose administration and indirect calorimetry. Compared to baseline, StudyI caused glycosuria (7.8[4.4] g over 3 hrs of fasting, median[IQR]), which led to an increase in endogenous glucose production (EGP, 13.8[5.2] to 17.6[4.8] µmol.kgFFM-1.min-1, p<0.0001) matching the glycosuria. These fasting-state changes were maintained in StudyII. Postmeal glycosuria rose to 29.0[12.5] and 28.2[15.4] g over 5 hrs in StudyI and II, respectively. Correspondingly, postmeal glucose AUC (51[11] and 51[10] vs. 57[16] g/dL) and insulin AUC (80[59] and 76[59] vs. 93[68] nmol/L) dropped, whereas the glucagon response rose (6.5[2.1] and 5.6[1.8] vs. 5.2[1.6] nmol/L) (all p<0.001). While appearance of oral glucose was unchanged, postmeal EGP was increased (AUC=40[14] and 37[11] vs. 34[11] g, both p<0.01). Tissue glucose disposal (=total glucose disposal minus glycosuria) was reduced (75[16] and 70[21] vs. 93[18] g, p<0.0001) due to a decrease in both glucose oxidation and non-oxidative glucose disposal, with a concomitant rise in lipid oxidation (all p<0.01). ß-cell glucose sensitivity improved (55[35] and 55[39] vs. 44[32] pmol.min-1.m-2.mM-1, p<0.0001), while insulin sensitivity was unchanged (9.1[6.7] and 8.6[8.0] vs. 8.2[5.8] ml.kgFFM-1.min-1.nM-1, p=ns). Conclusions: in T2D patients empagliflozin lowers fasting and postprandial glycemia by (a) increasing total glucose removal despite a compensatory increase in EGP, (b) improving ß-cell function, and (c) shifting substrate utilization from glucose to lipid.

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Novel Mechanism of Luseogliflozin, SGLT2 Inhibitor, Induced Beneficial Serum Uric Acid-Lowering Effect

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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 0.5-1.6 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 coupled to that of urinary glucose (Glc) excretion, but not to the pharmacokinetics of Luseogliflozin. From these findings, we focused attention 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 ^{14}C-UA by Xenopus oocytes expressing GLUT9-iso2. As a result, higher Glc concentration of over renal threshold of Glc clearly stimulated ^{14}C-UA efflux, suggesting that GLUT9-iso 2 plays an important role on facilitation of UA secretion by exchanging 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.

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Canagliflozin (CANA) Is Effective and Generally Well Tolerated in Subjects With Type 2 Diabetes Mellitus (T2DM) and Stage 3 Chronic Kidney Disease (CKD)

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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 (Wk 18, 1 study; Wk 26, 3 studies) with an eGFR ≥30 and <60 mL/min/1.73 m^2 (N = 1,085) and in subgroups with eGFR ≥45 and <60 (n = 721) or ≥30 and <45 mL/min/1.73 m^2 (n = 364). 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 ≥45 than <45 mL/min/1.73 m^2. For the pooled CANA group, overall AE rates were higher than PBO across populations (eGFR ≥30 to <60: 74.7% vs. 70.4%; ≥45: 71.0% vs. 66.9%; <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 (eg, pollakiuria, polyuria) were higher with CANA than PBO in subjects with eGFR ≥30 and <60 (4.0% vs. 3.7%) and <45 mL/min/1.73 m^2 (4.8% vs. 2.6%). Rates of AEs related to reduced intravascular volume (eg, 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.

Table. Summary of Efficacy Parameters

Parameter*,†	Baseline eGFR, mL/min/1.73 m²		
A1C	**≥30 and <60‡**	**≥45 and <60§**	**≥30 and <45‖**
PBO			
Change, %	−0.14 (0.06)	−0.10 (0.07)	0.05 (0.19)
CANA 100 mg			
Change, %	−0.52 (0.06)	−0.57 (0.07)	−0.18 (0.19)
Difference vs PBO	−0.38 (−0.50, −0.26)¶	−0.47 (−0.61, −0.32)	−0.23 (−0.45, −0.01)
CANA 300 mg			
Change, %	−0.62 (0.06)	−0.62 (0.07)	−0.34 (0.19)
Difference vs PBO	−0.47 (−0.60, −0.35)¶	−0.52 (−0.67, −0.38)	−0.39 (−0.61, −0.17)
Body weight	**≥30 and <60‡**	**≥45 and <60§**	**≥30 and <45‖**
PBO			
Change, kg	−0.5 (0.2)	−0.5 (0.2)	0.7 (0.6)
% change	−0.5 (0.2)	−0.6 (0.2)	0.9 (0.6)
CANA 100 mg			
Change, kg	−1.9 (0.2)	−2.1 (0.2)	−0.3 (0.6)
% change	−2.0 (0.2)	−2.3 (0.2)	−0.3 (0.6)
Difference vs PBO	−1.6 (−2.0, −1.1)¶	−1.8 (−2.3, −1.2)	−1.2 (−1.9, −0.5)
CANA 300 mg			
Change, kg	−2.3 (0.2)	−2.4 (0.2)	−0.8 (0.6)
% change	−2.4 (0.2)	−2.5 (0.2)	−0.9 (0.6)
Difference vs PBO	−1.9 (−2.3, −1.5)¶	−2.0 (−2.5, −1.5)	−1.8 (−2.5, −1.1)
Systolic BP	**≥30 and <60‡**	**≥45 and <60§**	**≥30 and <45‖**
PBO			
Change, mmHg	−1.6 (0.9)	−2.6 (1.0)	5.7 (3.2)
CANA 100 mg			
Change, mmHg	−4.4 (0.9)	−4.4 (1.1)	0.8 (3.2)
Difference vs PBO	−2.8 (−4.7, −0.8)	−1.8 (−4.1, 0.5)	−4.8 (−8.5, −1.2)
CANA 300 mg			
Change, mmHg	−6.0 (0.9)	−6.8 (1.1)	0.8 (3.3)
Difference vs PBO	−4.4 (−6.3, −2.4)	−4.3 (−6.5, −2.0)	−4.9 (−8.5, −1.2)

LS, least squares; SE, standard error; ANCOVA, analysis of covariance; CI, confidence interval. *LS mean (SE) change from baseline using ANCOVA and PBO-subtracted LS mean (95% CI) values; †*P* values are reported for pre-specified comparisons only; ‡Mean baseline age, 67.1 y; A1C, 8.1%; eGFR, 48.2 mL/min/1.73 m²; body weight, 90.9 kg; §Mean baseline age, 66.3 y; A1C, 8.1%; eGFR, 53.3 mL/min/1.73 m²; body weight, 90.4 kg; ‖Mean baseline age, 68.6 y; A1C, 8.1%; eGFR, 38.2 mL/min/1.73 m²; body weight, 91.7 kg; ¶*P* <0.001 vs PBO.

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Empagliflozin (EMPA) Increases Genital Infections But Not Urinary Tract Infections (UTIs) in Pooled Data from Four Pivotal Phase III Trials

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SGLT2 inhibitors increase urinary glucose excretion. It is important to identify whether this promotes UTIs and/or genital infections.

Four randomized, placebo-controlled Phase III trials investigated the effect of EMPA for 24 weeks as monotherapy, add-on to metformin, add-on to metformin + SU, or add-on to pioglitazone ± metformin in patients with T2DM. Using pooled data from these trials, which included 2477 patients (mean age 55.6 [SD 10.5] years) treated with EMPA 10 mg (n=830), EMPA 25 mg (n=822) or PBO (n=825), events consistent with UTI or genital infection were evaluated using prospectively defined search categories of 67 or 87 preferred terms, respectively.

The percentage of patients with events consistent with UTI was similar with PBO and EMPA (8-9%). More patients on EMPA than PBO reported events consistent with genital infection (4% vs. 1%). Both types of event were more common in women than men and more common in patients with a history of UTI or genital infection (table). Most patients who reported an event consistent with UTI or genital infection experienced only 1 such episode, very few patients discontinued due to such an event and most events were mild in intensity.

To conclude, in a pooled analysis of data from 2477 patients, EMPA was not associated with an increased frequency of UTIs, but was associated with an increased frequency of genital infections compared with PBO.

	Placebo (n=825)	Empagliflozin 10 mg (n=830)	Empagliflozin 25 mg (n=822)
Events consistent with UTI			
Patients with events consistent with UTI, n (%)	**68 (8.2)**	**77 (9.3)**	**62 (7.5)**
Male, n/N (%)	16/424 (3.8)	9/463 (1.9)	5/464 (1.1)
Female, n/N (%)	52/401 (13.0)	68/367 (18.5)	57/358 (15.9)
Patients without a history of chronic or recurrent UTIs, n/N (%)	57/772 (7.4)	66/788 (8.4)	51/776 (6.6)
Patients with a history of chronic or recurrent UTIs, n/N (%)	11/53 (20.8)	11/42 (26.2)	11/46 (23.9)
Number of episodes per patient, n (%)			
0	757 (91.8)	753 (90.7)	760 (92.5)
1	62 (7.5)	65 (7.8)	56 (6.8)
2	6 (0.7)	10 (1.2)	6 (0.7)
≥3	0	2 (0.2)	0
Intensity of worst episode, n (%)			
Mild	57 (6.9)	62 (7.5)	55 (6.7)
Moderate	9 (1.1)	14 (1.7)	7 (0.9)
Severe	2 (0.2)	1 (0.1)	0
Patients with events consistent with UTI leading to treatment discontinuation, n (%)	1 (0.1)	2 (0.2)	1 (0.1)
Events consistent with genital infection			
Patients with events consistent with genital infection, n (%)	**6 (0.7)**	**35 (4.2)**	**30 (3.6)**
Male, n/N (%)	2/424 (0.5)	12/463 (2.6)	5/464 (1.1)
Female, n/N (%)	4/401 (1.0)	23/367 (6.3)	25/358 (7.0)
Patients without history of chronic or recurrent genital infections, n/N (%)	5/818 (0.6)	33/826 (4.0)	27/809 (3.3)
Patients with history of chronic or recurrent genital infections, n/N (%)	1/7 (14.3)	2/4 (50.0)	3/13 (23.1)
Number of episodes per patient, n (%)			
0	819 (99.3)	795 (95.8)	792 (96.4)
1	5 (0.6)	30 (3.6)	25 (3.0)
2	1 (0.1)	3 (0.4)	4 (0.5)
≥3	0	2 (0.2)	1 (0.1)
Intensity of worst episode, n (%)			
Mild	5 (0.6)	24 (2.9)	20 (2.4)
Moderate	1 (0.1)	11 (1.3)	10 (1.2)
Severe	0	0	0
Patients with events consistent with genital infections leading to treatment discontinuation, n (%)	0	1 (0.1)	2 (0.2)

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Delayed-Release Metformin May Be Suitable for Use in Diabetes Patients With Renal Impairment that are Contraindicated for Currently Available Metformin Formulations

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We recently uncovered that metformin's (Met) glucose-lowering effects are predominantly due to actions on enteroendocrine L-cells which are more densely populated in the lower bowel and that plasma Met exposure is not required for efficacy (ADA 2013, #1087-P). Met is contraindicated in patients with renal impairment due to Met accumulation and associated risk of lactic acidosis. By targeting a daily dose of 1000 mg delayed-release metformin (Met DR) directly to the lower bowel of patients with type 2 diabetes, Met exposure was reduced by 68% relative to a daily dose of 2000 mg immediate release Met (Met IR). Despite lower Met exposure, Met DR produced a similar glucose lowering effect as Met IR and increased fasting and postprandial GLP-1 and PYY. We hypothesized that Met DR may be a viable treatment for diabetic patients with renal impairment. We used a population PK model based on data from 2 clinical studies (N=44) of Met DR, Met IR, and Met extended-release (Met XR) to predict Met exposure ($AUC_{0\text{-}48}$) following administration of each formulation in normal subjects and those with varying degrees of renal impairment (mild to severe). The median predicted AUCs (ng*h/mL) for 1000 mg daily Met DR in patients with normal, mild, moderate and severe renal impairment were 4669, 4984, 5524, and 6527. Predicted AUCs (ng*h/mL) were significantly higher for 2000 mg daily Met IR and Met XR (22659 and 20607 with normal renal function and 31606 and 29074 with severe renal impairment). Thus, in patients with severe renal impairment, 1000 mg daily Met DR is predicted to result in lower Met exposure than 2000 mg daily Met IR or Met XR in patients with normal renal function, while maintaining comparable glucose-lowering efficacy. Met

DR may provide a method to treat renally impaired type 2 diabetic patients with metformin without increasing the risk of Met associated lactic acidosis.

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

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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 m^2) or ≥65 y (n = 445; male, 51.5%; mean age, 69.3 y; A1C, 7.9%; body weight, 85.1 kg; eGFR, 76.9 mL/min/1.73 m^2). 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 (59.9%, 59.0%, 58.9%) and ≥65 y (61.0%, 60.4%, 61.3%). Serious AE and AE-related discontinuation 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 than PBO of genital mycotic infections in women and men and osmotic diuresis-related AEs; rates of AEs related to reduced intravascular volume were low in both age groups. UTI and renal-related AE rates were similar across 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. Summary of Efficacy Parameters and Selected AEs for Subjects <65 and ≥65 y

	Subjects <65 y			Subjects ≥65 y		
Efficacy Parameters*,†	PBO (n = 509)	CANA 100 mg (n = 674)	CANA 300 mg (n = 685)	PBO (n = 137)	CANA 100 mg (n = 159)	CANA 300 mg (n = 149)
A1C change, %	−0.15 (0.04)	−0.89 (0.03)	−1.08 (0.03)	−0.05 (0.07)	−0.69 (0.07)	−0.88 (0.07)
Difference vs PBO		−0.74 (0.05)	−0.93 (0.05)		−0.64 (0.10)	−0.82 (0.10)
Body weight % change	−0.6 (0.2)	−2.8 (0.1)	−3.4 (0.1)	−0.6 (0.3)	−2.9 (0.3)	−3.8 (0.3)
Difference vs PBO		−2.2 (0.2)	−2.9 (0.2)		−2.3 (0.4)	−3.2 (0.4)
Systolic BP change, mmHg	−0.3 (0.5)	−4.2 (0.4)	−4.8 (0.4)	−0.8 (1.1)	−4.6 (1.0)	−5.9 (1.0)
Difference vs PBO		−3.9 (0.6)	−4.5 (0.6)		−3.9 (1.4)	−5.1 (1.5)
Triglycerides % change‡	9.1 (2.2)	3.7 (2.0)	0.6 (2.0)	2.6 (3.0)	−2.4 (2.8)	−2.5 (2.9)
Difference vs PBO		−5.4 (2.9)	−8.5 (2.9)		−5.0 (4.1)	−5.2 (4.2)
LDL-C % change‡	1.5 (1.4)	6.3 (1.2)	9.8 (1.2)	0.7 (2.1)	3.5 (1.9)	6.8 (2.0)
Difference vs PBO		4.8 (1.8)	8.3 (1.8)		2.8 (2.9)	6.1 (2.9)
HDL-C % change‡	4.0 (0.8)	9.2 (0.7)	10.2 (0.7)	3.7 (1.4)	9.7 (1.3)	10.7 (1.4)
Difference vs PBO		5.2 (1.1)	6.2 (1.1)		6.0 (1.9)	7.1 (1.9)
LDL-C/HDL-C % change‡	−0.5 (1.4)	−0.7 (1.2)	1.3 (1.2)	−1.4 (2.3)	−4.2 (2.2)	−1.2 (2.3)
Difference vs PBO		−0.2 (1.8)	1.8 (1.8)		−2.8 (3.2)	0.2 (3.2)
Non–HDL-C % change‡	1.1 (1.0)	2.6 (0.9)	4.7 (0.9)	−0.5 (1.8)	0.8 (1.7)	2.9 (1.8)
Difference vs PBO		1.4 (1.4)	3.6 (1.4)		1.4 (2.5)	3.4 (2.6)
Selected AEs§	PBO (n = 509)	CANA 100 mg (n = 674)	CANA 300 mg (n = 685)	PBO (n = 137)	CANA 100 mg (n = 159)	CANA 300 mg (n = 149)
Genital mycotic infection						
Male‖	2 (0.8)	14 (4.3)	11 (3.4)	0	3 (3.7)	4 (5.2)
Female¶	10 (4.1)	37 (10.7)	44 (12.3)	0	7 (9.0)	5 (6.9)
UTI	20 (3.9)	41 (6.1)	29 (4.2)	6 (4.4)	8 (5.0)	7 (4.7)
Osmotic diuresis-related AEs	4 (0.8)	44 (6.5)	39 (5.7)	1 (0.7)	12 (7.5)	8 (5.4)
Volume-related AEs	5 (1.0)	6 (0.9)	8 (1.2)	2 (1.5)	4 (2.5)	3 (2.0)
Renal-related AEs	2 (0.4)	2 (0.3)	12 (1.8)	2 (1.5)	3 (1.9)	2 (1.3)

LS, least squares; SE, standard error; ANCOVA, analysis of covariance. *LS mean (SE) change from baseline using ANCOVA and PBO-subtracted LS mean (SE) values. †Data are reported for prior to rescue therapy except for lipid parameters, which are for regardless of rescue therapy. ‡% changes determined based on changes in conventional units; §Number (%) of subjects; ‖Males <65 y: PBO, n = 263; CANA 100 mg, n = 327; CANA 300 mg, n = 327; males ≥65 y: PBO, n = 71; CANA 100 mg, n = 81; CANA 300 mg, n = 77. ¶Females <65 y: PBO, n = 246; CANA 100 mg, n = 347; CANA 300 mg, n = 358; females ≥65 y: PBO, n = 66; CANA 100 mg, n = 78; CANA 300 mg, n = 72.

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

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Aims: To explore the effect of metformin and acarbose on islet α 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 24Kg/m^2, 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 glucagons 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 51years, HbA1c 7.7%, BMI 26.8Kg/m^2 were enrolled. 54 patients were assigned in 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: 2.27mmol/L and 2.87 mmol/L; 2h postprandial glucose: 3.19mmol/L and 3.25 mmol/L, respectively); The early-phase insulin secretion index ΔI30/ΔG30 was improved only in acarbose group; Body weight decrease (metformin:-2.3Kg vs. acarbose: -2.6Kg); Decrease of fasting and 0.5h postprandial glucagon in acarbose group was markedly greater than that in metformin group (fasting glucagon: -11.4pg/mL vs. -0.5pg/mL; 0.5h postprandial glucagon: 5.7 pg/mL vs. -10.7pg/mL, respectively. P<0.05).

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 α cell function better than metformin, representing by the greater decrease of fasting and 0.5h postprandial glucagon. Although the improvement of ΔI30/ΔG30 can decrease postprandial glucagon, further studies are needed to explore the related pathophisiology mechanism.

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Resveratrol Synergy in Pre-Diabetes

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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 rodents 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, n=13), 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 irisin levels, LeuRes increased circulating irisin by nearly 50% from 340±60 to 502±92 ng/mL (p=0.02). Thus, capitalizing on synergistic properties of HMB and Leu with resveratrol may be an attractive neutraceutical strategy to improve metabolism.

Supported by: NuSirt Sciences, Inc.

CLINICAL THERAPEUTICS/NEW TECHNOLOGY—PHARMACOLOGIC TREATMENT OF COMPLICATIONS

79-LB

FG-4592, a Novel Oral Hypoxia Inducible Factor (HIF) Stabilizer, Raises Hemoglobin (Hb) in Diabetic Subjects With Anemia of Chronic Kidney Disease (CKD)

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Diabetes is a leading cause of CKD. Anemia of CKD is associated with lower quality of life and increased rate of progression to ESRD and death, yet is undertreated outside the dialysis setting due to the cost, inconvenience, and safety concerns associated with use of erythropoiesis-stimulating agents (ESAs). FG-4592 is an oral HIF stabilizer that increases endogenous erythropoietin and utilization of iron (Fe). We report the effects of FG-4592 on Hb, total cholesterol (TC), and blood pressure (BP) in a subset of diabetic patients (n=107 [74%], majority T2DM) from an open-label, Phase 2 study of 145 adult ESA-naïve anemic non-dialysis CKD patients. Treatment was 16-24 weeks in six cohorts differing by starting dose (50-150 mg) and frequency (QW, BIW, TIW) during Hb correction/maintenance. Dose titration was allowed every 4 weeks to target Hb 11-13g/dL. Fe repletion at baseline (BL) was not required and intravenous Fe was not allowed. Across cohorts, the diabetic subjects were comparable in number (n=15-19/cohort) and BL traits, including Hb (mean 9.7 g/dL; cohort range 9.6-9.9g/dL). Treatment to Hb targets resulted in a mean peak Hb increase of 1.9 g/dL (cohort range 1.4- 2.3g/dL; p<0.0001) and an 83-100% response rate, defined as Hb ≥11g/dL and ≥1g/dL from BL. Across cohorts, there was a mean peak decrease from BL TC (170 mg/dL) of

15% (p<0.0001) (-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)

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The expression/actions of estrogen receptor α(ERα 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 ERα expression in T2D. Subjects with HbA1c >6.5, eGFR 25-80ml/min/1.73m^2, and albumin excretion >300mg/day were randomized to SevCarb (4800mg/day) or Ca_2CO_3 (1950mg/day) for 6 months in an intention-to-treat trial. At 3 months there was a robust increase of ERα 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*
ER α	1.503	3.515	**0.003**	-0.358	0.941	**0.011**	**0.003**
Nrf2	0.497	1.662	**0.025**	-0.131	0.991	0.350	**0.009**
AGER1	0.284	1.001	**0.035**	-0.045	0.446	0.532	**0.028**
TNFR1	-0.359	1.841	0.144	0.331	1.491	0.168	0.052
RAGE	-0.100	0.765	0.086	0.419	1.413	0.059	**0.011**

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

In conclusion, ERα restoration after sevelamer carbonate treatment may underlie and reduced 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.

Supported by: Sanofi

HEALTH CARE DELIVERY—ECONOMICS

81-LB

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

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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 $29,331 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 (<1.81%), enrolling fewer participants (<16), or increasing the hourly cost (>$90) or the annual 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 an economically reasonable intervention.

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82-LB

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

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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 outcomes of inpatient admissions, charges, length of stay, and severe aggregate outcomes (SOAs) involving mortality, amputation, sepsis, or surgical complications. Across the 5-year time period, 3,845 inpatient cases of DFI 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 bill of $208 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 state inpatient charges, and 86.1% more SAOs (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

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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 $8,880 in 1992 to $4,070 in 2012. This has been partially offset by a steady increase in per capita costs for outpatient care from $1,390 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

For author disclosure information, see page LB66.

medications and supplies has been of significant benefit. We conclude that the cost of diabetes resides mainly in the growing number of those affected. Continued, if not enhanced, attention to the delivery of quality outpatient diabetes care will likely be the best approach to controlling costs, until efforts to prevent diabetes become more widespread.

84-LB

Resource Utilization and its Impact on the Inpatient Diabetes Management in the Non-Critical Care Units

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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 756 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=<.001), female gender 41.2 vs. 45.0% (p=0.5), mean 4BG values 202.2±52.5 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=<.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. 5.3±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 SDT cared for sicker patients with higher A1C. This was also reflected in the observed wide variability of BG levels during hospitalization. In conclusion: utilizing PST for diabetes management in medical 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

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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-naïve 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 antidiabetic 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.

	LIRA	EXEN	SITA		
Sample size	234	182	1,757	p	p
Baseline A1c	7.8%	7.8%	7.9%	(LIRA-EXEN)	(LIRA-SITA)
Change in A1c from baseline	-1.08%	-0.75%	-0.68%	P<0.001	P<0.0001
Pct. achieving A1c<7%	64%	54%	49%	P<0.05	P<0.0001

Supported by: Novo Nordisk, Inc.

86-LB

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

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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 218±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 (55±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.

PEDIATRICS—OBESITY AND TYPE 2 DIABETES

87-LB

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

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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 universally labeled 13C-glucose/Kg dissolved in water, breath samples were taken at baseline, 30, 60, 90, 120, 150 and 180 minutes.

The cumulative percentage of oxidized dose of 13C-glucose was significantly different (p<0.05) between the study groups at the times previously described, however the greatest difference was found at 180 minutes (17.75% ± 5.64 in control group and 9.95% ± 4.73 in MS group).

A ROC curve was constructed obtaining a cutoff point of 14.45 of cumulative percentage of oxidized dose of 13C-glucose at 180 minutes which corresponded to a sensitivity of 82% and specificity of 84% (AUC: 0.87, p<0.001, CI95:0.78-0.95).

The results obtained demonstrate that the 13C-glucose breath test is a valid screening method to identify MS in Mexican pediatric population and represents an alternative in non-clinical settings where venipuncture and blood retrieval results impractical or impossible.

Supported by: CONACYT

88-LB

A Community-Based Intervention for Diabetes Risk Reduction in Inner-City Obese Adolescents

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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 of care with a parallel-group randomized controlled trial comparing BB with standard clinical care (CC) in obese adolescents (10-16 yo) 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; diff=-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 glucoses.

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89-LB

Even If Not Macrosomic, Children of Diabetic Mothers Tend to be Overweight at Age 17

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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 and1993 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 gestational diabetes (GDM), 97 children (51 males) from mothers with pre-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 length or weight between the groups. Our study shows that improved treatment of diabetes during pregnancy in the 1990's resulted in normally sized newborns. Nevertheless, children born to diabetic mothers tend to be overweight adolescents and are at risk to develop future metabolic syndrome.

Males	Number	GDM 159	PGDM 34	Control 198	P
Males	Birth length	49.7±2.0	49.3±1.8	49.6±1.6	0.37
Males	Birth weight	3423±527	3451±535	3344±372	0.18
Males	BMI≥85th percentile at age 17	27.0%	26.5%	16.1%	0.009*
Females	Number	113	23	147	
Females	Birth length	48.7±2.0	48.6±1.7	48.9± 1.9	0.610
Females	Birth weight	3230±510	3210±364	3228±324	0.978
Females	BMI≥85th percentile at age 17	15.9%	34.8%	15.6%	0.441*

* p<0.05 between the control group and the 2 other groups combined

90-LB

Metabolites as Novel Biomarkers for Childhood Obesity-Related Traits in Mexican American Children

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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 ($P \leq 0.018$) differences between obese and normal weight children by ANOVA. Higher levels of bradykinin, phosphocholine and phosphotidylethanolamine and lower levels of L-thyronine, naringenin, indole-3-propionic acid, 2-methylbutyroylcarnitine, 3-hydroxyquinine, 1α22-dihydroxy-23,24,25,26,27-pentanorvitamin D3, lysophosphatidylcholine (18:1), calciferol B, diglyceride, malvidin3-(6-acetyl glucoside) and linoleic acid were found in obese children. After adjustment for multiple testing (significance threshold = $P<5.9\text{x}10^{-5}$), L-thyronine (18.2 fold); bradykinin (4.2 fold); and naringenin (1.8 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-methylbutyroylcarnitine, all the metabolites were significantly ($P \leq 0.05$) 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 Diabetes: Comparisons With SMBG and YSI

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We studied the performance of the new Dexcom G4 PLATINUM (DG4P) CGM system in 176 youth (age 2 - 17, mean 11.5,29 < age 6; 57% male), at 6 centers. Most youth (72%) used CSII; mean A1C was 8.2 ± 1.3%; and mean zBMI was 0.5 (range -4.7 to 2.6). Youth wore 2 systems (1 blinded, 1 displayed) on either the abdomen and/or upper buttocks for 7 days of home use. Youth/parents calibrated CGM twice daily using fingerstick SMBG . There was a single in-clinic session on sensor days 1, 4, or 7. In youth ≥ 6 years, the session lasted up to 6 hrs in which "arterialized" (via a heating pad over an arm IV) venous YSI samples q15 mins and SMBG samples q30 mins were collected. In youth <6 years, 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 19% on day 1 to 12% on day 7. In a comparison of SMBG to YSI, MARD was 13% (n= 1296), 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. 85% 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.

92-LB

The Impact of Diabetic Ketoacidosis (DKA) History on Brain Microstructural White Matter (WM) and Memory Function in Young Children With Type 1 Diabetes (T1D)

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Children with T1D are at risk of developing neurocognitive complications but the extent and mechanisms of these remain unknown. Subtle learning and emotional problems and poor concentration have been reported in children following an episode of DKA and evidence suggests long-lasting decreases in memory function in school-aged children years after a DKA

episode. To determine if a history of DKA was associated with memory and brain changes in young children (ages 4 to 10 years) with T1D, we analyzed data from a DirecNet study assessing neuroimaging and cognitive differences in children in this age range. One hundred forty-two children with T1D completed subtests of the Children's Memory Scale and unsedated diffusion weighted MRI scans. There were 51 episodes of DKA, 46 at diagnosis of DKA, in 51 subjects. The median time from the DKA event to cognitive testing was 3.1 years (range 0.1 to 7.6 years). After controlling for age of onset and sex, a history of DKA was not related to memory scores. However, increasing clinical severity of DKA (mild=pH<7.3 or $C0_2$<15; moderate=pH<7.2 or $C0_2$<10; or severe=pH<7.1 or $C0_2$<5) was significantly correlated with higher radial diffusivity (RD) and lower fractional anisotropy (FA) ($p<0.05$, corrected) in widespread brain regions. In addition, more severe DKA was associated with higher axial diffusivity (AD) ($p=0.05$, corrected) in the right frontal, temporal and parietal brain regions. Longitudinal studies of these children may predict memory deficits seen later in older children. These data indicate that even a single episode of moderate to severe DKA in very young children with T1D can have long-term effects on brain white matter microstructure.

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93-LB

Ultrasensitive Measurement of C-Peptide in Serum and Urine Using Novel Electrochemiluminescent Technology

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C-peptide is a 31-aa protein that connects insulins' A-chain to its B-chain in the proinsulin molecule. The measurement of C-peptide in serum and urine are used to assess insulin secretory reserve levels, impaired glucose tolerance and insulin resistance.

We have developed an ultrasensitive two-site C-peptide immunoassay using electrochemiluminescent (ECL) detection technology (Meso Scale Discovery®, MSD). The assay is capable of measuring C-peptide down to 0.004 ng/mL in human serum and urine and is designed as a two-step reaction utilizing a pair of monoclonal antibodies raised against human C-peptide. One of the antibodies (capture) is immobilized on the carbon surface of the MSD 96-well plate while the other antibody is conjugated with an ECL signal (MSD Sulfo tag™) molecule to generate a signal antibody. C-peptide samples are allowed to react in the capture antibody coated plate, then incubated with the signal antibody. Bound 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 high assay performance and exceptional sensitivity that may exceed most commercial diagnostic C-peptide assays.

94-LB

Identify High Risk for Type 1 Diabetes: ECL-GADA and IAA Assays

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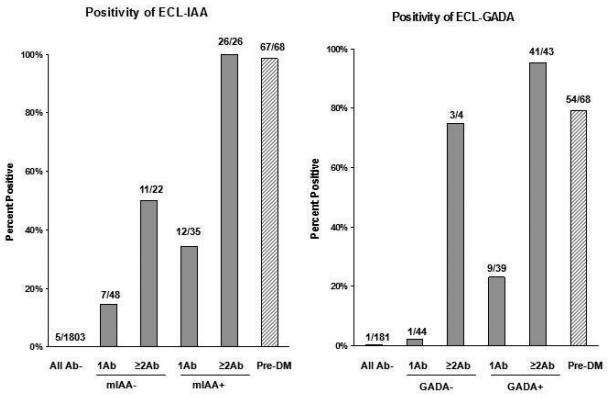
Detection of diabetes-specific islet autoantibodies is essential for prediction and prevention of type 1 diabetes. The present study aimed to identify high risk for typ1 diabetes with newly developed more specific assays for insulin and GAD65 autoantibodies. In addition to a previously reported non-radioactive electrochemiluminescense-based ECL-IAA assay, we recently developed an ECL-GADA assay. The assays were validated using serum samples from 227 newly diagnosed diabetic children, 68 pre-diabetic children who were prospectively followed to type 1 diabetes, 130 non-diabetic children longitudinally followed for years with confirmed islet autoantibodies to insulin, GAD65, IA2 and/or ZnT8, and 181 age-matched healthy antibody negative children. Both ECL-GADA and ECL-IAA were able to pick up 100% of antibody positivity detectable with current radioassay among newly diagnosed children with type 1 diabetes, pre-diabetic children, and high risk children with multiple positive islet autoantibodies. On the other hand, only a small portion of children positive for a single islet autoantibody by radioassay were positive for either ECL-GADA (9/39 [23%]; $p<0.0001$) or ECL-IAA (13/35 [37%]; $p< 0.0001$). Both IAA and GADA not detectable by ECL assay were shown to be of low affinity. In conclusion, new ECL-based assays for IAA and GADA were more disease specific and may help to differentiate high-risk from low-risk single islet autoantibodies for staging of type 1 diabetes.



95-LB

The Effect of Type I Diabetes Mellitus on Final Adult Height

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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.

Patients with T1DM seen in the pediatric endocrinology clinic at ECU between the ages of 8 and 18 years were included in a retrospective chart review. Parental heights were obtained and gender adjusted mid-parental heights were calculated using the formula described by Tanner. Each patient's final adult height was the height at age 18 if growth velocity in the preceding 6 months was zero. Final adult height was subtracted from the predicted height to determine the height difference (predicted minus final). 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.

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 if this 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 9%. The regression analysis of height difference on A1C had an r-squared = 0.06 and correlation of r = 0.25 (P = 0.21). Data from 111 surveys showed that on a scale of 1 to 10 (least to most important), average score for importance of final adult height was 7.3 for males and 6.3 for females. Ninety-one percent of adolescents surveyed believed they would work harder on their diabetes control if the complication of impaired growth was known.

Although our results were not statistically significant, the scatter plot of A1C to 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.

PREGNANCY—BASIC SCIENCE

96-LB

Circulating Markers of Endothelial Dysfunction and Glutathione Peroxidase Activity in Normal Pregnancy

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Endothelial dysfunction is positively related to insulin resistance and cardiovascular disease. Oxidative stress increases and antioxidant status 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 gravidae (African-American 35%, Hispanic 46%, Caucasian 19%) age 21.7±0.2 (yr), pregravid BMI (kg/m^2) 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 by multiple regression analysis 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 per mU/mg hemoglobin (Hb) GPx, p=0.009), sVCAM-1 (-2.687 ng/ml per mU/mg Hb GPx, p=0.026) and sE-selectin (-0.348 ng/ml per mU/mg Hb GPx, p=0.006). The relationship persisted at the 3rd trimester for sICAM-1 (-2.011 ng/ml per mU/ mg Hb GPx, p=0.027) and sE-selectin (-0.460 ng/ml per mU/mg Hb GPx, p=0.007) but not sVCAM-1 (-0.970 ng/ml per mU/mg Hb GPx, p>0.05).

In conclusion, the 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

97-LB

ACE C1237T Gene Polymorphism in Indian Women With Gestational Diabetes Mellitus

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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 8. Two previous studies involving (Keavney et al in 1998 and Zhu et al 2000) ACE-6 polymorphism and hypertension focused on 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 (α = 5.50, p=0.0190). Further analysis revealed that the ACE T/T 1237 genotype was positively associated (95 % CI=0.1135-0.9281, 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.

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PREGNANCY—CLINICAL

98-LB

Delivery Outcomes in Infants of Women Who Would Be Added to the Diagnosis of Gestational Diabetes by IADPSG Criteria

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Based on the NIH consensus conference regarding gestational diabetes (GDM), it is unclear if women who meet IADPSG criteria should be included as GDM. Therefore, we sought to assess perinatal outcomes with Carpenter/ Coustan (CC) criteria for GDM, those with normal glucose testing, and those who would be added to the diagnosis of GDM by IADPSG criteria. This is a retrospective cohort study of women who underwent screening and diagnostic testing for GDM. Women with preexisting diabetes were excluded. Subjects were divided into non-overlapping groups: GDM by CC, IADPSG GDM criteria but not CC, and normal GDM screening/testing (control). Outcomes included newborn birth weight (BW), BW z-score, ponderal index (PI) and percent >90%ile for gestational age (%LGA). Data was analyzed with one-way ANOVA with Tahmane method for multiple comparisons, t-tests, or Chi-squares. 8390 women were identified with 338 CC; 271 IADPSG; and 7771 controls. Maternal characteristics including age (29.0 vs. 28.5 vs. 25.0 yrs, p<0.01), BMI (35.9 vs. 35.6 vs. 32.3 kg/m^2, p<0.01) and race (Caucasian 41.1% vs. 47.0% vs. 36.4%, p<0.01) differed among the groups. Mean BW (3411 vs. 3240g, p<0.01), BW z-score (0.477 vs. 0.059, p<0.01), PI (2.79 vs. 2.73 g/cm^3, p=0.014), and %LGA (19.9 vs. 8.8%, p<0.01) were higher in IADPSG vs. controls with no difference in gestational age at delivery (39.1 vs. 39.3 wks, p=0.08). Compared to CC neonates, IADPSG had greater BW (3288 vs. 3411g, p<0.01) and gestational age at delivery (38.6 vs. 39.1 wks, p<0.01) with no difference in %LGA (16.0 vs. 19.9%, p=0.20) or BW z-score (0.330 vs. 0.477, p=0.06). Women who would be diagnosed as GDM by IADPSG criteria have newborns with greater BW, BW z-score and %LGA compared to women with normal glucose testing. No significant difference was found in BW z-score or %LGA between GDM by CC criteria or IADPSG.These data support a recommendation to consider including women with IADPSG criteria as GDM assuming that treatment improves outcome.

99-LB

Self-Report and Medical Record Agreement of Pregnancy Outcomes in Women With Diabetes

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Previous studies have shown that self-report may not be as accurate as medical records. The purpose of this analysis was to investigate the agreement between self-report (SR) and medical record (MR) documentation of pregnancy outcomes, e.g., length of hospitalization, infant birth weight, and maternal and fetal complications in women with T1D. An online SR follow-up survey to evaluate long-term reproductive health outcomes is being conducted with women who participated in a preconception counseling (PC) randomized control trial (*READY-Girls*) as an adolescent with a matched comparative group of women with T1D who did not receive PC as teens. Pregnancy outcomes are also being collected from MR. Agreement was assessed using kappa and intra-class correlation (ICC) coefficients. Ninety-one women (49 RCT, 42 matched) were recruited (age range 18-35yrs). Twelve (13.2%) of these women reported 28 pregnancies. These 12 had a mean age of 27.5 (±4.2) yrs., 100% were Caucasian, 25% had some college, and a mean duration of diabetes = 20.3 (±7.1) yrs. Perfect agreement (kappa=1.0) was observed between SR and MR for type of delivery, live birth, and infant birth weight >9lbs. Excellent agreement was observed for baby's actual weight (ICC=.943; mother's self-report was on an average 0.31 lbs lower than MR). Lower levels of agreement were found in duration of hospitalization [mothers reporting longer stays for themselves (ICC=.429) and their babies (ICC=.459)]. There is excellent agreement between the self-report and medical record data for several of the pregnancy outcomes in these participants with T1D. Self-report of pregnancy outcomes should be verified by medical record data for infant birth weight and length of hospitalization, suggesting that reporting of these types of variables may not be interchangeable. Time between delivery and self-report may influence mother's recall and should be included in multivariate analyses.

EPIDEMIOLOGY—AGING

100-LB

The Aging Islet of Langerhans

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The incidence of T2D increases dramatically with age. Because it is unclear how islet-specific factors as opposed to systemic factors (e.g. insulin resistance, vascular senescence) contribute to aging of the islet, we aimed at identifying the intrinsic mechanisms responsible for age-related changes in islet physiology. We compared islets from old (18-months, "old islets") and young mice (2-months, "young islets") and examined their function and structure longitudinally in vivo, in vitro, and after transplantation into young mice. Although glucose tolerance did not change with age, old mice were insulin resistant and had higher plasma insulin concentration under fed conditions. Old islets were larger than young islets but exhibited similar

vascular 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.

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EPIDEMIOLOGY—CARDIOVASCULAR DISEASE

101-LB

Pain Qualities and STEMI: The Croatian Experience: Can Type 2 Diabetics Benefit from Silent Myocardial Ischemia Screening?

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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 significantly later than nondiabetics; 56% 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 (8.1% 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

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This study aimed to evaluate the Finnish Diabetes Risk Score (FINDRISC) in detecting the undiagnosed diabetes and pre-diabetes in U.S. adults and to examine whether there was a gender and racial/ethnic difference. This cross-sectional analysis included 20,633 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, in non-Hispanic White than in Hispanics.

103-LB

Association of White Blood Cells Types With Incident Type 2 Diabetes: The Insulin Resistance Atherosclerosis Study

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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 (S_I) 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

	Neutrophils	Lymphocytes	Monocytes
2-h glucose	0.16 *	0.14 *	0.05
Body mass index	0.13 *	0.23 *	0.08 ‡
Insulin sensitivity index	- 0.21 *	- 0.24 *	- 0.10 †
C-reactive protein	0.28 *	0.17 *	0.12 *

* p <0.001; † p <0.01; ‡ p <0.05

Lymphocyte count predicted incident diabetes, whereas neutrophil and monocyte counts did not. S_I 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

	Adjustment model	1st tertile	2nd tertile	3rd tertile	p for trend
Neutrophils	Non-adjusted	Referent	1.27 (0.80, 2.02)	1.48 (0.94, 2.33)	0.088
Monocytes	Non-adjusted	Referent	1.30 (0.82, 2.04)	1.23 (0.78, 1.95)	0.373
Lymphocytes	Non-adjusted	Referent	1.45 (0.90, 2.33)	1.83 (1.16, 2.91)	0.010
Lymphocytes	Demographic, BMI, and glucose tolerance	Referent	1.48 (0.88, 2.50)	1.77 (1.05, 2.98)	0.034
Lymphocytes	Demographic, BMI, glucose tolerance, and S_I	Referent	1.34 (0.78, 2.32)	1.36 (0.78, 2.37)	0.295

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

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104-LB

An Evaluation of the Ipswich Touch Test for Peripheral Neuropathy Screening in a Developing Country: A Comparative Study

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Diabetic foot ulcers (DFU) are associated with substantial morbidity and mortality in persons with diabetes in in Dar es Salaam, Tanzania. Peripheral neuropathy (PN) is the major risk factor for DFU in this population. Thus, sensitive screening methods are desirable to identify at-risk persons. Recently, 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)

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 lpTT 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 in 1st, 3rd, and 5th toes, heel, and plantar surfaces with a sensitometer. Of 671 individuals screened, 579 (86%) 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, lpTT was 89%, 50%, 38%, and 10%, respectively. Using HCPT as the gold standard, the overall sensitivity of VPT, MF, lpTT was 53%, 42%, and 12%, respectively. The sensitivity of VPT for each DOD quartile was 37%, 41%, 69%; the corresponding sensitivity of MF was 40%, 36%, and 47%, respectively. In conclusion, the lpTT was not useful as a screening test for PN because of relatively low sensitivity. HCPT demonstrated a high prevalence of PN in the diabetes population in Dar es Salaam, did not vary much with duration of diabetes as VPT, and is therefore a better gold standard than VPT or lpTT for ascertaining DFU risk.

105-LB

Pancreatic Regenerating Protein: A New Predictor for the Development of Type 2 Diabetes and Diabetic Chronic Complications

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Type 2 diabetes mellitus (T2DM) is one of the most common non-communicable diseases globally, and its complications result in increasing disability, reduced life expectancy and enormous health costs. However, it is frequently not diagnosed until complications occur. Pancreatic regenerating protein (*reg*) is mitogenic to islet β-cells and associated with inflammation, while no data exist regarding the prognostic value of reg among T2DM patients. The aim of this pilot study was to evaluate the quantity of reg in different clinical stages of T2DM and its correlation with diabetic complications. We analysed serum reg and its correlation with clinical and biochemical parameters in 1004 subjects with T2DM at different clinical phases. reg values were measured by a newly developed ELISA. reg was correlated with the duration of diabetes (spearman's rank correlation coefficient 0.319 p<0.001). Compared to healthy controls, reg levels were elevated in high-risk patients (18.7[15.0-26.4] vs. 16.4[13.9-20.8], p=0.014), and patients with long-term diabetic mellitus (without complications: 26.4[17.4-38.2] vs. 16.4[13.9-20.8] p<0.001; with complications: 32.1[22.1-55.5] vs. 16.4[13.9-20.8] p<0.001). Interestingly, there is no statistically significant differences among the population of high-risk, IGR and incipient diabetic patients. The area under the curve (AUC) of reg for incidence of diabetes-onset and chronic complications were 0.640 and 0.754, separately. Two reg cut-offs potentially allow to identify individuals with a high risk to develop T2DM and its chronic complications. reg levels above 22 ng/ml in nondiabetes were associated with a high risk to develop T2DM in future, levels above 29 ng/ml among T2DM were the most significant parameter to predict the occurrence of diabetic chronic complications. reg might evolve as a promising new marker to predict the occurrence, development of T2DM and diabetic chronic complications.

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EPIDEMIOLOGY—DIABETES COMPLICATIONS

106-LB

Predictors of 30-Day Hospitalization Readmissions in Patients With Type 2 Diabetes Mellitus (T2DM): A Retrospective Database Study

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To understand factors predictive of 30-day hospital readmissions among patients with T2DM, this retrospective study used 2009-2011 deidentified Humedica U.S. electronic medical record data to identify patients ≥21 years old with ≥6 months of data prior to index hospitalization (pre-period) and ≥31 days of data post-discharge (post-period). Stepwise logistic regression, including demographics, clinical characteristics, and index hospitalizations, was used to identify factors associated with readmission. Among 31,615 patients with T2DM and an initial hospitalization for any reason, 3,531 (11.2%) were readmitted within 30 days and 28,084 (88.8%) were not. Most patients were 65 or older (56.4%), female (55.7%), Caucasian (60.6%), resided in the Midwest (50.5%), and were on no observed pre-period antidiabetic treatment agents (76.8%). Diabetic treatment escalation, region, T2DM diagnosis, pre-period congestive heart failure diagnosis, and number of post-period non-inpatient healthcare visits were the strongest predictors of readmission (Table). Other significant predictive variables included length of index stay, A1C testing, and pre-period emergency room visits. These data highlight the importance of appropriate recognition of and treatment for T2DM prior to and during a hospitalization as well as encounters with the healthcare system following discharge in predicting a subsequent hospitalization.

Variable	Total N	% Readmitted (n)	Adjusted OR (95% CI)
Treatment escalation	—	—	*OR vs. no escalation:*
Insulin-to-insulin	2,963	15.2 (450)	3.134 (2.715-3.618)
None-to-insulin	1,282	75.4 (966)	69.225 (59.120-81.056)
None-to-oral	675	28.6 (193)	8.338 (6.893-10.087)
Oral-to-insulin	446	56.7 (253)	35.549 (28.435-44.442)
Oral-to-oral	3,937	4.8 (190)	1.073 (0.905-1.272)
No treatment escalation	22,312	6.6 (1,479)	NA
Region			*OR vs. South:*
Northeast	33	6.1 (2)	1.368 (0.316-5.919)
Midwest	15,981	10.6 (1,689)	1.635 (1.442-1.853)
West	2,620	13.2 (347)	1.201 (1.015-1.421)
Other	1,052	33.2 (349)	4.356 (3.647-5.202)
South	11,929	9.6 (1,144)	NA
T2DM diagnosis status			*OR vs. undiagnosed:*
Diagnosed diabetes	21,074	11.5 (2,432)	0.514 (0.463-0.570)
Undiagnosed diabetes	10,541	10.4 (1,099)	NA
Pre-period CHF diagnosis			*OR vs. no CHF:*
No	28,586	10.3 (2,948)	NA
Yes	3,029	19.2 (583)	1.606 (1.421-1.815)
Variable	**Not readmitted, mean (SD)**	**Readmitted, mean (SD)**	**Adjusted OR (95% CI), not readmitted vs. readmitted**
Pre-period emergency room visits	0.61 (1.72)	0.96 (2.74)	1.058 (1.040-1.076)
Post-period non-inpatient visits	3.85 (4.14)	5.40 (7.07)	1.040 (1.032-1.049)
Index length of stay, days	4.17 (5.04)	5.60 (6.00)	1.039 (1.028-1.049)

Abbreviations: CHF=congestive heart failure; CI=confidence interval; NA=not applicable; OR=odds ratio; SD=standard deviation; T2DM=type 2 diabetes mellitus.

EPIDEMIOLOGY—NUTRITION

107-LB

Vitamin C and E Supplementation and Risk of Type 2 Diabetes in Men: Results from the Physicians' Health Study II Randomized Controlled Trial

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Background: Laboratory experiments and human observational studies have suggested potential benefits of antioxidant vitamins C and E in reducing the risk of type 2 diabetes. Direct evidence from randomized trials has been limited, however, especially among men.

Objective: To assess the direct effect of long-term vitamin C and E supplementation on the incidence of type 2 diabetes in a randomized factorial trial of men.

Design: In the Physicians' Health Study II randomized trial, a total of 14,641 U.S. male physicians initially aged 50 years and older were randomly assigned to receive vitamin C (500 mg ascorbic acid daily) and vitamin E (400 IU synthetic a-tocopherol every other day) or their respective placebos, and followed between 1997 and 2007.

Results: During a mean follow-up of 7.5 years, 775 incident cases of type 2 diabetes were diagnosed based on annual follow-up questionnaires among 13,651 men who were free of diabetes at baseline. There was no overall effect of vitamin C (relative risk [RR], 1.13; 95% CI, 0.97-1.31 [P=0.11]) or vitamin E (RR, 0.94; 95% CI, 0.81-1.09 [P=0.38]) on risk of type 2 diabetes. There was no evidence that diabetes risk factors, including age, body mass index, physical activity, alcohol intake, smoking status, or parental history of diabetes, modified the effects of vitamin C or vitamin E on the risk of developing type 2 diabetes. No significant interactions were observed between vitamin C and vitamin E on diabetes risk. These results remained unchanged after excluding either non-compliant participants or incident diabetes cases that occurred in the first two years of follow-up.

Conclusions: This large-scale, long-term randomized trial showed no overall effects of vitamin C or vitamin E on risk of developing type 2 diabetes in initially healthy men. These data do not support the use of these antioxidant supplements for the prevention of type 2 diabetes.

For author disclosure information, see page LB66.

108-LB

Alcohol Consumption, Plasma Fetuin-A and Risk of Type 2 Diabetes in Women

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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=0.006): Least-squares means±SE 476.8±5.7μg/mL for abstainers, 469.0±5.1 μg/mL for 0.1-4.9 g/d consumers, 456.4±6.9 μg/mL for 5.0-14.9 g/d, and 449.3±9.2 μg/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=0.0008). 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.

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109-LB

Prospective Study of Fast-Food Consumption and the Risk of Gestational Diabetes: The SUN Cohort

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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 1.77 (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.

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EPIDEMIOLOGY—OTHER

110-LB

The Risk of Fractures after Initiating Oral Anti-Diabetic Drugs: Results from the National Claim Registry

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Thiazolidinedione (TZD) increases 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 a 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 (269.6, 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=0.086). 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.

111-LB

Dipeptidyl Peptidase 4 Inhibitors and Comparative Pancreatic Cancer Risk

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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 sitaglitpin 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.

112-LB

Combination Therapy With Metformin Plus Sulfonylureas versus Metformin Plus DPP-4 Inhibitors and Risk of All-Cause Mortality

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Aims: The aim of this study was to evaluate the risk of all-cause mortality for patients exposed to dual therapy with metformin and sulfonylureas (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, 27,251 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=1.265, 95%CI 0.900-1.779). 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.

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113-LB

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

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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 myocardiac infarction, heart failure, cerebral infarction and stroke), malignant tumors and aggravation of diabetic nephropathy 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 baseline, the numbers of patients reaching good glucose control (HbA1c ≤7.0 %) in the low, medium and high educational group were 49.09%, 54.82% and 62.59%, respectively (P<0.001). (2) Logistic analysis showed that after adjusted for confounding factors, educational level was independently associated with glucose control(medium OR=0.772, High OR=0.589, p all <0.05). (3) After 42-month intervention, 2637 people were followed up. Fasting plasma glucose and HbA1c reached the highest in the low educational group (7.51±2.05 mmol/L, 7.20±1.27 %, respectively). The numbers of patients reaching good glucose control (HbA1c ≤7.0 %) in the low, medium and high educational group were 49.09%, 54.82% and 62.59%, respectively. The incidence of end events among the three educational level groups was 4.5%, 2.4% and 1.5%. (4) Cox regression analysis showed that educational level was related to the incidence of end events(medium HR=0.572, High HR=0.351, 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.

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114-LB

Metformin Effects on High- vs. Low-Grade Prostate Cancer

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While several *in vitro* studies suggested that metformin may reduce the risk of prostate cancer (PCa), epidemiologic studies have been inconclusive. A recent case control study suggested that metformin may slow PCa progression. We investigate whether the effect of metformin on low-grade PCa incidence differs from that on high-grade PCa in men with type 2 diabetes (T2DM).

We conducted a historical longitudinal study using a clinical cohort seen at the VA October 2002-September 2012. The cohort included men ≥40 years old, with T2DM, without statin use, and no cancer history. They either had metformin prescriptions ≥180 days, or were never on any diabetes medication. Cox proportional regression adjusting for covariates and the propensity for metformin use was used to estimate the hazard ratio (HR) of PCa due to metformin use.

The cohort included 13409 men with T2DM, where 2490 were metformin users. There were 423 low-grade PCa cases and 87 high-grade PCa cases. Overall, metformin was not increase the risk of PCa (HR=1.07, 95% CI: 0.95–1.21; p=0.266). Metformin was associated with an increased risk of low-grade PCa (HR=1.14, 95% CI: 0.99–1.30; p=0.053), and a decreased risk of high-grade PCa (HR=0.75, 95% CI: 0.55–1.02; p=0.067). The differential effect of metformin on high- vs. low-grade PCa was statistically significant (p=0.007).

The heterogeneity of metformin's pleiotropic effects on high- and low-risk PCa needs to be corroborated by large clinical trials.

	Non-Metformin (n=10919)		**Metformin** (n=2490)	
	mean	*s.d.*	*mean*	*s.d.*
study length (days)	2280.60	1300.36	2266.31	1146.93
low-grade PCa (%)	3.10		3.41	
high-grade PCa (%)	0.66		0.60	
age	69.85	10.87	67.23	11.06
Black (%)	18.12		19.00	
Hispanic (%)	4.77		7.07	
baseline A1c	6.34	1.35	6.59	0.95
mean A1c	6.29	1.34	6.48	1.27
baseline LDL	100.59	28.26	95.84	25.82
mean LDL	91.60	27.16	88.69	24.49
baseline BMI	30.31	62.25	30.79	6.11
mean BMI	29.25	62.00	29.30	6.19
baseline comorbidity	4.75	2.77	3.87	2.20
mean comorbidity	3.62	2.69	3.37	2.60

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EPIDEMIOLOGY—TYPE 1 DIABETES

115-LB

Prevalence of Diabetes in Veterans Stratified by Gender

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Background: Despite the large number of epidemiological data available on the high prevalence of diabetes, a paucity of studies explored the risk of diabetes in the veteran population.

Methods: The Integrated Health Interview Series (IHIS) is a harmonized data for the U.S. National Health Interview Survey (NHIS). Based on the IHIS 1997-2010 dataset, population-based prevalence of diabetes among veterans were compared to non-veteran civilians, further stratified by gender. Regression models were performed to evaluate the association between the veteran status and the risk for diabetes. Veteran status was defined if a participant answered 'Yes' to the question, "Have you ever been honorably discharged from active duty in the U.S. Army, Navy, Air Force, Marine Corps, or Coast Guard?" Diagnose of diabetes was self-reported including those who were in the borderline. Statistical analyses were performed by SAS version 9.0.

Results: From 1997 to 2010, overall 13.8% of veterans reported to have diabetes compared to 7.7% of non-veteran civilians (Rao-Scott chi square, p<.0001). Among men, 14.3% of veterans and 6.6% of civilians had diabetes (Rao-Scott chi square, p<.0001). For females, prevalence of diabetes (6.7%) was lower in veterans than civilians (8.4%) with statistical significance. Adjusting for age, race, marital status, Body Mass Index (BMI), alcohol drinking, education, poverty level, smoking and exercise, a regression model showed that male veterans were 1.4 times more likely to have diabetes than

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: 0.7-1.1).

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

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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 28,727 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 (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): 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).

Rs1556516, a proxy for the known CAD SNP rs1333049 in the well-established 9p21 locus, had a smaller OR in patients with T2D (T2D OR=1.10, p=3.9E-03 and non-diabetic OR=1.17, p=2.9E-10) and this was nominally significant for interaction (p=4.2E-02).

Investigation of the known T2D-risk loci revealed that the major allele (frequency=0.89) of rs7612463, in *UBE2E2*, decreased the risk of CAD in patients with T2D (OR=0.86, p=7.4E-03) but increased the risk of CAD in non-diabetic individuals (OR=1.11, p=3.7E-02). This interaction was significant after Bonferroni correction (p=7.2E-04).

This study suggests known CAD SNPs in *ADAMTS7* and 9p21, and known T2D SNPs in *UBE2E* may differentially modify CAD risk based on T2D status.

Supported by: Innovative Medicines Initiative

117-LB

eQTLs from Skeletal Muscle and Adipose Tissue Account for Most of the Heritability to Type 2 Diabetes Estimated in Mexican Americans, Mexicans and Europeans

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Previous studies have shown that top signals from genome-wide association studies (GWAS) on type 2 diabetes (T2D) are enriched for expression quantitative trait loci (eQTLs) identified in skeletal muscle and adipose tissue. We therefore hypothesized that such eQTLs might account for a disproportionate share of the phenotypic variance in liability to type 2 diabetes (T2D) estimated from all SNPs interrogated through GWAS. To test this hypothesis, we applied genome-wide complex trait analysis (GCTA) to GWAS on T2D from Mexican Americans living in Starr County, TX (SC), and Mexicans from Mexico City (MC), as well as to the GWAS on T2D from the WTCCC on subjects from the UK. We estimated the proportion of phenotypic variance attributable to additive effects of all variants interrogated in these GWAS (i.e. chip-based heritability), as well as from a much smaller set of variants identified as eQTLs for muscle or adipose tissue. Estimates of chip-based heritability were appreciable and statistically significant in all 3 samples (see table). Interestingly, the smaller sets of muscle and adipose eQTLs accounted for more of the variance in T2D liability (with stronger statistical significance) in the SC and MC GWAS than was observed using all SNPs. These results support our hypothesis that common eQTLs mapped in insulin-responsive tissues account for a substantial portion of the variance in liability to T2D.

Dataset	Cases (n)	Controls (n)	Total ± SE (%)	Muscle ± SE (%)	Adipose ± SE (%)	Percent of Muscle (Adipose) eQTLs out of Total Set
SC	837	781	58 ± 16	75 ±16	63 ± 16	15 (18)
MC	965	345	77± 23	84 ± 22	75 ± 23	16 (18)
WTCCC	1924	2938	55 ± 5	39 ± 4	40 ± 5	15 (17)

118-LB

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

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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 recombineered additional copies of Tcf7l2 into the mouse leading to global overexpression. Intraperitoneal 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 over-expression. Perifusion 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 [HDL/LDL/TC/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.

Joint analysis enabled identification of 26 signals with genome-wide significant (P_{LRT}<5.0x10^{-8}) multi-trait effects, including 9 loci with associations (P<5.0x10^{-8}) driven by the individual trait effects: a) *TRIB1* on BMI, b) *GCKR*, *FADS1*, *MLXIPL* on TG; c) *CEPT* on BMI/HDL, d) *LPL, APOA1* on BMI/TG; e) *LIPC* on HDL/TG, f) *APOE* on HDL/LDL/TG. At four loci, where association with obesity was identified for the first time, higher BMI was related to higher HDL and lower TG indicating complex relationships between adiposity and regulation of lipid levels. Effects on lipids at *CELSR2, ABCA1*, *HNF4A*, *MADD*, *PPP1R3B* and *RANB10* were determined by the association with HDL. At the remaining 11 pleiotropic loci multiple traits contributed to the signal.

We detected a substantial proportion of loci with complex patterns of genetic effects, highlighting the importance of modelling multiple T2D-related metabolic traits simultaneously for dissection of association signals.

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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 C57BL6 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. On glucose tolerance testing (GTT) in 8-week old females, area under the curve was 17% larger for P2x7-C57 vs. P2x7-129 mice (P<0.05); and at 16-20 weeks, 13% larger (P<0.05). On insulin tolerance testing (ITT), area above the curve was 27% larger (P<0.005) for P2x7-C57 females. Similar results on GTT and ITT were seen for males.

In humans, we mined the Meta-Analysis of Glucose and Insulin-Related Traits Consortium (MAGIC) and Diabetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium databases to examine purinergic signalling genes for association with glycemic traits and type 2 diabetes risk, respectively. We found associations of one SNP each in 5 genes with glycemic traits and 14 SNPs in 3 genes withT2D risk, using an a priori significance level based on the number of independent tests per gene. Testing these along with common missense variants in the Botnia Primary Prevention Program (n=3,000), we found a strong association for rs1718119 in P2RX7: carriers of the minor (A) allele have increased insulin sensitivity, increased insulin release, and improved disposition index. This SNP encodes an A348T amino acid change shown to have increased pore function compared to the major allele.

Together, our data show association of the purinergic signaling pathway in general and of hypofunctioning P2X7 variants in particular with impaired glucose homeostasis in both mice and humans.

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121-LB

Smoking-Genotype Interaction in Type 2 Diabetes Risk: The Framingham Heart Study

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Smoking is an important behavioral risk factor for type 2 diabetes (T2D), but not all smokers get T2D. Small studies have reported certain gene-smoking interactions that modify T2D risk. We performed a genomewide-by-environment interaction study (GEWIS) in the Framingham Heart Study (FHS), a community-based cohort study, to test whether smoking interacts with genetics to influence T2D risk and to identify genetic loci where such interaction occurs. We defined incident T2D as a fasting plasma glucose ≥7.0 mmol/L or use of diabetes medications and excluded T2D at baseline. We categorized baseline smoking status as never, current, or former based on validated self-report. Blood samples were genotyped on the Illumina CARe 50K iSelect chip. We performed a GEWIS across all single-nucleotide polymorphisms (SNPs) using Cox regression accounting for relatedness, modeling hazard of incident T2D as a function of age, sex, 4 principal components, smoking category, SNP, and smoking*SNP interaction. We used a joint test of (SNP + SNP*smoking) to test the hypothesis that genotype itself or its interaction with smoking increases risk for T2D, with p<2*10^{-5} defining statistical significance for the joint test. Among 2,987 individuals, 1,024, 842, and 1,121 were never, current, and former smokers, respectively. Over 2 to 24 years, there were 431 cases of incident T2D. No SNP met significance for SNP*smoking interaction, but four independent SNPs met significance for the joint effect: rs3091258 at the *TNF-LTB* locus, rs9267490 in *NFKBIL1*, rs1115764 in *SEZ6L*, and rs1019856 in *TGFBR2*. For rs1019856 in *TGFBR2*, the SNP main effect (p=9.8*10^{-6}) seemed to drive this joint effect, but SNP*smoking interaction (p 3.3*10^{-5} to 5.8*10^{-4}) seemed to drive the joint effect of the other three. Variants in *SEZ6L* 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, N=96,496 EU in 29 cohorts) and m-a the two results files using MANTRA, a Bayesian method accounting for allelic heterogeneity among population clusters that returns a Bayes Factor, with (logBF) &gt 6 suggesting g-w SNP-FG association. We evaluated associations for 23 known FG loci by testing reported Index SNPs (Dupuis 2010, PMID 20081858, Manning 2012) and also finding the Best SNP within +/- 250 kb of the Index SNP. We sought new loci for replication based on low METAL P values in AA and high MANTRA logBF in AA plus EU.

For 23 known FG loci, 1 Index (MTNR1B) and 1 Best SNP (GCK) were g-w significant (P &lt 2.5x10-8) in AA and 8 Index and 23 Best SNPs were nominally significant (P &lt 0.05). At 10/23 loci the r2 for Index vs. Best SNP was &lt 0.2. Seven AA loci had a SNP P &lt 10-6 and logBF &gt 6 and 7 more had a SNP P &lt 10-5 and logBF &gt 4, giving 14 high-interest SNPs to test for replication.

All 23 FG loci known in EU show at least nominal association in AA, suggesting some genetic determinants of FG are likely similar across AA and EU, but with allelic heterogeneity at many FG loci. Combining fixed effects m-a in an AA sample with trans-ethnic m-a in an AA-EU sample has identified a wealth of interesting new AA FG SNPs to test for replication.

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123-LB

Using the "Gene Mine" to Identify Novel Diabetes-Susceptibility Loci

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The Collaborative Cross is a next-generation genetic resource, developed to simplify discovery of genes controlling complex genetic traits. It consists of hundreds of inbred mouse strains descended from eight genetically diverse founders. To identify genetic loci associated with Type 2 diabetes susceptibility, we characterised basal blood glucose levels and insulin sensitivity using an insulin tolerance test (0.75IU/kg) in 50 Collaborative Cross strains followed by genetic linkage analysis using HAPPY modified for the Collaborative Cross. Strains with a high basal blood glucose level (above 20 mM) and thus strong genetic predisposition to β-cell dysfunction were further characterised using intravenous and oral glucose tolerance tests (IVGTT 1g/kg and OGTT 2g/kg). Linkage analysis localised several hotspots for diabetes susceptibility with 90% confidence. One locus was mapped to chromosome 8 with genome-wide significance at 95% confidence (F statistic p<0.05). Glucose tolerance tests in those with the high basal blood glucose showed several strains had abnormal glucose tolerance. Two of these strains provide novel mouse models of Type 2 Diabetes susceptibility with reduced insulin secretion. Our results validate the utility of the Collaborative Cross as a powerful genetic resource for the identification of diabetes susceptibility loci and for the generation and characterisation of novel mouse models for the study of Type 2 Diabetes.

IMMUNOLOGY

124-LB

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 PEPITEM (PEPtide Inhibitor of Trans-Endothelial Migration). Interestingly, PEPITEM did not act directly on T cells; rather it stimulated EC to release the lipid mediator sphingosine 1 phosphate, which in turn inhibited T cell migration. Synthetic PEPITEM could also effectively inhibit T cell migration in vitro (EC50=19pM). In zymosan induced peritonitis, T cell recruitment was significantly increased in mice lacking B cells when compared to wild type animals. This excess of T cell recruitment was ameliorated by treatment with PEPITEM 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 PEPITEM to T1D lymphocytes re-established the block on transmigration. We hypothesise that modulating the PEPITEM pathway has therapeutic potential for T1D.

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125-LB

WITHDRAWN

126-LB

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-65 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-65 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-65 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, antiproliferative, and immunomodulatory effects and its use has been associated with autoimmune diseases such as multiple sclerosis, systemic lupus erythematosis, thyroid disease, and Type 1 diabetes (T1D Subjects with IFN-associated diabetes have higher levels of C-peptide and GAD-65 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.

127-LB

NCoR KO Reprograms Macrophage Lipid Metabolism Increasing ω3 Fatty Acid Synthesis and Insulin Sensitivity

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Macrophage-mediated inflammation is a 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 de-repression 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.

128-LB

Augmentation of Leptin Receptor Signaling by Loss of Socs3 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 (SOCS3) is a key negative feedback regulator of ObR-mediated signaling in the hypothalamus. We now show that gastrointestinal epithelial cell-specific SOCS3 conditional knockout (T3b-SOCS3 ckO) mice developed gastric tumors by enhancing leptin production and the ObRb/signal transducer and activator of transcription 3 (STAT3) signaling

pathway. All T3b-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 T3b-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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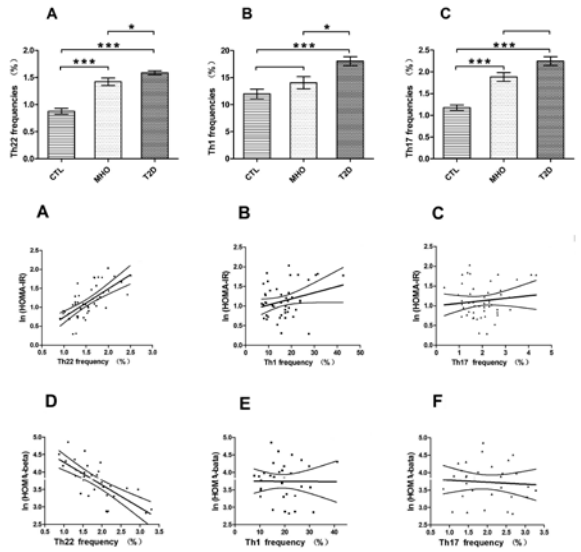
The chronic low-grade inflammation has long been recognized as the central link between obesity associated insulin resistance and type 2 diabetes. In light of the role of Th22 cells in the pathogenesis of chronic inflammation, we first identified increased frequencies of Th22 cells in peripheral blood 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 important role in the development of obesity associated insulin resistance and disease progression to type 2 diabetes.



Supported by: Shandong University

130-LB

Hyperglycemia Inhibits Complement-Mediated Immunological Control of S. Aureus in a Rat Model of Peritonitis

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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 toxin C5a. Thus, complement defenses were inhibited, which correlated with increased bacterial survival of neutrophil-mediated killing. These findings suggested that high-glucose inhibition of complement effectors against S. aureus may contribute to an increased risk of and severity of infections caused by S. aureus for patients with diabetes. We now report preliminary data testing whether hyperglycemia would inhibit complement-mediated control of S. aureus infection in a rat peritonitis model. Thirty rats were treated with streptozocin to induce diabetes and 10 were sham treated. After stable hyperglycemia was achieved, all rats were inoculated i.p. with 1 × 108 CFU S. aureus. After 2 hours, 20 rats (10 streptozocin-treated, 10 sham) were euthanized and subjected to peritoneal wash. Ten of the remaining 20 hyperglycemic rats were then rescued with insulin (NPH). These 20 rats were euthanized at 24 hours and subjected to peritoneal wash. The peritoneal concentration of the classical/lectin cascade component C4 and the central complement cascade component C3 were both increased in euglycemic rats at 2 hours as well as in the insulin-rescued rats at 24 hours compared to hyperglycemic rats. Peritoneal concentrations of the anaphylatoxin C5a was > 2-fold higher for insulin-rescued rats compared with hyperglycemic rats. These findings correlated inversely with colony counts of S. aureus recovered from the peritoneum. Thus, there were decreased bacteria in euglycemic and insulin-rescued rats compared to hyperglycemic rats.

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, 9 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 insulitis, 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 iAbs 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

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

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We have previously shown that a single injection of allogeneic newborn blood, functionally equivalent to umbilical cord blood, into un-preconditioned prediabetic 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. 184:3008-15). 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 CD62L, CD44 or FoxP3. Both the ability of $CD4^+CD25^+FoxP3^+$ T regulatory cells to exert suppression 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 *Cela3b* and enhancement of *Mif*, respectively implicated in diabetes manifestation and protection by our recent transcriptome analysis (Jayaraman et al. 2013; PloS One, 8: e55074). 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 CDs 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-/-STZ mice, but the anti-inflammatory cytokines was higher than WT STZ 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, TLR-2 and TLR-4 either in spleen or pancreatic islets Mo and CDs. 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 CDs 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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TRANSPLANTATION

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 8 (AAV8) 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 autoimmunity-mediated insulitis in PDL1-CTLA4Ig expressing islets. We then analyzed the effects of PDL1-CTLA4Ig expression on rejection of allo-islets in MHC-matched recipient mice. Streptozocin (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 alginate-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 transplant. The present study demonstrated the utility of the beta cell-targeted AAV8 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.

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136-LB

The Potential Contribution of Beta-Cell Purinergic Signaling and Ectonucleotidases in the Pathophysiology of Diabetes: Preliminary Rodent Study

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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 24hr 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 pancreas of C57BL/6, prediabetic NOD (pNOD) and NOD.SCID mice 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 6.1% 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; CD39+ was similar in all strains (40.1%, 42.5%, and 51.8%, respectively). CD31+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 CD3+ cells. ATP/P2X and E-NTPDases may contribute to islet immunity. Their modulation could be appealing to preserve β-cells in diabetes.

TABLE 1: FACS PROFILE IN VIABLE β-CELLS

TREATMENT	R2D6+P2X7+	R2D6+CD39+	R2D6+CD73+	R2D6+MHC-I+
CONTROL	6.8%	1.7%	21.4%	2%
IFN-γ (1000U/ml)	11.5%	6.5%	36%	46.3%
IL-1β (50U/ml) IFN-γ (100U/ml) TNF-α (2000U/ml)	17.8%	8.1%	54.7%	17.1%
HIGH GLUCOSE (25mM)	0.6%	4.6%	47.5%	1.6%

Supported by: DRIF

INSULIN ACTION—ADIPOCYTE BIOLOGY

137-LB

AKT2 Is Not Required for Insulin Regulation of Lipolysis In Vivo

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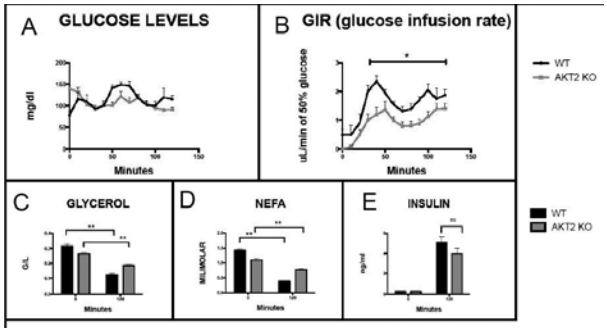
Regulation of lipolysis is under tight hormonal control mainly by catecholamines and insulin. The general consensus is that insulin antagonizes catecholamine-activated lipolysis through Akt phosphorylation and activation of PDE3B. Recently, this hypothesis was challenged as in vitro studies demonstrate 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 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.



Hyperinsulinemic Euglycemic Clamp. WT n=6 and AKT2 KO n=8.

NEFA – Non Esterified Fatty Acids

* = p<0.05; ** = p<0.005

INSULIN ACTION—CELLULAR AND MOLECULAR METABOLISM

138-LB

Amyloid β_{42} Administration Impairs Energy Metabolism *In Vivo* and *In Vitro*

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Amyloid β_{42} ($A\beta_{42}$) is a protein implicated in Alzheimer's Disease (AD), in part through causing impaired neuronal metabolism. Obese and diabetic patients have increased circulating $A\beta_{42}$, yet it is unknown whether circulating $A\beta_{42}$ contributes to altered metabolism in these conditions. The aim of this study was to determine whether $A\beta_{42}$ alters metabolism of insulin sensitive cells *in vitro*, and whole body metabolism *in vivo*. Monomeric $A\beta_{42}$ ($mA\beta_{42}$) increased glucose production in FAO hepatocytes, while aggregated $A\beta_{42}$ ($aA\beta_{42}$) had no effect. Similarly, $mA\beta_{42}$ impaired glucose uptake in 3T3-L1 adipocytes, while $aA\beta_{42}$ had no effect. We next investigated the effect of $mA\beta_{42}$ or scrambled $A\beta_{42}$ (control) administration to mice (1µg / day; I.P. injection) over 2 weeks. Administration of $mA\beta_{42}$ had no effect on bodyweight or food intake compared with control mice. However, administration of mAβ42 reduced oxygen consumption and total carbohydrate oxidation compared with control animals ($p \leq 0.05$).

This data shows that monomeric $A\beta_{42}$ impaired glucose metabolism while aggregated $A\beta_{42}$ had no effect. This data suggests that not only is $A\beta_{42}$ 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 $A\beta_{42}$ levels are elevated.

Supported by: NHMRC

INSULIN ACTION—GLUCOSE TRANSPORT AND INSULIN RESISTANCE IN VITRO

139-LB

Physiological Hemodynamics and Transport Restore Insulin and Glucagon Responses in a Normal Glucose Milieu in Hepatocytes *In Vitro*

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The use of primary hepatocytes as an in vitro tool for drug discovery and development in diabetes research is severely limited by their lack of insulin sensitivity. Routine culture conditions for hepatocyte survival in vitro mandate super-physiological levels of insulin and glucose in media, confounding their applicability and relevance. We previously described a system that applies physiological hemodynamics and transport to restore and retain hepatocyte phenotype, resulting in drug and hormone responses at in vivo levels. We cultured rat and human hepatocytes in this system at physiological insulin and glucose levels before testing them for insulin/glucagon responsiveness. The regulation of gluconeogenesis was tested by addition of substrates lactate/pyruvate or glycerol in the presence of glucagon or insulin, and measuring glucose in supernatants. Glucagon increased glucose output by 50-80% while insulin was seen to inhibit it by 25-40%. Hepatocytes were then cultured in the presence of high or low glucose/insulin levels for 7 days. Lipid accumulation (nile red stain), total triglycerides and metabolic activity (p450 glo assays) were assessed. High glucose/insulin levels resulted in a steatotic state characterized by lipid accumulation and increased triglycerides (3 fold) along with a reduction of CYP3A2 and CYP1A1 activity (3- 6 fold) relative to low glucose/insulin conditions. These changes were prevented by concomitant administration of pioglitazone (1.5µM). In conclusion, we demonstrate a novel liver platform that maintains hepatocytes at physiological glucose and insulin levels retaining their responsiveness to insulin and glucagon. A high glucose/insulin insulin environment in the system induces steatotic changes along with metabolic suppression similar to clinical non alcoholic fatty liver disease, which are preventable by administration of Pioglitazone at plasma Cmax levels.

INSULIN ACTION—SIGNAL TRANSDUCTION, INSULIN, AND OTHER HORMONES

140-LB

LRP6 Mutation Alters Expressions of Insulin and IGF Receptor and Causes Insulin Resistance in Humans

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We have identified a large kindred in whom a non-conservative mutation (R611C) in the Wnt co-receptor LRP6 underlies the development of autosomal dominant early onset CAD, type 2 diabetes and metabolic syndrome. Healthy nondiabetic LRP6 mutation carriers exhibited insulin resistance compared to noncarrier relatives during oral glucose tolerance test. The skeletal muscle biopsies and skin fibroblasts of the mutation carriers showed diminished 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 IGFR expression and subsequent activation of ERK1/ERK2 in LRP6R611C compared to wildtype fibroblasts. Further investigations revealed posttranscriptional regulation of IGFR by LRP6. In IGF-1 treated LRP6 knockdown cells IGFR was stabilized by sumoylation. 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-Inhibited Insulin Signaling Pathway

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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), Diphenyleneiodonium (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. ANG II-stimulated phosphorylaion 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 PKC δ which was inhibited by SGK inhibitor, GSK 650394. From these results, we conclude that ANGII-stimulated SGK/PKCδ/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 (AIRarg) and glucose-potentiated (AIRargMAX) 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. AIRarg was determined in first 5 min post Arg followed by a 60 min infusion of glucose and repeat injection of Arg (AIRargMAX). AIRargMAX - AIRarg = Insulin Secretory Reserve (ISR). All AIR parameters are adjusted for basal insulin (AIR/[basal insulin] in μU/mL). Table includes intraclass correlation coefficient (ICC), a measure of R. ICC > 0.8 = high R.

	N	BMI	AIRarg	AIRarg MAX	ISR	ICC AIRarg	ICC AIRarg MAX	ICC ISR
		(mean±SD)	Geometric mean (95% CI)	Geometric mean (95% CI)	Geometric mean (95% CI)			
NGT	23 (12M/11W)	31.5±2.8	9.9[a] (8.8,11.1)	47.9[a] (41.9,54.8)	37.6[a] (32.5,43.5)	0.93	0.94	0.87
PDM	8 (2M/6W)	33.0±2.6	5.9[b] (5.3,6.5)	23.6[b] (21.1,26.5)	17.6[b] (15.3,20.2)	0.92	0.87	0.96
T2DM	22 (11M/11W)	32.8±3.9	4.0[c] (3.6,4.3)	8.7[c] (7.9,9.6)	4.6[c] (4.0,5.3)	0.91	0.79	0.95
	ANOVA across populations (*P*). Superscripts that differ from one another indicate statistically separable parameters, *P*<0.005.		<0.001	<0.001	<0.001			

AIRarg, AIRargMAX, and ISR differ across the 3 populations (all *P*<0.001). Overall, AIRs had high R. AIRarg also correlates with AIRargMAX within each group and across all 3 populations (r=0.98), suggesting that the basal responses track with glucose-potentiated, 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

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 (PDM) to type 2 DM (T2DM) and to compare the BCF and IS responses to those from the FSIGT.

All subjects were tested after an overnight fast on 3 separate days. Each subject had 2 MMTTs and 1 FSIGT. Parameters for BCF were estimated using published methods. The table summarizes sample size, BMI, and respective MMTT/FSIGT measures for: IS (Si); insulin release (Φtot and AIRg); and disposition index (DItot and DI) by group. MMTT parameters and FSIGT Si summarized as geometric means (95% CI); all others as arithmetic means (95% CI).

			Insulin Sensitivity		Insulin Secretion		Disposition Index	
	N	BMI (kg/m2)	MMTT Si (10^{-4}/ ((μU/ml)(min))	FSIGT Si (10^{-4}/ ((μU/ml) (min))	MMTT Φtot (10^{-9}/min)	FSIGT AIRg (μU min/ ml)	MMTT DItot (10^{-13}/ ((μU/ml)(min^2))	FSIGT DI
NGT	23 12M/11W)	31.5 (30.3-32.7)	4.5[a] (3.83-5.33)	1.6[a] (1.23-2.08)	102.6[a] (89.0-118.4)	914[a] (594-1235)	464[a] (375-573)	1339[a] (970-1709)
PDM	8 (2M/6W)	33.0 (31.0-35.0)	2.3[b] (1.75-3.04)	1.2[a] (0.9-1.59)	108.4[a] (93.8-125.3)	412[b] (91-733)	250[a] (190-329)	520[b] (124-915)
T2DM	22 (11M/11W)	32.8 (31.1-34.5)	1[c] (0.74-1.35)	0.49[b] (0.29-0.8)	15.4[b] (12.8-18.6)	10.4[b] (-8.7-29.6)	16.8[b] (11.3-24.9)	9.3[b] (-13.8-32.5)
	ANOVA across populations (P) Superscripts that differ from one another are statistically separate for that parameter, *P*<0.05.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

ADA-Funded Research

For author disclosure information, see page LB66.

For MMTT and FSIGT, Si, Φtot and AIRg, and DI, values decline from NGT to T2DM (aII P<0.001). Correlation analysis for each MTT/FSIGT parameter pair ACROSS ALL 3 GROUPS: Si (r=0.69); Φtot/AIRg (r=0.73); and DItot and DI (r=0.74), suggesting that values from the tests tracked similarly across GT states. In conclusion, modeled results from the MMTT correspond to FSIGT-derived parameters across range of responses of GT spectrum.

Supported by: FNIH Biomarkers Consortium Beta Cell Project

145-LB

Arginine (Arg) Is Preferred to Glucagon (Glgn) in Stimulation Testing for Beta Cell Function (BCF)

DAVID A. FRYBURG, R. PAUL ROBERTSON, RALPH H. RAYMOND, DOUGLAS S. LEE, FOR BETA CELL TEAM OF FNIH BIOMARKERS CONSORTIUM, *East Lyme, CT, Seattle, WA, Skillman, NJ, Cambridge, MA*

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 objectives 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/m^2)) subjects (n=23 (12M/11W)) with normal glucose tolerance were studied twice with Arg or Glgn in a randomized cross-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.

	AIRarg	AIRarg MAX	ISRarg	AIRglgn	AIRglgn MAX	ISRglgn
Insulin (μU/mL) geometric means (95% CI)	95 (85,106)	460 (392,541)	361 (302,431)	113 (101,127)	606 (512,718)	465 (347,624)
ICC	0.93	0.92	0.91	0.82	0.75	0.32

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 to AIRglgnMAX r=0.84). 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

146-LB

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 ng/mL), samples and controls (5 μL ea) 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 r^2 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.

INTEGRATED PHYSIOLOGY—LIVER

147-LB

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 stearoyl 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 (htgCB1$^{-/-}$), but not in global CB1 knockout (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 URB597. 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.

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148-LB

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 triglyceride (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 (63%), 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.

149-LB

Dietary Iron Regulates the Circadian Rhythm of Hepatic Gluconeogenesis 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-Erbα with its cosuppressor NCOR. 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 gluconeogenic enzymes seen with variation of dietary iron. The differences among diets are also lost with inhibition of heme synthesis by treatment with isonicotinylhydrazine. Heme levels respond to dietary iron through modulation of the level of Peroxisome Proliferator-Activated Receptorγ 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.

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150-LB

Hepatic Fat Is a Determinant of Heaptic Insulin Sensitivity in Pre-Diabetes

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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/m^2, LBM 48±8 kg) with a 3 hour labeled [6,6-$^2H_2$ glucose] 75 g oral glucose tolerance test. [6,6-$^2H_2$ glucose] enrichment was measured by GCMS. Endogenous glucose concentration was calculated by established methods. 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_{Liver}) was determined by both model independent (iAUC endogenous glucose/iAUC insulin) method and our validated oral model(1). Total hepatic FA was negatively correlated to SI_{Liver}, the latter measured with either model independent (r=0.64; p<0.02) method or with the model (r=0.6; p<0.03). These data demonstrate that hepatic fatty acid content is a determinant of hepatic insulin sensitivity in individuals with prediabetes. Future studies are needed to determine whether interventions that lower hepatic fatty acid content alter hepatic insulin action.

1. Dalla Man C, Toffolo G, Basu R, Rizza RA, Cobelli C: Use of labeled 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

151-LB

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.

152-LB

Glycine Sensing in the Dorsal Vagal Complex Lowers Food Intake

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Glycine 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 stereotaxic 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 µmol/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 (766±29 vs. 1906±153 µmol/L, P<0.005), comparable to the increase in DVC tissue glycine levels achieved by direct DVC glycine administration. Dietary glycine supplement for 24 h lowered food intake by 25% compared with regular drinking water (17.0±1.9 vs. 22.6±0.9 g, P<0.04, n=5/group). Chronic glycine intake for 8 d lowered cumulative 4-d food intake by 5% (P<0.05).

We provide novel evidence that glycine triggers a sensing mechanism in the DVC to reduce appetite. Importantly, dietary glycine suppresses appetite in diet-induced obesity. These findings suggest that glycine or a glycine analogue may have nutraceutical benefits to lower food intake in obesity by triggering the CNS.

Supported by: CIHR

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 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 ablated 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). 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. DT-treated controls. Our results indicate that SFAs stimulate microglia-dependent hypothalamic

inflammation and reveal a novel way to modulate microglial content as a tool to test their role in diet-induced hypothalamic inflammation, neuronal injury, and leptin resistance.

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154-LB

Hypothalamic Mitochondrial Superoxide Accumulation Accompanies Inflammation After Short Term Exposure to High-Fat Diet in Rats or Palmitate in Clonal Hypothalamic Neurons

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Dietary saturated fat intake leads to low-grade hypothalamic inflammation and is associated with CNS hormone resistance and impaired energy balance regulation in rodents. Mitochondrial oxidative stress promotes inflammation and insulin resistance in numerous peripheral tissues of obese mice, by activating a family of redox sensitive inflammatory kinases including the c-Jun N-terminal kinase (JNK) pathway. The role of oxidative stress in high-fat (HF) induced hypothalamic inflammation has not been reported. We hypothesized that oxidative stress is an early feature of diet-induced obesity (DIO) that occurs within the first days after exposure to excess dietary fat. Long Evans rats were fed a lard based high-saturated fat (HF: 45% kcal fat) or low-fat (LF: 10% kcal) control diet for 1 week. HF diet consumption increased hypothalamic phosphorylation of JNK by 1.38±0.14-fold and elevated mRNA expression of the pro-inflammatory cytokine gene IL-6 by 4.0±0.8-fold. We determined hypothalamic superoxide (O2-) levels using the mitochondrial O2- specific probe MitoSOX and HPLC. Hypothalamic O2- levels increased by 2.2-fold in rats fed HF diet (LF: 6,158±1,380 vs. HF: 13,754±1,745; p<0.001). We extended these findings in an in vitro setting, using the clonal mouse hypothalamic cell line mHYPO E-42 treated with the saturated fatty acid palmitate (P) or vehicle (BSA 100uM) for 12 hours. Palmitate treatment (100uM) increased O2- by 1.45±0.07-fold and increased JNK phosphorylation by 1.29±0.09-fold. Reducing O2- production with oleate or MitoTEMPO (25nM) prevented palmitate induced inflammation and insulin resistance in vitro. Collectively, this data provides evidence that mitochondrial O2- accumulation is a feature of early DIO that may contribute to hypothalamic inflammation. More work is needed to ascertain the role of ROS in hypothalamic hormone resistance associated with the development of DIO.

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155-LB

Cardiometabolic Dysfunction After Just 2 Weeks of High Fat Feeding

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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% carbohydrate, 32% fat and 28% protein. For fat feeding, 6g/kg of rendered pork fat was added to control chow for a final composition of 52% fat. Food was presented each day from 9a-10a. Dogs were presented with 3,578 calories/day during control and 5,025 calories/day during fat feeding.

High fat feeding resulted in significant weight gain (5% at W2, 8% at W6, 13% at W24; p<0.001) in both visceral and subcutaneous depots (by MRI), as well as decreases in insulin sensitivity (-18%, -21% and -41% at W2, W6 and W24, respectively and reaching significance by W6; p<0.05). Left ventricular function (circumferential strain by tissue tagging) was also impaired with high fat feeding, with the largest decrease in function occurring at 2 weeks (-18% at W2, -26% at W6, -16% at W24 [n=4 for W24 cardiac data]; p<0.05). No changes were observed in left ventricular mass, dimensions, blood pressure or heart rate.

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.

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156-LB

Differential Effects of Sleeve Gastrectomy and Duodenojejunal Bypass on Fat Preference

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Sleeve gastrectomy (SGx) and Duodenojejunal bypass (DJB) are commonly performed bariatric procedures. In SGx, over 80% of the stomach is removed without surgical reconfiguration of the intestine, whereas in DJB the stomach is intact but ingested food bypasses the duodenum and upper jejunum. An important question is whether altered size of the stomach or rearrangement of the small intestine is more important in treating obesity and associated disorders. Thus, comparing these two operations in one experiment provides a unique opportunity to determine the relative importance of different segment of the gastrointestinal tract (GI) in the regulation of body weight, food intake and metabolism. We therefore performed SGx, DJB and sham procedures in SD rats fed a chow diet in order to minimize any changes of body weight. Neither SGx nor DJB induced significant weight loss or changes of resting or fasting energy expenditure relative to sham surgery over 20 weeks in these chow-fed rats. Additionally, SGx and DJB rats had similar daily food intake, and it was comparable to that of sham-operated controls. However, compared with sham-operated rats, SGx rats had a significantly reduced preference for Intralipid concentrations of over 10% as assessed using 2-bottle preference tests ($P < 0.001$). In contrast, DJB rats had comparable lipid preference as sham-operated controls. SGx but not DJB also significantly reduced consumption of a high-fat solid food ($P < 0.05$) and consequently had increased intake of standard low-fat chow consumption compared with sham controls ($P<0.05$). Thus, the present results demonstrate that loss of the stomach may underlie reduced dietary fat preference. The role of fat preference in mediating long-term maintained weight loss after SGx requires further investigation. Understanding the different physiologic mechanisms of these surgical procedures may help development of less invasive treatments against obesity and associated complications.

157-LB

Chronic Advanced Glycation Endproducts (AGE) Exposure Promotes Spinal Degeneration in Insulin-Resistant Non-Hyperglycemic Mice

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Intervertebral disc (IVD) degeneration is a major cause of back pain. Modern diets contain high levels of advanced glycation end products (AGEs), contributors to diabetes and aging complications. We previously showed diabetes related IVD degeneration in mice is associated with AGE accumulation. This study investigates the role of AGEs on IVD independent of hyperglycemia.

C57BL6 mice were fed isocaloric diets with standard amount of AGEs methylglyoxal (MG+ $1.8x10^4$ nmol/day) or reduced AGEs (MG- $0.65x10^4$ nmol/day $p<.01$) for 5 generations. F5 mice were sacrificed at 18 months. MG+, but not MG- mice, were insulin resistant but not hyperglycemic, and had higher serum MG (1.59±0.2 vs. 0.83±0.2 nmol/ml), and weight (35.1±1.8 vs. 29±0.52g) $p<.05$. Lumbar spines were analyzed using μCT, histology, and immunohistochemistry (MG).

MG+ spines had reduced proteoglycan content of the nucleus pulposus (Fig. 1) and higher vertebral cortical thickness and area than MG- ($p<.05$). AGE/MG+ mice exhibited greater MG accumulation in vertebral endplates, higher bone mineral density ($p<.01$) and lower connectivity density in superior endplates ($p<.01$) than MG- mice.

Chronic exposure to oral AGEs promotes early IVD degeneration in parallel with insulin resistance, independent of hyperglycemia. Reduced oral AGEs were IVD protective. More studies are needed to mechanistically establish the role of AGEs in IVD relative to metabolic syndrome and diabetes.

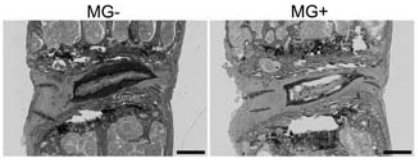

Figure 1. Extended FAST histology of representative lumbar spines of MG- mice (*left*) and MG+ mice (*right*). Safranin-O staining (red-orange) indicates reduced proteoglycan content in the nucleus pulposus of MG+ mice. scale bar = 200 μM

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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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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 four-fold increase over baseline [$P < 0.0002$]. In contrast, a 75 gram oral glucose load had no effect on FGF21 at this time point. We next 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 ± 2.4 min*ng/ml vs. MetS 141.7 ± 11.5, $P < 0.007$]. We also compared the FGF21 excursion following ingestion of a combined fructose + glucose load (37.5g of 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.

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INTEGRATED PHYSIOLOGY—MUSCLE

160-LB

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

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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 is critical in organelle function. We determined the effects of fasting-induced insulin resistance and AMPK activity on mitochondrial morphology and function using intravital microscopy. Subsarcolemma (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/μm^2). 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:65% decrease in NADH area $p<0.003$)] and decreased oxidation [SS:51%, IMF:57%, decrease in NADH AF ($p<0.03$, $p=0.34$; respectively)]. 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: NIH

161-LB

eIF2α 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) 7 520-532) requires PERK-mediated eIF2α 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 Fv2E-PERK, which promotes eIF2α 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 eIF2α phosphorylated 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 antidiabetic, antihyperlipidemic, and antiobesity effects. Consistent with this, plasma FGF21 concentration was markedly increased in TG mice. Promoter analysis identified that eIF2α regulates *Fgf21* in a downstream transcription factor, ATF4,-dependent manner. Our results show that eIF2α 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/eIF2α-induced myokine and that phosphorylation of eIF2α is a potential therapeutic target for diabetes and obesity.

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 (60 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 v. 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.

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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/mg.min) euglycemic clamp and vastus lateralis muscle biopsies were performed in 24 young, non diabetic (age= 26±3 y, BMI= 24.1±0.5 kg/m^2, VO_2max=25.4±2 ml/kg.min, M/I=16.87±1.7 mg/kg FFM.min/mU/ml x100) and 48 older, non diabetic (age=73±7, BMI=24.4±0.4, VO_2max=17.1±0.9, M/I=14.1±2.3) subjects. 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 level 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 a 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 VO_2 (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.

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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. TRB3 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 (Y612), Akt (T308), FoxO1 (S256), and FoxO3a (S253), demonstrating improved insulin sensitivity. Liver triglycerides were decreased by 57% and tissue weight 49% lower, suggesting 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 IκB Kinase (IKK) β Independently of Weight Loss

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We have recently demonstrated that HH occurs in one third of men with type 2 diabetes (T2D) and these patients have a significantly greater intensity (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

hyperinsulinemic-euglycemic clamp(80mU/m2/min) and expressed as mg/kg body weight/min. GIR increased by 30% after 6 months (4.1±2.0 vs. 5.3±2.3 mg/kg/min, p=0.005) of T therapy but did not change in placebo group(3.4±1.5 vs. 3.5±1.8 mg/kg/min, p=0.88). FFA, CRP and TNF-α concentrations decreased (0.63±0.10 vs. 0.41±0.04 mM/L, 3.78±1.34 vs. 2.90±1.08 mg/L and 2.56±0.39 vs. 2.18±0.31 pg/mL respectively, p<0.05 for all) after T therapy. There was a decrease in mRNA expression in mononuclear cells of SOCS-3 (-27%) and IKKβ (-23%), two proteins known to interfere with insulin signaling and to cause insulin resistance. PPARα increased by 38% but there was no change in c-Jun N-terminal kinase (JNK)-1 and PPARγ expression after T therapy. There was no significant change in any of the inflammatory mediators in the placebo group. There was no change in weight in either group. Thus, our data define for the first time that T restores insulin sensitivity in T2D men with HH independently of weight loss and due to the suppression of FFA, TNFα and CRP concentrations and the expression of SOCS-3 and IKKβ.

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167-LB

Potent, Insulin-Independent Glucose-Lowering Mediated by FGF19 Action in the Brain

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The gut-derived hormone fibroblast growth factor-19 (FGF19) has potent anti-diabetic effects in leptin-deficient ob/ob mice that are hypothesized to involve the FGFR1 receptor isoform. Since FGFR1 is concentrated in mediobasal hypothalamic areas involved in glucose homeostasis, we sought to determine whether the glucoregulatory effects of systemic FGF19 administration involves an action in the brain, and if so, to clarify the underlying mechanism(s). We performed a series of studies in ob/ob mice following implantation of a cannula in the lateral ventricle. As expected, systemic FGF19 administration (1 mg/kg) lowered blood glucose levels and improved glucose tolerance relative to vehicle-treated controls (P<0.05). This effect was attenuated by an intracerebroventricular (icv) pre-treatment injection of an FGFR inhibitor (PD1731074; 25μg) at a dose that had no effect when given alone, suggesting that the systemic effect involves a central mechanism. Consistent with this hypothesis, icv administration of a much lower dose of FGF19 (3μg) lowered blood glucose and improved glucose tolerance in ob/ob mice (p<0.05), while the same dose had no effect when administered peripherally. To clarify the mechanism of glucose lowering, we performed a frequently sampled IVGTT 2-h after icv injection of either FGF19 or vehicle, and analyzed glucose and insulin data using the Minimal Model. Central infusion of FGF19 induced a marked increase of glucose tolerance despite no change of either insulin secretion or insulin sensitivity. Rather, the glucose lowering effect resulted entirely from a 3-fold increase of glucose effectiveness (p<0.05), the insulin-independent component of glucose tolerance. We conclude that 1) the anti-diabetic effect of FGF19 involves a central site of action, 2) the brain has the capacity to rapidly and potently induce glucose lowering through an entirely insulin-independent mechanism, and 3) the beneficial effect of central FGF19 action is mediated via this mechanism.

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168-LB

Acute Reduction in Fetuin A, Retinol Binding Protein 4 and Several Metabolites After Gastric Bypass But Not Sleeve Gastrectomy in Obese Patients With Type 2 Diabetes

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Bypass of foregut secreted factors promoting insulin resistance is hypothesized to be one of the mechanisms by which diabetes resolution follows roux-en-y gastric bypass (GBP) surgery. We wished to identify proteins or metabolites linked with insulin resistance which reduced after laparoscopic GBP,compared to sleeve gastrectomy (SG) with intact foregut, using quantitative proteomic and metabolomic analyses. Plasma from 15 subjects with type 2 diabetes (T2D), matched for BMI, oral diabetes therapy and glycemic control, undergoing GBP or SG was analyzed 3 days before and after surgery. Insulin sensitivity was estimated using homeostatic model assessment (HOMA-IR). Samples were depleted of abundant plasma proteins, trypsin digested and labeled with iTRAQ™ isobaric tags prior to liquid chromatography-coupled mass spectrometry analysis. Gas chromatography and mass spectrometry were used for metabolomic analysis. Relative change after surgery was calculated for both proteomic and metabolomic data and compared between GBP and SG. Post-operative diabetes therapy was discontinued in all. Although mean reduction in HOMA-IR was greater following GBP than SG, this did not reach statistical significance (3.44 vs. 0.47, p=0.39). Proteomic analysis yielded 7 proteins which reduced after GBP only, including Fetuin A and Retinol binding protein 4 (RBP4), both known to be associated with insulin resistance. Reductions in Fetuin A (25.7±3.8%, p=0.02) and RBP4 (50.5±6.9%, p=0.02) after GBP were confirmed using ELISA and immunoassay respectively. Metabolomic analysis identified significant reduction of citrate, proline, histidine and decanoic acid specifically after GBP. Greater reduction of fetuin A, RBP-4, and several metabolites occur early after GBP compared to SG, independent of weight loss, and may contribute to enhanced T2D remission observed following foregut bypass (GBP) procedures.

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169-LB

One Week of Effective Treatment of Obstructive Sleep Apnea in Patients With Type 2 Diabetes Results in Enhanced Post-Prandial GIP Release

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Post-prandial release of Glucose-dependent Insulinotropic Polypeptide (GIP) is reduced in type 2 diabetes mellitus (T2DM). Approximately two-thirds of patients with T2DM have obstructive sleep apnea (OSA). An adverse impact of OSA on glycemic control has been documented but the underlying mechanisms are unclear. Continuous positive airway pressure (CPAP) treatment of OSA may improve glucose metabolism but efficacy has been limited by insufficient use. We investigated the impact of effective CPAP treatment on post-prandial GIP levels in patients with OSA and T2DM to determine whether reduced incretin release is involved in the adverse impact of OSA on glycemic control.

Individuals with T2DM (n=15; Age: 54.3±2.3 years, BMI: 38.7±2.5 kg/m2, HbA1c: 7.6±0.4 %, apnea hypopnea index: 44.7±6.1/hour) were randomized in a 2:1 ratio to 1-week of active CPAP (n=11) or sham-CPAP treatment (n=4). Both groups spent each night in the laboratory with optimum CPAP adherence. Body weight and medications did not change over the study period. Prior to randomization and after one week of treatment, the participants underwent a 24h period of blood sampling at 15-30 min intervals while identical high-carbohydrate meals were ingested at 0900h, 1400h, and 1900h. Each sample was immediately centrifuged at 4°C and kept frozen at -20°C until assay. Measurements of plasma GIP levels were performed using a multiplex technology (Millipore, Billerica, MA).

Mean 24-h GIP levels were significantly higher (p=0.005) after one week of rigorously controlled active CPAP treatment due to an elevation of post-prandial levels (p=0.01; + 17.1±5.4 %) without change in overnight fasting levels (p=0.5). No consistent changes in post-prandial GIP levels were observed following sham CPAP treatment.

The findings suggest that impaired GIP release is involved in the adverse impact of OSA on glycemic control in T2DM.

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170-LB

Exendin-4 (Ex4) Improves Cardiac Function in a Mouse Model of Inflammatory Cardiomyopathy

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Type 2 diabetes is associated with chronic inflammation that affects organs such as the heart. Monocyte chemotactic protein-1 (MCP1) 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. 0.6±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

For author disclosure information, see page LB66.

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 (ED_{30}) 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 (264W94) 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%-of-ED_{30} + 50%-of-ED_{30} mixture (KGA2727, 0.25 mg/kg; GSK2299027, 0.07 mg/kg), maximal effect was attained (plasma [glucose] 192 ± 29 mg/dL) without evidence of correlated increases in cecum 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 18-50yr) from a biobank to select 24 subjects (age 34.8±8.9yr), 12 each in the lowest (L-IGF) and highest (H-IGF) quartiles of age specific IGF-I SD scores. The evaluations were undertaken after a 24hr fast, and included glucose and glycerol appearance rates (Ra) using tracers and an IVGTT to estimate peripheral insulin sensitivity (IS) and acute insulin and C-peptide responses, indices of insulin secretion. Other evaluations included magnetic resonance spectroscopy to measure IMCL, DXA, calorimetry and a muscle biopsy.

The two groups were similar in age and body composition. Fasting glucoses were similar, however, L-IGF group had reduced levels of insulin ($p=0.032$) and C-peptide ($p=0.027$), and lower glucose Ra adjusted for insulin levels ($p=0.027$) suggesting increased hepatic IS. Acute insulin and C-peptide responses ($p<0.05$) were lower, however similar peripheral IS resulted in reduced insulin secretion adjusted for IS ($p=0.03$) in the L-IGF group. L-IGF group also had higher overnight levels of free fatty acids ($p=0.03$) and β-hydroxy butyrates ($p=0.027$), increased accumulation of IMCL from 12-24hrs of fast ($p=0.008$), and tended to have a lower respiratory quotient ($p=0.08$) indicating increased fat utilisation. Furthermore, fat oxidation pathways were upregulated ($p=0.001$) while GLUT-1 receptors down-regulated in the muscle on gene expression array. Overnight Growth Hormone secretion, lipolytic rates and basal metabolic rates were unchanged.

These data suggests that GF-I levels could be an important marker of β-cell function and substrate metabolism, and provide new insights into the links between IGF-I and T2D.

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173-LB

Comparable Gastrointestinally Mediated Glucose Disposal in South Asians and Caucasians—Preliminary Findings From a Study Investigating Racial Differences in the Incretin System in South Asians

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South Asians are at an increased risk of type 2 diabetes that develops early and at lower levels of obesity with an increased risk of complications. There is emerging evidence from clinical and genomic studies, to suggest a possible 'racial' variation in the incretin system. However most of the data comes from Japan & Korea with limited evidence of incretin studies in South Asians who differ metabolically from Far East Asians.

We hypothesised that the incretin system varies in South Asians, which may be due to a difference in the incretin effect, endogenous postprandial responses of individual incretin hormones, and/or insulinotropic or alpha cell responses to circulating incretin hormones. Preliminary data from our study comparing the incretin effect in both groups is presented below.

Nine South Asian subjects (age: 34+/-4 years (Mean+/- SEM); BMI 25+/-1.2 kg/m^2; waist-hip ratio (WHR) 0.87+/- 0.02) and eight age & BMI matched Caucasian subjects (age: 29+/-4 years; BMI 23+/-0.96 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- hr. 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 $100x(glucose_{OGTT}- glucose_{IGII})/glucose_{OGTT}$ with $glucose_{OGTT}$ being 50 gm. for all subjects.

There were no significant differences in the gastrointestinally mediated glucose disposal (GIGD%)(South Asians 60.3+/- 4.9; Caucasians 56.8+/-5.3).

GIGD 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 *Drosophilia* 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 Siah2 regulates the expression of over 100 genes involved in inflammatory processes. Genes encoding adipocyte-secreted pro-inflammatory proteins such as serpine-1 (PAI-1) and serum amyloid A3 (Saa-3) are significantly down-regulated in both visceral and subcutaneous adipose depots, identifying Siah2 as modulating obesity-related inflammation in adipose tissue via regulation of adipocyte biology.

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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/free 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 α/β-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 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 show 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 reduction in food intake, 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 O_2 consumption and thermogenesis, 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.

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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 (CTL) and high-fat diet by 5 consecutive weeks (DIO). During these 5 weeks, some mice of the DIO and CTL 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-Myd88 interaction, IKKβ and JNK phosphorylation and iNOS expression in DIO mice treated with probiotic. This treatment also improved the expression of ileal 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 (*Biochemical 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 Tg 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 γ-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.

179-LB

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

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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 (8mg/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-a, IL-6 and iNOS by 38% (p=0.036), 45% (p=0.035) and 32% (p=0.042), 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 ATM's. 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 Results in 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.

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181-LB

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

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The aryl hydrocarbon receptor (AhR), together with its partner AhR nuclear transloacator (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.

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182-LB

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

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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 the 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.

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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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The molecular mechanisms involved 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 GW311616A 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.

185-LB

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

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Pharmaceutical enhancement of uncoupling protein1 (UCP1) 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 (a β3-adrenergic receptor agonist, CL, 1mg/kg/day for 10 days) administration, compared to that of wild type (wt) mice. 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-deficent 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 incubated with CL (10uM) 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.

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186-LB

Prolylcarboxypeptidase Expression Is Altered in Experimental Animal Models of Obesity

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Proopiomelanocortin (POMC) neurons possess remarkable ability to regulate feeding behavior and promote energy expenditure. Activation of POMC neurons results in the release of alpha-melanocyte-stimulating hormone (α-MSH) and beta-endorphin which both inhibit feeding. Despite a detailed description of the roles of α-MSH, its mechanisms of regulation remain unknown within the feeding circuitry. Emerging evidence suggests that prolylcarboxypeptidase (PRCP) modulates the anorexic effects of α-MSH, the endogenous ligand for melanocortin 4 (MC4) receptors. PRCP-deficient mice have reduced food intake and a lean phenotype. In contrast, obese diabetic patients have elevated plasma levels of PRCP, suggesting a potential role for PRCP in diabetes and obesity. We hypothesized that PRCP contributes to regulation of food intake and that a novel inhibitor of PRCP (UM8190) would reduce blood glucose levels in obesity. To test this hypothesis, PRCP expression levels were determined in three experimental animal models, Zucker Diabetic Fatty rats (ZDF), Zucker Lean (ZL) rats, and wild type (WT) mice. After 16 weeks, PRCP protein levels were significantly lower ($p<0.05$, n=6) in left ventricles and kidneys of ZDF than in ZL rats (n=5). After 32 weeks, while PRCP protein levels in the kidney of ZDF rats were higher than in ZL rats ($P\leq0.005$, n=6), PRCP protein levels in the left ventricle of ZDF were significantly lower than in ZL rats ($P\leq0.005$, n=5). To test the effect on feeding behavior, UM8190 was infused i.v. in WT mice for 10 days. Food intake was reduced in UM8190 -treated mice compared to vehicle treated WT mice. Unlike the MC4 receptor agonist melanotan II (MTII), i.v. infusion of UM8190 in mice did not change heart rate or blood pressure. Our studies highlight the importance of PRCP in obesity. What remains elusive is the role of PRCP in diabetes. The details of diabetes-accelerated PRCP expression regulation in obesity and type 2 diabetes could open new therapeutic avenues to maintain glycemic control.

187-LB

Variations in Susceptibility to Diet-Induced Obesity Between C57BL6/J and C56BLKs/J Inbred Mouse Strains

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Impaired glucose tolerance (IGT) and Type 2 Diabetes (T2D) are polygenic disorders with complex pathophysiologies; recapitulating them with mouse models is challenging. Despite 70% genetic homology, C57BL/6J (BL6) and C57BL/KsJ (BLKS) inbred mouse strains differ in response to diet (DIO)- and genetic-induced obesity. We hypothesized differences would yield insight into IGT and T2D susceptibility and treatment responses. To define phenotypes in response to DIO, male BL6 and BLKS mice (8 wks) were fed normal chow, high fat diet (HFD; 42% kcal from fat), or HFD supplemented with PIO (20mg/kg/day) for 16 wks. Body composition, glucose metabolism, β cell function and gene expression, and energy metabolism were analyzed. As expected, HFD BL6 mice gained weight and visceral adiposity. They were hyperinsulinemic and insulin resistant with IGT, but did not develop T2D due to compensatory β cell proliferation. PIO did not attenuate HFD-induced weight gain but did decrease visceral fat mass, normalize serum insulin, and improve insulin sensitivity and IGT. Islet microarray revealed that PIO partially reversed changes in gene expression conferred by HFD. In contrast, BLKS-HFD mice gained less weight due to decreased consumption and increased activity. Visceral adiposity increased to a lesser extent; addition of PIO increased total fat but not visceral fat mass. Despite no significant increase in insulin resistance, HFD BLKS mice had IGT but no change in serum insulin levels, β cell proliferation, or glucose-stimulated insulin secretion. Because of distinct compensatory responses, both strains avoided HFD-induced T2D. BL6 mice met DIO with increased β cell proliferation and insulin production; BLKS mice responded by restricting food intake and increasing activity, limiting weight gain. Differences may reflect divergent responses of humans to a Western lifestyle and drug therapy and underscore the careful consideration needed when choosing mouse models of DIO and T2D treatment.

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OBESITY—HUMAN

188-LB

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

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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 in obese women. This is a double-blind, placebo-controlled study to investigate the safety/tolerability, 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 vs. placebo (N=38) twice-weekly for 12wks. Body weight (BW), sense of hunger, and cardiometabolic biomarkers were measured. Results are based on pre-specified interim analysis of first 19 patients who completed 12 weeks of treatment duration (n=5, 6, 3, and 5 for 0.6, 1.2, 2.4mg, and placebo, respectively). Patients were white females (mean age 40.3 yr, BW 101.2 kg, and BMI 37.9 kg/m^2). The most common adverse events (AEs) with higher incidence during beloranib treatment were sleep disturbance, nausea, and vomiting (resulting in 2 drop-outs from the 2.4 mg group). There were no severe AEs, serious AEs, or deaths. There were no clinically significant abnormal laboratory measures, vitals, or ECG findings. After 12wks, subjects on 0.6, 1.2, or 2.4mg lost an average (±SEM) BW of -3.8±0.8, -6.1±1.5, and -9.9±2.3 kg vs. +1.8±0.4 kg for placebo (all p<0.005 vs. placebo). Hunger, LDL-c, TG, and hs-CRP decreased in the beloranib groups vs. placebo. Beloranib treatment for 12wks was generally well-tolerated by SC administration, resulted in rapid and sustained clinically meaningful BW loss of up to ~10%, improved sense of hunger and cardiometabolic risk markers in this interim analysis of an ongoing Phase 2 study, which is scheduled to be completed by May 2013.

189-LB

Rescue of Intracellularly Retained Human Melanocortin-4 Receptor Mutants

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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 (Ipsen 5i and ML00253764) and 1 agonist (THIQ) were studied. Totally 14 human MC4R mutations were studied, including 10 (N62S, I69R, P78L, C84R, G98R, Y157S, W174C, P260Q, F261S, and C271Y) that are retained intracellularly, and 4 (Δ88-92, 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 were measured using radioimmunoassay. The results were similar in the two cell lines studied. With 10-6 M Ipsen 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 ML00253764 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.

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190-LB

Exercise Following Gastric Bypass Surgery Maintains Higher Fatty Acid Oxidation

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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 (EX, n = 26) or GPS only control (CON, n = 45). Percutaneous biopsies of the vastus lateralis were obtained, before and after the 6-month interventions. 14-C palmitate oxidation was measured in muscle homogenates. Resting metabolic rate (RMR) and respiratory quotient (RQ) were determined by indirect calorimetry. Aerobic capacity (VO_2 max) was determined by a graded exercise test. Cardiorespiratory capacity (VO_2) max increased in the EX group (+160 VS. -25 ml/min, P=0.026). In this subset of completers, the EX 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 EX group (Pre = 0.74: Post = 0.74, P = 0.97), suggesting a shift in substrate preference to carbohydrate in the CON group and a maintenance of higher fatty acid oxidation in the EX group. Palmitate oxidation in muscle homogenate decreased to a greater extent in the CON group compared to the EX group (-3.40 VS. 0.10 nmol/g tissue/min, P=0.05), again suggesting a shift towards CHO oxidation in the CON group and a maintenance/enhanced fatty acid oxidation in the EX 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

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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 adenylyl 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 agouti-related peptide (AgRP), MCL0020, Ipsen 5i and ML00253764), which are inverse agonists at the Gs-cAMP signaling pathway, to regulate the activity of phosphorylated 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 (D146N) had significantly increased pERK1/2 level upon stimulation with AgRP, MCL0020 or ML00253764, but not Ipsen 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 pathway. 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.

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192-LB

Improvement in Insulin Sensitivity and Beta Cell Function in Severely Obese Adolescents Following Gastric Bypass Surgery

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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·SI.

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.

	Metabolic parameters			
Timepoint (n)	Baseline (15)	2 weeks (11)	3 months (13)	12 months (15)
	Mean +/- SEM	Mean +/- SEM	Mean +/- SEM	Mean +/- SEM
BMI (kg/m2)	63.1 +/- 2.8	59.9 +/- 3.6	50.6 +/- 2.6	38.8 +/- 2.1
Fasting gluocose (mg/dl)	94.1 +/- 2.7	82.8 +/- 1.4	84.1 +/- 2.4	83.1 +/- 1.4
Fasting insulin (pM)	152.7 +/- 22.7	106.3 +/- 12.5	66.1 +/- 7.8	46.8 +/- 3.9
SI (x10-5) x min-1 x pM	1.26 +/- 0.52	1.41 +/- 0.31	1.89 +/- 0.38	4.91 +/- 0.83
AIRg (pM)	979 +/- 116	1,015 +/- 177	651 +/- 65	402 +/- 46
Disposition index	891 +/- 165	1,173 +/- 261	1,163 +/- 239	1,855 +/- 382

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193-LB

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

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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 effect 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= 36) 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 transnonchlordane 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

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Glucokinase (GCK) acts as a component of the "glucose sensor" in pancreatic β-cells and possibly in other tissues, including the brain. However, >99% of GCK in the body is located in the liver, where it serves as a "gatekeeper", determining the rate of hepatic glucose phosphorylation. Specific to the liver, as major end product of GCK is lactate, which can be measured as a surrogate for GCK activity. Recently, we introduced a novel model of lactate kinetics (1), during the frequently-sampled intravenous glucose tolerance test (FSIGT) to estimate *in vivo* hepatic glucokinase (GCK) activity (K_{GK}), glycolysis (K_{12}) and whole body lactate clearance (K_{01}). While the FSIGT is a gold standard, it is difficult to do in the clinical setting. The oral glucose tolerance test (OGTT) is more practical for the clinical assessment of glucose homeostasis. Our objective was to estimate the hepatic indices from OGTT glucose and lactate data in 20 Caucasian non-diabetic females (age: 31±1, BMI: 26.9±1.2). Once consented, Participants underwent a 72 hr carbohydrate load, followed by a standard 75g oral glucose tolerance test performed at our General Clinical Research Center. The model was uniquely identifiable in all 20 subjects. Estimates of GCK activity are inversely correlated to fasting insulin (r=-.59, P=.01), and percent body fat as calculated by BAI index (r=-.54; P=.02).

In conclusion, we show that in women, hepatic GCK activity is incrementally decreased with the increase in percent body fat and the subsequent rise in fasting insulin. One possible mechanism linking the decrease in GCK activity is the increased expression of glucose regulatory protein (GCKR) brought forth by the rise in fasting insulin.

1.Stefanovski D, Youn JH, Rees M, Watanabe RM, Ader M, Ionut V, et al. Estimating hepatic glucokinase activity using a simple model of lactate kinetics. Diabetes Care. 2012 May;35(5):1015-20.

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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.

To study the relevance of CoQ10 content in adipose tissue dysfunction, the abundance of the oxidized (Q10) and reduced (Q10H2) form of this molecule were determined in subcutaneous (SCAT) and omental (OAT) adipose tissues in healthy women (n = 29) with a wide range of body mass index (BMI 21.5 to 53.2 kg/m2). The expression of proinflammatory genes was also measured.

Although CoQ10 levels were similar in these compartments, the redox state of OAT favored the reduced form while the oxidized form was more abundant in the SCAT. A negative and non-linear association was observed between omental content in CoQ10 or Q10H2 and BMI, total body fat mass measured by dual energy x-ray absorptiometry and computed tomography-assessed visceral adipose tissue area. A depletion of CoQ10 content in SCAT is also observed in subjects with higher BMI and SCAT area. Greater omental adipocyte diameter was associated with lower CoQ10 content while hypertrophy of subcutaneous adipocytes negatively correlated with Q10H2 level. Although the degree of obesity did not appear to influence adipose tissues level of Q10, a negative relationship was observed between SCAT Q10 content and mRNA abundance of CD68 and cyclooxygenase-1. Also, the mRNA abundance of prostaglandin E2 receptors type 3 and type 4 (EP4 and EP3) increased in patients with low levels of CoQ10.

This study confirms that OAT and SCAT adipocyte hypertrophy is closely related to depletion of CoQ10. This could represent a mechanism contributing to oxidative stress and adipose tissue dysfunction in obesity.

196-LB

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

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This study was designed to investigate the effect of administration of liraglutide to patients with type I 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/m^2, mean total cholesterol 195.55 mg/dL and mean uptake for Slow and rapid acting insulin was 19.83 and 34,55 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	-22,63%
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 all patients for rapid-acting insulin, while slow insulin remained at the same levels. From these early results it appears that the administration of liraglutide in patients with type I diabetes can be beneficial in weight control as well as reduced uptake of insulin with marked decrease in incidences of hypoglycemia in spite of the weight loss.

197-LB

Two-Year Outcomes of a Randomized Controlled Trial of Behavioral Treatment for Comorbid Obesity and Depression 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=.01), 1-year (BA =66.4%; LI =47.4%; p =.03), and 2-years (BA =73.8%; LI =55.9%, p=.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 Adipogenesis 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 retinoic acid 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 and adipogenic markers in human adipose tissue from lean and from morbidly obese subjects.

Visceral 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 on VDR gene expression in ATL. No significant effects 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—APOPTOSIS

200-LB

Reporter 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 luminometric 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

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 XBP1 and ATF6, 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: FP7 BETABAT

202-LB

Protective Effect of Nicotinamide on High Glucose/Palmitate-Induced Glucolipotoxicity to INS-1 Beta Cells Is Attributed to Its Inhibitory Activity to 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-dependant 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 glucolipotoxicity 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 autologous, 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-specified 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 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 propensities 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 Rheb 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 STAT5 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 Prl-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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206-LB

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

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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.

CA19.9+ 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 pancreatospheres (92.4±0.45% duct; 0.19±0.07% c-peptide+ cells). Gene expression of krt19 was reduced on days 3, 7 and 30 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, sst, gcg 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.

Supported by: ISCIII (PI10/00636); CIBERDEM; Novo Nordisk A/S

ISLET BIOLOGY—BETA CELL—STIMULUS-SECRETION COUPLING AND METABOLISM

207-LB

Constitutive, Pulsatile Release of Gamma-Aminobutyric Acid Coordinates Insulin Secretion in Human Pancreatic Islets

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The insulin-secreting beta cell of the pancreatic islet releases the neurotransmitter gamma-aminobutyric acid (GABA) as a paracrine signal that inhibits surrounding alpha cells and promotes beta cell survival. It is unclear, however, how and when GABA is secreted from human islets. Here we show that the human islet is periodically pervaded by a wave of constitutively released GABA. This GABA secretion is independent of glucose concentration and does not require conventional Ca2+-dependent exocytosis but membrane transport of a metabolic pool of GABA. This secretory activity produces robust periodic GABA pulses that impose a rhythm on insulin secretion, thus helping increase the synchronicity of pulsatile insulin secretion. GABA secretion is disrupted in human islets from type 2 diabetic patients, which offers an explanation for the disorganized hormonal output under diabetic conditions. Our results indicate that loss of GABA release is due to excessive palmitoylation during hyperlipidemia, which sequesters the GABA-synthesizing enzyme GAD65 and reduces cytosolic GABA production in beta cells. Because restoring GABA signaling can be proposed as an intervention point to promote islet function, our study may have implications for a novel pharmaceutical strategy for the treatment of diabetes.

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208-LB

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 isoform (PK-hM1) in INS-1 832/13 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 trended 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 O_2 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, $P=3x10^{-7}$). 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-1ß Gene Expression in Pancreatic Islet ß-Cells

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Aryl hydrocarbon receptor nuclear translocator (ARNT) / hypoxia-inducible factor-1ß (HIF1- 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 *Wfs1*$^{-/-}$ *A*^{y}*/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 Zeitgever 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. Overexpression 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 2.5-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.

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 hasic insulin secretion are not known. Here, we examined the role of actin dynamics regulated by neuronal Wiskott-Aldrich syndrome protein (N-WASP) and cofilin in glucose-induced insulin secretion (GIIS). 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-K8 β-cells) 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-K8 β-cells or knockdown of N-WASP suppressed GIIS. We performed perifusion experiment using DN-N-WASP-introduced or N-WASP-knocked down MIN6-K8 β-cells and found that the second phase of GIIS 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-K8 β-cells by glucose stimulation, thereby promoting F-actin remodeling. In addition, perifusion experiment using MIN6-K8 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 GIIS, indicating that activity of cofilin is critical for the second phase. In contrast, the first phase of GIIS 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 GIIS.

211-LB

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

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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 (StaR®) 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 Zn^{2+} coupling via Gαs and Gαq signalling cascades to increase cAMP and IP_1/Ca^{2+} respectively. Using our approach we have identified novel GPR39 agonists that have been used to examine the potential of GPR39 activation for the treatment of metabolic diseases.

Zn^{2+} and the identified GPR39 agonists G#01 and G#89 stimulated the accumulation of cAMP and IP_1 in GPR39 transiently transfected HEK293 cells with $EC_{50(cAMP)}$ 0.84μM/4.4μM/2.4μM and $EC_{50(IP1)}$ 0.6μM/14.7μM/32.6μM (Zn^{2+}/G#01/G#89). The secretion of insulin and GLP-1 in response to GPR39 agonism was assessed using the pancreatic β- cell line, NIT-1, and primary mouse intestinal epithelial cells (mIECs). Zn^{2+} and G#01/G#89 dose-dependently stimulated glucose-dependent insulin secretion from NIT-1 cells (EC_{50} 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 Zn^{2+} and compounds G#01/G#89 also stimulated GLP-1 secretion from primary mIECs with EC_{50} 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 breeding the $Crif1^{flox/flox}$ mice with RIP2-cre mice. CRIF1 is a protein required for the intramitochondrial production of mtDNA-encoded 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 mice (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 mice model of mitochondrial diabetes with beta cell specific CRIF1 deficiency.

ISLET BIOLOGY—SIGNAL TRANSDUCTION

213-LB

Sulfonylureas 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 sulfonylureas (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

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 redifferentiation.

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 comparison to control 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 Oct-4 expressing cells 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

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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 95 ± 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 NAD(P)H 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 (t1/2) under 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 t/12 was ~24hr, while the mRNA t1/2 was ~6hr. IL-1β+HG reduced the protein t/12 to 16hr, but the mRNA t/12 was stable. IL-1β+HG led to induction of iNOS and cleaved caspase-3 and reduced SERCA2b protein t1/2 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 t1/2 and restored SERCA2b levels. Together, our data suggest β cell SERCA2b protein t1/2 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.

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