Late Breaking Abstracts

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72nd scientific sessions

JUNE 8-12, 2012 • PHILADELPHIA, PA

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COMPLICATIONS—HYPOGLYCEMIA

1-LB

Recurrent Hypoglycemia Leads to Loss of Glutamatergic Neurotransmission

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Activation of the counterregulatory response to hypoglycemia is achieved in part by increasing stimulatory glutamatergic (Glu) neurotransmission and decreasing inhibitory GABAergic neurotransmission within the ventromedial hypothalamus (VMH), an important glucose sensing region in the brain. It is this interplay that allows for appropriate activation of the glucagon and epinephrine responses. Loss of the counterregulatory response with recurrent hypoglycemia (RH) is due in part to defects in neurotransmission within the VMH. We showed previously that VMH GABAergic tone is elevated in RH animals and this contributes to suppression of counterregulatory responses. On the other hand, our pharmacological data indicates that Glu acts to stimulate counterregulatory responses in hypoglycemia-naïve rats, but changes in VMH Glu levels following RH have not been examined in detail. Hence, the current study uses two complementary techniques, microdialysis and biosensors, to assess changes in VMH Glu levels with progressive exposure to hypoglycemia. Two separate studies were performed. In the first study, rats made RH (n=8) for 3 consecutive days and their recurrent saline control (n=8) counterparts were subjected to a hypoglycemic clamp with microdialysis on day 4. In the second study, rats (n=3-4) were implanted with biosensors to monitor real-time changes in Glu levels in the VMH with each progressive episode of hypoglycemia. Our data showed that during the first episode of hypoglycemia, VMH Glu levels rose transiently during the initial onset of hypoglycemia and then steadily declined below baseline levels. With recurring exposure to hypoglycemia, baseline Glu concentrations decreased ~50% ($P<0.01$), but more importantly, Glu levels failed to increase at the outset of hypoglycemia and this was associated with a failure to counterregulate. Our data suggests that loss of Glu neurotransmission may be another factor contributing to counterregulatory failure following recurring exposure to hypoglycemia.

Supported by: JDRF

COMPLICATIONS—MACROVASCULAR—ATHEROSCLEROTIC CARDIOVASCULAR DISEASE AND HUMAN DIABETES

2-LB

Sulfonylureas Associated with Increased Risk of Cardiovascular Disease: Results from Meta-Analysis

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Sulfonylurea (SU) use has been previously linked with increased risk of cardiovascular disease (CVD); however, both clinical trials and observational studies have been inconsistent. Type 2 diabetes is an independent risk factor for CVD and therefore, understanding the link between antidiabetic medications and CVD risk has important clinical implications.

A systematic literature search in MEDLINE and CENTRAL was conducted through Dec 2011 to find observational studies which report the adjusted association between SU and CVD events. Ratios (relative risk (RR), odds ratios, or hazard ratios) adjusted for potential confounders (e.g. concomitant medications, baseline CV risk, diabetes severity) were pooled using random effects model to yield RRs and associated 95% confidence intervals (CIs).

This meta-analysis included a total of 20 observational studies representing a total of 1,311,090 patients, followed over a median of 4.6 years. Compared to other oral diabetes drugs, SU use was associated with a significantly increased risk of CV death (RR:1.26, 95%CI:1.18-1.34) and of a composite CV event (which includes myocardial infarction, stroke, CV-related hospitalization or CV death) (RR:1.12; 95%CI:1.06-1.19). When SU was compared to metformin, these RRs were 1.25 (95%CI:1.17-1.34) and 1.18 (95%CI:1.12-1.24), respectively.

This meta-analysis provides more precise estimates for CV mortality compared to prior observations by expanding the pool of studies and using adjusted estimates, while also assessing an additional outcome of a composite CV event. These results suggest that SU use may elevate the risk of CVD, and this association warrants consideration in clinical practice, especially among high risk patients, when other treatment options may be available.

Supported by: Merck Sharp & Dohme Limited

COMPLICATIONS—MACROVASCULAR—CELLULAR MECHANISMS OF ATHEROGENESIS IN DIABETES

3-LB

Role of NF-κB in the Endothelial Protective Action of Niacin

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Our laboratory and others have recently reported that niacin preserves endothelium-dependent relaxation and augments endothelial nitric oxide (eNO) bioavailability independently of cholesterol lowering actions. Data in the literature demonstrate that eNO exerts antiinflammatory actions in the cardiovascular system in part via inhibition of NF-κB and downregulation of leukocyte-endothelium interactions (LEI). Accordingly, we hypothesized that niacin prevents upregulation of NF-κB and LEI, independently of lipid modifying actions. NF-κB transcriptional activity in endothelial cells was assessed by luciferase assay. Niacin (100 μM) significantly attenuated NF-κB activity in endothelial cells stimulated with 20 ng/ml TNFα for 6 consecutive hours ($p<0.05$ vs TNFα alone). LEI were measured by intravital microscopy in mesenteric post-capillary venules of live C57BL/6 wild-type mice given a 14-day administration of 50 mg/kg/day L-NAME in the drinking water to induce subchronic endothelial dysfunction. Mice given L-NAME exhibited increased number of adherent leukocytes (6±2.1 vs 2.7±0.9 cells/100μm, respectively; $p<0.01$). Niacin significantly attenuated L-NAME-induced leukocyte adhesion to 3.4±1.5cells/100 μm vessel length (NS versus control mice). No significant changes were observed in body weights, white blood cell counts, and plasma glucose and lipids levels in all groups of mice. These data support the concept that inhibition of NF-κB plays an important role in the endothelial protective action of niacin and uncover new pleiotropic actions of niacin in the cardiovascular system

Supported by: Merck Sharp & Dohme Limited

4-LB

Non-Alcoholic Fatty Liver Disease and Peripheral Insulin Resistance are Associated with Increased Right Ventricular Oxidative Stress in Mice

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Diabetic cardiomyopathy is characterized by impaired myocardial function and ischemic tolerance in patients with type 2 diabetes (T2D). This has been related to reduced oxidative capacity and increased oxidative stress in cardiomyocytes. Non-alcoholic fatty liver (NAFL) and insulin resistance are associated with increased cardiovascular risk. Here, we aimed to investigate how NAFL and insulin resistance relate to cardiac oxidative stress and mitochondrial oxidative capacity in mice.

Female mice with adipose tissue-specific overexpression of the sterol regulatory-element binding protein-1c (aP2-SREBP-1c: AP2), a model with NAFL, and wild-type controls (CON) underwent hyperinsulinemic-euglycemic clamps to assess peripheral and hepatic insulin sensitivity (n=5-6). Mitochondrial oxidative capacity and oxidative stress were assessed ex vivo by high-resolution respirometry and Amplex Red method, respectively, in permeabilized heart ventricles (n=4).

Peripheral insulin sensitivity was decreased in AP2 mice as reflected by 43% lower insulin-mediated glucose uptake compared to CON ($p<0.05$), while hepatic insulin sensitivity was unchanged. AP2 mice showed greater heart weights than CON (178±24, CON: 132±21 mg; $p<0.05$). Ex vivo mitochondrial capacity of left and right ventricles on tricarboxylic acid cycle-derived and fatty acid-derived substrates did not differ between AP2 and CON. H_2O_2 production by mitochondrial complex III was approximately doubled ($p<0.05$) in the right ventricle of AP2 compared with CON.

In conclusion, peripheral insulin resistance in NAFL associates with increased H_2O_2 production in the right ventricle while cardiac mitochondrial function remains unchanged. This suggests that oxidative stress in the right ventricle could play an important role in the development of diabetic cardiomyopathy, which features diastolic relaxation dysfunction of the right ventricle as an early abnormality.

Supported by: DFG Feasibility Grant

COMPLICATIONS—NEPHROPATHY

5-LB

WITHDRAWN

6-LB

Loss of Kidney Respiratory Activity in Type 1 Diabetic Mice Correlates with Development of Nephropathy

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Diabetes mellitus causes a number of complications, in particular, diabetic nephropathy. Persistent hyperglycemia and polyuria put a huge energetic burden on kidneys which require a robust mitochondrial performance. The aim of this study was to investigate whether mitochondrial mechanisms are involved into development of nephropathy in conditions of Type 1 Diabetes.

We used diabetic mice of two strains (B6 and DBA) with the same mutation in Ins2 gene (the Akita mice) and age matched wild type (WT) controls. The Akita mice of both stains became hyperglycemic at 4 weeks, had profound polyuria and increased kidney mass as compared to WT controls. At the age of 24 weeks DBA-Akita, but not B6-Akita, mice developed profound albuminuria symptomatic of nephropathy. Respiratory activity of kidney homogenates with a number of mitochondrial substrates for Complex I and Complex II were measured. Tests with specific mitochondrial drugs showed that in kidney homogenates mitochondria are responsible for 98% of O2 consumption and well coupled. This approach allowed assessing mitochondrial activity normalized by tissue mass and to characterize energetic status of kidney as a whole. Respiratory activities of kidneys from 8 week old Akita mice of both strains were equal or up to 20% higher as compared to respective WT controls, apparently, to compensate for diabetic metabolic derangements. In aged 24 week old mice mitochondrial activity of kidneys from B6-Akita mice were still close to that of control group, while kidney mitochondrial activity from DBA-Akita mice dropped 5-20% depending on substrate (succinate and b-HOB most of all). Additionally, kidney mitochondria of aged DBA-Akita, but not B6-Akita, mice had elevated levels of Mn-SOD indicating higher level of oxidative stress. In conclusion, despite similar levels of hyperglycemia, DBA-Akita mice had more profound kidney dysfunction in form of albuminuria which was accompanied by loss in respiratory activity and symptoms of oxidative stress.

Supported by: Department of Defense (W81XWH-06-1-0317)

7-LB

Primary Care Detection of CKD in Adults with Type-2 Diabetes in the Awareness, Detection, and Drug Therapy in Type-2 Diabetes and Chronic Kidney Disease (ADD-CKD) Study

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Approximately 10–15% of the US population has chronic kidney disease (CKD). Diabetes is the leading cause of CKD. Early detection will encourage clinicians and patients to address factors that can improve outcomes.

ADD-CKD was a US, multicenter, observational study that assessed the prevalence of CKD in adult patients with type-2 diabetes (T2DM) and assessed and characterized the proportion of patients with detected and undiagnosed CKD in the primary care setting using the following: a clinician survey; a patient physical exam and medical history; a single patient blood draw for eGFR and glycosolated hemoglobin (HbA1c); a urine dipstick for protein; an albumin-creatinine ratio; two patient quality of life questionnaires; and a 15-month patient medical record review. The study consisted of 9339 adult patients with T2DM and 466 investigator sites. Of the 9339 enrolled, 9307 could be assessed using tests for urine protein and serum creatinine to establish the presence of CKD and to assess the sensitivity of the clinician in identifying the presence of CKD.

Of the 9307 patients, 5036 (54.1%) had Stage 1-5 CKD based on eGFR and albuminuria; however, only 607 (12.1%) of those patients were identified as having CKD. Clinicians were more successful in diagnosing patients with Stage 3-5 CKD than Stages 1 and 2. Of the 445 clinicians who enrolled at least 10 patients, 19 (4.3%) had a ≥50% likelihood of identifying patients with CKD, 217 (48.8%) had a likelihood of <50%, and 209 (47.0%) didn't identify any of their patients as having CKD. There were no differences in clinicians' likelihood of identification of CKD based on practice setting, number of years in practice, or self-reported numbers of patients seen per week.

CKD is significantly under-diagnosed in the T2DM population. Additional analyses will explore the impact of CKD detection on the implementation of evidence-based CKD interventions and outcomes.

COMPLICATIONS—NEUROPATHY

8-LB

Oxidative Stress in Diabetic Neuropathy—Source of Reactive Oxygen Species

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Diabetic neuropathy (DN) is the most common complication accompanying diabetes. Impairment in signalling mechanisms regulating neuron differentiation is hypothesised to be one of the main causes of neuronal dysfunction. There is evidence suggesting that oxidative stress (OS) is a significant mediator in the development of DN. Exposing neurons to elevated glucose increases OS, inhibits neurite outgrowth and cause neuronal cell death. We recently suggested the role for NADPH oxidase and not mitochondria derived ROS (mROS) in diabetic retinopathy. In the present study, we used SH SY-5Y neuroblastoma cells to explore the role of NADPH oxidase vs. mROS in DN.

Human neuroblastoma cells SH SY-5Y were treated with high glucose (HG, 25 mM) or normal glucose (NG, 5mM) for 6 days in the presence and absence of the specific NADPH oxidase inhibitor gp91ds-tat (1μM scrambled and unscrambled). Apoptosis was detected using a Homogeneous Caspases assay kit. Carboxy-H2DCFDA and MitoSOX™ were used as indicators of general OS, and mROS levels, respectively. NADPH oxidase activity was subsequently measured. Mitochondrial membrane potential ($\Delta\Psi$m) was measured using TMRM. The effects on neurite outgrowth was analysed using ImageJ.

Chronic exposure to HG increased caspases activity (138.6 ± 17.3 vs. 100 ± 6.1, P <0.001) cytoplasmic but not mROS levels (169.9 ± 35.8 vs. 100 ± 17.65, P = 0.0079), and reduced the number of neurites per cell (2.28 ± 0.63 vs. 3.65 ± 0.67, P <0.001) and the neurite outgrowth (151 ± 44.02 vs. 285.1 ± 71.9, P <0.001) compared to NG. Incubating with the NADPH oxidase inhibitor gp91ds-tat reversed the increased levels of ROS and prevented the glucose-induced neurite degeneration. $\Delta\Psi$m and mROS levels were unaffected at the end of the treatment.

Here we show that under in vitro hyperglycaemic conditions, ROS derived from the NADPH oxidase and not the mitochondria is responsible for the induction of apoptosis in SH SY- 5Y cells. The use of NADPH oxidase inhibitors may prove be of therapeutic value in the treatment of DN.

Supported by: PCMD Foundation

9-LB

Retinopathy in Relation to Cerebral Ischemic Lesion and Cognitive Decline in Older Patients with Type 2 Diabetes

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Retinal and cerebral arterioles are similar in anatomy, physiology and embryology, but the relationship between retinopathy and cerebral abnormalities and cognitive impairment remains unclear in persons with type 2 diabetes (T2D). The ACCORD trial is a randomized study designed to assess the impact on cardiovascular events intensive glycemic control compared to standard treatment. A total of 1,895 ACCORD participants underwent the assessment of retinopathy at baseline and the assessment of cognitive function at baseline and 40 months. The brain MRI was also conducted in a subset of 454 individuals at baseline. Participants had an average age of 62 years with a mean T2D duration of 10 years. During the 40-month follow-up, baseline retinopathy was associated with accelerated declines in the Digit Symbol Substitution Test of psychomotor speed (-0.8, -1.0 and -2.1 for none, mild and moderate/severe diabetic retinopathy respectively; p=0.0002) and Mini Mental State Exam of global cognitive function (0, -0.3 and -0.2 respectively; p=0.02), but not Rey Auditory Verbal Learning Test of memory and the modified Stroop test of executive function, after adjusting for baseline cognitive measures, age, gender, ethnicity, education, smoking and treatment assignment. These associations were attenuated but remained statistically significant with further adjustment for systolic blood pressure, lipids, hemoglobin A1c, fasting glucose, diabetes duration and visual acuity. In a cross-sectional analysis, retinopathy was also associated with a greater

amount of MRI-defined cerebral ischemic lesions (23% and 51% increment respectively compared to none; p=0.02) and less gray matter (470, 467 and 462 cm^3 respectively; p=0.02), but not white matter in the brain, after adjusting for intracranial volume, age, gender, ethnicity, education and smoking. In conclusion, retinopathy may indicate cerebral abnormalities and predict an accelerated cognitive decline in patients with T2D.

Supported by: NIH (ACCORD)

10-LB

Patient Global Impression of Change (PGIC) and Brief Pain Inventory-Short Form (BPI-SF) Assessments With Tapentadol Extended Release (ER) for Painful Diabetic Peripheral Neuropathy (DPN)

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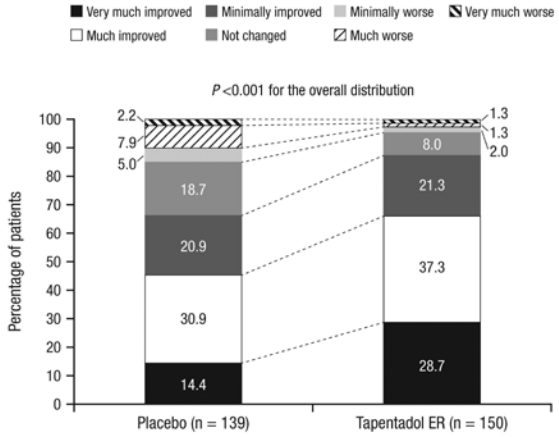
In this randomized-withdrawal, placebo-controlled study (NCT01041859) of tapentadol ER for moderate to severe, chronic, painful DPN, patients were titrated to an optimal dose of tapentadol ER (100-250 mg bid) during a 3-week, open-label period. Patients with ≥1-point reduction in pain intensity (11-point numerical rating scale) were then randomized to receive placebo (n = 152) or their optimal dose of tapentadol ER (n = 168) during a 12-week, double-blind, maintenance phase. At double-blind endpoint, the distribution of PGIC scores was significantly better with tapentadol ER versus placebo (P <0.001; Figure). From the open-label start to double-blind endpoint, tapentadol ER was associated with a significant reduction in the mean (SD) pain intensity subscale score of the BPI-SF (placebo, -2.3 [2.33]; tapentadol ER, -3.0 [2.16]; P = 0.003) versus placebo. The mean (SD) change in the pain interference score of the BPI-SF from open-label start to double-blind endpoint was -2.6 (2.38) for placebo and -3.0 (2.07) for tapentadol ER (P = 0.05). Nausea (21.1%) and vomiting (12.7%) were the most common TEAEs (≥10%) with onset or worsening in intensity during the maintenance phase in the tapentadol ER group. Compared with placebo, tapentadol ER (100-250 mg bid) provided significant improvements in PGIC and BPI-SF scores for the management of moderate to severe, chronic, painful DPN.



Supported by: Janssen Research & Development, L.L.C., Grünenthal GmbH

11-LB

Oral Methylglyoxal Promotes both Insulin Resistance and Inflammatory Brain Changes via AGER1 and SIRT-1 Deficiency; A Lifestyle-Related Link between Type 2 Diabetes and Brain Dysfunction

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Insulin resistance, often preceding T2D, is a risk factor for cognitive impairment and is preceded by chronic oxidative stress and inflammation (OS/Infl). Recent socio-economic changes include the widespread use of thermally-processed nutrients, replete with pro-OS/Infl advanced glycation endproducts (AGEs), which promote IR and T2D. Human studies have linked derivatives of a highly reactive AGE, methylglyoxal-H1 (MG) to cognitive impairment, while others indicated a link between dietary MG and insulin resistance. We assessed the mechanisms of these events in C57BL6 mice (n=15/group), pair-fed an isocaloric diet with or without synthetic MG-derivatives (MG^+ or MG^-) for life. Exposure to MG^+ led to increased OS/Infl, visceral adiposity and IR in MG^+. This was associated with elevated serum, brain hippocampal area (HC) tissue CML, MG and amyloid-β 1-42 (Aβ-42) (>2-fold, p=0.01, respectively) and was marked by a severe deficiency in anti-AGE receptor AGER1 (by >54%, p=0.02) and deacetylase SIRT1 protein (by >65%, p=.03) and a pro-inflammatory shift in both, peripheral insulin-responsive tissues and HC astroglia and microglia. These changes were absent in 18 mo MG^- mice. In conclusion, chronic exposure to orally delivered highly reactive AGEs present in food simultaneously destabilizes metabolic and inflammatory processes, via overt OS/Infl and a severe depletion in central defense mechanisms including AGER1 and SIRT1. Since AGE-restriction improves IR in T2D, targeting the presence and/or absorption of food-derived AGEs may hold significant therapeutic potential for both these conditions.

Supported by: NIA (AG23188)

12-LB

Prevalence of Sudomotor Dysfunction in Type 2 Diabetic Patients Attending Diabetes Clinics—"A Multicenter Study"

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To investigate the prevalence of sudomotor dysfunction (SD) in type 2 diabetic patients on a multicenter study and assess any relation to overall nerve fiber damage.

The study included 1010 type 2 diabetic patients randomly selected from those attending five (5) diabetes centers. There were 608 males (60,19%). Mean age and diabetes duration were 63,90±10,26 and 12,24±7,75 (yrs) respectively. A new indicator plaster method (neuropad) recently approved was used for the diagnosis of SD.

Assessment of overall nerve fibre dysfunction was performed and graded clinically using the Neuropathy Disability Score (NDS). The plaster (colour blue) was applied for 10 minutes on the plantar aspect of the feet and the results recorded as pink, patchy (blue/pink), and blue. The abnormal result defined as patchy and/or blue indicated patients with SD.

441 patients (43,7%) were found with SD - group A. They were older (66,74±8,78 vs 61,71±10,79 yrs p<0,0001), had longer duration of diabetes (14,42±7,63 vs 10,54±7,42 yrs, p<0,0001)) in comparison with those (n=569) without any sign of SD - group B. The signs of developed SD had 94,9% sensitivity and 70,2% specificity for overall fibre dysfunction. Furthermore more severe peripheral somatic neuropathy (NDS 6,85±5,59 vs 1,25±1,79, p<0,0001) was detected in SD patients in comparison with those expressing normal SD tests. In the logistic regression analysis age, diabetes duration but not sex were found to affect the development of SD. (p <0,05).

The findings of the present study indicate that Sudomotor Dysfunction affects a large proportion of diabetic patients. The study also identifies the most important risk factors for SD, e.g. age, long diabetes duration. Proper foot care and education for this population is of great importance since SD is associated with peripheral nerve fiber damage.

13-LB

A Pooled Analysis Evaluating Efficacy and Tolerability of Tapentadol ER for Chronic, Painful Diabetic Peripheral Neuropathy (DPN)

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The efficacy and tolerability of tapentadol ER were evaluated using pooled data from 2 randomized-withdrawal, placebo-controlled, phase 3 studies (NCT00455520; NCT01041859) of similar design in patients with moderate to severe, chronic, painful DPN. In each study, patients were titrated to their optimal dose of tapentadol ER (100-250 mg bid) during a 3-week, open-label (OL) titration period. Patients who had tolerated tapentadol ER and had ≥1-point improvement in pain intensity (11-point NRS) from the start to end of titration were randomized to placebo or tapentadol ER (dose determined during titration) for a 12-week, double-blind (DB) maintenance period. Average pain intensity over the previous 12 hours was recorded twice daily. The primary efficacy endpoint was mean change in pain intensity from the start to Week 12 (LOCF) of DB maintenance. Mean (SD) pain intensity for the overall population (n=1,034) was 7.29 (1.38) at the start of OL titration and decreased to 4.15 (2.10) at the end of titration. With placebo (n=343) and tapentadol ER (n=360), respectively, mean (SD) pain intensity scores were 3.48 (2.02) and 3.67 (1.85) at the start of DB maintenance and 4.76 (2.52) and 3.77 (2.19) at Week 12; mean (SD) changes from the start to Week 12 of DB maintenance were 1.28 (2.41) and 0.08 (1.87), indicating that pain intensity worsened with placebo but was relatively unchanged with tapentadol ER. The least-squares mean difference for the change from start to Week 12 of DB maintenance for tapentadol ER versus placebo was –1.14 (95% CI, –1.435 to –0.838; P<0.001). AEs led to treatment discontinuation for 16.3% (169/1,040)

of patients during OL titration and 8.2% (28/343) of patients receiving placebo and 14.2% (51/360) of those receiving tapentadol ER during DB maintenance. Results of this pooled analysis support those of the individual studies and indicate that tapentadol ER was effective and well tolerated for managing moderate to severe, chronic, painful DPN.

Supported by: Janssen Research & Development, L.L.C., Grünenthal GmbH

COMPLICATIONS—OCULAR

14-LB

The Roadmap to Close the Gap for Vision—Diabetes Related Eye Care in the Indigenous Australian Population

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Blindness from diabetic retinopathy is almost entirely preventable with regular eye examination and timely laser surgery. Limited strategies have been developed to address the alarming discrepancy in blindness from diabetic retinopathy between Indigenous and non Indigenous populations. The aim of this study was to assess the use of eye care services by Indigenous Australians with diabetes and to explore the barriers to service provision, delivery and utilisation and so identify strategies to reduce blindness from diabetic retinopathy. The study included 370 semi-structured interviews from twenty-one sites within capital cities, regional and remote areas across Australia and ten focus groups across seven urban and rural sites in Victoria. Semi-structured interview questions were designed to collect information about eye health services, pathways of care and the co-ordination of services. The analysis identified major gaps in the pathways for diabetic retinopathy screening, including poor knowledge of available services, lack of understanding of referal pathways and no accountability for retinopathy examinations or for image capture 'store and forward' applications. The implementation within primary health care of specific case management for patients with diabetes and the utilisation of e-health tracking and the improvement in applying needs based planning and assessment would lead to a dramatic improvement in Indigenous eye health outcomes among those with diabetes.

15-LB

A Novel Peptide (MTP-131) that Improves Mitochondrial Function Reverses Visual Decline in Mouse Models of Metabolic Dysfunction Leading to Diabetes

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A set of chronic conditions, including obesity and insulin resistance, define the metabolic syndrome, which is a major risk factor for untreatable diabetes and associated visual decline. There is growing evidence that mitochondrial dysfunction is the core cellular pathophysiology of the metabolic syndrome and diabetes. But no study has directly investigated whether improving mitochondrial metabolism improves visual dysfunction. Here we test whether a novel agent that reduces mitochondrial oxidative stress and improves ATP production, is able to reverse diabetic visual decline.

We modeled the metabolic syndrome with progression to diabetes in male C57BL/6 mice, with a diabetic diet from 1 month of age, with streptozocin (STZ) treatment at 2 months. Complementary age-matched groups were fed a diabetic diet alone, received STZ treatment alone, or were fed a normal diet. Over 6 months, body weight and glucose homeostasis were monitored but not controlled, and visual behavior was measured in a virtual optokinetic system (OptoMotry). At 3 months of age, half the animals in each group received daily injections of MTP-131, a small cell-penetrating peptide that selectively accumulates in the inner mitochondria membrane where it scavenges reactive oxygen species and augments ATP production. The rest of the animals received saline injections. Visual dysfunction emerged in the diabetic diet + STZ group after 9 weeks, and was reduced by 15% at 12 weeks of age. Visual decline was halted within 1 week of initiating MTP-131 treatment, and gradually improved thereafter, until normal visual function was restored by 6 months. Groups fed a diabetic diet, or treated with STZ alone displayed less visual decline, and which was also fully restored with MTP-131.

Since MTP-131 has favorable pharmacokinetics and positive human safety results, it may be a viable tool to treat human visual diseases related to metabolic dysfunction, such as diabetic retinopathy.

DIABETIC DYSLIPIDEMIA

16-LB

Rare *LPA* Genetic Variants Affect Lipoprotein (a) Level in the Old Order Amish

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Lipoprotein (a) [Lp(a)] is an independent risk factor for atherosclerosis-related events. Genetic variation in the *LPA* gene may be responsible for between 42% and >90% of variation in circulating Lp(a) concentrations. We performed a genome-wide association scan for genetic variants that associated with serum Lp(a) levels in the Old Order Amish. Using the Affymetrix 500K chip, we successfully genotyped 382,935 single-nucleotide polymorphisms (SNPs) in 1,200 subjects, and imputed all autosomal SNPs in HapMap by MACH. We identified 194 common variants significantly associated with serum Lp(a) levels ($P = 5 \times 10^{-8}$ to 5.02×10^{-33}) spanning ~8.8 Mb region on chromosome 6q25-26 that were within or flanking 26 genes including *LPA*. However, no common SNP in *LPA* itself was significantly associated with Lp(a) levels. To further investigate the effect of *LPA* variants on Lp(a) levels, we sequenced the 40 exons, all intron-exon boundaries and 2 kb of the promoter region of the *LPA* gene in 24 Amish subjects. We identified 23 variants including 6 missense variants in exons 26, 32, 37, 39 and 40. A nonsynonymous SNP (rs3798220, I1891M) in exon 26 was significantly associated with Lp(a) levels ($P = 8.13 \times 10^{-30}$). The minor allele frequency of rs3798220 was 0.9% in the Amish. Conditional association analysis using the SNP (rs3798220) as a covariate revealed that most loci were still significantly associated with Lp(a) levels albeit at lower significance thresholds ($P = 5 \times 10^{-8}$ to 6.56×10^{-15}) indicating that multiple loci in chromosome 6q25-26 regulate Lp(a) levels. Together, these variants in chromosome 6q25-26 explained 14.2% of the variation in Lp(a) levels in the Amish. In conclusion, we have confirmed that a rare variant in *LPA* and some common variants outside of *LPA* on chromosome 6q25-26 are significantly associated with Lp(a) levels. Studies characterizing further variation in the genes in this region and their functional consequences on Lp(a) levels are warranted.

Supported by: AHA

DIABETES EDUCATION

17-LB

Telehealth Program for Medicaid Patients with Type 2 Diabetes Lowers Hemoglobin A1C

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Type 2 diabetes (T2DM) is a chronic medical condition that affects 7.8% of the U.S. population. Only 7% of these individuals meet the guidelines for control of hyperglycemia and hypertension. While a diabetes team approach with nurse-led telephone support has been shown to be effective in helping patients attain treatment goals, prior diabetes telehealth research has included only minimal descriptions of the clinical and telehealth protocols developed by nurses for delivering diabetes self-management education (DSME) and care. In the current analysis, we describe the protocols and procedures employed in an effective diabetes telehealth program named *HouseCalls* that serves a Medicaid, urban dwelling population with T2DM. The degree of patient engagement and clinical outcomes associated with the program are examined, and we discuss the barriers to future scalability and long-term sustainability that our healthcare system will need to overcome if we are to provide realistic solutions in caring for the growing epidemic of T2DM. The 330 patients included in this analysis were 21 years of age or older, had a baseline HbA1c 7.0%, and enrolled in the *HouseCalls* program between January 1, 2008 and December 31, 2009. Mean HbA1c improved significantly for the entire population (mean change: -1.8%; SD = 2.2), with the greatest improvement observed in those patients who graduated from the program (mean change: -3.3%; SD: 2.3). Despite such positive outcomes, barriers exist that threaten the scalability and long-term financial viability of T2DM telehealth. Emerging models provide data to support telehealth and patient-centered care that improves patient access, quality of care, and healthcare costs.

EXERCISE—ANIMAL

18-LB

The Role of NFkB on Exercise Training-Induced Changes in Glucose Tolerance

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Nuclear factor -kB (NFkB) is a transcription factor that controls the gene expression of proteins involved in inflammation, metabolism and angiogenesis. Aerobic exercise has been shown to increase NFkB activity in muscle from rodents and human subjects. However, the physiologic relevance of NFkB activity changes caused by exercise is not known. In this study we tested the hypothesis that NFkB plays a role in glucose homeostasis improvement caused by exercise training. For this purpose, we studied transgenic mice in which NFkB is inhibited by overexpressing an IkB superrepressor mutant in muscle (MISR). We examined the following groups of mice (n=6-10 per group): Sedentary (SED), wild type (WT) and MISR mice on normal (ND) diet for 10 weeks (WT-SED-ND and MISR-SED-ND); Sedentary mice on a high fat diet (HFD) for 10 weeks (WT-SED-HFD and MISR-SED-HFD); trained (TR) mice (on a treadmill) on normal diet (WT-TR-ND and MISR-TR-ND); and trained mice on HFD (WT-TR-HFD and MISR-TR-HFD). Glucose tolerance tests were performed 24 hrs after the last exercise bout. Results: Inhibition of NFkB per se improved glucose tolerance in sedentary mice fed a normal diet (glucose AUCs: WT-SED-ND = 763±35 mg/dl.hr vs MISR-SED-ND = 645±32, $P < 0.05$) and the HFD further worsened glucose tolerance in sedentary MISR mice (AUCs: WT-SED-HFD = 1043±56 vs MISR-SED-HFD = 901±4, $P < 0.05$). Training improved glucose tolerance in WT mice fed both a normal (AUCs: WT-SED-ND = 763±35 vs WT-TR-ND = 672±31, $P < 0.05$) and a HFD (AUCs: WT-SED-HFD = 1043±56 vs WT-TR-HFD = 904±50, $P < 0.05$) diet. In contrast, training did not significantly improve glucose tolerance in MISR mice fed both a normal ($P = 0.2$) and a HFD ($P = 0.7$) diet. Consequently, the beneficial effect of exercise training on glucose tolerance was diminished in MISR mice fed normal and HFD. Summary: (i) Muscle-specific inhibition of NFkB signaling improves glucose tolerance in normal- and HFD-fed mice; and (ii) Intact NFkB signaling is important for training-induced improvements in glucose homeostasis.

19-LB

Endurance or Sprint Interval Exercise, and Metformin Treatment Differently Modify Insulin-Induced Vasodilation in Skeletal Muscle Arterioles of Obese Insulin Resistant Rats

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Insulin-induced vasodilation is obligatory for glucose disposal, and is impaired with insulin resistance. We examined the effects of endurance (EXT) and interval sprint (IST) exercise training with and without metformin (MET) treatment on acetylcholine (ACh) and insulin-induced vasodilation in skeletal muscle arterioles of high and low oxidative muscle from obese, insulin resistant OLETF rats. Rats remained sedentary (SED), or were treated with EXT, IST, MET, EXT+MET, or IST+MET from 20-32wks (n=11-13). At sacrifice, 2nd order arterioles from red (G2AR) and white (G2AW) gastrocnemius muscle were isolated for *in vitro* assessment of vasomotor responses to ACh (10^{-9}-10^{-4} M), insulin (1-1000 μIU/mL), and insulin (1-1000 μIU/mL) + tezosentan (ET-1 antagonist; 3 μM). EXT and IST enhanced ACh responses in both G2AR and G2AW to a greater extent than MET alone (all $p<0.05$). In the G2AR, EXT improved insulin-induced vasodilation compared to IST, while MET was greater than IST alone (all $p<0.05$). ET-1 blockade improved insulin-induced vasodilation in IST compared to EXT, and MET in the G2AW, whereas IST+MET exhibited less vasodilation compared to IST (all $p<0.05$). In conclusion, the effects of EXT and IST on insulin-induced vasodilation in 2A arterioles of low and high oxidative muscle fibers are similar; but the mechanisms appear to be different. EXT selectively improved insulin-induced vasodilation in G2AR, and was not affected by ET-1 blockade, suggesting it is mediated by nitric oxide. Conversely, no insulin-induced vasodilation was observed in the G2AW following IST, yet a marked vasodilation occurred with insulin + ET-1 blockade. Overall these data suggest that the type of exercise training, and treatment with MET, have differential effects on ACh- and insulin-induced vasodilation in skeletal muscle arterioles perfusing high vs. low oxidative muscle fibers in the obese, insulin resistant OLETF rat.

Supported by: NIH (RO1HL036088), VA (CDA-2 IK2BX001299-01)

20-LB

Impact of Exercise and Saxagliptin on Mitochondria in the Diabetic Vasculature

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Exercise decreases cardiovascular disease (CVD) risk and all-cause mortality. Mitochondrial adaptation is induced by exercise in many target organs, including the vasculature. In healthy vasculature, exercise upregulates mitochondria and its upstream regulators such as endothelial nitric oxide synthase (eNOS), sirtuins (SIRTs), and/or PPARγ co-activator alpha (PGC1-α). We tested the hypothesis that vascular mitochondrial adaptation to exercise is impaired by diabetes and that dipeptidyl peptidase-4 (DPP-4) inhibitor saxagliptin (SAX) will restore this response via the activation of eNOS, SIRTs, and/or PGC1-α. To test this hypothesis, we examined an exercise intervention with or without SAX in the Goto-Kakizaki (GK) rat, a model of lean, type 2 diabetes. Male rats in either exercise or sedentary groups were run on a treadmill for 8 days. Aortas were removed, processed for Western analysis, and probed for mitochondrial complexes I-V, as well upstream signaling molecules. In diabetes + exercise group, there was a decrease in the expression of mitochondrial complexes I-V. The interaction of exercise with SAX was significant ($p=0.0306$) and resulted in an increase (36%) of complex IV compared to the sedentary + SAX group. This trend of increased expression was also observed in total mitochondrial complexes of the exercise + SAX group. Consistent with these results, exercise + SAX also had a significant 29% increase effect on eNOS protein content ($p=0.0268$) compared to the sedentary + SAX group. Also, there was a significant effect of both exercise and SAX on PGC1-α expression (26% increase, $p=0.018$) and SAX alone on the increase of SIRT3 expression ($p=0.049$). In summary, there is no induction of eNOS, PGC1-α, SIRT3, or mitochondrial protein expression with exercise in diabetes; exercise plus SAX increased eNOS, PGC-1α, SIRT3 and the mitochondrial response to exercise in diabetes. Thus, our data suggest that saxagliptin restores the healthy mitochondrial adaptation to exercise in a diabetes model.

Supported by: Bristol-Myers Squibb, VA Merit (HL56481, RR025780)

21-LB

Exercise Training Induces Basal Autophagy and Autophagy is Required for Metabolic Adaptation in Skeletal Muscle

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Autophagy, a catabolic process for clearance of aggregate proteins and damaged organelles (e.g. mitochondria), is required for normal muscle function. Acute exercise activates autophagy in skeletal muscle; however, it is unknown whether exercise training promotes autophagy and whether autophagy is required for skeletal muscle adaptation. Here, we report that long-term (4 weeks) voluntary wheel running promotes basal autophagy in recruited plantaris muscle in mice as shown by increased protein expression of autophagy related genes (Atg6/Beclin1, Atg8/LC3), a mitophagy gene (Bnip3) and increased autophagy flux (increased LC3-II and decreased p62/Sqstm1). Similar directional changes were found when comparing slow-twitch, oxidative soleus muscle with intermediate plantaris and fast-twitch, glycolytic white vastus lateralis muscles. We also found that transgenic mice with muscle-specific overexpression of PGC-1*alpha* have increased basal autophagy with elevated Bnip3 expression, but not significant increases in Atg6 and LC3, suggesting that PGC-1*alpha* is sufficient to promote autophagy likely through enhanced Bnip3 expression. Finally, voluntary exercise-induced improvement of endurance capacity is absent in heterozygous Atg6 knockout mice ($Atg6^{-/+}$) along with attenuated increases in markers of mitochondrial biogenesis, autophagy and autophagy flux in muscle. These findings suggest that endurance exercise training promotes basal autophagy in skeletal muscle and that autophagy is required for mitochondrial biogenesis, as well as improved exercise capacity.

Supported by: APS Postdoctoral Fellowship (V.A.L.)

EXERCISE—HUMAN

22-LB

Enhancement of Physical Performance by 10 Weeks Exercise or rhEPO Injections Does Not Change VLDL-TG Turnover or Oxidation

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Lipids are important energy substrates at rest and during exercise. However, little is known about the role of VLDL triglyceride (VLDL-TG). We compared the effect of two performance-enhancing interventions on VLDL-TG fatty acid (FA)

oxidation (ox) and turnover in healthy, sedentary men; endurance exercise (ex) and recombinant human erythropoietin (EPO) treatment.

In a randomized, single-blinded, placebo (pl)-controlled design healthy, sedentary men were studied before and after 10 weeks of supervised endurance bicycle ex thrice weekly + pl (n=9); no ex + EPO (n=8); no ex + pl (n=9). A 4th group (ex + EPO) (no TG kinetics) was included to ensure blinding. Before and 3 days after the interventions basal VLDL-TG kinetics was examined using a 4h infusion of triolein labelled 14C-VLDL-TG in combination with indirect calorimetry and 14CO2 specific activity in expired air followed by a 2h hyperinsulinemic euglycaemic clamp to assess insulin sensitivity (M-value). VO2max was measured before and after interventions.

Ex and EPO both increased VO2max ($p<0.05$), but only ex improved the M-value (mg/kg/h) significantly (ex 3.5 ± 0.5 to 5.0 ± 0.5*, EPO 4.6 ± 0.8 to 5.6 ±1.1, pl 3.5 ± 0.5 to 3.9 ± 0.4, *$p<0.05$ vs. basal). BMR tended to increase in both intervention groups, although not significantly ($p=0.06$). RER was unchanged in each group. Basal VLDL-TG secretion (μmol/min) did not change significantly compared with pl following ex (41.1 ± 8.0 to 49.1 ± 7.2) or EPO (57.4 ± 9.1 to 49.1 ± 9.1) compared with pl (45.4 ± 5.9 to 51.4 ± 12.9). Moreover, no significant change was noted in VLDL-TG FA ox (μmol/min) (ex 54.8 ± 3.6 to 50.6 ± 2.7, EPO 44.6 ± 1.6 to 53.0 ± 4.7, pl 47.9 ± 1.8 to 53.9 ± 5.8) or clearance. Plasma VLDL-TGs were not different between the groups.

In conclusion: Despite improved aerobic capacity and insulin sensitivity VLDL-TG FA turnover and oxidation were unaffected by 10 weeks of ex or EPO treatment. VLDL-TG FA ox remained, however, a significant energy source for oxidative metabolism.

NUTRITION—CLINICAL

23-LB

Poor Diet Quality Associated with Increased Risk of Incident Colorectal Cancer in People with Type 2 Diabetes

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People with type 2 diabetes are at increased risk of development of colorectal cancer. The aim of this study was to investigate whether poor diet quality contributes to the risk of colorectal cancer in a cohort study of people with type 2 diabetes. Data from 27986 men and 13548 women, aged 50-71 years, with self-reported diabetes who participated in the NIH-AARP (National Institute of Health-American Association of Retired Persons) Diet and Health Study and were cancer free at baseline (1995-1996) were analyzed. Diet quality was measured with Healthy Eating Index-2005 (HEI-2005), with higher scores indicating a better diet quality. Stratified Cox regression models were constructed to estimate the sex-specific Hazard ratios (HRs) and 95% confidence intervals (95% CIs) of incident colorectal cancer associated with diet quality. Models were adjusted for age, body mass index (BMI), physical activity, multivitamin use, smoking status, race/ethnicity, family history of colon cancer, history of colorectal polyp, education, and replacement hormone therapy (for women). During an average follow-up of 9.1 years, we identified 876 (629 men and 247 women) new cases of primary colorectal cancer. The mean (SE) HEI-05 scores of the persons who subsequently developed colorectal cancer and that of the rest of the cohort were, respectively, 69 (0.06) and 68 (0.42), P = 0.02, among men and 72 (0.08) and 71 (0.61), P = 0.08, among women. Poor diet quality (tertile 1 vs. tertile 3 as reference) was associated with an increased risk of colorectal cancer both among men (HR: 1.36, 95% CIs: 1.11, 1.66) and among women (HR: 1.63, 95% CIs: 1.18, 2.24). Poor diet quality may play a role in incident colorectal cancer among people with type 2 diabetes.

24-LB

Reduction in Glycemic Variability and Hyperglycemia with a Low-Glycemic Index Portion-Controlled Diet in Persons with Type 2 Diabetes

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Although HbA_{1c} is usually the primary endpoint in diabetes intervention trials, this measure accounts for a modest portion of the variance in disease outcomes. Evidence suggests that glycemic variability promotes oxidative stress, which, in turn, increases risk of diabetes-related morbidity. This study compared glycemic variability in response to two diets: usual diet (UD) and a commercially available low-glycemic index portion-controlled diet (PCD; Nutrisystem D). Twelve obese adults with type 2 diabetes participated in this randomized cross-over trial, which was completed in March 2012. During each 2-week diet period (periods were separated by a 1-week washout), participants wore a continuous glucose monitoring (CGM) device and were blinded to output. Outcomes included the 14-day mean (M), standard deviation (SD), and interquartile range (IQR) of blood glucose values, as well as % of readings in the hypoglycemic (%hypo; < 3.9 mmol/l), euglycemic (%eu; 3.9-10.0 mmol/l), and hyperglycemic (%hyper; > 10.0 mmol/l) ranges. Two participants who were randomized to receive PCD first refused UD in the second test period and, thus, were excluded from analyses. The sample included 4 men and 6 women (age = 55.4 ± 11.6 years, BMI = 37.7 ± 2.9 kg/m^2, HbA1c = 6.8± 0.9%) with a history of diabetes for 4.9 ± 5.9 years. Paired t-tests revealed significantly less glycemic variability during the PCD vs. UD (SD: 1.77 ± 0.52 vs. 2.01 ± 0.57 mmol/l, $p < .04$; IQR: 2.16 ± 0.75 vs. 2.5 ± 0.93 mmol/l, $p < .05$) and significantly lower mean values (7.62 ± 1.62 vs. 8.27 ± 1.85 mmol/l, $p < .01$). There were no differences in %hypo (3.3 ± 5.5 vs. 1.3 ± 1.6%, $p = .19$) or %eu (83.3 ± 18.7 vs. 77.8 ± 23.0%, $p = .14$), but %hyper was significantly lower during the PCD period (13.4 ± 19.5 vs. 21.0 ± 23.8%, $p = .02$). These findings suggest a favorable, rapid-onset effect of the low-glycemic index PCD on glycemic variability and hyperglycemia as compared with usual diet in persons with type 2 diabetes.

PSYCHOSOCIAL—BEHAVIORAL MEDICINE

25-LB

The Relationships Among Help-Seeking Behavior and Cognition in Patients with Diabetes (ACCORD-MIND)

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Diabetes self-management requires the complex self-regulation of numerous distinct behaviors. Although it is well-established that patients with diabetes are at increased risk for cognitive decline, little research exists examining the extent to which diabetics seek help with self-management. The purpose of the present study was to examine the relationship between help-seeking behavior and cognition in patients with diabetes. Patients from the ACCORD MIND study (n = 2977, M age = 62.5 years; SD = 5.8) indicated how much help that they receive from others for a) following a doctor's plan for managing diabetes, b) remembering to take diabetes medication, and c) remembering to check blood sugar. The response scale consisted of "a lot," "some," "a little," and "none." Cognition was assessed with the digit symbol substitution task (DSST), the Rey Auditory Verbal Learning Test (RAVLT), the Mini-Mental State Examination (MMSE), and the Stroop test. Help-seeking and cognition were assessed at baseline and 20-months of follow up. A series of logistic regression models predicting help-seeking that included each measure of cognition separately indicated that higher levels of cognition at baseline significantly predicted less help-seeking at 20 months. Additionally, models that included all of the cognitive measures together indicated that the DSST (speed of processing and memory) was significantly related to help-seeking relative to checking blood sugar ($p<.05$) and taking diabetes medication ($p<.05$). The results of the present study indicate that cognition is related to one's ability to seek help with diabetes self-management and may have important implications for glycemic control.

Supported by: NIA, NHLBI (ACCORD-MIND)

26-LB

A Telephone Intervention for Diabetes Prevention: The Call-2-Health Trial

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Specific aims: 1) evaluate the feasibility and acceptability of a telephone-based weight loss and exercise intervention; and 2) conduct a preliminary evaluation of the intervention's effectiveness.

The 24-week Call-2-Health program includes 12 weekly 20-minute calls followed by 4 maintenance calls over 12 weeks. We used the electronic health record to identify potential participants who: 1) were not diabetic; 2) had a BMI >25 kg/m^2; and 3) had, within the prior 5 years, a FPG of 105-125 mg/dL, a HbA1C 5.7-6.4%, and/or a random glucose of 140-199 mg/dL. Potential participants were sent an invitation letter, screened via phone, and invited to participate. Those eligible and interested had blood drawn and those still eligible (FPG 100-125 mg/dL, HbA1c 5.7-6.4%) were invited to participate. From 438 potential participants, 10.7% were randomized, 45.7% were ineligible, and 43.6% refused. Characteristics of the control and intervention groups were similar except for baseline steps/day (7957 vs. 5654 respectively).

Among 24 participants randomized to the intervention, 93% of intervention calls and 85% of maintenance calls were completed. Twelve and 24 week visits were completed by 93.4%, and 85.4% of intervention and 87% and 82.6% of controls. Compared to usual care, the Call-2-Health intervention led to significant improvements in weight, BMI, HbA1c, and steps per day (Table 1).

Call-2-Health is a highly promising diabetes prevention intervention with enormous potential for translation in a wide range of diverse settings.

Table 1: 24-week findings, Call-2-Health Pilot Trial*

	Control	Intervention	Difference	
	Mean (95% CI)	**Mean (95% CI)**	**Mean (95%CI)**	**p-value**
Weight (kg)	94.9 (92.8, 96.9)	88.0 (85.3, 90.6)	-6.9 (-10.2, -3.5)	<.0001
BMI (kg/m²)	32.7 (32.0, 33.4)	30.3 (29.4, 31.2)	-2.4 (-3.5, -1.3)	<.0001
HbA1c (%)	5.8 (5.7, 5.9)	5.6 (5.5, 5.7)	-0.2 (-0.4, -0.1)	<0.005
Steps / day	6283 (4695, 7870)	8939 (7863, 10015)	2656 (729, 4584)	<0.01

*All values adjusted for baseline values

Supported by: NIDDK (R34 DK076555)

27-LB

GAPP2™: Global Survey Finds in the Last Month One in Four Type 2 Diabetes Patients Do Not Take Basal Insulin as Prescribed and Over a Third Suffer Hypoglycaemia

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Injection non-adherence and self-treated hypoglycaemia (hypo) are continued management challenges in insulin treated type 2 diabetes (T2DM). The frequency and impact of basal insulin (BI) taking behaviour and hypos were investigated in a large six-country internet survey (GAPP2™) from 3042 T2DM patients using insulin analogues (IA) and 1653 healthcare professionals (HCPs) (primary care, diabetes specialists and diabetes nurses/educators).

In the last 30 days, 22% of patients missed a BI (mean 3 times), 24% took a BI >2 h beyond the scheduled time (mean 4.2 occasions) and 14% reduced a BI (mean 4.2 times). 39% of patients worried if they missed their BI and 37% felt guilty about missed doses.

In the last 30 days, significantly more patients who missed a BI in this time period reported a hypo compared to those who did not (41% vs 34%). Overall, 36% of patients reported a hypo in the last 30 days, 26% of which were nocturnal. Nocturnal events worried patients (42%) more than diurnal hypos (23%) but HCPs said that only an average of 24% of patients reported this nocturnal hypo worry.

74% of HCPs routinely discussed BI dosing irregularities with IA patients and hypos were also discussed. However, only around a third of HCPs reported always discussing these events with their BI only (27%) or basal-bolus (37%) patients.

Dosing irregularities as well as hypos are not uncommon and impact diabetes treatment and management. Despite apparent HCP awareness, T2DM patients using IA still need further support to improve medication taking behaviour and reduce rates of hypoglycaemia.

Missed/mis-timed/reduced doses of BI, and self-treated hypoglycaemia in T2DM patients taking IA

	Missed dose (n=2883)	Dose taken >2 hrs earlier or later than prescribed (n=2741)	Reduced dose (n=2913)	Self-treated hypoglycaemia (n=2918)
Ever	48%	51%	38%	80%
More than 1 year ago	7%	6%	6%	13%
Within last year	10%	10%	9%	16%
Within last 90 days	9%	11%	9%	16%
Within last 30 days	22%	24%	14%	36%
# times in last 30 days (Mean ± SE)	3±0.16	4.2±0.21	4.2±0.24	3.1±0.09
5+ times in the last 30 days	17% (n=647)	27% (n=656)	27% (n=417)	19% (n=1042)

Supported by: Novo Nordisk A/S

28-LB

Lifetime Exposure to Racist Events and Heart Rate Variability During Acute Stress in Women with Type 2 Diabetes

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Exposure to racism has been linked to adverse cardiovascular functioning. Heart rate variability (HRV) is a measure of autonomic tone that predicts cardiovascular morbidity in diabetes. In a multi-racial sample of women with type 2 diabetes (T2DM), This study investigated self-reported exposure to racism and autonomic reactivity as measured by high frequency HRV (HF-HRV) during acute stress.

Measures: Exposure to racist events was measured by adding the lifetime frequency and stressfulness subscales of the Schedule of Racist Events (SRE; Landrine 1996). Beat-to-beat intervals were recorded on ambulatory ECG recorders (GE Medical); HF-HRV was calculated using spectral analysis.

Methods: 33 women (16 African-American, 17 White) with T2DM ate a standardized breakfast and completed a resting baseline, followed by 6-min public speaking stressor in which participants defended themselves against a false accusation of shoplifting. HF-HRV was assessed at baseline and during the speaking stressor.

Results: African-Americans and Whites did not differ significantly on A1c or resting heart rate, but as expected, African-Americans had significantly higher SRE scores than Whites. In zero order correlations, stressor HF-HRV was significantly related to SRE scores, resting HR, and marginally related to A1c. In multiple regression, higher SRE scores predicted lower HF-HRV during acute stress (R2=.23, F(1,32)=9.29, beta=-.48, *p<.01), but not at rest, p=.19. In regression controlling for A1c, resting HR, and race, SRE scores remained a significant independent predictor of HF-HRV (R2=.41, F(4,32)=4.76, *p<.01). A test for an interaction of race x SRE scores was not significant. HF-HRV was unrelated to hostility, depression, neuroticism, and general perceived stress.

Conclusions: Preliminary data suggest that among women with T2DM, exposure to racism is adversely associated with autonomic reactivity during acute stress. Findings should be replicated in a larger sample.

CLINICAL THERAPEUTICS/NEW TECHNOLOGY—GLUCOSE MONITORING AND SENSING

29-LB

Evaluation of the System Accuracy of 34 Blood Glucose Monitoring Systems According to EN ISO 15197

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Self monitoring of blood glucose (SMBG) is used for metabolic control and for therapy adjustment by patients. Therefore, high quality blood glucose monitoring systems are required. In this study, 34 currently available SMBG systems with a CE-label were evaluated for system accuracy according to the European Standard EN ISO 15197.

Measurements were performed in duplicate from 100 capillary blood samples with glucose concentrations ranging from 20 mg/dL to 600 mg/dL. Results were compared with the respective reference method (manufacturer's measurement procedure): glucose oxidase method (YSI 2300 STAT PLUS) or hexokinase method (Hitachi 917 / cobas c 501). According to EN ISO 15197, the minimum acceptable accuracy limits for SMBG systems are as follows: 95 % of the results shall fall within ± 15 mg/dl of the results of the reference measurement at glucose concentrations < 75 mg/dL and within ± 20 % at glucose concentrations ≥ 75 mg/dL.

Of the 34 investigated SMBG systems, 27 systems complied with the system accuracy requirements stated in EN ISO 15197. The other 7 systems did not meet the system accuracy requirements. The mean percentage of results within the required accuracy limits was 96.0 ± 6.2 % [± SD], ranging from 72 to 100 % for the different systems.

In this evaluation 7 of 34 SMBG systems did not meet the minimum accuracy limits required in EN ISO 15197. SMBG systems providing inaccurate results bear the risk of false therapeutic decisions by the patient with the risk of severe health injury. Regular and standardized evaluation of SMBG systems might be helpful to ensure adherence to quality standards.

Supported by: Roche Diagnostics GmbH, Germany

30-LB

Accuracy and Acceptability of the 6-Day Enlite Continuous Subcutaneous Glucose Sensor

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The Enlite subcutaneous glucose sensor (Medtronic) was previously evaluated in adults and children and shown to be accurate for 6 days, durable, and acceptable to patients and parents/caregivers. A pivotal 6-day trial in adults was conducted at 7 US investigational centers. Sensors were self-inserted and taped, and were calibrated 3-4 times/day. Accuracy was evaluated vs frequently-sampled YSI plasma glucose values. Hypoglycemia and hyperglycemia were induced on days 1 (immediately after initial calibration), day 3, and day 6. Patient satisfaction with Enlite was also evaluated with a 7-point Likert-type questionnaire. Adults with type 1 (N=65) or type 2 (N=25) diabetes (mean age 44, range 18-71) wore either 1 or 2 sensors on their abdomens for 6 days. The Table shows mean and median absolute relative differences (ARD) between sensor and YSI glucose values by day and by glucose range. Mean responses of patient satisfaction surveys were 5.9/7 for "ease of use," 6/7 for "comfort," 5.9/7 for "ease of insertion," and 5.8/7 for "would recommend." There were no device-related adverse events. We conclude that the Enlite sensor is accurate, durable, comfortable, safe, and easy to use. Improvements in continuous glucose sensing should expedite development of semi-automated insulin delivery features in modern pumps.

Table. Enlite sensor accuracy by mean and median absolute relative differences

By Day	Overall	Day 1	Day 3	Day 6
Mean (Median) ARD	13.6% (10.1%)	15.9% (12.2%)	11.8% (9.1%)	13.2% (9.6%)
By Glucose Range	Overall		75-180 mg/dL	>180 mg/dL
Mean (Median) ARD	13.6% (10.1%)	10.8* (8.5)*	12.7% (9.4%)	12.0% (9.0%)

* mean absolute differences

Supported by: Medtronic, Inc.

31-LB

3 Continuous Glucose Monitoring Systems: A Comparison with 6 Sensors per Subject in Parallel

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This study aimed to compare 3 commercially available needle-type continuous glucose monitoring (CGM) systems under daily life conditions.

Twelve people with type 1 diabetes (age: 47.7 ± 6.9 years (mean ± standard deviation (SD)), HbA1c 8.2 ± 1.4 %, duration of diabetes: 23.7 ± 9.9 years) wore 6 sensors in parallel, two sensors each of the three following CGM systems: Abbott Diabetes Care FreeStyle Navigator™, DexCom™ Seven® Plus 3rd Generation and Medtronic MiniMed Guardian® REAL-Time with Enlite Sensor. Each system was used for one sensor lifetime, which was 5 days for the FreeStyle Navigator™, 6 days for the Guardian® REAL-Time or 7 days for the Seven® Plus. CGM measurements were paired to capillary blood glucose (bG) measurements (approx. 30 per day and subject). Performance was characterized by mean absolute relative differences (MARD) between CGM and paired bG measurements and percentage absolute relative difference (pARD), calculated from the absolute relative differences between paired CGM measurements of the two sensors of each system.

Capillary bG measurements ranged from 35 to 446 mg/dL. MARD and pARD are reported in the table (mean ± SD [range]).

All systems could be worn for nearly 100 % of their estimated lifetime (FreeStyle Navigator™: 100.6 %, Seven® Plus: 98.3 % and Guardian® REAL-Time: 99.0 %) before the sensors had to be removed.

The 3 CGM system tested showed good overall performance, with the FreeStyle Navigator™ achieving lower sensor-to-bG differences and sensor-to-sensor differences than the DexCom™ Seven® Plus 3rd generation and the Medtronic MiniMed Guardian® REAL-Time with Enlite Sensor.

CGM system characteristics			
CGM system	MARD (in %, n = 24)	pARD (in %, n = 12)	Average data reporting percentage (in %)
FreeStyle Navigator™	12.4 ± 3.6 [8.4 - 19.0]	10.1 ± 4.1 [5.2 - 17.6]	97.3 ± 5.2
Seven® Plus	16.7 ± 3.8 [12.7 - 29.2]	15.4 ± 4.2 [9.2 - 23.5]	95.9 ± 4.6
Guardian® REAL-Time	16.4 ± 6.9 [7.5 - 23.9]	18.1 ± 6.5 [9.2 - 31.2]	98.9 ± 0.8

Supported by: Roche Diagnostics GmbH, Germany

32-LB

Randomized Outpatient In-Home Pilot Trial of Predictive Nocturnal Pump Shut-Off

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We have developed a nocturnal hypoglycemia prediction algorithm to suspend insulin pump delivery to avoid hypoglycemia that utilizes Kalman filtered glucose sensor data. This study was designed to test how the system and algorithm functions with home use and is among the first studies in the US of in home partial closed-loop control.

Each subject wirelessly linked a Medtronic Paradigm® insulin pump and Sof-Sensor® to a small bedside laptop computer nightly. The algorithm stops insulin infusion when the sensor glucose is predicted to become low. Once the glucose is past the nadir, basal insulin is restarted. The maximum single suspension time is 120 minutes, and the maximum suspension time each night is 180 minutes. Once the sensor glucose is past the nadir, basal insulin is restarted. A randomization occurs each night on initialization of the system, so that the algorithm is active on 67% of the nights and 33% of nights are control nights (algorithym off).

Five subjects (ages 18-32 yrs.; A1c's 6.4-7.7%) were studied for 101 nights with the algorithm active on 65 nights. When active a pump shut-off occurred on 77% of the nights with median a shutoff duration of 60 minutes.

Glycemic Changes with Nocturnal Pump Suspension		
Algorithm Status	Algorithm Off	Algorithm On
Mean Bedtime Glucose	167 +/- 53 mg/dl	154 +/- 47 mg/dl
Mean Fasting Glcuose	123 +/- 48 mg/dl	157 +/- 52 mg/dl
% Fasting <60 mg/dl	8%	2%
% Fasting <70 mg/dl	11%	5%
% Fasting >180 mg/dl	17%	33%
Mean CGM glucose Overnight	144 +/- 39 mg/dl	159 +/- 34 mg/dl
% nights with CGM <70	28%	18%

Predictive algorithms utilizing continuous glucose sensor data to suspend insulin delivery can be safely initiated in the home setting. The algorithm is being modified to decrease the length of pump suspension so there will be a smaller increase in mean overnight and mean fasting glucose levels when the algorithm is active.

Supported by: NIH

CLINICAL THERAPEUTICS/NEW TECHNOLOGY—INSULIN DELIVERY SYSTEMS

33-LB

Clinical Evaluation of a Fully-Automated Artificial Pancreas Using Zone-Model Predictive Control with Health Monitoring System

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We evaluated a fully automated closed-loop artificial pancreas in 12 subjects (8F:4M) with type 1 diabetes (age mean±SD 49.4±10.4, diabetes duration 32.7±16.0y, A1C 7.3±1.2). Subjects were studied in-clinic for 25 hours. One day prior to the session, two continuous glucose sensors (DexCom, San Diego) were inserted. CGM values were used as the input of the system. Subjects arrived at 4PM, their insulin pump was discontinued and a study

CSII pump (Animas, West Chester, PA) was inserted. Plasma glucose was measured every 30 min by YSI for safety. The subjects ate 2 mixed meals (50 gm CHO dinner/40 gm CHO breakfast) and underwent a 30 min exercise session at 50% of the predicted heart rate reserve at 11:30 AM. There were no meal announcements or pre-meal boli. Subjects were discharged at 6PM on the following day.

A zone-model predictive controller was used to control glucose concentration. The controller used an a priori model that was initialized with the subject's total daily insulin. The controller was designed to keep the glucose between 70-180mg/dl during the day and 80-140mg/dl overnight. A predictive hypoglycemia prevention algorithm (Health Monitoring System/HMS) designed to alarm if the glucose was predicted to cross 70 mg/dl in the next 15 min was used in conjunction with the zone controller to minimize the risk of hypoglycemia. The subject was given 16 gm CHO if the HMS alarm was triggered.

Time in range (YSI 70-180 mg/dl): Entire session = 81%, 12AM to 7AM = 92%, session minus postprandial/exercise periods = 92%. Time in range 80-140 mg/dl: 12 AM to 7 AM = 70%. Time spent < 70 mg/dl for entire session by YSI = 1%. During the overnight period, there were no YSI values below 70 mg/dl. There were no safety events. The HMS sent appropriate warnings to prevent hypoglycemia via SMS & MMS.

The combination of the zone-MPC controller and the HMS hypoglycemia prevention algorithm was able to safely regulate glucose, including challenges of unannounced meals and moderate of exercise.

Supported by: NIH

34-LB

Initial Clinical Experience with Hyaluronidase Preadministration in the Treatment of Type 1 Diabetes by Sensor Augmented Analog Insulin Pump Therapy

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Patients with type 1 diabetes participating in randomized, controlled, double-blind, 2-way crossover random sequence outpatient clinical pharmacology study were treated with sensor augmented insulin analog pump therapy (SAP) with and without hyaluronidase (rHuPH20; Hylenex® recombinant, Halozyme Therapeutics) between study visits. 150 units (1.0 ml) of Hylenex (or sham injection) were administered through the infusion cannula immediately following each infusion site change (every 72 hours) by trained personnel not otherwise involved in the study. The first three subjects completing the study represent the initial clinical experience using rHuPH20 preadminstration for outpatient control of blood glucose, through approximately 2 weeks of treatment covering 4 infusion set cycles each. Subject #1 (Caucasian Female; Age 22; BMI 26.7; A1C 8.4) was experienced in SAP while Subject #2 (Native Hawaiian Female; Age38; BMI 29.9; A1C 7.9) and Subject #3 (Caucasian Female; Age 21; BMI 21.9; A1C 8.4) were experienced pumpers new to SAP. All three patients were able to achieve tighter glucose control both lowering their mean CGM glucose and glucose variability, primarily by decreasing hyperglycemia. Hypoglycemic events (determined from symptoms and SMBG records with values ≤70 mg/dL) were mild, and of similar frequency, with 6 episodes during analog alone and 7 episodes after rHuPH20 pretreatment.

Sensor Glucose Values and Distribution

Glucose Level (mg/dL)	Sub#1 Analog alone	Sub#2 Analog alone	Sub#3 Analog alone	Sub#1 (+PH20)	Sub#2 (+PH20)	Sub.#3 (+PH20)
Mean	167	187	232	162	177	202
SD	74	85	104	63	74	84
# of Values	N=2723	N=2263	N=2547	N=2736	N=2205	N=2524
<70	129 (5%)	101 (4%)	43 (2%)	89 (3%)	53 (2%)	134 (5%)
70-180	1631 (60%)	1071 (47%)	941 (37%)	1810 (66%)	1314 (60%)	960 (38%)
>180	963 (35%)	1091 (48%)	1563 (61%)	837 (31%)	835 (38%)	1430 (57%)
>240	359 (13%)	541 (24%)	1123 (44%)	318 (12%)	408 (18%)	804 (32%)

CLINICAL THERAPEUTICS/NEW TECHNOLOGY—PHARMACOLOGIC TREATMENT OF DIABETES OR ITS COMPLICATIONS

35-LB

Pantoprazole Reduces A1C levels in Type-2 Diabetes: A Randomized Double-Blind Placebo Controlled Trial, PANDIT (PANtoprazole in Diabetes Trial)

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The role of entero-insular axis involving gastrin, insulin and beta cells regulating blood glucose was reported in animal and retrospective clinical studies. The proton pump inhibitors (PPIs) are known to augment gastrin levels by reducing gastric pH. Since there was no controlled clinical trial data available evaluating the efficacy of PPIs on glucose-insulin homeostasis in type-2 diabetes (T2DM), the present study was planned.

Out of the 49 screened, 31 eligible patients were randomized to receive either pantoprazole 40 mg twice daily (n=16) or matching placebo (n=15) in equal ratio. The investigators, patients, and study staff were blinded to treatments. Change in A1C was the primary end point while changes in fasting and post-prandial glucose, fasting insulin, GLP-1 and gastrin levels were the secondary end-points. Patients were evaluated at 0, 4, 8 and 12 weeks.

Twelve weeks of pantoprazole therapy significantly reduced A1C level (mmol/mol) from the baseline (± SD) 59.95 ± 8.55 to 51.17 ± 7.53 (95% CI; 5.48-12.08, P <0.001). In addition, there was significant reduction in blood glucose and increase in plasma insulin, beta-cell function, GLP-1, and gastrin levels (P <0.05 for all). A total of nine adverse events were reported during the trial, all of which were mild in nature and similar in both the groups.

The present proof of concept study, for the first time demonstrated the efficacy of the PPI pantoprazole on glycaemic control in T2DM in a randomized double blind manner and established its role on glucose-insulin homeostasis. The mechanism is through a possible feedback increase in gastrin, GLP-1 and improvement in pancreatic beta-cell function related to the incretin like effects. However, larger clinical trials in diverse patient populations using different PPIs are probably needed to establish their full therapeutic potential in diabetes.

Supported by: PGIMER, Chandigarh

36-LB

Impact of Subclinical Cognitive Impairments in Type 2 Diabetics on Achieving Glycemia Treatment Goals: The Action to Control Cardiovascular Risk in Diabetes Study (ACCORD)

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Subclinical cognitive impairment (SCI) is prevalent in community dwelling older adults. However, the impact of SCI on achieving and maintaining treatment goals in diabetes is unknown.

The ACCORD-MIND study enrolled 2977 type 2 diabetics. Approximately half of the participants were assigned a standard A1C goal of of 7.0 to 7.9 and the other half an intensive goal of <6.0. Persons with known cognitive impairment were excluded by their physician. At baseline and after randomization, cognitive function was assessed using the Digit Symbol Substitution Test (DSST), the Rey Auditory Verbal Learning Test (RAVLT), the Stroop test and the Modified Mini-Mental State Exam (MMSE). A composite cognition score (CCS) was calculated as the sum of z-scores of all four measures. SCI was defined as a score below 27 on the MMSE plus > 1.5 SD below the mean score on at least 1 of the other measures. The outcome assessed for this analysis was time from randomization to achieve glycemic treatment goals (AGTG). A goal of 7.5 was used for standard participants. Participants starting out with less than these values at baseline were excluded. Separate analyses were performed for standard and intensive groups. We examined the association of time to AGTG with DSST, RAVLT, Stroop, MMSE, CCS and SCI after varying levels of covariate adjustments.

In the standard group, none of the cognitive measures were predictive of AGTG. In the intensive group, those with SCI at baseline were 26% more likely to AGTG as compared with those with no SCI (HR: 1.26, 95% CI: 1.004-1.587, p-value: 0.0462). DSSST, RAVLT, Stroop MMSE and CCS were not statistically significant in predicting AGTG for the intensive group.

The link between SCI and glycemia treatment response implies that health care providers should assess diabetic patients for possible SCI and more closely monitor patients found to have CI to assist them with better management strategies.

Supported by: NHLBI (N01 HC 95178)

37-LB

β-Cell Function in Latent Autoimmune Diabetes in Adults (LADA) Treated with Linagliptin versus Glimepiride: Exploratory Results from a Two Year Double-Blind, Randomized, Controlled Study

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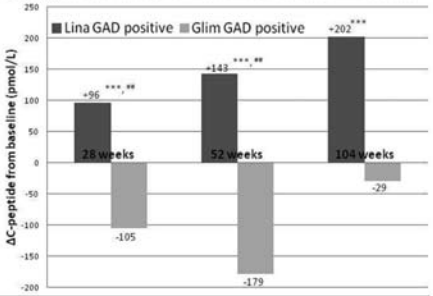
LADA is associated with a more rapid decline in β-cell function than common type 2 diabetes (T2D). Presently, no treatment modality is drug of choice in LADA. We compared the impact of treatment with the DPP4 inhibitor linagliptin (lina) 5mg/d and the sulfonylurea glimepiride (glim) 1-4mg/d on β-cell function in patients retrospectively identified with LADA who had insufficient glycemic control despite metformin therapy in a 2 yr study (NCT00622284). Patients were classified as LADA if ≥1 of the autoantibodies assessed (GAD65, ICA, IA-2A, IAA) were present at baseline or any on-treatment visit. GAD was assessed with RIA (cut-off 0.05 [sensitivity 82%/specificity 98.89% in the Diabetes Autoantibody Standardization Program 2010]).

Of 1519 patients (16 countries) with assumed T2D, 7.8% (n=118) had LADA. GAD65 was the most prevalent autoantibody (6.5%) (ICA 0.3%, IA-2A 1.2%, IAA 0.2%). Proportion of patients with ≥2 positive antibodies was 0.4%. At baseline, GAD65+ LADA patients treated with lina (n=65) or glim (n=53) were fairly well balanced (respective age 59/63 yrs, BMI 30.3/31.7 kg/m^2 and diabetes duration >5 yrs 62%/59%). C-peptide was available in a subset and GAD65+ patients treated with lina preserved C-peptide significantly better than those treated with glim over a 2 yr trajectory (Figure). HbA1c reductions were of similar magnitude in the groups.

In conclusion, lina or glim in LADA could have differing impacts on long term β-cell function. Further research is exploring the potential modulating impact of lina on LADA.

Figure: *Changes in fasting C-peptide over time by treatment and GAD65 autoimmune status*



	28 weeks		52 weeks		104 weeks	
	Lina	Glim	Lina	Glim	Lina	Glim
n	21	17	14	14	9	9
Baseline C-peptide	821	1326	835	1425	944	1374
Follow-up C-peptide	917	1221	978	1246	1146	1345
Baseline HbA1c (%)	7.62	7.75	7.50	7.46	7.40	7.40
Δ HbA1c	-0.25	-0.75	-0.49	-0.52	-0.41	-0.49

***: p<0.001 vs baseline, ##: p < 0.01 lina vs glim

Supported by: Boehringer Ingelheim Pharmaceuticals, Inc.

38-LB

Efficacy and Safety of Canagliflozin, a Sodium Glucose Co-Transporter 2 Inhibitor, Compared With Glimepiride in Patients with Type 2 Diabetes on Background Metformin

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Canagliflozin (CANA) is a sodium glucose co-transporter 2 (SGLT2) inhibitor in development for treating type 2 diabetes mellitus (T2DM). This large, randomized, double-blind, active-controlled, Phase 3 study compared CANA 100 and 300 mg with glimepiride (GLIM) in T2DM subjects (N = 1,450) inadequately controlled with metformin (MET) (mean characteristics: age, 56.2 yrs; A1C, 7.8%; fasting plasma glucose [FPG], 9.2 mmol/L; body weight, 86.6 kg). GLIM was titrated up to 6 or 8 mg/day, with a mean dose achieved of 5.6 mg. A1C (primary endpoint) was reduced at 52 weeks with CANA 100 and 300 mg and GLIM. Both CANA doses demonstrated non-inferiority to GLIM in reducing A1C; CANA 300 mg demonstrated superiority to GLIM. CANA provided consistently lower A1C values over 52 weeks; A1C increased with GLIM after Week 18. Both CANA doses were superior to GLIM in weight loss and had fewer hypoglycemia episodes. More GLIM-treated subjects received rescue therapy (10.6%) than with CANA 100 or 300 mg (6.6% and 4.9%).

Adverse event (AE) rates were similar with CANA 100 and 300 mg and GLIM (64.2%, 68.5%, and 67.6%). Serious AE and AE-related discontinuation rates were low across groups. CANA 100 and 300 mg showed higher rates than GLIM of AEs consistent with superficial genital fungal infections (women, 14.3% and 23.8% vs 3.7%; men, 6.7% and 8.3% vs 1.1%), urinary tract infections (6.4% for both vs 4.4%), and osmotic diuresis-related AEs (<3% per specific AE). In summary, CANA improved glycemia over 52 weeks and was well tolerated in T2DM patients on background MET.

Table. Summary of Efficacy Endpoints at Week 52 (LOCF)

Parameter[a,b]	CANA 100 mg	CANA 300 mg	GLIM
A1C change, %	–0.82 (0.04)	–0.93 (0.04)	–0.81 (0.04)
Difference vs GLIM	–0.01 (–0.11, 0.09)[c]	–0.12 (–0.22, –0.02)[c,d]	
FPG change, mmol/L	–1.4 (0.1)	–1.5 (0.1)	–1.0 (0.1)
Difference vs GLIM	–0.3 (–0.6, –0.1)	–0.5 (–0.7, –0.3)	
Documented hypoglycemia rate, %[e]	5.6	4.9	34.2
Difference vs GLIM	–28.6 (–33.6, –23.7)[d,f]	–29.3 (–34.1, –24.4)[d,f]	
Body weight % change	–4.2 (0.2)	–4.7 (0.2)	1.0 (0.2)
Difference vs GLIM	–5.2 (–5.7, –4.7)[d,f]	–5.7 (–6.2, –5.1)[d,f]	
Systolic BP change, mmHg	–3.3 (0.6)	–4.6 (0.6)	0.2 (0.6)
Difference vs GLIM	–3.5 (–4.9, –2.1)	–4.8 (–6.2, –3.4)	
Triglycerides % change	–3.7 (2.5)	2.3 (2.5)	9.5 (2.5)
Difference vs GLIM	–13.2 (–19.4, –7.0)	–7.2 (–13.4, –1.0)	
HDL-C % change	7.9 (0.8)	9.0 (0.8)	0.3 (0.8)
Difference vs GLIM	7.5 (5.6, 9.5)	8.6 (6.7, 10.6)	
LDL-C % change	9.6 (1.9)	14.1 (1.9)	5.0 (1.9)
Difference vs GLIM	4.5 (0.0, 9.1)	9.0 (4.4, 13.7)	

LOCF, last observation carried forward. [a]Least squares mean (SE) change from baseline using ANCOVA (except for documented hypoglycemia rate) and GLIM-subtracted mean (95% CI) values; [b]*P* values are reported for pre-specified comparisons only; [c]Upper limit of 95% CI less than pre-specified non-inferiority margin of 0.3% for the comparison to GLIM; [d]Upper limit of 95% CI <0.0% for the comparison to GLIM; [e]Defined as ≤3.9 mmol/L with or without symptoms; [f]*P* <0.001 vs GLIM.

Supported by: Janssen Research & Development, L.L.C.

39-LB

A Novel Agonist Antibody to the Bradykinin 2 Receptor for the Treatment of Type 2 Diabetes

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DM204 is a novel agonist murine mAb developed to stimulate the bradykinin 2 receptor (BK2R), a G-protein coupled receptor (GPCR). BK2R activation leads to the release of NO, translocation of GLUT4 to the cell surface, and inhibition of glycogen synthase kinase-3 beta. In a prior study, acute DM204 treatment in rodent models of type 2 diabetes (DM2) resulted in improved glucose-dependent insulin secretion, insulin sensitivity, and BP. Here we report the results of a 21 day chronic dose ranging study of DM204 in a rat model of DM2.

ZDF fa/fa rats (N=48) were divided into 6 groups of 8 based on FBG values at 11 wks of age. Three doses of DM204 (0.2, 0.04, 0.008mg/kg) were injected SC twice weekly vs. vehicle, sitaglipitin (10mg/kg), and exenatide (1μg/rat). OGTTs, fasting blood glucose (FBG), total cholesterol (TC), and BP were monitored on days 0, 7, 14, and 21. A1c was done on day 21.

A dose-dependent glucose lowering activity of DM204 was observed. DM204 exhibited potent activity as observed in AUC OGTTs after 3 wks (high: 2495, medium: 2935, low: 3026, neg: 3773, sitagliptin: 3549, exenatide: 2671). There was also a profound effect on FBG, with a significantly lower increase in FBG in the high dose group vs. vehicle (+33.1 vs. +111.0 mg/dL, p=0.0058). A1c was significantly lower at the end of the study in the high dose group vs. vehicle (6.2% vs. 8.8%, p=0.0103), as was TC with the high dose group demonstrating a greater reduction in TC from baseline compared to vehicle (-12.1 vs. +28.0mg/dL, p=0.0156). The A1c reduction with high dose DM204 was significantly greater compared to the sitaglitpin and exenatide treated rats. BP improved in the high dose DM204 rats by 25 mmHg (p=0.0004) compared to vehicle. There were no discernible changes in food intake or weight.

DM204, a GPCR agonist mAb which leverages a novel mechanism, improves glucose, blood pressure, and total cholesterol in ZDF fa/fa rats over 21 days. These effects make DM204 appealing as a candidate for the treatment of DM2 with its numerous cardiometabolic derangements.

40-LB

Once-Weekly Exenatide versus Once or Twice Daily Insulin Detemir: Randomized, Open-Label Clinical Trial of Efficacy and Safety in Patients With Type 2 Diabetes Inadequately Controlled with Metformin ± Sulfonylureas

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This multicenter, open-label, parallel-arm study compared the efficacy and safety of exenatide once weekly (EQW) with titrated insulin detemir in patients with type 2 diabetes inadequately controlled with metformin (± sulfonylureas [SFU]). Patients were randomized to add-on therapy with EQW (2 mg) or detemir (titrated to achieve fasting plasma glucose ≤99 mg/mL) for 26 weeks. The primary outcome was a composite of the proportion of patients with A1C ≤7.0% and weight loss ≥1.0 kg at endpoint and was analyzed by means of logistic regression including factors for treatment, use of SFU, and baseline A1C and weight. Secondary outcomes included glycemic control measures, cardiovascular markers, and safety and tolerability. Of 216 patients in the ITT population, 111 received EQW and 105 received detemir. Overall, 44.1% (95% CI: 34.7, 53.9) of EQW-treated patients vs. 11.4% (95% CI: 6.0, 19.1) of detemir-treated patients achieved the primary outcome (P<0.0001). Treatment with EQW resulted in significantly greater reductions than detemir in A1C (LS mean±SE, -1.30±0.08% vs. -0.88±0.08%; P<0.0001) and weight (-2.68±0.34 kg vs. +0.8±0.36 kg; P<0.0001). Gastrointestinal-related and injection-site-related AEs occurred more frequently with EQW than with detemir. There were no events of major hypoglycemia in either group. Five (6.4%) patients in the EQW group and 6 (6.6%) patients in the detemir group experienced hypoglycemia with SFU use; there was 1 event of hypoglycemia without SFU use in the detemir group. Treatment with EQW resulted in a significantly greater proportion of patients achieving target A1C and weight loss compared to treatment with detemir and was associated with a low risk of hypoglycemia. These results suggest that EQW may be a viable treatment option to insulin detemir in type 2 diabetes patients with inadequate glycemic control on oral antidiabetes drugs.

41-LB

Canagliflozin (CANA), a Sodium Glucose Co-Transporter 2 (SGLT2) Inhibitor, Improves Glycemia and is Well Tolerated in Type 2 Diabetes Mellitus (T2DM) Subjects With Moderate Renal Impairment

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This randomized, double-blind, placebo (PBO)-controlled, Phase 3 study evaluated the efficacy and safety of CANA, an SGLT2 inhibitor, in T2DM subjects (N = 269) with moderate renal impairment (estimated glomerular filtration rate [eGFR] ≥30 and <50 mL/min/1.73 m^2). Subjects received CANA 100 or 300 mg or PBO (mean baseline characteristics: age, 68.5 yrs; A1C, 8.0%; fasting plasma glucose, 9.1 mmol/L; body mass index, 33.0 kg/m^2; eGFR, 39.4 mL/min/1.73 m^2). Median albumin/creatinine ratio [ACR] was 30.0 µg/mg and 74.0% of subjects were on insulin. Compared with PBO, A1C was significantly reduced at Week 26 with CANA 100 mg (P <0.05) and 300 mg (P <0.001).

Adverse event (AE) rates were slightly higher for CANA 100 and 300 mg than PBO (77.8%, 74.2%, and 73.3%), with serious AE rates of 11.1%, 11.2%, and 17.8%, respectively. AE-related discontinuation rates were low. CANA 100 and 300 mg had higher rates than PBO of hypoglycemia (52.9% and 51.2% vs 36.4%), superficial genital fungal infections (women, 6.3% and 4.9% vs 0%; men, 1.7% and 2.1% vs 0%), and osmotic diuresis- and volume-related AEs (<5% per specific AE except hypotension [6.7%] and dizziness [5.6%] for CANA 300 mg). UTI rates were slightly higher with CANA 300 mg (7.9%) than other groups (5.6% for both). Compared with PBO, CANA 100 and 300 mg showed increases in serum creatinine (9% and 10% vs 4%) and blood UN (9% and 6% vs 2%), and a decrease in median ACR (-21.6% and -12.4% vs -2.5%). In summary, CANA improved glycemia and was well tolerated in T2DM subjects with moderate renal impairment.

Table. Summary of Efficacy Endpoints at Week 26 (LOCF)

Parameter[a,b]	CANA 100 mg	CANA 300 mg	PBO
A1C change, %	–0.33 (0.09)	–0.44 (0.09)	–0.03 (0.09)
Difference vs PBO	–0.30 (0.12)[c]	–0.40 (0.12)[d]	
% of subjects reaching A1C <7%	27.3	32.6	17.2
Difference vs PBO	10.0	15.3	
FPG change, mmol/L	–0.8 (0.3)	–0.7 (0.3)	0.03 (0.28)
Difference vs PBO	–0.9 (0.4)	–0.7 (0.4)[e]	
Body weight % change	–1.2 (0.3)	–1.5 (0.3)	0.3 (0.3)
Difference vs PBO	–1.6 (0.4)	–1.8 (0.4)	
Systolic BP change, mmHg	–6.1 (1.5)	–6.4 (1.5)	–0.3 (1.5)
Difference vs PBO	–5.7 (1.9)	–6.1 (2.0)	
Triglycerides % change	6.2 (4.6)	11.9 (4.6)	7.9 (4.8)
Difference vs PBO	–1.7 (6.2)	3.9 (6.1)	
HDL-C % change	4.0 (1.7)	3.0 (1.7)	1.5 (1.8)
Difference vs PBO	2.5 (2.3)	1.5 (2.3)	
LDL-C % change	6.4 (3.5)	–1.0 (3.4)	6.3 (3.6)
Difference vs PBO	0.1 (4.6)	–7.2 (4.6)	

LOCF, last observation carried forward. [a]Least squares mean (SE) change from baseline using ANCOVA for all parameters except for % of subjects reaching A1C <7%; [b]P values are reported for pre-specified comparisons only; [c]P <0.05 vs PBO; [d]P <0.001 vs PBO; [e]P = NS vs PBO; additional testing was not performed due to multiplicity control.

Supported by: Janssen Research & Development, L.L.C.

42-LB

WITHDRAWN

43-LB

Racial Variation in The Hemoglobin Glycation Index

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Racial variation due to factors other than blood glucose concentration complicates the use of HbA1c for the diagnosis and management of diabetes. The hemoglobin glycation index (HGI) measures biological variation in HbA1c controlled for blood glucose and was reported to be higher in black than white children with type 1 diabetes. We used data from the National Health and Nutrition Examination Survey (1999-2008) to test the hypothesis that racial variation in HGI exists in adults with type 2 diabetes or without diabetes. The analysis included 1449 type 2 diabetes subjects and 8884 non-diabetic subjects identified as white, African American, Mexican American or other race. All subjects were ≥20 y old and fasted overnight prior to blood draw. Predicted HbA1c were calculated for each subject by inserting fasting plasma glucose (FPG) into linear regression equations for HbA1c vs. FPG:

Type 2 diabetes HbA1c = 3.59 + 0.022*FPG
Non-diabetic HbA1c = 3.75 + 0.016*FPG
HGI = Observed HbA1c – Predicted HbA1c

The results show that mean HGI was significantly greater for African Americans compared to whites regardless of diabetes status. HGI was not different between white, Mexican American and other race subjects without diabetes but was significantly greater for Mexican Americans compared to whites with type 2 diabetes. These results may have implications for clinical practice in a multi-ethnic population and show that HGI may have clinical use for assessing variation in HbA1c.

	Type 2 Diabetes				Non-diabetic			
Race	**n**	**FPG**	**HbA1c**	**HGI**	**n**	**FPG**	**HbA1c**	**HGI**
White	627	147[c] (2.4)	6.7[b] (0.07)	-0.152[b] (0.040)	4585	97[a] (0.3)	5.3[b] (0.01)	-0.022[b] (0.011)
African American	321	160[b] (5.4)	7.5[a] (0.12)	0.300[a] (0.063)	1614	94[b] (0.3)	5.4[a] (0.01)	0.145[a] (0.011)
Mexican American	356	175[a] (4.9)	7.8[a] (0.14)	0.315[a] (0.076)	1857	97[a] (0.4)	5.3[b] (0.01)	-0.001[b] (0.013)
Other	145	162[abc] (7.0)	7.5[a] (0.23)	0.260[a] (0.152)	828	96[a] (0.5)	5.3[b] (0.02)	0.011[b] (0.018)
All subjects	1449	153 (2.0)	7.0 (0.06)	0 (0.032)	8884	96 (0.2)	5.3 (0.01)	0 (0.008)

[a,b,c] Means (SE) with different superscripts are significantly different, p<0.05.

Supported by: NIH (1R01HL110395-01)

44-LB

Anti-Hypertensive Drug Telmisartan Is a Selective PPARγ Agonist with Anti-Diabetic but Not Anti-Osteoblastic Activity

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Anti-diabetic drug rosiglitazone (RSG) improves insulin sensitivity by activating PPARγ nuclear receptor, however its prolonged use causes bone loss and increases fracture risk in part due to suppression of osteoblast differentiation from marrow mesenchymal stem cells (MSCs). Telmisartan (TEL), the anti-hypertensive drug and angiotensin receptor blocker, also acts as a selective PPARγ agonist. *In vitro*, in a model of MSC differentiation under the control of PPARγ2, TEL inhibited cell proliferation and induced brown-like adipocyte phenotype typified by significant increase in expression of FoxC2, UCP1 and Dio2, however and in contrast to RSG, TEL did not affect osteoblast phenotype as assessed by an alkaline phosphatase (ALP) activity and expression of Runx2 and Dlx5. Moreover, TEL did not suppressed the expression and activity of TGFβ/BMP signaling pathway, and even prevented RSG negative effect on the expression of members of this signaling pathway. *In vivo*, in a murine model of Type 2 diabetes, yellow agouti A^{vy}/a mice, at the dose which normalized glucose levels and glucose tolerance, TEL did not affect bone mass and body weight, and did not increase marrow fat. In contrast, a dose of RSG, which equally to TEL normalized diabetic phenotype, resulted in 60% loss of trabecular bone, significant increase in body weight, and massive accumulation of fat in bone. Simultaneous treatment with RSG and TEL of A^{vy}/a mice prevented bone loss and decreased levels of RSG-induced bone turnover markers, but did not counteract RSG effect on body weight. These results indicate that TEL may be a safe for bone alternative to treat hyperglycemia and provide *in vivo* evidence that the anti-diabetic and the anti-osteogenic activity of PPARγ may be differentially activated by selective agonists.

Supported by: NIH, NIA

45-LB

Melatonin Supplementation Improves Glycemic Control While Lowering Oxidative Stress in Type 2 Diabetes Mellitus

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Complications of type 2 diabetes mellitus (T2D) are believed to result from hyperglycemia-driven increases in oxidative stress. A limited amount of in-vivo evidence indicates a potential role for melatonin (N-Acetyl-5-Methoxytryptamine) in improving glucose regulation, lipid metabolism and oxidative stress in T2D. Thus, the purpose of this investigation was to evaluate the effect of a commercially available preparation of melatonin on glycemic control, lipid metabolism, and oxidative stress in uncomplicated T2D.

Fourteen subjects with T2D (10 female, 4 male; 52.5 ± 5.0 years) were randomly assigned to a melatonin group (MEL) or placebo group (PLA) for 42 days, followed by 42 days in the other group in a crossover design. During each supplementation period, subjects ingested either 10 mg of melatonin in capsule form 30-60 minutes prior to sleep (MEL) or an identical capsule containing white flour (PLA). Fasting blood draws occurred on three mornings: prior to supplementation, after 42 days, and after 84 days. Mean differences were analyzed using a 2-way ANOVA with repeated measures on one factor (time), whereas one-tailed, paired t-tests were used to assess change from baseline between groups. Results are presented as mean ±SEM.

After supplementation, levels of plasma malondialdehyde (MDA), a marker of oxidative stress, were significantly lower for MEL (-6.25±2.10 nmol/ml) compared to PLA (0.72±3.30, p=0.028). The change in glycated hemoglobin (HbA1c) resulted in a total improvement in HbA1c of 0.33% after 42 days of melatonin supplementation compared to placebo (-0.24±0.23 % for MEL vs. 0.09±0.21 % for PLA, p=0.01). No significant changes were noted for blood lipids or fasting plasma glucose.

In conclusion, daily melatonin supplementation may play an important role in enhancing glycemic control and diminishing oxidative stress in individuals with T2D.

46-LB

High Dosage RAS Inhibition and Beta Blockade Significantly Reduce Death and Hospitalization in Diabetic Patients with Elevated NTproBNP Levels (PONTIAC Study)

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Recent studies failed to show cardiovascular outcome benefit beyond standard treatment in diabetic patients. The reason for this might be a sub-optimal selection of patients at high CV risk.

Since NT-proBNP is an indicator of subclinical cardiovascular disease and diastolic dysfunction we aimed to evaluate a therapy with RAS-antagonists and beta-blockers at maximum tolerated dosages in patients with type II diabetes mellitus and increased NT-proBNP levels(> 125 pg/ml) without a history of cardiac disease, normal ECG, and normal LV function. Group 1 (150 patients) was treated at four specialized diabetes care units, group 2 (150 patients) was treated by cardiologists with RAS-antagonists and beta-blockers at maximum tolerated dosage and this was compared to standard dosages in the conventional therapy group. The primary endpoint was hospitalization/death due to cardiac disease after 2 years.

At baseline, the age of the patients was 68±9 years, duration of diabetes 15±1 years, 37% were male, HbA_{1c} was 7±1.1%, BMI 30±7, blood pressure 151±2 mmHg, heart rate 72±1 bpm, NT-proBNP 314±236 pg/ml (all n.s. between groups). Blood pressure was significantly reduced in both groups ($p<0.05$, but no difference between groups). The primary endpoint cardiac hospitalization and death was significantly reduced in the high dosage treatment group only: HR 0.334, CI 0.118-0.942, p=0.038 (adjusted for duration of diabetes, blood pressure, heart rate, BMI, NT-proBNP, albuminuria, LDL-cholesterol, HbA_{1c}, age, gender). The same was found for other endpoints like all-cause hospitalization and heart failure hospitalizations ($p<0.05$ for all).

In diabetic patients without known cardiac disease but increased NT-proBNP levels cardiovascular events were significantly reduced by high dosage RAS and beta blockade.

47-LB

Soluble Non-Aqueous Glucagon Formulations for the Treatment of Severe Hypoglycemia

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Severe hypoglycemia remains a significant unmet medical need. Recent studies estimate that 6% to 10% of deaths of patients with type 1 diabetes are attributable to hypoglycemia. Administration of glucagon is effective in reversing severe hypoglycemia. However, development of a simple, ready-to-use glucagon product has been hampered by the property of glucagon to spontaneously assemble into fibrils in aqueous solution. Thus, currently approved products (Lilly, Glucagon for Injection; Novo Glucagen®) are based on lyophilized formulations. The need for reconstitution has made these products difficult to administer in emergency situations, and thus, they are infrequently used. We have developed a soluble glucagon formulation based on biocompatible, non-aqueous solvents. These formulations effectively suppress the fibrillation of glucagon observed in aqueous solutions, even at high concentrations and temperatures. Further, the chemical stability of glucagon in these formulations is similar to that of dry powders. Non-aqueous solutions of glucagon (5 mg/ml) have been demonstrated to be free of fibrillation after incubation at 40 degrees C for two months (compared to just hours for aqueous solutions). Additionally, minimal chemical degradation of glucagon is observed in non-aqueous solutions (apparent degradation rate at room temperature is ~0.25%/month). Comparative pharmacology studies in a rodent model show the non-aqueous solutions of glucagon to have equivalent pharmacokinetics and pharmacodynamics to aqueous formulations. Similar to aqueous solutions, injection of non-aqueous formulations of glucagon show rapid absorption (Tmax ~ 5 min) and elevation of glucose levels (within 15 minutes). These data support the development of a ready-to-use rescue pen for severe hypoglycemia as well as a glucagon formulation suitable for a bi-hormonal (insulin-glucagon) infusion pump.

Supported by: NIH, NIDDK

48-LB

Development of a Stable Formulation of Liquid Glucagon for Bihormonal Pump Use

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We and others have reported that automated closed loop delivery of glucagon with insulin markedly reduces the hypoglycemic frequency in type 1 diabetes. Commercially-available glucagon is prepared at pH 2.5 and is suitable for immediate use, but cannot be maintained in aqueous form due to rapid amyloid fibril formation and cytotoxicity.

We sought to determine whether aqueous glucagon aged at a high pH would be more feasible for a closed loop system. Several assays were used to measure stability and gel formation, including occlusion of low caliber pump catheters. Native glucagon was prepared at pH 2.5-3 and at pH 10 in glycine buffer; both were aged in OmniPod patch pumps (Insulet) at 37° C. Boluses were delivered regularly, and the duration until occlusion noted. Aged samples delivered by the pump were analyzed by mass spectrometry to determine preservation of intact glucagon. The amount of amyloid formation was measured by intrinsic tryptophan (TRP) fluorescence. To assess whether alkaline preparations of protein cause discomfort, 10 healthy subjects were given human albumin SC injections at pH 7.4 and pH 10 in a double-masked fashion.

Low pH glucagon delivered without a pump occlusion for 47.40 ± 9.78 hr (n= 5, mean ± SE), whereas high pH glucagon had not caused occlusion at the 72 hr pump expiration (p = 0.036, n= 5). TRP fluorescence of low pH glucagon showed rapid formation of amyloid within 8-10 hours, while high pH glucagon showed no amyloid signature, even after 14 days. Mass spectrometry showed the native peak largely maintained after 72 hr of aging at pH 10 (AUC= 91% of the fresh glucagon peak). In clinical studies, slow injections of human albumin at alkaline pH resulted in a mild degree of discomfort vs. neutral injections (2.2 ± 0.5 on a 6-point scale vs 0.8 ± 0.4, p= 0.010). For fast injections, there was minimal discomfort and no difference between neutral and alkaline injections.

These biochemical and clinical results suggest that a high pH native glucagon will be suitable for delivery in a closed loop bihormonal pump.

Supported by: JDRF

49-LB

Safety and Efficacy of Empagliflozin as Monotherapy or Add-On to Metformin in a 78-Week Open-Label Extension Study in Patients with Type 2 Diabetes

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This Phase IIb, randomized, open-label extension study investigated safety and efficacy of empagliflozin (EMPA), an SGLT-2 inhibitor, as monotherapy or add-on to metformin IR (MET) for 78 weeks in patients with T2DM. After completing one of two 12-week, randomized, controlled trials, patients who took 1, 5 or 50 mg EMPA or placebo in the first trial were randomized to 10 mg or 25 mg EMPA (monotherapy or add-on to MET). Patients who took 10 mg or 25 mg EMPA, MET only, or sitagliptin as add-on to MET (SITA) in the first trial continued the same treatment. In the 78-week extension, 272 patients received 10 mg EMPA (166 as add-on to MET), 275 received 25 mg EMPA (166 as add-on to MET), 56 received MET only and 56 received SITA.

Of all patients in the 78-week extension, adverse events (AEs) were reported in 63.2-74.1% on EMPA and 69.6% on MET only or SITA. Over 90% of AEs were mild or moderate. Hypoglycemic events were reported in 0.9-3.6% of patients on EMPA, 7.1% on MET only and 5.4% on SITA. AEs related to UTIs were reported in 3.8-12.7% of patients on EMPA, 3.6% on MET only and 12.5% on SITA. AEs related to genital infections were reported in 3.0-5.5% of patients on EMPA, 1.8% on MET only and none on SITA.

Efficacy is reported for patients who took EMPA, MET only, or SITA over 90 weeks (including preceding trials) (Table). Compared with baseline of the preceding trial, patients on EMPA showed reductions in HbA_{1c}, FPG and body weight at the end of the extension study (Table).

In conclusion, long-term EMPA treatment was well tolerated and provided sustained glycemic control and weight loss in patients with T2DM.

Parameter	Type of therapy	EMPA, n (10/25 mg)	Comparator (n)	Adjusted mean change from baseline (95% CI) [mean baseline]		
				Comparator	10 mg EMPA	25 mg EMPA
HbA_{1c} (%)	Monotherapy	80/88	MET (56)	-0.56 (-0.79, -0.33) [8.15]	-0.34 (-0.54, -0.14) [7.88]	-0.47 (-0.66, -0.27) [7.99]
FPG (mg/dL)	Monotherapy	80/88	MET (56)	-26.0 (-33.5, -18.4) [176]	-30.4 (-37.1, -23.7) [181]	-27.8 (-34.3, -21.3) [178]
Body weight (kg)	Monotherapy	80/88	MET (56)	-1.28 (-2.30, -0.26) [85.8]	-2.24 (-3.12, -1.36) [83.4]	-2.61 (-3.46, -1.77) [83.5]
HbA_{1c} (%)	Add-on to MET	137/139	SITA (56)	-0.40 (-0.60, -0.20) [8.03]	-0.34 (-0.47, -0.21) [7.92]	-0.63 (-0.76, -0.50) [7.89]
FPG (mg/dL)	Add-on to MET	137/139	SITA (56)	-15.6 (-23.6, -7.62) [179]	-21.3 (-26.4, -16.2) [177]	-31.8 (-36.8, -26.7) [179]
Body weight (kg)	Add-on to MET	137/139	SITA (56)	-0.41 (-1.49, 0.67) [88.6]	-3.14 (-3.89, -2.38) [90.7]	-4.03 (-4.77, -3.29) [89.7]

Supported by: Boehringer Ingelheim Pharmaceuticals, Inc.

50-LB

Efficacy and Safety of Canagliflozin, a Sodium Glucose Co-Transporter 2 Inhibitor, Compared with Sitagliptin in Patients with Type 2 Diabetes on Metformin Plus Sulfonylurea

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Canagliflozin (CANA) is a sodium glucose co-transporter 2 inhibitor in development for treating type 2 diabetes mellitus (T2DM). This randomized, double-blind, active-controlled, Phase 3 study assessed CANA 300 mg compared with sitagliptin (SITA) 100 mg in subjects (N = 755) with T2DM inadequately controlled with metformin (MET) plus a sulfonylurea (SU) (mean baseline characteristics: age, 56.7 years; A1C, 8.1%; fasting plasma glucose [FPG], 9.3 mmol/L; body weight, 88.3 kg). At 52 weeks, A1C was reduced with CANA 300 mg and SITA 100 mg. CANA 300 mg demonstrated non-inferiority as well as superiority to SITA in reducing A1C, with consistently lower A1C values over 52 weeks (SITA showed increases in A1C after Week 12). CANA 300 mg showed greater reductions in body weight, FPG, and systolic BP compared with SITA.

Overall adverse event (AE) rates were similar with CANA 300 mg and SITA 100 mg (76.7% vs 77.5%). Serious AE (6.4% vs 5.6%) and AE-related discontinuation rates (5.3% vs 2.9%) were low for CANA and SITA. AEs consistent with superficial genital fungal infections were more frequent with CANA than SITA (women, 15.3% vs 4.3%; men, 9.2% vs 0.5%). Incidences of urinary tract infections were similar for CANA and SITA. There were no other notable differences in AEs between CANA and SITA. More subjects had ≥1 hypoglycemic episode with CANA (43.2%) than with SITA (40.7%). In conclusion, CANA 300 mg showed improved glycemic control, weight reduction, and BP compared with SITA 100 mg over 52 weeks and was well tolerated in subjects with T2DM on background MET + SU.

Table. Summary of Efficacy Endpoints at Week 52 (LOCF)

Parameter	CANA 300 mg[a]	SITA 100 mg[a]	Difference vs SITA[b,c]
A1C change, %	–1.03 (0.05)	–0.66 (0.05)	–0.37 (–0.50, –0.25)[d,e]
% of subjects reaching A1C <7%	47.6	35.3	12.3 (4.9, 19.6)
FPG change, mmol/L	–1.7 (0.1)	–0.3 (0.1)	–1.3 (–1.7, –1.0)[e,f]
Body weight % change	–2.5 (0.2)	0.3 (0.2)	–2.8 (–3.3, –2.2)[e,f]
Systolic BP change, mmHg	–5.1 (0.7)	0.9 (0.7)	–5.9 (–7.6, –4.2)[e,f]
Triglycerides % change	9.6 (2.8)	11.9 (2.9)	–2.3 (–9.8, 5.3)[g]
HDL-C % change	7.6 (0.9)	0.6 (0.9)	7.0 (4.6, 9.3)[h]
LDL-C % change	11.7 (1.8)	5.2 (1.8)	6.4 (1.7, 11.2)

LOCF, last observation carried forward. [a]Least squares mean (SE) change from baseline using ANCOVA for all parameters except % of subjects reaching A1C <7%; [b]SITA-subtracted mean (95% CI) values; [c]*P* values are reported for pre-specified comparisons only; [d]Upper limit of 95% CI less than pre-specified non-inferiority margin of 0.3% for the comparison to SITA; [e]Upper limit of 95% CI <0.0% for the comparison to SITA; [f]*P* <0.001 vs SITA; [g]*P* = NS vs SITA; [h]Not significant due to multiplicity control.

51-LB

Ultra-Concentrated Insulins: Design and Pre-Clinical Validation of a Rapid-Acting U-500+ Insulin Analog Formulation

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The pandemic of Type 2 diabetes highlights an urgent need for concentrated insulin formulations for obese patients with marked insulin resistance. Large-volume injections of U-100 formulations (Humalog®, Novolog®, and Apidra®) lead to inconsistent PK/PD, injection-site pain, and high cost. The only ultra-concentrated formulation is Lilly U-500 regular, which exhibits prolonged PK/PD (8-16 hours) due to self-association as the insulin concentration is raised to 3.0 mM (U-500). Small clinical studies suggest that glycemic control in patients with marked insulin resistance may be improved by novel rapid-acting U-500 insulin analog formulations. Clinical benefits may be particularly notable among under-represented minorities.

To address this need, we designed an active ultra-stable insulin monomer with rapid action at a protein concentration of 3.0 mM. Desigated *Fluorolog*, the analog contains a single fluorine atom at B24 and standard amino-acid substitutions at dimer- and hexamer-forming surfaces; a structural framework was provided by insulin *lispro*. Its NMR structure is essentially identical to insulin *lispro*. The duration of Fluorolog at 3.0 mM (after subcutaneous injection in anesthetized Yorkshire pigs; body masses 25-30 kg) is indistinguishable from that of Humalog® at a concentration of 0.6 mM (U-100) and markedly faster *on* and faster *off* than Lily U-500 R. Remarkably, the AUC of Fluorolog at this concentration was twofold higher than that of Lilly U-500 R, indicating that its strength was greater than U-500 as measured by biological response. We thus designate this ultra-concentrated rapid-acting formulation as *U-500+*. The mitogenicity of the analog and its affinity for the IGF receptor are indistinguishable from human insulin. Clinical trials are planned.

Supported by: NIH (DK040949)

CLINICAL THERAPEUTICS/NEW TECHNOLOGY—TREATMENT OF INSULIN RESISTANCE

52-LB

The Dual PPARα/δ Agonist GFT505 Improves Hepatic and Peripheral Insulin Sensitivity in Abdominally Obese Subjects

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The development of new insulin-sensitizers is an unmet need in the treatment of type 2 diabetes and non-alcoholic steatohepatitis. GFT505 is a liver-targeted dual PPARα/δ agonist, which improves multiple metabolic parameters in patients with pre-diabetes or combined dyslipidemia.

Here, we determined the effect of GFT505 on insulin sensitivity in 22 male subjects with insulin resistance (HOMA-IR: 5.6 ± 2.3 IU) and abdominal obesity (BMI: 32.2 ± 3.4 kg/m2, waist circumference: 109 ± 9 cm), using a 2-step (0.2 and 1.0 mU insulin/kg.min) hyperinsulinemic-euglycemic clamp with [6,6 2H2]-glucose infusion. This randomised, placebo-controlled study was performed in a cross-over design: two 8-week treatment periods (GFT505: 80 mg once daily or placebo), with a 6-week wash-out period. Skeletal muscle biopsies were performed at the end of the first treatment period.

Compared to placebo, GFT505 significantly increased the glucose infusion rate at the 2nd step of the clamp (primary outcome) by 21% (3.79 ± 1.29 vs 3.13 ± 1.48 mg/kg.min; p=0.048), reflecting an improvement in peripheral insulin sensitivity. Moreover, GFT505 potentiated insulin-inhibition of hepatic glucose production by 44% (-49.2% ± 19.3 vs -34.3% ± 17.4; p=0.0016), indicating an improvement of hepatic insulin sensitivity. There was no significant induction of either PPARα or δ target genes in skeletal muscle, suggesting a liver-targeted action of GFT505. GFT505 improved the plasma lipid profile with a decrease of triglycerides (-21.0%, p=0.003), free fatty acids (-11.0%, p=0.03) and LDL-C (-13.1%, p=0.006). GFT505 also reduced fibrinogen (-15.0%, p=0.04), haptoglobin (-10.1%, p=0.03) and the liver enzymes γGT (-30.4%, p=0.003) and ALT (-20.5%, p=0.004) in plasma. There was no serious adverse event related to GFT505.

This study demonstrates that GFT505 is an insulin-sensitizer, which is an attractive and safe candidate for the treatment of metabolic disorders related to insulin resistance.

HEALTH CARE DELIVERY—ECONOMICS

53-LB

Cost-Effectiveness of Real-Time Continuous Glucose Monitoring (RT-CGM) in Type 2 Diabetes (T2DM)

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We have shown that intermittent "doses" of RT-CGM (unblinded use) were associated with a sustained reduction in A1C over 52 weeks as compared with daily self-monitoring of blood glucose (SMBG) (RT-CGM: -1.1% vs. SMBG: -0.5%) in patients with T2DM not on prandial insulin. The current analysis modelled the cost-effectiveness of RT-CGM of this type of intervention.

Using the validated IMS CORE Diabetes Model, we projected the lifetime clinical and economic outcomes for RT-CGM vs. SMBG. In base-case analysis, the frequency and duration of RT-CGM use was modelled after the clinical study, i.e. 2 weeks on/1 week off RT-CGM for 12 weeks. A scenario analysis was conducted to simulate the additional costs and health benefits of a second "dose" of RT-CGM in year 2 assuming maintenance of the A1C achieved at the end of year 1. In both analyses we assumed that A1C converged within 21 months following the active intervention. Analyses were conducted from a U.S. third-party payer perspective, including only direct costs obtained from published sources and inflated to US$2011. Outcomes were discounted at 3% per annum, with sensitivity analyses on time horizons and key clinical variables.

Base-case: RT-CGM results in an incremental 0.09 life years (LY) and 0.07 quality-adjusted life years (QALY) with an incremental cost of $250.The cost of RT-CGM is offset by lower SMBG and reduced complication rates. The incremental cost-effectiveness ratios were $2,903 per LY gained, and $3,735 per QALY gained. Scenario analysis: similar use of RT-CGM in year 2 results in a greater QALY gain (0.165 or 2 months), at an incremental cost of $1,217, with the cost-effectiveness ratio of $10,071 per QALY gained.

In summary, the results show that RT-CGM is a cost-effective disease management option in the US for people with T2DM not on prandial insulin. Repeated use of RT-CGM may result in additional cost-effective health benefits, due to longer-term impact on physiological parameters.

Supported by: Dexcom, Inc.

54-LB

Return on Investment (ROI) from the Online Diabetes Prevention Program (DPP)

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Virtual Lifestyle Management (VLM), based on NIH's DPP, is a year-long online weight management program with education, guided self-discovery, goal setting, monitoring, tracking, barrier mitigation & internet-based coaching. Overweight & sedentary patients with or at risk for type 2 diabetes were recruited by newsletter mailed to health plan members. This analysis assesses impact of VLM on healthcare expenditures examining intervention participants (N=242) & overweight comparison subjects (N=320) covered continuously by the health plan year before (2008), year of (2009), & year after (2010) the intervention.

VLM participants were matched to a weighted composite of 5 members in the non-VLM group using the STATA nnmatch function to maximize similarity between study subjects & comparison subjects designed to predict program

participation & minimize selection bias. All subjects were overweight or obese & were matched on 2008 health insurance claims, gender, age, age-squared, median zip income, median zip income-squared, presence of diabetes, obesity, hypertension, sleep apnea.

Average age 50.7 years; 69 % female, BMI 32.6, 9.9% diabetes, 8.3% obese, 20.7% hypertension, 3.3% sleep apnea; 27.7% dyslipidemia; average family income $56,544. Total cost for year-long program (recruitment, enrollment, online coaching, incentives, technology) was $367/patient. Savings, measured by reduction in claims for intervention group during study year & year post study (compared to pre-study year) were $566 & $893 respectively. Two year savings was $1,593 representing 2 year ROI of 4.3 to 1. Impact on expenditures was similar for users who were overweight & obese but dramatically higher for morbidly obese patients.

The intervention had positive ROI within 2 years from the beginning of the program. Technology-enabled interventions using DPP can have significant clinical & economic impact & should be considered in a population of overweight adults who need to lose weight to improve health.

55-LB

Collaborative Case Management Between Primary Care and Insurance Company Case Managers

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Case management (CM) is a process that assists the patient with meeting treatment goals. CM is often administered by insurers and referral is based on healthcare utilization criteria rather than clinical or laboratory criteria. We evaluated the effectiveness of a pilot collaborative referral process between a primary care practice and insurance company case managers by identifying high risk patients with diabetes (HgbA1C>8.0%) for CM referral. Patients referred for CM by the group practice were compared with a patient cohort from the same group practice previously identified for CM using insurer CM triggers of high resource utilization and/or recent hospitalization. We measured HgbA1C, weight, BMI, BP, lipids, number of medications, presence of insulin therapy, influenza, pneumococcal immunization and eye exam up to date. Differences between groups were measured through Mann-Whitney test; frequency data was compared through Chi-Square test and Fisher's Exact Test. Our new referral method proved more successful in engaging patients with CM. The intervention group had 18 of 48 patients accept and complete CM (37.5%); the comparison group had 4 of 34 patients accept and complete CM (11.8%, p=0.021). Intention to treat analysis revealed that practice-based CM referral resulted in greater drop in HgbA1C than did health plan CM referral CM (-1.91% vs. +.221%, p<0.001). Adherence to other process measures (vaccines and eye exam) was low both before and after the intervention. Intervention patients completing CM had greater drops in HgbA1C when more goals were achieved through CM (Pearson correlation -0.692, p=0.001) Implementation barriers included technology problems, staff engagement, and buy-in with the new process. Our pilot collaboration resulted in greater acceptance of CM when identifying the "high risk" patient referred by the primary care provider (i.e. based on poor glucose control) vs. the "high utilization" patient referred by the insurer (i.e. based on insurers' utilization data).

56-LB

Pharmacist-Led Group Medical Visits for Cardiovascular Risk Reduction in Type 2 Diabetes

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We conducted a multi-site, randomized, controlled unblinded trial of parallel design involving 3 VA hospitals to determine whether a pharmacist-led group medical visit program for Cardiovascular Risk Reduction in Type 2 Diabetes added to usual care, could improve cardiac risk factors in participants with type 2 diabetes mellitus (DM) compared to usual care alone after 13 months.

Using stratified randomization based on # of uncontrolled risk factors, 250 participants (117 cases, 133 controls) were enrolled. The cases received 4 weekly group sessions followed by booster session held every 3 months. Each 2 hour session included diabetes education and behavioral modification by nutritionist and pharmacist and 1 hour of medication management by a clinical pharmacist, in addition to usual primary care. We compared change from baseline in A1C, total cholesterol/HDL ratio, systolic blood pressure (SBP) and cost. Participants were similar in age (65.8 ± 8.7 vs. 65.0 ± 9.8 years) and gender (4.5 vs 3.9% female) and baseline risk factors. After 13 months, both cases and controls had significant improvement in SBP and lipids, but only cases have significant reductions in A1c (Table). Health care system costs included provider time, medications, hospitalization, ER visits and others. The intervention reduced an average of $3263.1 ± 66032.9 (p=0.59) per patient vs. $887.7 ± 23277.5 in controls (p=0.66) compared to the year prior to the study.

A pharmacist-led group medical visit program for Cardiovascular Risk Reduction in Type 2 Diabetes added to usual care significantly improved diabetes control without additional cost increase.

Table: Baseline and Change in Risk Factors by Randomization

	Cases N = 117		Controls N = 133	
Parameter	Baseline (SD)	Mean change from baseline (SD)	Baseline +/- SD	Mean change from baseline +/- SD
Hemoglobin A1C (%)	8.20 (1.41)	-0.31 (1.31)*	8.14 (1.31)	-0.16 (1.27)
Total-Cholesterol to HDL ratio	3.65 (1.85)	-0.25 (1.03)*	4.13 (1.85)	-0.35 (1.21)*
Systolic blood pressure (mm Hg)	144.4 (33.8)	-6.4 (20.4)*	140.6 (26.6)	-9.4 (17.7)*

* indicates significant change from baseline at P value <0.05
There were no significant differences in the baseline or mean change between the 2 groups
SD = Standard deviation

Supported by: VA Health Services (IAB06-269)

PEDIATRICS—TYPE 1 DIABETES

57-LB

Trends in Hospitalization for Diabetic Ketoacidosis in Youth, U.S. 1993–2009

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Diabetic ketoacidosis (DKA) is a major acute complication of diabetes, particularly among youth. However, no national estimates exist for DKA hospitalizations for U.S. youth. This study examined national trends in DKA hospitalization rates among youth aged < 20 years. We obtained the number of DKA hospitalizations from the Agency for Healthcare Research and Quality's Nationwide Inpatient Sample. We calculated DKA hospitalization rates among youth from 1993 to 2009 using census youth population as denominator. Because trends among the general youth population changes with diabetes prevalence, we also calculated DKA hospitalization rates among youth with diabetes from 2001 to 2009. We estimated the number of youth with diabetes by applying diabetes prevalence from the SEARCH for Diabetes in Youth study to the census youth population. We used Joinpoint regression to assess changes in trends.

The number of DKA hospitalizations for youth increased from 17,000 in 1993 to 31,000 in 2005 and then leveled off, with 29,000 occurring in 2009. DKA hospitalization rates for youth increased from 1993 to 2005 (p<0.01), then leveled off (p=0.8). DKA hospitalization rates among youth with diabetes changed little from 2001 (16.1%±1.1%) to 2009 (15.8%±1.2%, p=0.3). Trends were unchanged among youth aged 5-14 years and among boys and girls. In children aged <5 years, DKA hospitalization rates increased from 2001 to 2005 (p=0.01), then leveled off (p=0.10). In youth aged 15-19 years rates decreased over the entire time period (p=0.03). In 2009, a substantial proportion of youth with diabetes experienced DKA hospitalizations, specifically 30.2% (±4.5%), 12.0% (±1.5%), 13.4% (±1.5%) and 17.7% (±1.0%) in youth aged <5 years, 5-9 years, 10-14 years, 15-19 years, respectively.

In conclusion, except among older youth, DKA hospitalization rates have not improved among youth with diabetes in the U.S. in past decade. Better prevention programs for this serious but potentially preventable complication are urgently needed.

58-LB

Older Diagnostic Age and Black Race Predict Glycemic Deterioration Among Children and Adolescents with Type 1 Diabetes (T1DM)

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Poor glycemic control (GC) early in the course of T1DM raises the long-term risk for vascular complications. However, predictors of GC deterioration after

diagnosis of T1DM have not been well described, making it difficult to identify high-risk patients and proactively provide more aggressive interventions. To identify such predictors, we examined whether diagnostic age, gender, and race were associated with worsening GC in the initial years (yrs) after T1DM diagnosis among 2218 pediatric patients seen in a large tertiary referral center from 1993-2009. Within-patient HbA1c (A1c) trajectories were constructed using 3rd order polynomials and using all available A1c values within 5 yrs of diagnosis. 52.6% of patients were male; 86.1% were non-Hispanic White. Mean diagnostic age was 9.0 yrs (± 4.1). Mean # of A1c values/yr/subject was 2.4 ± 0.9. A1c trajectory curves differed in shape across age strata at the time of diagnosis (Fig. 1A; P < 0.001). A1c trajectory curves stratified by diagnostic age were qualitatively similar across sex (P for sex * diagnostic age interaction = 0.01) but not across race (P for race * diagnostic age < 0.001). Black patients experienced higher initial A1C after insulin therapy (8.7% ± 2.8 vs. 7.6 ± 1.7; P < 0.001), and greater deterioration in A1c than Whites across diagnostic age strata (Fig. 1B). Older diagnostic age and Black race are major risk factors for GC deterioration early in the course of T1DM. These findings form the foundation for future work to explore the reasons behind these differences and develop effective preventive interventions for high-risk patients.

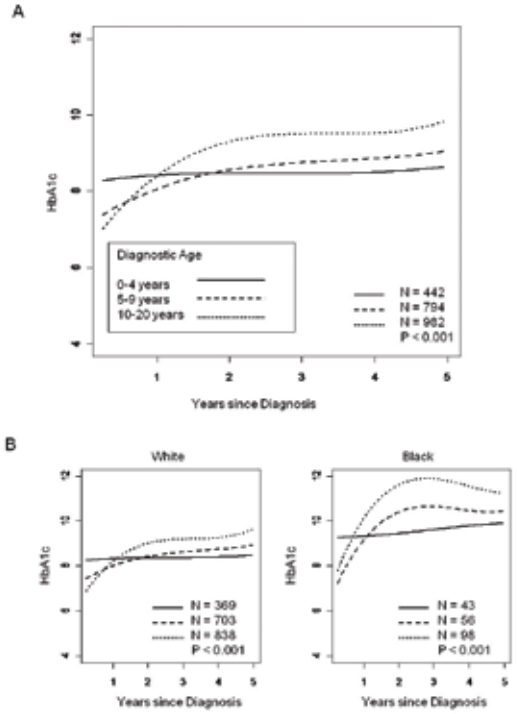

Figure 1. (A) HbA1c trajectories across diagnostic age strata. (B) HbA1c trajectories across race * diagnostic age strata. Key for all graphs shown in panel A. P values shown represent comparisons of trajectory curves across diagnostic age subgroups within each graph.

Supported by: Physician Scientist Award, Children's Mercy Hospitals & Clinics

PEDIATRICS—TYPE 2 DIABETES

59-LB

Liraglutide Trial in Pediatric Type 2 Diabetes: Safety, Tolerability and Pharmacokinetics/Pharmacodynamics

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Increasing prevalence of type 2 diabetes (T2D) in adolescents is escalating the need for more treatment options. We evaluated the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics of liraglutide QD in adolescents (10-17 yrs) with T2D, treated with either diet/exercise or metformin, in a randomized, double-blind, placebo-controlled trial. We randomized 21 subjects 2:1 to liraglutide (14) or placebo (7). Starting at 0.3 mg, doses were escalated weekly to 0.6, 0.9, 1.2, 1.8 mg/d (or placebo) for 5 weeks. 19 subjects completed the trial. Groups were matched at baseline, with mean (SD) age 14.8 (2.2) yrs, weight 113.2 (35.6) kg, diabetes duration 1.7 (1.4) yrs, A1C 8.1% (1.2%). No serious adverse events (AEs), including severe hypoglycemia, were reported; AEs were mild in liraglutide subjects and mild to moderate in placebo subjects. Transient, dose- and weight-independent gastrointestinal AEs were most common at low liraglutide doses (Table). There were no significant changes in other measures of safety, tolerability. There was no evidence for pancreatitis and calcitonin levels were within normal range in all subjects and did not increase meaningfully with liraglutide. After administration of 1.8 mg liraglutide, mean $t_{½}$ was 12 h and CL/F 1.7 L/h, similar to observations in adults ($t_{½}$ 13 h, CL/F 1.2 L/h). After 5 weeks, A1C reduction was greater with liraglutide vs placebo (-0.86 vs 0.04%, p=0.0007). Mean weight remained stable (-0.50 vs -0.54 kg, p=0.9703). Liraglutide was well tolerated in a pediatric cohort with T2D, with safety, tolerability and PK parameters similar to adults.

Supported by: Novo Nordisk A/S

Table. Summary of gastrointestinal AEs, hypoglycemic events by liraglutide dose and by randomized group and calcitonin levels.

	Lira 0.3 mg	Lira 0.6 mg	Lira 0.9 mg	Lira 1.2 mg	Lira 1.8 mg	Total lira	Total placebo
	N (%) E	N (%) E	N (%) E	N (%) E	N (%) E	N (%) E	N (%) E
Subjects (N)	14	12	9	9	9	14 (100)	7 (100)
Gastrointestinal disorders*	5 (35.7) 9	3 (25.0) 4	1 (11.1) 1	1 (11.1) 1	2 (22.2) 2	8 (57.1)17	2 (28.6) 7
Diarrhea	3 (21.4) 3	3 (25.0) 3	0 (0.0) 0	0 (0.0) 0	1 (11.1) 1	6 (42.9) 7	1 (14.3) 1
Nausea	3 (21.4) 3	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	1 (11.1) 1	3 (21.4) 4	1 (14.3) 1
Vomiting	0 (0.0) 0	1 (8.3) 1	1 (11.1) 1	0 (0.0) 0	0 (0.0) 0	2 (14.3) 2	2 (28.6) 3
Hypoglycemic events							
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Symptomatic	1 (7.1) 1	1 (8.3) 1	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	1 (7.1) 2	0 (0.0) 0
Asymptomatic	1 (7.1) 1	3 (25.0) 8	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	3 (21.4) 9	1 (14.3) 1
Calcitonin levels (pmol/L) **						Mean (SD)	Mean (SD)
Males:							
Baseline						0.60 (0.54)	0.33 (0.12)
Post-treatment						0.55 (0.50)	0.18 (0.11)
Females:							
Baseline						0.13 (0.10)	0.10 (0.0)
Post-treatment						0.17 (0.14)	0.10 (0.0)

N = number of subjects, % = proportion of subjects, E = number of events; lira = liraglutide; SD = standard deviation
*Total numbers include 7 classes of events; 3 classes with the highest numbers of events are presented
**Calcitonin levels were measured before treatment and after the end of treatment for total liraglutide and placebo arms
Normal calcitonin range (pmol/L): males (3–18 yrs) ≤3.51; females (3–18 yrs) ≤1.46
Safety parameters were not statistically analyzed

PREGNANCY

60-LB

WITHDRAWN

EPIDEMIOLOGY

61-LB

Differentially Segmented Association Between HbA1c and CHD Risks and FPG Across Ethnicities

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HbA1c has been advocated for the diagnosis of diabetes and pre-diabetes and is also a marker for vascular disease risk. However, close examination of the relationship between HbA1c levels, diagnosis of diabetes based on fasting plasma glucose (FPG), and CHD risks for each segment of HbA1c levels across the whole HbA1c spectrum has not been well studied in different racial/ethnic groups. Therefore, we assessed the association between HbA1c levels, diabetes diagnosis, and estimated 10-year and lifetime CHD risks among 22704 adults (20-79 y, 10,649 men and 12055 women) from the recent NHANES 1999-2010.

CHD risk elements, including mean systolic blood pressure, mean diastolic blood pressure, BMI, total cholesterol and HDL, and estimated 10-year and lifetime CHD risks were all significantly associated with rising HbA1c between HbA1c 5-5.9%, whereas at other segments of HbA1c levels (~4%, 4~4.9%, 6~6.9% and above 7%), these CHD risk elements and estimated CHD risks were not significantly correlated with changing HbA1c levels, although elevated HbA1c (>6%) levels were associated with higher CVD risks. Every 0.1 percent increase in HbA1c between 5% and 5.9% was associated with higher percent increase of CHD risks in non-Hispanic White than in non-Hispanic Black and Mexican American. HbA1c was not associated with FPG, 2-hour glucose, and insulin resistance with HbA1c <5%, but above 5%, the associations were strong. In ROC curve analyses, the current HbA1c cutoffs for diabetes and pre-diabetes provided comparable prevalence with FPG criteria in non-Hispanic

Black with optimal sensitivity and specificity; lowering current HbA1c cutoffs for diagnosis of diabetes and pre-diabetes by 0.2% in non-Hispanic White and Mexican American would provide comparable prevalence with FPG criteria and optimize sensitivity and specificity.

In conclusion, to optimally predict prevalence of diabetes, and CVD risk, HbA1c should be lowered from 6.5% to 6.3% in non-Hispanic White and Mexican American.

Supported by: NIDDK (P60 DK 079626)

62-LB

Macronutrient Intake as a Mediator with FTO to Increase Body Mass Index

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The purpose of this study was to examine whether fat mass and obesity-associated (FTO) gene polymorphisms are associated with body mass index (BMI) and whether macronutrient intake (carbohydrate, protein, total fat, total calories) mediates or moderates the association between FTO single nucleotide polymorphisms (snps) (rs1421085, rs17817449, rs9939609, rs8050136) and BMI. Baseline data from the Atherosclerosis Risk in Communities (ARIC) study for whites (n=10176) and African Americans (n=3641) aged 45 to 64 years were analyzed. The ARIC food frequency questionnaire provided the macronutrient indices. In linear regression models with BMI as the dependent variable, FTO snps were significantly associated with higher BMI after adjusting for energy-adjusted macronutrients and other covariates (range: (β=0.41; 95% confidence interval: 0.21, 0.61) for rs17817449 CA vs. AA carriers in whites to (β=1.89; 0.06, 3.72) for rs1421085 GG vs. AA carriers in African Americans. In models with macronutrient as the dependent variable, FTO snps were associated with lower carbohydrate and total caloric intake, and higher protein and fat intake after adjusting for BMI and other covariates; range: (β= -45.36; -77.56, -13.16) for rs1421085 GG vs. AA carriers for total calories in whites to (β=2.48; 0.79, 4.18) for rs8050136 AA vs. CC carriers for protein in African Americans. Associations were stronger and more consistent in whites than African Americans; however power to detect significant associations was limited for African Americans. In mediational analysis, macronutrients were found to have small mediational effects for the relationship between FTO snps and increased BMI. Further effects were seen in whites with ≥1 and ≥4 risk-raising alleles for protein and carbohydrate intake respectively, when snps were combined to create a genetic risk score. These findings highlight the potential importance of the role of macronutrient intake in mediating gene polymorphisms to increase BMI.

63-LB

Smoking Cessation Among Diabetes Patients: Results of a Pilot Randomized Controlled Trial in Kerala, India

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India has the second largest diabetes population (61 million) and tobacco users (275 million). Kerala, the Indian state most advanced in epidemiological transition and the state with the highest prevalence of diabetes, was reported as the harbinger of what is going to happen to the rest of India in the near future. Data on smoking cessation among diabetic patients are limited in low and middle income countries. In our parallel-group randomized controlled trial, we selected 224 adult male diabetes patients aged 18 years or older who smoked in the last month from two diabetes clinics in Kerala. Using a computer generated random sequence with block size four; the patients were randomized equally into intervention-1 and intervention-2 groups. Patients in both groups were asked and advised to quit smoking by a doctor and distributed education materials on smoking and diabetes. The intervention-2 group received an additional diabetes specific 30 minutes counseling session using the 5As (ask, advise, assess, assist, arrange for follow-up) from a non-doctor health professional. Follow up data were available for 87% of patients at six months. The primary outcome was quit rate (abstinence of smoking for at least seven days) at six months. The trail was registered with Clinical Trial Registry of India, number CTRI/2012/01/002327. Quit rate of 51.8% in the intervention-2 group, based on intention to treat analysis, was 8.6 times higher [Odds Ratio (OR) 8.6, 95% confidence interval (CI) 4.2-17.6] than intervention-1 group. Even among high level smokers (those who smoked > 10 cigarettes/bidis per day) the quit rate was surprisingly similar. Diabetes specific tobacco cessation counseling by a non-doctor health professional was found to be efficacious and has the potential to significantly increase quit rates among diabetes smokers after a strong quit advise by their doctors.

Supported by: Fogarty International Centre of the U.S., NIH (TW005969-01)

64-LB

Prevalence of Diabetic Foot Complications and Lower Extremity Amputation Trends at a Tertiary Diabetes Center in South India: 2001–2010

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Annually ~3% of all diabetic patients have been estimated to develop a foot ulcer and many of these patients require a lower extremity amputation (LEA). In the west it has been shown that with improved foot care and by using a multi-disciplinary approach to the management of diabetic foot ulcer, the incidence of amputations can be lowered. The efficiency of such implemented foot care strategies can also be studied by looking at the temporal trends of amputation rates among diabetic foot ulcer patients. In this study we report on the prevalence of diabetic foot complications and compare the temporal trends in the prevalence of major and minor lower extremity amputation among type 2 diabetes patients registered at a diabetes center in south India between 2001 and 2010. Among 116,629 type 2 diabetes patients registered, 2677 (2.29%) had foot infection, 1870 (1.60%) had foot ulcers with most being neuropathic (83.7%); and an additional 346 (0.30%) patients had gangrene. The prevalence of major amputation decreased significantly from 11.6% of the total foot infection cases seen at the center during the years 2001-2002 to 3.8% during the years 2009-2010 ($p<0.001$). The ratio of major: minor amputations also decreased. At this diabetic center, major amputations are declining perhaps due to the comprehensive foot care services provided.

65-LB

Common Variants In and Near *IRS-1* and Subclinical Cardiovascular Disease in the FHS SHARE Study

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Background: Common variants at the 2q36.3-*IRS1* locus, near the gene encoding insulin receptor substrate-1, are associated with insulin resistance (IR), type 2 diabetes (T2D) and coronary artery disease (CAD) in large scale association studies. We tested the hypothesis that variants at this locus are associated with subclinical atherosclerosis traits.

Methods: We studied 2740 Framingham Offspring Study participants (54.9% women; mean age 57.8 years) without clinical cardiovascular disease and with measures of coronary artery or abdominal aortic calcium, internal and external carotid intima-media thickness, and ankle-brachial index (ABI). We tested for association with subclinical atherosclerosis traits 1) four SNPs (rs2943634, rs2943641, rs2972146 and rs2943650) previously shown to be associated with IR, T2D or CAD and 2) any SNP at 2q36.3-*IRS1*. Models were adjusted for age, sex, and cardiovascular risk factors. We set type 1 error rate for test 1) as 0.05/5 traits = $P < 0.01$, and for test 2) as 0.05 divided by the effective number of tests, divided by 5. We had 72% power to detect association with a SNP that explained 0.5% of the total variance in the traits after accounting for the effective number of independent SNPs tested at the locus.

Results: We found no association between the four known SNPs and subclinical atherosclerosis traits. We identified one SNP (rs10167219) at 2q36.3 that was significantly associated with ABI (corrected $P = 0.02$). However, rs10167219 was not associated with ABI ($P = 0.7$) in a validation look up in a published ABI association study with N=35,404.

Conclusion: Common variants at the 2q36.3-*IRS1* locus are not associated with subclinical atherosclerosis traits in a study adequately powered to find true negative results. Although IR and T2D may be mechanistically linked to CAD via subclinical atherosclerosis, an alternate mechanism for the IR-T2D-CAD associations at 2q36.3-*IRS1* must be postulated.

66-LB

What Next After Metformin? A Retrospective Evaluation of the Outcome of Second-Line, Glucose-Lowering Therapies in People with Type 2 Diabetes

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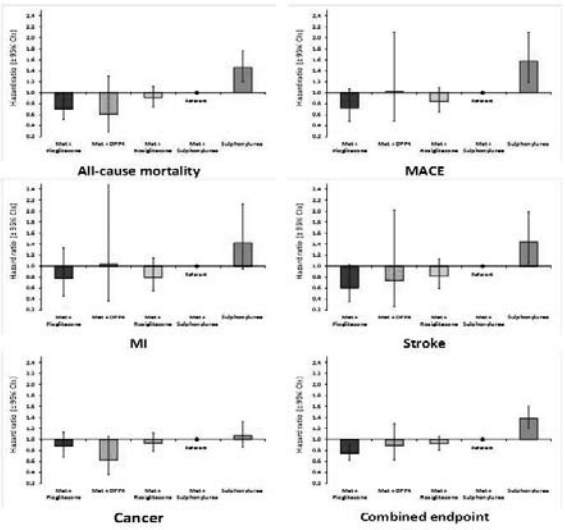
The aim of this study was, for the first time, to compare outcomes for all commonly used second-line, glucose-lowering regimens. Retrospective data from the UK General Practice Research Database was used. Patients initiating treatment between 2000 and 2010 were selected. By comparison with metformin plus sulfonylurea combination therapy, the primary endpoints were all-cause mortality, major adverse cardiovascular event (MACE [MI and

stroke]), cancer, and a combined endpoint of the first of any of these. Time to each endpoint was compared using Cox proportional hazards models. A secondary endpoint was change in HbA1c between baseline and 1 year. 26,278 patients initiated a common regimen. Sulfonylurea monotherapy had significantly higher adjusted hazard ratios (aHR) for all-cause mortality, MACE, stroke and the combined endpoint. Metformin plus pioglitazone combination therapy had significantly lower adjusted aHRs for all-cause mortality and the combined endpoint. Mean HbA1c improved between baseline and 12 months for all regimens other than sulphonylurea monotherapy. Metformin plus sulfonylurea was the most common second-line therapy but metformin plus pioglitazone was significantly superior for both all-cause mortality and the combined endpoint. DPP-4s or rosiglitazone in combination with metformin also had lower HRs for most endpoints but none achieved statistical significance. Sulfonylurea monotherapy resulted in worst outcome. Figure. Summary of adjusted hazard ratios across the various endpoints by alternative glucose-lowering regimen.



Supported by: BMS, EASD, Cardiff University, Pharmatelligence

67-LB

Prediabetes Prevalence among U.S. Adults, NHANES 2005–2010: Comparisons of 2- and 6-Year Averaged Estimates

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Prediabetes has been defined by at least 3 different measures: HbA1c 5.7-6.4% (A1c); fasting plasma glucose 100-125 mg/dL (IFG); and 2-hour oral glucose tolerance test 140-199 mg/dL (IGT). The National Health and Nutrition Examination Survey (NHANES) is the nationally-representative source for data on these measures in U.S. civilian, non-institutionalized adults. Combining 2-year NHANES cycles is recommended to improve estimates' precision and to minimize sampling error. However, examining the variation in 2-year estimates is needed before calculating averaged estimates. We used NHANES 2005-2010 data to compare 2-year prevalence estimates for the 3 prediabetes measures and their combinations with 6-year averages. Data were from the 7,806 participants aged ≥18 years who had all 3 prediabetes measures. The table shows prevalence estimates (95% confidence interval) by measure and survey period.

Survey years (n) by prediabetes measure	Cycle 1 2005–2006 (2213)	Cycle 2 2007–2008 (2686)	Cycle 3 2009–2010 (2907)	All Years 2005–2010 (7806)
A1c	14.0 (13.0-15.1)	18.9 (16.6-21.5)	23.2 (21.1-25.3)	18.8 (17.6-20.0)
IFG	25.5 (22.5-28.7)	31.7 (28.7-35.0)	25.8 (23.0-28.8)	27.7 (25.9-29.5)
IGT	13.4 (11.2-15.9)	15.1 (13.3-17.1)	11.6 (10.7-12.4)	13.3 (12.3-14.4)
A1c or IFG	30.5 (27.9-33.3)	38.1 (35.3-41.0)	36.2 (33.1-39.4)	35.0 (33.3-36.7)
A1c or IGT	21.3 (19.5-23.2)	26.3 (24.1-28.6)	26.5 (24.7-28.3)	24.7 (23.6-25.9)
IFG or IGT	28.9 (26.0-32.0)	35.8 (32.5-39.3)	27.7 (25.5-29.9)	30.8 (29.1-32.5)
A1c or IFG or IGT	32.8 (30.1-35.6)	40.5 (37.8-43.3)	36.5 (33.9-39.1)	36.6 (35.1-38.2)

There was a significant increase in prediabetes prevalence between 2005-2006 and 2007-2008 for all measures (p<0.01), except IGT. Between 2007-2008 and 2009-2010, prevalence increased only for the A1c measure alone (p=0.012). Increases in prediabetes prevalence based on A1c did not correspond to changes in individual or combined IFG and IGT estimates. Regardless, prevalences averaged over 6 years were less variable than 2-year estimates. In all, using both multiple years of data and measures helps to stabilize prediabetes estimates but requires careful decision-making.

68-LB

Pathobiology of Prediabetes in a Biracial Cohort (POP-ABC) Study: Baseline Characteristics and Glycemic Outcomes During 5.5 Years of Follow-Up

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The POP-ABC study assessed incident prediabetes (IFG/IGT) or diabetes (T2D) among offspring of parents with T2D. We enrolled 376 offspring (217 black, 159 white) and followed them for 2.25-5.5 years (mean 3.9 yr), with repeated assessments (including OGTT, body composition, euglycemic clamp, IVGTT, energy expenditure). Among whites, 85.9% had one parent with T2D and 14.1% had both; among blacks, 86.8% had one and 13.2% had both parents with T2D. At baseline, the cohort (70% female) had a mean (±SD) age of 44.2 ± 10.6 yr. Compared with whites, black offspring had lower triglycerides and FPG levels but higher BMI and systolic BP. As at March 30, 2012 (mean follow-up 3.9 yr), 111 subjects (29.5%) had developed prediabetes (N=100 or 26.6%) or T2D (N=11 or 2.9%). Among whites, 48 of 159 offspring (30.2%) developed prediabetes compared to 52 of 217 blacks (24%). The T2D rates were 3.14% in whites and 2.76% in blacks. Glycemic progressors were ~2 yrs older and had higher BMI, waist, trunk fat, BP, FPG, 2hrPG and triglycerides at baseline compared to nonprogressors. The median time (days) to occurrence of prediabetes was 524 [103-1588] in whites and 503 [228-1575] in blacks. Using Cox regression model (after adjusting for age, FPG, 2hPG, BMI), there was no effect of race (P= 0.7494) on time to occurrence of prediabetes. Baseline total fat, trunk fat, waist, and BP were similar between black and white glycemic progressors. Insulin sensitivity (Si-clamp, umol/kg.min-1/pmol/L) also was similar in black vs. white progressors (0.061 ± 0.008 vs. 0.066 ± 0.007) but lower than the cohort mean Si-clamp, 0.085 ± 0.003 (P=0.01). We here demonstrate that among offspring of T2D parents a) direct transition to T2D is rare (0.73%/yr), b) ~27% developed prediabetes (6.7%/yr), c) 50% of prediabetes events occurred within 1.5 yr, and d) the occurrence of prediabetes/T2D is independent of race/ethnicity during a mean follow-up of 4 years. These are novel data for non-Pima populations.

Supported by: NIH (R01 DK067269, MO1 RR00211)

69-LB

Cheiroarthropathy in the DCCT/EDIC Cohort

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The development of skin thickening of the hands and limited joint mobility (cheiroarthropathy) is associated with diabetes and can lead to disability. The purpose of this study is to describe the prevalence of cheiroarthropathy (cheiro) in the large, well characterized DCCT/EDIC (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications) type 1 diabetes (T1DM) cohort, examine risk factors (including glycemia) and diabetes microvascular complications associated with cheiro, and to examine impact of former DCCT therapy (intensive vs. conventional).

Cheiro has been assessed thus far in 873 DCCT/EDIC subjects using a medical history and physical examination performed by certified DCCT/EDIC staff. A self-administered questionnaire (Disabilities of the Arm, Shoulder and Hand: DASH) assessed function of the upper limbs.

Characteristics of those with cheiro (defined as presence of any one of the following: frozen shoulder, carpal tunnel, trigger finger, Duptryen's contracture, or a positive prayer sign) and without cheiro are described (Table 1). Cheiro was present in 66% of subjects (63% of the former intensive group, 70% conventional; p=0.031) and was associated with age, gender, diabetes duration, HbA1c, neuropathy and retinopathy (P < .05). DASH functional disability scores were worse among subjects with cheiro (P<.0001).

This preliminary evaluation of cheiro in the DCCT/EDIC cohort shows that it is common in people with T1DM of long duration (~ 30 years), less frequent with prior intensive therapy, and is related to glycemia and other factors.

Results

	Total	Cheiroarthropathy Present	Cheiroarthropathy Absent	p-value
N	**873**	**577**	**296**	
Type of Cheiroarthropathy * Frozen Shoulder		269		
Carpal Tunnel		264		
Trigger Finger		242		
Dupytren's Contracture		78		
Positive Prayer Sign		171		
Age (years)	51.9±6.9	52.5±6.7	50.8±7.3	0.001
Gender (female)	412 (47)	301 (52)	111 (38)	<0.0001
Duration DM (years)	30.6±4.9	31.4±5.0	29.2±4.4	<0.0001
Therapy (Int. treatment)	451 (52)	283 (49)	168 (57)	0.031
Mean A1c (%)				
Time-weighted DCCT/EDIC	8.0±1.0	8.0±1.0	7.9±0.9	0.019
During DCCT	8.1±1.4	8.1±1.4	8.0±1.3	0.093
During EDIC	8.0±1.0	8.0±1.0	7.9±1.0	0.018
Neuropathy	245 (30)	187 (35)	58 (21)	<0.0001
Nephropathy	120 (14)	78 (14)	42 (14)	0.785
Retinopathy	190 (22)	151 (26)	39 (13)	<0.0001
DASH Disability/Symptom score (N=863)	10.8±13.2	13.2±14.2	6.1±9.4	<0.0001
DASH Work Module score (N=738)	7.5±14.2	9.2±15.7	4.3±10.2	<0.0001
DASH Sports/Performing Arts Module score (N=347)	14.0±23.1	17.6±24.6	8.3±19.3	<0.0001

Data are mean±std or N (%).
*Participants could have more than one type of cheiroarthropathy.
Neuropathy defined as the presence of confirmed clinical neuropathy at EDIC year 13/14 (N=662 had a neurologic exam).
Nephropathy defined as an AER>30mg/24 hours at two consecutive visits.
Retinopathy defined as scatter laser treatment to one or both eyes by self-reported history.

Supported by: NIH, NIDDK (NCT003680815, NCT00360893)

70-LB

Apparent Rapid Rise in Glycemia Prior to Diagnosis of Diabetes May be an Artifact

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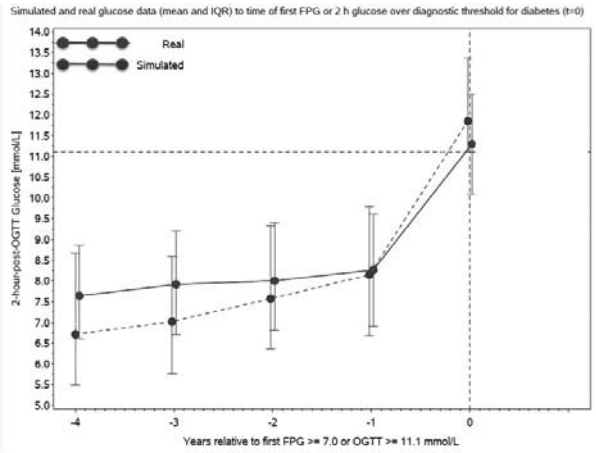
Retrospective analyses report that glucose levels increase more rapidly as people approach the time when their diabetes is diagnosed. We postulated that day-to-day biological, assay and random variation in glucose values lead to selective over diagnosis of diabetes in those who happen by chance to have higher glucose values at any particular time point.

Glycemic data from 534 NAVIGATOR participants who were randomized to placebo study medication and who progressed from impaired glucose tolerance to new-onset diabetes were analyzed. 2-hour post-challenge glucose (2HPG) trajectories, plotted backwards from the time diabetes was diagnosed, showed a rapid rise in the last year (Figure-Real). Applying WHO glycemic diagnostic criteria for diabetes to simulations of a solely linear glucose increase over time also showed apparent acceleration of glucose values, with the slope increasing as the variability of the simulated data was increased. NAVIGATOR glycemic data were used to estimate the slope of a putative solely linear increase in glycemic values, as well as their covariance structure. Simulated mean FPG and 2HPG glycemic trajectories utilising these estimates were similar to those observed from the real NAVIGATOR data (2HPG shown in Figure-Simulated).

Previously reported apparent accelerations in the rate at which glucose trajectories appear to increase immediately prior to diagnosis of type 2 diabetes are likely to be due largely to an artifact of the repeated diabetes categorization process, and not to reflect a substantive metabolic deterioration immediately preceding the diagnostic FPG and 2HPG thresholds for diabetes.



Supported by: Novartis Pharmaceuticals Corporation

GENETICS—TYPE 2 DIABETES

71-LB

An E3-Ubiquitin Ligase Regulates the Activity of an Inhibitor of Insulin Secretion

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We previously mapped a fasting glucose locus on chromosome 16 (Chr16) in an F2 intercross from the BTBR T (+) tf (BTBR) Lepob/ob and C57BL/6 (B6) Lepob/ob mouse strains. Using interval-specific congenic strains, where BTBR Chr16 fragments were introgressed in B6 mice, we narrowed the locus to a 1.6 Mb region. The islets from these mice (B6.16BT36-38) were defective in the second phase insulin secretion, suggesting that the 1.6 Mb region encodes for a regulator of insulin secretion. Within the 1.6 Mb region we discovered a single nucleotide polymorphism (SNP) (Ser912→Leu) in the protein coding for tomosyn-2. The coding SNP negatively impacted its protein turnover. The B6 allelic product of tomosyn-2 (Ser912) was susceptible whereas the BTBR allelic product (Leu912) was resistant to proteasomal degradation. We demonstrated a reduced capacity for insulin secretion in β-cells from mice congenic for the tomosyn-2 SNP, directly correlating with the hypoinsulinemic/hyperglycemic phenotype of the animals. Overexpression of b-tomosyn-2 isoform in human pancreatic β-cells attenuated glucose-stimulated insulin secretion. Using mass spectroscopy we have identified phosphorylation sites and a potential E3-ubiquitin ligase that may play a role in regulating tomosyn-2 abundance. To understand the mechanism by which tomosyn-2 abundance is regulated we hypothesize that phosphorylation of tomosyn-2 targets it for proteasomal degradation. Loss and gain of function experiments are being performed to establish the role of the E-3- ubiquitin ligase. While these preliminary results are quite exciting, the mechanisms by which tomosyn-2 regulates insulin secretion remain unknown. Understanding the role of tomosyn-2 in insulin secretion will provide further insight in the regulation of insulin secretion in type 2 diabetes.

Supported by: NIDDK (66369, 52037)

72-LB

Trans-Ethnic Meta-Analysis Reveals Novel Loci for Type 2 Diabetes Susceptibility

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Recent meta-analyses of genome-wide association studies (GWAS) of type 2 diabetes (T2D) in European, South Asian and East Asian ancestry populations have demonstrated substantial overlap of loci contributing effects to the disease. These results imply that the underlying causal variants at these loci are shared across multiple ancestry groups, and suggest that trans-ethnic meta-analysis has the potential to reveal additional T2D susceptibility loci.

We considered T2D GWAS meta-analyses in 26,488 cases and 83,964

controls from four ancestry groups: European, East Asian, South Asian, and Mexican American. We combined summary statistics for all SNPs with strong evidence of association (p<10-4) in at least one ancestry group. We employed two approaches to combining these summary statistics: (i) fixed-effects, inverse-variance weighted meta-analysis; and (ii) Bayesian trans-ethnic meta-analysis (MANTRA) that allows for heterogeneity in allelic effects between ancestry groups according to a prior model of relatedness between them.

Our trans-ethnic meta-analysis revealed three novel loci for T2D susceptibility that have not been previously reported in any ancestry group: MC4R (rs571312, fixed-effects p=2.0x10-10, MANTRA log10BF=8.17); ZMIZ1 (rs810517, fixed-effects p=2.6x10-10, MANTRA log10BF=8.22); and TMEM154 (rs7686797, fixed-effects p=1.5x10-8, MANTRA log10BF=6.53). The lead SNP at the MC4R locus is the same as that previously associated with body-mass index in European ancestry populations.

We observed nominal evidence of heterogeneity (p<0.05) in allelic effects between ancestry groups at lead SNPs at just 4 of 30 novel and established T2D loci in our trans-ethnic meta-analysis (CDKAL1, CDC123, HHEX/IDE and TCF7L2). These results suggest that combining GWAS data across ancestry groups is a powerful strategy for T2D susceptibility locus discovery, and inclusion of additional populations may reveal further association signals at genome-wide significance.

IMMUNOLOGY

73-LB

CT Perfusion Monitoring of Pancreas in Streptozotocin-Induced Diabetic Rats

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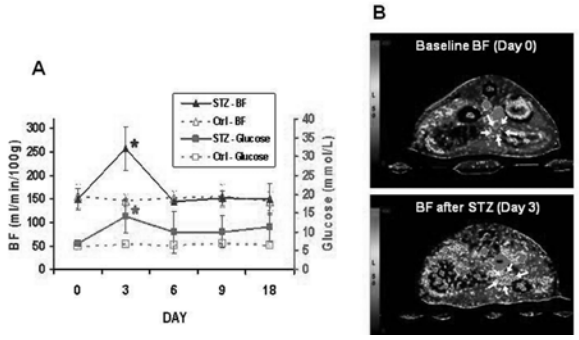
Diabetes mellitus (DM) is an autoimmune-mediated disease destroying beta-cells in the islets of Langerhans throughout the pancreas. A common feature of immune-mediated diseases is the build-up of an inflammatory infiltration in the target organ. The subsequent inflammatory response dilates the blood vessels around the site of inflammation, allowing increased blood flow to the area. We have developed a dynamic contrast enhanced-CT (DCE-CT) technique to assess quantitatively blood flow (BF) in microvessels. In this study, we measured DCE-CT derived BF in the pancreas of STZ-induced diabetic rats to investigate how BF correlated with change of blood glucose and whether it could be used as a surrogate imaging marker for the inflammatory responses involved in the development of DM of the diabetic rat. After collecting blood via tail vein for fasting blood glucose (FBG), each rat (male Wistar) was scanned with infusion of iodinated contrast agent at D0 (baseline) and D3, 6, 9 & 18 after STZ treatment (55 mg/kg i.p.). Both FBG and pancreatic BF were significantly increased at D3 in STZ-treated rats, compared to those in control rats (Figure). Even though the transient increase in BF of the diabetic rat arised from the acute toxicity of STZ on the insulin-secreting beta-cells, prior histopathological studies showed that the toxicity elicited only a mild inflammatory response in the pancreas at D3. These results suggest the potential feasibility of using DCE-CT to monitor for early and ongoing autoimmune-mediated inflammatory reaction in the pancreas of an autoimmune-mediated diabetic animal model.

A. Changes in fasting blood glucose (red lines, right axis) and DCE-CT derived BF (blue lines, left axis) in the pancreas of control (Ctrl, dotted lines, N = 3) and STZ-induced diabetic rats (N = 3) before (baseline, Day 0) and after STZ treatment (upto Day 18) (mean ± SD, *; $p \leq 0.05$ *vs.* corresponding control group, Mann-Whitney U test). B. Representative transaxial BF maps at baseline and Day 3 after STZ treatment (arrows indicate the pancreas).



Supported by: Ontario Research Fund (ORF)-Small Animal Imaging Consortium, GE Healthcare

74-LB

A Distinct Population of Lipid Sensing iNKT Cells are Enriched in Fat and Protect Against Diet-Induced Obesity and Insulin Resistance

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Immunometabolic interactions regulate adipose tissue inflammation in obesity and subsequent development of insulin resistance and diabetes. We have shown that human and murine fat contains the largest population of iNKT cells in the body. Fat resident iNKT cells are a distinct subset with unique phenotype and anti-inflammatory cytokine profile. These iNKT cells are depleted in diet-induced obesity and removal of high fat diet results in iNKT restoration in mice and humans. Furthermore, mice lacking iNKT cells have enhanced weight gain, larger adipocyte size, fatty liver infiltration and insulin resistance. Adoptive transfer of iNKT from WT mice into obese mice or activation of remaining iNKT in obese fat by through injection of alpha-galactosylceramide (aGC) had a profound improvement in insulin sensitivity and glucose handling, a decrease in serum triglycerides, leptin, body fat, fatty liver infiltration and proinflammatory macrophage levels. It is therefore beneficial to identify what causes iNKT cells depletion in obesity. We have purified lipids from obese and lean adipose tissue, to identify which, if any, lipid fraction may activate iNKT cells. Early preliminary data suggest that a HFD abherrently activate iNKT cells in obesity, leading to their loss, and subsequent development of insulin resistance. In summary, we have identified a distinct population if iNKT cells in fat and describe a unique role for iNKT in protection against obesity and related metabolic disease.

75-LB

Anti-Inflammatory Effects of Exendin-4 on Human Peripheral Lymphocytes in Patients With Type 1/2 Diabetes

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Chronic infiltration of diabetic islets with immune cells and their secreted pro-inflammatory cytokines/chemokines are characterized in diabetes. We hypothesize that exendin-4 may reduce inflammatory response in both T1 and T2DM.

Using peripheral blood mononuclear cells (PBMC) sampled from 10 T1DM, 10 T2DM patients and 10 sex- and age-matched control subjects and supernatants from cell culture, we 1) applied flow cytometry to analyze expression of phospho-mitogen activated protein kinase (MAPK) signaling pathways in CD4+ T lymphocytes and monocytes; 2) cytometric bead arrays to measure cytokines/chemokines and 3) chemiluminesence assay to determine superoxide dismutase (SOD) activity before and after treatment with exendin-4.

Compared to control subjects, PBMC from both T1 and T2DM patients showed significantly activated p-p38, reduced SOD activity and increased proinflammatory cytokines/chemokines in both T1/T2 DM patients. These changes were attenuated by exendin-4, possibly through suppression of p38 MAPK. Data are expressed as median (IQR). (* p<0.05). These results suggest that exendin-4 might down-regulate proinflammatory responses and reduce oxidative stress by suppressing MAPK signaling pathways in diabetes.

Effects of exendin-4 on ex vivo production of cytokines/chemokines of patients with diabetes

Cytokine/ Chemokine	Group	Medium(pg/ml)	Exendin-4(pg/ml)
TNF-α	Control	22.5 (8.7-38.7)	21.3 (9-71.9)
	T1DM	41.0 (13.7-215.8)	32.9 (13.3-144.1)*
	T2DM	56.65 (7.5-236.6)	48.75 (7.7-127.0)*
IL-1β	Control	35.5 (22.2-61.0)	43.2 (13.6-133.5)
	T1DM	161.6 (78.4-246.2)	90.2 (63.3-154.5)*
	T2DM	111.0 (6.9-184.3)	70.55 (9.3-190.5)*
IL-6	Control	55 (20.7-105.5)	47.9 (7.5-80.6)
	T1DM	217 (41.6-413.8)	234.9 (51.8-473.4)
	T2DM	114.4 (7.5-204.8)	90.6 (11.1-177.2)*
IL-10	Control	6.8 (4.1-20.6)	8.7 (2.5-45.1)
	T1DM	27.2 (1.8-54.4)	18.8 (1.2-56.8)*
	T2DM	42.9 (10.3-64.8)	31.2 (9.3-54.4)*
CXCL10	Control	24.2 (15.6-37.6)	18.9 (18.6-21.0)
	T1DM	26.6 (20.1-67.2)	17.0 (12.2-34)*
	T2DM	76.4 (10.4-155.7)	65.2 (19-86.8)*
CCL5	Control	32.3 (14.9-98.1)	34.3 (17.6-95.9)
	T1DM	138.3 (71.1-331.8)	150.2 (27-257.9)
	T2DM	329.8 (52.5-694.4)	191.1 (71.4-376.2)*

Expression of phospho-p38 in exendin-4 treated CD4+ T lymphocytes and monocytes

Cell type	Group	Medium (MFI/104 leucocytes)	Medium (MFI/104 leucocytes)
Th cells	Control	2.97 (2.4-4.1)	2.7 (2.3-3.9)
	T1DM	5.5 (3.4-7.5)	4.4 (3.3-4.9)*
	T2DM	6.65 (5.3-7.1)	4.4 (3.9-6.1)*
Monocytes	Control	14.8 (13.5-24.2)	14.6 (13.4-23.1)
	T1DM	25.3 (15.9-25.8)	20.7 (15.8-22.1)*
	T2DM	32.9 (25.7-64.9)	26.2 (20.7-53.2)*

76-LB

The Role of TRIF in the Development of Type 1 Diabetes (T1D) in Nonobese Diabetic Mice

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TIR-domain-containing adapter-inducing interferon-β (TRIF) is an adaptor for activation of some Toll Like Receptors (TLRs). It mediates one of two TLR-associated signaling cascades, where TRIF mediates a more delayed cascade and the other is dependent upon the MyD88 adaptor. In addition to the common MyD88-dependent pathway, TLR3 & 4 utilize a MyD88-independent signaling pathway that leads to the activation of IRF-3 and induction of IFN-β. To investigate whether TRIF plays a role in T1D development in the NOD mice, we generated TRIF-/-NOD mice. We found that female TRIF-/-NOD mice (n=15) had delayed onset of spontaneous T1D compared to TRIF+/-NOD (n=22) and TRIF+/+NOD mice (n=10) after 8 months observation. Glucose tolerance test showed that TRIF-/- mice were more insulin sensitive compared to TRIF+/+ mice ($P<0.05$). In adoptive transfer using irradiated NOD mice, we found that the recipients (n=15/group) injected with the diabetic TRIF-/- slpenocytes had delayed onset of induced T1D ($P<0.01$). To further investigate the role of T cells and antigen presenting cells (APCs), we used NOD.scid (N/S) mice (n=6 per group) as recipients of TRIF+/+ splenic T cells together with TRIF-/- splenic APCs or TRIF-/- splenic T cells together with TRIF+/+ splenic APCs (T:APCs=1:1). Interestingly, N/S mice that received TRIF-/- T cells and TRIF+/+ APCs developed delayed onset of diabetes ($P<0.05$). We further performed bone marrow (BM) chimera experiments and our results showed that the TRIF-/- recipients (n=10) that received TRIF+/+ BM cells developed accelerated diabetes compared with the TRIF+/+ recipients (n=10) that received the TRIF-/- BM cells. Our data demonstrate that in the absence of TRIF, both spontaneous and induced T1D development was significantly delayed. Our study suggests that TRIF might be a novel target for T1D prevention and possibly also for immunotherapy.

77-LB

In Vitro and in Vivo Effects of the GLP-1 Receptor Agonist Liraglutide on Immune Competent Cells in Healthy Subjects

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Incretin-mimetic, glucagon-like peptide-1 receptor agonists (GLP-1RA) are used as a novel therapy in T2D. Immunomodulatory effects of GLP-1RA were suggested after improved psoriasis following GLP-1RA treatment and anti-inflammatory effects in preclinical studies had been observed. In this study, we investigated the in vitro effect of liraglutide in human immune cells as well as the in vivo effect on the frequency of immune competent cells in the peripheral blood of healthy subjects.

Proliferation of PBMCs from healthy donors was tested in vitro after exposing cells to increasing concentrations of liraglutide (0-80 µg/ml). Unstimulated cells and cells stimulated by adding anti-CD3/CD28 dynabeads were tested after 96 h of liraglutide exposure. In vivo tests were done in a pilot trial including 5 healthy subjects (2f/3m; age: 35±9 years; BMI: 25.1±4.9) who were initially treated with 0.6 mg liraglutide/day followed by 1.2 mg/day for a total of 4 weeks. A multi-color FACS analysis for Treg, B-, iNKT-, NKT-, NK-, Th1-, Th2-, Th-17 cells and DCs was performed at baseline (BL), after 2 and 4 weeks.

In vitro PBMC proliferation of unstimulated cells increased 2-3 fold upon addition of liraglutide (20, 40, 80 µg/ml) whereas proliferation of stimulated cells did not change. In vivo Helios expression of Treg remained stable but peripheral Treg in CD4posTc increased significantly from 4.64%±1.06 (BL) to 5.74%±1.14 after 2 wks (p=0.001) and 5.52%±0.83 after 4 wks (p=0.004). The % of Th1-cells decreased significantly (7.50%±2.42 at baseline to 4%±1.96 after 4 weeks, p=0.002) whereas all other cell types remained stable.

Our study is first to show immunomodulatory effects of liraglutide treatment in healthy human subjects and our results advance the mechanistic insight into the immunomodulatory potential of incretin hormons in vitro and in vivo. Our findings provide further rationale to investigate potential beneficial effects of GLP-1RA treatment in autoimmune diseases such as T1D.

78-LB

Reversal of Autoimmune Diabetes by Restoring Antigen-Specific Tolerance Using Genetically Modified Lactococcus Lactis

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Current interventions for arresting autoimmune diabetes have yet to find the balance between sufficient efficacy, minimal side-effects, and lack of generalized immunosuppression. Introduction of antigen via the gut represents an appealing method for induction of antigen-specific tolerance. Here, we present a novel strategy for tolerance restoration using mucosal delivery by live biologically-contained genetically-modified *Lactococcus lactis* (LL) of the whole pro-insulin (PINS)-autoantigen along with the immunomodulatory cytokine IL10 (2×10^9 cfu, 5 days per week for 6 weeks). We show that a combination therapy with low-dose systemic anti-CD3 ((2.5 µg, day 0-4, iv) stably reverted diabetes in almost 60% of recent-onset diabetic NOD mice and increased local regulatory T-cell frequencies which not only accumulated in the pancreatic islets, but also suppressed in an autoantigen (PINS)-specific way. Cured mice remained responsive to disease-unrelated antigens, arguing against excessive immunosuppression. Application of this novel therapeutic tool achieved gut mucosal delivery of a diabetes-relevant autoantigen (PINS) and a biologically active immunomodulatory cytokine IL10, and when combined with a low-dose of systemic anti-CD3, was well-tolerated and induced autoantigen-specific long-term tolerance allowing reversal of established autoimmune diabetes.

Treatment of recent-onset diabetic NOD mice

treatment (number of mice)	diabetes incidence (%)
untreated (n = 9)	100
LL-PINS+IL10 (n = 13)	15
Anti-CD3 (n = 32)	25
anti-CD3+LL-pT1NX (n = 22)	23
anti-CD3+ LL-IL10 (n = 30)	40
anti-CD3+ LL-PINS (n = 35)	49
anti-CD3+LL-PINS+IL10 (n = 61)	59

Supported by: JDRF (17-2011-524)

79-LB

Longitudinal, Live Imaging of Islet Autoimmune Destruction in Mice

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Autoimmunity progression was assessed by transplanting NOD.SCID islets into either spontaneously diabetic female NOD (recurrence model) or NOD.SCID mice (autoimmunity adoptive transfer model). Antigen-specific immunity was studies using RIP-OVA islets implanted into C57BL/6-Rag1-/- mice receiving adoptive transfer of C57BL/6-GFP-OT-I lymphocytes. Recurrence of diabetes occurred with a median of 10 (range 5-12) and 8 (5-13) days in the ACE (n=6) and kidney capsule (KDN; n=12), respectively. Adoptive transfer of splenocytes from newly diabetic NOD mice induced diabetes within 30-35 days. Similarly, antigen-specific CD8 T-cells from GFP-OT-I mice accumulated and progressively destroyed RIP-OVA islets in the ACE. Longitudinal assessment of individual islet volume and granularity in the ACE of reconstituted NOD.SCID mice demonstrated islet swelling starting one week before onset of overt hyperglycemia followed by relatively quick volume reduction within a week. Live time-lapse studies performed after direct cytolabeling and cell viability dye injection in the ACE in selected animals allowed assessing the behavior of infiltrating B and T cells and cell death in the target tissue with single-cell resolution prior, during and after the onset of diabetes. Preliminary assessment of islet grafts explanted after diabetes onset from ACE and KDN showed infiltrating T and B lymphocyte populations similar to native pancreas by immunofluorescence staining. In conclusion, islet transplantation into the ACE represents a valuable model to study islet immunity and offers unprecedented advantages compared to

other transplantation sites, particularly the possibility to perform longitudinal in vivo noninvasive imaging on the very same islets with cellular resolution to characterize the effector phase kinetics of the infiltrating cells on the site of immune attack.

Supported by: Diabetes Research Institute Foundation, NIH (5U19AI050864-10)

TRANSPLANTATION

80-LB

Extrahepatic Tissue Engineering Enhances Islet Transplantation

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Intrahepatic infusion is the only accepted site for clinical pancreatic islet transplantation (PIT), in spite of marginal clinical success. Here we describe development of an extrahepatic site for PIT. Recipient rodents are implanted with a 3D, hollow cylindrical device fabricated from a clinical grade mesh coated with matrix proteins and adsorbed with FGF-1. The response to the implanted device included an immediate luminal influx of macrophages followed by the appearance of mesenchymal stromal cells (MSCs). As MSCs migrate into the device, they are followed by angiogenesis, ultimately demonstrating a mature MSC tissue interdigitated with an abundance of neovascular structures. In vitro, device-recovered MSCs function as endothelial cell precursors and express soluble factors competent to stabilize islet function. Marginal mass transplantation studies demonstrated that the microenvironment of the vascularized device promoted more efficient syngeneic islet function, compared to intrahepatic transplantation. In vitro, the shift from proliferation to quiescence by MSCs was accompanied by a change in cytokine expression from a Th1 to a Th2 microenvironment. MSCs and MSC-derived soluble mediators were able to deviate Th1 cytokine responses from a diabetogenic T cell to a regulatory phenotype. Early preliminary results suggested that islets in co-culture with MSCs are resistant to rapamycin cytotoxicity. Extrahepatic transplanted syngeneic islets also were resistant to rapamycin therapy in vivo. Following extrahepatic allogeneic rat PIT, immunostaining confirmed that: (i) activated T cells readily traffic to the extrahepatic site; (ii) MSC-dependent expression of Th2 cytokines alone are unable to modulate, acute immune responses; (iii) the device microenvironment exhibited the appearance of Tregs; and (iv) rapamycin monotherapy was not able to prevent islet rejection. These studies predict that the extrahepatic site will adapt to modern tolerogenic strategies associated with allo/xeno PIT.

81-LB

Continuous Glucose Monitoring Identifies Reduced Hyperglycemia Post Kidney Transplantation with Split Dose Prednisolone

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Avoiding excessive glucocorticoid exposure by divided twice daily (BID) prednisolone dosing and monitoring of free (active) prednisolone may reduce post transplant hyperglycemia. We aimed to determine if BID dosing reduces hyperglycemia versus daily (QD) and if free prednisolone exposure correlates with glycemia, using a validated limited sampling strategy. At transplantation, 14 subjects without diabetes were randomised to BID or QD prednisolone. In week 3, total daily dose was fixed and a continuous glucose monitor (iPro2® Medtronic) applied for 5 days. Subjects continued randomised regimens for Days 1-2 before crossover (Day 3) to the alternative dosing regimen for Days 4-5 (the crossover day was disregarded). Free prednisolone exposure, mean glucose, peak glucose (time, level), exposure to hyperglycemia (AUC>140mg/dl) and Athens insomnia scale were assessed. Mean (SD) age was 47 (12) years and 11/14 were male. Mean daily dose was 22.5 (2.6) mg. BID was associated with decreased glucose (mean 138 vs 145mg/dl, p<0.0001), peak glucose (mean 181 vs 215mg/dl, p=0.0007), and exposure to hyperglycemia (mean 117 vs 486mg/dl/hr, p=0.007). Mean time of day peak glucose occurred was 14:00 (BID) and 15:55 (QD) (Fig. 1). BID dosing did not increase insomnia (mean BID 6.0 (4.2), QD 4.5 (3.5), p=0.14). Free prednisolone exposure correlated with peak glucose (r^2=0.55, p=0.0002), mean glucose (r^2=0.27, p=0.018) and exposure to hyperglycemia (r^2=0.38, p=0.004). We conclude free prednisolone exposure correlates with glycemia and split dosing reduces hyperglycemia post kidney transplantation.

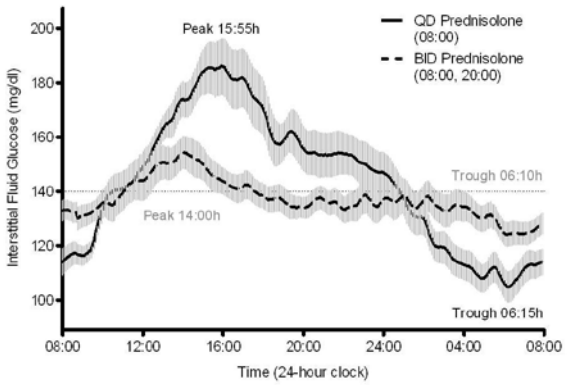

Fig. 1 Mean glucose (SD) for prednisolone dosing regimens, p<0.0001

82-LB

Prolonged Allogeneic Islet Survival by Oxidized Adenosine Tri-Phosphate Treatment

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Short-course Lymphocyte Function-associated Antigen (LFA)-1 blockade is partially successful in preventing murine allograft rejection in mice. Early post-transplant inflammation negatively impacts engraftment and survival of allografts. Oxidized Adenosine Tri-Phosphate (oATP) is largely utilized as nonselective blocker of the purinergic receptors (P2XRs) with anti-inflammatory and analgesic proprieties. We evaluated the effects of oATP therapy alone or combined with LFA-1 blockade on islet allograft survival in a fully MHC-mismatched transplant combination [DBA/2 (H2d)->streptozotocin-diabetic C57BL/6 mice (H2b)]. Single or combined treatments included: oATP (Medestea Research) 0.25mg/mouse intravenously daily on day 0-4, then biweekly for 4 weeks; 7-day course of anti LFA-1 antibody 100ug/mouse/day intraperitoneally from day 0. In vitro, proliferative response to mitogenic stimulation of naïve splenocytes (both mixed lymphocyte reaction, MLR, and anti-CD3) was suppressed in a dose-dependent by oATP, and paralleled by an increase in Treg proportions. LFA-1 blockade was synergistic with oATP in suppressing MLR. Control animals rejected allogeneic islets within 15 days (n=12). Long-term allograft survival (>100 days) was observed in 25% of animals receiving oATP treatment alone (median graft survival=63 days; n=8; p=0.04), and in 57% of animals treated with anti-LFA-1 (n=7). Combination therapy based on oATP+LFA-1 blockade yielded 100% long-term graft survival (n=6; p<0.001). Explanted grafts showed well-preserved islets and limited non-infiltrating mononuclear cells. Collectively, a direct immunomodulatory effect of oATP was demonstrated both in vitro and in vivo. Administration of oATP enhanced the efficacy of LFA-1 blockade in inducing long-term islet allograft survival in our model. Combinatorial strategies targeting purinergic receptors and co-stimulatory blockade may allow for the development of efficient tolerogenic clinical protocols.

Supported by: Diabetes Research Institute Foundation, Converge Biotech, Inc.

83-LB

Immunomodulation with Pancreatic Islets Engineered to Display on Their Surface a Novel Form of FasL Protein Induces Auto and Allotolerance in Spontaneously Diabetic NOD Mice

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We have recently demonstrated that immunomodulation with cells or pancreatic islets engineered with a chimeric streptavidin (SA) and FasL protein (SA-FasL) induces allotolerance in rodents. In this study, we tested if this tolerance protocol is effective in spontaneously diabetic NOD mice.

C57BL/6 mouse islets were modified with biotin followed by engineering with SA-FasL protein. Newly diabetic (BGL > 250 mg/dl) female NOD mice were used as recipients of SA-FasL or control streptavidin (SA) protein engineered allogeneic islets transplanted under the kidney capsule. Graft recipients were treated transiently with rapamycin administered at 3 mg/kg daily starting on the day of transplantation for 20 doses. Unmodified pancreatic islets without

rapamycin treatment were used to access the normal rejection rate. Select groups of long-term (>100 days) recipients underwent surgery to remove islet-bearing kidney to test the regeneration of endogenous beta cells.

All, but one, SA-FasL-engineered allogeneic grafts (n = 7) enjoyed prolonged survival in new onset diabetic NOD recipients over a 150-day observation period. Control SA-engineered (n=6) and unmanipulated islets (n=3) were rejected acutely with MST = 21.8±3.5 and 13±1 days, respectively. Most importantly, the removal of kidney harboring the transplanted islets did not result in hyperglycemia, suggesting the recovery/regeneration of endogenous beta cells that was confirmed by immunohistochemistry.

Immunomodulation with SA-FasL-engineered islet grafts under transient cover of rapamycin results in robust tolerance to allo and autoantigens that allows for the recovery of endogenous beta cells and cure of diabetes with significant clinical implications.

INSULIN ACTION—INSULIN RESISTANCE IN VITRO

84-LB

Comparative Proteome Analysis of Secreted Proteins from Insulin-Resistant C2C12 Cells

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Skeletal muscle accounts for majority of insulin-stimulated glucose disposal and has a high capacity to metabolize fatty acids. Impaired glucose metabolism and lipid metabolism in skeletal muscle are hallmark features of insulin resistance associated with obesity, type 2 diabetes, and metabolic syndrome. Elevated plasma free fatty acids (FFA) are thought to be responsible for development of insulin resistance however the mechanisms by which FFA cause insulin resistance are not clear.

Skeletal muscle has been suggested to be source of secreted proteins which can influence metabolism and other biological processes in a systemic manner. Here we report a secretome of an insulin- resistant muscle cell line. To analyze proteins secreted from insulin-resistant murine C2C12 skeletal muscle cells, we applied a stable isotope labeling by amino acids in cell culture (SILAC) based quantitative proteomics platform. Exposure of isotopicaly labeled C2C12 cells to 0.5 mM palmitate results in development of insulin resistance (confirmed by impairment in insulin signaling). Thereafter, we compared the secretome of control vs insulin-resistant cells. Protocols were optimized to efficiently derive secreted proteins from cells in culture. In supernatants, we identified and quantified 2205 proteins including 36 cytokine signaling proteins, 60 growth factors and 46 metallopeptidase. We found that 182 of these proteins were significantly different in palmitate treated cells compare to non-treated cells. In addition to previously reported secreted protein, we identified many novel proteins that have not been shown to be released from skeletal muscle.

These proteins may act as signaling mediators to other cells and tissues and supporting a role of skeletal muscle as an important secretory organ.Moreover some of these proteins might play a role in development of palmitate-induced insulin resistance.

Supported by: Federation of European Biochemical Society

INSULIN ACTION—METABOLISM

85-LB

Cellular Mechanism by which Estradiol Protects Mice From Diet-Induced Hepatic and Muscle Insulin Resistance

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Estrogen replacement therapy reduces the incidence of type 2 diabetes in postmenopausal women, however the mechanism by which this occurs remains unknown. In order to address this question we examined the metabolic effects of estradiol (E2) replacement therapy in an experimental mouse model of menopause. At 8 weeks of age, female mice were ovariectomized (OVX) or sham (SHAM) operated. OVX mice were treated with vehicle or E2. All mice were fed with a high-fat diet (HFD - fat 60%) for 4 weeks. After 4 weeks of HFD feeding and E2 or vehicle treatment, metabolic cage and euglycemic-hyperinsulinemic clamp experiments were conducted. Whole-body energy expenditure and insulin sensitivity were reduced by 30% and 36%, respectively, in OVX compared with SHAM mice (both $P<0.05$ compared to SHAM). E2 treatment reversed both of these effects, increasing energy expenditure by 45%, ($P<0.05$) and improving whole-body insulin sensitivity by 130%, ($P<0.001$). Improved whole-body insulin sensitivity in E2 treated mice was due to both increased hepatic and muscle insulin sensitivity, while SHAM mice displayed only increased muscle insulin sensitivity compared with OVX mice. Insulin resistance in OVX mice was associated with a 150% ($P<0.05$) increase in diacylglycerol (DAG) content in muscle compared to SHAM, and E2 treatment reduced intracellular DAG accumulation in muscle and liver by 60% compared to OVX ($P<0.001$). Reduced tissue DAG content in the E2 treated mice was associated with decreased PKCε and PKCθ activity and increased insulin-stimulated AKT2 phosphorylation in liver and muscle, respectively, compared to OVX mice. Taken together these data support the hypothesis that E2 protects OVX mice from lipid-induced liver and muscle insulin resistance by increasing energy expenditure and reducing ectopic lipid (DAG) content in these organs, leading to reduced PKCε and PKCθ activation in liver and muscle, respectively.

86-LB

Local Knockdown of Ventromedial Hypothalamus PTP1B Improves Peripheral Insulin Sensitivity and Glucose Tolerance

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It is generally believed that insulin regulates glucose homeostasis via its effects on liver and peripheral tissues. Little attention has been given to the possibility that insulin's glucoregulatory effects are also mediated at the level of the ventromedial hypothalamus (VMH), a key brain glucose-sensing region. Our previous studies have shown that the suppression of VMH insulin receptor expression leads to hepatic insulin resistance and glucose intolerance. Whether enhancement of VMH insulin signaling could improve glucose tolerance and insulin sensitivity remains to be determined. To address this question we determined the effect of targeted knockdown of VMH PTP1b, a negative regulator of insulin signaling, on glucose homeostasis in non-diabetic rats. This was achieved via microinjection of a lentivirus for specific knockdown of the PTP1B or a control lentiviral vector directly into the VMH. Following microinjection animals were observed for 5 wks. An oral glucose tolerance (OGT) test was conducted at 3 wk post-knockdown and insulin sensitivity was assessed using hyperinsulinemic euglycemic clamps (4mU/kg/min) in conjunction with 3H-glucose at 5 wk. The fasting blood glucose was unaltered in both groups. However, VMH PTP1b knockdown rats exhibited a significant improvement in glucose tolerance with glucose levels at 30 min (213±20 control vs 176±4 mg/dL PTP1B Kd) and 1 hr (220±10 control vs 181±13 mg/dL PTP1B Kd). Euglycemic clamp studies demonstrated that VMH PTP1b knockdown rats were more insulin sensitive. The capacity of the insulin infusion to stimulate peripheral glucose utilization was significantly increased (30 ± 3 controls vs 39 ± 3 mg/kg/min PTP1B kd). We conclude that the knockdown of VMH PTP1b expression improves glucose tolerance and peripheral insulin sensitivity in non-diabetic rats. These data suggest that improving hypothalamic insulin signaling may have therapeutic value in alleviating peripheral insulin resistance in type 2 diabetes.

INSULIN ACTION—SIGNAL TRANSDUCTION

87-LB

Identification of PPP1R12A Interaction Partners in L6 Myotubes by Co-IP and HPLC-MS/MS

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The type 1 protein phosphatase (PP1) is a heterotrimeric holoenzyme, comprising a catalytic subunit (PP1c), a targeting/regulatory subunit (PPP1R12A) and a 20-kDa subunit (M20) of unknown function. PPP1R12A binds PP1c at the N terminus, resulting in increased catalytic activity and higher affinity for phosphorylated substrates. The activity of PP1 is diminished or activated through phosphorylatin of PPP1R12A by various serine/threonine kinase. PP1 is widely expressed and abundant in animal cells, responsible for a large number of Ser/Thr dephosphorylation reactions. In addition to regulation of muscle relaxation by dephosphorylation of myosin regulatory light chain, PP1 has been found to participate in insulin signaling based on recent study that PPP1R12A and PP1c are endogenous interaction partners of insulin receptor substrate-1 (IRS-1) in L6 myotubes upon insulin stimulation. To understand how PPP1R12A involves in insulin signaling pathway we identified its interaction partners in L6 myotubes with or without insulin stimulation. The pull-down assays were done with antibodies against PPP1R12A or Serine/threonine-protein kinase akt-2 (negative control). The isolated partners were in-gel digested and analyzed by HPLC-ESI-MS/MS. We found five insulin-stimulated PPP1R12A

partners, including low molecular weight phosphotyrosine protein phosphatase (LMW-PTPase), a novel partner. In addition, eukaryotic translation initiation factor 3 subunit B (eIF3b), Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform (PP2A-alpha), and 26S protease regulatory subunit 8 were among the eleven novel insulin-independent PPP1R12A partners. We also found some previously reported PPP1R12A interaction partners, such as PP1c beta and ubiquitin protein ligase E3 component n-recognin 4 (UBR4). Our study provides potential novel targets for understanding the role of PPP1R12A in insulin signaling pathway.

Supported by: NIH (R01DK081750)

88-LB

Label-Free Quantitative Phosphoproteomics Analysis of Insulin-Stimulated Phosphorylation in L6 Myotubes

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Insulin resistance (IR) constitutes a common and broadly prevalent metabolic disorder, which is a key factor for the development of the type 2 diabetes mellitus (T2D). Insulin resistance in skeletal muscle is vital since it is normally responsible for more than 75% of insulin-mediated glucose disposal. Here, we attempted to identify and characterize the phosphor-peptides of the skeletal muscle in response to insulin stimulation. To conduct this study, phosphor-peptide enrichment with TiO beads combined with HPLC-ESI-MS/MS were adapted to analyze phosphor-peptides from L6 myotube cells with or without insulin stimulation. A total of 848 unique phosphor-peptides from 2312 phosphor-spectrum in 482 proteins were identified. Based on label-free quantification, 144 proteins with at least two peptides showed 1.5-fold change. Among them, ten kinases (MAPK1, MAPK3, WNK1, PAK1, SPEG, KCC2G, AKT2, PDPK1, KKN2 and MAP2K2) of all sixteen kinases showed increasing pattern and the other six (AAK1, RAF1, PGK1, PRP4B, KAP2 and ERBB2) showed decreasing pattern upon insulin stimulation. Functional analyses suggested that most of the proteins differentially expressed were in the pathways of EIF2and EIF4 signaling, insulin receptor signaling and FAK signaling. Our results provide the novel insulin-induced molecular in muscle cells, and will contribute to the diagnosis and treatment of the type 2 diabetes mellitus and other insulin resistance related diseases.

89-LB

Site-Specific Identification of Protein S-nitrosylation in Mouse Muscle and Implications in Metabolism and Insulin Action

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S-nitrosylation, an important reversible thiol oxidation on protein cysteine residues, represents a prototypical redox-based signal transduction mechanism that modulates a broad spectrum of cellular pathways. The potential significance of S-nitrosylation in insulin action has been well recognized based on the observation of this modification on several key insulin signaling proteins including insulin receptor, insulin receptor substrate proteins, and Akt. However, site-specific identification of s-nitrosylation remains an analytical challenge due to the labile and low-abundance nature of S-nitrosylation. Here we present an optimized mass spectrometry (MS)-based approach for enriching S-nitrosylated peptides and its application for quantitative profiling of site-specific S-nitrosylation in mouse muscle, the major tissue for glucose metabolism. Our approach involves free thiol blocking by alkylation, resin-specific enrichment of cysteine-containing peptides after converting S-nitrosylation to free thiols, and quantification by isobaric labeling. This approach was applied to identify S-nitrosylation sites in mouse muscle treated with nitric oxide (NO) donor S-nitrosoglutathione at two concentrations (10 and 100 μM). The results revealed ~500 cysteine sites modified by S-nitrosylation and ~265 sites (from 140) are particularly sensitive to S-nitrosylation at low concentration of stimulation. Many of proteins sensitive to S-nitrosylation are preferentially localized in mitochondria, contractile fiber, and actin cytoskeleton. Moreover, many S-nitrosylated proteins are key enzymes involved in a number of metabolic pathways including the TCA cycle, glycolysis/gluconeogenesis, glutathione metabolism, fatty acid metabolism as well as insulin signaling pathway, suggesting the potential significance of protein S-nitrosylation as a regulatory mechanism for metabolism and insulin action.

Supported by: NIH (R01 DK074795, DP2OD006668)

90-LB

WITHDRAWN

91-LB

A Novel Lysine Methyl-Transferase Negatively Regulates Hepatic AKT Signaling during Fasting

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In liver, signaling from the insulin receptor through AKT represents a major mechanism regulating responses to dietary fluctuations in carbohydrate load. Aberrant hepatic AKT signaling contributes to major diseases in humans including diabetes. We have uncovered a previously unrecognized mechanism of negative regulation of hepatic AKT signaling through SETDB2, a novel enzyme with a putative lysine methyl-transferase function. Preliminary studies show mouse liver expression of SETDB2 is induced by fasting and repressed by refeeding, a characteristic shared with gluconeogenic genes. Primary hepatocytes overexpressing SETDB2 show increased glucose production with upregulation of PEPCK and glucose-6 phosphatase. Overexpressed SETDB2 in primary hepatocytes also inhibits insulin stimulated AKT signaling and keeps FOXO1, an important transcription factor of gluconeogenesis, in an active unphosphorylated state. Furthermore, upregulation of SETDB2 during fasting is blunted in liver-specific FOXO1 knockout mice putting SETDB2 downstream of FOXO1. In vitro, SETDB2 and AKT physically interact suggesting a direct regulation that may occur in liver. Thus, we hypothesize that SETDB2 regulates hepatic insulin signaling through inhibiting AKT to optimally induce fasting dependent gene expression and hepatic glucose production. Future studies will evaluate the effects of manipulating SETDB2 levels on hepatic metabolism by increasing or decreasing SETDB2 levels in livers of mice through recombinant adenoviruses expressing SETDB2 protein or a shRNA designed to reduce SETDB2. The mechanism by which the liver regulates insulin signaling through AKT is of primary importance in diabetes. The identification of a new mechanism for regulating hepatic AKT signaling exposes a previously unknown area of diabetes research to explore with a novel biomarker, which is also a new potential therapeutic target.

INTEGRATED PHYSIOLOGY—ADIPOCYTE BIOLOGY

92-LB

Effects of Growth Hormone Treatment Beyond the Body Fat Changes in GH Deficient Adults

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The mechanisms underlying the effects of growth hormone (GH) on fat metabolism in GH-deficient (GHD) patients are not fully understood. This study was to evaluate the effects of GH replacement before the body fat changes and their relevant parameters in GHD patients.

In total, 14 (5 males and 9 females) GHD adults (mean 34.4 ± 8.2 yr, BMI 23.1 ± 1.0 kg/m^2) received GH treatment for 12 weeks. Body composition by dual-energy X-ray absorptiometry and fasting serum analyses were assessed before and after treatment.

GH replacement did not affect body weight (60.9±3.0, *vs.*60.3± 3.3 kg, *p=0.30*), body fat mass (19.7±1.5, *vs.* 18.4±1.8 kg, *p=0.10*) or HOMA IR (1.65±0.3, *vs.*1.94±0.4, *p=0.34*). However, serum adiponectin and leptin levels were distinctly reduced after GH administration (6.86± 1.2, *vs.* 5.51 ± 0.8 mg/L, *p<0.01*; 8.7 ±1.1, *vs.*6.5 ± 1.0 ng/mL, *p<0.01*, respectively). Interestingly, correlations between body fat mass and adipokines got stronger (adiponectin, before r= -0.502, *p=0.07 vs.* after r= -0.634, *p=0.02*; leptin, before r=0.378, *p=0.18 vs.* after r=0.711, *p=0.00*) after GH administration. After 12 weeks-GH replacement, the reductions in serum adiponectin and leptin levels without significant body fat changes and the stronger correlations between body fat mass and these adipokines are reflective of the metabolic effects of GH probably due to the amelioration of compensatory mechanism of adiponectin like GHRH to overcome GH deficiency and leptin resistance in GHD patients.

93-LB

WITHDRAWN

94-LB

Defining the Switch Controlling Commitment of Progenitors to the Brown Adipocyte Lineage Over Vascular Cell Lineage

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Enhancing the development and/or activity of brown adipose tissue (BAT) is of potential therapeutic benefit for obese individuals. Progenitors for both adipocytes and vascular cells have vascular origins; however the mechanisms regulating commitment to these distinct lineages are not understood. BMP7 and TGFβ, both members of the TGFβ family, direct progenitors to distinct lineages_brown adipocytes and smooth muscle (SM), respectively. Our study aims to identify key early events regulating the commitment of mesenchymal stem cells (MSCs) to the BAT lineage, and to characterize the contrasting effects of BMP7/TGFβ on commitment. To address these questions, we employed multipotent C3H10T1/2 MSCs that are able to differentiate to distinct cell lineages. We find that while BMP7 promotes the differentiation of MSCs to brown adipocytes, TGFβ strongly induces SM cell-like differentiation, even in the presence of adipogenic cocktail. Microarray analyses identified a set of genes that are selectively regulated by BMP7 during commitment, which encode the transcription factor Zic1, Gremlin1, a secreted BMP antagonist, and Rho kinases ROCK1/2. Overexpression of either Zic1 or Gremlin1 attenuates BAT lineage commitment. TGFβ, however, induces expression of both proteins during SM differentiation. It is established that TGFβ activates Rho Kinase (ROCK1/2), and induces SM-like morphology in MSCs. We observe that BMP7 induces a distinct cell morphology and consistent with this, we find that inhibition of ROCK1/2 activity promotes BAT over SM lineage commitment. Our study has defined a set of genes involved in a SM/BAT switch that is controlled by members of the TGFβ family. Insight into mechanisms regulating MSC commitment to the BAT lineage will likely lead to the identification of targets for potential anti-obesity drug development.

Supported by: USPHS (DK51586, DK58825, DK086629)

95-LB

Adipocyte-Released Insulin Like Growth Factor-1 is Regulated by Glucose and Fatty Acids and Controls Breast Cancer Cell Growth

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It has recently become clear that obesity and type 2 diabetes are associated with an increased frequency of many cancers. The adipocyte and their precursor cells are largely represented in microenvironment of several tumors and may represent a candidate to integrate energy and nutrient metabolism with cancer cell growth by providing a number of signals and resources to tumor cells.

We have investigated whether metabolic alterations at the level of adipose-derived differentiating cells may affect specific phenotypes of breast cancer cells.

We have obtained evidence that co-cultures with either differentiated 3T3-L1 or human mammary adipocytes increased viability of MCF-7 cells, at a larger extent, compared to their undifferentiated precursors. Adipocytes cultured in 25 mmol/l glucose were 2-fold more effective in promoting cell growth, compared to those grown in 5.5 mmol/l glucose, and activated mitogenic pathways in MCF-7 cells. Growth promoting action was also enhanced when adipocytes were incubated in the presence of 10 umol/l palmitate or 0.5 umol/l oleate. Interestingly, 3T3-L1 and human adipocytes released higher amounts of KC/IL-8, RANTES and IGF-1, compared to their precursor cells. Their levels were reduced upon incubation with low glucose and enhanced by fatty acids. Moreover, both undifferentiated cells and differentiated adipocytes from obese individuals displayed IGF-1 release and MCF-7 cell growth induction about 2-fold higher than lean subjects. Finally, inhibition of IGF-1 pathway almost completely prevented growth promoting effect of adipocytes on breast cancer cells.

In conclusion, we have demonstrated that IGF-1 release by adipocytes is regulated by glucose and fatty acids and may contribute to control of cancer cell growth in obese individuals.

Supported by: EFSD

SIGNAL TRANSDUCTION (NOT INSULIN ACTION)

96-LB

Genome-Wide Analysis of Glucocorticoid Receptor Binding Sites in Myotubes Identifies Gene Networks Modulating Insulin Signaling

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Glucocorticoids elicit a variety of biological responses in skeletal muscle, including inhibiting protein synthesis and insulin-stimulated glucose uptake and promoting proteolysis. Thus, excess or chronic glucocorticoid exposure leads to muscle atrophy and insulin resistance. Glucocorticoids propagate their signal mainly through glucocorticoid receptors (GR), which, upon binding to ligands, translocate to the nucleus and bind to genomic glucocorticoid response elements (GRE) to regulate the transcription of nearby genes. Using a combination of chromatin immunoprecipitation sequencing (ChIPseq) and microarray, we identified 173 genes in mouse C2C12 myotubes. The mouse genome contains GR binding regions (GBR) in or near these genes and the genes' expression was regulated by glucocorticoids. Eight of these genes encode proteins known to regulate distinct signaling events in insulin/insulin-like growth factor 1 (IGF-1) pathways. We found that overexpression of p85α, one of these eight genes, caused a decrease in C2C12 myotube diameters, mimicking the effect of glucocorticoids. Moreover, reducing p85α expression by RNA interference in C2C12 myotubes significantly compromised the ability of glucocorticoids to inhibit Akt and p70 S6 kinase activity and reduced glucocorticoid induction of IRS-1 phosphorylation at serine 307. This phosphorylation is associated with insulin resistance. Furthermore, decreasing p85α expression abolished glucocorticoid inhibition of protein synthesis and compromised glucocorticoid-induced reduction of cell diameters in C2C12 myotubes. Finally, a GRE was identified in the p85α GBR. In summary, our studies identified GR-regulated transcriptional networks in myotubes and showed that p85α plays a critical role in glucocorticoid-induced insulin resistance and muscle atrophy in C2C12 myotubes.

INTEGRATED PHYSIOLOGY—INSULIN SECRETION IN VIVO

97-LB

Investigating the Relationship of Activator-Enzyme Binding Kinetics to *In Vivo* Hypoglycemia Risk for Glucokinase Activators

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Glucokinase activators represent a promising strategy for the treatment of type 2 diabetes; however, hypoglycemia has emerged as a key risk for this class leading to interest in the design of second generation glucokinase activators with inherently reduced hypoglycemia risk. Glucokinase functions as a physiological glucose sensor, and herein we evaluate whether activator-enzyme binding kinetics (k_{on}, k_{off}) influence *in vivo* efficacy and hypoglycemia safety. Activator binding kinetics were postulated to be relevant to pharmacodynamics since the affinity of activator binding to the glucokinase enzyme is cooperative with glucose such that the rate at which an activator dissociates off of the enzyme may influence its binding sensitivity to changes in physiological glucose concentrations. To experimentally inform this question, a series of structurally diverse glucokinase activators spanning a range of *in vitro* potencies (EC_{50} = 10 - 391 nM) were selected and the binding kinetics of these activators were characterized against recombinant glucokinase using surface plasmon resonance. Observed on rates (k_{on}) ranged from $6.21x10^4$ to $6.45x10^5$ ($1/M*s$) while off rates (k_{off}) ranged from $1.0x10^{-3}$ to $8.15x10^{-2}$ ($1/s$) providing a range of dissociative half-lives from 686 to 8.5 sec for these activators. These activators were also evaluated for *in vivo* efficacy and hypoglycemia safety in Wistar rats during an oral glucose tolerance test (OGTT). Correlation of the pharmacodynamic and binding kinetic data suggested that activators with faster off rates, and hence shorter residence time on glucokinase, had similar efficacy but reduced hypoglycemia risk relative to activators with slower off rates. This observation indicates that activator-enzyme binding kinetics may be a relevant parameter for the optimization of glucokinase activators.

INTEGRATED PHYSIOLOGY—LIVER

98-LB

Overexpression of G0/G1 Switch Gene 2 Promotes Hepatic Steatosis Through Inhibition of Triglyceride Turnover and Fatty Acid Oxidation

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Hepatic steatosis is often associated with obesity-induced insulin resistance and can lead to steatohepatitis and cirrhosis. In this study, we have demonstrated that G0/G1 switch gene 2 (G0S2), a specific inhibitory protein for adipose triglyceride lipase (ATGL), contributes to lipid turnover in the liver and functions to promote hepatic accumulation of triglycerides (TGs). Immunoblotting experiments revealed a robust increase in G0S2 expression in livers isolated from ob/ob and high fat-fed mice relative to normal chow-fed animals. Adenoviral overexpression of G0S2 decreased TG hydrolase activity by 65% and increased TG content by over 4 folds in mouse liver. Moreover, G0S2 gain of function significantly increased hepatic TG secretion in vivo. The effect on TG secretion coincides with elevated fasting TG levels in plasma and increased expression of lipid droplet coat protein adipophilin in liver. Furthermore, circulating β-hydroxybutyrate levels were markedly decreased after a 24-h fast in G0S2-overexpressing mice. In parallel, G0S2 overexpression in primary mouse hepatocytes led to a marked decrease in the rates of fatty acid oxidation. As demonstrated by gene microarray and real time PCR analyses, the impact of G0S2 on fatty acid oxidation is consistent with decreased hepatic expression of peroxisome proliferator-activated receptor α (PPAR-α) in mice with G0S2 overexpression. In summary, hepatic overexpression of the lipolytic inhibitor G0S2 can impede fatty acid oxidation and promote hepatic steatosis. Our study suggests a direct functional role for G0S2 in hepatic lipid homeostasis and identifies this protein as a potential therapeutic target for ameliorating hepatic steatosis associated with insulin resistance and obesity.

Supported by: NIH

INTEGRATED PHYSIOLOGY—MACRONUTRIENT METABOLISM AND FOOD INTAKE

99-LB

Hyperglycemia Mediates a Shift from Cap-Dependent to Cap-Independent mRNA Translation Through a 4E-BP1 Dependent Mechanism

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Association of 4E-BP1 with the mRNA cap-binding protein eIF4E plays a major role in the regulation of gene expression by controlling the overall rate of mRNA translation as well as the selection of mRNAs for translation. Phosphorylation of 4E-BP1 releases it from eIF4E, allowing eIF4E to associate with eIF4G in a manner that promotes ribosome loading onto the mRNA 5′ cap end of mRNA. Previous studies from our laboratory have established that 4E-BP1 is also modified by addition of N-acetylglucosamine to Ser and/or Thr residues (O- GlcNAcylation) in the liver of diabetic mice concomitant with increased association with eIF4E. In the present study, we evaluate the hypothesis that hyperglycemia causes increased flux through the hexosamine biosynthetic pathway, leading to elevated O-GlcNAcylation of 4E-BP1 and increased association with eIF4E, which in turn causes a shift from cap-dependent to cap-independent translation. In both diabetic mice and cells in culture exposed to 25 mM compared to 5 mM glucose both the expression of 4E-BP1 and its association with eIF4E were elevated in a manner that was associated with a downregulation of cap-dependent and concomitant upregulation of cap-independent mRNA translation, as assessed using a bicistronic luciferase reporter assay. Phlorizin treatment of diabetic mice lowered blood glucose concentrations and returned the activity of cap-independent reporter in the liver of diabetic mice to a level that was not significantly different than those observed in non-diabetic mice. Notably, the glucose-induced shift from cap-dependent to cap-independent mRNA translation did not occur in cells lacking 4E-BP1, demonstrating the essential role of 4E-BP1 in this shift. The extensive nature of this translational control mechanism was revealed using pulsed stable isotope labeling by amino acids in cell culture (pSILAC) to identify proteins that undergo altered rates of synthesis upon exposure to hyperglycemic conditions.

Supported by: NIH (13499 (L.S.J.)), NIH Postdoctoral Fellowship (M.D.D.)

100-LB

Markers of Lipogenesis and Sterol Absorption are Predictors of Diabetes and Insulin Sensitivity in IRAS

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Recent reports show that serum metabolite levels predict incident diabetes, but have been silent on the independence of metabolite types in establishing risk. Is metabolism altered globally, yielding few independent predictors, or do some pathways have primacy? To address this, we quantified 93 fatty acids, sterols, bile acids, amino acids and acylcarnitines in baseline serums from 690 subjects in the Insulin Resistance Atherosclerosis Study (IRAS). IRAS is a five-year prospective multi-ethnic cohort with frequently-sampled IVGTT measures taken at baseline. We identified associations between each marker and insulin sensitivity (IS) or the 5-year risk of incident diabetes. Although branched chain amino acids (BCAA), sterol synthesis markers and mid-chain acylcarnitines were correlated with IS and predictive of diabetes ($p < 0.05$), these markers lost significance after controlling for any of fasting glucose, insulin or BMI. In contrast, fatty acids linked to increased lipogenesis or its inhibition (16:0, 16:1n7 and 18:2n6, ORs = 1.60, 1.60 and 0.66, respectively), absorbed phytosterols (β-sitosterol and campesterol, ORs = 0.63 and 0.64, respectively) and the amino acid glycine (ORs = 0.71), all remained significant after controlling for clinical variables. No metabolite remained significant for predicting diabetes after controlling for palmitate (16:0) concentrations. However, β-sitosterol, campesterol, 16:1n7, 18:2n6 and glycine remained significant predictors of diabetes after controlling for each of the other metabolites except 16:0. The results indicate that many metabolic markers are significant predictors of diabetes, but markers such as BCAA, sterol synthesis intermediates and acylcarnitines do not retain independent predictive power after controlling for clinical or metabolic measures. The markers of lipogenesis, sterol absorption and the amino acid glycine appear strongly and independently associated with diabetes risk.

101-LB

Effect of High Protein vs. High Carbohydrate Diets on Incretins in Obese, Nondiabetic, Premenopausal Women

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Our studies on the effect of dietary macronutrients on metabolic parameters have shown that a high protein (HP) diet [30% protein, 30% fat, and 40% CHO] compared to a high carbohydrate (CHO) diet [(HC) (55% CHO, 30% fat, 15% protein] results in greater reduction in oxidative stress (DCF), lipid peroxidation (MDA), cardiovascular risk factors and less insulin resistance (HOMA-IR) in the HP group than the HC group at 6 months on the diets compared to baseline.

Since GIP and GLP-1 are incretins that have important roles in both insulin secretion and maintenance of pancreatic β-cells, we now report additional studies on the incretins (GLP-1 and GIP) in response to 75 gm oral glucose tolerance test (OGTT) and Meal Tolerance Test (MTT) using a HP or HC meal at 0, 30, 60, 90 and 120 min of the HP and HC diet groups. Area under the curve (AUC) was calculated for GIP and GLP-1 with the following results.

	HP (n=12)			HC (n=12)			
Parameters	Baseline	6 months	p*	Baseline	6 months	p*	p**
Incretin AUC							
GLP-1 (pmol/l)/min) MTT	7860 ± 31	8970 ± 37	0.001	6570 ± 29	7110 ± 35	0.05	0.001
GLP-1 (pmol/l/min) OGTT	4920 ± 28	5880 ± 34	0.01	4890 ± 30	5220 ± 38	0.12	0.06
GIP (pg/ml/min) MTT	22825±517	27031±571	0.014	20025±640	22005±616	0.06	0.005
GIP (pg/ml/min) OGTT	16,263±371	17,854±316	0.05	15893±352	16079±328	0.13	0.07

The * indicates Wilcoxon Signed Rank Test and ** indicates Wilcoxon Rank Sum Test for 6 months HP vs HC

We conclude that although equal weight loss and changes in BMI are noted in both groups, they are not significantly different. The incretins, however, exhibited significantly greater increase with the HP diet than the HC diet group with the MTT but not with OGTT.

INTEGRATED PHYSIOLOGY—MUSCLE

102-LB

Upregulation of PGC-1α and Fat Transport/Oxidation Genes in Skeletal Muscle is Linked to Improved Insulin Sensitivity Following a Lifestyle Intervention in Obese Humans

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Defects in molecular pathways regulating skeletal muscle glucose and fat metabolism contribute to insulin resistance and type 2 diabetes. We hypothesized that exercise training in combination with various glycemic index (GI) diets would differentially alter gene expression of nuclear regulators of fat transport and oxidation in insulin resistant skeletal muscle. Older obese subjects (65±1 yrs; 34±1 kg/m2) were randomized to receive either a high-GI (HiGIX; 80±0.6 units, N=10) or low-GI diet (LoGIX; 40±0.3 units, N=8) and 12-wks of supervised exercise (1h/d at ≈ 85% HRmax). Insulin sensitivity was determined via 40 mU.m-2.m-1 hyperinsulinemic euglycemic clamp. Vastus lateralis muscle was obtained pre- and post-intervention; gene expression was determined by quantitative-real time PCR. Insulin sensitivity increased following both interventions (P<0.003), as did expression of nuclear regulators, peroxisome proliferator-activated receptor-gamma (PPARγ) [HiGIX and LoGIX: 1.7±0.3 and 1.8±0.2, P=0.004 (fold, post-pre)], PPARγ-coactivator-1-alpha (PGC-1α) [1.8±0.4 and 1.5±0.2, P=0.02], fatty acid translocase, CD36 [1.5±0.3 and 1.8±0.3, P=0.009], fatty acid binding protein 3 (FABP3) [1.6±0.3 and 1.2±0.3, P=0.04], and carnitine palmitoyltransferase-1B (CPT1B) (trend; P=0.06). The improvement in clamp-derived insulin sensitivity correlated with fold induction of PGC-1α (r=0.52, P=0.03), and decreases in HOMA-IR negatively correlated with increases in FABP3 (r=-0.61, P=0.01) and CPT1B (r=-0.55, P=0.02). Further, increases in FABP3 were positively correlated with increases in basal whole body fat oxidation (r=0.57, P=0.02). We conclude, that independent of the glycemic content of the diet, lifestyle-induced improvements in insulin sensitivity are associated with molecular changes controlled by PGC-1α that upregulate downstream fat transport and oxidation genes in skeletal muscle.

Supported by: NIH (R01 AG12834)

INTEGRATED PHYSIOLOGY—OTHER HORMONES

103-LB

The Novel GLP-1-Gastrin Dual Agonist ZP3022 Improves Glycemic Control in ZDF Rats

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Combination treatment with exendin-4 and gastrin has been shown to improve diabetes and preserve β-cell mass by stimulating β-cell growth and differentiation in diabetic mice. Here we investigated the anti-diabetic effects of a novel GLP-1-gastrin dual agonist ZP3022 in Zucker Diabetic Fatty (ZDF) rats.

ZDF rats aged 11 weeks were dosed s.c., bid for 8 weeks with either vehicle, ZP3022 (10, 40 nmol/kg), liraglutide (40 nmol/kg), exendin-4 (30 nmol/kg), gastrin17 (80 nmol/kg), or exendin-4 + gastrin17 (30 + 80 nmol/kg). HbA1c was measured at treatment start and at termination, an oral glucose tolerance test (OGTT) was performed after 5 weeks of treatment, and non-fasting blood glucose (BG) was measured every other week.

The changes in HbA1c levels can be seen in Figure 1. ZP3022 clearly improved glycemic control as did all treatments, except gastrin17. Notably, ZP3022 caused a significantly greater reduction in HbA1c levels than liraglutide at equimolar dose.

Only ZP3022 high dose, exendin-4 and exendin-4 + gastrin17 significantly improved glucose tolerance after an OGTT ($P < 0.001$, $P < 0.05$, $P < 0.001$ vs. vehicle, respectively). Moreover, ZP3022 BG lowering effect was already present after 2 weeks of treatment and persistent throughout the study ($P < 0.001$ vs. vehicle). Other treatments, except gastrin17, also had a significant BG lowering effect. However, the effect of liraglutide tended to be transient.

In conclusion, treatment with ZP3022 markedly improved glycemic control in diabetic ZDF rats, which indicate GLP-1-gastrin dual agonism as a possible target for the treatment of diabetes.

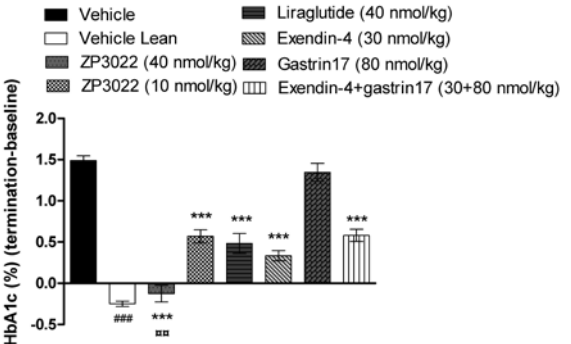

*Figure 1. HbA1c changes after 8 weeks of treatment. Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test, *** $p < 0.001$ vs. vehicle; or Mann Whitney test ### $p < 0.001$ vs. vehicle, ¤¤ $p < 0.01$ vs. liraglutide. n = 10-12. Data are mean ± SEM.*

104-LB

Underlying Mechanisms of Regulation of Adiponectin in an Obesity/Type 2 Diabetic Mouse Model

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Adiponectin is an adipose tissue-derived hormone with anti-diabetic, anti-atherogenic and anti-inflammatory functions. Defects in adiponectin are associated with diabetes/obesity. In this study, we investigated the alterations in adiponectin in an obesity/type 2 diabetic mouse model and its underlying regulation mechanisms.

Our results showed that both 10-week and 27-week OB mice had glucose and insulin intolerance with increased serum insulin level (ng/ml, 10-week: 58 ± 10 and 27-week: 47 ± 10, n = 10/group). 10-week OBs had increased blood glucose level, whereas 27-week OBs showed normal level. While 10-week OBs had increased serum adiponectin level (μg/ml, C57: 17.1 ± 0.8 vs OB: 24 ± 2, n = 10/group, $P = 0.003$), 27-week OBs maintained this level which was comparable to their age-matched C57 mice (μg/ml, C57: 21 ± 1 vs OB: 24 ± 1, n = 10/group, $P = 0.14$). Compared with 10-week C57s, 27-week C57s had slightly but significantly increased serum adiponectin level, so was their serum insulin level (ng/ml, 10-week: 1.1 ± 0.1 vs 27-week: 4.1 ± 1.6, n = 10/group) accompanying with insulin intolerance. Further studies on differentiated 3T3-L1 adipocytes showed that decreased insulin signaling by blocking the insulin receptor with an anti-receptor antibody stimulated adiponectin level in the medium, coexisting hyperinsulinemia counteracted this effect. The effect of insulin alone showed dose-dependent with higher insulin level having significant depression effect. However, high glucose showed stimulation effect. Interestingly, OB mice in both age groups had decreased mRNA expression level of adiponectin in their visceral lipid tissues compared with their age-matched C57 mice. This supports the posttranscriptional regulation of adiponectin.

In conclusion, this study points to the existence of posttranscriptional regulation of adiponectin. Both decreased insulin signaling and high glucose have stimulation effect on adiponectin, whereas hyperinsulinemia has counterpoised effect.

Supported by: Health Research Council of New Zealand

105-LB

γ-Glutamyl Carboxylase Regulates Whole Body Energy Metabolism Through Its Expression In Osteoblasts

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Osteocalcin is a hormone produced by osteoblasts that regulates energy metabolism by enhancing insulin secretion, insulin sensitivity and energy expenditure. While osteocalcin can exist in two forms, γ-carboxylated and undercarboxylated, only the undercarboxylated form of this molecule appears to function as a hormone. We have shown that it is the osteoclast, the bone-resorbing cell, that is responsible of decarboxylating and activating the osteocalcin trapped in the extracellular matrix (ECM) through the low pH generated during bone resorption. Thus, the results obtained so far imply that γ-carboxylation of osteocalcin should have an inhibitory effect on the bioavailability of this hormone and, indirectly, on whole body glucose homeostasis. To determine if it is the case in vivo we generated osteoblast-specific γ-glutamyl carboxylase (*Ggcx*)-deficient mice ($Ggcx_{osb}$-/-). In contrast to the *Ggcx* null mice, which died shortly after birth form haemorrhage, the $Ggcx_{osb}$-/- mice were viable and obtained at the expected Mendelian ratio. These mutant mice however, displayed a 5 fold increase in their serum levels

of uncarboxylated osteocalcin. This resulted in a significant improvement of glucose handling in $Ggcx_{osb}$-/- as measured by a glucose tolerance test (GTT). We also observed an increase in insulin sensitivity in these mutant mice when we subjected them to an insulin tolerance test (ITT). Lastly, $Ggcx_{osb}$-/- mice presented a 50% decrease in their fat mass. Importantly, when fed a high fat diet the $Ggcx_{osb}$-/- mice gained significantly less weight than control mice and were protected against glucose intolerance. Taken together, these results establish that γ-glutamyl carboxylase negatively regulates glucose homeostasis through it expression in osteoblasts. This work further underscores the importance of bone in glucose homeostasis and suggests that Vitamin K, an essential cofactor of γ-glutamyl carboxylase, may play a role in the control of energy metabolism by bone.

Supported by: Canadian Diabetes Association Fellowship (M.F.)

OBESITY—ANIMAL

106-LB

Effect of Diet-Induced Paternal Obesity on Glucose and Insulin Levels of Offspring

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Epidemiological studies have shown that parental high fat diet (HFD) and increased body mass index correlate with abnormal metabolic profile and increased risk of obesity and insulin resistance in offspring. Based on this we studied glucose and insulin levels during glucose tolerance test (GTT) and insulin sensitivity test (IST) in murine offspring that were born from obese male parents.

To develop obesity, C57BL/6 male mice were fed with HFD (45% kcal fat) for 12 weeks starting at the age of 4 weeks. After that they were mated with females on low fat diet (LFD, 10% kcal fat). Control group was formed of 8 mouse pairs with both parents on LFD. After weaning, all offspring were maintained on normal lab chow. At the age of 5-6 weeks, glucose levels were measured at different time points after intraperitoneal injection of glucose (GTT) or insulin (IST). Blood was also collected at the 30 minute time point after injection of glucose to measure insulin levels.

Insulin sensitivity is greater in male and female offspring from male parents on HFD than in control group ($p < 0.05$). A similar trend is seen in GTT for male and female offspring.

To summarize, offspring from obese male parents are more sensitive to insulin than control group. More samples need to be tested in GTT to determine if the results are significantly different.

107-LB

Chemerin Signaling via CMKLR1 Does Not Impair Glucose Homeostasis in Obese Mice

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Chemerin is a secreted protein highly expressed in liver and white adipose tissue (WAT), and acute administration of chemerin has been shown to exacerbate glucose intolerance in obese mice. Chemerin interacts with and/or activates multiple receptors: CCRL2, CMKLR1 and GPR1, but it is unclear which receptor(s) mediates its effect on glucose homeostasis. Since CMKLR1 is the most highly expressed chemerin receptor in WAT, we sought to characterize the consequences of CMKLR1 deletion on glucose and energy homeostasis in lean and diet-induced obese mice. Mice lacking CMKLR1 (*Cmklr1*$^{-/-}$) and their wild-type (WT) littermates were fed either a low-fat (LF) or high-fat (HF) diet for 26 weeks. As expected, mice fed a HF diet gained more weight than those fed a LF diet, but no genotype-dependent differences in body weight were found. Although *Cmklr1*$^{-/-}$ mice fed a HF diet had slightly higher fasting glucose levels than WT mice, deletion of CMKLR1 did not affect glucose tolerance or circulating insulin levels after glucose challenge in both LF- and HF-fed mice. We next evaluated whether exogenous chemerin administration could modulate lipid and glucose metabolism in mice and whether these effects are mediated in a CMKLR1-dependent manner. Administration of a stable analog of $chemerin_{148\text{-}156}$ (saChem-9) led to a rapid reduction in circulating free fatty acid in WT but not *Cmklr1*$^{-/-}$ mice. In contrast, injection of saChem-9 had no effect on fasting glucose levels, glucose tolerance and insulin sensitivity in diet-induced obese mice and, consistently, was without effect in HF-fed *Cmklr1*$^{-/-}$ mice. We finally examined the effect of CMKLR1 activation in ob/ob mice and found that treatment with saChem-9 did not affect their glucose tolerance or circulating insulin levels after glucose administration. Taken together, our data suggest that chemerin signaling via CMKLR1 does not play a major role in mediating glucose intolerance in obese mice suggesting that other receptor(s) are involved in chemerin's effect on glucose homeostasis.

108-LB

Effect of Vertical Sleeve Gastrectomy in Melanocortin Receptor 4-Deficient Rats

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Bariatric surgery is currently the most effective treatment for obesity. Vertical sleeve gastrectomy (VSG), a commonly applied bariatric procedure, involves surgically incising most of the volume of the stomach. In humans, partial loss of melanocortin receptor-4 (*MC4R*) activity is the most common monogenic correlate of obesity regardless of lifestyle. At present it is unclear whether genetic alteration of MC4R signaling modulates the beneficial effects of VSG. Following VSG, we analyzed body weight, food intake, glucose sensitivity, and macronutrient preference of wild-type and MC4R-deficient (*Mc4r*$^{+/-}$ and *Mc4r*$^{-/-}$) rats as compared to sham-operated controls. VSG reduced body weight and fat and improved glucose metabolism, and also shifted preference towards carbohydrates and away from fat. All of this occurred independently of MC4R function. In addition, *MC4R* was resequenced in forty-six human subjects who underwent VSG. We observed common genetic variations in the coding sequence of *MC4R* in five subjects. However, none of those variations affected the outcome of VSG. We conclude that the beneficial effect of VSG on body weight and glucose metabolism in rats is independent of MC4R function. Taken together, our rodent and human data suggest that humans with partial or full loss of MC4R may be good candidates for VSG.

109-LB

Large Scale High-Fat Diet Induced Metabolic Syndrome Model Development in Cynomolgus Monkeys and Characterization of Diabetic Progression by Intravenous Glucose Tolerance Test

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Monkeys serves as best animal model for human metabolic disease as monkeys and human share similarities in metabolism and metabolic disease progression path. Therefore, development of a large scale high fat diet (HFD) induced metabolic disease monkey model will lead to a better understanding of mechanism of metabolic disease and facilitate new drug discovery.

In this study, 161 aged cynomolgus monkeys (age: 12.3±1.3 years; body weight: 9.6±1.7 kg) were fed with a HFD (18% protein, 30% fat and 15% carbohydrate) for 10 months. The average body weight was increased 30g/day in the first 45 days. The average fasting glucose (FG) was increased (64 to 73mg/dl, P<0.0001) after 35 days. After 80 days on HFD, these monkeys were evaluated by IVGTT. Normal FG (68±7mg/dl) and fasting insulin (FI) (26±13uU/ml) were determined by Normal Distribution analysis, and 51 monkeys (32%) were in this category. A multi-factorial analysis method was developed to classify them into different diabetic stages. Eighty-seven monkeys (54%) were characterized into 3 stages: diabetic/prediabetic (16%); impaired glucose tolerance (IGT) (22%); and insulin resistance (IR) (17%). After 6 months on HFD, these monkeys were re-evaluated by IVGTT to follow up diabetic progression with results: 25% normal monkeys advanced into IR/IGT with increase of FG/FI (16%/269%); 40% IR became IGT/diabetic with increase of FG/FI (18%/52%), and 30% IGT advanced into diabetic stage with increased FG/FI (17%/224%).

Lipid profiling was conducted after 4 months on HFD with results: high cholesterol (66%), high triglyceride (32%), high LDL (32%), and low HDL (1.2%). Blood pressure was measured after 10 months on HFD: pre-hypertension (16%) and hypertension (25%). In summary, we successfully developed a robust HDF-induced metabolic disease monkey model that will contribute to novel diabetes drug discovery.

110-LB

Neonatal Exendin-4 Promotes Long-Term Browning of White Adipose

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Increased risk for obesity has origins during fetal and neonatal development. Exendin-4 (Ex-4) is a treatment for adult human type 2 diabetes that can be associated with weight loss. We find that neonatal Ex-4 administration in mice

results in long-term effects on body weight, fat mass and the "browning" of specific white adipose tissue (WAT) depots. Reduced body weight is apparent in regular chow-fed males by postnatal day (p)28, whereas the most dramatic effect is observed in high fat (HF; 45% kcal fat)-fed females, resulting in 19% reduction in body weight at 6 months that persists until at least 10 months. Dissection of specific fat depots at p28 reveals 21% and 32% reductions in male inguinal and perigonadal fat, respectively, and a 48% reduction in female perigonadal fat after neonatal Ex-4 ($p \leq 0.05$). By NMR, total body fat of adult female mice treated with neonatal Ex-4 is 20% lower ($p \leq 0.05$). Notably, perigonadal WAT of Ex-4 treated females expresses a population of multilocular UCP1 expressing cells, first observed at postnatal day 28. These brown-like cells persist into adulthood in both LF- and HF-fed females treated with neonatal Ex-4. p28 adipose explants from neonatal Ex-4 females exhibit a 47% ($p \leq 0.05$) greater rate of oxygen consumption after *ex vivo* isoproterenol (IPT) stimulation than IPT-stimulated explants from vehicle treated females. At 10 months, neonatal Ex-4 HF-fed females expend 26% ($p \leq 0.05$) more energy. There was no change in total food intake or fluid intake. Enrichment of GLP1R transcript in the stromal vascular fraction (SVF) of perigonadal WAT suggests that Ex-4 may act through GLP1R to promote the formation of brown-like adipocytes. Indeed, Ex-4 treatment of female perigonadal SVF results in a 52% increase in IPT-stimulated UCP1 and PGC1alpha expression ($p \leq 0.05$). Thus, neonatal Ex-4 has long-term metabolic consequences on body weight regulation that results at least in part from a resetting of pathways that regulate energy homeostasis and the browning of specific white adipose depots.

Supported by: R01 801830

111-LB

Protection Against Diet Induced Obesity in Insulin-Like Growth Factor Binding Protein-1 Knockout Mice

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Insulin-like growth factor I (IGF-I) is a single chain peptide growth factor/hormone that shares similar metabolic actions with insulin, including promoting cellular glucose uptake, glycogen synthesis, lipogenesis, amino acid uptake and free fatty acid uptake into adipocytes. IGF-I is also a potent mitogen stimulating cell growth and differentiation in multiple cell types. The biological actions of IGF-I are regulated primarily on the level of bioavailability by a family of 6 high-affinity binding proteins (IGFBPs). IGFBP-1 forms a binary complex with IGF-I and is the primary acute regulator of free IGF-I within serum. IGFBP-1 knockout (KO) mice were used as a model of increased IGF-I bioavailability to investigate susceptibility to diet-induced obesity and alterations in metabolic homeostasis. This study consisted of IGFBP-1 KO and wildtype (WT) mice maintained on a high-fat diet consisting of either 45% calories from fat (HF45%) or 60% calories from fat (HF60%) for 16 weeks. From this study, we obtained growth curves and food intake measurements, performed metabolic assessments, and generated an endocrine and adipokine profile. IGFBP-1 KO mice 1) demonstrate enhanced sensitivity to exogenous IGF-I 2) are 7.4% heavier than WT mice at 8 weeks of age 3) after 16 weeks of feeding gain 33.3% less weight than WT mice on HF45% diet, and 16.6% less weight than WT mice on HF60% diet 4) exhibit increased insulin sensitivity and decreased leptin levels when maintained on a normal chow diet 5) have decreased free fatty acid and inflammatory cytokine levels when maintained on a high-fat diet. These findings suggest that increased IGF-I bioavailability has beneficial actions in attenuating diet induced obesity and maintaining normal glucose metabolism.

112-LB

Splenectomy Improves Insulin Sensitivity in Diet-Induced Obese Mice

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A high-fat diet intake induces obesity and chronic subclinical inflammation, which play important roles in insulin resistance. Increased circulating levels of proinflammatory cytokines and free fatty acids activate innate immune system, which triggers inflammation and cytokine expression, leading to insulin resistance. It is important to study novel sources of these inflammatory components that could promote this phenomenon, and the influence of the spleen in obesity has not yet been investigated. In order to investigate the role of spleen in insulin resistance, we evaluated metabolic parameters, insulin sensitivity, glucose tolerance, insulin signaling activation, inflammation, and liver and adipose tissue macrophage infiltration in splenectomized obese mice and after lipolysis induction. Insulin sensitivity was significantly increased in splenectomized obese mice, measured by hyperinsulinemic-euglycemic clamp, when compared to obese mice with spleen. On the other hand, blood glucose, serum insulin and glucose intolerance were reduced. Systemic, liver, muscle and adipose tissue inflammation was prevented by splenectomy, as seen by the reduction of circulating TNF-alpha levels and inhibition of JNK and IKK-beta activation, and IRS-1 Ser307 phosphorylation. Consequently, under insulin stimulation, insulin signaling proteins phosphorylation were strikingly increased in splenectomized obese mice compared to diet-induced obese animals. Attenuation of liver steatosis and macrophage infiltration and also a vast reduction in adipose tissue crown-like structures (CLS) and infiltrated macrophages were observed in splenectomized obese mice compared to obese animals submitted to sham surgery and in lipolysis promoter treated mice against its control. In summary, the spleen has a potential role on the metabolism and insulin resistance as a source of inflammatory components and macrophages that infiltrate and promote inflammation in metabolically active tissues in obesity.

Supported by: FAPESP, INCT-CNPq

113-LB

WITHDRAWN

114-LB

Topiramate Reduces Obesity and Increases Hypothalamic Insulin and Leptin Signaling in Mice

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Topiramate (TPM) treatment has been shown to reduce adiposity in humans and rodents. The reduction in adiposity is related to decreased food intake and increased energy expenditure. However, the molecular mechanisms through which TPM induces weight loss are contradictory and remain to be clarified. Whether TPM treatment alters hypothalamic insulin, or leptin signaling and action, is not well established. Thus, we investigate herein whether short-term TPM treatment alters energy balance by affecting insulin and leptin signaling, action or neuropeptide expression in the hypothalamus of mice fed with a high fat diet. As expected, short-term treatment with TPM diminished adiposity in obese mice which may be due to an improvement in anorexigenic signaling and high energy expenditure. TPM enhanced the activation of the insulin induced IRS/Akt/FoxO1 pathway and the leptin induced OBR/JAK2/STAT3 pathway in the hypothalamus of obese mice independent of body weight. TPM also raised POMC, TRH and CRH mRNA levels in obese mice. In addition, TPM increased the activation of the hypothalamic MAPK/ERK pathway induced by leptin, accompanied by high O2 consumption and UCP-1 levels in BAT. Together, these results provide novel insights into the molecular mechanisms through which TPM treatment reduces adiposity.

Supported by: FAPESP, CNPq, INCT

115-LB

Insulin and Leptin Signaling in Striatum and Amygdala are Crucial to Maintain Energy Homeostasis and They Are Impaired in DIO and Binge Eating Rats

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Feeding is controlled by a complex circuit, including the hormones insulin (INS) and leptin (LEP) and is also influenced by food reward. Striatum (STRI) and amygdala (AMY) are key regions in the reward system, which express abundant leptin receptor (LEPR) (STRI); and insulin receptor (IR) (STRI, AMY). However, whether INS and LEP signaling in these regions contribute to overconsumption of palatable food is poorly understood. Thus, the aim of the present study was to investigate (1) whether INS and LEP signaling in STRI and AMY have a role in the regulation of feeding behavior in chow, diet-induced obese (DIO) and binge eating rats; (3) in addition, whether INS and LEP signaling are impaired in STRI and AMY of DIO and binge eating rats. INS injection in the STRI or in the AMY reduced 8, 12, 24h-food intake (FI) in rats on chow. This result was associated with higher IR/Akt phosphorylation in those regions. Similarly, LEP injection in the STRI decreased 8, 24h-FI which was associated with higher LEPR/STAT3 phosphorylation in this brain area in rats on chow diet. INS and LEP did not decrease FI nor increase IR/Akt or LEPR/STAT3 phosphorylation in STRI and AMY in DIO and binge eating rats. To examine whether obesity induces endoplasmic reticulum stress (ER stress)

in the STRI we analyzed PERK phosphorylation in this area. Preliminary results indicated that PERK phosphorylation was increased in STRI of obese animals, suggesting that ER stress may be is involved in the development of LEP and INS resistance in this brain region. In summary, our data provide evidence, in vivo, that INS and LEP act in STRI and AMY controlling feeding behavior and this regulation is impaired in DIO and binge eating rats. Also, we showed that obese animals have an increased ER stress in the STRI, which may contribute to INS and LEP resistance in this area. Thus, insulin and leptin signaling in STRI and AMY are crucial to maintain energy homeostasis and those signals are altered in obese rats.

Supported by: Fapesp, CNPq

116-LB

Effects of Non-Absorbable Green Tea Extract on Body Weight and Glucose Tolerance in Obese-Type Diabetic Mice

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Obesity is an important risk for type II diabetes (T2DM). Some components of green tea extract (GTE) have been shown to inhibit intestinal glucose and lipid uptake. Nevertheless, the application of GTE for obesity and T2DM is still debated. The present study was aimed at providing evidence of the effect of a non-absorbable GTE on obese-type T2D, compared with absorbable natural GTE.

To evaluate acute blood glucose-lowering effects, oral glucose tolerance test was performed after oral GTE and/or polyethylene glycol (PEG; MW3350) administration in normal C57BL/6 mice. To determine the long-term effects, PEG, GTE, or GTE plus PEG was administered in the diet of db/db mice for 4 weeks (n=10 in each group).

In the normal mice, orally-administered GTE acutely elevated blood glucose levels compared to control, when glucose was orally loaded 1 h after the GTE administration ($P<0.05$), whereas GTE plus PEG decreased blood glucose levels than control. In db/db mice ingested with the regimen for 4 weeks, measured obesity parameters and diabetic parameters were significantly ameliorated in GTE plus PEG-treated groups ($P<0.05$) than the control without any regimen treatment. Groups with PEG alone or GTE alone did not exhibit different results from the control.

GTE ingested together with the polymer PEG, but not absorbable natural GTE, has beneficial effect on ameliorating obese-type T2D, probably by PEG blocking GTE entrance into the circulation but leaving the beneficial effect of GTE in the intestinal lumen.

Supported by: iPET, Ministry for Food, Agriculture, Forestry, Fisheries, Korea (110135-3)

117-LB

Acyl-CoA Synthase 5 (ACSL5) Ablation Attenuates Diet-Induced Obesity and Impairs Enteric Lipid Transport

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Members of the long-chain acyl-CoA synthase (ACSL) family catalyze the initial step in fatty acid (FA) metabolism: the conversion of FA to acyl-CoAs. Different ACSL isozymes are thought to direct FA to physiologic fates such as triacylglycerol (TAG) formation or oxidation. The ACSL5 isozyme is expressed in intestinal mucosa, liver, and brown and white adipose tissue of mice, and has been suggested to promote esterification of fatty acids into TAG and to be regulated by high carbohydrate diets. Thus, we tested the effects of ACSL5 ablation on adiposity and lipid metabolism. Female wild type (WT) and ACSL5 knockout (ACSL5KO) mice were provided a high fat (60% kcal) high sucrose (24% kcal) diet. For 12 weeks, ACSL5KO mice had significantly less fat mass by MRI (94.8 ± 16.8g n=4 ACSL5KO vs. 168.4 ± 13.9g n=9 WT, $p<0.05$), and at sacrifice had reduced adipose tissue depot weights (subcutaneous: 1.88 ± 0.21g n=4 ACSL5KO vs. 2.84 ± 0.23g n=8 WT, $p<0.05$; and gonadal: 2.26 ± .24g n=4 ACSL5KO vs. 3.87 ± 0.44g n=8 WT, $p<0.05$). Fasting serum insulin was also reduced in ACSL5KO mice (0.70 ± 0.08 ng/ml n=4 ACSL5KO vs. 1.76 ± 0.27 ng/ml N=9 WT, $p<0.05$). We hypothesized that ACSL5 was critical for esterification of fatty acids into TAG. Coherent anti-Stokes Raman scattering microscopy revealed a dramatic reduction of TAG within enterocytes of male ACSL5KO mice compared to WT 4h after an olive oil oral gavage or in the fed state of high fat (60% kcal) fed mice. Additionally, plasma TAG secretion was reduced in ACSL5KO mice after an olive oil gavage in the presence of a lipoprotein lipase inhibitor, tyloxapol. Interestingly, fecal fat content was similar in WT and ACSL5KO mice. These data demonstrate that ACSL5 regulates incorporation of dietary fatty acids into TAG in enteroyctes for storage and secretion and that this may contribute to obesity.

Supported by: USDA (1950-51000-071-02S)

118-LB

Consumption of a Single High Fat Meal Acutely Upregulates P-selectin Expression and Actions in the Visceral Microcirculation: Metabolic Implications

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Recent studies have indicated that deletion of the glycoprotein ligand-1 (PSGL-1) attenuates adipose tissue inflammation and insulin resistance in chronically obese mice. PSGL-1 contributes to leukocyte extravasation in inflamed tissues by binding several selectins, including endothelial expressed P-selectin and/or E-selectin. Constitutively expressed P-selectin is the first selectin upregulated on the endothelial cell surface during inflammation. Accordingly, we hypothesized that P-selectin is responsible for initiating leukocyte endothelium interactions (LEI) in the microcirculation of visceral fat depots following consumption of obesogenic high-fat meals (HFM). LEI were measured by intravital microscopy in splanchnic post-capillary venules of C57BL/6 wild-type mice gavaged with a liquid HFM (60% fat, 20% carbohydrate, and 20% protein). Administration of a single HFM increased LEI in the visceral microcirculation with peak levels of leukocyte rolling and adhesion reached at 1 and 2 hours post-gavage ($p<0.01$ versus mice given control liquid meal). The HFM failed to increase leukocyte rolling and adhesion in P-selectin deficient mice. Immunohistochemistry studies demonstrated increased P-selectin expression in the visceral microcirculation, but not the subcutaneous microcirculation, of mice given the HFM. Following 10 week high fat feeding, both wild-type mice and P-selectin deficient mice experienced a comparable degree of weight gain and visceral fat expansion. However, obese P-selectin deficient mice were protected from the insulin resistance and adipose tissue inflammation seen in wild-type mice, as demonstrated by nearly normal fasting plasma insulin and glucose levels, and TNFα fat tissue levels ($p<0.05$ versus wild-type). Taken together, these data uncover a role for endothelial expressed P-selectin in the initiation of adipose tissue inflammation and insulin resistance in obesity

Supported by: NIH, NIDDK

119-LB

Cdc2-like Kinase 2 (Clk2) in Arcuate Nucleus has a Key Role in Energy Balance and Hepatic Glucose Metabolism in Mice

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Cdc2-like kinase 2 (Clk2) is a kinase and an insulin-regulated suppressor of hepatic gluconeogenesis, glucose output. This kinase is downregulated in the liver of db/db mice. In vitro studies have shown that insulin is able to induce Clk2 threonine 343 phosphorylation (Clk2Th343), and this phosphorylation seem to be important for Clk2 signaling and activity. However, whether insulin and leptin modulate Clk2 phosphorylation in arcuate nucleus (ARC) and the role of this kinase in ARC regulates energy balance and hepatic glucose production were not yet investigate. Thus, the aim of the present study was to investigate, in vivo, (1) the effect of insulin and leptin on Clk2Th343 in ARC; (2) the role of Clk2 in the regulation of energy homeostasis; (3) whether Clk2 in ARC regulates hepatic glucose production. Refeeding after fasting or injection of insulin and leptin in ARC induced Clk2Th343 in control mice. This regulation was blunted in diet induced obesity (DIO) and in db/db mice. Insulin or leptin induced Clk2Th343 in ARC was abolished by LY294002 or Akt VIII inhibitor. We confirmed these results using GT1-7 cells. In addition, the inhibition of Clk2 in ARC for 5 days with TG003 (pharmacological inhibitor) or siRNA enhanced adiposity and food intake. In contrast, the inhibition of Clk2 by siRNA decreased O2 consumption and UCP1 expression in the brown adipose tissue. Furthermore, siRNA injected in ARC was associated with higher fasting glycaemia and hepatic glucose output, measured by an IP pyruvate tolerance test, and enhanced Pepck expression in the liver. In summary, our data provide evidence, in vivo, that insulin and leptin induced Clk2Th343 in the ARC. This phenomenon is dependent on PI3K/Akt pathway and is impaired in DIO and db/db mice. Moreover our findings suggest that Clk2 in ARC has a key role in the regulation of energy balance and hepatic glucose metabolism in mice, and may be a new target to treat obesity and type 2 diabetes.

Supported by: FAPESP, FAEPEX, CNPq

120-LB

Manipulations of Gastrointestinal Anatomy Reveal Physiologic Mechanisms of GLP-1 Regulation

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Gastric Bypass (GBP) combines gastric restriction and a shortcut for nutrients to the distal bowel, which is believed to increase secretion of GLP-1 levels. However, GLP-1 also increases after sleeve gastrectomy (SG), a gastric restrictive procedure that does not involve intestinal bypass. To understand the role of gastric vs intestinal mechanisms in GLP-1 regulation, we compared proximal intestinal bypass alone (duodenal-jejunal bypass -DJB) and gastric resection alone (SG) in Zucker Diabetic Fatty (ZDF) Rats. DJB improved oral glucose tolerance (GT) without significant increase in insulin or GLP-1 levels. In contrast, SG had only marginal effect on GT but it significantly increased insulin and GLP-1 (3-fold). These findings do not support a role for rapid stimulation of distal small bowel by nutrients as a primary mechanism for increased GLP-1 after gastrointestinal bypass. Since both SG and GBP involve exclusion/resection of gastric fundus and accelerated gastric emptying, we hypothesized that increased GLP1 levels after such surgeries may result primarily from alteration of gastric physiology, not from distal intestinal stimulation. We therefore investigated GLP-1 response to mixed meal test after selective, direct injection of meal stimulus in the stomach (physiological route), duodenum or distal jejunum of normal anesthetized mice. Blood sampling was obtained from the inferior vena cava. Direct duodenal stimulation (without gastric stimulation) significantly increased GLP-1 levels whereas neither gastric nor distal-jejunal injection increased GLP-1 response. We then injected a mixed meal into a short segment of duodenum isolated by proximal and distal clamping. This was sufficient to significantly increase GLP-1 levels in absence of nutrients passage into the distal small bowel, supporting an important physiologic role of the duodenum in GLP-1 regulation. These findings may help develop novel strategies to pharmacologically increase endogenous GLP-1.

121-LB

CD40 Promotes MHC II Expression on Adipose Tissue Macrophages and Regulates CD4+ T Cells in Adipose Tissue of Mice with Diet-Induced Obesity

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CD40 is a TNF receptor superfamily member that is expressed on macrophages, endothelial cells and adipocytes. CD40 expression is elevated on monocytes from patients with metabolic syndrome, and CD40 activation promotes atherogenesis in mice. We found that Cd40 mRNA levels were elevated in whole epididymal adipose tissue (eAT) and adipose tissue macrophages (ATMs) from male C57BL/6 mice fed a high fat diet (HFD; 60%) for 20 weeks. Therefore, we tested the hypothesis that CD40 on ATMs contributes to the progression of obesity-induced inflammation and insulin resistance. Male Cd40-knockout (KO) and wild type (WT) mice were fed a HFD for 20 weeks. Compared to WT mice, KO mice showed a trend (P=0.10) towards protection from HFD-induced weight gain despite having increased visceral adiposity. Stromal vascular cell number and ATM (CD11b+F4/80+) content were similar in eAT from obese WT and KO mice; however, the number of MHC II+ ATMs and the expression of CD86 on ATMs were reduced in KO mice. Obese KO mice had fewer conventional CD4+Foxp3- T cells in eAT, whereas the number of regulatory T cells (Tregs; CD4+Foxp3+) was unaltered. Despite these changes, there were no significant effects of CD40 deficiency on measures of HFD-induced insulin resistance. To examine the contribution of hematopoietic cell derived CD40, we generated bone marrow chimeric mice with (BMT-WT) and without (BMT-KO) CD40 expression on leukocytes. BMT-WT and BMT-KO mice gained similar body weight in response to HFD. Similar to the whole body KO mice, MHC II+ ATMs and CD4+Foxp3- T cells, but not Tregs, were reduced in eAT from obese BMT-KO mice. However, hematopoietic disruption of CD40 did not ameliorate insulin resistance. Collectively, these data indicate CD40 plays a role in obesity induced T cell-mediated inflammation in adipose tissue with obesity, but that CD40-independent mechanisms contribute to the development of HFD-induced metabolic disease in mice.

Supported by: NIH (DK091976 (D.L.M), DK090262 (C.N.L.), DK078851 (C.N.L.))

OBESITY—HUMAN

122-LB

One Year Diabetes Remission in the Helping Evaluate Reduction in Obesity (HERO) Study

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Remission of type 2 diabetes (T2D) after gastric bypass surgery has been shown to be related to the duration of diabetes, severity of disease, and amount of weight loss/regain. Few studies have reported the impact of adjustable gastric banding (AGB) on diabetes remission. The overall objective of the HERO study is to examine the clinical effectiveness and safety of LAP-BAND® AP AGB over five years. Here we seek to assess diabetes remission after AGB and identify factors associated with T2D remission 1 year after AGB.

300 subjects out of 1,123 HERO enrollees who reported either having a history of T2D or taking an anti-diabetic medication at baseline (BL), were defined as T2D at BL. The analysis sample included the 199 subjects who provided complete BL and 1 year data. Using the American Association of Clinical Endocrinologists (AACE) guidelines for T2D, diabetes remission was defined as HbA1c level of ≤ 6.5% and no anti-diabetic medication usage at 1-yr. Multivariate logistic regression was used to examine factors associated with remission at 1-yr, controlling for region (US vs OUS) and years of diabetes duration.

There were no differences in mean BL weight (kgs) (128 vs 131) between remission and non-remission groups. About 39% (77/199) had remission of T2D at 1-yr. Patients with remission had significantly shorter T2D duration (5.5 years vs 8.6 years, p=.002). At 1-yr, patients with remission had significantly greater percent weight loss than those without remission (19.2% vs 12.2%, p<.001). Multivariate analysis showed that %WL (OR=1.11, p<.001) was the most significant factor in predicting T2D remission at 1-yr. Controlling for region (OR=2.6, p=.045) and diabetes duration (OR=.89, p=.003), subjects with 20%WL were 8.6 times more likely to have diabetes remission at 1-yr.

AGB was associated with significant weight loss and diabetes remission by 1-yr. Further analysis of this study will examine the durability of remission and the association of remission with continued weight loss.

123-LB

STAMPEDE Metabolic Substudy: Effects of Bariatric Surgery vs. Intensive Medical Therapy on Glycemic Control, β-Cell Function and Body Composition in Type 2 Diabetes

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STAMPEDE is a single center, randomized control trial comparing glucose control after intensive medical therapy (IMT) alone, versus IMT plus Roux-en-Y gastric bypass (RYGB), or IMT plus sleeve gastrectomy (SG). This substudy examined glucose metabolism and beta cell function (Mixed Meal Tolerance Test with insulin and c-peptide kinetics) in relationship to weight loss and body composition (iDXA) changes at baseline and 1, and 2 yrs later. The first 60 subjects (20 per group) were randomized (age 49±1 y, 60% F, 71% Cauc, BMI 36±2.4 kg/m^2, HbA1c 9.7±0.2%, DM duration 8.6 ± 5 y) to IMT, RYGB and SG, respectively. At 2 yrs, 10% dropped out with17 subjects remaining in IMT, 18 in RYGB, and 19 in SG. Glycemic control significantly improved in all three groups. At 2 years, HbA1c was 6.7±1.2% in RYGB, 7.1±0.8% in SG, and 8.4±2.3% in IMT (P<0.05 for each surgical group vs IMT). Remission (HbA1c ≤ 6%) at 2 years was achieved in 41%, 10% and 6% of patients in RYGB, SG and IMT, respectively. Weight loss was similar in RYGB and SG (-25.4±10 and -22.5±8 kg), and both were markedly greater than IMT (-0.5±4 kg). Reduction in total fat mass was similar for RYGB and SG (-10.6±7 vs -7.7±4%, P=0.11), with greater absolute reduction in percent truncal fat in RYGB vs. SG (-16 vs -10%, P=0.04). Insulin sensitivity (Matsuda Index), increased 3.5-fold in RYGB from 1.5 at baseline to 5.2 at 2 years (P<0.001), 3.9 to 5.7 for SG (P=0.05), and did not change after IMT (P=0.85). Beta cell function, measured with the oral disposition index, increased 5.3-fold in RYGB (P<0.001), and by 2-fold in both SG (P=0.03), and IMT (P=0.19). We conclude that in moderately obese patients with poorly controlled diabetes, bariatric surgery, particularly RYGB, provides more durable glycemic control compared to IMT at 2 yr. Despite similar weight loss, RYGB is more effective in restoring pancreatic beta cell function and targets truncal fat compared to SG, the key metabolic defects in diabetes.

Supported by: Ethicon-Endo Surgery IIS 19900

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A Metabolically Healthy Obese Phenotype in Hispanic Participants in the IRAS Family Study

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Some obese individuals appear to be protected from developing type 2 diabetes mellitus and cardiovascular disease. This has led to characterizing different body size phenotypes based on standard cardiometabolic risk factors. The aim of this study was to measure the prevalence and describe fat distribution across these different phenotypes in a minority population. Hispanic participants (N=1054) in the IRAS Family Study were categorized as obese or overweight, and as metabolically healthy (MH) or metabolically abnormal (MA) based upon a previously published algorithm (Wildman, et al. Arch Intern Med 2009). Computed tomography scans were evaluated for measures of non-alcoholic fatty liver disease (NAFLD) and abdominal fat distribution. Statistical models adjusting familial relationships were estimated. Seventy percent of the Hispanic cohort was overweight (32%) or obese (38%). Forty-one percent (n=138) of overweight participants and 19% (n=74) of obese participants met criteria for MH. In addition to expected differences in metabolic factors used to define these groups, MH individuals were, on average, younger than MA groups, more physically active, less likely to be on medications, had smaller waist circumference, and had higher levels of circulating adiponectin. Adjusted analyses showed the MH phenotype was associated with lower visceral adipose tissue (VAT) and liver density in obese participants (p=0.0004 and p=0.0002), and lower VAT but not liver density in overweight participants (p=0.0069 and p=0.15) compared to their MA counterparts. Odds of NAFLD were reduced in MH obese (OR=0.34, p=0.0007) compared to MA obese. VAT and liver density did not differ between MH overweight (or obese) and normal weight groups. These findings suggest that lower levels of visceral and liver fat, despite overall increased total body fat, may be a defining feature of MH obesity in Hispanic Americans. Further, obesity as defined by BMI may not have the same physiologic significance for every individual.

125-LB

Long-Term Effects of Lifestyle Intervention on Weight and Cardiovascular Risk Factors in Individuals with Type 2 Diabetes in Real World Clinical Practice: 4-Year Longitudinal Results

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Clinical studies showed that obese patients with type 2 DM (T2DM) who lose weight through intensive lifestyle interventions (ILI) frequently regain most of that weight within a year. However, no study evaluated the impact of long-term weight maintenance versus weight regain on cardiovascular risk factors. We longitudinally evaluated 119 patients with T2DM (mean age 54.5±10.1 yrs) who enrolled in the Weight Achievement and Intensive Treatment (Why WAIT) program, a 12-wk of ILI in real-world clinical practice. After 1 yr, we divided them into Group A, who maintained ≥7% of weight loss and group B who regained weight. We followed them for 4 yrs. Group A (55 patients, mean weight 249.8 lbs) lost an average of -29.8± 8.9 lbs (-11.9%) at 12 wks and maintained -31.6±14.8 lbs (-12.6%) at 1 yr. Group B included (64 patients, mean weight 241.5 lbs) lost an average of -19.4±8.4 lbs (-8.0%) but was down to -6.2±9.0 lbs (-2.6%) at 1 yr. At 2, 3 & 4 yrs, group A maintained a weight loss of -24.4, -23.8 & -24.4 lbs respectively. Group B maintained -7.1, -6.5 & -8.2 lbs respectively. In group A, HbA1c dropped from 7.2±1.1% to 6.2±0.9% at 12 wks ($p<0.001$) and was 6.9±1.0% at 4 yrs ($p<0.05$). In group B, HbA1c dropped from 7.5±1.4% to 6.6±0.8% ($p<0.001$) but rebounded to 8.0±1.8% at 4 yrs ($p<0.05$). At 4 yrs, group A had greater % weight loss (-9.6% vs -3.4%; $p< .001$) and HbA1c reduction (-0.36% vs .55%; p=.002). There was no difference in systolic (-3.73 vs -1.49 mm Hg; P=.4) & diastolic (-1.75 vs -1.18 mm Hg; p=.8) BP, and levels of HDL-cholesterol (+7.38 vs +7.6 mg/dL; p=.9), triglycerides (-1.33 vs 29.62 mg/dL; p=.05) and LDL-cholesterol (-3.98 vs -3.88 mg/dL; p=.3). We concluded that ~50% of patients enrolled in ILI in clinical practice maintain weight loss for 4 yrs. A weight loss of ≥7% at 1 yr is predictor of long-term weight maintenance and improvement in HbA1c. Patients who regain weight retain some benefits on other cardiovascular risk factors.

126-LB

Gastric Bypass Surgery Improves Insulin Action by Reducing BCAA and mTOR Activation in Muscle and Adipose Tissue

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Recent studies suggested a potential key role of branched-chain amino acids (BCAA) metabolism early in the pathogenesis of type 2 diabetes and also indicate that BCAA profiles could aid in diabetes risk assessment. Moreover it was demonstrated that gastric bypass surgery (GBP) was able to induce both reduction in BCAA levels and improvement ininsulin resistance and glycemic control. In the same line, it is well established that chronic activation of mammalian target of rapamycin (mTOR) promotes insulin resistance by mechanism involving degradation and also serine phosphorylation of IRS-1. Since mTOR can respond to nutritional status and amino acid availability we hypothesized that GBP-induced reduction in BCAA levels could result in an important reduction in the mTOR activation. Thus the current study was performed to determine BCAA levels, the mTOR signaling pathway and also insulin sensitivity and signaling in insulin target tissues before and 1 and 6 months after the GBP. Twelve obese subjects (mean BMI 46.2 ± 3.3 kg/m^2; 4 male and 8 female) who were scheduled to undergo GBP participated in this study. We also included 9 apparently healthy lean subjects as a control group (mean BMI 24.7 ± 2.8 kg/m^2, 5 male and 4 female).Our data show that before the GBP the obese subjects displayed higher circulating levels of BCAA and increased phosphorylation levels of mTOR, p70S6K and IRS-1^{ser312} in muscle and adipose tissue, compared to lean subjects. We next demonstrated that BCAA levels were decreased in both situation 1 and 6 months after the GBP and that these reduction was accompanied by lowering phosphorylation levels mTOR, p70S6K and IRS-1^{ser312} in the adipose tissue. In summary, GBP can induce an important reduction in BCAA levels, which in turn decreases mTOR activation resulting in an improved insulin action in these subjects. Altogether these results suggest a possible mechanism by which the bariatric surgery can improve insulin sensitivity and glycemic control in obese patients

Supported by: FAPESP, INCT

127-LB

Effect of Body Weight and Adipose Tissue Partitioning on Inter-ethnic Variations in Insulin Sensitivity

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We have previously shown that ethnicity modifies the relationship between body mass index (BMI) and insulin resistance (IR). While lean Chinese had the lowest IR compared to Malays or Asian-Indians, the detrimental impact of increasing BMI on IR was greatest in the Chinese. We investigated whether variation in fat partitioning mediates this phenomenon. We used hyperinsulinemic euglycemic glucose clamp to measure insulin sensitivity index (ISI) and compared 148 lean (64 Chinese, 40 Malays, 44 Asian-Indians; BMI <25) to 110 overweight (34 Chinese, 41 Malays, 35 Asian-Indians; BMI ≥25-30) male subjects, aged 21-40 years. Fat depots were assessed by MRI [visceral (VAT) and subcutaneous abdominal fat (SAT)] and MRS [hepatic triglycerides (HTG) and intramyocellular lipids (IMCL)].

For the lean, ISI (mg/min/kg) was highest in Malays (10.5±5.2), followed by Chinese (8.7±3.6) and Asian-Indians (6.7±2.5)(p=0.001). ISI was similar among overweight Chinese (5.4±2.7), Malays (4.7 ± 2.2) and Asian-Indians (5.0±3.5)(p=0.508). In the univariate analysis, there were significant negative correlations between ISI with VAT, SAT, IMCL and HTG for all 3 ethnic groups. In the multivariate analysis, there was significantly greater reduction in ISI with increasing BMI in Chinese and Malays than Asian-Indians (p-interaction for ethnicity x BMI=0.009). No significant ethnicity x BMI interaction was observed in all fat depots. For the ISI-fat depot relationship, there was significant ethnic influence only for SAT, with lower decline in ISI as SAT increases in Asian-Indians relative to Malays or Chinese (p=0.001) after adjusting for age and BMI.

Hence, fat depot expansion with increasing BMI was similar in all 3 ethnicities. However, the negative impact of increasing SAT on ISI is greater in Chinese compared to Asian-Indians.

Supported by: Translational and Clinical Research (NMRC) Grant

128-LB

Associations between Common Variants in CYP24A1 and Risk of Obesity in Chinese Hans

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The 24-hydroxylase (encoded by CYP24A1) is a key enzyme involved in degradation of vitamin D metabolites, and gene variants in CYP24A1 may modify vitamin D metabolism. Recently, emerging evidence suggested a role of vitamin D in the development of obesity. Therefore, the objective of the present study was to investigate whether variants in CYP24A1 were associated with obesity and its related phenotypes in Chinese Hans. Eight tag single-nucleotide polymorphisms (SNPs) in CYP24A1 (rs2245153, rs927651, rs3787554, rs2181874, rs2762941, rs3886163, rs2248461 and rs2296241) were genotyped by using ABI Prism 7900 HT Sequence Detection System in a population-based sample including 474 obese (BMI ≥28kg/m2), 1221 overweight (24 ≤BMI <28kg/m2) and 1515 normal-weight subjects (BMI <24kg/m2). After adjustment for age, sex, residential region, season of blood collection and physical activity, five SNPs (rs3787554, rs2181874, rs2762941, rs3886163 and rs2296241) showed significant associations with BMI, waist circumference and/or risks of overweight and obesity (P ≤0.04). After Bonferroni corrections, the associations of rs3886163 A-allele (OR = 1.17, P=0.0060) and rs2762941 A-allele (OR = 1.20, P = 0.0017) with risk of overweight remained significant (P <0.0063, 0.05/8 tests). In conclusion, the variants in CYP24A1 might contribute to risk of obesity and related traits in Chinese Hans.

Supported by: CAS (2009KIP401), INS Clinical Nutrition Research Center (CRC2010008)

129-LB

Insulin, Decision Making, and Dopamine Receptors in Obesity

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Insulin acts in the brain to regulate dopamine (DA) tone and signaling. Altered DA functioning in the brain may underlie obesity-related phenotypes, including altered decision making. While the effect of insulin on DA and DA-mediated behaviors has been shown in animal studies, research in human populations is lacking. In this preliminary analysis, we examined the relationship among insulin resistance, beta-cell function, decision making and D2 receptor measurements in obese humans.

Participants were obese adults (n=19; BMI>30, ages 21-39), without type 2 diabetes or other significant medical conditions. Insulin resistance was evaluated using the Homeostasis Model of Insulin Resistance (HOMA-IR) and beta-cell function was evaluated by using the disposition index (insulin secretion relative to insulin sensitivity) derived from an oral glucose tolerance test. Decision making was measured with the probabilistic reward discounting (PRD), delayed reward discounting (DRD), and Iowa Gambling (IGT) tasks. A subset of participants (n=10) also underwent positron emission tomography scans using a D2-specific receptor ligand, [^{11}C]NMB. D2 receptor binding potentials (BPs) were computed for putamen and caudate using the Logan graphical method with cerebellum as the reference region.

Results showed that DI and HOMA-IR correlated to each other (r=-.80, p<.001). PRD task measures correlated with DI, such that lower DI was related to less risky decision making (k, r=.71, p=.004; AUC, r=-.56, p=.04), controlling for age and BMI. Lower D2 BP in the putamen correlated with lower DI (r=.71, p=.046) and higher HOMA-IR values (r=-.76, p=.03), controlling for age and BMI. In addition, lower D2 BP in the caudate was correlated with lower DI (r=.72, p=.045), controlling for age and BMI.

These preliminary findings indicate that in obese patients, changes in insulin sensitivity and beta cell function are associated with decision making and with D2 receptor binding in the striatum, independent of BMI.

Supported by: R01DK085575, T32HL007456

130-LB

Dysregulation of Fat and Glucose Metabolism is Worsened by Bedrest in Older, Obese Adults

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The combined influence of geriatric obesity and unanticipated bedrest on fatty acid and glucose metabolism has not been determined. Therefore, we measured fatty acid kinetics, and hepatic and peripheral insulin resistance prior to and immediately following ten days of bedrest in a group of eight older, obese (64±3 y/age; 39±2% fat) individuals. Under pre- and post-bedrest conditions, an octreotide, basal glucagon replacement, multi-stage insulin infusion (MSI) was performed to determine insulin-mediated suppression of glucose production (ISGP) and insulin-stimulated glucose disposal (ISGD) in a sequential fashion. The MSI was combined with the infusion of stable isotope tracers, [6,6 $^{2}H_{2}$]glucose and [1-^{13}C]palmitate, and they were utilized to derive glucose R_a and free fatty acid (FFA) R_a, respectively. Body weight, % body fat and basal energy metabolism were not altered by bedrest. Surprisingly, bedrest was associated with a significant decrease (-2291±316 cm^2) in visceral fat without the loss of lean mass. ISGP was impaired prior to bedrest, and there was a further reduction (>15±2%) in ISGP under post-bedrest conditions. There was also a concomitant reduction (~40%) in the insulin-mediated suppression of FFA R_a, and an increase in plasma FFA. There was also a decline (>2.0±0.6 0.41 mg · kgFFM^{-1} · min^{-1}) in ISGD from pre- to post-bedrest conditions. As indicated by data derived from the 1st stage of the MSI, short-term bedrest exacerbated the defective regulation of lipolysis, and promoted the further development of hepatic insulin resistance in geriatric obesity. While bedrest also promoted peripheral insulin resistance, FFA R_a and plasma FFA were not increased during the 2nd stage of the MSI. Therefore, bedrest-induced insulin resistance may originate by separate mechanisms in liver and skeletal muscle, and unanticipated hospitalization will likely increase metabolic risk through a multi-factorial etiology in older, obese individuals.

Supported by: NIH (PO1 AG023591-01, KO1 DK 64716-01, M01 RR14288)

131-LB

DNA Damage is Increased in Lymphocytes of Patients with Metabolic Syndrome

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Metabolic syndrome (MetS) is serious and growing health care problems worldwide, leading an increased risk for diabetes and cardiovascular disease. We aimed to assess possible DNA damage in patients with MetS. Twenty-eight subjects with MetS and 20 controls were enrolled in this study.

This study was conducted between August 2011 and January 2012 in the Erzurum Training and Research Hospital. Micronuleus (MN) test was carried out with the blood-cell cultures from subjects. Micronuclei frequencies were assessed in cytokinesis-blocked lymphocytes. Twenty-eight patients, diagnosed with MetS and 20 healthy controls of corresponding ages, abdominal obesity (waist circumference), levels of triglycerides, HbA1c, HDL-C and fasting glucose and blood pressure and body-mass index (BMI) were included in the study.

Serum levels of triglycerides, fasting glucose, HbA1c and waist circumference, systolic blood pressure, diastolic blood pressure and BMI in the MetS group were significantly higher than those of the control group (for each, P<0.001). However, the mean level of HDL-C in the MetS group was lower than in control group (P<0.001). Our result showed a MN frequency significant increase in Mets patients (3.62 ± 1.19 per 1000 cells) relative to that of the control group (1.83 ± 0.71 per 1000 cells) (p<0.001). There were positive correlations between micronucleated cell rate and waist circumference, BMI and plasma and triglycerides (for each, P<0.01). In addition, there was negative correlation between micronucleated cell rate and level of HDL-C (P<0.01). However, there was no correlation between micronucleated cell rate and level of systolic blood pressure, diastolic blood pressure and plasma fasting glucose (for each, P>0.05).

These findings suggest that patients with MetS have a high incidence of DNA damage. MN may constitute a possible component of a panel of biomarker for the risk of diabetes and cardiovascular disease.

ISLET BIOLOGY—BETA CELL—DEVELOPMENT

132-LB

The Nkx2.2 NK2-SD Domain is Required for β-Cell Development and Islet Cell Identity

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Type I and type II diabetes mellitus are associated with a loss of functioning insulin-producing β cells in the pancreas. Understanding the mechanism of normal islet and β cell development will be an important step in developing possible treatments for the disease. Nkx2.2 is essential for proper β cell differentiation. Nkx2.2 null mice show a complete absence of insulin-producing β cells, a 90% reduction of glucagon-producing α cells, and an increase in ghrelin-producing cells. Nkx2.2 contains three conserved domains: the tinman domain (TN), homeodomain (HD), and NK2-specific domain (SD). The SD

domain is highly conserved among Nk2 family members and across species. However, its function remains largely unknown. In order to further understand the molecular interactions involving Nkx2.2 in the developing mouse pancreas, we have generated a mouse line containing mutations in the NK2-SD domain. We show that SD mutant mice have a decrease in β cell numbers as well as a decrease in the β cell markers, NeuroD, Nkx6.1, Ins1 and Ins2. However, there is no change in α cell numbers or the α cell markers, glucagon and Irx2. We also show an early increase in ghrelin cell numbers and expression which normalizes by birth. Additionally, polyhormonal cells are seen as early as e12.5 and persist postnatally. Postnatally, the mice show morphological changes in islet size and the proximity of their islets to the ducts. Moreover, they show a continuing loss of β cells resulting in severe hyperglycemia. The data suggest that the SD domain plays an important role specifically in β cell development and the maintenance of normal islet cell identities.

133-LB

In Vivo Reprogramming of SOX9 Positive Progenitor Cell Population within the Billiary Tree Stem Cell Niche Forms Insulin-Secreting Ducts and Relieves Diabetes

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In embryonic development, the pancreas and liver share developmental history up to the stage of bud formation. On the basis of this developmental similarity, we postulated that direct reprogramming of liver to pancreatic cells will occur when suitable transcription factors are over expressed. Using a polycistronic vector we overexpressed Pdx1, Ngn3 and MafA in the livers of NOD-SCID mice rendered diabetic by treatment with streptozotocin. Upon delivery of the pancreatic transcription factors the diabetes is relieved long term, and the glucose tolerance and glucose induced serum insulin are restored towards normal. Many ectopic duct-like structures appear in the liver, which express beta cell markers and produce insulin. Use of a vector also expressing GFP shows that the ectopic ducts persist long after the viral gene expression has ceased, indicating that this is a true irreversible cell reprogramming event. We have recovered these insulin-positive ductal cells from the livers by cell sorting and shown that they display glucose-sensitive insulin secretion and express many beta cell markers (Ins1, Ins2, Sur1, Kir6.2, Gck, Glut2, Pdx1, Rfx6, Nkx2.2, Pax4, MafA). The insulin positive ductal structures were not found to co-stain for albumin but were found to co-localise with SOX9 positive cells indicating that these novel ectopic reprogrammed ducts did not arise from the hepatocytes but arised from the SOX9 positive progenitor population in the billiary tree. Thus we presume that our polycistronic adenoviral construct underwent targeted reprogramming of the SOX9 positive progenitor population located within the billiary tree stem cell niche. This niche essentially present in the small bile ducts and peribiliary glands was reprogrammed towards a beta cell type to relieve diabetes and suggested a new type of therapy for diabetes.

Supported by: NIH

134-LB

Deciphering the Role of HIF1 Alpha in Pancreatic Beta-Cell Development

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During embryonic life, oxygen tension (pO2) controls proper organ development. For example, at early stages of pancreas development, the blood flow is weak and pO2 is low. Later, the blood flow increases, correlating with beta-cell differentiation. In the present study, we hypothesized that Hypoxia Inducible Factor 1 alpha (HIF1a), a crucial mediator of cell adaptation to hypoxia, plays a critical role during pancreatic beta-cell development. The aim was to decipher the role of HIF1a. Using an in vitro bioassay where beta-cells develop from rat fetal pancreas, we first demonstrated that increasing pO2 enhances beta-cell differentiation. This effect was independent of epithelio-mesenchymal interactions and neither oxidative nor energetic stress occurred. Moreover, the induction of beta-cell differentiation by increased pO2 could also be observed in human and mouse fetal pancreas. To define the role of HIF1a in the effects of pO2, we deleted in vitro the gene encoding pVHL (Von Hippel Lindau), a protein necessary for the proteasomal degradation of HIF1a at normoxia. For this purpose, E13.5 Vhl floxed mouse pancreases were infected with Cre-expressing adenoviruses (Ade-Cre) and next cultured at 21% (percent) O2. Deletion of vhl induced the stabilization of HIF1a. The rate of epithelial cell proliferation was similar in wt/Ade-Cre and Vhl/Ade-Cre pancreases. However, NGN3 expression and beta-cell development decreased in Vhl/Ade-Cre pancreases compared to wt/Ade-Cre pancreases. These data demonstrate that HIF1a exerts a negative control over beta-cell differentiation. Modulating the HIF pathway could thus represent a new approach for amplifying pancreatic endocrine precursors and beta cell mass.

Supported by: EFSD, Novo Nordisk A/S, JDRF, SFD, AFD

135-LB

Pregnancy Induces Beta-Cell Proliferation in Transplanted Islets

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During pregnancy, proliferation of islet beta-cells is driven by lactogenic hormones and serotonin, peaking mid-pregnancy (day 12.5-14.5) in mice. Islets represent ~1-2% of pancreatic tissue and molecular studies aimed at identifying the transcriptome pattern of proliferating beta-cells requires isolation and enrichment for the endocrine pancreas. The islet isolation per se induces cellular stress responses that may alter basal expression profiles. We developed a model of syngeneic islet transplantation under the kidney capsule of chemically-induced diabetic female mice. Following completion of engraftment (≥4 wks), to allow recovery from islet isolation and implantation-related stress, animals are mated. Using this approach, the large mass of endocrine pancreatic tissue (that is, purified islets) can be easily retrieved without harsh manipulation for analysis. Incorporation of bromodeoxyuridine in transplanted beta-cells by immunohistopathology showed proliferation rates comparable to those of the native pancreas during pregnancy. Proliferating beta-cells in the native pancreas was 7.4±2.9% (n=5; baseline) and 22.8±7.2% (n=5) in control and 12.5-day pregnant mice, respectively ($p<0.01$). A similar pattern was observed in transplanted islets, with 4.5±2.1% in control (n=6) and 30.8±6.8% in pregnant mice (n=6; $p<0.001$). Microarray analysis of mRNA expression performed on islet grafts obtained from control and pregnant mice (day 12.5) showed that 296 of the ~25,000 genes detected were significantly differentially expressed in islet grafts of pregnant vs. control mice (n=3 independent microarrays with a $p<0.08$). 68 genes resulted significantly differentially expressed both in our analysis and a previous report on isolated islets (Rieck and coll. 2009). Differential expression of multiple genes was confirmed by qRT-PCR. Transplanted islets proliferate under physiological conditions (e.g., pregnancy) in an ectopic site. This may open new avenues for in depth molecular studies.

Supported by: Diabetes Research Institute Foundation, Converge Biotech, Inc.

ISLET BIOLOGY—BETA CELL—POSTNATAL GROWTH

136-LB

New β-Cells are Not Generated by Prolonged Administration of Incretin-Based Therapies (Exendin-4 or Sitagliptin) in Adult Mouse Pancreas

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The β-cell mitogenic response of incretin-based therapies remains one of the most intensely controversial topics in diabetes research. Multiple studies indicate that short-term administration of incretin-based therapies can increase β-cell proliferation. Based on these findings, incretins have been widely proposed as β-cell mitogens that could be utilized to expand β-cell mass. However, few (if any) long-term treatment studies have been performed to rigorously test if incretins expand β-cell mass. We set out to quantitatively measure β-cells after prolonged administration of incretin therapies (exendin-4 or sitagliptin) in young adult mice. Treatment was given in combination with a high fat (60% kcal) or a low fat control diet (10% kcal), to additionally evaluate differences effectiveness of incretin therapies in association with higher metabolic demand. To accurately quantify total β-cells, we employed a high-throughput approach to pancreatic morphometry, which combines large-scale microscope image acquisition with computer-assisted automated image processing. This strategy allows quantification of vast numbers of microscopic slides and biological samples, and thus improves the overall accuracy of β-cell quantification. Here we show that exendin-4 and sitagliptin treatment of young adult mice for 4 months does not increase the total number of β-cells in association with high or low fat diets. Morphometry with serial optical tomography of the entire pancreas revealed that β-cell mass was unaltered by long-term administration of sitagliptin or exendin-4. Thus, new β-cells are not generated in response to incretin therapies, regardless of metabolic need.

Supported by: NIH (1R01DK064101, 1R01AG040110), Merck Sharp & Dohme Limited, Robert and Janice McNair Foundation

137-LB

Engaging Canonical and Non-Canonical Wnt Signaling with Wnt3a and R-Spondin3 and Inhibition of RhoA/ROCK Signaling Increases Proliferation of Adult Human β-Cells

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Our previous studies demonstrated that Wnt/GSK-3/β-catenin and mTOR signaling are necessary for increasing regenerative processes in adult human β-cells. Direct inhibition of GSK-3 increased β-catenin nuclear translocation and β-cell proliferation but resulted in a loss of insulin content. Our current goal was to engage canonical and non-canonical Wnt signaling at the receptor level, identify additional pathways involved in β-cell proliferation, and maintain β-cell phenotype.

Treatment of human islets with a GSK-3 inhibitor or conditioned media (CM) containing Wnt3a and R-spondin3, enhanced DNA synthesis ~2 fold. However, treatment with CM+ [containing CM, ROCK inhibitor (Y-27632) and SB-431542 (that results in RhoA inhibition)] significantly enhanced DNA synthesis ~6 fold in a rapamycin-sensitive manner. Moreover, CM+ increased human β-cell proliferation ~20 fold above glucose alone and enhanced β-catenin-mediated gene expression as well as IRS1/2 gene expression. PKA signaling is also important for enhancement of Wnt signaling. Inhibition of PKA by H89 significantly blocked CM+ mediated human islet DNA synthesis. DNA synthesis was not enhanced by exendin-4 alone, but significantly increased in the presence of RhoA/ROCK inhibitors. However, these effects were significantly lower than the CM+ treatments. Activation of PKA with forskolin increased Wnt signaling through phosphorylation of GSK-3 and CREB, and led to increased expression of CREB target genes, NR4A2 and IRS2. Treatment of human islets with CM or CM+ also increased expression of NR4A2 and IRS2 and resulted in activation of Akt.

Our data demonstrate that engaging Wnt signaling at the receptor level leads to crosstalk between Wnt/β-catenin, PKA/CREB and mTOR signaling. Regulation of these pathways with CM+ or exendin-4 plus RhoA/ROCK inhibitors, substantially enhances human β-cell proliferation and may maintain β-cell differentiation.

Supported by: NIH

138-LB

Alpha-Cells are Dispensable for Post-Natal Islet Morphogenesis and Maturation of Beta-Cells

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Glucagon-producing α-cells are the second most abundant cell type in islets. While α-cells make up less than 20% of the cells in a mature islet, they occupy a much larger proportion of the pancreatic endocrine cell population during the early post-natal period, the time when extensive β-cell proliferation and dynamic morphogenesis occur to form mature islets. To determine if α-cells have a role in this postnatal process of islet development, we have established a diphtheria toxin-mediated α-cell ablation mouse model in which α-cells can be ablated by toxin injection. Rapid and persistant depletion of α-cells was achieved by daily injections of the toxin for 2 weeks starting at post-natal day1 (P1). Total pancreatic glucagon content was undetectable at P14, and still only 2.8±0.7ng at 4 months of age in the α-cell ablated mice, compared with 393±39ng and 1,087±18ng, respectively, at those ages in control mice. Histological analyses revealed that formation of spherical islets from primitive aggregations of endocrine cells occurred normally despite the near total lack of α-cells. Furthermore, islet size distribution, which was evaluated histologically was not changed by the lack of α-cells in the 4-month old mice. Insulin content of the α-cell ablated pancreata was not significantly different from control pancreata during the post-natal period, but it was somewhat higher (a 26% increase) at 4 months of age. Blood glucose and plasma insulin levels in the α-cell ablated mice were slightly lower than that of the control mice, but the differences were not significant. Both glucose and insulin tolerance tests indicated that the α-cell ablated mice had normal glucose homeostasis physiology. In perifusion experiments, glucose-stimulated insulin secretion in islets isolated from the α-cell ablated mice was not different from control islets. Taken together, these results indicate that α-cells do not play a critical role in post-natal islet morphogenesis nor functional maturation of β-cells.

139-LB

MicroRNA-7a Regulates the mTOR Pathway and Proliferation in Adult Pancreatic β-Cells

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Elucidating the mechanism underlying the poor proliferative capacity of adult pancreatic beta cells is critical to regenerative therapeutic approaches for diabetes. We find that the miR7/7ab family member microRNA-7a (miR-7a) is enriched in adult pancreatic islets and, remarkably, targets five components of the mTOR signaling pathway, including the two main downstream effectors of TORC1, p70S6K and eukaryotic translation initiation factor 4E (eIF4E), two MAPK-interacting kinases Mknk1 and Mknk2 that phosphorylate eIF4E, and an essential TORC2 component, Mapkap1. miR-7a acts on the 3′-UTR of these genes. Inhibition of miR-7a increases protein levels of these components as well as the activity of their downstream signaling targets (p-Akt S473, pS6, and pEIF4E) in Min6 cells and primary mouse islets. Expression of the mitosis marker, phospho-histone H3, was increased, and immunofluorescence analysis further showed a 2.6 and 2.9 fold increase in the number of insulin+ cells expressing p-HH3 and Ki67, respectively, in dispersed adult murine islets transfected with anti-miR-7a, demonstrating that miR-7a negatively regulates adult primary beta cell proliferation. There was no change in cell survival as measured by TUNEL or in glucose stimulated insulin secretion in miR-7a deficient and control islet cells. Importantly, this miR-7a-mTOR-beta cell proliferation axis is conserved in primary human beta cells. Dispersed human islets transfected with control oligonucleotide showed no detectable replication, whereas miR-7a inhibitor transfection resulted in 0.5% insulin+ Ki67+ human islet cells. To establish the causal role of mTOR signaling in this response, we used the mTOR inhibitor rapamycin and found complete abrogation of the enhanced beta cell replication induced by miR-7a deficiency in both mouse and human islets. These data suggest that miR-7a acts as a brake on adult beta cell proliferation by inhibiting mTOR signaling and they implicate miR-7a as a therapeutic target for diabetes.

Supported by: NIH (P01DK49210), State of PA (4100043362)

140-LB

Signaling Pathway of β Cell Proliferation in Pancreatectomy is Distinct from that in High-Fat Diet-Loading in Mice

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High-fat (HF) diet-loading and partial pancreatectomy are good models for the evaluation of adaptive proliferation of β cells in rodents. Here we compared the role of glucokinase (Gck) and insulin receptor substrate 2 (IRS-2) in these mouse models. As we previously reported, both β cell-specific Gck haploinsufficient (*Gck*$^{+/-}$) mice and IRS-2-deficient (*IRS-2*$^{-/-}$) mice failed to increase β cell mass on a HF diet in association with insufficient upregulation of IRS-2, Pdx1 and cyclin D2 in islet. Next, *Gck*$^{+/-}$ mice, *IRS-2*$^{-/-}$ mice and wild type (*WT*) mice were subjected to 60% partial pancreatectomy (Px) or a sham operation (Sham). Both *Gck*$^{+/-}$ mice and *IRS-2*$^{-/-}$ mice, as well as *WT* mice, exhibited sufficient expansion of β cell mass and significant increase in β cell proliferation after Px, compared to Sham. There were no significant differences in the expression levels of Gck, IRS-2, Pdx1, cyclin D1 and cyclin D2 in islets between Sham and Px in all genotypes. Gene expressions of FoxM1, cyclin B1, Aurora kinase B and Birc5 in islets were increased by Px in *WT* mice and *Gck*$^{+/-}$ mice, while those were not increased by Px in *IRS-2*$^{-/-}$ mice. Thus, neither Gck nor IRS-2 was required for β cell proliferation after Px. Furthermore, the gene expression profiles of transcriptional factors and cyclin families demonstrated quite different patterns between Px and HF. Since *IRS-2*$^{-/-}$ mice demonstrated β cell expansion without upregulation of FoxM1 and its downstream molecules, β cell proliferation after pancreatectomy in *IRS-2*$^{-/-}$ mice might be mediated by independent pathway of both FoxM1 and insulin signaling. In conclusion, varying signaling pathways of β cell proliferation exist in respective experimental models. Partial pancreatectomy in mice may be an attractive model for therapy development by exploring the unique nature of β cell proliferation.

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

141-LB

Selective Activation of Gq Signaling in Pancreatic Beta Cells In Vivo Improves Beta Cell Function and Whole Body Glucose Homeostasis

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Impaired function of pancreatic beta-cells is one of the hallmarks of type 2 diabetes (T2D). Beta-cell function is modulated by various nutrients, hormones and neurotransmitters most of which act through G-protein coupled receptors (GPCRs). Pancreatic beta cells express many GPCRs which are linked to different functional classes of heterotrimeric G proteins including Gq. In order to be able to selectively stimulate beta-cell Gq signaling in vivo, we generated transgenic mice that expressed a Gq-coupled designer GPCR (Rq) in pancreatic beta-cells only (b-Rq mice). Importantly, this designer receptor can only be activated by an exogenously administered drug, clozapine-N-oxide (CNO), an otherwise pharmacologically inert compound. Prolonged activation of beta-cell Gq signalling by chronic CNO treatment of b-Rq mice was associated with elevated serum insulin and decreased blood glucose levels, increased pancreatic insulin content, increased beta-cell mass and rate of beta-cell proliferation, and elevated expression of several genes important for the maintenance of beta-cell function and mass, including IRS-2 and the transcription factors Pdx1, MafA, NeuroD1 and Ngn3. Chronic activation of beta-cell Gq signalling also protected b-Rq mice against hyperglycemia and glucose intolerance induced by consumption of a high-fat diet or treatment with low doses of streptozotocin. Studies with b-Rq-mice lacking IRS2 strongly suggested that IRS2 plays a central role in mediating the beneficial metabolic effects resulting from prolonged activation of beta-cell Gq signalling. In vitro studies demonstrated that the enhanced expression of IRS-2 triggered by activation of beta-cell Gq signaling required PKC-dependent ERK activation. These results suggest that agents aimed at enhancing Gq signaling in pancreatic beta-cells could become clinically useful as antidiabetic drugs.

142-LB

GABA Secretion from Human Pancreatic Islets is Pulsatile

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Gamma-aminobutyric acid (GABA) is found in high concentrations in the endocrine pancreas, the islets of Langerhans. It is generally believed that GABA is a paracrine signal regulating insulin and glucagon secretion. It is not known, however, what cells within the islet release GABA, what stimulates its release, and how it is secreted. Here we used CHO cells expressing GABAB receptors (GABA biosensors) to record GABA secretion from isolated human islets in real time. Islets were placed on a layer of GABA biosensors loaded with the Ca2+ indicator Fura 2. GABA secretion was detected by performing Ca2+ imaging of GABA biosensors. In the presence of islets, GABA biosensors showed repeated periods of activation, indicating pulsatile GABA secretion. The periods depended on the human islet preparation and ranged from 4 to 18 minutes, with an average of 9 minutes. GABA biosensor responses were blocked by CGP55845 (10 uM), a GABAB receptor antagonist, demonstrating that biosensors detected GABA. Changing the glucose concentration (1, 3, 11 mM), a major stimulus for islet endocrine cells, did not produce changes in the amplitude and frequency of GABA release. To test for the source of GABA, we sorted beta and alpha cells from human islets and placed them on biosensors. In the presence of beta cells, but not of alpha cells, biosensors recorded robust GABA secretion that also showed a pulsatile pattern. To determine that GABA metabolism is directly coupled to GABA secretion, we applied allylglycine to inhibit glutamate decarboxylase (GAD), the main enzyme producing GABA. This reduced both the amount of released GABA and the pulse frequency. Applying gamma-vinyl GABA to inhibit intracellular GABA conversion via GABA transaminase increased GABA secretion. These results indicate that GABA release from human islets depends on the metabolic pool of GABA. Moreover, the frequency at which GABA was released depended on GAD activity, suggesting a central role for this enzyme in the islet's oscillatory behavior.

Supported by: NIH, NIDDK (R01DK084321)

143-LB

Acute Deletion of SirT1 in Pancreatic Beta Cells Causes Decreased Insulin Secretion in Mice

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SirT1 functions as an (NAD)-dependent deacetylase and is involved in the regulation of cell metabolism. Previous studies have shown that SirT1 is a positive regulator of insulin secretion and conferred protection against diabetes in mice. These findings suggest that SirT1dysfunction is involved in the etiology of diabetes although the precise mechanism is largely unknown.

Using the inducible CreLox system, we aim to metabolically characterize a novel, inducible pancreatic beta cell-specific SirT1 knockout mouse (SirT1BKO) and determine the effect of SirT1 deletion on beta cell function.

When orally challenged with glucose, SirT1BKO mice displayed glucose intolerance which correlated with decreased insulin secretion. Isolated SirT1BKO islets secreted less insulin (6.2±0.6 ng per 10 islets vs. 10.0±0.9 control) at 20mM glucose. Islets were assessed for beta cell mass and insulin content however no significant difference was observed suggesting reduced insulin secretion is due to an intracellular defect. When islets were stimulated with KIC (a substrate for the TCA cycle) SirT1BKO cells still secreted less insulin indicating the defects are downstream of glycolysis. Distally, SirT1BKO cells exhibited normal insulin granule exocytosis revealed by capacitance measurements. Interestingly, preceding this step, SirT1BKO cells displayed decreased influx of calcium at 20mM glucose. This may reflect defects upstream, namely mitochondrial metabolism. SirT1BKO cells displayed a 38±2.1% decrease in glucose-induced hyperpolarization compared to controls by measuring mitochondrial membrane potential, indicating the proton motive force is reduced. To gain mechanistic insight, SirT1 knockdown in Min6 cells resulted in increased expression of mitochondrial genes, Pgc1α, Cox2, and Pparγ, thereby altering mitochondrial dynamics ultimately affecting insulin secretion.

These data highlight SirT1's role in insulin secretion and its potential for therapeutic use in diabetic patients.

ISLET BIOLOGY—SIGNAL TRANSDUCTION

144-LB

Toll-Like Receptor 3 Mediates Coxsackievirus B4 Acceleration of Type 1 Diabetes in NOD Mice

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Viruses play an important role in the pathogenesis of Type I Diabetes Mellitus (T1DM). Specifically, coxsackievirus B4 (CVB4), cytomegalovirus, and rubella have been implicated in triggering T1DM in genetically susceptible individuals. Viral infection of beta cells initiates disease by direct beta cell damage, beta cell toxicity from the acute antiviral response, or release/induction of beta cell self-antigens triggering autoimmune destruction. A pancreatrophic strain of CVB4, which is associated with the development of T1DM in humans, accelerates the development of T1DM in non-obese diabetic (NOD) mice. Toll-Like Receptors (TLRs) are transmembrane receptors, which are activated by distinct pathogenic signature molecules and mediate an innate immune response. TLR3, in particular, is activated by viral dsRNA or polyinosinic-polycytidylic acid, a synthetic dsRNA, and functional TLR3 is broadly expressed by NOD mice and human pancreatic beta cells, suggesting that TLR3 signaling may be important in CVB4 acceleration of T1DM. We used NOD mice deficient in TLR3 (TLR3 KO NOD mice) to test the hypothesis that TLR3 signaling is important in CVB4 acceleration of T1DM in NOD mice. We report that TLR3 KO NOD mice are markedly protected from CVB4 acceleration of T1DM compared to wild type NOD mice. Although that it has been shown that there is no difference in the incidence of spontaneous diabetes between TLR3 KO NOD and TLR3 heterozygous mice, suggesting TLR3 is not important for spontaneous development of T1DM in NOD mice, this result clearly indicates the distinct importance of TLR3 signaling in environmental (i.e. viral) induction of T1DM. Thus, these findings reveal, for the first time, that TLR3 signaling mediates CVB4-triggered T1DM in genetically susceptible NOD mice, and lend additional support to the idea that therapies targeting abnormal TLR3 signaling may be an effective novel approach to the treatment of viral-induced T1DM.

Supported by: NIH, NIDDK (1R15 DK081192-01 (K.D.M.))

145-LB

Paracrine Regulation of Human Delta Cells is Impaired in Type 2 Diabetes

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The delta cell of the islet of Langerhans secretes somatostatin to inhibit the secretion of the gluco-regulatory hormones insulin and glucagon and thus indirectly impacts glucose metabolism. Despite its influence on islet hormone output, the delta cell has been barely investigated. We have found that paracrine signaling from human beta and alpha cells stimulates somatostatin secretion. Beta and alpha cells respectively release the neurotransmitters GABA and glutamate and activate GABAA and AMPA/kainate receptors on delta cells. In response, delta cells secrete somatostatin, providing negative feedback to beta and alpha cells. These feedback loops restrain insulin and glucagon secretion and likely avoid exacerbated islet hormone secretory responses. Because islet hormone secretion is deranged in type 2 diabetes, we investigated delta cell function in islets derived from type 2 diabetic donors. We exposed islets from healthy and diabetic donors to KCl depolarization, acetylcholine, GABA, and kainate (a glutamate receptor agonist) and measured somatostatin secretion. Whereas somatostatin responses to acetylcholine were not affected, those to KCl and kainate were significantly diminished by 50%. Strikingly, responses to GABA were reduced by 90%. This suggested that the GABA signaling pathway may be more susceptible to pathological conditions associated with type 2 diabetes. We therefore examined GABA secretion in real time using GABA biosensor cells. GABA secretion could be readily measured from islets from healthy donors, but could not be detected from islets from type 2 diabetes donors. Our results indicate a selective impairment of GABA paracrine signaling within the human islet in type 2 diabetes and suggest specific pathological mechanisms acting on this signaling pathway. While this awaits further exploration, the loss of delta cell responsiveness to paracrine signals may help explain why delta cells become refractive to stimulation with nutrients in type 2 diabetes.

Supported by: DRIF, NIDDK (F32DK083226, 5U19AI050864-10, R01DK084321), JDRF, Swedish Research Council

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