COMPLICATIONS—HYPOGLYCEMIA

1-LB

A Phase 2 Comparative Safety PK/PD Study of Stable Nonaqueous Glucagon (G-Pen) vs. Lilly Glucagon for Treatment of Severe Hypoglycemia

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Severe hypoglycemia remains a significant unmet medical need in patients with diabetes. Currently approved rescue products (Lilly Glucagon for Injection [rDNA origin], Novo Nordisk GlucaGen® [glucagon [rDNA origin]] HypoKit®) are based on lyophilized formulations, which require reconstitution at time of use, complicating administration in emergency situations. Xeris is developing a ready-to-use, soluble liquid glucagon formulation in a pre-filled syringe, based on biocompatible, non-aqueous solvents that suppress the fibrillation of glucagon typically observed in aqueous solutions. The G-Pen® (glucagon injection) formulation has demonstrated excellent chemical stability after storage as a liquid at room temperature. A Phase 2, double-blind, cross-over, comparative pharmacology study in fasted, healthy, non-diabetic volunteers (n = 30) demonstrated that subcutaneously (SC) injected G-Pen® glucagon (1 mg) resulted in bioequivalent mean glucose AUC, Cmax, and Tmax as compared to 1 mg SC doses of Lilly Glucagon reference drug. The TOST procedure indicated significance (p<0.05) for all pairwise contrasts and all 95% confidence intervals for the ratio of means were contained in the interval 0.80 to 1.2, the FDA standard for bioequivalence. Injection of both 1 mg G-Pen® [Cmax 148.0 (24.9) mg/dL] and Lilly Glucagon [Cmax 154.9 (28.0) mg/dL] showed rapid, marked elevation of blood glucose levels from baseline. Despite therapeutic equivalence, pharmacokinetic serum glucagon parameters (AUC, Cmax, Tmax) were significantly different between Xeris and Lilly preparations. There were no apparent safety or tolerability issues with any of the glucagon treatments; all AEs observed were those expected with rescue injections of glucagon. No serious adverse reactions were reported. Overall these data support the development of G-Pen® as a commercial rescue treatment for severe hypoglycemia in a single-use auto-injector pen format.

Supported By: NIDDK (5R44DK086839-03)

2-LB

Different Clinical Predictors of Nonsevere and Severe Hypoglycemia during Treatment with Glargine or Standard Care in the ORIGIN Trial

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Hypoglycemia limits treatment of diabetes and is associated with increased risks. This large, long-term, randomized trial (NCT000809784) compared use of insulin glargine with standard care in people with dysglycemia (IGT, IFG, or T2DM) and high cardiovascular risk. We analyzed data from 12,537 participants to identify baseline and on-treatment factors associated with hypoglycemia, including treatment assignment (Figure), NHW continued to have 32% and 31% (p<0.01) more frequent hypoglycemic episodes compared to non-Hispanic Blacks (NHB) and 303 Hispanics (His). Total number of hypoglycemic episodes per year differed across major race/ethnic groups. The total numbers of interim hypoglycemia episodes with symptoms were reported at each visit. The current analysis included 1096 non-Hispanic Whites (NHW), 303 non-Hispanic Blacks (NHB) and 303 Hispanics (His). Total number of hypoglycemic episodes per year were more frequent in NHW compared with NHB and His (mean [95% CI]: NHW, 10.9 [11.1]; NHB, 6.5 [7.0]; His, 7.0 [7.8]; P<0.001). After adjustment for other predictors of hypoglycemia, including treatment assignment (Figure), NHW continued to have 32% and 31% (P<0.01) more frequent hypoglycemic episodes compared with NHB and His, respectively. Very similar patterns were seen for severe and nocturnal hypoglycemia. Although intensive treatment was a significant predictor of hypoglycemic events, there was no interaction between treatment effect and ethnicity. Together with our previous report, these data suggest that the risk/benefit ratio for intensive glycemic control differs between major racial/ethnic groups in the VADT.

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4-LB

Hypoglycemic Episodes among Race/Ethnicity Groups in the VA Diabetes Trial (VADT)

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We have previously shown that the effect of intensive glycemic control on CVD outcomes differed across major race/ethnic groups in VADT. We now determined whether the frequency of hypoglycemic episodes in response to this therapy differed across major race/ethnic groups. The total numbers of intermittent hypoglycemia episodes with symptoms were reported at each visit. The current analysis included 1096 non-Hispanic Whites (NHW), 303 non-Hispanic Blacks (NHB) and 303 Hispanics (His). Total number of hypoglycemic episodes per year were more frequent in NHW compared with NHB and His (mean [95% CI]: NHW, 10.9 [11.1]; NHB, 6.5 [7.0]; His, 7.0 [7.8]; P<0.001). After adjustment for other predictors of hypoglycemia, including treatment assignment (Figure), NHW continued to have 32% and 31% (P<0.01) more frequent hypoglycemic episodes compared with NHB and His, respectively. Very similar patterns were seen for severe and nocturnal hypoglycemia. Although intensive treatment was a significant predictor of hypoglycemic events, there was no interaction between treatment effect and ethnicity. Together with our previous report, these data suggest that the risk/benefit ratio for intensive glycemic control differs between major race/ethnic groups in the VADT.

Supported By: ADA (1-06-CR-32), NIH (R01DK067690, R01HL094775)

Means and SE (error bars) adjusted for treatment assignment, prior-CVD events, baseline variables, and 4-LB treatment allocation, and on-treatment A1C. Sulfonylurea use ( Hazard Ratio [HR] 2.07 for non-severe, 1.35 for severe) and glargine treatment (HR 4.53 for non-severe, 3.57 for severe) were independently associated with higher risk in both categories. Risk of non-severe events was lower with older age (HR [95%CI] 0.98 [0.98-0.99]), and higher BMI (HR 0.97 [0.96-0.98]), and higher with presence of diabetes (HR 1.52 [1.21-1.92]) and higher baseline A1C (HR 1.24 [1.14-1.35]). Risk of severe events was higher with older age (HR 1.04 [1.03-1.06]), hypertension (HR 1.51 [1.41-2.00]), higher serum creatinine (HR 1.01 [1.01-1.02]), lower with higher MMSE score (HR 0.96 [0.93-0.99]), and unrelated to baseline glycemic status. With glargine treatment, risk of both non-severe and severe events was higher at lower on-treatment A1C, with standard care risk of severe events increased at higher A1C. Conclusions: Overall incidences of hypoglycemia were low. Sulfonylurea and glargine are associated with both non-severe and severe events, but other independent predictors differ between categories. Non-severe and severe events affect different people and occur in different settings. Awareness of predictors may guide individualized therapy.

Supported By: Sanofi

ADA-Funded Research

For author disclosure information, see page LB1.
COMPLICATIONS—MACROVASCULAR—ATHEROSCLEROTIC CARDIOVASCULAR DISEASE AND HUMAN DIABETES

5-LB
Natural Language Processing of Clinical Notes in Electronic Health Records to Improve Capture of Hypoglycemia

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Hypoglycemia is underascertained in healthcare billing data, especially for mild or moderate events. Clinical notes in electronic health records (EHR) include details of medical encounters that may not be represented in structured data fields. We assessed whether natural language processing (NLP) of clinical notes increases capture of hypoglycemia events and hypoglycemia severity. The Humedica statistically deidentified EHR database includes information on over 25 million patients from 195 hospitals throughout the United States. We identified all patients in Humedica with an International Classification of Diseases, Ninth Revision (ICD-9) diagnosis code for diabetes mellitus between January 2007 and September 2013. Hypoglycemia was identified via NLP of clinical notes and ICD-9 codes within structured data fields. The hypoglycemia NLP algorithm was developed iteratively by specifying, reviewing, and updating term lists that originated from standard clinical nomenclature. Term analogies were excluded to account for differences in spacing, hyphenation, and spelling. A clinical nurse specialist manually identified additional terms from notes and the algorithm searched for expressions that were highly correlated with known hypoglycemia terms. Of 1,914,324 patients with diabetes, 286,388 (15.0%) had ≥ 1 hypoglycemia event identified via NLP and 148,158 (7.7%) had ≥ 1 event identified via ICD-9. Only 49,544 patients had an event identified by both NLP and ICD-9. Information on severity was available for ≥ 1 event for 39,241 patients (13.4%) with NLP-identified hypoglycemia; 19,984 patients had ≥ 1 event described as mild to moderate and 23,237 had ≥ 1 event described as severe. NLP of clinical notes broadened the capture of hypoglycemia events relative to ICD-9 diagnoses alone and identified a largely different set of events. Mild-moderate events were underrepresented and may not be reported to providers or may not include descriptions of severity when noted.

6-LB
Enhanced Prediction of Cardiovascular Events by Adding Novel Biomarkers to Clinical Risk Factors in the ORIGIN Trial

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The measurement of large numbers of biomarkers in stored blood, using new assay technologies, from carefully phenotyped and prospectively followed individuals may identify new biomarkers and physiologic pathways for cardiovascular (CV) events in people with dysglycemia and improve the ability of clinical risk factors to identify susceptible individuals. The concentration of 237 out of 284 biomarkers that were assayed in the stored baseline serum samples from 8401 ORIGIN trial participants using the Human Discovery Multi-Analyte Profile (DiscoveryMAP® 250+) platform (Myriad RBM, Inc., Austin, TX, USA) were detectable in >99% of people. Participants were divided into a model building group (N=5630) and a model validation group (N=2771). The levels of these biomarkers were added to the following risk factors in the model building group (male sex, age [male≥55 or female ≥65], prior CV event, albuminuria, smoking, established diabetes, LDL/HDL, established hypertension) in a Cox regression model if the P value for their inclusion was < 0.05/237 (i.e. < 0.00021). The following biomarkers were identified as independently adding to the ability of clinical risk factors to predict the first occurrence of nonfatal MI, nonfatal stroke or CV death during a median follow-up period of 6.2 years: a) trofile factor 3; b) angiotensin-Z; c) N Terminal pro BNP; d) glutathione S-transferase alpha; e) osteoprotegerin; f) alpha 2 macroglobulin; g) peroxiredoxin 4; and h) apolipoprotein B; the largest P value for inclusion of any biomarker was 0.000083. Inclusion of these biomarkers increased the area under the receiver operating characteristic curve from for predicting CV events from 0.62 (95%CI 0.60, 0.64) to 0.72 (95%CI 0.71, 0.74). If validated in the validation subset, we will have identified 8 novel biomarkers that together strongly increase the ability to predict CV events in people with dysglycemia.

7-LB
WITHDRAWN

8-LB
Effect of Baseline Atherosclerosis on Long-term Consequences of Intensive Glycemic Control on Cardiovascular Outcomes: A Subset Analysis of Coronary Artery Calcification (CAC) in the Veterans Affairs Diabetes Trial (VADT)

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We previously reported that intensive glucose lowering (INT) significantly reduced a composite cardiovascular outcome in those with low baseline CAC, but not in those with high CAC, over the median 5.6 years follow-up of the VADT. We now report the results of nearly 10 years of combined intervention and observational follow-up of this subset of VADT study subjects that were randomized to INT or standard (STD) therapy and received baseline measures of vascular calcification. 301 participants from 7 VA sites had baseline CT measures of CAC at the beginning of the VADT. Data were collected on these subjects during the VADT and during approximately 4 more years of observation utilizing the VA, CMS and NDI databases for procedures, hospitalizations and death. The pre-specified primary outcome was a composite of major cardiovascular events including non-fatal MI or stroke occurring in hospitalization, new CHF, amputation for ischemic diabetic gangrene, or cardiovascular-related death. All outcome assessments were fully blinded. HbA1c separation between the INT and STD arms in the subset was 1.4% at the conclusion of the VADT (medians of 7.1% vs. 8.5%, respectively), declined to 0.9% one year after the trial ended (7.3% vs. 8.2%, respectively), and to 0.4% three years after the trial (7.7% vs. 8.1%, respectively). Blood pressure, lipid and lipoprotein concentrations were maintained at similar levels during and after the trial in the INT and STD arms. In individuals with low CAC (< 100 Agatston units) or high CAC (>100 units), INT was associated with a lower incidence of major cardiovascular outcomes (< .05 for both groups).

After nearly 10 years of follow-up of this subset of VADT study subjects, the effects of INT on major cardiovascular events appeared favorable regardless of the degree of baseline atherosclerosis.

Supported By: 5R01HL094775

9-LB
Accelerated but Compositional Unaltered Carotid Atherosclerosis Assessed by MRI in Newly Diagnosed Type 2 Diabetes

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Type 2 diabetes is associated with macro-vascular complications such as cerebral infarctions, myocardial infarctions, and peripheral vascular disease. It is feasible that this may be caused by not only accelerated atherosclerosis, but also by altered composition of the arterial wall contents, as it is well-known that certain characteristics of the atherosclerotic plaque are associated with increased risk of a clinical event. We aimed to investigate whether there are differences in the morphology and composition of atherosclerosis in the carotid arteries assessed by MRI in newly diagnosed type 2 diabetic patients compared to non-diabetic control subjects.

One hundred type 2 diabetic patients diagnosed within the last 5 years and 100 age- and gender-matched non-diabetic control subjects underwent magnetic resonance imaging of carotid arteries bilaterally in a 1.5 Tesla Phillips Achieva MRI scanner with a dedicated carotid coil. Scans were performed with four different contrast weightings and subsequently analysed in a software tool to assess atherosclerosis morphology and composition.

In the diabetes group 142 carotid arteries and in the control group 172 carotid arteries were available for analysis. In diabetic patients the minimal lumen area was 29.8% smaller (P<0.001) and maximal normalized wall index was 3.7% higher (P=0.046) than in the control subjects. This remained significant after adjusting for LDL-cholesterol and smoking habits (minimal lumen area P<0.001 and maximal normalized wall index P=0.030). Relative maximal calcification was not significantly different between groups (P=0.497), as was the case for relative maximal necrotic core (P=0.086), relative maximal hemorrhage (P=0.172) and relative maximal loose matrix (P=0.876) (all volume percentages).
Clear signs of accelerated carotid atherosclerosis assessed by MRI were found at a very early stage of type 2 diabetes, but no sign of altered arterial wall compositional contents was found.

**10-LB**

**Ranolazine, Ethnicity, and the Metabolic Syndrome (REMS) Study: A Pilot Study Showing Differential Effects amongst Caucasians, African Americans, and Asians in Exercise Tolerance Time and Glycemic Control but Not Angiina Scores**

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Background: Ranolazine is a well proven and effective drug for the treatment of angina. It may also have some favorable arrhythmogenic and glucose metabolism properties. There is however limited data on the drug’s safety and efficacy in non-Caucasian ethnic patient population.

Methods: Single center, prospective, randomized, 6 month open label trial of ranolazine (RAN) vs. standard of care (SOC) in stable CAD pts.

Results: Of the 122 pts randomized, there were 55 Caucasians, 50 African Americans and 17 Asians. Mean age 62+11 yrs, 59% males. Hypertension (93%) was the most common metabolic syndrome component followed by abdominal obesity (81%), elevated glucose (46%) and low HDL (44%) or high triglycerides (28%).

Comparing RAN to SOC pts, more RAN pts improved their exercise treadmill (ETT) time (67% vs. 45%, p=0.03) while duration improved an average of 40 sec (p=0.13). Pts with angina improved ETT time more than those with angina equivalents (88% vs. 19 sec, p=0.03). Lipid parameters (mean HDL, 49, LDL 85 g/ml) and anthropometric measurements (BMI=30, WHR=0.97) did not change. HgbA1c however was significantly less in the RAN group (6.2 vs. 7.1, p=0.02) after 6 months.

Baseline characteristics between the 3 ethnic groups differed significantly (p<0.001). ETT duration improved in more Caucasians (64%) and Asians (56%) compared to African Americans (42%). Similarly glycerol control tended to improve in Caucasians and Asians while it worsen in African Americans. Adverse events trended higher in RAN group (25% vs. 15%, p=0.08) with Asians tolerating the drug best.

Conclusions: Our pilot study findings suggest that ranolazine improves exercise duration and glyceric control in CAD pts over 6 months. There may however be a differential effect on these parameters in pts with an African American or Asian ethnicity. These findings warrant further validation in larger sample sizes.

**11-LB**

**Autoantibodies in Adult Type 2 Diabetes Having Atrial Fibrillation Cause Acute Intracellular Ca2+ Increase in HL-1 Adult Atrial Cardiomyocytes by IP3 Receptor-Mediated Mechanism**

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Type 2 diabetes mellitus (T2DM) is associated with increased occurrence of atrial fibrillation (AF). Increased Ca2+ release from sarcoplasmic reticulum contributes to AF in animal models; however, the mechanism underlying the association between AF and T2DM is unknown. Since in our prior work, circulating T2DM autoantibodies (AA) caused elevated intracellular Ca2+ in endothelial cells, we hypothesize that T2DM AA perturb Ca2+ homeostasis in atrial cardiomyocytes contributing to AF. Protein A-purified IgG was obtained in a cohort of adult T2DM participants from the Veterans Affairs Diabetes Trial (mean: age 64 yrs, diabetes duration 11 yrs) and other subjects. To test the acute effects of AA on Ca2+ signaling, we used Ca2+ fluorescent dye fura-2 and cultured HL-1 adult mouse atrial cardiomyocyte cells that exhibit rhythmic Ca2+ oscillations. IgG (1 g/ml) from 14/18 diabetic AF patients caused acute intracellular Ca2+ elevation in HL-1 cells compared to 12/22 diabetic and 1/9 non-diabetic subjects without AF; left ventricular hypertrophy and another arrhythmia (IP=0.001). The T2 DM, AF IgG-induced Ca2+ release in HL-1 cells was insensitive to verapamil (20 μM), nifedipine (25 μM) or BTP-2 (5 μM), indicating that the elevation of intracellular Ca2+ is not through voltage-gated Ca2+ channels or store-operated Ca2+ entry. On the other hand, Xestospongin C (10 μM) a membrane-permeable IP3 receptor antagonist, significantly decreased elevation of intracellular Ca2+ stimulated by the T2DM, AF IgG (60% reduction, P < 0.01, n = 5 experiments). 2-Aminoethoxydiphenyl borate (2-APB, 100 μM), another IP3R inhibitor, completely blocked IgG-induced Ca2+ elevation. These data suggest that AA cause IP3 receptor activation which may be involved in the mechanism for AF in some older type 2 DM subjects.

AA preceded AF occurrence in some subjects suggesting they exert more than a bystander role in T2DM atrial fibrillation.

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**12-LB**

**Rotating Night Shift: Risk of Type 2 Diabetes and Metabolic Disorders**

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Compromised Quality and Quantity of sleep may be a novel risk factor for metabolic syndrome and type 2 diabetes mellitus due to interference with diet, circadian metabolic rhythms, and lifestyle. The long-term elevated cortisol leads to the high blood sugar level and decreased insulin associated with higher levels of cholesterol, triglycerides and BMI may contribute to the increased risk of metabolic syndrome and CVDs.

The aim of the present study was to investigate the risk of type 2 diabetes and metabolic disorders in rotating night shift workers.

In the present case-control study, we recruited 30 healthy nursing professionals, aged 20-40 year, performed day and night shift duties (continuous 9 days night shift with alternate day shifts) and were randomly selected from the Trauma Center, GM and Associated Hospitals, King George Medical University and 30 age sex matched controls were also recruited in this study. In the Present study, we have investigated the effect of rotating night shift on Fasting blood glucose level and Insulin resistance.

Data were analysed by unpaired t-test. BMI was higher in cases (23.69±1.96) as compared to controls (21.66±4.04) (p=0.05) found in fasting blood sugar between night workers (78.38 ± 9.40) and controls (75.14 ± 14.77). Fasting insulin level was increased in night workers (4.05± 2.45) than controls (2.75± 2.53) and was statistically significant (p<0.05). Insulin resistance was slightly increased among night workers (0.80± 0.50) than controls (0.53± 0.51) which was statistically significant (p<0.05).

Night shift work is associated with increased risk of insulin disturbance leading to insulin resistance making them more prone for metabolic syndrome and type 2 diabetes.

**Supported By:** India Council of Science & Technology

**13-LB**

**Determinants of Metabolic Control in the Early Phase of Diabetes**

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Dietary habits and subclinical inflammation relate to insulin sensitivity and secretion. However, their impact on the early development of metabolic control of type 2 (T2D), but also type 1 diabetes (T1D) is largely unclear. We analyzed parameters possibly affecting metabolic control in diabetes patients during the first two years after diagnosis. Insulin secretion (ratio of C-peptide release (6 min/0 min) from the glucagon stimulation test) and glycemic control (A1c) were measured in 103 (31% T1D) diabetes patients. Insulin sensitivity (SI) from the intravenous glucose tolerance test was assessed in T2D (n=61). Multivariable regression models were used to assess the prospective associations of food consumption frequencies and cytokine concentrations at baseline with changes in glycemic control, insulin sensitivity and secretion after two years. Patients with T1D and T2D exhibited good glycemic control (A1c 7.1±1.6% and 6.4±1.0%) at diagnosis. Within two years, insulin secretion did not change in T1D (1.7 (Q25, 75: 1.4; 2.0) vs. 1.6 (Q25, 75: 1.3; 2.1)). In T2D, insulin secretion increased (1.9 (Q25, 75: 1.6; 2.2) vs. 2.1 (Q25, 75: 1.8; 2.4); p<0.001) and insulin sensitivity (2.0 (1.3; 2.8) 10^{-4} min^{-1} μmol/ml^{-1} vs. 1.8 (1.2; 3.0)) was unchanged during the first two years. In T1D, a more frequent baseline consumption of non-whole-grain foods related to lower insulin secretion (-15% (95% CI: -26; -2), p=0.028) and increased A1c (0.81% (95% CI 0.20; 1.41), p=0.011) at follow-up, adjusted for age, sex, BMI, glucose-lowering medication. Also in T1D, higher baseline interleukin (IL)-6 predicted subsequently increased A1c (0.77% (95% CI 0.13; 1.42), p=0.021). In T2D, a more frequent consumption of meat/meat products related to lower insulin sensitivity (-9% (95% CI: -15; -1), p=0.029) after two years. During the initial course of the disease, intake of non-whole-grain foods and subclinical inflammation negatively affect insulin secretion in T1D, whereas intake of meat/meat products impairs insulin sensitivity in T2D.

**Supported By:** German Center for Diabetes Research

**14-LB**

**Beneficial Effect of Multifactorial Intervention on the Prognosis of Patients with Type 2 Diabetes Mellitus and Critical Limb Ischemia/Peripheral Arterial Disease**

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The combination of critical limb ischemia/peripheral artery disease (PAD) and diabetes mellitus type 2 (T2DM) is known for poor survival. The last major publication on successful management of such a patient population reported a...
50% mortality and 25% amputation rate after six years of follow-up. We have analyzed whether more recent treatment advances of T2DM and PAD in the last five years have ameliorated those detrimental effects.

In a prospective study we enrolled 366 patients (34% female) with PAD, 38% had T2DM, 33% impaired glucose tolerance (IGT) and 29% normal glucose tolerance (NGT). As expected the patient cohort had a high cardiovascular risk factor (CRF) burden: 92% hypertension, 37% hyperlipidemia, 74% active or former smoker, Connary heart disease (CHD) was known in 32% and carotid artery disease (CAD) in 39% of the patients. Within 6 months the target values of CRF control - LDL-Cholesterol <100 mg/dL, blood pressure <140/80 mm Hg, HbA1c in DM <7.0% were reached in 58%, 69% and 89%. Patients followed a strict control visit program in the center for 5 years.

The overall survival of this cohort was 89.3% after 4.9 years. MACCE (combination of death, non-fatal myocardial infarction or stroke) free survival was 84.3% and event free survival including interventional or surgical procedures due to critical limb ischemia/PAD was 88%. Patients with T2DM showed a survival of 87.8% compared to 89.3% PVE, and 95.2% NGT (p=0.161). MACE free survival was 81.3% for T2DM, 87.6% for PVE, and 92.4% NGT (p=0.059). Additionally, event free survival was 85.5% for T2D, 71.5% for PVE, and 77.1% for NGT (p<0.155).

In summary, strict multifactorial management induced a dramatic reduction in the annual death rate (2.8 for patients with T2DM and PAD), MACE and amputation. Thus, management of such patients should be restricted to centralized centers to improve outcome for the patients.

### 15-LB

**Impairment of Autophagy in Endothelial Cells Prevents Shear-Stress-Induced Increases in Nitric Oxide Bioavailability**

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Autophagy is a lysosomal catabolic process by which cells degrade or recycle their contents to maintain cellular homeostasis, adapt to stress, and respond to disease. Impairment of autophagy in endothelial cells studied under static conditions results in oxidative stress and impaired nitric oxide (NO) bioavailability. We tested the hypothesis that vascular autophagy is also important for induction of NO production caused by exposure of endothelial cells to shear stress (i.e., 3 h x 20 dyn/cm²). Atg3 is a requisite autophagy pathway mediator. Control cells treated with scrambled, non-specific siRNA to Atg3 (Atg3 siRNA) showed increased autophagy, mitochondrial turnover, reactive oxygen species (ROS) production, endothelial NO synthase (eNOS) phosphorylation, and NO production upon exposure to shear stress (p<0.05 for all). In contrast, cells with >85% knockdown of Atg3 protein expression (+Atg3 siRNA) exhibited less mitochondrial turnover, a profound impairment of eNOS phosphorylation, and were incapable of increasing NO in response to shear stress. These findings reveal that autophagy not only plays a critical role in maintaining NO bioavailability, but may also be a key regulator of oxidant / antioxidant balance and inflammatory / anti-inflammatory balance that ultimately regulate endothelial cell responses to shear stress.

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### 17-LB

**Protein Kinase C Theta Is Involved in Angiotensin II-stimulated PAI-1 Expression in Vascular Smooth Cells**

HONG-CHI CHEN, YI-CHUN LIAO, YUNG-CHEH LIN, Hualien City, Taiwan

Protein kinase C (PKC), a diverse family of serine/threonine kinase, is involved in many important physiological events in various cells, including development, memory, differentiation, proliferation, apoptosis, survival, migration and carcinogenesis. Increased expression/activity of PKC isoforms in vascular smooth muscle cells (VSMC) have been shown to cause vasconstriction, leading to hypertension. Furthermore, the activation of PKC resulted from high concentrations of glucose and nonesterified fatty acids has been shown in vascular cells of diabetic and insulin resistant patients, and of animal models, suggesting that it has significant roles in microvascular complication, cardiac hypertrophy, and in promoting atherosclerosis. PKC-theta, a member of nobile PKC, is expressed in mouse skeletal muscle, human 9 lymphocytes, thymocytes, T cell lines, megakaryoblastic cells and platelets. Studies on T cell activation recognize PKC-theta as a master inducer of T cell proliferation and IL-2 production, ascertaining its essential role for the activation and survival of 1 cells. However, understanding of PKC-theta’s role in VSMC is very limited. Our studies revealed that Angiotensin II (Ang II) stimulated PKC-theta phosphorylation in rat VSMC and that both Ang II-stimulated mRNA and protein expressions of plasminogen activator inhibitor-1 (PAI-1), the major regulator of both tissue and urokinase plasminogen activators, were inhibited by a myristoylated PKC-theta pseudosubstrate, suggesting a functional role of PKC-theta in VSMC. In addition, the expression of a constitutively active PKC-theta induced PAI-1 promoter activity, and the PKC-theta inhibition reduced Ang II-induced nuclear factor-kB (NF-kB) activation. In summary, our data strongly suggest that PKC-theta-NF-kB signaling plays an important role in mediating Ang II-stimulated PAI-1 transcriptional activation in VSMC.

**Supported By:** ADA-Funded Research

### 18-LB

**Protein Phosphatase 2A Activation Contributes to Cardiovascular Complications that Occur in mice with Diet-induced Obesity**

LEENA PANNEERSEELAN-BHARATH, TING RUAN, YOU LI LI, LANCE DEETER, DAVID KUNZ, QUAN-JIANG ZHANG, E. DALE ABEL, J. DAVID SYMONS, Salt Lake City, UT

Cardiovascular complications exist in individuals with diet-induced obesity (DIO) and type 2 diabetes (T2DM). Our results from endothelial cells treated with palmitate, lipid-infused mice, and obese mice indicate that protein phosphatase 2A (PP2A) binds directly with eNOS and disrupts interactions among Akt-Hsp90-eNOS. When this occurs, eNOS enzyme function and NO bioavailability are impaired, and endothelial dysfunction and hypertension exist. In each model system indices of NO bioavailability are restored by pharmacological and genetic approaches that limit production of the FFA metabolite ceramide. Further, indices of NO bioavailability are preserved in endothelial cells and lipid-infused mice when PP2A activation is suppressed. We hypothesized that arterial dysfunction and hypertension that occur in obese vs. lean mice is prevented by PP2A inhibition. First we verified the ability of Lixte Biotechnology 100 (LB1, Setauket, NY) to suppress (p<0.05) arterial PP2A activity in mice after 3 and 14 days. Next, 7 week-old mice consumed standard (CON, n=20) or high-fat (HF, n=20) chow for 12 weeks. Subgroups (n=10) of CON and HF mice received IP injections of vehicle (V) or LB1 (1 mg/kg/day) for the last 14-days. HF mice had higher body mass, gonadal fat pad mass, and area under the curve during a glucose tolerance test vs. CON mice (all p<0.05) regardless of LB1 treatment. Furthermore, arterial function in HF vs. CON was not impaired (p<0.05). p-eNOS to total eNOS was impaired (p<0.05) in homogenates of aorta, iliac, femoral arteries from HF mice vs. all groups. In the same admixture, Akt and Hsp90 co-immunoprecipitation with eNOS was less (p<0.05) in samples from HF-V mice vs. all groups. In the same model system, indices of NO bioavailability are restored by pharmacological and genetic approaches that limit production of the FFA metabolite ceramide. Further, indices of NO bioavailability are preserved in endothelial cells and lipid-infused mice when PP2A activation is suppressed. We hypothesized that arterial dysfunction and hypertension that occur in obese vs. lean mice is prevented by PP2A inhibition. First we verified the ability of Lixte Biotechnology 100 (LB1, Setauket, NY) to suppress (p<0.05) arterial PP2A activity in mice after 3 and 14 days. Next, 7 week-old mice consumed standard (CON, n=20) or high-fat (HF, n=20) chow for 12 weeks. Subgroups (n=10) of CON and HF mice received IP injections of vehicle (V) or LB1 (1 mg/kg/day) for the last 14-days. HF mice had higher body mass, gonadal fat pad mass, and area under the curve during a glucose tolerance test vs. CON mice (all p<0.05) regardless of LB1 treatment. Furthermore, arterial function in HF vs. CON was not impaired (p<0.05). p-eNOS to total eNOS was impaired (p<0.05) in homogenates of aorta, iliac, femoral arteries from HF-V mice vs. all groups. In the same admixture, Akt and Hsp90 co-immunoprecipitation with eNOS was less (p<0.05) in samples from HF-V mice vs. all groups. Endothelium-dependent vasorelaxation was attenuated (p<0.05) in femoral arteries from HF-V vs. all groups. Suppression of PP2A activity in vivo proved critical due to arterial function in obese mice.

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**For author disclosure information, see page LB91.**
Disruption of Mitochondrial Quality Control by Myo-Inositol Oxygenase (MIOX) in Diabetic Kidney Disease
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Diabetic kidney disease (DKD) is believed to be associated with oxidative stress and mitochondrial injury. MIOX, a specific tubular enzyme, modulates redox imbalance and apoptosis in tubular cells in diabetes, but the mechanisms remain unclear. We investigated the role of MIOX in perturbation of mitochondrial quality control machinery including mitochondrial dynamics and selective autophagy (mitophagy) under high glucose (HG) ambiance both in vitro and in vivo. In HK-2/LLC-PK1 cells with HG treatment or cells stably transfected with MIOX, upregulation of MIOX was accompanied with mitochondrial fragmentation and depolarisation, inhibited autophagy and mitophagy, and altered expressions of mitochondrial dynamic and mitophagic proteins. As a result, dysfunctional mitochondria without autophagic removal generate excessive ROS and initiate apoptotic pathway, as indicated by increased Mitosix intensity, Bax activation, cytochrome C release and apoptosis. MIOX siRNA or D-glucarate, an inhibitor of MIOX could partially reverse these perturbations. The mechanism by which MIOX disrupt mitochondrial integrity may be via its modulation on ROS production and Pin1-dependent MnSOD-Parkin interaction. In proximal tubules of STZ-induced diabetic mice, an increased MIOX expression and mitochondrial fragmentation but defective autophagy was observed. Dietary supplementation of D-glucarate to diabetic mice decreased MIOX expression, which also attenuated tubular damage and improved renal functions. Importantly, the tubular cells with drug treatment showed partial restoration of mitochondrial quality control, together with decreased oxidative stress and apoptosis. These results suggest a novel mechanism linking MIOX to mitochondrial dysfunctions in the pathogenesis of DKD, and D-Glucarate may be a potential therapeutic agent for the treatment of this disease.
Supported By: NIDDK

Nucleobindin-2 Regulates Insulin-stimulated Glut4 Translocation in Podocyte
Shuichi Okada, Tsugumichi Saito, Ejiru Yamada, Kihachi Oshihama, Koshi Hashimoto, Tetsuro SATOH, Masatomo Mori, Masanobu Yama-da, Maebashi, Japan, Fukuoka, Japan
Insulin resistance and diabetes are strongly associated with kidney complications leading to impaired podocyte function and microalbuminuria. Although normal and pathophysiology of insulin signaling is well studied in classical insulin target tissues, the molecular pathways in podocytes remains poorly described. Previous studies have identified Septin 7 as a negative regulator of insulin stimulated Glut4 translocation and glucose uptake in podocytes. We have found that Nucleobindin 2 is a constitutive binding partner for Septin 7, although Nucleobindin 2 over expression or knockdown had no significant effect on 2-deoxyglucose uptake or Glut4 translocation. However fenofibrate treatment had a dramatic effect on podocyte morphology associated with a 10% reduction in Septin 7 mRNA and increases in Nucleobindin 2 mRNA (10%), Nephrin mRNA (20%), Syntaxin4 mRNA (50%), and Clic5 mRNA (50%). In the presence of fenofibrate, Nucleobindin 2 knock down significantly inhibited Glut4 translocation. We hypothesize that Nucleobindin 2 can function as scaffolding protein that suppresses Septin 7 negative function to promote insulin signaling lead to glucose uptake in podocytes.

Optimal Blood Pressure Targets for Favorable Renal Outcomes in Patients with Type 2 Diabetes: A Systematic Review and Meta-analysis
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Most large clinical trials and epidemiologic analyses have proven that lowering blood pressure (BP) improved cardiovascular outcomes. Most guidelines generally recommend a systolic BP goal of 140 mmHg, and a diastolic BP goal of < 90 or 80 mmHg. However, there is uncertainty about optimal BP targets improving renal outcomes in patients with type 2 diabetes. We searched PUBMED, EMBASE, and Cochrane Library for randomized controlled trials between 1965 and October 2013, and performed a systematic review and meta-analysis. We identified 13 randomized clinical trials enrolling 26703 type 2 diabetic patients and comparing prespecified BP targets. Outcome measures were development of microalbuminuria, macroalbuminuria, doubling of serum creatinine, and end-stage renal disease (ESRD)/dialysis. Overall, intensive BP control was associated with a significant decrease in the risk for composite renal outcome (odds ratio (OR), 0.74, 95% CI, 0.61-0.90), and this effect was largely dependent of reducing development of micro- and macroalbuminuria (OR, 0.66, 95% CI 0.53-0.82). In the analyses according to the prespecified BP targets, systolic BP targets < 140 mmHg was associated with significant reduction of composite renal outcome (OR, 0.77, 95% CI, 0.72-0.83), and albuminuria (OR, 0.76, 95% CI, 0.70-0.83), but not doubling of serum creatinine and ESRD/dialysis. Similar results were shown with systolic BP < 135 mmHg. However, there was no significant benefit with lowering BP < 130 mmHg for any renal outcome measures.
In patients with type 2 diabetes, lowering systolic BP < 140 mmHg, or < 135 mmHg may be beneficial in terms of renal outcomes, especially reduction of development of albuminuria. However, more intensive systolic BP lowering < 130 mmHg did not reduce adverse renal outcomes.
24-LB
Improved Urinary Inflammatory Profile in GFO-Adherent Adolescents with Type 1 Diabetes and Celiac Disease
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Recent data implicate Celiac Disease (CD) as a risk factor for microvascular complications in Type 1 diabetes (T1D), including diabetic nephropathy (DN). Urinary inflammatory cytokine/chemokines have been implicated as early markers of DN. We characterized the urinary cytokine/chemokine excretion in adolescents with T1D and CD following a Gluten Free Diet (GFD) and evaluated if adherent CD and T1D patients (T1D-CD) represent a distinct group with altered urinary inflammatory markers compared to T1D without CD (T1D) and healthy controls (HC). T1D-CD and T1D patients aged 10-19y, with duration of T1D > 1 year and no vascular complications were included. Eighteen T1D-CD biopsy-positive patients were matched 2:1 for age, sex, T1D duration and HbA1C to 36 T1D patients and 36 HC. T1D-CD patients were adherent with a GFD; confirmed by levels of anti-tissue transglutaminase. Urine and serum levels of cytokines/chemokines as well as baseline clinical and laboratory variables were assessed. T1D alone had higher systolic blood pressure and Albumin Creatinine Ratio (ACR) than HC. Other baseline clinical characteristics were similar between the groups. T1D-CD patients exhibited lower levels of urinary TNF-α, IL-1α, IL-4, IL-5, IL-1B and G-CSF compared with T1D (p<0.05). Urinary biomarker levels between T1D-CD and HC were similar. In contrast, urinary FGF-2, GM-CSF, IL-12F, MCP-3, MDC, MIP-1β, sCD40L excretion was higher in T1D vs. HC (p<0.05). Therefore, “Dual Diagnosis” T1D-CD patients, who were adherent to a GFD, demonstrate decreased urinary excretion of inflammatory cytokine/chemokines which was similar to HC, suggesting a modulatory role of Celiac Disease and a GFD on urinary biomarkers.

25-LB
Gait Speed as an Indicator of Microvascular Disease and Inflammation in Older Adults with Type 2 Diabetes
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Type 2 diabetes mellitus (DM) is associated with slower walking, chronic inflammation and endothelial dysfunction negatively affecting the brain, kidney and eye. In DM patients, slower walking is linked to impaired cerebral vasoreactivity (CVR). However, the link between walking with central and peripheral microvascular disease remains unclear. We investigated the association between gait speed, serum soluble vascular and intercellular adhesion molecules (s-VCAM and s-ICAM), CVR, diabetic retinopathy (DR) and UACR (urine albumin/creatinine ratio) in older adults with DM. 143 participants, 72 DM, age 65.1±8.4 years, 74 F, DM duration 13.1±10.3 years. Global CVR was calculated as the slope of the regression between perfusion measured at baseline and in response to hyper- and hypoxia conditions using perfusion MRI. Slower gait speed correlated with increased s-ICAM levels (rs = -0.01, p = 0.04) and with higher DR scores (rs = 0.1, p = 0.03). In DM patients, slower gait speed was associated with reduced global CVR (rs = -0.09, p = 0.04), with higher levels of s-ICAM (rs = 0.05, p = 0.01), s-VCAM (rs = 0.03, p = 0.04) and UACR (rs = -0.04, p = 0.03) independent of BMI, DM duration and HbA1c. Slower gait speed may indicate chronic inflammation, microvascular disease and decreased vascular reserve in diabetic patients. UACR, along with s-VCAM, could reflect the detrimental microvascular changes affecting different vascular beds in the DM population.

26-LB
mTOR/p70S6K Pathway-mediated Hyperphosphorylation of Tau Is Involved in Cognitive Dysfunction of STZ-induced Diabetic Mice
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Abnormal levels of mammalian target of rapamycin (mTOR) signaling have been recently implicated in the pathophysiology of neurodegenerative diseases, such as Alzheimer’s disease (AD). The implication of mTOR in diabetes mellitus (DM)-related cognitive dysfunction still remains unknown. In the present study we detected alterations of mTOR/p70S6K signaling and increased phosphorylation of tau in the hippocampus of streptozocin (STZ)-induced diabetic mice. The expression of phosphorylated mTOR (p-mTOR), phosphorylated p70S6K (p-p70S6K) and phosphorylated tau (p-tau) in the hippocampus of diabetic mice significantly increased when compared with control mice. A low dose of rapamycin was used to elucidate the role of mTOR signaling in DM-related cognitive deficit. Rapamycin restored normal mTOR/p70S6K signaling and attenuated the phosphorylation of tau protein in the hippocampus of diabetic mice. Furthermore, the spatial learning and memory function of diabetic mice significantly impaired compared with control mice, was also reversed by rapamycin. These findings indicate that mTOR/p70S6K signaling pathway is hyperactive in the hippocampus of STZ-induced diabetic mice and inhibiting mTOR signaling with rapamycin prevents the DM-related cognitive deficits partly through attenuating the hyperphosphorylation of tau protein.

27-LB
The Role of Proxynitrite in Peripheral Diabetic Neuropathy
JOSHUA F. EDWARDS, IRINA G. OBROSOVA, CAROLINA M. CASELLINI, HENRI PARSON, MARK A. YOREK, AARON I. VINIK, Norfolk, VA, Baton Rouge, LA, Iowa City, IA
Accumulation of nitrotyrosine (NT) has been associated with sympathetic nerve and endothelial dysfunction. NT can be measured in the peripheral circulation as a marker of peroxynitrate action. The objective of this study was to determine NT levels in healthy controls (HC), diabetic patients without diabetic peripheral neuropathy (DM-Non-DPN) and diabetic patients with DPN (DM-DPN). We hypothesized that a correlation would exist between NT levels and severity of neuropathy. This was a cross-sectional study of 49 patients (15 HC, 12 DM Non-DPN, 11 possible/probable (PP) DM-DPN, and 11 definite DM-DPN). Neuropathy diagnosis was stratified according to the Toronto Consensus guidelines. Severity of neuropathy was classified according to Total Neuropathy Scores (TNS) which assess neuropathy symptoms as well as motor and sensory function.
Mean NT levels (pmol/ml protein) in serum were 3.14±0.31 for HC, 4.32±0.44 for DM-Non-DPN, 4.47±0.46 for PP DM-DPN, and 4.81±0.46 for Definite DM-DPN. Mean levels in all diabetic subgroups were significantly higher than controls. NT levels correlated significantly with TNS (r = 0.374, p = 0.0001), total symptom score (TSS) (r = 0.353, p = 0.013), motor score (MS) (r = 0.485, p = 0.0004), and sensory score (r = 0.318, p = 0.026) for the whole group, and with TSS (r = 0.673, p = 0.026) and MS (r = 0.708, p = 0.003) for the PP DM-DPN group. Linear regression analysis revealed a significant correlation between TNS and NT levels for all subjects (r = 0.0032) and PP neuropaths (r = 0.023). There were no significant correlations found between NT and weight, BMI, BP, HbA1c or lipoprotein levels.
Our findings suggest that peroxynitrite production may have a pathogenic role in DM independent of glycemic and metabolic control. The significantly steeper slope of the PP DM-DPN group compared to the HC group suggests that circulating levels of NT are increased in patients with and without DPN. In conclusion, NT could serve as a biomarker for the presence and severity of neuropathy.

28-LB
Effects of Plasma Kallikrein on the Neuroretina in Diabetic Rats
GONGXING WU, EDWARD FEENER, Boston, MA
Diabetic retinopathy is often associated with manifestations such as microcirculatory abnormalities and impairment of neuroretinal structure and function primarily due to reduced thickness of retinal layer, particularly the ganglion cell layer leading to visual impairment. However, the mechanisms underlying neuro-retinal injury and dysfunction in diabetes and DME are not fully understood. Here, we report that the Plasma Kallikrein (PK) engages with retinal tissue and contributes to retinal injury. PK inhibitor (PK ASO) decelerates this retinal neuron degeneration. In vitro, PK-ASO group shows significantly lower expression of phosphorylated Kallikrein (PK) in the retina and the PK-ASO group shows significantly lower expression of phosphorylated PK (p-PK) in the retina. Together, these results indicate that PK plays a critical role in retinal neuro-degeneration in diabetes and suggest that PK may serve as a potential therapeutic target for clinical intervention and treatment of diabetic retinal neuro-degeneration.

For author disclosure information, see page LB91.
The Role of Neospecific Proteins in the Diagnosis of Cognitive Dysfunction in Patients with Diabetes Mellitus Type 1

Maria Novoselova, Julia Samoylova, Tomsk, Russian Federation

One of the targets of diabetes mellitus type 1 (DM1T) is the central nervous system with the further formation of cognitive dysfunction. In the case of timely diagnosis and treatment of cognitive impairment associated with metabolic changes that can partially or completely regress.

The aim of this study was to identify neospecific proteins as biomarkers of the brain damage in patients with DM1T.

We examined 58 patients at the age of 22.45 ± 4.827 years, disease duration 6.6 ± 3.951 years, the control group was consisted of 29 healthy people at the same age and sex. To assess mental status used Montreal Assessment Scale cognitive dysfunction (MoCa test). Statistical processing was carried out using an application software package R-system. It was found that DM1T may manifest cognitive impairment of the central nervous system according MoCa test. Analysis of the results showed that patients with DM1T had cognitive impairment (total score of 25 points ) to 72.2 % while in the control group cognitive functions were normal in 100 % ( total score of 30 points ).

When evaluating MoCa test recorded a statistically significant reduction of parameters that assess short-term memory and attention in patients with DM1T compared with the control group.

The study found a significant increase in all neospecific proteins: S100, myelin basic protein and glial fibrillary acidic protein in patients with DM1T compared with the control group, which were correlated with Hba1c (p<0.001). The most important finding was the reduction in memory functions while increasing neospecific proteins. The final total score reflecting the total value of cognitive function had negative correlation with all the studied biomarkers.

As a consequence, it is recommended indication of neospecific proteins in patients with DM1T who have not achieved the target values of carbohydrate metabolism, there is a decrease of compliance, as well as cognitive impairment.

Comparing Technical Failure Rates in Diabetic Retinopathy Screening between RETeval, a 30 Hz Flicker Electroretinogram Device, and Hydratric, 7-Field, Stereo Fundus Photography

April MAA, Ensa Pillow, William Feuer, Tara Brown, Rachel Caywood, Quentin Davis, Joel Chasan, Stephen Franzen, Decatur, GA, Oklahoma City, OK, Miami, FL, Garthsburg, MD

Diabetic retinopathy (DR) is a cause of preventable blindness and screening reduces vision loss. There are limitations to current screening methods. ERG implicit time correlates with extent of DR but using ERG for DR screening is impractical because of difficulty performing and interpreting ERG results. This implicit measured the performance of RETeval, a handheld ERG device. 500 diabetic patients were selectively recruited to obtain 80 patients in each category: 1) no DR, ETDRS level 10. 2) Mild DR without CSME, ETDRS levels 14-35. 3) Moderate DR without CSME, ETDRS levels 43-47. 4) Mild/moderate DR with CSME, ETDRS levels 10-47. 5) Severe DR or PDR, ETDRS levels 53 and higher. The RETeval test was performed. Patients were dilated and ETDRS-compliant 7 field fundus photographs were taken. These photographs were double-read in a masked fashion in a reading center. photographic results differing by more than 1 ETDRS step were sent to adjudication where 2 readers and a retinal specialist determined the final ETDRS level. the photography results served as the gold standard to which the RETeval findings were compared. 392 patients completed the study. There were 340 male and 52 female. The RETeval device had a technical failure rate (no results generated) of 0.5% (2/392 patients) whereas ETDRS fundus photography (unreadable images) had a significantly higher (p<0.001, exact McNemar test) technical failure rate of 15% (57/392 patients). The RETeval device is a new handheld ERG device. Compared to fundus photography, the RETeval device had a low technical failure rate that was statistically significant. This study shows that the RETeval device promise as a new screening tool for DR because its easy to use and has low failure rates.

Supported By: NIH

Compliance with Recommended Follow-up for Diabetic Retinopathy in a County Hospital Population

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The purpose of this study is to assess the association between insufficient follow-up and demographic, clinical, and social parameters among diabetic patients at an inner-city county health system. This is an IRB-approved, case series of the Wishard-Eskemian Teleretinal Diabetic Retinopathy Screening Program using retinal cameras conducted at four primary care clinics from June 2009 through February 2013. Patients identified with diabetic retinopathy (DR) were referred for eye clinic examination based on the American Academy of Ophthalmology’s Guidelines. Compliance was determined by adherence to recommended eye clinic follow-up interval through medical records review. Analysis applied Pearson’s chi-squared test or Fisher’s exact test for categorical variables and Student’s t-test for continuous variables. Multivariate logistic regression model was employed to obtained adjusted odds ratios (ORs) for compliance with the significant variables. Of 258 patients referred, 93 were compliant and 165 were noncompliant. We observed significant association between compliance with DR severity alone (p=0.033) and when combined with diabetic macular edema (p=0.025), as well as with White and Asian vs. Black and Hispanic patients (p=0.043). In the final multivariate logistic regression model, the effects of DR severity and its interaction with age were found to be significant: Odds of compliance of those with severe or proliferative DR were much greater than those with mild or moderate DR (OR=8.4); Odds of compliance for elderly (older than 50 years) is slightly greater than for younger patients (OR=1.2). History of “no-show” to medical appointments (0-1 vs. 2 or more) trended toward significance (p=0.084). We conclude that patients with poor follow-up adherence were significantly more likely to have less severe DR and younger age. Targeted education campaign at the primary care level in this vulnerable population to prevent future vision loss would be beneficial.

Supported By: ADA

Complications—Ocular

A New Mechanism in Regulation of RPE Tight Junctions in Diabetic Retinopathy

Sarah X. Zhang, Jacey H. MA, Josh J. Wang, Buffalo, NY

The retinal pigment epithelium (RPE) is a monolayer of pigmented epithelial cells located adjacent to photoreceptors cells and plays an essential role in supporting the structural and functional integrity of the neural retina. One of the major functions of the RPE is to form the outer blood-retinal barrier (BRB). Damage of the RPE barrier leading to increased permeability is believed to contribute in part to retinal edema in diabetic retinopathy. Herein, we investigate the role of ER stress and X-box binding 1 (XBP1), a central coordinator of cellular response to ER stress, in regulation of RPE tight junctions. Exposure of differentiated human RPE cells to pharmacological ER stress inducers thapsigargin and tunicamycin results in increased ER stress, evident by elevated levels of GRP78, P58IPK, CHOP, and splicing of XBP1 mRNA. Pharmacological inhibition of XBP1 splicing prevented the upregulation of GRP78 and P58IPK, but did not affect CHOP expression. Sub-lethal dose of thapsigargin, but not tunicamycin, reduced ZO-1 expression and impaired tight junction formation. Interestingly, pretreatment with XBP1 inhibitor sensitizes the cells to tight junction damage in the presence or absence of tunicamycin. In contrast, forced expression of active XBP1 gene largely reversed the tight junction damage caused by pharmacological XBP1 inhibition. In line with the results from in vitro study, conditional knockout of XBP1 in RPE cells leads to defective tight junction formation, which was exacerbated in diabetic condition. Taken together, our results have revealed that tunicamycin and thapsigargin differentially regulate ER stress and tight junction formation, suggesting that other mechanisms independent of ER stress are involved in RPE barrier regulation. Furthermore, our study suggests that activation of XBP1 by ER stress may play a protective role in the RPE barrier function and in diabetic retinopathy.

Supported By: ADA (7-11-B-182); NIH (EY201949)

31-LB

For author disclosure information, see page LB91.

Complications—Ocular

29-LB

Acute and Chronic Complications

POSTERS

ADA-Funded Research
Identification of a Putative Glucocorticoid Receptor Element in Human Cholesteryl Ester Transfer Protein Gene

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Transcriptional regulation of human cholesteryl ester transfer protein (CETP) by glucocorticoid was investigated using a transient reporting system. A series of deleted vectors generated from 5′-upstream region (3434 bp) of the CETP gene linked to the cholangiomeracn acetyltransferase (CAT) reporter gene was individually transfected to HepG2 cells. Promoter analysis revealed an essential regulatory machinery in the -138/-101 region of the upstream sequence of the human CETP gene. When the cells, transfected with the reporter vectors with the -138/-101 region deleted, were treated with dexamethasone (Dexa), the CAT activity was increased, suggesting that there may be a glucocorticoid receptor element (nGRE) in the region. Competition analyses on the gel mobility shift assay, using the consensus GRE and a purified glucocorticoid receptor as competitors, confirmed the -138/-101 region as a GRE. Footprinting analyses showed that the DNA segment at the -133/-108 is protected by the nuclear extract from HepG2. The identified GRE sequence did not show homology with the consensus GRE sequences. It may be a unique negative GRE existing in the CETP gene.

Serum Triglyceride Levels are Correlated with Markers of Inflammation in Isolated Hyperlipidemia Patients

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Hyperlipidemia is characterized by elevation of serum cholesterol, triglyceride and LDL cholesterol and decreased level of HDL cholesterol. Hyperlipidemia leads to atherosclerosis and cardiovascular diseases. Studies have shown that there was a correlation between hematological parameters and atherosclerotic cardiovascular diseases. We aimed to investigate some markers of inflammation (Red Cell Distribution Width and Nutrophil to Lymphocyte Ratio) in newly diagnosed and isolated hyperlipidemia patients.

The study recruited 82 patients (46 females, 56.1%) with a newly diagnosed and isolated hyperlipidemia applied in our outpatient clinics. The patients had no other documented atherosclerotic cardiovascular disease, hypertension, diabetes or renal failure. The mean ages of the male and female groups were 41.36 and 53.87 years, respectively. Mean serum triglyceride (Trig), low density lipoprotein, and high density lipoprotein levels of the whole group were 277.2±290, 186.3±45.1, 50.6±13.3 mg/dL, respectively. As markers of inflammation, mean red cell distribution width (RDW) and neutrophil to lymphocyte ratio (NLR) in newly diagnosed and isolated hyperlipidemia patients. The study recruited 82 patients (46 females, 56.1%) with a newly diagnosed and isolated hyperlipidemia applied in our outpatient clinics. The patients had no other documented atherosclerotic cardiovascular disease, hypertension, diabetes or renal failure. The mean ages of the male and female groups were 41.36 and 53.87 years, respectively. Mean serum triglyceride (Trig), low density lipoprotein, and high density lipoprotein levels of the whole group were 277.2±290, 186.3±45.1, 50.6±13.3 mg/dL, respectively. As markers of inflammation, mean red cell distribution width (RDW) and neutrophil to lymphocyte ratio (NLR) in newly diagnosed and isolated hyperlipidemia patients.

Diabetic foot infections (DFIs) are common and serious complications affecting worldwide diabetes population. Few randomised studies have assessed antibiotic regimens in Asian populations. Our objective was to assess the efficacy and safety of ertapenem versus piperacillin/tazobactam (TZP) for DFIs in Chinese population.

Diabetic adults (n=665) with moderate-to-severe DFIs requiring intravenous (IV) antibiotics were randomly assigned either ertapenem (1g daily) or TZP (4.5g every 8h) for a minimum of 5 days. Oral amoxicillin/clavulanate (625mg every 12h) could be given for 23 days at maximum IV therapy. Vancomycin may be allowed for bacterial species known resistant (i.e. Enterococcus spp) to study therapy. The primary outcome was the proportion of patients with a favourable clinical response on the day that IV antibiotic was discontinued (DCIV). An evaluable-patient population was identified for primary analysis. Safety was assessed across the study.

At DCIV, 443 patients were assessed clinically evaluable and 533 MITT qualified. Baseline characteristics between groups were comparable. Findings on primary outcome were summarised in table. Safety was similar by ertapenem versus TZP based on drug-related AE (13.5% vs. 16.0%) and AE leading to discontinuation (4.0% vs. 5.6%).

Ertapenem is non-inferior to piperacillin/tazobactam and may be an option for DFIs in China.

Results of DCIV study primary outcome in Clinical Evaluable/Missing (CE/M) and MITT/Missing (MITT) populations.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Ertapenem (n=234)</th>
<th>Piperacillin/Tazobactam (n=210)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Discontinued Response</strong></td>
<td>191 (81.6%)</td>
<td>166 (79.3%)</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Ertapenem (n=224)</strong></td>
<td>179 (79.8%)</td>
<td>151 (71.9%)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Piperacillin/Tazobactam (n=201)</strong></td>
<td>179 (81.6%)</td>
<td>151 (71.9%)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**DIABETES EDUCATION**

Microsocial Network Interventions for Obesity and Diabetes in Jordan: A Three-Armed Cluster Randomized Controlled Trial

Andrea B. Feigl, Daniel E. Zoughie, Kathleen T. Watson, Nancy Bui, Leila Makarech, Yazeed M. Ibrahim, Eric L. Ding, Boston, MA, San Francisco, CA

Background: Diabetes and obesity are suggested to propagate within social networks, with diabetes a concern in the Middle East. Leveraging pre-existing social networks to propagate healthy behaviors, we conducted the first ever social-network randomized trial to improve obesity and diabetes in a developing country.

Methods: Based in community health clinics in Amman, Jordan, we tested the effects of various microsocial Network Systems (MSNS) behavioral interventions in collaboration with the Jordanian Ministry of Health and Royal Health Awareness Society. A 3-armed 28-week cluster randomized trial was designed. Arm A) enhanced MSN social network program in weekly interactive sessions. Arm B) enhanced MSN social network program in weekly interactive sessions. Arm C) control (usual care).

Results: 944 participants (45.7% female, 22.1% BMI >30) from 6 clinics were randomized. Both groups receive arm A) enhanced MSN social network program in weekly interactive sessions. There were no significant differences in outcomes between groups. Safety was acceptable in all arms.

Conclusion: Our study suggests that enhancing social networking within existing social networks to propagate healthy behaviors is a promising approach to improving diabetes and obesity in a developing country.
sessions led by health-educators; Arm B) basic MSN social network program led by health-educators; and Arm C) controls with standard care. Participants were 66% women, mean age 55.1 years (10.2), mean BMI 33.6 (3.2). After 12 weeks, Arm B reduced weight vs. C (-0.99 kg, 95% CI: -1.93 to -0.06; P=0.037), while A yielded borderline weight change vs. C (-0.59 kg, P=0.296). However, by end of 28-weeks of intervention, Arm A showed the strongest sustained weight reduction versus control (-1.11 kg. -1.87 to -0.35; P<0.004), while B did not (-0.64, -1.69 to 0.41; P=0.23), with overall P for program*time interaction=0.019. HbA1c showed borderline significant drop at 28 weeks for vs. A control (-0.20, P=0.08), but not B vs. control (-0.15, P=0.27). Waist circumference and blood pressure were not significant.

Conclusions: Results demonstrate the effectiveness of MSN health interventions in a resource-limited, high chronic disease burdened, developing-country setting.

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38-LB

Improved Diabetes Control Using SMBG Pattern Management in High-Risk Minorities (HRM)

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SMBG in a diabetes education program improves T2D outcomes. This study was done to evaluate if use of SMBG data taught by clinical pharmacists or educators improved A1C compared to diabetes classes or medication review in HRM. A pretest-posttest format was used. Subjects (n=807) were referred by providers from 5 federally qualified health centers and a practice group. HEDIS measures collected included: A1C, BP, FPG, BMI, total cholesterol (TC), LDL-C, HDL-C, and TGs. Subjects received a validated knowledge test initially, 3 months after taking a diabetes class and at study end. A psychosocial inventory was collected initially and at study end. This multi-component model was a four-arm study comparing Diabetes Classes (DC), Pharmacist Medication Management (MTM), Pattern Management (PM) using a glucose meter data management system, and control subjects who did not participate (Ctl). Subjects had at least two interventions prior to assignment to PM. 258 subjects attended the diabetes classes, 71 in the MTM arm, 44 in the PM arm, and 138 Ctls. 296 were lost due to incomplete and/or lost data. Demographics revealed 77% in the 20-44 y.o. range, 21% in the >65 age group, and 16% in the <20 age group, with 65% female subjects. The mix was 86% non-Hispanic black and 13% non-Hispanic white. 60% of subjects were on Medicare/ Medicaid, 30% employer-insured, and 16% uninsured. Mean A1C levels in the DC group decreased from 7.93 to 7.80, 8.06 to 7.32 in the MTM group, 7.70 to 7.28 in the PM group, while Ctls increased from 7.00 to 7.36. The PM group had the greatest mean changes in multiple measures including BMI, BP, FPG, TC, and TG. The MTM group had the greatest improvement in A1C. Conclusions: The PM group had the largest changes in overall measures of T2D control. There was increased diabetes knowledge and reduced ER visits. Thus, in HRM using PM from SMBG, the improvements suggested better monitoring of multiple metrics of relevance in T2D control.

39-LB

Can Promotoras Reconnect Individuals to the Healthcare System?

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Pathways to Better Health through Workforce and Community Engagement (Pathways) in New Mexico is part of a larger CMS Health Care Innovation Project (HCIP) to test the feasibility, reproducibility, and clinical impact of a model using local community health advocates to deliver well-established health information classes. The overall HCIP program has enrolled 1,111 participants in the first year, with over 70% completing the 3-month follow-up and data collection. The program has produced significant improvements in BP (75.8% < 140/90), follow-up rates were 82.9% baseline, 80.0% (p < .001). Of 156 people with pre-diabetes (A1C 5.7%-6.4%) at baseline, 28.5% moved to the non-pre-diabetes range at 12 months (A1C < 5.7%) follow-up, p < .001. In the past the Pathways program had used dietitians and New Mexico State University Extension agents for delivery of this program, but has now switched to a promotora model to extend the reach of the program, and to reduce costs. Changes in BP, A1C, and pre-diabetes are similar when the program is delivered by promotoras, and, when compared to non-promotoras, there is an improvement in participants’ re-engagement with the healthcare system. Re-engagement is measured by looking at those participants who had not seen a healthcare provider (HCP) in more than six months. Of these “healthcare-disconnected” participants, 33% said at follow-up that they had made an appointment with a HCP, while 33% said that they had shown their A1C and BP results to a HCP. In the prior incarnation of the program, not delivered by promotoras, only 16% of “disconnected” participants had shown these values to an HCP. p < .001. Thus, promotoras can effectively and efficiently reconnect individuals to the healthcare system.

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40-LB

Impact of Ramadan Fasting on People with Diabetes

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Objectives: To examine whether fasting during Ramadan, compared to non-fasting periods, affects glycemic control and other related variables in people with diabetes.

Methods: A cross-sectional study of 1,745 adults with diabetes during Ramadan (n=1,016) and an age-matched control group (n=729) was conducted. The study was conducted in 6 countries (Eritrea, Jordan, Egypt, Saudi Arabia, Turkey, and United States). Participants were recruited through snowball sampling. A total of 2,480 participants were included in the analysis. Participants were asked to report their dietary habits, medication adherence, and blood glucose measurements during Ramadan and non-Ramadan periods. The impact of Ramadan fasting on glycemic control was assessed using the glucose control index (GCI).

Results: The GCI was significantly lower during Ramadan compared to the non-Ramadan period (p<0.001). Additionally, there was a significant decrease in the percentage of patients with good glycemic control (HbA1c < 7%) during Ramadan compared to the non-Ramadan period (p<0.001). A significant decrease in the percentage of patients with normal blood pressure (BP < 130/80 mmHg) during Ramadan was also observed (p<0.001).

Conclusions: Fasting during Ramadan is associated with a significant decrease in glycemic control and other related variables in people with diabetes. These findings highlight the need for diabetes education and support during Ramadan.

41-LB

Cognitive Function and Self-Efficacy in Type 2 Diabetes with Poor Glycemic Control

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Objectives: To evaluate the relationship between cognitive function and self-efficacy in people with type 2 diabetes with poor glycemic control.

Methods: A cross-sectional study of 84 consecutive patients with type 2 diabetes and poor glycemic control was conducted. Participants completed a self-efficacy questionnaire, the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA), the Five-Word Test (5WT), and the Frontal Assessment Battery (FAB). Cognitive function was assessed using the MMSE, MoCA, 5WT, and FAB.

Results: The study sample consisted of 84 consecutive patients with type 2 diabetes and poor glycemic control. The mean age was 60.7 ± 9.3 years. A total of 31 patients (36.9%) had cognitive complaints (McNair) as defined by scores below the 10th percentile of the control group. Cognitive complaints were present in 31 patients (36.9%) as defined by scores below the 10th percentile of the control group. In conclusion, in this study is being tested this year in 6 countries.

ADA-Funded Research

For author disclosure information, see page LB91.
A Comparison of Two Methods of Foot Care Education: The Fremantle Diabetes Study

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The purpose of this study was to compare the effectiveness of two methods of diabetic foot care education on foot health, behaviours and attitudes in a community-based cohort of patients with type 2 diabetes. Community-based patients enrolled in Phase II of the Fremantle Diabetes Study were randomly allocated to receive either written foot care education (Group A) or an interactive 90-minute foot care education program presented by a credentialed diabetes educator (Group B). A quantitative foot score (maximum 90 points scored based on graded severity of pathology ranging from skin abnormalities to ulcers/gangrene in both feet), the Nottingham Assessment of Functional Foot Care (NAFFC) survey score (maximum 30 points based on foot care behaviours) and a 6-question survey of attitudes to diabetes-related foot complications were recorded at baseline and 3 months. 154 patients (mean±SD age 68±10 years, 59.7% males, median interquartile range diabetes duration 11.5 [5.6-18.9] years) were recruited. There was a significantly greater change (△) in foot score in Group A vs. Group B at 3 months (8.3±6.5 at baseline, △-1.8 [95% CI -2.4 to -1.2] vs. 6.8±2.6, △-0.1 [0.7 to 0.4], P<0.001) that persisted after adjustment for baseline values, but no change in NAFFC survey score (P>0.13). In the attitudes survey, Group B felt they better understood how to prevent foot complications than Group A (P=0.031). Written information was more effective at improving foot health, while interactive education improved participants' confidence in undertaking preventive measures. These data suggest that the most effective foot care education should include both written information and interaction with a qualified diabetes health care professional.

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Nonvisual Foot Examination for People with Visual Impairment

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People with diabetes and visual impairment have high risk of foot problems. In diabetes self-management education (DSME), usual care is to teach them to seek sighted assistance for regular home foot exams, yet many report not doing this. A simple nonvisual technique for inspecting feet, using touch and smell, may help people detect foot problems in early, treatable stages, ultimately decreasing ulcers and amputations.

The purpose of this pilot study was to compare the efficacy, acceptability, and feasibility of nonvisual foot examination with usual care (requiring sighted assistance for examination of feet).

Fifty-seven visually impaired adults with diabetes were recruited, consented, and assigned to experimental or comparison groups. Both groups received comprehensive DSME, with emphasis on foot care. The experimental group was taught nonvisual foot examination, the comparison group to ask for sighted assistance for regular foot checks. All had a baseline podiatric evaluation, with visits at 3 months and 6 months. Focus groups were conducted for all at the end of the study.

Analysis included total frequency of home foot checks and frequency of home foot checks according to instructions given each group; number of symptoms reported per podiatrist visit; number of foot problems documented by podiatrists per visit; and qualitative acceptability analysis from focus groups.

Total number of foot checks was similar between the two groups. There was a large difference in frequency of home checks by the instructions given each group (p < .001). The experimental group did more nonvisual examinations, while many in the comparison group had others check their feet rarely or never and checked their own feet “as best they could.” The podiatrists discovered slightly fewer problems in the experimental group. In the experimental group, overall response was positive to nonvisual foot examination. In the comparison group, many were reluctant or unable to ask for sighted assistance.

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Understanding the Effect of Cooking on Glucose Availability: Glycemic Index Analysis of Korean High-Carbohydrate Food with Different Cooking Methods

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Although glycemic response to carbohydrates in different foods has been quantified, the changes in glycemic index after cooking is not well-understood. This study aimed to investigate the effects of cooking by different methods on the glycemic index of Korean food. The most popular carbohydrate foods, including white rice (62.9g), glutinous rice (61.1g), barley (84.4g), brown rice (65.0g), corn (170.0g), potato (359.7g) and sweet potato (160.3g), from Korean National Health and Nutrition Examination Survey nutrient database were cooked using various conventional domestic methods. Sixty young healthy adults were recruited to participate in the feeding trial and consumed each test food on 8 separate days. Blood glucose and insulin levels were subsequently measured at times 0, 15, 30, 60, 90, and 120 min for each test period. Blood glucose and each test food containing 50g of carbohydrates. Glycemic indices calculated from different cooking methods for boiled, steamed, baked and puffed food and incremental areas under the curve were calculated by weighing geometrically. Depending on cooking methods, steamed rice cake (50.6 ± 7.2), boiled glutinous rice (75.7 ± 10.6), boiled barley (35.4 ± 9.2), puffed corn (68.9 ± 11.4), grinded and pan-fried potato (128.0 ± 5.1), fried sweet potato (57.7 ± 10.9) may be considered the low and medium glycemic index foods, which may prove beneficial for diabetic or insulin-resistant consumers. Further investigation for glycemic index and glycemic load analysis in different food groups, especially fruit, is required to identify the beneficial food items and cooking methods for dietary therapy for diabetes management.

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Population Focused Peer Support to Reach Those Not Receiving Recommended Diabetes Services

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As reported by the CDC, 46% of people with diabetes are estimated not to receive the diabetes self management education and support they need and this number is higher among ethnic minorities. Most published research in the field reports that patient education and support interventions are reaching only select samples of patients. Here we report population focused peer support strategies designed to reach and engage all diabetes patients at a federally qualified health center in Chicago serving a predominantly Latino population. Peer support is delivered through a tiered program in which all patients with diabetes receive Regular Care that includes quarterly contacts, group classes, activities, “point of care marketing” by which peer supporters are present in the waiting rooms and at clinic visits, all promoting pursuing self management goals as well as regular clinical care. A High Need group (n=469; HbA1c>9%, elevated psychosocial needs, or physician referral) receives bimonthly contact for 6 months and then monthly until they no longer meet criteria or progress has stabilized. Flexible, nondirectional strategies are used to engage patients in peer support and include: 1) low demand - an initial call to describe and offer services, not push to accept, 2) repeat calls in 2-4 weeks to “check in with” not “check up on” patient, 3) two-year availability to patient - not considered refusal unless they clearly request no further contact, 4) after patient is engaged, begin working on individually chosen goals from set of low self management behaviors such as health eating, etc. Results to date (after 18 months since program initiation): of the High Need patients, we have reached 375 (80%) with 260 (69%) up-to-date on contacts. In the Regular Care group, we have reached 1,731 (48%) with 1,463 (85%) up-to-date on contacts. These preliminary results demonstrate that peer supporters can reach and engage an entire patient population into educational, support and clinical services.

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The Effect of Lifestyle Modification Program Reduces Fasting Plasma Glucose in Overweight Children: A Systematic Review and Meta-analysis

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The increase in childhood obesity and young type 2 diabetes mellitus has been determined globally. Lifestyle modification may be helpful for weight and glycemic control. We aimed to determine the effects of lifestyle modification program on fasting plasma glucose (FPG) in overweight children. Data was obtained in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis statement, including 4 relevant electronic databases searched from 1965 to June 2013 of the publication date. We included overweight children between the age of 5 and 18 years from whom measurements for FPG were obtained and lifestyle modifications such as diet control, healthy nutrition, exercise or fitness, or physical activities were attempted. After an initial search and full text reviewed, 5 studies were identified containing FPG for inclusion into qualitative synthesis and meta-analysis. A total of 1291 children in the intervention and 1288 in the control group completed. The point estimate for the mean difference in effect size

For author disclosure information, see page LB91.
was -2.02 (95% CI, -3.51 to -0.55, Z = -2.68, p = 0.007), which indicated that lifestyle modifications would decrease significantly 2.0 mg/dl of FPG. This study concludes that lifestyle modification is effective for reducing FPG in overweight children. A large cohort study can be expected to prevent the onset of adolescent type 2 diabetes.

EXERCISE

47-LB

Exercise Effects on Postprandial Glucose Metabolism and Insulin Mobilization in Type 1 Diabetes

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A better understanding of effects of exercise on postprandial glucose metabolism, insulin availability and action in type 1 diabetes (T1D) would help inform next generation closed loop control algorithms. We therefore studied 14 recreationally active T1D subjects (age 44.9±12.5 yrs., BMI 28.6±5.8 kg/m², HbA1c 7.6±0.7%) on insulin pump before, during and after 75 min of moderate intensity exercise (50% VO₂ max) that started 120 min after a mixed meal containing 75 g glucose. Pancelinal insulin bolus was administered as per each subject’s customary insulin:carbohydrate ratio adjusted for the light physical activity. Basal insulin infusion rates were not altered. There were no episodes of hypoglycemia during the study. Over the next six hours, glucose turnover was measured with the triple tracer technique. Rates of endogenous glucose production (EGP) fell 98% within 75 min then rose rapidly during exercise to baseline levels. Whole body glucose uptake (Rd) peaked at 75 min after the meal. During exercise, rates of Rd rose gently before returning to baseline levels within 45 min after completion of exercise. Interestingly, plasma insulin concentrations rose by 31% during exercise despite no changes in insulin pump infusion rates implying increased mobilization of insulin from subcutaneous depots. In contrast to healthy subjects undergoing the same protocol, Rd and glucose clearance were lower (p<0.01) and integrated rates of EGP higher, despite higher plasma glucose (p<0.01) concentrations in the T1D during exercise. Also, the rise in plasma glucagon concentrations during exercise was lower in T1D (33% vs. 210%) than healthy subjects implying a combined effect of hypoglycemia and persistent alpha cell dysfunction in T1D. Closed loop control algorithms will need to account for the effects of exercise on glucose turnover, insulin mobilization and suboptimal glucagon response in next generation artificial pancreas systems to improve outcomes related to both hypoglycemia and hyperglycemia.

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48-LB

Clinical Results of Smart Detection of Physical Activity in Adults with Type 1 Diabetes

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Physical activity has a significant effect on glucose metabolism in individuals with type 1 diabetes (T1D), and often results in hypoglycemia. Consequently, early detection and classification of activity will improve glucose control and minimize the risk for immediate and latent hypoglycemia. The purpose of this study was to develop a novel activity detection method based on CGM, activity and heart rate sensors to detect and classify events as part of future smart glucose management system.

Eight T1D adults (4F: 4M; 44±18 y, 80±13 kg) were monitored for a total of 4 days using a detailed diary, tri-axial accelerometer, heart-rate monitor and CGM. After 24 hours of home data collection, subjects participated in an 8-hour in-clinic exercise session: 60 and 30 minutes at 30% and 50% predicted maximal heart rate reserve (HRR), respectively, using a treadmill or a recumbent bicycle. Subjects then continued in-home data collection for 48 hours. In-home data were used to develop the detection method based on principal component analysis, while in-clinic data were used to identify false positives. The proposed detection method flags an activity if consecutive samples exceed the threshold (6σ confidence limit), determined for the non-exercise data.

The detection method was able to identify the 30% HRR exercise at a median of 8 min [range 3-17 min], and the 50% HRR exercise at a median of 4 min [range 3-7 min]. The glucose drop from the start of exercise to the detection time ranged between -2.2 mg/dl to +17.8 mg/dl with a median of 3 mg/dl for 30% HRR exercise. For 50% HRR exercise it ranged from -4.0 to +9.6 mg/dl with a median of 2.4 mg/dl. This detection method based on different types of sensors provided good robustness to sensor dropouts and data outliers.

EXERCISE

The novel personalized method for reliable fast detection of exercise was validated on clinical data. Early detection of exercise is a critical factor in minimizing immediate and nocturnal hypoglycemia episodes in people with T1D.

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49A-LB

A Novel Method of Electrical Pulse Stimulation Mimics the Effects of Exercise in Human Myotubes

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Human satellite cells retain the phenotypic characteristics of the skeletal muscle from which they have been derived, and as such are well-established models for in vitro manipulations and cell studies. Here, we describe a novel method of in vitro exercise mimetics and demonstrate that the effects of this method recapitulate the effects of exercise observed in the whole body organism. Human myoblasts derived from lean, healthy individuals were cultured and differentiated over a 7 day differentiation time course. On days 5 - 7 of differentiation, myotubes were subjected to electrical pulse stimulation (EPS) using the C PACE, Cell Culture Stimulator by ION OPTIX®. Cells were pulsed for 3 hours/day with 11.5 V at a frequency of 0.2 Hz, in a 2 ms field. Myotubes were also switched to antibiotic-free differentiation media, which was replaced just prior to the start of EPS on each day of stimulation. This low frequency, 3 hour-long bout of EPS, repeated over a 3 day period most closely mimics a low intensity training program. On Day 7 of differentiation, cells were harvested at 0, 1, 2, 3, 4, 12 and 24 hour time-points at the end of the 3-hour EPS time period. Samples were also collected from a control plate not subjected to EPS at these same time-points. RNA was isolated from each sample and analyzed by qRT-PCR.

We have shown that the exercise-linked cytokine, IL-6, as well as several genes involved in muscle metabolism and exercise, including PGC1-alpha and PPAR alpha were significantly upregulated in the myotubes subjected to EPS, compared to the control cells. In addition, we found that PDK4, which is associated with lipid metabolism, was also significantly upregulated. These findings strongly indicate that this novel method of muscle stimulation is an effective exercise mimetic. With variations in frequency and intensity of stimulation, EPS may also be modified to examine the effects of different types of exercise programs in vitro, and is therefore a valid and useful means of examining the effects of exercise in cells.

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Effects of Fish or Meat Intake Before and After Rice on Postprandial Glucose Excursions and Incretin Secretion in Type 2 Diabetes: Meal Sequence as a Novel Target in Dietary Therapies for Diabetes

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We investigated the effects of fish or meat intake before and after rice intake on postprandial glucose excursions as well as incretin secretion in patients with type 2 diabetes (T2DM). In experiment A, untreated T2DM patients were subjected to 2-way crossover tests on two separate days, in which they received, with a 15-min interval, steamed rice before or after canned mackerels in brine. Postprandial glucose was significantly reduced when they received mackerels before rice. Strikingly, GLP-1 was significantly elevated when they received the mackerels before the rice, while GIP was hardly affected by changing the order of intake. In experiment B, untreated T2DM patients were subjected to 3-way crossover tests on three separate days, in which they received, with a 15-min interval, rice before or after grilled beef or rice after mackerels. Postprandial glucose was significantly reduced when they received beef before rice. GLP-1 was significantly elevated when they received beef before rice. The profiles of postprandial glucose and GLP-1 were similar when they received beef or mackerels before rice. Interestingly, GIP secretion was significantly elevated when the patients received the beef, but not the mackerels, before the rice. Experiment C, a nutrition component analysis, revealed that the beef was rich in oleate and stearate, strong enhancers of GIP secretion, while the mackerels were rich in eicosapentaenoic acid and docosahexaenoic acid, which are poor enhancers of GIP secretion. Our results show that meal-sequence can play an important role in controlling postprandial glucose and incretin secretion. While eating meat before rice in T2DM might ameliorate postprandial glucose and enhance GLP-1 secretion in a fashion similar to eating fish before rice, beef’s concomitant enhancement GIP secretion could promote obesity when such meal sequence is taken chronically.

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Complementary and Alternative Medicine Use among Diverse, Low-Income Patients with Diabetes: A Practice with Clinical Significance

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The degree to which diabetes patients employ complementary and alternative medicine (CAM) is poorly understood, and whether CAM is associated with health outcomes is not known. We report prevalence of CAM use and disclosure to clinicians among diverse diabetes patients, and explore independent associations between CAM and HbA1c. A Medicaid managed care plan enrolled 362 patients in a self-management program from 2009-11. Six month phone interviews collected self-reported CAM use in the prior 30 days using a 12-item validated instrument in English, Spanish and Cantonese. Oral hypoglycemic medication adherence was measured using pharmacy claims. HbA1c was obtained from electronic records. 278 patients (77%) completed follow-up interviews, and were predominantly Asian (80.1%), Latino (22.6%) and non-English-speaking (71.9%). Mean age was 55 yrs, with mean of 7 yrs with diabetes. Any CAM use was reported by 51.4%. One third (30.0%) used CAM for diabetes. Specific modalities included vitamins/ nutritional supplements (25.9%), natural remedies/herbs (24.5%), massage/acupuncture (11.5%), and meditation/yoga/taichi (10.4%), at a median monthly cost of $33 (range 0-$1200). While 47.2% did not disclose CAM to a clinician, 24% of CAM users reported putting off buying medications to pay for food, vs. 13% of non-CAM users (p<.05). Based on pharmacy claims, 21% of CAM users had poor adherence vs. 12% of non-CAM users (p<.05). CAM users were more likely to have poor glycemic control (A1c 8% or more)compared to non-CAM users (34% vs. 16%, P<0.05). CAM use is common among diverse, low income patients with diabetes, but disclosure to clinicians is inconsistent. CAM use appears to be clinically relevant, being associated with poor glycemic control. While CAM use may be a marker for disease burden or non-Western beliefs, that CAM use was associated with cost-related non-adherence suggests there may be a causal path between CAM and poor glycemic control.

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54-LB

Review of Smartphone Applications Designed to Improve Latino(a) Diabetes’ Self-Care Behaviors: “Opportunity” or Risk?

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Diabetes disproportionately affects Latinos in the U.S. Latinos are 1.7 times more likely to have diabetes compared to Whites. Increased diabetes risk among Latinos may be attributed to challenges in access to quality health care, language barriers, & genetic makeup. Latinos are significantly more likely than other groups to download apps to track their health. Consequently, Smartphone apps present a low cost opportunity to improve diabetes self-care among Latino diabetics. However, little is known about whether apps follow evidence-based guidelines or are grounded in behavioral theory. Using AADE7TM measures for diabetes self-care behaviors & tools for evidence-based evaluations of health apps, we examined Spanish language apps for diabetes self-care apps on iPhones™. On February 11, 2014, all apps (n=426) that resulted from the search of “diabetes” in the iPhones™ store were screened for Spanish language options. 130 apps that indicated availability in Spanish, & were classified as “Medical” or “Health & Fitness” were included in this review. All apps were first reviewed by three independent reviewers to determine whether content was designed for diabetes self-care. A total of 54 apps meeting this criterion were downloaded & evaluated. Overall, we found many discrepancies between the information in the description of each app, & their attributes upon downloading. Only 30 (55%) of the apps downloaded were in Spanish. Reliability between the number of AADE7TM behaviors found in the description & upon downloading was poor, only 20% of the number of behaviors claimed were available on the apps (kappa=.032). Results suggest the need for a revision on content or language availability for apps targeting Spanish-speaking individuals with diabetes. Diabetes apps available in Spanish are not grounded in behavioral theory or follow evidence-based guidelines, limiting their long-term impact for diabetes self-care, & potentially putting users at risk.

55-LB

Illness Identity and Self-Management of T1DM in Adolescents

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As glycemic control tends to deteriorate during adolescence, the purpose of this current study was to investigate how the process of identity development is related to self-management in adolescents with Type 1 diabetes (T1DM). Semi-structured interviews and validated measures of self-efficacy, social competency, and self-care were completed with 81 adolescents with T1DM, ages 13 to 21. Participants were predominantly ethnic minorities (47% Latino, 28% Black) had a mean age of 15.9 (SD=2.1) and had been diagnosed for 6.7 (SD=3.7) years; 53% were female and 55% were on insulin pump. Overall, glycemic control was poor (HbA1c: M=10.0, SD=2.4). Thematic content analysis determined whether adolescents had successfully “incorporated” T1DM in their identity, based on criteria informed by Charmaz’s (1998) theory of identity development in chronic illness. Those classified as “incorporated” described: acceptance of illness/treatment as a necessary part of daily life, sharing of T1DM status and knowledge with peers, and success in managing stigma. Routines of an ANCOVA, controlling for age, gender, insulin method, and duration of illness, indicated that those successfully incorporating had significantly lower HbA1c (difference: -1.4%, p<0.007), better self-care (p=0.005), higher self-esteem (p=.001), and greater perceived social competency (p=.015). Logistic regressions controlling for the same covariates were used to test HbA1c, self-care, self-esteem, and social competency as predictors of successful incorporation. In separate models, all predictors were significant (p<.02). However, a multivariate model showed that only social competency was a significant independent predictor of incorporation of T1DM into identity (p=0.04), with self-esteem trending toward significance (p=0.08). These results suggest that social competency is a significant predictor of successful incorporation of T1DM into the sense of self, and positive integration of T1DM in their identity, based on criteria informed by Charmaz’s (1999) theory of identity development in chronic illness.

56-LB

Willfulness to Initiate/Intensify Medications in the Second Diabetes Attitudes, Wishes, and Needs (DAWN2) Study

MARK PFEYRITZ, SIMREN E. SKOVLYND, Baltimore, MD, Bagsværd, Denmark

Delaying initiation or intensification of antihyperglycemic medication is a barrier to achieving optimal outcomes of diabetes care. DAWN2 examined the beliefs of people with diabetes (PWD) in 17 countries about medication and their willingness to initiate antihyperglycemic medications and increase insulin injections. Respondents included those with: type 1 diabetes mellitus (DM) (T1 = 1332), type 2 DM insulin-medicated (T2i = 2471), type 2 DM oral medication (T2o = 2771), type 2 DM no medication (T2n = 1700). Unweighted data were analyzed with no adjustment for country-level effects or individual-level respondent characteristics. Among T2n, 27% were unwilling to initiate oral medication if recommended by their healthcare provider (HCP), but significantly (p<.05) more reported that they would not be willing to start insulin (43%) or other injectable medication (46%). Among T2o, 38% were unwilling to initiate insulin or other injectable medication (41%) if recommended by HCP. Significantly (p<.05) fewer users using T1 (T1 = 99, T2i = 38%) were unwilling to initiate insulin or other injectable medication other than insulin; their unwillingness to increase insulin injections was similar (39% and 38%, respectively).

In multivariate analysis, T2o were less willing and T2i more willing than T1 to initiate/intensify insulin or initiate other injectable medication. Several beliefs such as, ability to avoid complications, current medication effectiveness, and understanding of current medication were associated (p<.05) with increased willingness to initiate/intensify both types of medication. Perceived injection pain was associated with lower willingness to initiate both types of medication. Weight worry and dietary restrictions were associated with an increased willingness to initiate injectable medication other than insulin. Our results suggest that psychological barriers to medication enhancement represent a significant barrier to effective diabetes care.

57-LB

Understanding the Influence of Low Income and Education Level on Glycemic Control: A Mediation Analysis

JANIE HOULE, MARIE-DOMINIQUE BEAULIEU, SOPHIE MEUNIER, JEAN-LOUIS CHASSON, FRANÇOIS LESPERANCE, JOSÉ CÔTÉ, IRENE STRYCHAR, JEAN LAMBERT, Montréal, QC, Canada

Living in poverty and low educational level have consistently been associated with poorer glycemic control. It is important to better understand how these two determinants of health influence diabetes control if we want to successfully intervene with these client groups. This study examines the contribution made by a variety of mediating factors known to be important in diabetes control: cognitive variables (illness representations, motivation, self-efficacy, cognitive functioning), behavioral variables (coping strategies, self-management), social variables (support from family, friends and peers) and medical variables (working alliance, care concordant with the Chronic Care Model). We conducted a 1-year cohort study in which 237 patients with type 2 diabetes were evaluated at baseline, 6 months and 12 months, using self-administered and HbA1C measures. Using the Preacher and Hayes approach, statistical analyses were performed to test the indirect effect of each mediator on the dependent variables: living in poverty and educational level. Mediation analyses revealed that depressive symptoms, avoidance coping strategies, and the representation that diabetes is unpredictable all mediate the relationship between living in poverty and glycemic control. Educational level has a negative association with glycemic control, mediating through lower levels of cognitive functioning and avoidance coping strategies. Social and medical variables were not identified as mediators in our analyses. Our results suggest that illness representations and coping strategies need to be explored and addressed, paying more attention to individuals with diabetes who live in poverty or have a lower education level.

58-LB

Hyperamylinemia Promotes Amylin Deposition in the Brain and Affects Brain Function

SARAH SREDLUSKI, SAVITA SHARMA, ADAM BACSTETTER, JENNIFER BRELSFORD, CONRADO PASCUAL, XINMIN XIE, KATHRYN SAATMAN, NELSON PETER, LINDA VAN ELDIK, FLORIN DESPA, Lexington, KY, Redwood, CA

Chronic hypersecretion of the pancreatic hormone amylin is common in humans with prediabetes and leads to amyloid deposition and proteotoxicity in pancreas. We recently showed that amylin deposits are also present in failing diabetic hearts and brain samples from patients with type-2 diabetes (T2D) and dementia. Here, we investigated whether amylin deposition impacts brain function.

Because rodent amylin is neither amyloidogenic nor cytotoxic, we used rats that overexpress human amylin in the pancreas (HIP rats) and wild-type (WT) rats as controls to assess mechanistically how a “human” hyperamylinemia affects brain function. Cage activity, rotorod and novel object recognition tests were performed on all animals. Brain amylin deposition was documented in cerebrovascular amylin antibody. We also assessed the level of lipid peroxidation in cortical arteries by confocal microscopy and cerebral inflammation by immunohistochemistry, qRT-PCR and cytokine protein levels. HIP rats, but not WT littermates, display deposition of amylin in the brain. Compared to WT rats, HIP rats show i) changes in active/inactive rhythm, motor coordination and balance, ii) impaired recognition memory and iii) no ability to improve the performance on the rotorod. Neurologic deficits in HIP rats may be due to oligomerized amylin-induced oxidative stress and inflammation. We For author disclosure information, see page LB91.
found elevated lipid peroxidation in smooth muscle cells isolated from HIP rat cortical arteries. Amylin deposits are co-localized with macrophages and activated microglia. Multiple inflammatory markers are expressed in HIP rat brains as opposed to WT rats, confirming that amylin deposition in the brain induces a neuroinflammatory response.

We therefore conclude that accumulation of aggregated amylin in the brain leads to neurological deficits through mechanisms that involve oxidative stress and inflammation. A novel brain amylin pathology could be a mechanism by which T2D predisposes to brain injury and cognitive decline.

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59-LB

Investigation of the Presence and Impact on Patients of Diabetes Social Stigma in the USA

ALEXANDRA FOLKAS, ADAM S. BROWN, JASMINE CARVALHO, VINCENT WU, KELLY L. CLOSE, RICHARD WOOD, San Francisco, CA

Does diabetes come with social stigma? Is diabetes management affected? Diabetes requires monitoring of blood glucose, adherence to demanding therapies, and regulated lifestyle regimes: difficult and unwelcome additions to patients’ lives. How patients feel socially about their diabetes can significantly impact adherence to - and efficacy of - their therapies.

To measure how perceptions affect people with diabetes, we surveyed 5,410 patients in the USA with type 1 diabetes (T1D) and type 2 diabetes (T2D) in December 2013, to learn whether they felt diabetes came with social stigma. The answer was a strong ‘yes’ from those with T1D (76%), particularly in parents of children with diabetes (83%). Respondents with T2D were equivocal, at 52% overall, and 55% amongst those taking insulin (A).

How social stigma is experienced did not vary by demographics (age, income, education, location). However, insulin patients were 72% more likely than patients on oral agents only (31% vs. 18%), to experience guilt, embarrassment, shame, blame or isolation (B). This likelihood increased with intensive therapy (83% more likely for rapid-acting insulin users) (C), and with poor glucose control (116% more likely for patients with an A1c>8%) (D) (33% and 39%). These data highlight where the social stigma associated with diabetes is felt most, and presents opportunities to better support subpopulations of patients who are most in need.

Meta-analysis showed a significant non-linear dose-response relation between nap time and the risk of type 2 diabetes, with a J-curve relation between nap time and the risk of diabetes, with longer nap time increasing the risk of diabetes at longer times. In conclusion, there was a non-linear dose-response relation.

Meta-analysis revealed that a longer nap time (>60 min/day) significantly increased the risk of type 2 diabetes (relative risk 1.46 (1.23-1.74, p<0.001)), while shorter nap time (<60 min/day) did not (0.95 (0.75-1.21, p=0.68)).

Meta-analysis showed a significant non-linear dose-response relation between nap time and the risk of diabetes (P for non-linearity=0.01) (Figure), with no effect of nap time up to about 40 minutes/day followed by a sharp increase in the risk of diabetes at longer times. In conclusion, there was a J-curve relation between nap time and the risk of type 2 diabetes, with longer nap times being associated with an increased risk.
challenges physicians may encounter at T2D diagnosis and the Jefferson Scale of Physician Empathy. Across countries, 76-100% agreed that diagnosis conversations impact on pts’ disease acceptance/treatment adherence. Factor analysis of the 12 challenges yielded 2 factors (Table). Discouraged with Pts at Diagnosis (DPP; α = 0.87) and Frustrated with Situation at Diagnosis (FSD; α = 0.72). Correlation between factors suggested related but distinct groups of challenges (r = 0.64, p < 0.0001). Factor scores were higher (DPP highest in France; FSD in Japan). Upon adjusting for demographic/clinical practice variables, regression models showed a negative relationship between physician empathy and perceived challenges for total score (all 12 items) as well as DPP and FSD (p < 0.0001). Thus many physicians, especially those scoring lower on empathy, report significant challenges and frustrations with diagnosis conversations. 92% wanted tools to help pts sustain behavioral change. Supporting use of empathy-related skills may contribute to better pt outcomes.

<table>
<thead>
<tr>
<th>12-item Questionnaire: “Challenges at Diagnosis”</th>
<th>% physicians answering</th>
<th>No</th>
<th>In ...</th>
<th>Some</th>
<th>Most</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortly after diagnosis, patients fail to keep up with the required behavioral changes and return to old habits</td>
<td>9</td>
<td>22</td>
<td>47</td>
<td>26</td>
<td>2</td>
<td>EPP</td>
</tr>
<tr>
<td>The patient does not understand the seriousness of the situation</td>
<td>9</td>
<td>25</td>
<td>48</td>
<td>21</td>
<td>2</td>
<td>EPP</td>
</tr>
<tr>
<td>It is frustrating to work with T2D patients that don’t follow my recommendations</td>
<td>14</td>
<td>34</td>
<td>33</td>
<td>15</td>
<td>5</td>
<td>EPP</td>
</tr>
<tr>
<td>It is difficult to convince patients that they can take control of their disease</td>
<td>7</td>
<td>33</td>
<td>42</td>
<td>16</td>
<td>2</td>
<td>EPP</td>
</tr>
<tr>
<td>It is difficult to develop a treatment plan with patients that they feel follow</td>
<td>13</td>
<td>39</td>
<td>36</td>
<td>13</td>
<td>2</td>
<td>EPP</td>
</tr>
<tr>
<td>It is difficult to convince patients to stay positive</td>
<td>9</td>
<td>41</td>
<td>37</td>
<td>12</td>
<td>2</td>
<td>EPP</td>
</tr>
<tr>
<td>Patients do not see the benefits/need to collaborate with me to manage the disease</td>
<td>12</td>
<td>42</td>
<td>34</td>
<td>10</td>
<td>2</td>
<td>EPP</td>
</tr>
<tr>
<td>Patients leave the visit without having a clear idea of what they are supposed to do</td>
<td>20</td>
<td>43</td>
<td>26</td>
<td>9</td>
<td>2</td>
<td>EPP</td>
</tr>
<tr>
<td>I don’t have enough time</td>
<td>25</td>
<td>25</td>
<td>27</td>
<td>17</td>
<td>6</td>
<td>EPP</td>
</tr>
<tr>
<td>I do not receive enough support from others (my team, nurses, etc.)</td>
<td>49</td>
<td>25</td>
<td>15</td>
<td>8</td>
<td>3</td>
<td>EPP</td>
</tr>
<tr>
<td>It is difficult to deal with patients’ emotional responses to the diagnosis</td>
<td>17</td>
<td>41</td>
<td>31</td>
<td>11</td>
<td>2</td>
<td>EPP</td>
</tr>
<tr>
<td>It is difficult to explain diabetes to these patients</td>
<td>25</td>
<td>28</td>
<td>27</td>
<td>8</td>
<td>2</td>
<td>EPP</td>
</tr>
</tbody>
</table>

Supported By: Boehringer Ingelheim/Eli Lilly and Company

63-LB

Examining the Relationship between Physical Activity, Psychological Mediators of Physical Activity, and Negative Symptoms in Individuals with Psychosis and Diabetes

Paul Gorczynski, Hirun Patel, Rohan Gandhi, Toronto, ON, Canada

Individuals with psychosis and Type 2 Diabetes Mellitus (T2DM) are physically inactive and are at risk for cardiovascular disease and premature mortality. Becoming physically active can mitigate this risk. Researchers have suggested that behavioral interventions designed to increase physical activity in this population would be theoretically sound and account for the symptoms of psychosis. This study examined the relationship between physical activity, psychological mediators of physical activity, and negative symptoms in people with psychosis and pre-diabetes or T2DM to better understand what variables to target in future interventions. Forty-nine individuals with psychosis and pre-diabetes or T2DM participating in a randomized controlled trial examining a weight loss intervention were included in the analysis. Negative symptoms were evaluated using the Scale for the Assessment of Negative Symptoms (SANS); physical activity was assessed using the International Physical Activity Questionnaire; while psychological mediators of physical activity, including self-efficacy, barriers to and benefits of physical activity, were assessed using the Patient-Centered Assessment and Counseling for Exercise questionnaire. Spearman’s correlations showed a significant association between physical activity and negative symptoms (r = -0.35, p < 0.05). Significant correlations existed between physical activity and self-efficacy (r = -0.37, p < 0.01) and perceived barriers to physical activity (r = -0.35, p < 0.05), but not perceived benefits of physical activity (r = -0.11, p = 0.45). The best predictor of physical activity was self-efficacy (r = -0.31, p < 0.05). Results suggest that interventions should aim to increase an individual’s confidence to be active in an attempt to improve physical activity. For individuals with high negative symptom scores, personalized engagement approaches may be necessary.

64-LB

Relationship of Self-Esteem to Glycemic Control among Minority Adolescents with Type 1 Diabetes

Joslyn Kenowitz, Persis Commissariat, Jeniece Traust, Rubrina Hep-Tulla, Jeffrey S. Gonzalez, Bronx, NY

The purpose of this study was to examine relationships between psychosocial factors and self-management in adolescents with type 1 diabetes. Eighty-five adolescents ages 13-21 years (54% female; 47% Latino, 29% Black; 55% used insulin pump) completed validated self-report measures of self-esteem, quality of life and self-care. Diabetes-specific self-esteem (DSSE) and three subscales on the Diabetes-Specific Quality of Life (GQoL) Scale (life satisfaction, disease impact and disease worry) were examined in relation to self-care and HbA1c using Pearson correlation and linear regression. The group had a suboptimal HbA1c (10.2% ± 2%). DSSE (p < 0.001) was associated with all GQoL subscales (r = 0.0001) and DSSE (r = 0.0001). Males reported higher satisfaction, lower impact, and lower worry than females (p < 0.002). Lower HbA1c was significantly correlated with lower disease impact (r = 0.008), greater life satisfaction (r = 0.003) and higher DSSE (p < 0.001). Regression analyses, controlling for age, gender, duration of illness and insulin method, indicated that DSSE was the strongest predictor of better self-care (B = 0.001, p < 0.001) and lower HbA1c (B = -0.54, p < 0.001). When self-care was added into the model, only DSSE was a significant predictor of HbA1c (B = -0.35, p < 0.004), while self-care, was not (B = -0.19, p = 0.086). These findings suggest gender differences in diabetes-specific QoL, and demonstrate that QoL and DSSE are important correlates of self-care and glycemic control across ethnically diverse adolescents. Assessment of adolescents’ subjective sense of how well they are meeting their diabetes self-management goals (DSSE) may identify those at risk for poor glycemic control. Results suggest that DSSE may be more closely linked with glycemic control than assessments of self-care behavior frequency.

65-LB

How Do Depressive Symptoms Influence Diabetes Self-Management and Glycemic Control? The Contribution of Mediating Variables

Janice Houle, Marie-Dominique Beaulieu, Sophie Meunier, Jean-Louis Chasson, François Lesferrance, José Côté, Irene Strychar, Jean Lamber, Montréal, QC, Canada

It is well known that depressive symptoms have a negative impact on diabetes self-management behaviors and glycemic control. This study seeks to provide a better understanding of this influence by examining mediating variables: illness representations, diabetes self-efficacy, motivation, social support, the physician-patient relationship, care concordant with the Chronic Care Model, coping strategies, and general health condition. In an observational prospective study, we assessed 237 adult patients with type 2 diabetes at baseline, 6 months and 12 months. Using the Preacher and Hayes approach, statistical analyses were performed to test the indirect effect of each mediator on both dependent variables: self-management behaviors, as measured by the Summary of Diabetes Self-Care Activities - Revised, and glycemic control, using HbA1c level. Our results indicate that diabetes self-efficacy, working alliance, chronic illness care, self-management support from family and friends, and coping strategies all mediate the relationship between depressive symptoms and self-management behaviors. However, the belief that diabetes is cyclical and unpredictable as well as a negative emotional reaction to diabetes mediate the relationship between depressive symptoms and glycemic control. The results suggest specific targets for intervention to achieve better self-management behaviors and glycemic control in patients with type 2 diabetes and depressive symptoms. The negative impact of depressive symptoms on diabetes outcomes may be lessened by improving self-efficacy and coping strategies, further developing the working alliance, and providing care concordant with the Chronic Care Model.

Supported By: CIHR

66-LB

Association of Medication Adherence with Psychological Distress in Relation to Types of Antidiabetic Medications among Patients with Uncontrolled Type 2 Diabetes

Melanie Siaw, Yu Ko, Daniel Malone, Yilun Lee, Elaine Tan, Joyce Yu-Chia Lee, Singapore, Singapore, Singapore, AZ

Although studies have focused on medication adherence and diabetes distress, little is understood about how different types of antidiabetic medications may play a role in this intricate relationship between adherence and diabetes distress. In this cross-sectional, multicenter study, we aimed to examine the association of medication adherence with psychological distress among patient taking different types of antidiabetic agents. All patients with HbA1c > 7% were included in this study while patients with limited language proficiency were excluded. A questionnaire which included an 8-item Morisky Medication Adherence Score (MMAS) and a 20-item Problem Areas in Diabetes Scale (PAID) were administered to all eligible patients. Of the 349 patients approached, 312 (89.4%) were eligible for the study. The mean age was 59.4 ± 8.1 years with 42.9% female and 57.1% male. The average HbA1c was 8.4 ± 1.3%. In addition, 211 (67.6%) were on oral hypoglycemic agents while 101 (32.4%) were on insulin-containing regimen. Overall, PAID scores
were 23.3 ± 16.5 while the adherence rate for low, medium and high were 121 (38.8%), 123 (39.4%) and 67 (21.5%) respectively. Using general linear model, adjusted for age, gender, ethnicity, education level, marital and employment status, duration of diabetes and number of comorbidities, our study showed that lower adherence to medications was associated with higher psychological distress (β = 0.033, p = 0.001). Interestingly, types of antidiabetic medications did not significantly influence the relationship between medication adherence and psychological distress (p > 0.05). In conclusion, the association of medication adherence with psychological distress was not related to the types of antidiabetic medications taken by patients.

**67-LB**

**A New Validated Measure of Diabetes Distress for Adults with Type 1 Diabetes**

**LAWRENCE FISHER, WILLIAM POLONSKY, DANIELLE HESSLER, USA STROCKER, UMESH MASHARANI, IAN BLUMER, ANNE L. PETERS, VICKY BOWYER, SAN FRANCISCO, CA, SAN DIEGO, CA, EUGENE, OR, WHITBY, ON, CANADA, LOS ANGELES, CA**

Several measures have been used to assess diabetes distress (DD) but none has targeted the unique worries and fears of T1D adults that are linked to clinical outcomes. We developed and validated a new survey instrument to address this need.

Items (58) were developed from interviews with 25 T1D adults and 10 providers. The validation sample consisted of 478 eligible patients identified from local clinics and diabetes registries in the U.S. and Canada, 412 completed an online survey (86%) that also included measures of Quality of Life (WHO5), depression (PHQ8), number of complications, Hypo Fear Survey-Worry, and HbA1C. The final sample contained 303 U.S. and 109 Canadian patients. Each received a $15 gift card.

Patient age (U.S./Canada) = 43.2 / 41.9, % female 55.4 / 54.1, years with T1DM = 22.5 / 26.0, HbA1C = 7.45 / 7.99 (9.3/10.1). Exploratory principal components analysis with promax rotation was undertaken with the U.S. sample, and a confirmatory analysis was performed with the Canadian sample. The same stable and clinically meaningful 7-factor solution (28 items) emerged in both analyses, and internal reliability and construct validity coefficients were highly significant (Table 1).

The T1-DDS is a reliable and valid measure of DD for use with adults with T1D. The 7 subscales reflect a comprehensive profile of worries and concerns that lower adherence to medications was associated with higher psychological distress, and psychological distress (p > 0.05). In conclusion, the association of medication adherence with psychological distress was not related to the types of antidiabetic medications taken by patients.

**T1-DDS sub scales**  

<table>
<thead>
<tr>
<th>No. items</th>
<th>PHQ 8</th>
<th>WHO 5</th>
<th>HbA1C r</th>
<th>Hypo worry s r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powerlessness</td>
<td>5</td>
<td>.87</td>
<td>.45</td>
<td>.43</td>
</tr>
<tr>
<td>Management, distress</td>
<td>4</td>
<td>.78</td>
<td>.23</td>
<td>.15</td>
</tr>
<tr>
<td>Hypo, distress</td>
<td>4</td>
<td>.78</td>
<td>.34</td>
<td>.24</td>
</tr>
<tr>
<td>Negative social perceptions</td>
<td>5</td>
<td>.85</td>
<td>.35</td>
<td>.04</td>
</tr>
<tr>
<td>Eating distress</td>
<td>3</td>
<td>.73</td>
<td>.30</td>
<td>.21</td>
</tr>
<tr>
<td>Physician/ friend distress</td>
<td>4</td>
<td>.80</td>
<td>.16</td>
<td>.10</td>
</tr>
<tr>
<td>Family/ friend distress</td>
<td>4</td>
<td>.79</td>
<td>.20</td>
<td>.12</td>
</tr>
</tbody>
</table>

*1 p < .05, *2 p < .01, *3 p < .001

Supported By: DK094683

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**68-LB**

**Emergence of Emotional Support in Peer Support Interventions: A Cross-Cultural Study**

**SARAH KOWITT, DIANA URLAUB, LAURA GUZMAN-CORRALES, MELISSA MAYER, JUANA BALLESTROS, DAVID SIMMONS, EDWIN B. FISHER, Chapel Hill, NC, Chicago, IL, Cambridge, United Kingdom**

Emotional support is commonly reported in peer and social support interventions to assist diabetes management. However, individuals often initially demand wanting to obtain emotional support and modes of emotional support vary across cultures. This study examined how emotional support emerged in interactions between peer supporters and participants in two distinct cultural settings. 7 Latino peer supporters serving a low-income, Hispanic population in Chicago, and 9 retired, middle class supporters in a program for an older, predominately Caucasian population in the United Kingdom (UK) completed semi-structured interviews focusing on their relationships with those they helped. Coding field notes used deductive and inductive codes and consensus among 3 coders to ensure accuracy. Consistencies across both cultures included a) gradual emergence of emotional support and b) emphasis on implicit support. Type of support varied over time. Initially, peer supporters provided information for diabetes management; over time, they came to provide substantial emotional support. Emotional support was frequently conveyed not explicitly (e.g., by reassurance or discussing stressors) but implicitly, in the manner in which information was shared. Implicit modes of support include non-verbal actions that convey emotional acceptance, e.g., a walk together, but do not involve discussion of problems. Cross-cultural differences did appear for barriers to diabetes management. Social concerns were more likely in the U.S. than the UK. Regarding the role of peer support, those in the U.S. were more likely informally to include family and provide directive support whereas UK peer supporters reported more nonDirective support. These findings suggest that peer supporters gradually provide emotional support through similar strategies across cultures, but that support is tailored to problems facing participants and to cultural factors, including the role of family and style (nonDirective, directive) of support.

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**69-LB**

**Continuous Sensing of Glucose at the Site of Insulin Delivery in Swine**

**WILLIAM K. WARD, SHEILA BENWARE, MATTHEW BRENN, KRISTIN MORRIS, XIAOSONG DU, CHRISTOPHER DURGAN, DAVID MATTHEWS, JOHN CONLEY, GREGORY HERMAN, ROBERT CARILL, PORTLAND, OR, CORDALIS, OR**

People with type 1 diabetes (T1D) who use an insulin pump and a continuous sensor must insert two devices. To address the issue of whether glucose can be measured at the site of insulin delivery, we developed a catheter on which sensing elements are disposed, allowing continuous glucose measurement at the site of insulin delivery. We hypothesized that glucose sensing catheters could provide glucose measurement directly at the site of subcutaneous (SC) insulin infusion.

Microfabrication and photolithographic techniques were used on a flat substrate to create multiple redundant indicating electrodes and a single, common reference electrode. Each substrate was then wrapped around, and adhered to, a 21-gauge steel needle. Compounds required for amperometric sensing were applied after wrapping.

Sensing catheters were tested in octreotide-treated, anesthetized Yucatan pigs. Several sensing catheters, connected to transceivers, were placed in the SC tissue. After initial stabilization, a euglycemic glucose clamp was carried out for 3.5 h. High dose insulin was infused through some of the sensing catheters (total rate, 0.7 u/ h). The final hour consisted of a hyperglycemic clamp. A notebook computer remotely collected transmitted data. A one-point calibration was performed at the start of insulin delivery. Data were available for 10 sensors through which insulin was infused and for 15 sensors in which no insulin was given.

Despite an increasing and large insulin effect, there was no clear time-related decline of the glucose signal from the sensing catheters that delivered insulin. There was a non-significant trend for sensed glucose levels to be slightly lower in the insulin catheters vs. non-insulin catheters. Insulin and non-insulin sensors performed similarly during hyperglycemia. Accuracy was improved by 30% with redundant sensing (2-6 sensing units per catheter) as compared to individual sensing units.

Our data suggest that it is possible to measure glucose continuously from the site at which insulin is delivered into SC tissue. Multiple sensing units on a single catheter appear to improve sensing accuracy.

** Supported By: NIDDK; Leona M. and Harry B. Helmsley Charitable Trust

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**70-LB**

**Time Lag of Glucose Transport from Intravascular to Interstitial Compartment in Type 1 Diabetes**

**SIMMI DUBE, SUNA VEETIL, MICHAEL SAMA, YOGISH C. KUDVA, CLAUDIO COBELLI, ANANDA BASU, RITA BASU, ROCHESTER, MN; PADUA, ITALY**

In the overnight fasted state, the physiological delay of glucose transport from vascular to interstitial space is ~6 min in healthy adults. The current studies were undertaken to assess the time lag in glucose appearance from intravascular to interstitial compartment in type 1 diabetes mellitus (T1D). Microdialysis catheters were placed in the abdomen of six T1D (age 44 ± 14 years, BMI 25.2 ± 3.6 kg/m², HbA1c 7.8 ± 0.9%) following an overnight fast.
**CLINICAL THERAPEUTICS/NEW TECHNOLOGY—GLUCOSE MONITORING AND SENSING**

[1-13C], [6,6-D2]-H2, [2,13C] glucose were administered sequentially at two hour intervals to achieve ~4% enrichment. Plasma/microdialysate samples were collected periodically for isotopic enrichments. Regular insulin was infused starting at 9 PM to maintain glucose concentrations (~113 mg/dL) throughout the study period. Figure 1 illustrates subject-specific profiles for [6,6-D2]-H2 glucose enrichments in plasma (A) and microdialysate (B) after tracer bolus (left panels) and with more resolution (right panels). Profiles of [1-13C] and [2-13C] glucose were similar to [6,6-D2]-H2 glucose. After accounting for catheter dead space transit time (6.2 min) and assay noise (MR > 0.3%), mean time lag of tracer appearance in the interstitial space was ~6 min, which is similar to healthy adults. Model derived equilibration time needs to be estimated for T1D. This data will help optimize future generation glucose sensor algorithms for the artificial endocrine pancreas systems.

Supported By: Leona M. and Harry B. Helmsley Charitable Trust (2012PG-T1D005); Dexcom, Inc. (DK29953)

**71-LB**

**Dulce Wireless Tijuana: A Randomized Control Trial Studying the Impact of the Project Dulce™ Model and Mobile Technology on Metabolic Outcomes, Quality of Life, and Behaviors**

MARIA CECILIA ANZAÑADO-CAMPOS, SONIA CONTRERAS, ADRIANA CAROLINA VARGAS-OJEDA, RIUPINO MENCHACA-DIAZ, ATHENA PHILIS-TSIMIKAS, Tijuana, Mexico, National City, CA, La Jolla, CA

The prevalence of type 2 diabetes mellitus (T2DM) is rapidly rising in the U.S./Mexico border regions (15.7% in adults). Efficient and cost-effective diabetes treatment and education.

**Table 1.**

<table>
<thead>
<tr>
<th>HbA1c (%)</th>
<th>QOL</th>
<th>LS</th>
</tr>
</thead>
<tbody>
<tr>
<td>81</td>
<td>16.88 (18.88)</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>19.28 (18.88)</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>54.08 (41.08)</td>
<td></td>
</tr>
</tbody>
</table>

**72-LB**

**Systematic Performance Evaluation of Five Blood Glucose Meters at Stable Low, Normal, and High Blood Glucose Levels**

ERIC ZIJLSTRA, ANNELEI FISCHER, CHRISTOPH KAPITZA, Neuss, Germany

Accuracy of a blood glucose (BG) meter is determined by the system’s random error (measurement variability) and systematic error (measurement bias). We performed a systematic evaluation of 5 commercially available BG meters to investigate their measurement performance at 3 stable BG levels, 60 - 100 - 200 mg/dL. Sixteen subjects with type 1 diabetes participated in this open label, single center trial. The accuracy analysis was performed for each of the 3 levels by variable rate infusions of glucose and insulin. Once BG was stabilized, medical staff performed regular fingerpricks (up to 10 per BG level) to obtain capillary blood samples for paired BG meter and YSI reference measurements. Each sample was measured in duplicate (on 2 devices) to investigate the precision absolute relative difference (PARD). One subject was excluded from the analysis due to problems with repeated capillary blood sampling.

Key results are shown (Table). At each BG level and overall, the BGStar, iBGStar and Accu-Chek meters showed the lowest bias and the highest measurement accuracy. Measurement variability, as well as PARD was similar for most meters at the 3 BG levels and overall.

<table>
<thead>
<tr>
<th>Meters</th>
<th>Measurement Accuracy—MARD (%)</th>
<th>Measurement Variability—SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGStar</td>
<td>5.7</td>
<td>4</td>
</tr>
<tr>
<td>iBGStar</td>
<td>6.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Accu-Chek</td>
<td>6.3</td>
<td>9.4</td>
</tr>
<tr>
<td>One Touch</td>
<td>6.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Freestyle</td>
<td>6.5</td>
<td>6.4</td>
</tr>
</tbody>
</table>

**73-LB**

**Accuracy of BG Detection in Diabetes Alert Dogs (DADs)**

JACLYN SHEPARD, JESSE GRABMAN, DALLAS DUCAR, ANAND TRIPATHI, CHARLOTTEVILLE, VA

Use of DADs to monitor BG extremes in type 1 diabetes is growing, but there is little data on their accuracy. This study investigated DAD accuracy using owner diaries of daily BG levels and DAD alerts. Participants were 18 DAD owners (44.4% female; 77.8% children) with T1D, all of whom obtained a DAD from the same training organization. Adults ranged in age from 40-47 yrs (M = 44.3 ± 4.4) and children ranged from 2-15 yrs (M = 9.1 ± 4.9). Participants (or parents) completed diaries, recording all daily BG readings and DAD alerts. Number of days of completed diaries ranged from 5-134 and number of entries ranged from 34-560. For each DAD, % Hits (alert with BG ≥ 11.1 mmol/L), % Misses (no alert with BG ≥ 11.1 mmol/L), and % False Alarms (alert with BG > 5.0 and < 11.1 mmol/L) were computed.

Table 1 shows an overview of results. Comparison of DAD Hits to Misses found significantly more Hits for both low and high BGs. There were significantly more Hits than False Alarms (p2 = 0.65, p65% and 44.4% hit >70%. For high BG, 16.7% of DADs hit >85% and 5.6% hit >70%

For author disclosure information, see page LB17.
Results indicate that DADs may be an effective tool for detecting out of range BG values. However, more research is needed to establish DAD accuracy and identify factors influencing variability of DAD accuracy in BG detection.

Table 1. Overview of Results.

<table>
<thead>
<tr>
<th>Test</th>
<th>Hit %</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Total Number</th>
<th>Chi Square</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>54.4%</td>
<td>38.0%</td>
<td>73.7%</td>
<td>2014</td>
<td>43.34</td>
<td>&lt;.0005</td>
</tr>
<tr>
<td>Low BG</td>
<td>65.6%</td>
<td>33.3%</td>
<td>100.0%</td>
<td>584</td>
<td>19.24</td>
<td>&lt;.0005</td>
</tr>
<tr>
<td>High BG</td>
<td>52.1%</td>
<td>29.4%</td>
<td>76.9%</td>
<td>1430</td>
<td>24.19</td>
<td>&lt;.0005</td>
</tr>
</tbody>
</table>

74-LB

Use of Structured Self-Monitoring of Blood Glucose Improves Glycemic Control in Australians with Non-Insulin-Treated Type 2 Diabetes: First Results of the SteP IT UP Trial

JANE SPEIGHT, JESSICA L. BROWN, GEORGE KOUAMANTAKIS, ALAN BARCLAY, HILTON SHAPIRO, GARY DEED, TRACY HUNTER, WALTER KOEHLER, CHRISTOPHER PARKIN, LUISA CAPEZIO, MATTHIAS A. SCHWEITZER, BETTINA PETERSEN, Melbourne, Australia, Castle Hill, Australia, Paddington Heights, Australia, Mascot, Australia, Coorparoo, Australia, Indianapolis, IN, Frankfurt, Germany, Boulder City, NV, Mannheim, Germany

Structured self-monitoring of blood glucose (SMBG) is an approach in which blood glucose data are gathered according to a defined regimen, interpreted and utilized to make appropriate pharmacologic and/or lifestyle adjustments. Its benefits have been demonstrated in efficiency and effectiveness studies in the U.S. and Europe but the generalizability of these findings have not yet been shown in Australia. The Structured Testing Program Implementation Trial (SteP IT UP) assessed the impact of structured SMBG on HbA1c and diabetes-related distress in 136 adults with non-insulin-treated type 2 diabetes managed in primary care settings across Australia: mean [SD] HbA1c 8.7 [1.2]% age 60.8 [12.2] years, 39.7 % women, BMI 32.1 [6.3]. In this 24-week, multi-center, open-label, controlled study, Australian clinicians with structured SMBG experience trained patients to use and interpret structured SMBG (3-day, 7-point profiles), using the Accu-Chek 360° View, a tool paper tool. Patients completed the tool prior to their visits at weeks 4, 12, and 24, results were discussed at each visit. Data from preliminary analyses of 25% of enrolled patients (n=77) showed reductions in HbA1c from week 4 to weeks 12 and 24 [-0.3[1.2], P<0.0001, -1.1[4.1], P<0.0001, respectively], with no increase in hypoglycemia [<72 mg/dL, <4 mmol/L]. Reductions in percentage of high glucose values (>180 mg/dL / >10 mmol/L) were seen at weeks 12 and 24 [-7.2[1.5], P=0.0114, -11.1[25.6], P=0.0049, respectively]. Diabetes-related distress showed no increase at weeks 12 or 24 (0.1[0.77], P=0.2294; -0.16[0.93], P=0.1811, respectively). In this preliminary analysis, use of structured SMBG by Australian adults with non-insulin-treated type 2 diabetes supported by primary care clinicians is associated with significant improvements in glycemic control without increasing hypoglycemia or diabetes-related distress.

75-LB

CGM Is Not a Limiting Factor in Artificial Pancreas Systems

TIMOTHY S. BAILEY, KATHERINE NAKAMURA, ANNA CHANG, MARK CHRISTIANSEN, DAVID A. PRICE, ANDY BALO, Escondido, CA, San Diego, CA, Concord, CA, Walnut Creek, CA

CGM used for Artificial Pancreatic (AP) systems requires low glucose accuracy for safety, euglycemic and hypoglycemic accuracy to optimize insulin dosing determinations, and consistent performance across sensors and over time. A modified CGM designed specifically for the AP project was assessed in a clinical research study.

The study enrolled 51 subjects from 3 U.S. centers, 86% with T1D. Subjects wore sensors for up to 7 days and used self-monitored blood glucose to calibrate their CGM twice daily. Each subject was in-clinic for 12 hours on day 1, 4, or 7 to collect YSI reference venous glucose every 15 minutes and capillary SMBG test every 30 minutes; glucose was manipulated to provide sufficient data in low and high glucose ranges.

The study concluded that the CGM readings were highly correlated with YSI with correlation coefficient of 0.97 comparing 0.99 of that for SMBG. Using YSI reference, the CGM performed similarly as the SMBG meter. The study showed that the overall point accuracy clinical, accuracy over the duration of wear, accuracy across the glycemic ranges, and reliability (98% of sensors lasted 7 days) were unmatched by current CGM systems. Accordingly, the CGM accuracy should not limit AP system development.

76-LB

A Multicenter Evaluation of the Accuracy of the Contour XT Glucose Meter Following ISO 15197:2013 Accuracy Criteria

JOSE LUIS BEDINI, JANE WALLACE, THORSTEN PETERSCHKE, BARBARA STOLL-SUSS, SCOTT PARDO, Barcelona, Spain, Mishawaka, IN, Leverkusen, Germany, Whippany, NJ

A multicenter study was carried out in 21 Spanish Hospitals to evaluate the performance of the Contour XT (Bayer) glucose meter, under daily routine conditions, in comparison with the hexokinase method.

A total of 76,446 samples were included, of which 56,213 samples were used to perform the ISO 15197:2013 accuracy criteria. At each site, the Contour XT results were compared to those of the respective hexokinase method, to determine whether they were within either ±15 mg/dl of the analyzer result, for samples ≤100 mg/dl, or within ±5% for samples >100 mg/dl. A Consensus Error Grid analysis was performed.

Overall blood testing results showed that 99.43% (2088 out of 2100) of the Contour XT results met the ISO 15197:2013 accuracy criteria. As for individual sites, 14/21 sites had 100% of results within the criteria; five had 99%, one had 98%, and one had 95%.

2096 out of 2100 results (99.8%) were inside the Consensus Error Grid zone A, while the remaining four results (0.2%) were in zone B.

This is one of the few multicenter studies performed with a blood glucose meter. Despite the inclusion of a high number of samples and sites, and the use of different hexokinase analyzers for the comparative glucose measurements, overall results exceed the system accuracy requirements of the ISO 15197:2013 regulation (99.43%). Moreover, individual results from each site also met these requirements. In conclusion, Contour XT tested under daily routine conditions was found to be a highly accurate and robust blood glucose monitoring system.

77-LB

A Wireless Continuous Glucose Monitoring System for Subcutaneous Implantation in Rodents

DANIEL V. AILLON, ERIK NAYLOR, HANS P. HARMON, BRIAN S. BARRET, DONNA A. JOHNSON, DAVID A. JOHNSON, PETER A. PETILLO, Lawrence, KS

Continuous monitoring of glucose concentrations is critical for the study and management of diabetic models in animals. We have developed a continuous glucose monitoring system specifically designed for use in rats. Subcutaneous implantation of the glucose biosensor is achieved using a minimally invasive surgical procedure and the telemetry system is attached to a jacket. Five biosensors were implanted in wild-type Sprague-Dawley rats for at least two...
weeks (Figure 1A). Each sensor was calibrated daily to Accu-Chek readings of tail-vein blood. Daily injections of glucose (1 g/kg p) were administered. No statistical difference was noted between the calibrated sensor reading and the Accu-Chek value taken during the bolus (calibrated value: 174 +/- 14 mg/dL, Accu-Chek Value: 189 +/- 12 mg/dL, P=0.05, two-tailed t-test) after two weeks of continuous monitoring. In a second cohort of animals, the femoral vein was catheterized and blood samples were taken every 8 minutes over the course of a single glucose (1 g/kg iv) or insulin bolus (2 U/kg iv). Using a two-point calibration, responses were accurately tracked by the biosensor (e.g. Figure 1B). The telemetry system integrates seamlessly with a software suite that provides both real-time monitoring of the CGMS signal and the ability to readily process the data.

**CLINICAL THERAPEUTICS/NEW TECHNOLOGY—INSULINS**

**78-LB**

**The Ultra-Rapid BioChaperone Insulin Lispro (BC LIS) Shows a Faster Onset of Action and Stronger Early Metabolic Effect than Insulin Lispro (LIS)**

GRIT ANDERSEN, BERTRAND ALLUIS, GREGORY MEKFRENE, NYMERIEC RANSION, OLIVIER SOULA, GERARD SOULA, REMI SOULA, ANNELIE FISCHER, LESZEK NOSEK, FREIMUT SCHLIESS, TIM HEISE, Neuss, Germany, Lyon, France

In this double-blind, crossover study we investigated the pharmacodynamic characteristics of BC LIS, a novel insulin lispro formulation with BioChaperone aimed at accelerating the absorption from the subcutaneous tissue. Thirty-six people with type 1 diabetes completed this study and received 0.2 U/kg of BC LIS or LIS under automated euglycemic clamp conditions (ClampArt®, target blood glucose 100 mg/dL, clamp duration 6h post-dosing). Mean glucose infusion rates [GIR] are given in the figure. Compared with LIS, BC LIS showed ultra-rapid properties with a faster onset of action (23.1 +/- 7.0 (means:SD) vs. 34.4 +/- 15.3 min, p=0.0001), an earlier maximum effect (Tmax, 99.4 +/- 42.2 vs. 133.3 +/- 45 min, p=0.0002) and a stronger early metabolic effect in the first hour (AUCmax, 218 +/- 88 vs. 129 +/- 63 mg/kg, p<0.0001) and maximum metabolic effect (GIRmax, 34.4 +/- 15.3 min, p<0.0001), an earlier maximum effect (Tmax, 78 +/- 18 vs. 86 +/- 37.5 min, p=0.0041). Total (AUC0-24h, 809 +/- 261 vs. 1434 +/- 576 mg/kg, p=0.0002) and first 2 hours (AUC0-2h, 99 +/- 14 vs. 126 +/- 28 mg/kg, p=0.0001) and a stronger early metabolic effect in the first 2 hours than native insulin lispro. BC LIS has the characteristics of an ultra-fast acting insulin with the potential to be injected at mealtime with excellent glycemic control.

**80-LB**

**Glycemic Control and Hypoglycemia with New Insulin Glargine 3000 U/ml in People with T1DM (EDITION 4)**

PHILIP D. HOME, RICHARD M. BERGENSTAL, MATTHEW C. RIDDLE, MONIKA ZIEMEN, MARIA ROJESKI, MELANIE ESPINASSE, GEREMIA B. BOLLI, ERROL DE SOUZA, Danbury, CT

EDITION 4 studied the efficacy and safety of new insulin glargine (300 U/ml - Gla-300) vs. glargine 100 U/ml (Gla-100) in people with T1DM. In this 6-month, multinational, multicenter, open-label study, participants (n=549, BMI 27.6 kg/m², T1DM duration 21.0 yr, HbA1c 8.12%) were randomized 1:1:1:1 to once-daily Gla-300 or Gla-100, morning or evening, while continuing meal-time insulin. Overall, Gla-300 was non-inferior to Gla-100 for HbA1c change from baseline (primary endpoint) (LS mean change [SE] -0.40 [0.05] % and -0.44 [0.05] %; LS mean difference 0.04 [95% CI: -0.10 to 0.19] %). Event rate of confirmed (0.04 [0.05] % and 0.04 [0.05] %; LS mean difference 0.00 [95% CI: -0.40 to 0.40] %) or severe hypoglycemia at any time of day (24 h) was similar for the two groups, while nocturnal hypoglycemia was lower in the Gla-300 vs. Gla-100 group in the first 8 weeks of the study (Table). Neither glycemic control nor hypoglycemia differed between morning and evening injection groups. Severe hypoglycemia was observed in 0.6% (Gla-300) and 9.5% (Gla-100) of participants. Total insulin dose increased to a somewhat greater extent for Gla-300 vs. Gla-100 group in the first 8 weeks of the study (Table). Neither glycemic control nor hypoglycemia differed between morning and evening injection groups. Severe hypoglycemia was observed in 0.6% (Gla-300) and 9.5% (Gla-100) of participants. Total insulin dose increased to a somewhat greater extent for Gla-300 vs. Gla-100 (change from baseline +0.19 vs. +0.10 U/kg). Weight gain was significantly lower with Gla-300 (difference -0.56 [-1.09 to -0.03] kg, p=0.037). There was no difference in adverse events. In conclusion, Gla-300 provided comparable glycemic control vs. Gla-100 and nocturnal hypoglycemia was reduced during the first 8 weeks.
Clinical Diabetes/Therapeutics

81-LB

Sustained Glycemic Control and Less Hypoglycemia with New Insulin Glargine 300 U/mL Compared with 100 U/mL: One-Year Results in People with T2DM Using Basal + Mealtime Insulin (Edition 1)

Matthew C. Riddle, Geremia B. Boll, Hanneke Yki-Jarvinen, Monika Ziemen, Isabel Muehlen-Bartmer, Sophie Cssonho, Philip D. Home, Portland, OR, Perugia, Italy, Helsinki, Finland, Frankfurt, Germany, Levallois-Perret, France, Newcastle uppon Tyne, United Kingdom

In Edition 1, 887 people with elevated HbA1c using basal + mealtime insulin were randomized to titrated insulin glargine 300 U/mL (Gla-300) or glargine 100 U/mL (Gla-100) once daily in the evening for 6 months, continuing the mealtime insulin. In a 6-month open-label extension, participants continued Gla-300 or Gla-100, 89% and 88% completed 12 months of treatment. Improved glycemic control was maintained over 12 months in both groups (LS mean difference Gla-300 vs. Gla-100: −0.17 [95% CI: −0.30 to −0.05] % for HbA1c, and −0.34 [95% CI: −0.69 to 0.01] mmol/L for FPG) (Figure). Basal insulin doses were higher with Gla-300 than Gla-100 after 12 months (1.03 vs. 0.90 U/kg). During the 12 months of treatment, a similar % of participants had mean difference Gla-300 vs. Gla-100: −0.17 [95% CI: −0.30 to −0.05] % for HbA1c, and −0.34 [95% CI: −0.69 to 0.01] mmol/L for FPG) (Figure). Basal insulin doses were higher with Gla-300 than Gla-100 after 12 months (1.03 vs. 0.90 U/kg). During the 12 months of treatment, a similar % of participants had

Table – Hypoglycemic events per participant-year in the two insulin groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gla-300 (N=274)</th>
<th>Gla-100 (N=279)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total participant-years</td>
<td>124.10</td>
<td>126.83</td>
<td></td>
</tr>
<tr>
<td>Nightly (00:00-05:59 h) confirmed (53.9 mmol/L [≥70 mg/dL]) or severe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline to month 6</td>
<td>8.00</td>
<td>8.95</td>
<td>0.90 (0.71 to 1.14)</td>
</tr>
<tr>
<td>Baseline to week 8</td>
<td>7.75</td>
<td>11.20</td>
<td>0.69 (0.53 to 0.91)</td>
</tr>
<tr>
<td>Week 9 to month 6</td>
<td>8.13</td>
<td>7.85</td>
<td>1.04 (0.80 to 1.36)</td>
</tr>
<tr>
<td>Any-time (24 h) confirmed (53.9 mmol/L [≥70 mg/dL]) or severe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline to month 6</td>
<td>78.42</td>
<td>72.53</td>
<td>1.09 (0.94 to 1.25)</td>
</tr>
<tr>
<td>Baseline to week 8</td>
<td>87.39</td>
<td>89.49</td>
<td>0.98 (0.85 to 1.13)</td>
</tr>
<tr>
<td>Week 9 to month 6</td>
<td>73.93</td>
<td>64.18</td>
<td>1.16 (0.98 to 1.37)</td>
</tr>
</tbody>
</table>

RR, risk ratio; CI, confidence interval

Supported By: Sanofi (NCT01683266)

82-LB

Concentrated Insulin BIOD-531 Is Associated with Accelerated Onset of Action Compared to Humulin® R U-500 and Humalog® Mix75/25® and a Basal Duration Profile

Linda Morrows, Lori Conney, Philip Pickotta, Marcus Hounpesch, Alan Krasner, Errrol de Souza, Oula Vista, CA, Danbury, CT

Formulations of insulin containing citrate and EDTA have been shown to be more rapidly absorbed than conventional formulations of recombinant human insulin (RHI) or rapid acting insulin analogs. BIOD-531, a concentrated (400 U/ml or U-400) formulation of RHI containing EDTA, citrate and MgSO4, has been shown in diabetic swine to be associated with rapid onset and extended duration of action. In this single-center, randomized, double-blind four-period crossover study employing 24-hour euglycemic clamps, the pharmacokinetics (PK) and pharmacodynamics (PD) of BIOD-531 at two doses (1 U/kg and 0.5 U/kg) were compared to Humulin® R U-500 (1.0 U/kg) and Humalog® Mix75/25® (0.5 U/kg) in 13 obese non-diabetic subjects. All study drugs were well tolerated. Key PK and PD parameters are summarized in Table 1. This study demonstrates that BIOD-531 has a more rapid onset of action and rises to higher peak effect than either comparator. The duration of action of BIOD-531 (~18 hours) while slightly shorter than that of either comparator is commensurate with a basal insulin. In addition, BIOD-531 delivers significantly greater glucose lowering activity over a 24 hour period than Humalog® Mix75/25®. These PK/PD profiles suggest BIOD-531 has the potential to deliver ultra-rapid prandial and basal insulin coverage in small injection volumes.

Table 1. Data Represent the Mean ± SEM; Median Values are Presented in Parentheses.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Early % HbA1c (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>min</td>
<td>11.0±1.9</td>
<td>13.5±3.94</td>
<td>0.001</td>
<td>16.4±4.9</td>
<td>47.5±2.6</td>
<td>0.002</td>
</tr>
<tr>
<td>95% CI</td>
<td>(8.2)</td>
<td>(98.6)</td>
<td>(10.4)</td>
<td>(46.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>223.8±62.3</td>
<td>393.3±68.3</td>
<td>0.006</td>
<td>131.3±43.4</td>
<td>190.0±11.9</td>
<td>0.846</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(195.0)</td>
<td>(360.0)</td>
<td>(37.5)</td>
<td>(190.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC0,50 (mg/kg)</td>
<td>108±22.0</td>
<td>40.4±10.0</td>
<td>0.001</td>
<td>88.8±13.4</td>
<td>15.0±4.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(101.1)</td>
<td>(44.9)</td>
<td>(52.8)</td>
<td>(6.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset of Action (min)</td>
<td>7.2±1.4</td>
<td>22.4±8.7</td>
<td>0.023</td>
<td>14.6±6.0</td>
<td>36.5±9.8</td>
<td>0.033</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(1.0)</td>
<td>(12.5)</td>
<td>(11.0)</td>
<td>(33.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of Action</td>
<td>1165.0±56.9</td>
<td>1383.6±38.0</td>
<td>0.002</td>
<td>1078.5±53.7</td>
<td>1294.9±47.7</td>
<td>0.002</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(1180.5)</td>
<td>(1440)</td>
<td>(1028.5)</td>
<td>(1231.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Effect (ESR, mg/kg/min)</td>
<td>6.2±0.66</td>
<td>5.5±4.07</td>
<td>0.032</td>
<td>4.8±0.72</td>
<td>3.1±0.55</td>
<td>0.009</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(5.21)</td>
<td>(5.60)</td>
<td>(5.40)</td>
<td>(2.29)</td>
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<tr>
<td>Total glucose lowering activity (AUC0-1440 mg/kg)</td>
<td>4229±329.0</td>
<td>4471.7±512.4</td>
<td>0.765</td>
<td>2913.3±315.2</td>
<td>2748.3±264.7</td>
<td>0.016</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(4552.7)</td>
<td>(4800.9)</td>
<td>(3061.9)</td>
<td>(2137.7)</td>
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</tbody>
</table>

For author disclosure information, see page LB91.
Pharmacokinetic (PK) and Pharmacodynamic (PD) Characteristics of BioChaperone Combo (BC Combo), the First Fixed Combination of Glargine and Lispro, in Type 1 Diabetes

ULRIKE HÖVELMANN, BERTRAND ALLUIS, GREGORY MEIFRREN, AYMERIC RANSON, OLIVIER SOULA, GÉRARD SOULA, RÉMI SOULA, BIRGIT KRONSHAGE, LESZEK NOSEK, SUSANNE FAMULLA, TIM HEISE, Neuss, Germany, Lyon, France

In this double-blind, crossover study we investigated the PK/PD characteristics of BC Combo, a novel insulin combining lispro (25%) and glargine (75%) in a lipidl-neutral formulation. Twenty people with type 1 diabetes participated in automated euglycemic clamps (ClampArt®). Target blood glucose (BG) (100 mg/dL, clamp duration 30h) and received 0.8 U/kg of BC Combo or Humalog Mix25 (MIX). Mean glucose insulin rate (GIR) curves (Figure) illustrate an earlier onset of action (25±11 vs. 40±13 min) consistent with earlier T1/2 (2.8±0.8 vs. 3.4±0.4 h) of BC Combo vs. MIX. Likewise, the early metabolic effect (AUC[0-2.5h]) 504±210 vs. 325±183 mg/kg) and the early PK exposure (AUC[0-5h]) 38±39 vs. 34±19 h·mg/L/L) were higher, and BC Combo showed a more pronounced late metabolic effect (AUC[12-24h] 1480±900 vs. 961±553 mg/kg) consistent with a higher basal exposure (AUC[0-12h] 563±409 vs. 286±233 h·mg/L/L). Duration of action (time to BG > 118 mg/dL, 29.8±0.7 vs. 25.5±4.3 h) and half-life (17.6±8.7 vs. 7.7±5.0 h, p=0.05 for all comparisons) were longer with BC Combo indicating the potential for once daily dosing. Both formulations were well tolerated, no local reactions occurred. Both PK and PD demonstrate faster prandial and longer basal action for BC Combo supporting the potential for improved BG control vs. MIX with only one daily injection.

Recombinant Human Hyaluronidase Pretreatment of CSI Cannula Sites Provides Comparable Glycemic Control with Reduced Hypoglycemia in T1DM Compared to Usual CSI


Recombinant human hyaluronidase (rHuPH20) is FDA-approved to enhance dispersion and absorption of injected drugs. In CSI, a single pretreatment of the cannula site with rHuPH20 accelerates exposure and action of bolus doses of rapid analogs for up to 3 days of catheter use. 456 subjects with T1DM (mean age 48±13 years, BMI 28.5±5.1, screening A1C 7.8±0.7) were randomized 3:1 to rHuPH20 or 1:1 to CSII alone. The protocol specified primary HE analysis was based on event rates (p=.76). There were fewer hypoglycemic events (HEs) with rHuPH20 than for CSII alone. The primary endpoint of A1C noninferiority (p=.01; **P<0.001). *Data collected from two health insurance plans, associated with Optum® (OP) and HealthCore® (HC). Statistical significance denotes differences between treatment cohorts within each health plan. Among patients with ≥1 A1C result or ≥1 weight reading in the follow-up period. Within each treatment group, hospitalizations and costs during the second half year of follow-up (HY2) were assessed and compared with HY2: 65 (5.1) ** vs. 74 (4.9) **. Mean overall 90 min post-meal glucose excursion was 18.6 mg/dL with rHuPH20 and 19.6 for CSI alone (p=0.76). There were fewer hypoglycemic events (HEs) with rHuPH20 than for CSI alone. The protocol specified primary HE analysis was based on event rates after a month of active titration following randomization. Documented HEs >70 mg/dL obtained from SMBG uploads of 259,666 records were reduced 11% from 13.7/subject-month for standard CSII vs. 12.2 with rHuPH20 (p=1.1). While documented HEs ≤56 mg/dL were reduced 21% from 4.0/mo to 3.1 (p=0.033). Nocturnal HEs (>70 mg/dL between 23:00 and 06:00 hrs) were reduced 20% from 1.1±0.8 to 0.8±0.6 (p=0.33). This study demonstrates comparable glycemic control with reduced hypoglycemia in T1DM patients initiating GLA or LIRA. The study highlights challenges in translating clinical trial findings into the real world, and conducting comparative effectiveness studies when important BL group differences exist.

<table>
<thead>
<tr>
<th>Table.</th>
<th>INITIATOR Characteristics and Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OP-GLA</strong></td>
<td><strong>LIRA</strong></td>
</tr>
<tr>
<td>(n = 1,278)</td>
<td>(n = 1,468)</td>
</tr>
<tr>
<td>BL Age (years), Mean (SD)</td>
<td>53.3 ± 9.8</td>
</tr>
<tr>
<td>BL Female, n (%)</td>
<td>593 (46.1)</td>
</tr>
<tr>
<td>Bl Quan-Charlson Comorbidity Score, Mean (SD)</td>
<td>0.89 ± 1.53</td>
</tr>
<tr>
<td>Bl A1C</td>
<td>9.72 ± 2.08</td>
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<tr>
<td>Follow-up A1C change (%)</td>
<td>1.24 (2.26)</td>
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<tr>
<td>Bl Weight/ Follow-up Weight change (lbs), Mean (SD)</td>
<td>22.1 ± 3.25</td>
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<tr>
<td>DL: 15</td>
<td>-5.71 (1.15)</td>
</tr>
<tr>
<td>DL: 15</td>
<td>1.52 (2.7)</td>
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<tr>
<td>DL: 15</td>
<td>3.30 (1.94)</td>
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<tr>
<td>DL: 15</td>
<td>0.90 (1.36)</td>
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<tr>
<td>DL: 15</td>
<td>3.13 (0.47)</td>
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<tr>
<td>DL: 15</td>
<td>2.88 (0.47)</td>
</tr>
<tr>
<td>DL: 15</td>
<td>0.98 (1.36)</td>
</tr>
<tr>
<td>DL: 15</td>
<td>3.13 (0.47)</td>
</tr>
<tr>
<td>DL: 15</td>
<td>2.88 (0.47)</td>
</tr>
</tbody>
</table>

* P≤0.01; ** P≤0.001. •Data collected from two health insurance plans, associated with Optum® (OP) and HealthCore® (HC). Statistical significance denotes differences between treatment cohorts within each health plan. Among patients with ≥1 A1C result or ≥1 weight reading in the follow-up period. Within each treatment group, hospitalizations and costs during the second half year of follow-up (HY2) were assessed and compared with HY2: 65 (5.1) ** vs. 74 (4.9) **. Mean overall 90 min post-meal glucose excursion was 18.6 mg/dL with rHuPH20 and 19.6 for CSI alone (p=0.76). There were fewer hypoglycemic events (HEs) with rHuPH20 than for CSI alone. The protocol specified primary HE analysis was based on event rates after a month of active titration following randomization. Documented HEs >70 mg/dL obtained from SMBG uploads of 259,666 records were reduced 11% from 13.7/subject-month for standard CSII vs. 12.2 with rHuPH20 (p=1.1). While documented HEs ≤56 mg/dL were reduced 21% from 4.0/mo to 3.1 (p=0.033). Nocturnal HEs (>70 mg/dL between 23:00 and 06:00 hrs) were reduced 20% from 1.1±0.8 to 0.8±0.6 (p=0.33). This study demonstrates comparable glycemic control with reduced hypoglycemia in T1DM patients initiating GLA or LIRA. The study highlights challenges in translating clinical trial findings into the real world, and conducting comparative effectiveness studies when important BL group differences exist.

Supported By: Sanofi

For author disclosure information, see page LB1.
86-LB  
Efficacy and Safety of Once-Daily Biphasic Insulin Aspart 70/30 (BIAsp 30) with Sitagliptin and Twice-Daily BIAsp 30 With or Without Sitagliptin in Patients with Type 2 Diabetes: The Sit2Mix Trial  
SULTAN LINJAWI, RADHIKRISHNA SOTHIRATNAM, RAMAZAN SARI, HENNING ANDERSEN, LINE CONRADSEN HIORT, PATRIK V. RAÖ, CoRis, Harbour, NSW, Australia, Seremban, Malaysia, Antalya, Turkey, Seborg, Denmark, Hyderabad, India  
Sit2Mix is a 24-week, randomized, controlled, open-label trial comparing efficacy and safety of twice-daily BIAsp 30 + sitagliptin (BIAsp BID+Sit, n=195), once-daily BIAsp 30 + sitagliptin (BIAsp QD+Sit, n=193) and twice-daily BIAsp 30 without sitagliptin (BIAsp QD, n=194), all with metformin, in patients with type 2 diabetes (T2D) inadequately controlled on sitagliptin and metformin. At baseline, age, diabetes duration, BMI, FPG, and A1C 8.4% [88 mmol/mol] were similar across groups. After 24 weeks, A1C reduction [% (mmol/mol)] was statistically superior with BIAsp BID+Sit vs. BIAsp QD+Sit (-1.15 [-1.65] vs. -1.15 [-1.65], difference: -0.06 [-0.36 to -0.09], difference: -0.06 [-0.36 to -0.09], p<0.001) and vs. BIAsp BID vs. -1.27 [-1.38], diff: 0.02 [0.12 to 0.18], 0.02 [0.12 to 0.18], p=0.11). BIAsp QD+Sit and BIAsp QD were not significantly different. A trend was seen for A1C responders >7.0%: 59.8% of patients achieved target with BIAsp BID+Sit, 46.5% with BIAsp QD+Sit and 49.7% with BIAsp QD. Severe or minor hypoglycemia (plasma glucose <56 mg/dL ± symptoms) was significantly different with BIAsp QD+Sit vs. BIAsp BID (p=0.015); rates were 1.17 events/patient-year with BIAsp QD+Sit, 1.50 with BIAsp BID+Sit and 2.24 with BIAsp QD. Other adverse events were similar across groups. Treatment difference in body weight change significantly favored BIAsp QD+Sit vs. both BID groups (vs. BIAsp BID+Sit 1.51 [0.82;2.21], p=0.001; vs. BIAsp BID 2.19 [1.49;2.89], p=0.001). Total final daily insulin dose was 0.39, 0.66 and 0.72 U/kg, respectively (baseline 0.16 U/kg). To conclude, addition of BIAsp 30 to T2D inadequately controlled with sitagliptin and metformin is efficacious and has a good tolerability profile; however, while BIAsp BID+Sit was superior in glycemic control, BIAsp QD+Sit had a lower hypoglycemia risk vs. BIAsp BID and less weight gain vs. both BID groups.  
Supported By: Novo Nordisk A/S  
87-LB  
Bedtime Oral Insulin Lowers Fasting Blood Glucose Levels in T2DM Patients  
JOEL NEUTEL, MIRIAM KIDRON, EHUD ARBIT, KENNETH HOMER, Tustin, CA, Jerusalem, Israel, Cedar Knolls, NJ  
Bedtime insulin administration has been suggested to best counteract abnormal morning fasting blood glucose (FBG) levels, a harbinger of diabetes and a key obstacle to optimal glycemic management in T2DM patients. However, many early-stage patients resist introduction of insulin injections into their routine. The pursuit of an orally bioavailable insulin formulation has been driven by the notion that it can both increase patient compliance, and better mimic the physiological route of naturally secreted insulin, consequently lowering risk of hypoglycemia. In this randomized, double-blind, placebo-controlled study, the pharmacokinetics and pharmacodynamics of bedtime administration of the ORMD-0801 oral insulin were assessed in 30 adult T2DM patients inadequately controlled with diet and exercise and/or metformin. Following a 5-day placebo run-in period, a blinded continuous glucose monitor (CGM) was implanted and patients received a single placebo dose on day 1, followed by a 7-day, bedtime placebo or ORMD-0801 (80 IU) or 690 IU treatment in an imitating set. Plasma insulin and c-peptide levels were monitored for 5-hours postdosing. A manufacturing fault limited 690 IU dose efficacy; the data were excluded from the analysis. No hypoglycemic events were recorded throughout the entire study period. ORMD-0801-treated patients showed consistently higher mean plasma insulin levels throughout the 180 min Day 6 postdosing period, when compared to baseline. Moreover, in the first 60 min postdosing, plasma insulin exposure was 20.53 ± 7.9 U/mL higher among ORMD-0801-treated patients when compared to the placebo arm and followed a concentration-time course similar to that of plasma c-peptide. Fasting CGM data demonstrated a mean -30.24 mg/dL difference between the last two days of active versus placebo treatment. Overall, ORMD-0801 led to a stable, consistent and short-acting rise in plasma insulin levels, which positively impacted FBG concentrations in the treated T2DM patients.  
Supported By: Sanofi (NCT01689129)  
88-LB  
New Insulin Glargine 300 U/mL: Glycemic Control and Hypoglycemia in Japanese Patients with T1DM (EDITION JP 1)  
MUNEHIDE MATSUHASHI, MASAYOSHI KOYAMA, XI CHENG, SHIN SHIMIZU, TAKAHISA HIROSE, ON BEHALF OF THE EDITION JP 1 STUDY GROUP, Tokushima, Japan, Tokyo, Japan, Beijing, China  
EDITION JP 1 was a 8-month multicenter, open-label, phase 3 study to compare the efficacy and safety of new insulin glargine 300 U/mL ([Gla-300]) vs. glargine 100 U/mL ([Gla-100]) in Japanese patients with T1DM using basal plus mealtime insulin. Participants (n=243; mean age 45.2 yr; T1DM duration 13.0 yr; HbA1c 8.1%) were randomized to Gla-300 or Gla-100 in combination with mealtime insulin. Basal insulin was titrated to target FPG 4.4-7.2 mmol/L (80 to 130 mg/dL). Primary endpoint was HbA1c change from start to treatment to month 6; similar HbA1c decreases were seen with both Gla-300 and Gla-100 (LS mean (SE) -0.30 [-0.08] % and -0.31 [-0.08] %, LS mean difference 0.13 [-0.03 to 0.29] %). Fewer participants who received Gla-300 experienced confirmed or severe nocturnal hypoglycemic events vs. Gla-100 over the 6-month study period with the greatest difference observed during the first 8 weeks (Table). Rate of hypoglycemic events per participant-year at any time of the day was lower with Gla-300 vs. Gla-100. Severe hypoglycemia was infrequent in either group. Comparable numbers of adverse events were recorded in both groups. In conclusion, in Japanese people with T1DM using basal and mealtime insulin, Gla-300 provides comparable effective glycemic control with less nocturnal hypoglycemia, particularly in the first 8 weeks, and no increase in daytime hypoglycemia vs. Gla-100.  
<table>
<thead>
<tr>
<th>Table: Confirmed or severe hypoglycemia in the EDITION JP 1 study (safety population)</th>
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<tbody>
<tr>
<td><strong>Gla-300</strong></td>
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<tr>
<td>N=122</td>
</tr>
<tr>
<td>Rate per participant-year</td>
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<tr>
<td><strong>Baseline to week 8</strong></td>
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<tr>
<td>Rate per participant-year</td>
</tr>
<tr>
<td><strong>Week 9 to month 0</strong></td>
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<tr>
<td>Rate per participant-year</td>
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<tr>
<td><strong>Baseline to week 8</strong></td>
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<tr>
<td>Rate per participant-year</td>
</tr>
<tr>
<td><strong>Week 9 to month 0</strong></td>
</tr>
<tr>
<td>Rate per participant-year</td>
</tr>
<tr>
<td><strong>Confirmed (≥30 mmol/L, ≥7.0 mmol/L) or severe hypoglycemia</strong></td>
</tr>
<tr>
<td>Rate per participant-year</td>
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<tr>
<td><strong>Confirmed (&lt;0.3 mmol/L, ≤40 mg/dL) or severe hypoglycemia</strong></td>
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<tr>
<td>Rate per participant-year</td>
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<tr>
<td><strong>Fasting Plasma IC 50%</strong></td>
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<tr>
<td>Rate per participant-year</td>
</tr>
</tbody>
</table>

RR, relative risk; CI, confidence interval; *p<0.05, **p<0.01  
88-LB  
A Novel Very Long-acting Insulin Analog (HM12470) with Potential for Once-Weekly Dosing Has a Favorable PK, PD, and Mitogenic Profile  
SANG YOUN HWANG, IN YOUNG CHOI, JIN YOUNG KIM, SUNG-YOUNG JUNG, DAE JIN KIM, YOUNG-MI LEE, YOUN HOON KIM, MICHAEL TRAUTKANN, MARCUS HümPERSCH, JEOWKONG SON, SE CHANG KWON, Gyungy, Republic of Korea, Gyeonggi, Republic of Korea, Hamburg, Germany, Chula Vista, CA, Seoul, Republic of Korea  
Long-acting insulins have the potential to improve treatment compliance and opportunities to start an insulin therapy earlier in the disease. To develop insulins with a longer half-life various formulations of insulin, and various insulin analogues are under investigation. The long-acting basal insulin, HM12470 is developed for once-weekly injection by conjugating an insulin analog with the constant region of a human immunoglobulin fragment via non-peptidyl linker. The objective of this study was to investigate the in vitro properties, pharmacokinetics and pharmacodynamics of HM12470 in normal and diabetic animal models to evaluate the once-weekly dosing and the metabolic/mitogenic effects. In a pharmacodynamic study, subcutaneously injected HM12470 exhibited a half life of ~ 43 hr in normal rats, while insulin degludec showed 2.9 hr of half-life. The extended half-life was also confirmed in
other species such as mice, dogs, and monkeys. The improved pharmacokinetic profile had contributed to prolonged glucose lowering efficacy in db/db mice. Moreover, the prolonged glucose lowering efficacy was even observed at lower dose levels when compared with a native human insulin conjugate (HM12460A). Based on the results from these three species, human pharmacokinetics was projected by the Wajima C–MRT method. The half-life in humans is expected to be 132 hr and the peak-to-though ratio was calculated to be 1.6 on once weekly dosing. In vitro mitogenic potency of HM12470 was assessed by using cell proliferation in MCF-7 and Saos-2 cells. Compared to its lipogenic efficacy assessed in adipocyte-induced 3T3-L1 cells, the mitogenic to lipogenic potency ratio was significantly lower than that of human insulin. These observations suggest that HM12470 has a once-weekly dosing potential with a sufficiently extended half-life and a low mitogenic risk.

**90-LB**

New Insulin Glargine 300 U/mL: Glycemic Control and Hypoglycemia in a Meta-analysis of Phase 3a EDITION Clinical Trials in People with T2DM

ROBERT RITZEL, RONAN ROUSSEL, GEREMIA B. BOLLI, LAETITIA VINET, HANNELE YKI-JÄRVINEN, München, Germany, Paris, France, Perugia, Italy, Nanterre, France, Helsinki, Finland

The EDITION 1, 2 and 3 studies compared the efficacy and safety of new insulin glargine 300 U/mL (Gla-300) with insulin glargine 100 U/mL (Gla-100) in people with T2DM on basal and mealtime insulin, basal insulin and OADs, and no prior insulin, respectively. A meta-analysis of these three studies enabled glycemic control and hypoglycemia to be examined over 6 months in a large, heterogeneous T2DM population (Gla-300, N=1247; Gla-100, N=1249). Mean change in HbA1c was comparable for Gla-300 and Gla-100 (Δ−1.02 [SE 0.03]). Gla-300 was associated with a reduced risk of experiencing a hypoglycemic event vs. Gla-100 (noclur and at any time of day; Table). Rates of nocturnal hypoglycemia were consistently lower with Gla-300 than Gla-100. Severe hypoglycemia was rare in both treatment groups (2.3% with Gla-300 vs. 2.6% with Gla-100). Weight gain with Gla-300 and Gla-100 was slight (mean change: 0.49 [SE 0.10] kg, 0.75 [0.10] kg, respectively), with a trend for less weight gain with Gla-300 (−0.26 [95% CI −0.52 to 0.01] kg, p=0.058). In conclusion, Gla-300 provides comparable glycemic control and hypoglycemia to Gla-100 in T2DM population (Gla-300, N=1247; Gla-100, N=1249). Mean change in HbA1c was comparable for Gla-300 and Gla-100 (Δ−1.02 [SE 0.03]). Gla-300 was associated with a reduced risk of experiencing a hypoglycemic event vs. Gla-100 (noclur and at any time of day; Table). Rates of nocturnal hypoglycemia were consistently lower with Gla-300 than Gla-100. Severe hypoglycemia was rare in both treatment groups (2.3% with Gla-300 vs. 2.6% with Gla-100). Weight gain with Gla-300 and Gla-100 was slight (mean change: 0.49 [SE 0.10] kg, 0.75 [0.10] kg, respectively), with a trend for less weight gain with Gla-300 (−0.26 [95% CI −0.52 to 0.01] kg, p=0.058). In conclusion, Gla-300 provides comparable glycemic control to Gla-100 in T2DM, with consistently less hypoglycemia at any time of the day and less nocturnal hypoglycemia.

Table – Glycemic control and hypoglycemic events over 6 months in a meta-analysis of the EDITION 1, 2 and 3 studies

<table>
<thead>
<tr>
<th></th>
<th>Gla-300 (N=1247)</th>
<th>Gla-100 (N=1249)</th>
</tr>
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<tbody>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8.30</td>
<td>8.31</td>
</tr>
<tr>
<td>Change from baseline to Month 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Change in HbA1c</strong></td>
<td>−1.02 (0.02)</td>
<td>−1.02 (0.02)</td>
</tr>
<tr>
<td><strong>Weight gain (kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safety population</td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>90.90</td>
<td>90.90</td>
</tr>
<tr>
<td>Change from baseline to Month 6</td>
<td>0.49 (0.20)</td>
<td>0.75 (0.35)</td>
</tr>
</tbody>
</table>

**92-LB**

Transition Therapy for Inpatient to Outpatient Glycemic Control: Results of the Veterans Inpatient Insulin Study and Transition Algorithm (the VISTA Study)

DENNIS G. KAROUNOS, VIISTA STUDY GROUP, Lexington, KY

Little data exists regarding optimal transition from inpatient (ipt) to outpatient (outpt) insulin therapy after hospitalization for acute noncritical illness. The purpose of this phase IV, randomized, open-label study of 120 patients with T2D was to determine the efficacy of both basal-bolus insulin (detemir & aspart) during the inpt hospitalization for treatment of hyperglycemia as well as the transition to pre-mixed insulin after discharge, randomized to receive either NPH/regular 70/30 insulin Group A (Grp A) or NPH/aspart 70/30 insulin analog (Grp B) twice daily. There were 6 screen failures or withdrawals during inpt phase, leaving an intention-to-treat cohort of 112 males and 2 females, age 63.8 ± 8.8 y, duration of DM 13.4 ± 9.3 y with 50 randomized to group A and 64 to group B. The 20 week outpt phase consisted of bi-weekly phone calls and monthly clinic visits using blood glucose (BG) profiles to adjust therapy. Of 104 completing the study, mean total daily insulin dose was 0.72 ± 0.49 U/kg, which was a 15% increase since hospital discharge. In Grp A there was a 6.7% improvement in glycemic control (HbA1c 8.91% ± 2.0 at baseline to 8.32% ± 1.5 at 16 weeks, p=0.051 & BG improving from 198 ± 2.9 to 185 ± 0.8 mg/dl, p=0.0001). There was a greater effect in Grp B with a 12.2% improvement in glycemic control (HbA1c 9.51% ± 2.3 at baseline to 8.35% ± 1.95 at 18 weeks, p=0.0004 & BG improving from 208 ± 3 mg/dl to 184 ± 0.7 mg/dl, p<0.0001). With 70/30 asapart analog (Grp B) there was better glycemic control compared to NPH/regular 70/30 (Grp A) with no difference in occurrence of hypoglycemia, body wt, serious adverse events or patient-reported distress. Thus, we provide new data on transition therapy from inpatient to outpatient care. This strategy of transitioning from basal bolus therapy to premixed twice daily insulin is particularly useful when treating older individuals with type 2 diabetes after hospitalization for an acute non-critical illness.

**93-LB**

Less Nocturnal Hypoglycemia and Weight Gain with New Insulin Glargine 300 U/mL Compared with 100 U/mL: 1-Year Results in People with T2DM Using Basal Insulin with OADs (EDITION 2)

HANNELE YKI-JÄRVINEN, RICHARD M. BERNSTEIN, ROBERT RITZEL, RONAN ROUSSEL, MONIKA ZIEMEN, MAREK WARDECKI, ISABEL MUEHLEN-BARTMER, MAGALI MAROCCIA, MATTI HAMBERG, MATTEO SPILIO, VILMA RUKKA, MARIANNA VIROLA, MARI KANIA, MARIANNE VÄRRIES, KAI EHLERT, JIN R. CHO, MATTHIAS GASSEN, JÚLIA FREITAS, YU-HSIAO YEN, MARIANNE MARTIN, MARIE-ÉLÉONORE DESLARS, MIRELLA PASQUETTO, MARIAM TAYEB, KIM S. FANG, AZIZ A. ALI, STEPHEN A. BURDEN, FRANK BODE, ABDEL M. EL-MAZHI, TIMOTHY J. HALL, FREDERIK V. NIELSEN, TIMOTHY S. PIKE, WILLIAM E. REID, JOHN R. SMITH, CLAUDE S. TROJAN, JAMES P. VANCE, CHANDRA R. VAIDYANATHAN, MARCO YAMBROVIC, CANDICE ZICH, MARIETTA ZAMBRANO, JOHN ZWEIBACH, MINNEAPOLIS, MN

EDITION 2 investigated glycemic control and hypoglycemia in 811 adults with T2DM and inadequate control of HbA1c, using basal insulin and OADs randomized to receive either insulin glargine 300 U/mL (Gla-300) or glargine 100 U/mL (Gla-100) for 6 months. In this 6-month open-label extension, participants continued to receive Gla-300 or Gla-100 once daily plus OADs; 315 (78%) in Gla-300 and 314 (77%) in Gla-100 completed 12 months of treatment. Improved control of HbA1c was maintained at 12 months with each regimen. Over 12 months, per-participant-year event rates of confirmed (≥3.9 mmol/L [≥70 mg/dL]) or severe nocturnal hypoglycemia were 37% lower with Gla-300 than Gla-100 (1.74 vs. 2.77, RR 0.63, 95% CI 0.42 to 0.96). Fewer participants experienced ≥1 confirmed (≥3.9 mmol/L [≥70 mg/dL]) or severe nocturnal hypoglycemia with Gla-300 than Gla-100 (RR 0.84, 95% CI 0.71 to 0.99). Severe hypoglycemia was infrequent. Body weight increase was observed in both groups, and was significantly less with Gla-300 than Gla-100 (mean: 0.42 vs. 0.80 kg, p=0.0091). No between-treatment differences in adverse events were seen. Over 1 year of treatment, people with T2DM using Gla-300 and OADs had comparable glycemic control, experienced fewer nocturnal hypoglycemic events and less weight gain compared with those using Gla-100.

For author disclosure information, see page LB91.
94-LB
Glycemic Control and Hypoglycemia in Japanese People with T2DM Receiving New Insulin Glargine 300 U/mL in Combination with OADs (EDITION JP 2)
YASUO TERAUCHI, MASAYOSHI KOYAMA, XI CHENG, SHIN SHIMIZU, TAKAHISA HIROSE, ON BEHALF OF THE EDITION JP 2 STUDY GROUP, Yokohama, Tokyo, Japan, Beijing, China
In this multicenter, randomized, open-label, phase 3 study (EDITION JP 2), people with T2DM on basal insulin plus OAD(s) (n=241; mean age 60.8 yr; mean BMI 25.3 kg/m²; mean duration of T2DM 14.0 yr; mean HbA1c 8.0%) were randomized to receive new insulin glargine 300 U/mL (Gla-300) or glargine 100 U/mL (Gla-100) plus OAD(s). Insulin was titrated to target FPG 4.4-5.6 mmol/L (80-100 mg/dL). Primary endpoint was HbA1c change from baseline to month 6. HbA1c decreased similarly in both groups [LS mean (SE): -0.45 (0.08)% for Gla-300 and -0.55 (0.06)% for Gla-100; LS mean difference 0.10 (CI: -0.08 to 0.27)%]. Fewer participants experienced any hypoglycemic events during 6 months with Gla-300 vs. Gla-100. The number (% of participants with ≥1 confirmed (≥3.9 mmol/L) or severe hypoglycemic event (24 h and nocturnal), LS mean difference (95% CI): -0.62 (-0.19 to 0.05) kg for Gla-300 vs. Gla-100. Similar safety profiles were observed in both groups. In conclusion, in Japanese people with T2DM using basal insulin plus OAD(s), Gla-300 provides comparable effective glycemic control with fewer hypoglycemic events, particularly during the first 8 weeks, vs. Gla-100.

95-LB
Inspire Diabetes: A Pulse of Basal Bolus Analog Insulin as the First Treatment of T2DM
JAY H. SHUBROOK, AIRANI SATHANANTHAN, MASATO NAKAZAWA, NISHITA PATEL, RUCHA J. MEHTA, FRANK L. SCHWARTZ, Athens, OH, Pomona, CA
Most people with type 2 diabetes (T2DM) require increasing medication over time to maintain glucose control due to progressive beta cell failure. INSPIRE Diabetes is a multicenter randomized open label clinical trial that evaluated the treatment of adults with newly diagnosed T2DM. Participants (10 men, 13 women. Mean age of 44.5 yrs) were randomized to (1) a pulse of basal bolus analog insulin (glargine, glulisine) that was used for a total of 12 weeks with weight based initialisation and twice weekly titration with forced down titration (EIT) versus (2) routine care (RC) as recommended by the 2009 ADA treatment recommendations. Primary end points were need for and time to rescue therapy, There was no difference in time to rescue (16.4 weeks RC vs. 24.0 weeks EIT) or need for rescue therapy (2/10 RC vs. 6/13 EIT). The A1c were improved in both groups significantly overtime. The A1c at baseline was 10.1% +/- 1.2% RC vs. 9.9% +/- 1.2% EIT. This improved to 7.01% +/- 0.8 RC vs. 6.7% +/- 0.8 EIT. At 12 weeks the routine care group continued oral anti-glycemic therapy, but the insulin group stopped all diabetes treatments until rescue was needed. At 15 months A1c for RC was 8.7% +/- 0.8 and E1G 6.8% +/-0.4. The EIT group lost weight (2.4 kg) vs. weight gain of 2.1 kg for RC (NS). When excluding participants with a BMI >50 the EIT arm lost significantly more weight than RC (p<0.05). The EIT group also had significantly higher fasting and stimulated-cpeptide levels (p<0.001). There were only 10 hypoglycemic episodes in the study (7 EIT, 3 RC) and zero severe hypoglycemic episodes. Twelve weeks of EIT was as effective as RC for people with newly diagnosed T2DM and is not associated with the weight gain we typically see later in the disease. Further, there is some evidence that EIT improves beta cell function over 15 months. This study is limited by its size and the fact that the RC group did substantially better than what is normally seen in real clinical practice. EIT produced not only rapid control of glucose but may have a legacy effect on beta cell function.

Supported By: Sanofi

For author disclosure information, see page LB91.
Improved Oral Insulin Bioavailability when Delivered in Soft Capsules

MIRIAM KIDRON, CAMIL FUCHS, EHUD ARBIT, SHIOSHI SHITZEN, DANIEL SCHURR, Jerusalem, Israel, Tel Aviv, Israel

One of the established clinical advantages of soft gel capsules is the potential to enhance active ingredient bioabsorption and bioavailability, which often translates to lower required drug doses. In this study, the bioavailability of insulin orally administered to five type 1 diabetes mellitus (T1DM) patients by way of a hard versus soft gelatin capsule was compared. Patients received an 8 mg or 16 mg (2 x 8 mg capsules) dose of insulin packaged in either soft or hard gelatin, enteric-coated capsules, 15 min before a standard meal. Plasma insulin and glucose concentrations were monitored over the ensuing 5 hour period and the ratios of responses in the baseline (0-20 min postdose) vs. treatment (20-300 min postdose) periods were computed. The soft gelatin capsules generated consistently higher concentration-time insulin curves when compared to the hard gelatin capsules, and demonstrated a dose-dependent effect on blood glucose levels. More specifically, the 8 mg and 16 mg doses delivered in a soft capsule were associated with 31% and 38% respectively higher mean plasma insulin concentration and area under the curve (AUC) baseline vs. response ratios, when compared to identical doses delivered in hard capsules. Moreover, upon dose doubling, mean plasma insulin concentration and AUC ratios increased by 13.8% and 14.5%, respectively, when delivered in soft capsules, but only by 7.4% when delivered in hard gelatin capsules. In parallel, the mean plasma glucose concentration ratio following treatment with the 8 mg insulin soft gel capsule was 19.1% higher than that measured after a similar dose delivered in a hard capsule, while a 31% difference was observed following dosing with 16 mg insulin in a soft vs. hard gelatin capsule. The improved bioavailability and bioefficacy observed upon insulin delivery in soft gelatin capsules will be valuable in further clinical development of oral insulin.

Evaluation of Safety of Insulin Degludec on Undergoing Total Colonoscopy Using Continuous Glucose Monitoring

SOICHI TAKEISHI, AKIHITO MORI, NOBUTOSHI FUSHIMI, HIROKI HACHIYA, TAKAYUKI YUMURA, SHUN ITO, TAKASHI SHIBUYA, NORITSUGU OASHI, HIROMI KAWAI, Ichinomiya, Japan

Screening of colon cancer with total-colonoscopy (TCS) in type 2 diabetic patients is a significant clinical approach. Anti-diabetic agent should be reduced or discontinued because preparation for TCS forces the patient to be long fasting. However, there is little information regarding how to adjust insulin degludec (D) having an ultra-long action profile. Therefore, we investigated glucose variability of twelve patients with type 2 diabetes mellitus treated with D scheduled to undergo TCS, using continuous glucose monitoring (CGM). In admission, CGM was attached from the previous day to the following day upon dosing of insulin.

In even patients with normal glucose control, adjustment of D dose on the previous day seems to be unnecessary, because degree of decreased glucose level with fasting was lower in patients with nearer normal glucose level.
The Relationship between Insulin Dosing and Patient Outcomes among Patients with Diabetes: Evidence from a Medicare Cohort


The prevention of hypoglycemia, especially nocturnal hypoglycemia, is one of the early achievements of the Artificial Pancreas (AP) and Low Glucose Control Systems. However, when and how long to suspend basal delivery, and when and to resume it, are very much open questions. These studies were conducted to evaluate the AP's ability to control hyperglycemia and make decisions concerning basal delivery. The study results are presented in the form of abstracts. The level of control and clinical outcomes were assessed using continuous glucose monitoring (CGM) devices. The primary endpoint was the change in HbA1c level from baseline to 6 months. A total of 331 subjects were randomized (45.6% women, mean age 56.0±9.6 yr, BMI 33.4±7.3 kg/m²) at 3 sites. Participants had 3 announced meals (30-90g), unannounced responses to hypo- and hyperglycemia, was evaluated in an ambulatory multi-centered trial. Results: The proposed AP performed predictive pump suspensions, and subsequent, heuristics, auxiliary safeguards, or user interaction. A total of 202 outpatient closed-loop trials (~25 hr) were completed by 12 adults with type 1 diabetes (8F; age 25-62 [av. 50]; 4-45 [av. 28] years T1D, 60-119 [av. 78] kg) at 3 sites. Participants had 3 announced meals (30-90g), unannounced exercise (30-60 min), and an overnight sleep. The system: On average, attenuated insulin delivery by 1.5 IU (2.85%) from basal to basal hour, and 0.49 IU (0.41% of basal) at night, and decreased pump suspensions by >15 min, >60 min, and >120 min, respectively. The proposed AP performed predictive pump suspensions, and subsequent, timely predictive pump resumptions to ameliorate glucose rebounds, based on the AP's predictions and user inputs. The supported by NIH (RP1600093231).
CLINICAL THERAPEUTICS/NEW TECHNOLOGY—INSULIN DELIVERY SYSTEMS

104-LB
Multinight “Bedside” Artificial Pancreas for Patients with T1D Improves Glycemic Control
SUE A. BROWN, DANIELA BRUTTOMOSSO, MARC D. BRETON, SIMONE DEL FAVERO, STACEY ANDERSON, CLAUDIO COBELLI, BORIS P. KOVATCHEV, Charlestonville, VA, Padova, Italy

Objective: Test the feasibility of multi-night closed loop control (CLC) aiming for tight glycemic control in the morning to effectively “reset” the patient to normoglycemia before waking up.

Methods: N=10 subjects with T1D were enrolled in a randomized cross-over trial: sensor-augmented pump therapy (SAP) vs. CLC of 5 consecutive nights (23:00 to 07:00) in outpatient setting. Subjects wore a DexCom Platinum CGM, Roche Accu-Chek Combo Pump and the Diabetes Assistant (DiAs) - a cell-phone CLC platform running the USS Virginia control-to-range algorithm.

Results: Subjects (mean age 46±9, A1c 7.0±1.1%) completed 49 nights of CLC and 49 nights of SAP. The system functioned 98.3% of time with no adverse events. CLC vs. SAP improved significantly: mean glucose at 07:00am (119.3 ± 24 vs. 152.9 ± 59.5 mg/dL, p<0.001); overnight mean glucose (139 vs. 170.3 mg/dL, p<0.001); and percent time in 80-150 mg/dL (64.3 vs. 38.3%, p<0.001), 70-180 mg/dL (95.4 vs. 59.1%, p<0.001) - see Figure - using similar amount of insulin (6.1 vs. 6.8U, p=0.1). Time in hypoglycemia <70 mg/dL was low: 0.55% in CLC vs. 1.65% in SAP; 0.12 episodes/night in each. Overnight control correlated with following daytime control (r=0.47, p=0.002).

Overnight CLC results in significant improvement in morning and overnight glucose levels, and time in target range, with the potential to improve day-time control when glucose levels were “reset” to normoglycemia each morning.

105-LB
Provincially-Funded Insulin Pump Therapy and Health Care Utilization in Adults with Type 1 Diabetes in Ontario, Canada
SELINA L. LIU, BALU R. SHAH, STEWART B. HARRIS, London, ON, Canada, Toronto, ON, Canada

In September 2008, Ontario became the first province to publicly fund insulin pump therapy (IPT) for adults with type 1 diabetes (T1DM), via the Assistive Devices Program (ADP). This study characterized the ADP-funded adults on IPT and assessed the clinical impact of ADP enrollment on selected health care utilization outcomes.

Data from all adult ADP applicants from September 1, 2008 to December 31, 2012 were linked to provincial administrative databases detailing all hospitalizations and physician service claims.

There were 7,220 adults who started IPT. At enrollment, the mean age was 40.5 years, the mean A1C was 7.0±1.1% and 74.8% had T1DM duration ≥10 years, 75.0% had endocrinologist care, and few had any diabetic ketoacidosis (2.4%) events the year prior. Of note, 49.1% were from the 2nd highest income quintiles.

The frequency of health care utilization within 1 year pre- and post-ADP enrollment was compared (Table 1). Post enrollment, DM-related emergency room visits and family physician visits significantly decreased.

In Ontario, provincially-funded IPT in adults with T1DM was associated with positive changes in health care utilization, but there was evidence of disparity in access to funding in those of lower income.

106-LB
Real Time Remote Monitoring with Artificial Pancreas: A Family-Centered Pilot Trial
NATHAN LAU, MOLLY MCELWEE, CHRISTIAN WAXEMAN, MARK DEBOER, DANIEL R. CHERNAVSKY, Charlottes ville, VA

Adolescents with Type 1 Diabetes (T1D) struggle with blood glucose (BG) control while assuming more responsibility for their health. Supporting this transition with real-time remote monitoring (RM) enables joint parent-child review of BG levels and insulin delivery.

We evaluate the parent-child dyad’s acceptance and confidence using RM in a randomized, crossover artificial pancreas (AP) trial which studied 17 adolescents ages 13-18 on 2 separate days: sensor-augmented pump vs. AP. Parents remotely monitor their child’s BG control and rated their experience before and after the AP session. They also filled a Potential Action Questionnaire (PAQ) every 30 minutes during AP day to comment on how or if they would intervene given RM indications. In post-trial focus groups (FG), parents and adolescents provided feedback on their experiences and envisioned future uses of RM and AP.

The PAQ indicated that parents would intervene less frequently if their child was wearing the AP (26.4±102.6, p<0.01). The post-trial survey ratings on a scale from 1 (Very Uncomfortable) to 5 (Very Comfortable) suggested that the dyads were comfortable using the AP (N=17, M=4.25, SD=0.44) and the RM (N=17, M=4.32, SD=0.80) respectively. The FG highlighted that the systems were easy to learn and trusted and also indicated that customization features would be useful.

Parents wished to have RM when their child was at college while adolescents wanted to customize alerts for roommates, significant others, and healthcare providers. All expressed desire to adopt the AP and RM immediately, and noted that bringing the current technology to market should take precedence over any potential future improvements.

This first family-centered study indicates ease of use and trust in the AP and RM, demonstrates promise for transferring health responsibilities for adolescent independence, and shows potential for improvement of glycemic control. Future studies with larger patient populations will be necessary to confirm these findings.

107-LB
“Learning” Can Improve the Closed-Loop Blood Glucose (BG) Control Performance
CHAO FENG YAN, JINPING ZHANG, CLAUDIO COBELLI, DONG ZHAO, NA WANG, XIAOPING CHEN, BO ZHANG, SHAO LING WEI, CONG HAO, WEN YING YANG, YOU QING WANG, Bei jing, China, Padova, Italy

There exists repetitiveness in glucose-meal-insulin dynamics, but no clinical trial considers the possibility of learning from one day to another. To clinically evaluate the capability of “learning”, a learning-type closed-loop BG control algorithm, termed as L-MPC, has been tested on ten T1DM adult (age=16) subjects in China-Japan Friendship Hospital (6M & 4F: age= 35.8±13.2; BMI= 68.7±14.4).

With insulin therapy optimization and model identification in advance, the closed-loop clinical trials last six days for each subject. In each day, the trial starts at 8am and ends at noon with 50g CHO diet at 8am. To study the influences of alcohol and exercise, subjects drink 50mL beer and/or ride 15-min bike on the fourth and/or sixth day. The order of drinking and riding was random. The learning gain in L-MPC was chosen as 0.5.

Test results show that L-MPC can learn from an individual’s lifestyle and improve the blood glucose control performance from day to day. By comparing...
108-LB

Engineering a Thin-Film Cell Encapsulation Device for Treating Type 1 Diabetes
RYAN CHANG, CRYSTAL NITRAY, GAETANO FALEO, OUIZH TANG, TEJAL DESAI, San Francisco, CA

Replacement of insulin-producing cells using islet transplantation is a proven effective therapy for Type 1 Diabetes. The use of an encapsulation device as a physical barrier between the recipients and xenogeneic islets or hESC-derived beta cells may enable the safe use of these therapies while eliminating the need of immunosuppression. Here we present a novel thin-film cell encapsulation device with stringently defined porosity for immunoprotection as well as a favorable microenvironment that promotes cell survival. Polycaprolactone (PCL) thin films were fabricated by casting PCL and Polyethylene Glycol (PEG) dissolved in 2,2,2-trifluoroethanol and casted onto nanotemplated silicon wafers. PEDG particles were dissolved away in deionized water to release the thin film from the wafer. The device is assembled by heat sealing two PCL thin-films together with firely luciferase expressing islets encapsulated in the inner layer. Devices containing islets or free islets were transplanted in the subcutaneous space of syngeneic and allogeneic mice models. Islet viability was assessed by monitoring bioluminescence over time while therapeutic efficacy was evaluated by measuring blood glucose concentration.

Free islets and encapsulated islets survived equally well in the 30-day post-transplantation period. Syngeneic free islet transplants survived while allogeneic free islet transplants failed. However, encapsulated islets survived in both syngeneic and allogeneic transplants. Blood glucose levels dropped from 400 mg/dL to under 200 mg/dL in mice transplanted with the cell encapsulation device. The implanted devices demonstrated superior vascularization along the outer surface of the membrane as seen after 30 days in vivo.

These studies demonstrate proof of principle using our cell encapsulation technology to treat diabetes in allogeneic models. We look forward to further evaluate our technology by characterizing the immunoprotection capability in more stringent autoimmune animal models.

Supported By: JDRF

109-LB

ISIS-GCCRRX, an Antisense Glucagon Receptor Antagonist, Caused Rapid, Robust, and Sustained Improvements in Glycemic Control without Changes in BW, BP, Lipids, or Hypoglycemia in T2DM Patients on Stable Metformin Therapy
ERIN MORGAN, ANNE SMITH, LYNNETTA WATTS, SHUTING XIA, WEI CHENG, RICHARD GEARY, SANJAY BHANOT, CARSTIJN, CA

Excessive glucagon and/or dysregulation of postprandial glucagon secretion contributes to hyperglycemia in pts with T2DM. ISIS-GCCRRX (GR) is an antisense drug that reduces hepatic GCGR mRNA expression. We reported that GR was safe and produced significant increases in total GLP-1 levels without affecting BP or lipids in healthy volunteers (Diabetologia 2013 56: Suppl 1: 89P). In this double-blind study, T2DM pts on stable MET therapy were randomized to placebo, 100 or 200 mg GR injected SC as a loading dose (4 in 14 days) then once wkly for 11 wks. Mean baseline (BL) glycemic values were HbA1c (8.3 ± 9.1%), FPG (180.6 - 227.5 mg/dL) and fructosamine (290 - 311 µmol/L). GR treatment caused robust improvements in glycemic control that were sustained for many weeks after the last dose. Significant increases were observed in total GLP-1 (up to 4-fold), accompanied by OGTT improvements consistent with a GLP-1 effect. No hypoglycemia, no changes in vital signs, ECG, renal function, TGs, LDL-c, BV or BP were observed. As reported with GCP small molecules, some GR 200 mg pts had mild increases in ALT/AST without elevation in bilirubin, alk phos or clinical symptoms. The GR efficacy and safety profile supports further development in T2DM pts uncontrolled on existing therapies.

Efficacy Results.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HbA1c (%)</th>
<th>FPG (mg/dL)</th>
<th>Fructosamine (µmol/L)</th>
<th>GLP-1 AUC (µg/mg/min mL)</th>
<th>C-peptide AUC (µg/mg/min mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>9.0 ± 0.6</td>
<td>16.4 ± 8.4</td>
<td>-20.5 ± 11.4</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>GR 100mg</td>
<td>9.0 ± 1.7</td>
<td>59.9 ± 62.2</td>
<td>59.8 ± 37.3</td>
<td>161.7 ± 40.0</td>
<td>139.6 ± 63.0</td>
</tr>
<tr>
<td>GR 200mg</td>
<td>8.0 ± 2.5</td>
<td>40.9 ± 74.4</td>
<td>141.0 ± 100.0</td>
<td>139.6 ± 63.0</td>
<td>13.9 ± 40.0</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.01; ***p<0.001; N=9.

Supported By: EFSD/CDS/Eli Lilly and Company; NSFC (61374099)

110-LB

Efficacy and Safety of Once Weekly Dulaglutide vs. Once Daily Liraglutide in Type 2 Diabetes (AWARD-6)
KATHLEEN M. DUNGAN, SANTIAGO TOFE POVEDANO, THOMAS FORTS, JOSE G. GONZALEZ, CHARLES ATISSO, WHITNEY SEALLS, JESSE L. FAHRBACH, Columbus, OH, Palma de Mallorca, Spain, Mainz, Germany, Montemrey, Mexico, Indianapolis, IN

This Phase 3, randomized, open-label, parallel-arm 26-week (wk) study compared efficacy and safety of once weekly dulaglutide (DU) 1.5 mg, a long-acting GLP-1 receptor agonist, vs. once daily liraglutide (LIRA) 1.8 mg in metformin-treated (∼1500 mg) patients with type 2 diabetes. Patients (N=599) had a mean baseline age of 57 years; A1C of 8.1%; and weight of 94.1 kg. The primary objective was A1C change from baseline at 26 wk tested for noninferiority (margin 0.4%), DU 1.5 mg vs. LIRA 1.8 mg.

DU 1.5 mg was noninferior to LIRA 1.8 mg at 26 wk as measured by A1C change from baseline (between-group A1C change: -0.06; 95% CI [-0.19, 0.07]) (Table). While both groups experienced significant weight reduction, LIRA-treated patients demonstrated a 0.71 kg greater reduction than DU-treated patients (p=0.01). The most common treatment-emergent GI adverse events for DU 1.5 mg and LIRA 1.8 mg, respectively, were nausea (20.4%, 18.0%), diarrhea (12.0%, 12.0%), dyspepsia (8.0%, 6.0%), and vomiting (7.0%, 8.3%). Patients who discontinued study and/or study drug due to GI adverse events were similar (DU 1.5 mg [3.0%], LIRA 1.8 mg [4.3%]). Hypoglycemia rate was 0.34 (DU 1.5 mg) and 0.52 (LIRA 1.8 mg) events/pt/yr. No severe hypoglycemia was reported.

In conclusion, once weekly DU 1.5 mg demonstrated noninferior glycemic control compared to once daily LIRA 1.8 mg with a comparable safety and tolerability profile.

Efficacy Measures (26 wk, ITT)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DUL 1.5 mg</th>
<th>LIRA 1.8 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=299</td>
<td>N=300</td>
<td></td>
</tr>
<tr>
<td>A1C change</td>
<td>-1.42 (0.05)</td>
<td>-1.36 (0.05)</td>
</tr>
<tr>
<td>% of patients with A1C ≥7.0%</td>
<td>68.3</td>
<td>67.9</td>
</tr>
<tr>
<td>Weight change</td>
<td>-2.90 (0.22)</td>
<td>-3.61 (0.22)</td>
</tr>
</tbody>
</table>

1-sided p < 0.001 for noninferiority vs. LIKA for A1C change. *p = 0.01 vs. LIKA. †MMRM. ‡ANCOVA LOCF.

Supported By: Eli Lilly and Company

Clinical Effects of Metrolepin in Partial vs. Generalized Lipodystrophy: The Role of Baseline Abnormalities
TALIA DIKER-COHEN, ELAINE K. COCHRAN, PHILIP GORDEN, REBECCA J. BROWN, Bethesda, MD

Lipodystrophies (LD) are rare diseases of subcutaneous fat loss, leptin deficiency, insulin resistance and high triglycerides (TG). Leptin replacement therapy has just been FDA-approved for generalized LD (GLD), but not for partial LD (PLD) due to uncertain benefit. We compared effects of metrolepin (ML, a recombinant leptin analog) on metabolic abnormalities in PLD vs. GLD, with subgroup analyses in patients with severe metabolic disease (A1C ≥8% or TG ≥500 mg/dL at baseline).

86 patients (31 PLD; 55 GLD) completed ≥6 months in an open-label trial of ML. Inclusion criteria were low leptin (<8 ng/mL in men; <12 in women) and ≥1

Supported By: ADA-Funded Research

For author disclosure information, see page LB91.
of fasting TG >200 mg/dL, diabetes, or fasting insulin >30 uU/mL. A1c and TG were measured at baseline (N=86) and after 6 (N=74) and 12 (N=72) months.

There were no baseline differences for GLD vs. PLD. In the total cohort, A1c fell from 8.1 to 7.3 (PLD) and 8.4 to 6.4 (GLD). TG fell from 971 to 524 (PLD) and 1021 to 276 (GLD). In the severe subgroups, A1c fell from 10.1 to 8.3 (PLD, N=14) and 9.8 to 6.9 (GLD, N=34); TG fell from 1953 to 592 (PLD, N=13) and 2175 to 486 (GLD, N=22). FGF-21 (p<0.05 for all).

In LD patients selected for the presence of more severe metabolic disease, ML led to significant improvements in A1c and TG in both PLD and GLD, and should be considered as a potential therapy for PLD. This is especially true in patients with severe high TG, for which other effective treatments are not available.

A Novel Dual Action GIP/GLP-1 Coagonist Peptide Shows Enhanced Activity on Weight Loss and Energy Utilization Whilst Maintaining Its Efficacy for Glycemic Control

KRIST R. BOKVIST, TAWIJA COSKUN, ROBERT CUMMINS, THOMAS B. FARH, JAMES VIORICURILL, THOMAS FITCH, OLIVER GHOBRAL, LILU GLD, JOHN P. MAYER, LIBBEY O’FARRELL, XIANJU PENG, JORGE ALISNA-FERNANDEZ, Indianapolis, IN

It is well known that the incretins GIP and GLP-1 released from the gut in response to food dramatically enhance glucose removal following a meal. Although both incretins have been studied in great detail individually it remains largely unknown if GIP and GLP-1 produce additional benefits when combined. Here we have characterized a novel balanced GIP and GLP-1 receptor coagonist (Cpd86) with regard to glycemic control, blood lipids, weight loss, body composition and energy utilization. Cpd86 was a balanced full dual agonist with binding K of 5.3 and 4.4 nM at GIPR and GLP1R, respectively and >100x selective over GlucR. It enhanced insulin secretion from rat pancreatic islets with an EC50 of 5.4 nM. When given s.c. 16h prior to a glucose challenge Cpd86 enhanced insulin secretion in response to an i.v. bolus of glucose. The half-maximally efficacious dose was 2.5 nmol/kg. In a two-week weight loss study in DIO mice 10 nmol/kg of Cpd86 resulted in a 11% weight loss whereas vehicle was associated with a slight weight gain of +2.7%. As a comparison, in the same study, 10 nmol/kg of long-acting GLP-1 resulted in a 6.3% weight loss and combining the GLP-1 analogue with 100 nmol/kg long-acting GIP increased the weight loss seen to 11.3% suggesting that the added GIP pharmacology may enhance weight loss. The coagonist was able to suppress food intake to a similar extent, the inclusion of GIP pharmacology either in the form of a coagonist or as a combination treatment resulted in increased fat metabolism at rates that could explain the additional weight loss seen. Weight loss was predominantly fat mass (>80%), the remainder being water and lean mass. All compounds improved plasma lipids as well as glucose tolerance in an OGTT administered at the end of the 2-week study.

In conclusion, our observations indicate that a GIP/GLP-1 coagonist may have additional benefits in treating diabetes compared to a pure GLP-1 analogue.

Liraglutide Added to High-Dose Basal/Bolus Insulin in Type 2 Diabetes

WENDY S. LANE, STEPHEN WEINRIB, JONATHAN RAPPAPORT, Asheville, NC

Adding a GLP-1 receptor agonist to basal insulin is an effective treatment option in type 2 diabetes; the addition of GLP-1 to basal/bolus insulin therapy has not been studied. In this prospective trial 40 subjects with type 2 DM using >100 units per day of basal/bolus insulin therapy were randomized to receive liraglutide plus insulin (LIRA) or insulin only (control) for 6 months. Controls then crossed over to receive liraglutide plus insulin while the LIRA group remained on both agents for an additional 8 months. HbA1c, weight and total daily insulin dose (TDID) were measured at 2, 6, 9 and 12 months. Statistical comparisons within and between groups were made by analysis of variance (ANOVA) for repeated measures.

At 6 months, HbA1c improved from a baseline of 7.8% in both groups to 7.1% in the LIRA group (p=.002) and 7.4% in controls (p=.088). HbA1c in the LIRA group at 12 months was 7.1%. Adding liraglutide to insulin in controls further improved HbA1c from 7.4% at 6 months to 6.3% at 12 months (p=.019).

At 6 months the LIRA group experienced an average weight loss of 5.27 kg (p=.069) whereas controls had gained 0.37 kg (p=NS). By month 12, the LIRA group had regained 3.26 kg for a net loss of 2 kg (p=NS vs. baseline). Adding liraglutide from month 6 to 12 in controls resulted in weight loss of 9.78 kg (p=.001).

TDID was reduced by 34% in the LIRA group at 6 months from 200 to 132 U/d (p<.0001); controls had a small increase in TDID from 171 to 178 U/d (p=NS). From month 6 to 12, the LIRA group had a slight increase in insulin dose from 132 to 148 U/d (p=NS). Adding liraglutide to insulin in controls resulted in a 36% reduction in TDID from 178 U/d at 6 months to 114 U/d by 12 months (p<.0001).

Adding liraglutide to basal/bolus insulin therapy improved glycemic control over 12 months while lowering weight and TDID in patients with type 2 DM requiring > 100 units of insulin per day, but subjects regained some weight in the second 6 months of treatment. Additional weight loss and insulin-sparing treatments are needed in the management of this challenging population.

Efficacy and Tolerability of ITCA 650 (Continuous Subcutaneous Exenatide) in Poorly Controlled Type 2 Diabetes with Baseline A1C 10%

ROBERT R. HENRY, JULIO ROSENSTOCK, MICHELLE BARON, San Diego, CA, Dallas, TX, Cambridge, MA

ITCA 650, the injection-free GLP-1 receptor agonist that provides continuous SC exenatide for up to 12 months from a single sub-dermal placement, is undergoing extensive clinical evaluation in multiple Phase 3 double-blind studies. This report represents the first 6 month, open-label experience with ITCA 650 mini-pumps from an ongoing multicenter study in subjects with type 2 diabetes who did not meet enrollment criteria for the double-blind placebo controlled trial because of A1C >10%. Enrollment criteria for this open-label trial were: A1C >10% to ≤12%, age 18-80 years, BMI 25-45 kg/m2, and on stable ≤2 months diet and exercise and/or monotherapy or any combination of metformin, sulfonylurea, and thiazolidinedione. Treatment was initiated by placing a 3-month ITCA 650 mini-pump delivering 20 mcg/day, which was then replaced by a 6-month ITCA 650 mini-pump delivering 60 mcg/day for 26 weeks. Pre-study oral antidiabetic agents (OADs) were maintained unchanged for the 39 weeks of treatment. The primary endpoint was change in A1C from baseline to week 39. At the time of this interim analysis, 50, 39, and 25 of the 60 subjects enrolled had completed 13, 19, and 26 weeks of treatment, respectively. Mean baseline characteristics for the entire cohort (n=60) were A1C 10.7%, age 52.1 yrs, BMI 32.1 kg/m2, duration of diabetes 8.9 yrs, OADs use 69%. Mean reductions of A1C at Weeks 13 (n=50), 19 (n=39), and 26 (n=25) were -2.5%, -2.9%, and -3.2%, respectively. A1C reductions ≥3% were achieved by 78% of subjects who completed at least 13 weeks of treatment; 50% achieved ≥3% and 22% achieved ≥4% reductions. A1C targets of <7% were achieved in 22% of subjects who had completed at least 13 weeks of treatment. Adverse events were consistent with previous trials with ITCA 650. In conclusion, ITCA 650 has the potential to markedly improve glycemic control in patients with severe hyperglycemia and longstanding diabetes.

Direct Vascular Protection against Glycemic- and Lipid-induced Endothelial Dysfunction by Exenatide

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We previously showed that improvement of endothelial function by exenatide (Ex) in vivo was largely independent of metabolic action of Ex and involved activation of vascular GLP-1 receptors. In the present study we tested whether Ex prevents glucose and lipid induced endothelial dysfunction in human peripheral arteries and assess which signaling pathways contribute to this vascular effect of Ex.

Vasodilation response of isolated subcutaneous adipose tissue arterioles to increasing dose of acetylcholine (Ach) and papaverine was measured before (control) and after exposure to high glucose (HG, 33 mM) or hydrolyzed VLDL (150 µM of fatty acids), with or without 10nM Ex. Phosphorylation of protein kinases (PK) A and B, and AMP-activated protein kinase (AMPK) by Ex was assessed by Western blot in human aortic endothelial cells (HAEC).

Ach vasodilatation was attenuated with HG and VLDL (p<0.001 vs. control) and restored with Ex (p=1.0 vs. control, p=0.001 vs. HG or VLDL) (Figure).

In HAEC, Ex activated AMPK but not PKA or PKB. The AMPK inhibitor Compound C significantly attenuated Ex rescue of Ach vasodilatation (p<0.001 vs. [HG or VLDL]+Ex; p=0.1 vs. HG or VLDL).

Our data indicate that Ex directly protects peripheral arteries from glycemic and lipid-induced endothelial dysfunction and that activation of the AMPK pathway appears to play a key role in preservation of vascular function by Ex.

Support By: Novo Nordisk, Inc.
Pramlintide-insulin Fixed-Dose Combination: A Phase 1 Dose Ratio—Glucagon Dual Agonist

YOUNG JIN PARK, SUNG-YOUB JUNG, JUNG KUK KIM, JONG SOO LEE, YOUNG-MI LEE, YOUNG HOON KIM, JAHDOON KANG, MICHAEL TRAUTMANN, MARCUS HUMFREY, SC CHANG KWIN, HWASENG-SI, Republic of Korea, Seoul, Republic of Korea, Hamburg, Germany, Chula Vista, CA,

Oxymodulin, an alternative cleavage product of proglucagon, is a gut hormone which can lead to enhanced body weight loss and improved glycemic control by activating GLP-1 (GLP-1R) and glucagon receptor (GCGR), respectively. However, its clinical application is limited due to low potency at the individual receptors and a short half-life. We developed a high potency GLP-1/glucagon dual agonist peptide and ultra-long acting dual agonist, HM12525A, by conjugating a novel GLP-1/glucagon dual agonist with the constant region of human immunoglobulin via a non-peptidyl linker. In a previous pre-clinical study, we demonstrated that once weekly administration of HM12525A exerted potent body weight loss and improved glycemic control in obese and/or diabetic animal models. The aim of this study was to investigate the molecular basis for the beneficial effects of HM12525A in adipocytes and pancreatic β-cells. Since HM12525A administration significantly reduced the fat mass in diet-induced obesity (DIO) mice, we firstly checked whether HM12525A has lipolytic effects in adipocytes. Interestingly, HM12525A dose-dependently inhibited the intracellular lipid droplet formation in 3T3-L1 adipocytes. In addition, phosphorylation of hormone-sensitive lipase (HSL), a key enzyme for lipolysis, and following glycerol release were significantly increased upon HM12525A treatment in 3T3-L1 adipocytes, suggesting stimulating effects of HM12525A on lipolysis. As to the effects in pancreatic β-cells, HM12525A increased insulin secretion in RINm5F cells. In line with this, HM12525A administration significantly increased insulin secretion as well as insulin sensitivity, thereby lowering glucose excursion during ipGTT in normal mice. Taken together, these results demonstrate that dual agonism of HM12525A mediates lipolytic and insulinotropic effects in adipocytes and β-cells, conferring both anti-obesity and anti-diabetic potentials.

Lipolytic and Insulino tropic Effects of HM12525A, a Novel Long-acting GLP-1/Glucagon Dual Agonist

LIPOLYTIC AND INSULINO TROPIC EFFECTS OF HM12525A, A NOVEL LONG-ACTING GLP-1/GLUCAGON DUAL AGONIST

YOUNG JIN PARK, SUNG-YOUB JUNG, JUNG KUK KIM, JONG SOO LEE, YOUNG-MI LEE, YOUNG HOON KIM, JAHDOON KANG, MICHAEL TRAUTMANN, MARCUS HUMFREY, SC CHANG KWIN, HWASENG-SI, Republic of Korea, Seoul, Republic of Korea, Hamburg, Germany, Chula Vista, CA,

Oxymodulin, an alternative cleavage product of proglucagon, is a gut hormone which can lead to enhanced body weight loss and improved glycemic control by activating GLP-1 (GLP-1R) and glucagon receptor (GCGR), respectively. However, its clinical application is limited due to low potency at the individual receptors and a short half-life. We developed a high potency GLP-1/glucagon dual agonist peptide and ultra-long acting dual agonist, HM12525A, by conjugating a novel GLP-1/glucagon dual agonist with the constant region of human immunoglobulin via a non-peptidyl linker. In a previous pre-clinical study, we demonstrated that once weekly administration of HM12525A exerted potent body weight loss and improved glycemic control in obese and/or diabetic animal models. The aim of this study was to investigate the molecular basis for the beneficial effects of HM12525A in adipocytes and pancreatic β-cells. Since HM12525A administration significantly reduced the fat mass in diet-induced obesity (DIO) mice, we firstly checked whether HM12525A has lipolytic effects in adipocytes. Interestingly, HM12525A dose-dependently inhibited the intracellular lipid droplet formation in 3T3-L1 adipocytes. In addition, phosphorylation of hormone-sensitive lipase (HSL), a key enzyme for lipolysis, and following glycerol release were significantly increased upon HM12525A treatment in 3T3-L1 adipocytes, suggesting stimulating effects of HM12525A on lipolysis. As to the effects in pancreatic β-cells, HM12525A increased insulin secretion in RINm5F cells. In line with this, HM12525A administration significantly increased insulin secretion as well as insulin sensitivity, thereby lowering glucose excursion during ipGTT in normal mice. Taken together, these results demonstrate that dual agonism of HM12525A mediates lipolytic and insulinotropic effects in adipocytes and β-cells, conferring both anti-obesity and anti-diabetic potentials.
120-LB
Glucagon-like Peptide 1 Modulates Reductions in Myocardial Blood Flow Reserve during Euglycemia and Hyperglycemia in Both Type 2 Diabetics and Non-Diabetics

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We evaluated the effect of Glucagon-like peptide-1 (GLP1) on myocardial blood flow reserve (MBFR) in subjects with & without type 2 diabetes (T2D & non-T2D) under eu- or hyperglycemia with somatostatin pancreatic clamp. 21 subjects (8 T2D;813 non-T2D) were enrolled. Both groups underwent 2 study visits each (GLP1: 1.2 pmol/kg/min GLP1 infusion, NS. Normal Saline infusion). During each visit, 2 stage pancreatic clamp (somatostatin, glucagon & insulin 0.75 μU/kg/min) was conducted. Glucose was infused to maintain euglycemia (5μM) followed by hyperglycemia (14 μM) (each 2 hr stages). Real time myocardial perfusion echocardiography (RT-MPE) was performed during each glycemic state using diluted Definity (200 mHr), at rest and during regadenoson stress (400 μg IV bolus). MBFR (stress/rest) was quantified. Non-T2D (85% female, age 48±6 yrs, BMI 25±3 kg/m², HbA1C 5.4±3.1%) & T2D (75% male, age 54±6 yrs, BMI 32±4 kg/m², HbA1C 7.2±1.1%). Mean MBFR was reduced at hyper vs. euglycemia in T2D-NS (p=0.038) & non-T2D-NS (p=0.031). GLP1 infusion prevented this reduction, Figure. MBFR was lower in T2D-NS vs. non-T2D-NS (euglycemia: p=0.010, hyperglycemia: p = 0.003), but was not different in T2D-GLP1 vs. non-T2D-GLP1. GLP1-1 modulates the magnitude of MBFR reduction in T2D, both during euglycemia and hyperglycemia, suggesting a protective cardiovascular effect.

Supported By: Novo Nordisk A/S

121-LB
Beneficial Effects of Liraglutide in Type 1 Diabetes

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The diagnosis of Metabolic Syndrome has been frequently done in type 1 diabetes, as found in 40-50% in U.S. Larkin et al found 30% of obesity in type 1. The need of a further therapy for metabolic syndrome and obesity in type 1, made liraglutide an option for these patients. This study aimed to evaluate the effect of weight loss associated with liraglutide in body weight and metabolic control in type 1 diabetes. Materials and Methods: We evaluated 15 patients with type 1 diabetes before and after liraglutide with the following parameters: body mass index (BMI) hemoglobin glycated (A1c) and lipid profile. We also evaluate side effects. Results: The average age was 36.2 years and duration of diabetes of 19.1 years (3-33 years), the majority was female 12/3 (F/M). Forty percent was in insulin pump and 80% using analogs. Comparing before to after liraglutide 3-5 months, we note an improvement in A1C (7.9 ± 7.0%), weight loss (4.09±6.97 kg, p<0.0001) were observed from baseline. Gastrointestinal events was lower (-4.09±6.97 kg, p<0.0001) were observed from baseline.

Supported By: Eli Lilly and Company

122-LB
Safe and Effective Use of the Single-Use Pen for Injection of Once-Weekly Dulaglutide in Injection-Naïve Patients with Type 2 Diabetes

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Many patients with type 2 diabetes (T2D) fail to achieve adequate glycemic control with oral antihyperglycemic therapy alone, but patients and clinicians often avoid initiating injectable therapy-fearing pain and complexity. The single-use pen (SUP) device contains a pre-filled syringe and automates needle insertion and retraction, and drug delivery. It was designed for subcutaneous delivery of 0.5 ml of dulaglutide, a once weekly glucagon-like peptide-1 receptor agonist to treat T2D. The objective of this 4 week, Phase 3b, multicenter, open-label, single-arm, outpatient study was to demonstrate the safe and effective use of the SUP containing 0.5 ml of placebo in injection-naïve T2D patients as demonstrated by the final injection success rate (primary outcome) and the initial injection success rate following training (key secondary outcome). Patient-reported outcomes for pain, ease of use of the SUP, willingness to continue using the SUP, and fear of self-injecting were also reported. Mean baseline patient demographics (N=211) were: age 61 yr, duration of diabetes 7.7 yr, and BMI 31.7 kg/m². The primary objective was met, with a final injection success rate of 99.1% (95% CI: 96.6, 99.7). The initial injection success rate was 57.2% (95% CI: 53.9, 58.7), meeting the key secondary objective. On a scale of 1 (no pain) to 10, the mean (SD) of pain scores across injections was 1.0 (1.1). 99.0% of patients found the device easy to use and 96.7% of patients indicated they would be willing to continue to use the SUP after the study. There was a significant reduction (p<0.001) in patients’ fear of self-injecting, as measured by the self-injecting subscale of the modified Diabetes Fear of Injecting and Self-testing Questionnaire. The single-use pen was a safe and effective device for T2D patients who were injection naïve. Improvements in injection experience may be an important factor for some patients and providers when initiating injectable therapy.

Supported By: Eli Lilly and Company

123-LB
Effects of Allogeneic Mesenchymal Precursor Cells in Type 2 Diabetes: A Randomized, Placebo-Controlled Study

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Type 2 diabetes (T2D) is a chronic metabolic disease with inflammatory underpinnings that may be responsive to therapies which have anti-inflammatory attributes. This study assessed the safety and tolerability as well as exploratory metabolic effects of allogeneic, bone-marrow derived mesenchymal precursor cells (MPCs) in T2D subjects insufficiently controlled on metformin +/- another oral antidiabetic agent. Subjects were enrolled in a dose-escalating randomized, placebo (PBO) controlled trial to receive a single intravenous (IV) infusion of 0.3 million (M) MPCs/kg (0.3M; n=15), 1.0 M MPCs/kg (1M; n=15), 2.0 M MPCs/kg (2M; n=15) or placebo (n=16). Study duration was 12 wk. Sixty-one subjects (21 women, 40 men) with meansSD baseline A1c 8.3±1.0%, BMI 33.5±5.5 kg/m² and diabetes duration 10.1±6.0 years were

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For author disclosure information, see page LB91.
enrolled at 18 U.S. sites. No acute adverse events (AEs) were associated with infusion. There were no serious AEs, serious hypoglycemia AEs, or discontinuations due to AEs over 12 wk. The rate and pattern of adverse events were comparable among groups. No AEs were deemed treatment-related. No subjects developed donor specific antibodies.

A single IV infusion significantly reduced A1C (%) from baseline at 8 wk in 2M MPC compared to PBO. The adjusted least squares mean (LSM) difference from PBO (95% confidence interval) at 8 wk were -0.2 (0.0, 0.4), -0.1 (0.5, 0.3) and -0.4 (-0.5, 0.0) for 0.3M, 1M and 2M groups (p8% with MPC compared to placebo at 12 wk -0.2 (-1.1, 0.6), -0.2 (-1.0, 0.6) and -0.5 (-1.4, 0.5) ±0.4 for 0.3M, 1M, and 2M (NS). Target A1C <7% was achieved by 5/15 2M vs. 0/15 PBO subjects (p<0.05).

In conclusion, infusion of MPCs showed no safety issues. Suggestive beneficial effects of 2M/kg MPC on glucose control need to be evaluated in a properly powered long-term controlled study.

**Clinical Therapeutics/New Technology—Oral Agents**

**124-LB**

**A Novel Circadian Clock Modulator Improves Insulin Resistance in Diabetic Mice**

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Circadian rhythms are important for regulating physiology, and disruption of circadian rhythms has been associated with diverse changes in immune responses, behavior and metabolism. The bidirectional interaction between circadian rhythm and metabolism is well established, and metabolic diseases are associated with dysregulation of circadian rhythms.

Previous work by Hirota et al. (Science, 2012) resulted in the identification of compounds that interact with and stabilize Cryptochromes (Cry) proteins, which are key regulators of the intracellular circadian machinery. These compounds were found to modulate both core clock and metabolic gene transcription in vitro. We developed a series of Cry stabilizers with improved drug-like properties and tested them in two mouse models of diabetes: diet-induced obese (DIO) and db/db.

In vivo, Compound A alters expression of the core clock genes as determined by quantitative reverse transcription PCR (RT-PCR), reducing Period2 (Per2) gene expression and causing a phase delay in Bmal1 gene expression. After 7 days of oral QD dosing of Compound A, we found significant changes in glucose metabolism as measured by Fasted Blood Glucose (FBG) and Oral Glucose Tolerance Test (OGTT). These results were comparable to the efficacy of rosiglitazone and sitagliptin in these models, but were not associated with weight gain. Reductions in plasma insulin levels and increased insulin sensitivity were also observed in both models. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) values were significantly reduced, indicating re-sensitization. Compound A was also tested in the rat ZDF model of diabetes and significant changes were again found in glucose metabolism, comparable in efficacy to rosiglitazone.

Taken together, these data suggest that circadian rhythm modification represents a compelling new approach to treating type 2 diabetes and other metabolic disorders.

**125-LB**

**Energy Balance Following Sodium-Glucose Co-Transporter-2 (SGLT2) Inhibition**

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SGLT2 inhibitors lower glycaemia by inducing urinary glucose excretion (UGE), with the attendant calorie loss. Evidence suggests that the resulting weight loss (WL) is less than expected from UGE. To quantify this phenomenon we analyzed data from 86 type 2 diabetic (T2D) patients (39 women, age = 58 ± 9 years, BMI = 29.8 ± 4.5 kg/m², HbA1c = 7.8 ± 0.8%, FPG = 183 ± 41 mg/dl, eGFR = 89 ± 19 ml/min/1.73m², µ ± SD), the per-protocol completers cohort of a clinical trial who received empagliflozin (25 mg/day) for 80 weeks with frequent (n=11) assessments of body weight, eGFR, and FPG. Time-dependent glucose filtration was calculated as the product of eGFR and FPG, time-dependent UGE was estimated by assuming - from previous direct measurements - a quasi-linear relationship between fractional UGE and glycaemia. At week 90, WL averaged -3.2 ± 4.2 kg (range -17.0 to +5.5); over 90 weeks, UGE averaged 54 ± 15 g/day (fractional UGE = 45 ± 4%). The relation of calorie-to-weight changes was estimated using a mathematical model (http://bwsimulator.niddk.nih.gov) that simulates the time-course of WL for a given change in calorie balance. The observed WL corresponded to a calorie deficit of ~78 ± 103 kcal/day. On the other hand, the observed calorie loss (~217 ± 59 kcal/day) predicted a WL of -8.7 ± 2.4 kg (range -4.0 to -15.3 kg) over 90 weeks. Thus, patients lost only 38 ± 53% of the WL predicted by their glycaemia. As previous studies showed that empagliflozin does not affect either resting or meal-induced energy expenditure, patients likely increased their energy intake (by an estimated ~138 ± 116 kcal/day). This excess calorie intake was inversely related to baseline BMI (partial r = -0.33, p<0.01) and positively to baseline eGFR (partial r = 0.30, p<0.01). In conclusion, chronic glycosuria elicits an adaptive increase in energy intake, particularly in leaner patients with preserved renal function. Combining SGLT2 inhibition with strategies to maintain energy intake or curb appetite is expected to be associated with major WL.

**Supported By:** Boehringer Ingelheim

**126-LB**

**Factors Associated with Progression of Type 2 Diabetes and Impact of Treatment with Saxagliptin in the SAVOR-TIMI 53 Study**

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In T2D, glycemic control often deteriorates over time, requiring intensification of treatment. We aimed to identify factors associated with progression of diabetes and studied the impact of saxagliptin, a DPP-4 inhibitor, on diabetes progression. In addition, we evaluated the effect of saxagliptin on beta cell function as reflected by a decline in HOMA2-β.

We studied the association of clinical and biochemical parameters with diabetes progression in the SAVOR-TIMI 53 study, a randomized clinical trial of 16,492 patients with T2D treated with saxagliptin vs. placebo added to current anti-diabetic medications for a median of 2.1 years. Diabetes progression was defined by 1) HbA1c increase ≥0.5%, 2) initiation of new anti-diabetic medications, 3) increase in oral medication dose or 4) ≥25% increase in insulin dose for ≥3 months.

HOMA2-β was measured at baseline and at year 2 in 4134 patients (25.1% of trial). Progression of diabetes during the study occurred in 54.7% of all subjects. Compared with placebo, treatment with saxagliptin decreased the risk of diabetes progression (OR 0.80; 95% CI 0.57-0.65, p<0.001). The occurrence of an HbA1c increase of ≥0.5% was decreased by 30%; initiation of insulin was decreased by 30% and the increase in dose for an oral hypoglycemic medication or insulin by 19% in patients treated with saxagliptin compared with placebo. At 2 years, HOMA2-β was decreased by 7.6% with placebo, compared with 2.7% with saxagliptin (p=0.0004). A multivariate analysis that included baseline demographics, biochemical parameters, and medical treatments showed that older age, lower HLD, lower baseline HOMA2-β, and baseline sulfonylurea use were significantly associated with diabetes progression.

Saxagliptin decreased the progression of diabetes via improved glycomic indices and fewer concomitant anti-hyperglycemic agents compared with placebo, which may be related to reduced natural decline in β-cell function.

**Supported By:** AstraZeneca

**127-LB**

**Dual Add-On Therapy in Poorly Controlled Type 2 Diabetes on Metformin: Randomized, Double-Blind Trial of Saxagliptin+Dapagliflozin vs. Saxagliptin and Dapagliflozin Alone**

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SGLT2 and DPP-4 inhibitors have complementary mechanisms of action that can potentially improve glucose control with weight loss and low risk of hypoglycemia. We compared the efficacy and safety of dual add-on of saxagliptin (SAXA) and dapagliflozin (DAPA) to SAXA and DAPA alone. In this 24-week, multicenter, randomized, double-blind, active-controlled trial, adults with type 2 diabetes (T2D) and A1C ≥8.0% and ≤12.0%, received SAXA 5 mg and DAPA 10 mg once daily compared to SAXA and placebo (PBO) or DAPA and PBO on background of metformin XR ≥1500 mg/d. Primary end point was the change in A1C from baseline to week 24. Safety and tolerability assessments included adverse events (AEs) and hypoglycemia. 534 patients baseline were randomized. Mean ± SD A1C at baseline in SAXA+DAPA, SAXA+PBO, and DAPA+PBO groups was 8.9 ± 1.2%, 9.0 ± 1.1%, and 8.9 ± 1.2%, respectively. Adjusted reduction from baseline in A1C was -1.47% in SAXA+DAPA compared to -0.88% in SAXA+PBO (difference -0.59%, 95% CI [-0.81, -0.37], P<0.001) and -1.20% in DAPA+PBO (difference -0.27%, 95% CI [-0.48, -0.05], P<0.02). The adjusted proportion achieving A1C <7% was 41% in SAXA+DAPA compared to 18% in SAXA+PBO (difference of 23%; 95% CI [15, 32]) and 22% for oral disclosure information, see page LB91.
in DAPA+PBO (difference of 19%, 95% CI [10, 28]). AEs occurred in 48.6%, 52.8% and 48.6% in SAXA+DAPA, SAXA+PBO and DAPA+PBO, respectively. Urinary and genital infections occurred with the expected frequency previously reported. Incidence of hypoglycemia was 1.1%, 0.6% and 1.1%, respectively with no episodes of major hypoglycemia. In conclusion, this first report of triple therapy adding a well-tolerated combination of DPP-4 and SGLT2 inhibitors to poorly controlled metformin-treated T2D demonstrated that the combination of SAXA and DAPA had greater improvements in glucose control than each component alone, bringing >40% of poorly controlled T2D to goal, with weight loss as DAPA alone and very low hypoglycemia risk.

Supported By: AstraZeneca/Bristol-Myers Squibb

128-LB


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Trelagliptin is a novel dipeptidyl peptidase-4 (DPP-4) inhibitor, which is currently under development as a once-weekly oral anti-diabetic agent.

A Phase 3, multicenter, randomized, double-blind, parallel-group, non-inferiority study was conducted to evaluate the efficacy and safety of once-weekly trelagliptin for 24 weeks with daily DPP-4 inhibitor (alogliptin) as a comparator in Japanese patients with type 2 diabetes mellitus (T2DM) with inadequate glycemic control despite diet and/or exercise therapy. A placebo group was also set a reference group.

Patients were randomly assigned (allocation ratio 2:2:1) to receive either trelagliptin 100 mg once-weekly, alogliptin (Nesina) 25 mg daily or placebo (reference group).

A total of 243 patients were enrolled to either trelagliptin once-weekly group (n=101), alogliptin daily group (n=92) or placebo group (n=50). At baseline, patients had mean age (SD) of 58.9 (10.39) years, mean BMI of 24.96 (4.161) kg/m² and mean HbA1c of 7.88 (0.837)%.

Baseline characteristics among the treatment groups.

As for efficacy, HbA1c was decreased significantly in trelagliptin group (-0.32%) and alogliptin group (-0.46%) compared to placebo group (0.24%) at the end of the treatment period (p<0.0001). The least square mean difference (-0.32%) and alogliptin group (-0.46%) compared to placebo group (0.24%) at the end of the treatment period was 0.11% (95% CI: -0.054 to 0.281). Non-inferiority of baseline characteristics among the treatment groups.

As for safety, the frequency of adverse events in trelagliptin group was similar to those in alogliptin group and in placebo group. No hypoglycemia was reported.

Once-weekly trelagliptin may provide a new treatment option for T2Dm patients.

129-LB

Fixed Dose Combinations of Empagliflozin/Linagliptin for 24 Weeks in Drug-Naïve Patients with Type 2 Diabetes (T2DM)

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A 52-week Phase III study evaluated the efficacy and safety of fixed dose combinations (FDCs) of empagliflozin/linagliptin (EMPA/LINA). Drug-naive subjects with T2DM were randomized to EMPA 25 mg/LINA 5 mg (n=137), EMPA 10 mg/LINA 5 mg (n=136), EMPA 25 mg (n=135), EMPA 10 mg and LINA 5 mg (n=135). Primary endpoint was change from baseline in HbA1c at week 24. Key secondary endpoints were changes in fasting plasma glucose (FPG) and weight, and percentage of subjects with baseline HbA1c ≥7% who had HbA1c <7% at week 24. Efficacy was evaluated in 687 subjects (mean [SD] age 56.4 [10.2] years; BMI 31.8 [5.6] kg/m²).

At week 24, FDCs of EMPA/LINA reduced HbA1c, FPG and weight vs. LINA 5 mg. EMPA 10 mg/LINA 5 mg reduced HbA1c vs. EMPA 10 mg (table). Adverse events (AEs) were reported in 58.8%, 63.2%, 57.8%, 62.2% and 64.4% of subjects on EMPA 25 mg/LINA 5 mg, EMPA 10 mg/LINA 5 mg, EMPA 25 mg and LINA 5 mg, respectively, over 24 weeks. Confirmed hypoglycemic AEs (glucose ≤70 mg/dL, and/or requiring assistance) were reported in 2 subjects on EMPA 25 mg and 1 each on EMPA 10 mg and LINA 5 mg, none reported assistance.

In subjects with T2DM, FDCs of EMPA 25 mg or LINA 10 mg/LINA 5 mg for 24 weeks significantly reduced HbA1c, FPG and weight vs. LINA 5 mg. HbA1c reductions were greater with EMPA 10 mg/LINA 5 mg than EMPA 10 mg, but similar with EMPA 25 mg/LINA 5 mg and EMPA 25 mg. All treatments were well tolerated.

As add-on to metformin in subjects with T2DM, FDCs of EMPA 25 mg/LINA 5 mg and EMPA 10 mg/LINA 5 mg for 24 weeks significantly reduced HbA1c and

130-LB

Fixed Dose Combinations of Empagliflozin/Linagliptin for 24 Weeks as Add-On to Metformin in Patients with Type 2 Diabetes (T2DM)

RALPH A. DEFRONZO, ANDREW LEWIN, SANJAY PATHEL, DACHENG LIU, RENE KASTE, HANS J. WOERLE, ULI C. BROEDL, ANDREW LEWIN, RALPH A. DEFRONZO, SANJAY PATEL, DACHENG LIU, RENEE INAGAKI, Kurashiki, Japan, Osaka, Japan, Kyoto, Japan

A 52-week Phase III study evaluated the efficacy and safety of fixed dose combinations (FDCs) of empagliflozin/linagliptin (EMPA/LINA) as add-on to stable-dose metformin in subjects with T2DM. Subjects were randomized to EMPA 25 mg/LINA 5 mg (n=137), EMPA 10 mg/LINA 5 mg (n=136), EMPA 25 mg (n=141), EMPA 10 mg and LINA 5 mg (n=132). Primary endpoint was change from baseline in HbA1c at week 24. Key secondary endpoints were changes in fasting plasma glucose (FPG) and weight, and percentage of subjects with baseline HbA1c ≥7% who had HbA1c <7% at week 24.

In 674 subjects (mean [SD] age 56.2 [10.2] years; BMI 31.0 [5.5] kg/m²), FDCs of EMPA/LINA reduced HbA1c and FPG vs. the respective monotherapies (table). Adverse events (AEs) were reported in 54.7%, 54.4%, 63.1%, 57.1% and 54.5% of subjects on EMPA 25 mg/LINA 5 mg, EMPA 10 mg/LINA 5 mg, EMPA 25 mg, EMPA 10 mg and LINA 5 mg, respectively, over 24 weeks. Confirmed hypoglycemic AEs (glucose ≤70 mg/dL, and/or requiring assistance) were reported in 2 subjects each on EMPA 25 mg/LINA 5 mg, EMPA 10 mg/LINA 5 mg, EMPA 10 mg and LINA 5 mg, and 4 on EMPA 25 mg, none reported assistance.

As add-on to metformin in subjects with T2DM, FDCs of EMPA 25 mg/LINA 5 mg and EMPA 10 mg/LINA 5 mg for 24 weeks significantly reduced HbA1c and

For author disclosure information, see page LB91.
4. eGFR in the full study pool: From BL (108.11 mL/min), there was a small mean reduction in eGFR for PBO (-1.8), 2.5 mg (-2.3) and 5 mg SAXA (-3.0), with 95% CI excluding zero, and a small increase in eGFR for 10 mg SAXA (2.0). 4.0).

In summary, as observed in SAVOR, SAXA reduced the proportion with albuminuria. Though limited by the size of the albuminuria subpopulations, the data suggest: 1) in those without albuminuria SAXA prevents the upward drift observed with PBO and 2) in those with microalbuminuria SAXA treatment reduces albuminuria dose proportionately.

Supported By: Bristol-Myers Squibb/AstraZeneca

132-LB

**LX4211, a Dual Inhibitor of SGLT1/SGLT2, Reduces Postprandial Glucose in Patients with Type 2 Diabetes Mellitus and Moderate to Severe Renal Impairment**

PABLO LAPUTERA, ARTHUR SANDS, IGE OGBAA, PAUL STRUMPH, DAVID R. POWELL, PHILLIP BANKS, BRIAN ZAMBROWICZ, the Woodlands, TX

The prevalence of renal impairment (RI) in type 2 diabetes mellitus (T2DM) is ≥20%. Since selective sodium-glucose co-transporter 2 (SGLT2) inhibitors target only the kidney, they have reduced efficacy in T2DM patients with RI. Because LX4211 blocks both SGLT2-mediated renal glucose reabsorption and SGLT1-mediated gastrointestinal glucose absorption, it should benefit patients with T2DM and RI by significantly reducing postprandial glucose (PPG) levels.

The primary objective was to evaluate the effect of LX4211 on 4-hour PPG AUC change from baseline to Day 7 in patients with T2DM and baseline renal function (eGFR) ≥15 and ≤59 mL/min/1.73 m2 (calculated by MDRD). Patients (N=31) were randomly assigned to receive LX4211 (400 mg, n=16) or placebo (n=15) 15 minutes before a standard breakfast on 7 consecutive days. Glucose and GLP-1 were measured 15 minutes prior to breakfast and 1, 2, 2.5, 3, and 4 hours post breakfast at baseline and on Day 7.

LX4211 significantly reduced mean PPG AUC change from baseline to Day 7 (p<0.003). In patients with baseline eGFR values <45 mL/min/1.73 m2, the LX4211-treated patients (N=5) had a 259.6 mg/hr/dL mean PPG reduction compared to the placebo patients (N=9), p=0.002. Compared to placebo, LX4211 also showed significant elevations in the mean change in incremental AUC between baseline and Day 7 for total GLP-1 of 9.7 pmol/hr/L (p=0.017) and for active GLP-1 of 4.7 pmol/hr/L (p=0.042) in all patients. There were no serious adverse events (SAEs) and no discontinuations due to AEs. There were 3 mild cases of hypoglycemia reported as treatment-emergent adverse events during the trial: 1 in the LX4211-treated patients and 2 in placebo patients.

These results indicate that LX4211 may enhance glycemic control in patients with moderate to severe RI.

Support By: Bristol-Myers Squibb/AstraZeneca

131-LB

**Saxagliptin Effect on Urinary Albumin/Creatinine Ratio (ACR) and eGFR: Analysis of Pooled Phase 3 Studies**

ROBERT FREEDERICH, NAYYAR IOBAIL, MIKAELA SJÖSTRAND, WILLIAM COOK, BOAZ HIRSHBERG, Princeton, NJ, Wilmington, DE

Saxagliptin (SAXA) significantly reduced the proportion with micro- (ACR 30-300 mg/g) and macro- (>300 mg/g) albuminuria in the SAVOR outcome study. This prompted a post-hoc pooled analysis of 5 phase 3, double-blind, placebo (PBO) controlled studies (2 drug-naive, plus add-on to metformin, SU, and TZD).

At 24 weeks there was a net shift in the size of the population with albuminuria (-4.6%, -6.9%, -15.0%, -28.9% for PBO, 2.5, 5, & 10 mg SAXA respectively). Further analysis revealed (Figure):

- Nonalbuminuric (geometric mean ACR = 8-9 mg/g across arms): All SAXA doses prevented the rise in ACR vs. baseline (BL) seen with PBO.
- Microalbuminuric (ACR = 61-76 mg/g): There was a dose linear reduction in ACR vs. BL.
- Macroalbuminuric (ACR = 823-1054 mg/g): All 4 arms had a reduction in ACR vs. BL. The wide 95% CI (excluding 1) prevented seeing a clear pattern.

Support By: Boehringer Ingelheim/Eli Lilly and Company

133-LB

**Effect of Empagliflozin (EMPA) Monotherapy on Postprandial Glucose (PPG) and 24-h Glucose Variability Using Continuous Glucose Monitoring (CGM) in Japanese Patients with Type 2 Diabetes (TZDM)**

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A Phase IIIb randomized study evaluated the effect of EMPA on PPG and 24-h glucose variability in Japanese patients with TZDM.

Patients (N=60; baseline mean [SD] HbA1c 7.91 [0.80%]; age 62.7 [8.5] years; BMI 24.3 [3.2] kg/m2) were randomized to EMPA 10 mg (n=20), EMPA 25 mg (n=15), EMPA 50 mg (n=15), or placebo (n=10). The primary objective was to evaluate the effect of EMPA on PPG change from baseline to Day 7 in patients with TZDM and baseline renal function (eGFR) ≥15 and ≤59 mL/min/1.73 m2 (calculated by MDRD). Patients (N=31) were randomly assigned to receive LX4211 (400 mg, n=16) or placebo (n=15) 15 minutes before a standard breakfast on 7 consecutive days. Glucose and GLP-1 were measured 15 minutes prior to breakfast and 1, 2, 2.5, 3, and 4 hours post breakfast at baseline and on Day 7.

LX4211 significantly reduced mean PPG AUC change from baseline to Day 7 (p<0.003). In patients with baseline eGFR values <45 mL/min/1.73 m2, the LX4211-treated patients (N=5) had a 259.6 mg/hr/dL mean PPG reduction compared to the placebo patients (N=9), p=0.002. Compared to placebo, LX4211 also showed significant elevations in the mean change in incremental AUC between baseline and Day 7 for total GLP-1 of 9.7 pmol/hr/L (p=0.017) and for active GLP-1 of 4.7 pmol/hr/L (p=0.042) in all patients. There were no serious adverse events (SAEs) and no discontinuations due to AEs. There were 3 mild cases of hypoglycemia reported as treatment-emergent adverse events during the trial: 1 in the LX4211-treated patients and 2 in placebo patients.

These results indicate that LX4211 may enhance glycemic control in patients with moderate to severe RI.

Support By: Bristol-Myers Squibb/AstraZeneca

For author disclosure information, see page LB91.
Hypothesis for missing data (42.4 vs. -30.3 (3.8) -24.5 0.3 (2.1) 7.9 ( 9.9) -23.6 (2.2) -89.2 (10.4) -20.8 (-27.0, -18.1 (13.9) 1–4h -71.7 (11.3) -62.9 (11.1) 41.2 (9.8) -54.8 -5.8 (3.7) -31.9 (10.3) -8.1 (10.8) -29.2 (11.1) -71.7 (11.3) -54.8 (-46.4, -23.2)** (-94.6, -32.2)**

Change from baseline at day 28
Difference vs. placebo (95% CI)
Baseline
-97.1 (-126.5, -67.8)*** (-120.4, -62.9)*** -9.5 (-126.0, -65.4)*** (-148.6, -85.0)***
Change from baseline at day 28
-18.1 (13.9) -103.9 (14.2) -122.9 (14.4)
Difference vs. placebo (95% CI)
-85.5 (-104.9)

24-h mean glucose (mg/dL)
Baseline
181.4 (6.4)
Change from baseline at day 1
0.3 (2.1)
Difference vs. placebo (95% CI)
-20.8 (-27.0, -14.7)*** -23.9 (10.0, -17.9)***
Change from baseline at day 28
-5.8 (3.7)
Difference vs. placebo (95% CI)
-24.5 (-35.4, -13.6)*** (-42.5, -20.3)***

AUC (≥180 mg/dL) within 3 h of breakfast (mg/dL)
Baseline
107.5 (21.5)
Change from baseline at day 1
41.2 (9.8)
Change from baseline at day 28
-8.1 (10.8)
Difference vs. placebo (95% CI)
-73.1 (-102.2, -44.0)*** (-114.5, -57.4)***

Risk of New Onset Heart Failure in Patients Using Sitagliptin
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Recent randomized controlled trials have suggested that DPP-4 inhibitors may be associated with an increased risk of incident heart failure (HF); although results are inconsistent. Thus, we examined whether patients using sitagliptin, the most widely prescribed DPP-4 inhibitor in the U.S., at the time of acute coronary syndrome (ACS) are at greater risk for incident heart failure (HF) than those not exposed.

Using a large commercially insured U.S. claims database, diabetes subjects without a history of HF in the 3 years prior to admission to hospital for an ACS event were identified based on ICD-9-CM codes between 2004 to 2010. We used a nested case control design whereby cases were patients who developed incident HF within 30 days of admission to hospital for ACS and matched (using risk set sampling) by age and sex with up to 10 controls with no HF prior to the index date for their given case. Subjects exposed or not exposed to sitagliptin in the 90 days prior to ACS admission were compared using conditional logistic regression adjustment for demographics, clinical & laboratory data, pharmacy claims, health care utilization and propensity scores (conditional probability of being treated with metformin or sulfonylurea or insulin or sitagliptin).

In total, 457 HF cases were matched to 1,457 controls. Average age of subjects was 55 years, 65% were male, 71% had a history of dyslipidemia and 81% had a history of hypertension. Overall, 11 of 147 sitagliptin users (7%) developed HF compared to 446 of 4,880 non-users (9%) (adjusted odds ratio [aOR]: 0.75, 95% CI: 0.38 – 1.46; p=0.40). Similarly, sitagliptin use pre-ACS was not associated with an increased risk of death or HF combined (7% vs. 9%, aOR: 0.66, 95% CI: 0.34-1.26).

In our large population based cohort, sitagliptin use was not associated with an increased risk of HF following admission to hospital for ACS, raising doubts around the hypothesis that DPP-4 inhibitors have adverse cardiovascular effects in patients with type 2 diabetes.

Treatment Duration of Dual Therapy with Metformin and Sitagliptin in Type 2 Diabetes: The Odyssey Observational Study
PAUL VALENIS, GERARD DE POLI-DURVILLE, NATHALIE BENARD, CÉLINE CHANUT-VOGEL, CHRISTINE MOISAN, CHRISTIAN KEMPF, JEAN DALLONGEVILLE, BORDY, France, Paris, France, Courbevoie, France, Lille, France

Sitagliptin and DPP4 inhibitors are usually prescribed for T2DM patients in combination with metformin. Odyssey, a prospective, real-world, observational study conducted in France in primary care practices, compared the duration of maintenance of treatment without modification (withdrawal, substitution or add-on therapy) in T2DM patients in whom dual therapy with metformin + sitagliptin (MetSita) or metformin + sulfonylurea (MetSu) was initiated, based on physician choice. Patients were not randomized and were followed for a period of up to three years.

At baseline, differences between the two arms [MetSita [n = 1874] and MetSu [n = 733]] were modest (mean age: 62.4 vs. 64.2 years, BMI: 30.3 vs. 29.6 kg/m2, diabetes duration: 6.4 vs. 7 years, respectively). HA Matthews levels were similar (7.5 vs. 7.6%).

The median treatment duration for patients in the MetSita group was longer than the MetSu group (median treatment duration 43.2 vs. 20.2 months, respectively, between-group difference 23 months, log-rank p <0.0001). This difference persisted after adjustment for baseline differences with propensity score and application of maximum bias hypothesis for missing data (42.4 vs. 20.2 months). A similar reduction in HbA1c was noted in both arms (4.5%) and the incidence of hypoglycemia (prior to treatment modification) was lower in the MetSita arm (9.7% vs. 21%).

In real-life conditions, the Odyssey study shows that combined therapy MetSita is maintained without treatment modification longer than combined therapy MetSu. In addition, the study confirms that glyemic efficacy is similar, with a lower incidence of symptomatic hypoglycemia with MetSita compared to MetSu.
**137-LB**

**Metformin Action Prevents Sedentariness-induced Damages in Mice**

**LIVIO LUZI, ILEANA TERRUZZI, PAMELA SENESE, ANNA MONTESSANO, ROBERTO CODDELLA, STEFANO BENEDINI, Milan, Italy**

Metformin (Mef), a widely prescribed drug to treat type 2 diabetes, is being increasingly considered for treatment and prevention of sedentariness damages, insulin resistance and obesity, as well as for the extension of healthy lifespan. Recent data demonstrate that long-term treatment with Metf in middle-aged male mice extends healthy lifespan in male mice. In order to determine if Metf action was limited to middle age condition, our group studied Metf effects on sedentary adult young mice. To achieve this aim, C57BL/6 mice male at 12 weeks of age were treated with Metf (250 mg/kg per day, in drinking water) for 3 months. Control mice group drank water only. A muscular performance, evaluated by a submaximal running test prior and upon completion of the study, revealed that Metf treated mice exhibit an enhanced performance respect to the control mice. To assess how Metf enhanced physical performance and healthy lifespan of the sedentary animals, we analyzed the principal target tissues of insulin resistance: skeletal muscle, liver and visceral adipose tissues. Western Blot results revealed that Metf activated AMPK in these tissues, suggesting how this drug could prevent dysregulation of glucose and lipid metabolism. In liver, Metf decreased the levels of the principal kinases involved in hepatic stress conditions, ERKs. In skeletal muscle, Metf increased the activation of AKT, a central kinase involved not only in insulin signaling but also in cellular mechanisms of skeletal muscle function maintenance. Moreover, we would clarified this Metf molecular role on skeletal muscle using an immortalized model of satellite cells, C2C12 cells line. Immunofluorescence and Western Blot analysis revealed that Metf does not modify the C2C12 proliferation capacity, while positively influenced the differentiation process and the myotube maturation. Together, our novel results suggest that Metf may have a positive action not only on the promotion of healthy aging but also on the prevention of sedentariness damages.

**HEALTH CARE DELIVERY—ECONOMICS**

**140-LB**

**Hospitalization Costs, Resource Utilization, and Clinical Outcome in Patients Undergoing CAGB Receiving Intensive vs. Conservative Glucose Control**

**DAWN SMILEY, SAUMETH CARDONA, JEFF WEAVER, KENYON REGISTER, LIMIN PENG, FRANCISCO FASQUEL, GUILLERMO E. UMPIERREZ, Atlanta, GA**

The GLUCO-CABG trial reported that intensive control (IC) targeting a BG of 100-140 mg/dl in the ICU vs. conservative control (CC) targeting BG of 141-180 mg/dl did not reduce a composite of hospital complications including wound infection, pneumonia, acute respiratory or renal failure, major cardiovascular events, bacteremia and death (42% vs. 52%, p=0.08) in hyperglycemic patients undergoing CAGB surgery. The financial impact of this intervention, however, is unknown. Accordingly, we conducted a post-hoc, cost analysis to compare hospitalization costs using 2011-2013 cost-charge ratios from Centers for Medicare & Medicaid Services, as well as resource utilization and hospital complications in CAGB patients receiving IC vs. CC. A total of 288 of 302 patients (IC: 144, CC: 144) had financial data for analysis. The mean age was 64.2±9.5 with 50% prevalence of diabetes in each group. Median total hospitalization costs in the IC group were lower at $39.4K compared to $42.2K in the CC group (p=0.043), with a median cost savings of $2,699 (95% CI: $557-6,750). Median resource utilization, expressed as instances, was higher in the IC group for radiology (20 vs. 15, p=0.001), laboratory (248 vs. 213, p=0.018), consult service (14 vs. 9, p=0.017), and ICU use (3 vs. 2, p=0.013) which resulted in higher median total resource costs compared to the IC group ($18.9K vs. $14.2K, p=0.006). The CC group had more complications (52% vs. 42%, p=0.078) compared to the IC group. A multivariate analysis adjusted for

**ADA-Funded Research**

For author disclosure information, see page LB91.
treatment group, DM status and complications suggested that the observed cost benefit of IC is primarily due to the reduced complication rate.

In summary, intensive glucose control compared to conservative control in ICU patients who have undergone CABG procedures is associated with fewer complications and this in turn results in significantly lower hospitalization costs and resource utilization.

Supported By: ADA (7-07-CT-56); Sanofi; Glytec, LLC.

### 141-LB

**Comparative Effectiveness of Patient Participation Training vs. Diabetes Education in Low Socioeconomic Status Patients with Type 2 Diabetes: A Pragmatic Randomized Trial of Coached Care**

**SHEROLD GREENFIELD, JOHN BILLMEK, DARA H. SORKIN, SHERRIE H. KAPLAN, Irvine, CA**

We compared the impact on glycemic control of two community health worker (CHW) interventions: Coached Care, where the CHW teaches patients skills to participate more actively in their care, versus Diabetes Education, where the CHW presents information about diabetes but no training on participation skills.

An ethnically diverse, low-income sample of type 2 diabetes patients with HbA1c >7.5% was recruited. Participants (N=595) were randomized to either Coached Care or Diabetes Education. In both arms, the CHW met the patients at the clinic before every diabetes-related medical visit during the study period to conduct a 20-minute session. Change in HbA1c from baseline to one-year follow-up was estimated using a linear mixed model adjusting for age, sex, race and education.

Reduction in HbA1c was greater in patients randomized to Coached Care (-0.43% 95% CI -0.59, -0.28; p<0.0001) versus Diabetes Education (-0.10% 95% CI -0.28, 0.08; p=0.27), in spite of similar intensity of medication therapy.

CHWs teaching patient participation skills improved glycemic control in this diverse, low-income sample.

**Figure 1.** Reduction in hemoglobin A1c from baseline to one year follow-up. Results are estimated means (error bars represent 95% confidence intervals from linear mixed-effects models, adjusted for age, sex, race/ethnicity and education. Two for condition by time interaction shows that the source of the change in HbA1c from baseline to 12 months differs between the Diabetes Education and Coached Care groups (p=0.009).

### 142-LB

**Costs of Diabetes in the U.S.: 1996-2030**

**HONGYU CHEN, MAN LI, VENKAT, LISA S. ROTENSTEIN, JESSICA P. DOING, NINA RAN, MARK YARCHOAN, RICHARD KAHN, KELLY L. CLOSE, San Francisco, CA, Boston, MA, Philadelphia, PA**

Diabetes is responsible for substantial healthcare expenditure in the U.S., and prevalence continues to rise. More robust data on the past and future costs of diabetes are needed to inform public health policy and influence cost management strategies.

The purpose of this study was to assess U.S. healthcare costs directly attributable to diabetes from 1996 to 2010, and to forecast future cost trends through 2030. Expanding upon the strong methodology of the ADA's five-year cost-of-illness study, we calculated more granular cost data for every year from 1996 through 2010, drawing from the most robust longitudinal data sources available. We used this data to forecast future costs of diabetes through 2030.

Our analysis showed that the total annual healthcare costs directly attributable to diabetes in the U.S. rose from $64 billion in 1996 to $167 billion in 2010; we project costs to reach $494 billion by 2030. Broken down by components, we found that inpatient hospitalization declined from 58% of all costs in 1996 to 46% in 2010; we project a further decrease to 36% in 2030. The fastest growing cost segments were non-insulin prescription medications (7% in 1996, 16% in 2010, and a projected 26% in 2030) and diabetes supplies (3% in 1996, 10% in 2010, and a projected 12% in 2030). To explore the effects of diabetes prevention, we modeled the impact of a 1%, 5%, and 10% reduction in annual diabetes incidence from 2010 through 2030. Such reductions would save a cumulative projected total of $87 billion, $427 billion, and $788 billion, respectively, during that time period.

We conclude that, based on historical trends, the future costs attributable to diabetes in the U.S. will climb significantly, to levels greater than those projected by existing literature. Stemming this rise will likely require more successful diabetes prevention, as the total costs of diabetes are proportional to the size of the affected population. The dataset developed in this analysis opens exciting opportunities to study costs segmented by population demographics, complications, and care setting.

**143-LB**

**The Synergy to Control Emergency Department Hyperglycemia in Type 2 Diabetes Project: STEP-Diabetes**

**MICHELLE F. MAGEE, CARINE M. NASSAR, MIHRIYE METE, JEFFREY S. DUBIN, Washington, DC**

We assessed the impact of an intervention focused on glycemic management for adults with type 2 diabetes (T2DM) presenting to the emergency department (ED) with uncontrolled hyperglycemia.

A 4 week randomized controlled trial provided algorithm-based antihyperglycemic medications management; survival skills diabetes self-management education (DSME); and navigation to primary care for adults presenting to the ED with BG>200mg/dL. Medications were titrated and DSME content delivered by endocrinologist-supervised certified diabetes educators at each visit. Controls received standard ED care.

One hundred and one patients were consented (96%, Black, 62.3% Medicaid and/or Medicare insurance, and no prior DSME (65.4%)). Seventy-eight (77.2%) completed the week 4 visit.

In both the intervention (INT) and control (CON) groups mean BG decreased (403±132 to 192±93 mg/dL and 412±120 to 259±124mg/dL, respectively), p<0.001. Post-BG was significantly lower for the INT, p<0.01. A1C went down by 65% of INT and 29% of CON subjects, p=0.002. Hypoglycemia rates between groups did not differ and no severe hypoglycemia was reported.

Modified Morrisy Medication Adherence Scale: total scores decreased from 3.2±2.0 (low adherence) pre- to 1.4±1.4 (medium adherence) at 4 weeks (p<0.001) for the intervention and were sustained to 12 weeks. Improvement was greater for the intervention (-2.0±2.5 vs. -0.3±2.43), p=0.001.

The STEP-Diabetes study results demonstrate that ED visits made by adults with uncontrolled T2DM can be used to initiate a focused intervention providing timely titration of antihyperglycemic medications and survival skills DSME to improve medication adherence and short-term glycemic outcomes, without increasing risk for hypoglycemia.

Supported By: ADA (7-11-CT-23).

### 144-LB

**WITHDRAWN**

For author disclosure information, see page LB91.
PEDIATRICS—OBESITY AND TYPE 2 DIABETES

145-LB
Bridging Income Generation through Provision of Incentives for Care (BIGPIC)
SONAK PASTAKIA, SIMON MANVARY, JEMIMA H. KAMANO, DIANA MENYA, BENJAMIN ANDAMAA, JEREMIAH LAKTABA, Eldoret, Kenya, Webuye, Kenya

In resource-constrained settings, chronic diseases have been neglected leaving patients with limited prospects for a healthy life. Through the BIGPIC program we have piloted a holistic approach which directly addresses socioeconomic barriers while encouraging positive health seeking behaviours. This pilot project will establish whether the provision of group based healthcare combined with microfinance leads to improved chronic disease control and access for resource-constrained patients in rural western Kenya. Screen positive patients form community based microfinance groups where they receive portable care and are trained on various aspects of diabetes and hypertension self-care. Patients are required to pay subsidized user fees for all services and medications. The distinct groups are then assessed and incentivized based on their utilization of services and clinical outcomes.

917 individuals were screened for diabetes and hypertension of which 170 (18.5%) were screen positive with 147 for hypertension and 23 with diabetes and/or hypertension. 112 (65.9%) returned for confirmatory diagnosis, with 85 (81%) of those patients being confirmed positive and subsequently forming microfinance groups. After six months, 69 (65.7%) of the patients were retained in care with the overall group demonstrating a 12mmHg decline in systolic blood pressure and patients with diabetes having a 1 point reduction in HbA1C. Through the groups’ microfinance activities, they were able to generate a cumulative savings of $3,690 with an accrued interest of $1065 after six months. This approach demonstrated statistically improved linkage (65.9% compared to 20%, P<0.01) and retention (65.7% compared to 21%, P<0.01) compared to the standard of care in the public sector of Kenya.

By linking provision of health to microfinancing groups, we have been able to sustainably improve traditional elements of health and assist the population with economic opportunities to break the poverty cycle.

Supported By: Purdue University

PEDIATRICS—OBESITY AND TYPE 2 DIABETES

146-LB
Metabolic Changes in Severely Obese Adolescents Eight Years after Gastric Bypass
THOMAS INGE, TODD JENKINS, TAWNY W. BOYCE, SHELLEY KIRK, JESSICA WOO, ROBERT SIEGEL, STAVRA XANTHAKOS, MICHAEL HELMARATH, LAWRENCE M. DOLAN, Cincinnati, OH

Background: Severe adolescent obesity is associated with marked metabolic dysfunction. Little is known about long-term metabolic outcomes after roux en Y gastric bypass (RYGB) performed for adolescent severe obesity. The follow-up of Adolescent Bariatric Surgery-5+ (FABS-5+) assessed BMI and metabolic variables >5 yrs postoperatively.

Methods: Adolescents and young adults who underwent laparoscopic RYG from 2001-2007 were targeted for follow-up between 2011-2013. Baseline (pre-surgery) data were abstracted from charts. Patients were re-located to participate in a standardized research visit including a fasting blood draw. Body mass index (BMI) and biochemical changes were evaluated using Wilcoxon signed rank sum tests, McNemar’s test and Bowker’s test of symmetry.

Results: 80% of all subjects eligible for FABS-5+ were enrolled. The cohort (n=46) for this analysis included 32 females (70%), 39 Caucasians (85%) and 1 Hispanic (2%). Mean interval from surgery was 7.9 yrs. BMI declined by 33%, plasma insulin by 83%, and fasting glucose (FG) by 18% (all P<0.01, Table). The proportion with normal FG (<100mg/dL) increased significantly from 59% at baseline to 93%. Diabetes remitted in 7 of 8 subjects with no incident cases.

Conclusion: In severely obese adolescents with metabolic dysfunction, these data strongly suggest that RYGB is associated with major, sustained weight loss and marked improvement in glucose homeostasis.

Baseline and follow-up Metabolic Characteristics.

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Follow-Up</th>
<th>Paired p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age ± SD (range), yrs</td>
<td>17.5 ± 1.9 (14.0, 21.4)</td>
<td>25.2 ± 2.4 (20.7, 29.9)</td>
</tr>
<tr>
<td>Median BMI (IQR), kg/m2</td>
<td>37.2 (26.5, 48.2)</td>
<td>39.7 ± 4.9 (27.3, 51.5)</td>
</tr>
<tr>
<td>Median Insulin (IQR), uUI/mL</td>
<td>34.4 (28.2, 48.4)</td>
<td>45.1 (37.4, 56.3)</td>
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<tr>
<td>Median Glucose (IQR), mg/dL</td>
<td>90.8 (80.0, 100.0)</td>
<td>79.7 (75.0, 90.0)</td>
</tr>
<tr>
<td>Diabetes†, n (%)</td>
<td>8 (17%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Fasting Glucose</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(1) 0-126 mg/dL, n(%)</td>
<td>23 (59%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>(2) 126-199 mg/dL, n(%)</td>
<td>13 (33%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>(3) ≥ 200 mg/dL, n(%)</td>
<td>3 (8%)</td>
<td>0 (0%)</td>
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† Provider Dx or (HgbA1c ≥ 6.5 or Fasting Glucose ≥ 126mg/dL) or Meds for Diabetes.

Supported By: Ethicon Endo-Surgery, Inc.

147-LB
Examining Family Planning Vigilant Behavior in Adolescent Females with Type 2 Diabetes
JESSICA J. CHOI, NEIL H. WHITE, SUSAN SHEREKA, PATRICIA SCHMITT, DENISE CHARRON-PROCHOWNIK, Pittsburgh, PA, St. Louis, MO

Unplanned pregnancies in teenagers, especially with diabetes, could cause severe maternal and fetal complications. Preconception Counseling (PC) provides family planning information to prevent unplanned pregnancies. ADA recommends PC beginning at puberty. Adolescent females with type 2 diabetes (T2D) avoiding pregnancies should be vigilant in using effective family planning. This study reports levels of vigilance with family planning behaviors (e.g., consistent use of birth control (BC), abstinence, and seeking family planning advice/information from health care professionals (HCP)) in adolescent females with T2D. A subsample of 112 female subjects from the TODAY Study cohort completed a reproductive health questionnaire. The questionnaire measured reproductive health and diabetes knowledge, intentions, and behaviors. At baseline (of the TODAY Study), subjects had a mean age of 14.0±2.0 yrs and only 19.6% were non-Hispanic white. During the study, 62% had ever been sexually active, with a mean age of sexual debut of 16.5 yrs (range 12-22 yrs). Of these, 97% had used some form of BC, but only 31% were vigilant about using BC every time they had sex while they were not planning a pregnancy. Only 21% intended to be abstinent in the future. Although 74% of the teens reported having gotten information from their HCP about the importance of planning a pregnancy with diabetes, only 39% intended to get preconception counseling for planning future pregnancies, and only 29% ever actually discussed diabetes and birth control with their HCP. With regards to knowledge of family planning vigilance, 23% did not know that a condom is a form of BC, and 22% believed that women with diabetes have very limited choices of BC. Deficiencies were noted in family planning vigilant behaviors; these deficiencies could lead to unplanned pregnancies. Adolescents with diabetes could benefit from PC starting at puberty and booster sessions at routine diabetes clinic visits.

Supported By: NIH

148-LB
Obese Adolescents with T2DM Have a More Atherogenic Lipoprotein Pattern at a Given Insulin Sensitivity Compared to Those with Insulin Resistance or Prediabetes
SHELA N. MAGGE, RAY C. BOSTON, JUSTINE SHULTS, NICOLAS STEITTLER, LORRAIN KATZ, DANIEL J. RADER, Philadelphia, PA, Falls Church, VA

Obesity and T2DM are risk factors for metabolic dyslipidemia, but it is unknown if T2DM increases dyslipidemia risk beyond obesity and IR. We compared 5 pubertal adolescent groups: lean controls n=42, and obese insulin sensitive (OIS) n=44; insulin resistant (OIR= fasting insulin=20U/Ml) n=36, pre-diabetic (OPreT2 by OGTT) n=23, diabetic (OT2) n=11. NMR lipoproteins and FIVGT (in obese) were measured. Linear regression measured associations between Si (Minmod) and lipoproteins, with further inclusion of group by lipoprotein interactions. Lincan in Stata identified associations differing between OT2, and OIR or OPreT2.

For author disclosure information, see page LB91.
Higher Skin Autofluorescence in Youth with Type 1 Diabetic Retinopathy

YOON HI CHO, MARIA E. CRAIG, ANDRZEJ S. JANUSZEWSKI, STEPHEN HING, ALCIA J. JENNINGS, KIM C. DONAGHUE, Westmead, Australia, Sydney, Australia

Skin autofluorescence (AF) provides a non-invasive measure of accumulation of advanced glycation end-products (AGEs) in skin collagen. Skin AF is associated with vascular complications in older adults with diabetes, independent of HbA1c.

Our aims were: (1) to compare accumulation of skin AGES, as measured by skin AF, in young people with type 1 diabetes (T1D) vs. controls, and (2) the association between skin AF and retinopathy in T1D.

Skin AF was measured as a mean of 6 readings at the forearm using the DiagnOptics AGE-Reader in 78 youth with T1D (mean age 17.4 yrs ±3.8, duration 10.1 yrs ± 4.0, HbA1c 8.9% ±1.6; 73mmol/L) and 70 age-matched controls (mean 17.8 yrs ±6.0). Retinopathy was assessed using 7-field stereoscopic fundal photography and graded using Modified Airlie House Criteria, defined as ≥1 in any eye.

Age-adjusted mean skin AF was higher in diabetes vs. controls (1.43 ±0.04 vs. 1.22 ±0.04, p<0.001). Retinopathy was seen in 22% of diabetic patients. Age-adjusted mean skin AF was higher in retinopathy-free diabetes vs. controls (p<0.05) and tended to be higher in diabetes with retinopathy vs. retinopathy-free (p<0.08). ROC analysis showed skin AF as a strong screening tool for presence of retinopathy (AUC 0.78, p=0.001). Skin AF was associated with older age (p= 0.08, 95% CI 0.04-0.08; p<0.001) and higher HbA1c (0.1, 0.04-0.15; p=0.001), or longer duration (0.04, 0.02-0.06; p=0.002) and higher HbA1c (0.1, 0.04-0.18; p=0.02). Highest quartile skin AF (≥1.62) was associated with retinopathy (6.3, 1.9-20.5, p=0.003), which remained significant after adjusting for HbA1c (4.3, 1.2-15.3; p=0.03).

Accumulation of skin AGES in youth with diabetes is associated with retinopathy in cross sectional analysis. Longitudinal studies will determine the utility of skin AF as a non-invasive screening tool to predict future retinopathy risk and potentially provide a measure of “metabolic memory” in diabetes complications, which cannot be accurately measured by serial HbA1c alone.

Supported By: NIH (K23PA05143, UL1RR024134)
152-LB
Protective Effect of Sulforaphane on Type 1 Diabetes-induced Testicular Apoptosis Is Associated with Upregulation of Nrf2 Expression and Function
JIANG XIN, YANG BAI, YING XIN, LU CAI, Changshun, China, Louisville, KY
Infertility is a common complication in diabetic men, mainly due to the loss of germ cells by apoptotic cell death. Diabetes-induced testicular apoptosis is predominantly due to increased oxidative stress. The nuclear factor-erythroid 2-related factor 2 (Nrf2), as a master transcription factor in controlling anti-oxidative systems, is able to be induced by sulforaphane (SFN). To examine whether SFN could prevent testicular apoptosis through up-regulation of Nrf2, type 1 diabetic mouse model was set up with multiple intraperitoneal injections of low-dose streptozotocin. Diabetic and age-matched control mice were treated with or without SFN at 0.5 mg/kg daily in five day of each week for 3 months and then kept until 6 months. At 3 and 6 months of diabetes, testicular apoptosis, fibrosis, inflammation, and oxidative damage were assessed by Western blot, real-time qPCR, and histopathological examination. Diabetes significantly induced testicular apoptosis that was associated with ER-stress and mitochondrial cell death pathways, shown by increased expression of CHOP, cleaved caspase-12, Bax to Bcl2 ratio and cleaved caspase-3. Diabetes also significantly increased testicular oxidative damage (3-NT and 4-HNE), inflammation (ICAM and PAI-1) and fibrosis (TFG-1b and CTGF), as well as decreased the germ cell proliferation (PCNA). All these diabetes-induced testicular damages were significantly prevented by 3-month SFN treatment that up-regulated Nrf2 function, reflected by increased Nrf2 phosphorylation and its downstream antioxidants (Catalase, HO-1 and NQO1) at mRNA and protein level. These results suggest that SFN is able to prevent testicular oxidative damage and apoptosis in type 1 diabetes, which was associated with the up-regulated Nrf2 expression and transcription function.
Supported By: ADA (1-11-BS-17); NSFC (81201218)

153-LB
Does Frequent, Extended Use of an Automated Bolus Advisor Reduce Hypoglycemia in Pediatric Patients Treated with Insulin Pump Therapy? First Results of the BABE Study
RALPH ZIEGLER, CHRISTEN REES, NEHLE JACOBS, CHRISTOPHER PARKIN, MAUREEN R. LYDEN, BETTINA PETERSEN, ROBIN S. WAGNER, Münster, Germany, Indianapolis, IN, Boulder City, NV, Tampa, FL, Mannheim, Germany
The Bolus Advisor Benefit Evaluation (BABE) study was a single-center, prospective cohort study that assessed the impact of frequent use of the Accu-Chek Aviva Combo system bolus advisor (BA) feature on glycemic control among 104 pediatric type 1 diabetes patients on insulin pumps treated at a pediatric diabetology clinic in Germany. At 6 months, frequent use of an automated bolus advisor was associated with significant improvements in glycemic control with no increase in hypoglycemia. We further assessed the impact of frequent BA use at 12 and 24 months in a consistent cohort of 40 study patients (mean (SD) baseline: HbA1c 7.6 (1.0)%, age 13.4 (4.3) years, diabetes duration 47.2 (40.4) months, and 57.5% female): 28 high frequency (HF) users (≥50%); 12 low frequency (LF) users (<50%). ANCOVA controlled for baseline differences in HbA1c, diabetes duration and age. Clinically significant between-group differences in HbA1c were sustained in type 1 diabetes, which was associated with the up-regulated Nrf2 expression and transcription function.

154-LB
Correlation of Continuous Glucose Monitoring Profiles with Pregnancy Outcomes in Non-Diabetic Women
JOYCE SUNG, ELIZABETH KIGUT, HENRY LEE, KASRA NAVABI, MARK TASLIMI, YASSER EL-SAYED, Aurora, CO, Stanford, CA, Albuquerque, NM
We wished to determine whether hyperglycemic excursions detected by continuous glucose monitoring (CGM) correlate with birth weight percentile and other pregnancy outcomes, and whether CGM correlates better with these outcomes than a single glucose value from a 1-hour glucose challenge test (GCT).
This was a prospective observational study of 55 pregnant women without pre-existing diabetes, who wore a CGM device for up to 7 days, between 24-28 weeks’ gestation. The area under the curve (AUC) of hyperglycemic excursions above various thresholds (110, 120, 130, 140, and 180 mg/dL) was calculated. These AUC values, and results from a standard 50-g glucose challenge test (GCT), were correlated with our primary outcome of birth weight percentile, and secondary outcomes of unplanned operative delivery, pregnancy complications, delivery complications, fetal complications, and neonatal complications. A consistent correlation was seen between all AUC thresholds and birth weight percentile (r=0.29, p=0.05 for AUC-110, -120, -130, and -140; r=0.25, p=0.07 for AUC-180). This correlation was stronger than that of 1-hour oral GCT (r=-0.02, p=0.68). There was no association between AUC values and other outcomes.
In conclusion, among non-diabetic pregnant patients, hyperglycemic excursions detected by CGM show a stronger correlation to birth weight percentile than blood glucose values obtained 1-hour after a 50-g oral GCT.
Supported By: Dexcom, Inc.

155-LB
WITHDRAWN

156-LB
Identifying Japanese Americans at Risk for Prevalent or Incident Type 2 Diabetes by BMI, Waist, or Intra-Abdominal Fat
YUKIKO ONISHI, TOMOSHIRO HAYASHI, KYOKO K. SATO, MARGUERITE J. MONETT, DONNA L. LEONETTI, STEVEN E. KAHN, EDWARD J. BOYKO, WILFRED Y. FUMIOMOTO, Tokyo, Japan, Osaka, Japan, Seattle, WA
To determine the optimal approach using body anthropometrics to identify Japanese Americans at risk for prevalent or incident type 2 diabetes (T2D), we performed receiver operating characteristic (ROC) curve analysis using BMI, waist circumference (WC) and intra-abdominal fat area (IAFA) by computed tomography. Of 658 Japanese Americans, 139 had prevalent T2D. Of those without T2D at baseline, 100 out of 426 followed for 10-11 years developed T2D, diagnosed from a 75-g oral glucose tolerance test (1997 ADA criteria). For prevalent T2D, area under the ROC curve (AUROC) was: IAF 0.745 (95% CI 0.699-0.790) [men (m) 0.699 (0.628-0.752), women (w) 0.798 (0.726-0.869)]; WC 0.668 (0.618-0.717) [m 0.652 (0.555-0.871), w 0.711 (0.641-0.782)]; and BMI 0.644 (0.594-0.694) [m 0.618 (0.550-0.786), w 0.659 (0.580-0.736)]. For incident T2D, AUROC was: IAF 0.706 (0.648-0.763) [m 0.698 (0.619-0.791), w 0.724 (0.639-0.808)]; WC 0.655 (0.594-0.715) [m 0.651 (0.564-0.738), w 0.673 (0.591-0.755)]; and BMI 0.625 (0.560-0.690) [m 0.666 (0.577-0.755), w 0.600 (0.530-0.670)].

For author disclosure information, see page LB91.

XIANCHAO XIAO, CHENGLIN SUN, YUIJA LIU, SUYAN TIAN, ZHONGHUA SUN, YING GAO, YAZHEN LI, JIE CHENG, YOO LV, MII LI, ZHUO LI, YUMIN ZHANG, GANG WANG, YANG LIU, YUAN GAO, LIWEN ZHU, YAN LIU, BUDUO WANG, Changshu, China

Objective: To evaluate the sensitivity and specificity of fasting plasma glucose (FPG), 2-h post-load plasma glucose (2h-PGG), and glycated hemoglobin (HbA1c) measurements in the screening of diabetes and prediabetes in a Chinese population, and to determine the cutoff point of HbA1c in the diagnosis of diabetes and prediabetes in a Chinese population.

Research Design and Methods: A total of 7,611 individuals aged over 40 years who did not have a prior history of diabetes were randomly selected in the Changshu area. For each subject, a questionnaire was completed and a physical examination and an oral glucose tolerance test were performed. For data analysis, FPG, 2h-PGG, and HbA1c values were compared by area under the receiver operating characteristic (ROC) curves. The sensitivity, specificity, and Youden index for different measurements were also compared by statistical analysis. Results: The prevalence of newly diagnosed diabetes and prediabetes was 12.71% and 29.39%, respectively. For subjects with newly diagnosed diabetes, the area under the ROC curve was 0.8368 for FPG, 0.9330 for 2h-PGG, and 0.8064 for HbA1c; whereas for prediabetes, these values were 0.8022, 0.9288, and 0.6895, respectively. The sensitivity and specificity for 2h-PGG were the highest among all three indices. Conclusions: As a screening tool for diabetes and prediabetes, the 2h-PGG measurement demonstrated the highest sensitivity and specificity; thus, it is the optimal method for a Chinese population. In addition, HbA1c at 6.3% (45 mmol/mol) and 5.8-6.2% (40-44 mmol/mol) were the optimal cutoffs for the diagnosis of diabetes and prediabetes, respectively.

Supported By: Chinese Medical Association (3D512W993428); Science Technology Department of Jilin Province (20512J593428)

A Novel Testing Model for Screening of Prediabetes and Diabetes among U.S. Adults

LIWEI CHEN, YURONG ZHANG, LU ZHANG, RACHEL MAYO, GANG HU, Clemson, SC; Xi’an, China; Metairie, LA; Baton Rouge, LA

Historically, routine screening for diabetes in primary practice is challenging, largely for cost and time-consuming considerations. Hemoglobin A1c (HbA1c) is an attractive diagnostic test for diabetes because it is quick and does not require fasting. However, the sensitivity of using HbA1c alone is unsatisfactory low. The objective of this study was to evaluate whether a new model combining a diabetes risk score and HbA1c would be an acceptable tool in screening prediabetes and undiagnosed diabetes in general populations. This cross-sectional analysis included 3,896 adults (age ≥ 20 years) from the National Health and Nutrition Examination Survey who attended the morning sessions and had an OGTT. The Finnish Diabetes Risk Score (FINDRISC) was selected because it is simple, non-invasive, and has been validated in the U.S. population in our previous study. The FINDRISC score was developed based on 8 variables (age, BMI, waist circumference, use of antihypertensive drug, history high blood glucose, family history of diabetes, daily physical activity and fruit & vegetable intake). The crude prevalence of diabetes was 7.0% for undiagnosed diabetes and 43.1% for prediabetes (27.7% for isolated impaired fasting glucose (IFG), isolated 5.1% for impaired glucose tolerance (IGT), and 10.3% for having both IFG and IGT). The sensitivity and specificity of using the HbA1c alone was 24.2% and 99.6% for diabetes (cutoff: ≥6.5%), and 35.2% and 89.4% for prediabetes (cutoff: ≥5.7%). The sensitivity and specificity of using the FINDRISC alone (cutoff: ≥9) was 79.1% and 48.6% for diabetes and 60.2% and 61.4% for prediabetes. Using the simultaneous testing model with a combination of FINDRISC and HbA1c improved the sensitivity to 88.2% for diabetes and 74.2% for prediabetes. This simultaneous testing model is a practical and valid tool in diabetes screening in the general U.S. population and further study is warranted to evaluate the cost-effectiveness of this screening model in primary practice.

Increased Hemoglobin Concentration Is Associated with Future Development of Diabetes: The Insulin Resistance Atherosclerosis Study (IRAS)

CARLOS LORENZO, ANTHONY J. HANLEY, STEVEN M. HAFFNER, San Antonio, TX, Toronto, ON, Canada

For each chronic kidney disease stage, diabetes is associated with a 1 g/dl decrease in hemoglobin (Hb) concentration. Since Hb concentration tends to be lower in inflammatory conditions, we hypothesized that lower Hb concentration may precede the development of diabetes. We examined this issue in 888 non-diabetic participants in the IRAS. We assessed diabetes status by oral glucose tolerance test at baseline and after a 5-year follow-up period, and insulin sensitivity (S) and acute insulin response (AIR) by the frequently sampled intravenous glucose tolerance test. After controlling for age, sex, ethnicity, and clinic, Hb concentration was inversely related to log-transformed S (r = -0.11, p = 0.002), but was not related to S-adjusted log-transformed AIR (r = 0.02, p = 0.622). Participants in the upper tertile of Hb concentration had greater odds of developing diabetes than those in the lower tertile (Table). Sex did not have an interaction effect on the relationship between Hb concentration and incident diabetes. In summary, higher rather than lower Hb concentration is associated with more insulin resistance. Higher Hb concentration may also precede the development of diabetes.

<table>
<thead>
<tr>
<th>Table. Odds of Developing Incident Diabetes by Hb Tertiles.</th>
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<td>Participants, Adjustment model</td>
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<td>In men</td>
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<td>All</td>
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<tr>
<td>All + BMI</td>
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<td>All + HbA1c, IGT, and AIR</td>
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Supported By: NHLBI (HL-47887)

Discontinuation of Oral Antihyperglycemic Agents among Diabetes Patients

KRYSTI REYNOLDS, JAEJIN AN, TERESA N. HARRISON, RONG WEI, JUN WU, CATHERINE S. WLODARCZYK, JOHN MARTIN, SWAPNIL N. RAJPATHAK, Pasadena, CA, Downey, CA, Whitehouse Station, NJ, Los Angeles, CA

Oral antihyperglycemic agents (OHAs) are commonly prescribed in the treatment for type 2 diabetes mellitus. Many studies have examined medication adherence to OHA therapy, but few studies have examined OHA discontinuation rates in clinical practice. Using electronic health record data from a large, integrated healthcare delivery system in the U.S., we estimated rates of OHA discontinuation and examined factors associated with OHA discontinuation among patients with diabetes on dual therapy. We identified adult patients aged ≥ 18 years with a diagnosis of type 2 diabetes who initiated dual therapy (dispensed 2 different classes of OHAs) between 1/1/2005 and 6/30/2010. The index date was defined as the date of initiation of the second OHA. Patients using insulin, those on 3 or more classes of OHAs, and those who died or left the health plan during a 3 year follow-up were excluded. Discontinuation was defined as a gap of ≥1.5 times the last days’ supply without subsequent reinstitution during follow-up. Multivariable log-binomial regression models were used to investigate factors associated with OHA discontinuation. Among 28,458 eligible patients with diabetes (mean ± SD age: 58±12 years, 44% female, 34% white, 36% Hispanic, 12% black, 12% Asian/Pacific Islander, 6% other), 38.7% discontinued one (26.4%) or both (12.3%) of their OHAs. The mean ± SD time to discontinuation was 573.8±368.5 days (median time, 625 days). Patients who discontinued their OHA were more likely to be female, younger, black or of Hispanic ethnicity, have a higher Charlson co-morbidity index, higher medication co-pays, fewer concomitant medications, more likely to have started both OHAs at the same time, and to have higher health care utilization in the year before the index date. Discontinuation of OHAs is common among patients with diabetes and is associated with several patient factors. Future research should further examine the reasons for OHA discontinuation and evaluate the impact of discontinuation on health outcomes.
EPIDEMIOLOGY—DIABETES COMPLICATIONS

161-LB
Optimum BMI Cut-Points to Screen Asian Americans for Type 2 Diabetes
MARIA ROSARIO G. ARANETA, ALKA KANAYA, WILFRED FUMOTO, WILLIAM C. HSU, HEALANI CHANG, ANDREW GRANDINSETTI, EDWARD J. BOYKO, TOMOSHIGE HAYASHI, STEVEN E. KAHN, DONNA L. LEONETTI, MARGUERITE J. MCNEELY, YUKIKO ONISHI, KYOKO K. SATO, LA JOLLA, CA, SAN FRANCISCO, CA, SEATTLE, WA, BOSTON, MA, HONOLULU, HI, OSAKA, JAPAN, TOKYO, JAPAN

Lower BMI cutpoints have been suggested to identify Asian Americans (AA) for diabetes (DM) screening but few studies have evaluated BMI cut-points using sensitivity, specificity, and receiver operating characteristic (ROC) curve analysis. We used data from 1663 asymptomatic AA, ages 45+ years, without prior DM diagnosis. Participants were of South Asian, Filipino, Japanese, Chinese, Korean, or mixed Asian ancestry, without non-Asian admixture from the MASALA, UCSD Filipino, North Kohala and Seattle JACDS studies. Clinical measures included a 2-h 75g oral glucose tolerance test, BMI and HbA1C (except in Seattle). Mean age was 60 years, mean BMI was 25.4 kg/m², 58% were women, and the prevalence of undiagnosed DM (by ADA criteria) was 16.4%. At BMI≥25, sensitivity (63.7%) and specificity (52.8%) were most similar and area under the ROC curve was 0.583 (Table), but limiting screening at this BMI cut-point would miss 36.3% of AA with DM. For screening purposes, higher sensitivity is desirable to minimize missing cases, especially if the diagnostic test is relatively simple and inexpensive. The BMI≥23 kg/m² cut-point had a high sensitivity (84.7%) and would fail to identify only 15.3% of AAs with DM. Results were similar at age ≥35 (n=2042) or ≥40 years (n=1899). We conclude from these findings that the BMI cut-point for identifying AA who should be screened for undiagnosed DM should be lower than 25 and ≥23 may be the most practical.

Withdrawn:

162-LB

EPIDEMIOLOGY—DIABETES COMPLICATIONS

163-LB
Physical Activity, Sedentary Behavior, and All-Cause Mortality among Blacks and Whites with Diabetes
KIMBERLY R. GLENN, LOREN LIPWORTH, Nashville, TN

Previous studies of the relationship between physical activity (PA) and all-cause mortality (ACM) among individuals with diabetes were conducted primarily in white male populations.

We examined the association between PA and sedentary behavior and ACM risk in a racially diverse population of 15,945 low-income black and white men and women with diabetes from the Southern Community Cohort Study. Self-reported total PA and sedentary time (ST) were classified as metabolic equivalent tasks hours per day and total hours per day, respectively. Hazard ratios (HR) and 95% confidence intervals (95% CI) for ACM risk associated with total PA and total ST were estimated from multivariate Cox proportional hazards models. During follow-up (median 6.2 years, 2,370 participants died).

Overall, the multivariate ACM risk was 37% lower among participants in the highest quartile of PA compared to those in the lowest (HR 0.63 [95% CI 0.56-0.71]). ACM was significantly increased for participants in the highest quartile of ST compared to those in the lowest after adjusting for PA (HR 1.18 [95% CI 1.05-1.32]). Significant trends of decreasing ACM with rising PA and increasing ACM with rising ST were observed among both blacks and whites.

164-LB
Effect of Randomisation to Intensive Multifactorial Cardiovascular Treatment on Serum Methylglyoxal Levels: The Addition Trial
TROELS M. JENSEN, DANIEL R. WITTE, DORTE VISTISEN, THOMAS H. FLEMING, PETER P. NAVROTH, ANNELE SANDBAEK, TORSTEN LAURITZEN, MARIT E. JØRJENSEN, Gentofte, Denmark, Strassens, Luxembourg, Heidelberg, Germany, Aarhus, Denmark

Elevated methylglyoxal (MG) has been implicated in the development of micro- and macrovascular diabetic complications, but it remains unclear how current treatments for type 2 diabetes affect its circulating levels. In a secondary analysis of the Danish arm of the ADDITION trial, we examined the effect of intensive multifactorial treatment of people with screen-detected type 2 diabetes on serum levels of MG, compared to routine care. Serum MG was measured in baseline (n=1304) and 6-year follow-up (n=1153) samples. We observed a significant decrease in MG in both treatment arms, with no effect of allocation to intensive treatment. At baseline Mg was associated with current smoking and fasting glucose levels. In observational analyses of all patients adjusting for treatment allocation, a 1 mmol/L higher LDL cholesterol level at followup was associated with a 5.8% lower MG level (95% CI: -11.3; -1.0, p=0.03). No associations were observed between baseline risk factors and 6-year change in MG or between 6-year change in risk factors and change in MG. Patients receiving lipid lowering treatment at followup had higher MG, and those who initiated lipid lowering treatment during the trial period experienced a larger increase in MG (Table 1). No other treatment effects were observed. Our results suggest a potential interplay between MG, LDL cholesterol and lipid-lowering treatment.

Table 1. Treatment Status vs. Log-Methylglyoxal.

% difference 95% CI P
Any lipid-lowering treatment at baseline (n=863) vs. no treatment at baseline (n=1144) 3.0 [-8.4 to 18.1] 0.61
Any lipid-lowering treatment at followup (n=898) vs. no followup at followup (n=235) 28.4 [13.7 to 45.0] <0.001
Change in lipid-lowering treatment vs. change in MG during followup (reference group n=217) Treatment initiated (n=766) 15.8 [2.7 to 30.5] 0.02
Treatment continued (n=142) 9.6 [-6.4 to 28.3] 0.25
Change in glucose-lowering treatment vs. change in MG during followup (reference group n=498) Treatment initiated (n=825) 0.2 [-8.3 to 9.1] 0.96

[All analyses adjusted for age, sex, cluster randomisation, randomisation arm and treatment target]
Trends in Emergency Department Visit Rates for Hypoglycemia and Hyperglycemic Crisis among Adults with Diabetes, United States, 2006-2011

JING WANG, LINDA S. GEISS, DESMOND WILLIAMS, EDWARD W. GREGG, Atlanta, GA

Recent studies raised concern about the frequency of hypoglycemia in the diabetic population and the morbidity that may result. We examined the trends in emergency department (ED) visit rates for hypoglycemia and hyperglycemic crisis among adults with diabetes in United States from 2006 to 2011. Using the Nationwide Emergency Department Sample, visits for hyperglycemic crisis were determined by ICD-9-CM 250.1 or 250.2 and visits for hypoglycemia were determined via a validated algorithm among visits with diabetes identified by ICD-9-CM 250. We estimated the number of diabetic adults from the National Health Interview Survey. In 2011, ED visits for hypoglycemia and hyperglycemic crisis together comprised 3.6% of all visits by diabetic adults, declining from 4.7% in 2006.

Rates for hypoglycemia displayed a J-shape curve across age with the highest rates in persons aged ≥75 years (2.4 per 100 persons) while rates for hyperglycemic crisis presented an L shape with the highest rates among persons aged 65-74 years, 75+ years, and 45-64 years respectively (all p<0.05) but were unchanged for persons 18-44 years (p=0.2). Rates declined particularly among older adults while rates for hyperglycemic crisis remained stable overall and across all age groups (p>0.05 for all) with the exception of persons aged 65-74 years for whom rates increased 17% (from 0.18 (95% CI 0.16-0.19) per 100 persons in 2006 to 1.4 (95% CI 1.3-1.5) per 100 persons in 2011 (p<0.01). The rates decreased 33%, 22%, and 22% for persons aged 65-74 years, 75+ years, and 45-64 years respectively (all p<0.05) but were unchanged for persons 18-44 years (p=0.2).

In contrast to hypoglycemia, rates of hyperglycemic crisis remained stable overall and across all age groups (p>0.05 for all) with the exception of persons aged 65-74 years for whom rates increased 17% (from 0.18 (95% CI 0.16-0.19) per 100 persons in 2006 to 1.4 (95% CI 1.3-1.5) per 100 persons in 2011 (p<0.01). The rates decreased 33%, 22%, and 22% for persons aged 65-74 years, 75+ years, and 45-64 years respectively (all p<0.05) but were unchanged for persons 18-44 years (p=0.2).

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In contrast to hypoglycemia, rates of hyperglycemic crisis remained stable overall and across all age groups (p>0.05 for all) with the exception of persons aged 65-74 years for whom rates increased 17% (from 0.18 (95% CI 0.16-0.19) per 100 persons in 2006 to 1.4 (95% CI 1.3-1.5) per 100 persons in 2011 (p<0.01). The rates decreased 33%, 22%, and 22% for persons aged 65-74 years, 75+ years, and 45-64 years respectively (all p<0.05) but were unchanged for persons 18-44 years (p=0.2).
therapy in many patients (pts). Sitagliptin (SITA) and sulfonylureas (SU) are commonly prescribed after metformin as dual therapy in T2DM. This study assessed the time to insulin initiation among pts treated with MET+SITA vs. MET+SU.

This retrospective cohort study used a sample from the GE Centricity database. Included were pts with T2DM, ≥18 years (yrs), with continuous medical records. Index was the date of the 1st prescription of SITA or SU used as monotherapy or in combination with metabolic outcomes with variance components models. After adjustment for age, sex, percent body fat, and seasons, one standard deviation increase of 1-month average ambient air PM_{2.5} was associated with 4.9% decrease of insulin sensitivity (S) measured by FSIGT (p<0.001). Higher 1- and 12-month average PM_{2.5} were also associated with higher fasting glucose and insulin, HOME-IR, LDL, and lower HDL (all p<0.01). Higher freeway-related NOx was associated with higher fasting glucose and insulin, and lower acute insulin response (p=0.001, 0.001, and 0.043, respectively). Adjustment for each in the joint model including both ambient air PM_{2.5} and freeway-related NOx did not change the conclusion. Results were robust to further adjustment for weekly PA minutes and daily calorie intakes. We concluded that ambient air and traffic-related pollutants may adversely impact insulin and glucose homeostasis, and lipid profiles. Our findings suggest that ambient air and traffic pollution may play a role in T2D pathophysiology.

Supported By: NIH (DK-61628)

172-LB

Development of Diabetes Among the Body Phenotype in Korean Adults: The Korean Genome and Epidemiology Study

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Introduction: Longitudinal studies evaluating the relevance of metabolically healthy obese (MHO) or metabolically obese but normal weight (MONW) phenotype at risk for diabetes are few and results are contradictory. We aimed to investigate associations between combinations of body mass index (BMI) categories and metabolic syndrome and risk of the development of diabetes in Korean adults.

Methods: We studied 3,723 participants without diabetes, aged 40-69 years at baseline from the Korean Genome and Epidemiology Study. Participants were divided into four groups based on the BMI and metabolic syndrome: metabolically healthy normal weight (MHNW), MONW, MHO, and metabolically abnormal obese (MAO) subjects. Diabetes was diagnosed by 75g oral glucose tolerance test and medication history. The incidence of diabetes was identified by biennial health examinations during the 8-years of follow-up.

Results: The proportion of MHNW, MONW, MHO, and MAO subjects were 36.2, 19.7, 17.8, 26.4% of the baseline population. After 8 years, those were changed into 28.4, 29.0, 10.2 and 32.4%, respectively. The cumulative incidence of diabetes was 7.9%, 23.3%, 29.0% and 32.4% in MHNW, MONW, MHO, and MAO subjects. In age- and sex-adjusted time-dependent Cox proportional hazards models, the risk for diabetes was increased in MONW (hazard ratio 2.98 [95% confidence interval: 2.29-3.63]) and MAO (3.78 [3.05-4.69]), while it was not increased in MHO (1.37 [0.98-1.90]), compared with the MHNW subjects. In this population, the risk factors for the development of diabetes were systolic blood pressure (1.01 [1.00-1.02]), triglyceride (1.88 [1.57-2.26], and fasting glucose levels (1.96 [1.05-3.2]), but not BMI.

Conclusion: Metabolically unhealthy phenotypes were increased during the 8-years of follow-up, and those were more important risk factors for diabetes than obesity itself in Korea.

173-LB

A Systematic Review and Meta-analysis of the Association between Hyperglycemia and Surgical Site Infections

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Hyperglycemia is frequently hypothesized to be a risk factor for surgical site infection (SSI) in adults; however the magnitude of this effect is difficult to discern with an adequate sample size. Our objective was to conduct a systematic review and meta-analysis of the association between hyperglycemia and SSI. We conducted a systematic literature search of relevant articles published from December 1985 through April 2013. Articles were reviewed for eligibility and the most-adjusted estimate was abstracted. Only studies which assessed a threshold definition for hyperglycemia were included in the analysis. Summary estimates and predictive intervals were calculated by random-effects meta-analysis. Our initial search terms yielded 2,371 articles. All abstracts were screened per inclusion criteria and 98 articles were reviewed in depth. Twelve articles were eligible for analysis, encompassing 30,199 patients.

For author disclosure information, see page LB91.
174-LB
Cancer among T1D Patients: 8,800 Cases in 3.7 Million Person-Years of Follow-up in Four Countries
BENDICK CARSTENSEN, SOREN FRIS, JESSICA HARDING, DIANNA J. MAGLIANO, REJIO SUND, ILMO KESKIMAKI, RICKARD LJUNG, ANN-MARIE SVENSSON, SARAH WILD, SOFFIA GUBBÖRJUSSON, GENTOFTE, COPENHAGEN, DENMARK, MELBOURNE, AUSTRALIA, HELSINKI, FINLAND, STOCKHOLM, SWEDEN, GOTHENBURG, SWEDEN, EDINBURGH, UNITED KINGDOM
Diabetes (DM) patients carry an excess risk of cancer in the order of 20-25%; this risk mainly derived from follow-up of type 2 patients (T2D). The excess risk of cancer among type 1 (T1D) patients is described here as it is anticipated to be different from T2D. T1D patients from four countries with nationwide diabetes registers: Australia (1997-2008), Denmark (1995-2009), Finland (1972-2010), and Sweden (1987-2011) were followed for cancer occurrence. T1D was defined by diagnosis of DM before age 30. Cancer incidence rates were compared to population cancer incidence rates from national cancer registers. We used Poisson-models for rates, adjusting for age and date of follow-up, and date of birth. We estimated the overall rate ratio (RR) for all T1D patients and the effect of time since DM diagnosis. There were a total of 8,807 cancers among T1D patients during 3.7 million person-years of follow-up with median age at cancer diagnosis 51.1 (IQR: 43.5, 59.5). Overall, we found an RR of any type of cancer of 1.90 (95% CI: 1.89-1.90) among men and 1.05 (95% CI: 1.02-1.08) among women. The highest RRs were found for colorectal cancer (RR=1.13 (M), 1.14 (F), liver cancer (RR=2.14 (M), 1.50 (F), pancreatic cancer (RR=1.74 (M), 1.31 (F), endometrial cancer (RR=1.4 kidney cancer (RR=1.29 (M), 1.46 (F)), thyroid cancer (RR=1.29 (M), 1.46 (F)), all significant. We found a strong effect of diabetes duration, with an RR of 2.5 during the first year, decreasing to 1.2 (M) and 1.1 (F), after 2-5 years. Some of the observed excess risk may be explained by risk factors for cancer being more frequent in T1D patients (obesity), however this effect is presumably smaller than in T2D patients, and hence consistent with the smaller excess risk. The long-term RR (5-years of DM) is less than 1.2, which means that some small effect of exogenous insulin cannot be excluded, but the study is also consistent with an assumption of no such effect.
Supported By: EP3D

175-LB
Change in A1c One Year after Continuous Subcutaneous Insulin Infusion (CSII) Initiation in Adults with Type 1 Diabetes (TID): The Joslin (JDC) and Steno (SDC) Experience
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Adults with T1D often consider CSII to improve glycemic control. We performed a retrospective analysis of adults ≥18 years with T1D seen at 2 diabetes centers since 2002 to assess change in A1c (∆A1c) 1 year after CSII start. All JDC and SDC patients were analyzed for age, T1D duration, weight (kg), and A1c (%) before and 1 year after CSII initiation to identify independent variables explaining ∆A1c at 1 year. Data were available for 871 patients (271 JDC, 45% male; 620 SDC, 38% male). Mean age and T1D duration at CSII initiation were 46±15 years and 19±13 years (JDC) and 40±14 years and 22±13 years (SDC). Baseline A1c was 7.8±1.1% (JDC) and 8.4±1.6% (SDC). Overall, ∆A1c was -0.2% (JDC) and -0.6% (SDC). The table shows ∆A1c and weight change (∆Wt) according to baseline A1c. ∆A1c was inversely correlated with ∆Wt at SDC (r=-0.12, p<0.05), but not JDC. At JDC, higher baseline A1c (p<0.05) was the only independent predictor of ∆A1c at 1 year. At SDC, higher baseline A1c, older age, and female sex were independent predictors (p<0.05 for all of ∆A1c at 1 year. Both models explained 26% of the variability of ∆A1c. CSII initiation resulted in lower A1c, most notably in those with the highest baseline A1c; greater A1c reduction at SDC may reflect the higher baseline A1c observed in this group. Modest weight gain was observed in adults with A1c<9% prior to CSII initiation.

176-LB
Glycemic Control in Patients with Type 1 Diabetes: The Role of Insulin Pump
NOEMI GONZALEZ, LUCREZIA HERRANZ, ROSALIA SANCHEZ, CRISTINA GRANDE, NATALIA HILLMAN, BEATRIZ BARQUIEL, FELIPE PALLARDO, MADRID, SPAIN
To assess glycemic control in type 1 patients at a Diabetes Unit, evaluate the effectiveness of CSII, and examine the relationship of A1c to long-term diabetes. A1c and clinical data from patients in 2012 were collected from clinical reports. Adequate control was defined as A1c <7% and long-term diabetes > 10 years. Pump/non pump users were compared by Student’s t test and χ2 test. Predictive factors of adequate A1c were assessed by multiple logistic regression. Patients were further stratified according to diabetes duration. 833 patients were included. Clinical characteristics are shown in table 1.

177-LB
Differential Transcriptome Analysis of Diabetes Resistant and Sensitive Mouse Islets Reveals Significant Overlap with Human Diabetes Susceptibility Genes
DANIELA MATZKE, OLIVER KLUTH, ANNE KAMITZ, GUNNAR SCHULZE, MARKUS JÄHNERT, HANS-GEORG JOOST, ANNETTE SCHÜRMANN, POTSDAM, GERMANY, NUTHAL, GERMANY
Type 2 diabetes in humans and in obese mice is polygenic. However, the majority of genetic markers discovered in recent genome-wide association studies (GWAS) explain only a small fraction of the genetic impact to the disease. New Zealand Obese (NZO) mice are diabetes susceptible and show beta-cell failure, whereas obese mice on C57BL/6-background (B6-ob/ob) do not develop diabetes, because they are able to compensate by enhancing beta-cell proliferation. Our aim was to identify responsible genes mediating beta-cell failure in NZO mice and to validate human diabetes genes, which have been identified in GWAS. RNA-sequencing based transcriptome analysis of islets from NZO and B6-ob/ob mice that received a short glucose challenge identified 2146 differentially expressed genes. Pathway enrichment studies indicate major differences in cell cycle regulation (G1 to S transition), cell adhesion, cytoskeleton remodeling, and glutathione metabolism between NZO and B6-ob/ob islets.
and B6-ob/ob islets. Projection of differentially expressed genes to QT of a F2 (NZD×C57BL/6) population depicted 5 genes hypomorphic in B6 and 9 genes hypomorphic in NZO on Chr. 1, 12, 13, and 19. One gene exclusively expressed in NZO, the Interferon-activated gene 202b (Ifit202b), is located within the diabesity QTL Not3 on Chr.1. Overexpression of Ifit202b significantly inhibited cell proliferation in MING cells indicating that it participates in disability of Nzo islets to compensate. Alignment of the differentially expressed mouse genes to 108 human diabetes candidate genes revealed an overlap of 20 genes, including TCF2, GFBP2, CDKN2A, CDKN2B, GRB10 and PRC1, that can be linked to the regulation of the cell cycle. Our data provide a functional validation of human diabetes candidate genes including those involved in regulating islet cell recovery and proliferation and furthermore deliver additional candidates that might be involved in human beta-cell failure.

Supported By: German Center for Diabetes Research

178-LB
Meta-analysis of Birth Weight Genome-Wide Association Studies Identifies Two Novel Loci Extending Links between Early Growth and Adult Metabolic Diseases
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Lower birth weight (BW) is associated with increased risk of future type 2 diabetes (T2D) and cardiovascular diseases. Based on HapMap2 imputation, we previously reported 7 loci associated with BW, of which two (ADCA5, CDKAL1) have been implicated in T2D and one (ADRB1) in hypertension. Here we report reanalysis based on an increased sample size and imputation up to 20.8M SNPs from the more dense 1000 Genomes Project reference panels. Our aims were to: discover novel loci; detect BW associations involving low-frequency (LF) variants (MAF<5%) of larger effect sizes; and fine-map established and novel loci to identify potential causal variants by constructing credible sets of variants with 99% overall posterior probability of being causal.

For analysis, we considered 41,551 European singletons (17 studies) born at ≤37 weeks’ gestation with genome-wide association (GWA) and imputation data. Standardized sex-specific 2-scores of BW were tested for association with each SNP assuming an additive genetic model. Association summary data. Standardized sex-specific Z-scores of BW were tested for association with each SNP assuming an additive genetic model. Association summary data. Standardized sex-specific Z-scores of BW were tested for association with each SNP assuming an additive genetic model. Association summary data. Standardized sex-specific Z-scores of BW were tested for association with each SNP assuming an additive genetic model. Association summary data.

We detected two novel common variant loci at genome-wide significance: near MARF2 (p=3.1x10^-4) and SREBF2 (p=2.3x10^-4). MARF2 has been implicated in hyperlipidemia and SREBF2 is involved in cholesterol biosynthesis. There was no evidence for causal LF variants explaining common GWA study signals. The 99% credible sets defined by fine-mapping at known GWA study signals included fewer than 20 SNPs at 4 loci. At ADRB1, the credible set included just 5 variants, including CDCA20R.

Collectively, 4 of the 9 known and novel loci provide genetic links between BW and T2D, hypertension and hyperlipidemia, highlighting complex non-linear relationships between genetic variation, early growth and later metabolic disease including T2D.

179-LB
Regulators of Mendelian Disease Genes Are Enriched among T2D-associated Variants
JASON M. TORRES, KAANAN SHAH, NANCY J. CDX, CHICAGO, IL

Thousands of associations between Mendelian and complex diseases have been recently detected through extensive data mining of medical records from more than 110 million patients and constitute a “non-degenerate Mendelian code” (Blair et al., Cell, 2013). Given that common variants within Mendelian disease (MD) genes implicated by this code were found to be significantly associated with common diseases (Blair et al, 2013), we hypothesized that regulators of MD gene expression would be overrepresented among top signals from GWAS on T2D. We evaluated single nucleotide polymorphisms (SNPs) associated with gene expression (eSNPs) mapped in human adipose, skeletal muscle, lymphoblastoid cell lines (LCLs) and nine additional tissues mapped by the GTEx Consortium for enrichment among T2D-associated variants in the Wellcome Trust Case Control Consortium T2D GWAS dataset. We excluded eSNPs for genes underlying monogenic forms of diabetes to ensure that any observed enrichment would not be attributable to effects from established diabetes genes. The proportion of eSNPs for MD genes with false discovery rate (FDR) q-values ≤ 5% is 2x10^-4 whereas the proportion for all GWAS-interrogated SNPs is 3x10^-5. The MD eSNPs most associated with T2D correspond with myeloid differentiation primary response gene (MyD88). MyD88 deficiency caused by loss of function mutations has been observed in patients with a syndrome where coding variation could explain the infections. Moreover, the L265P mutation is common in patients diagnosed with Waldenstrom macroglobulinemia. Interestingly, we did not observe an enrichment of eSNPs for MD genes previously associated with T2D. The narrow-sense heritability explained by eSNPs of MD genes is disproportionate relative to the proportion of all SNPs in this set by a factor of 2.18. Taken together, these results support an important yet complex role for genetic regulators of MD genes in T2D susceptibility.

180-LB
Analysis of a Cardiovascular Disease Genetic Risk Score in the Diabetes Heart Study
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Recent studies have examined genetic risk scores of single nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWAS) for their cumulative impact on cardiovascular disease (CVD) related traits. Most analyses have used SNPs associated with a single trait; in this analysis, we instead examined a more comprehensive risk score of SNPs associated with blood pressure, arterial stiffness, C-reactive protein, body mass index, electrocardiogram traits, stroke, coronary heart disease, fasting plasma glucose, glomerular filtration rate, lipids (triglycerides, low-density and high-density lipoprotein, or total cholesterol), and type 2 diabetes (T2D). This risk score was analyzed for potential associations with subclinical CVD, prior CVD events, and mortality in 1175 individuals of European descent from 467 families in the Diabetes Heart Study (DHS), a T2D-enriched cohort at elevated risk of CVD. 83.7% of participants were affected by T2D, with average diabetes duration of 10.5 ± 7.2 years. Genetic scores were derived by adding the number of risk alleles across 215 SNPs; scores ranged from 186 to 245 (212.9 ± 8.6, mean ± SD). Associations were examined using marginal models with generalized estimating equations for subclinical CVD and prior CVD events and Cox proportional hazards models with sandwich-based variance estimation for mortality in SAS 9.3. Analyses were adjusted for age, sex, and T2D affected status. An increase in genetic risk score was modestly associated with increased coronary artery calcification (p=0.0044), with a trend for association with increased carotid artery calcification (p=0.11). No significant associations with aortic calcification, prior CVD events, or all-cause or CVD mortality were observed. These results indicate that a comprehensive genetic risk score for CVD-related traits does not have compelling predictive power for CVD in the DHS, highlighting the limits of current knowledge of the genetics of CVD in individuals affected by T2D.

181-LB
Quantitative Glycemic Trait Genetic Loci Are More Enriched for Common Variants with Regulatory Potential Compared with Type 2 Diabetes Risk Loci
SRICHARAN RAGHAVAN, AARON S. LEONIG, MARCO DAURIZ, CHHING-TI LIIU, JOSÉE DUPUIS, JOSE C. FLOREZ, JAMES B. MEIGS, BOSTON, MA, VERONA, ITALY

Background: Many common single nucleotide polymorphisms (SNPs) identified through genome-wide association studies, including for glycemic quantitative traits (QFs, fasting glucose [FG], fasting insulin [FI]) and type 2 diabetes (T2D), are neither protein coding nor in linkage disequilibrium (LD) with coding variants, suggesting that regulatory variation plays a prominent role in the genetic basis of common diseases. Here, we examined regulatory variation at or around SNPs associated with FG, FI, and T2D.

Methods: We used RegulomeDB to classify SNPs at 108 QT- or T2D-associated loci as having strong evidence for regulatory function (RegulomeDB score 1-3) or weak/no evidence for regulatory function (RegulomeDB score 4-7). We excluded loci at which QT and T2D SNPs were in strong LD (r2≥0.8) with implicated SNPs. SNPs with coding variation could explain the associations. For remaining loci, we counted the number of SNPs with strong or weak evidence for regulatory potential in strong LD with the lead SNPs, and compared the proportion of SNPs with strong regulatory potential at QT, T2D and overlapping loci.

Results: After excluding 16 loci harboring coding variants (6 QT, 7 T2D, and 3 overlapping), 92 loci (30 QT, 46 T2D, and 16 overlapping) were examined for regulatory variation. Of these, 19 QT, 17 T2D, and 7 overlapping loci had ≥1 SNP.
with RegulomeDB scores 1-3 (54 of 553 SNPs; 9.8%) than T2D (34 of 878 SNPs; 3.9%) and overlapping (11 of 304 SNPs; 3.6%) loci. The lower RegulomeDB scores at T2D loci compared to T2D loci appear driven by enrichment for transcription factor binding sites (16.5% vs. 10.8%) and eQTLs (2.7% vs. 0.1%).

Conclusions: Glycemic QT and T2D loci harbor regulatory variation with T2D loci associated with a greater proportion of predicted regulatory SNPs based on current annotation of the genome.

182-LB
Integration of Genomic and Expression Data Confirms 40 Known Loci to Be Cosmopolitan Disease Susceptibility Loci for Type 2 Diabetes in Both European and African American Populations
TOBY ANDREW, WINSTON LAU, NIKOLAS MANIATIS, London, United Kingdom

We aim to assess if the established 65 disease susceptibility loci for type 2 diabetes (T2D) identified in Europeans are also risk loci for African Americans. We conducted a genome-wide association study using fine-scale population-specific genetic maps derived from HapMap Phase II data, applied to 1) genomic array data for Type 2 diabetes (T2D) cases and controls for European (WTCCC1 and WTCCC2) and African-American T2D (NIDDK) and 2) fine map genomic and adipose and skin expression data for healthy European samples with T2D cases excluded [EBL, E-TABM-1140].

The method utilizes a powerful multi-marker test of association based upon the MaLeoct model to assess approximately 5000 analytic windows across the human genome, each of equal genetic size. The same analytic window co-ordinates were used for both disease and cis-eQTL expression mapping to provide commensurability between populations and to assess evidence that identified disease loci are eQTLs and hence confer risk by regulating gene expression. For each window, a test statistic is obtained along with a genome location estimate and 95% confidence intervals for the location of the putative functional variant.

We have been able to establish: 1) that the same loci for over 50% of the 65 loci in Europeans are also disease susceptibility loci for African-American samples; 2) more refined functional variant location estimates and 3) based upon the adipose expression data (and depending upon threshold criteria used), we estimate approximately 25% of all the identified susceptibility loci are themselves expression quantitative trait loci (eQTLs). We conclude that there are still likely to be many common genomic disease susceptibility variants that can be usefully characterized and that integrative genomic methods have the potential to provide important mechanistic clues about gene function and disease susceptibility.

Supported By: MRC (G090010/172)

183-LB
Functional Connectivity and Annotation on Fasting Plasma Glucose Risk-associated Variants in East Asians
JOO-YEON HWANG, MIN JIN GO, BOY-GHEE HAN, BONG-JO KIM, Chung-buk, Republic of Korea

Fasting plasma glucose (FPG) has been recognized as an important indicator of their suggested association with cardiovascular disease or related traits. We genotyped DNA from 988 cases and 987 controls using Metabochip arrays. The association between SNPs and T2D was assessed via logistic regressions adjusted for age, sex, body mass index and principal components for population stratification, under an additive model.

We found significant associations between T2D and several SNPs (not in linkage disequilibrium) in or close to GLIS3, JAZF1, FGDE2, ARRP21 and CYBRD1 (Table). When analyzing a genetic risk score from 60 T2D-associated SNPs in Europeans, we found a strong association of this score with T2D (effect size by risk-allele of 0.065; P=3.92x10^-9).

The involvement of GLIS3 or JAZF1 into T2D has been already reported in other populations. The association between CYBRD1 rs13392929D and T2D was only suggested. However, the contributions of FGDE6 rs65838592 and ARRP21 rs7613472 to T2D risk are novel. Both SNPs were present in the array because of their suggested association with cardiovascular disease or related traits.

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<th>AA*** (%)</th>
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*Adjusted for age, sex and BMI. **Adjusted for age and sex only. ***AA Ancestral Allele / AAF AAF frequency.

184-LB
Metabochip Genotyping in a Mexican Case-Control Study Identified New Loci Associated with Type 2 Diabetes
MARIA AURORA MEJA BENITEZ, AMELIE BONNEFOND, LOIC YENGO, STEPHANE LOBBENS, ADAN VALLADARES SALGADO, JESUS PERALTA, JAIME GARCIA MENA, MIGUEL CRUZ LOPEZ, PHILIPPE FROGUEL, Delniito Federal, Mexico, Lille, France

The prevalence of type 2 diabetes (T2D) is very high in Mexico (14%). Performing genetic studies in Mexicans could identify risk alleles that are not observed in other populations. The association between GLIS3 or JAZF1 into T2D has been already reported in other populations. The association between CYBRD1 rs13392929D and T2D was only suggested. However, the contributions of FGDE6 rs65838592 and ARRP21 rs7613472 to T2D risk are novel. Both SNPs were present in the array because of their suggested association with cardiovascular disease or related traits.

185-LB
Transcriptional Profiling of Polarized Macrophages Using RNA Sequencing
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Macrophages display remarkable plasticity and can display an array of activation states in response to environmental cues. Macrophages (M0) are polarized to classical pro-inflammatory (M1) or alternative anti-inflammatory (M2). The adipose tissue macrophages (ATMs) of obese individuals are major inflammatory mediators in white adipose tissue and crucial contributors to adipose tissue inflammation and insulin resistance. However, modulatory networks governing ATMs polarization have been investigated but the full picture remains vague.

For author disclosure information, see page LB91.
To explore genome wide signaling network in controlling ATM polarization, we generated transcriptome profiles from macrophages with various activation status - M0, M1 and M2. After analysis with multiple algorithms, we identified 13400 aligned unique loci in the mm10 database. Expression of 1803 transcripts are induced at least 2 fold during M1 and 765 during M2 activation, whereas 1612 are downregulated upon M1 and S2 by M2 stimuli. Gene ontology studies revealed adipokine signaling and antigen presenting and processing pathways are enriched in gene sets that are altered in M1 activation (>2 folds). Further, our study also identified several membrane proteins that are differentially presented on either M1 or M2 macrophages, thus may serve as potential cellular markers for identifying macrophage populations with polarized activation status. Our analysis found ZA0 long non-coding (IncRNAs) RNAs are actively regulated during macrophage polarization as annotated in the mm10 dataset. Scanning the chromosome region of 132 differentially expressed IncRNAs, we found that several IncRNAs are located in the loci with enriched gene clusters involved in inflammation, lipid metabolism and insulin resistance. Thus, this study provides a comprehensive profile of transcriptome that can be of great importance to understand the functions of ATMs in regulating adipose tissue function, especially obesity associated adipose tissue inflammation and insulin resistance.

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ERK Regulates TLR4 Endocytosis and Pro-inflammatory Responses in Macrophages
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Type 2 diabetes (T2D) is associated with low circulating levels of lipopolysaccharide (LPS) resulting in endotoxemia that has been linked to insulin resistance. Binding of LPS to TLR4 leads to the activation of two TLR4 signaling pathways: MyD88-dependent and TRIF-dependent. Activation of the TRIF-dependent pathway requires TLR4 endocytosis. TLR4 endocytosis in macrophages leads to the activation of pro-inflammatory signaling pathways and production of factors linked to the development of T2D. However, the molecular mechanisms involved in regulating TLR4 endocytosis remain elusive. The extracellular signal-regulated kinase 1 and 2 (ERK1/2) module is activated downstream of TLR4 and associated with insulin resistance. We examined whether inhibition of ERK activity blocked TLR4-mediated inflammatory responses. RAW 264.7 macrophages were pre-treated with a MAPK inhibitor, U0126 (50 microM, 1 hr), and then treated with LPS (100 ng/ml, 6 hr). Inhibition of LPS-induced ERK activity decreased the release of MyD88-dependent MCP-1 (15±0.3 fold) and TRIF-dependent RANTES (40±4.0 fold) compared to macrophages exposed to LPS alone. Because inhibition of LPS-induced ERK activity did not affect total TLR4 protein or mRNA, we examined if ERK modulates the cell surface expression of TLR4. By using the loss of cell surface expression by flow cytometry as readout for TLR4 endocytosis, LPS decreased TLR4 surface expression by 65±0.3%, but when ERK was inhibited with 10, 25, or 50 microM U0126 in the presence of LPS, TLR4 surface expression increased in a dose-dependent manner (34±1.0%, 54±1.3%, and 63±1.6%, respectively). In addition, incubation of macrophages with U0126 blocked LPS-induced activation of IRAK3, a TRIF-dependent transcription factor. In summary, ERK regulates TLR4 signaling pathways and endosomal trafficking in macrophages. We propose that ERK positively regulates TLR4-mediated inflammatory responses and inhibition of ERK signaling will protect against insulin resistance.

ERK regulates TLR4 endocytosis and pro-inflammatory responses

CHRISTINA KRIEGEL, KEVAN C. HEROLD, TAREK FAHMY, New Haven, CT

Type 1 diabetes (T1D) is a complex interplay of immune cells and pancreatic β cells. Upon activation T-cells releases proinflammatory cytokines viz. IFNγ, IL-17 and induce β cell dysfunction and eventual death. Deoxypseudamine synthase catalyses the crucial hypusine modification of eIF5A which promotes the translation of proinflammatory cytokines and induces intracellular stress in the pancreatic beta cell microenvironment. Inducible Double-transgenic mice carrying DAS-DAS-GAD65 gene were immunized with adenoviral vectors carrying GAD65 for diabetes induction. Animals were subsequently treated with deoxypseudamine synthase (DHS) inhibitor GC7 and monitored for diabetes development over time. Our result show that down regulation of eIF5A through inhibition of DHS, reduces the potent inflammatory cytokine CD3+CD4+IL17 population and help in reducing the ER stress in pancreatic beta cells micro-environment, leads to significantly increase in the Fasting insulin production. Results of glucose tolerance test also explain that administration of GC7 provide tolerance and maintain the insulin production upto 30 mins in an animal model that closely resembles human T1D.

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Nanoparticle Biodistribution in a Mouse Model of Type 1 Diabetes: The Role of Macrophage Uptake and Homing in the Pancreatic Micro-environment
CHRISTINA KRIEGEL, KEVAN C. HEROLD, TAREK FAHMY, New Haven, CT

Imaging inflammation and monitoring the course of disease after an intervention remains a highly sought after goal for diagnostics and drug development. For this purpose, we designed novel “theranostic” reagents using fluorescently labeled biodegradable nano-scale polymeric particles (NP). We observed that fluorescing NP, but not the fluorophore in its free form, substantially accumulated in the pancreatic microenvironment of diabetic animals. Notably, only a moderate to low signal was detected in pre-diabetic or non-inflamed control mice. We hypothesized that NP accumulation in inflamed tissues may be due to either retention in the inflamed microvasculature or due to macrophage trafficking to these sites. To elucidate the mechanism of NP retention in sites of inflammation, NPs were either incubated with macrophages of NOD mice and systemically administered or directly injected into either non-inflamed NOD/SCID control animals, pre-diabetic or diabetic NOD mice, or diabetic NOD mice subjected to in vivo macrophage depletion. Biodistribution of NP was evaluated using optical fluorescence on a Multispectral Bruker In Vivo imaging system. We observed an equally strong signal when NP were directly injected or delivered by macrophages. Most importantly, NP retention was dependent on macrophages since macrophage-depleted mice showed only minimal uptake in the pancreas after direct administration, whereas injection of ex vivo loaded macrophages led to significant NP accumulation in this group. Our results indicate that macrophages play a crucial role in NP trafficking to the site of inflammation in a model of type 1 diabetes. Therefore, utilization of macrophages as a “Trojan Horse” might be advantageous in the development of innovative, targeted therapies for treatment of immune-mediated conditions at the site of inflammation, reducing the risk of undesirable off-target effects.

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190-LB
Preservation of Beta-Cell Function following Pancreatic Islet Auto-transplantation
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The aim of the study was to assess the number of patients remaining insulin free after total pancreactectomy and islet autotransplantation in our center. Total pancreactectomy followed by islet autotransplantation was performed in 10 patients with the age of 34 (11-53) and BMI of 28 (16-35). Eight patients had chronic pancreatitis with intractable pain, remain patients had a benign pancreas tumor or small ampullary cancer. PSSQ1 or SPINK1 gene mutations were present in 3 patients. Exogenous insulin therapy was implemented for at least 6 weeks after the autotransplantation to support islet graft recovery and engraftment and subsequently weaned off, if possible. Follow up was 28 (2-68) months.

The islet tissue pellet volume was 6ml (2-30). Viability was 95% (81-98). Five (50%) patients are currently off insulin with excellent glucose control and HbA1c below 6. The remaining 5 patients still require insulin injections, however none of them experiences “brittle” form of diabetes mellitus; no severe hypoglycemic episodes were reported. Transplanted beta cell mass was significantly higher in patients currently insulin free comparing to those with insulin therapy. 202kIEQ (149-340kIEQ) vs. 84kIEQ (48-260), respectively. Islet mass per kilogram of patient body weight was also substantially higher in the same group: 3,300IEQ/kg (1,811-4,800) vs. 1,078kIEQ/kg (556-2,771), respectively. Islet gradient purification was applied in 3 cases and resulted in insulin independence in 2 individuals. BMI as well as time of chronic pancreatitis prior to operation did not differ in patients who became insulin free and insulin dependent. None of the patients developed long-term complications related to the islet transplant procedure.

Islet autotransplantation efficiently preserved beta cell function in patients after total pancreactectomy allowing for insulin independence in half of them and stable glucose control in remaining. The success was correlated with higher islet mass transplanted.

191-LB
Composition and Function of Macro-Encapsulated Human Embryonic Stem Cell-derived Implants Meet Characteristics of Clinical Human Islet Cell Grafts
EVI MOTTÉ, EDIT SZEPESSY, KRISTA SJUENS, GEERT STANGE, MYRIAM BOMANS, DANIEL JACOBS-TULLENEERS-THEVISSEN, ZHIDONG LING, EVERT KROON, DANIEL PIPELEERS, Jette, Belgium, San Diego, CA

Shortage of good quality human pancreatic tissue for use in organ and islet cell transplantation has led to development of large-scale laboratory sources that generate insulin-producing implants. Prior work showed that human embryonic stem (hES) cells can be differentiated in vitro to pancreatic endoderm that forms beta cell containing implants in immune-deficient mice. The present study reports a higher endocrine purity in encapsulated versus non-encapsulated subcutaneous implants, with enrichment in alpha-beta-delta cells when placed in TheraCyte-macro-devices and in alpha cells when alginate-micro-encapsulated. We compared endocrine composition and glucose-regulated functions of macro-huES-implants with the characteristics of cultured human islet cells as used in clinical transplantation. At post-transplant week 20-30, macro-huES-implants generated higher plasma C-peptide levels than human islet cell grafts with similar cell number at start. Their endocrine purity was higher, containing single-hormone-positive alpha and beta cells that exhibited rapid secretory responses to increasing and decreasing glucose concentrations, as well as the case during perfusion of cultured human islet cell preparations. Their insulin secretory amplitude was however lower, in part attributable to a lower cellular content; it was associated with lower rates of glucose-induced insulin biosynthesis, but not with lower glucagon-induced release, signs that are indicative for an immature functional state of the hES-derived beta cells at the time of analysis. These data support the therapeutic potential of macro-encapsulated huES-implants.

Their comparison with clinical-grade human islet cell grafts sets references for data support the therapeutic potential of macro-encapsulated huES-implants.

Identification of key regulators of lipid metabolism and thermogenic functions has important therapeutic implications for the current obesity and diabetes epidemic. Here we show that Grb10, a newly identified direct substrate of mechanistic/mammalian target of rapamycin (mTOR), is expressed highly in brown adipose tissue, and its expression in white adipose tissue is markedly induced by cold exposure. In adipocytes, mTOR-mediated phosphorylation at Ser501/503 switches the binding preference of Grb10 from the insulin receptor to raptor, leading to the dissociation of raptor from mTOR and down-regulation of mTOR complex 1 (mTORC1) signaling. Fat-specific disruption of Grb10 increased mTORC1 signaling in adipose tissues, suppressed lipolysis, and reduced thermogenic function. The effects of Grb10 deficiency on lipolysis and thermogenesis were diminished by rapamycin administration in vivo. Our study has uncovered a novel feedback mechanism regulating mTORC1 signaling in adipose tissues and identified Grb10 as a key regulator of adiposity, thermogenesis, and energy expenditure.

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194-LB
Acute Loss of Insulin and IGFl-1 Signaling in Adipose Tissue Results in a Severe, but Transient, Metabolic Syndrome
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Both obesity and lipodystrophy are accompanied by insulin resistance and inflammatory changes in adipose tissue. To investigate the effect of acute insulin resistance in adipose tissue, we created mice carrying IR and IGF1R floxed alleles and the tamoxifen-inducible Cre ERT2 recombinase under the adiponectin promoter. Within 2-3 days of tamoxifen treatment these mice displayed a major loss of insulin and IGF1R in both white and brown fat tissues. This resulted in acute, severe resistance insulin with marked hyperglycemia (>400 mg/dl) and hyperinsulinemia (6.8-fold increase), which may in turn contribute to AT inflammation.
after 2 weeks, serum glucose levels returned toward normal, and by 4 weeks glucose tolerance and insulin tolerance tests began to return toward normal in FindIGIRKO mice, despite the continued loss of adipose tissues. These data indicate the critical role of insulin and IGFR-1 signaling in maintenance of adipose mass and function, but also demonstrate difference in acute versus chronic response to insulin and IGFR-1 resistance of fat tissues, and the ability of these alterations in adipose tissue to initiate systemic compensatory responses, including β-cell hyperplasia. This new model of adipose tissue insulin resistance allows an opportunity to dissect these acute and chronic responses.

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197-LB

Browning and Inflammation of Subcutaneous White Adipose Tissue in Rhesus: Wellness vs. Dysmetabolism

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The presence of BAT and browning of subcutaneous white adipose tissue (SubQ WAT) in rodents and humans has been correlated with changes in expression of many different genes known to contribute to energy metabolism, nutrient uptake and inflammation. Indeed, recent publications suggest that induction of browning correlates with repression of inflammation in SubQ WAT in high-fat diet mice. To establish the status of browning and inflammation in Rhesus we have evaluated gene signatures of browning and inflammation in SubQ AT of dysmetabolic/insulin resistant versus lean Rhesus. Analysis of the BAT gene signature showed a significant reduction in uc1p, adrb-3, ppca1a, cidea, diolz2 and bmp-7 (ranging from 5 to 2-fold) in dysmetabolic rhesus compared to healthy. In addition, a striking decrease in browning gene markers such as tmem25, cost17, epsilon1 and cide-3 (ranging from 4 to 2-fold) was observed in SubQ WAT. This loss of a browning phenotype in SubQ WAT was accompanied by more than 10-fold increase in pro-inflammatory genes such as pai-1, c4a, ccl3 and osteopontin (abstract 2014-A-1921-Diabetes), and decreased adiponectin and insulin receptor gene expression. This apparent “vascularization” of the SubQ WAT might reflect the pathophysiologic state of these animals. Dysmetabolic Rhesus compared to healthy had increased body weight (16.97 ± 3.2 vs. 9.6 ± 0.86 g), increased percent of body fat (44.2 ± 5.9 vs. 5.3 ± 0.979%) and increased pro-inflammatory plasma markers (IL-6, MCP-1 and CRP). In addition, they had decreased fasting levels of HDL (64.7 ± 23 vs. 89.7 ± 17.1 mg/dl), increased triacylglycerols (114.8 ± 59.3 vs. 30 ± 7.8 mg/dl) insulin (33 ± 32.8 vs. 20.3 ± 11.9 mg/dl) and glucose (75.2 ± 9.5 vs. 69.3 ± 8 mg/dl). In summary, we report for the first time the presence of a BAT/browning gene signature in SubQ WAT of Rhesus that negatively correlates with increased systemic inflammation and insulin resistance.

198-LB

Adipose-specific Inhibition of BCKDH Activity Alters Substrate Flux in Metabolic Tissues to Mimic the “Metabolic Signature” of Insulin Resistance (IR)

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A “metabolic signature” associated with IR includes higher circulating branched-chain amino acids (BCAAs) and their metabolites C3/C5 acrycarnitines (ACs), and muscle medium-long-chain ACs (products of incomplete B-oxidation). BCAA metabolism in white adipose tissue (WAT) is down-regulated in obesity and IR. To determine whether impaired WAT BCAA metabolism contributes to the “metabolic signature” associated with IR we generated adipose-specific branched chain ketoacid dehydrogenase E1b subunit knockout (AdBCKDHKO) mice. WAT BCKDHE1β expression was decreased by 97% and valine oxidation by 74% in AdBCKDHKO mice vs. controls (wild type and BCKDH flx mice). High fat/high sucrose (HFHS) feeding caused obesity and elevated fasting blood glucose (FBG) but not overt glucose intolerance or IR in either genotype. HFHS control mice had 1.7-2X higher circulating BCAAs and branched-chain ketoacids (BCKAs) vs. Chow controls. HFHS AdBCKDHKO mice had similar adiposity and FBG to HFHS controls and further elevations in plasma BCKAs but not BCAAs. HFHS feeding did not raise C5-DHH3-DC ACs but this analyte was 2.2X and 1.3X higher in liver and muscle of HFHS AdBCKDHKO mice vs. Chow and HFHS controls. Some WAT long-chain ACs were decreased < 50% in HFHS AdBCKDHKO vs. HFHS controls. Yet muscle medium-long-chain ACs and liver medium-chain ACs showed modest elevations with diet in controls and were 1.5-2X higher in HFHS AdBCKDHKO vs. HFHS controls. In sum, HFHS feeding caused obesity, impaired FBG, higher plasma BCAAs and changed the tissue AC profile in controls. HFHS AdBCKDHKO mice have similar obesity and FBG to HFHS controls, yet higher plasma BCKAs, C3/C5 ACs in muscle and liver, and they accumulate β-oxidation intermediates in a tissue- or species-specific manner. Thus, down-regulation of WAT BCAA metabolism is sufficient to alter substrate flux in metabolic tissues to induce elements of the “metabolic signature” associated with IR.

For author disclosure information, see page LB91.
Regulation of Insulin Resistance and Adiponectin Signaling in Adipose Tissue by Liver X Receptor Activation Highlights Cross-Talk with PPAR?

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Liver X receptors (LXRs) have been recognized as a promising therapeutic target for atherosclerosis; however, their role in insulin sensitivity is controversial. Adiponectin plays a unique role in maintaining insulin sensitivity. Here, we investigated the role of LXR activation in insulin resistance based on adiponectin signaling and mechanisms.

C57BL/6 mice maintained on a regular chow received the LXR agonist, T0901317 (30 mg/kg/d) for 3 weeks with intraperitoneal injection, and differentiated 3T3-L1 adipocytes were treated with T0901317 or GW3965. T0901317 treatment induced significant insulin resistance in C57BL/6 mice. It decreased adiponectin gene transcription in epididymal fat, as well as serum adiponectin levels. Activity of AMPK, a key mediator of adiponectin signaling, was also decreased, resulting in decreased Glut-4 membrane translocation in epididymal fat. In contrast, adiponectin activity was not changed in the liver of T0901317 treated mice. In vitro, both T090137 and GW3965 decreased adiponectin expression in adipocytes in a dose-dependent manner, an effect which was diminished by LXRx silencing. ChIP-qPCR studies demonstrated that T0901317 decreased the binding of PPARγ to the PPAR-responsive element (PPRE) of the adiponectin promoter in a dose-dependent manner. Furthermore, T0901317 exerted an antagonistic effect on the expression of some PPARγ-target genes both in EP fat and adipocytes.

We generated 3T3-L1 adipocytes expressing endogenous levels of Akt isoforms. We accomplished this by overexpressing WT or MK-2206-resistant forms of Akt1 or Akt2. Drug-resistant isoforms. We generated 3T3-L1 adipocytes expressing drug-resistant Akt1 or Akt2. Our model system revealed that both Akt1 and Akt2 drug-resistant forms control insulin-mediated FoxO1 signaling. These findings indicate that the potential of LXR activation as a therapeutic target for atherosclerosis may be limited by the possibility of exacerbating insulin resistance-related disease.

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A New Model System to Study Akt Isoform Metabolic Functions

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Insulin metabolic action is largely mediated by the Akt kinase family. Akt2 is the most abundant Akt isoform in metabolic tissues and deletion or impairment of Akt2, but not Akt1, leads to altered glucose metabolism and insulin resistance in mice and humans. However, due to differential expression levels of Akt isoforms and the potential contribution of compensatory mechanisms upon deletion of Akt kinases, Akt isoform specific effectors and metabolic functions are still poorly understood. To elucidate Akt1 and Akt2 function in insulin regulated metabolism we combined the MK-2206 Akt pharmacological inhibitor and engineered drug-resistant forms of Akt1 and Akt2 to develop a new model system that allows for acute and specific inhibition of Akt isoforms. We generated 3T3-L1 adipocytes expressing endogenous levels of epitope Flag-tagged WT or MK-2206-resistant forms of Akt1 or Akt2. Drug-resistant Akt kinases were phosphorylated in response to insulin similar to their WT counterparts. MK-2206 treatment led to a dose-dependent reduction in phosphorylation of WT Akt kinases and their targets AS160 and FoxO1, however insulin-induced phosphorylation of AS160 and FoxO1 was preserved in adipocytes expressing drug-resistant Akt1 or Akt2. Our model system revealed that both Akt1 and Akt2 drug-resistant forms control insulin-mediated FoxO1 nuclear exclusion and the translocation of GLUT4 glucose transporters to the plasma membrane. On the contrary, only Akt1 activation promotes 3T3-L1 adipocyte differentiation, as assed by lipid content and the expression profile of adipogenic genes. EdU (5-ethylidene 2'-deoxyuridine) incorporation analyses revealed that Akt1, but not Akt2, regulates the mitotic clonal expansion that precedes terminal adipocyte differentiation, supporting a specific role for Akt1 signaling in adipogenesis. Our studies provide a new model system to elucidate Akt isoform metabolic functions, revealing overlapping and specific roles for Akt isoforms in adipocyte differentiation and metabolic function.

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Regulation of Insulin Resistance and Adiponectin Signaling in Adipose Tissue by Liver X Receptor Activation Highlights Cross-Talk with PPAR?
Exercise Improves Insulin Sensitivity and Glucose Metabolism in Myotubes from Severely Obese Subjects

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Exercise improves insulin sensitivity and oxidative capacity in skeletal muscles. However, molecular mechanisms underlying these adaptations are poorly understood. The purpose of the present study was to examine glucose and lipid metabolism after electrical pulse stimulation (EPS) as an in vitro model of exercise of myotubes established from healthy, lean subjects (LD) and myotubes originating from severely obese subjects (BMI ≥ 40) with (SO-T2D) or without type 2 diabetes (SO-nD).

In conclusion, EPS improved insulin sensitivity in myotubes, and this effect was most evident in myotubes established from severely obese subjects with T2D. EPS enhanced oxidative capacity of glucose in myotubes from all subjects, while OA oxidation was only improved in myotubes from lean subjects.

INSULIN ACTION—GLUCOSE TRANSPORT AND INSULIN RESISTANCE IN VITRO

KL4F Up-regulating MFN2 Expression Contributes to the Alleviation of High-Fat-induced Insulin Resistance in Hepatic Cells

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Kruppel-like factor 4 (KL4F) is a crucial transcription factor implicated in multiple cell events, while mitofusin 2 (MFN2) protein is involved in mitochondrial metabolism and insulin resistance (IR) regulation. However, the role of KL4F in high-fat-induced IR and MFN2 expression in hepatic cells remain unknown.

For author disclosure information, see page LB91.
Hepatic cells HepG2 were treated with control or palmitic acid, the cell insulin sensitivities were evaluated by glucose consumption measured with glucose oxidase method. Then cells were infected with control or KLF4 expression adenovirus and/or MFN2 siRNA expression adenovirus. The expression levels of KLF4, MFN2, insulin receptor (INSR), insulin receptor substrate 2 (IRS2) and glucose transporter type 2 (GLUT2) were detected by quantitative RT-PCR and Western-blot. MFN2 promoter luciferase reporter plasmid was constructed and reporter gene assays were performed to detect its transcriptional activities by cotransfection in HepG2 cells. The interaction of KLF4 with MFN2 promoter region in HepG2 cells was assayed by chromatin immunoprecipituation (ChIP).

The results show, that KLF4, MFN2, INSR, IRS2 and GLUT2 were down-regulated and the insulin sensitivities of HepG2 cells decreased by palmitic acid incubation. The over-expression of KLF4, the insulin sensitivities of HepG2 cells improved, and the expression levels of MFN2, INSR, IRS2 and GLUT2 were up-regulated. While after knock-down the expression of MFN2 by specific siRNA expression adenovirus, KLF4 over-expression could not ameliorate the impaired insulin sensitivities of HepG2 cells effectively. The results of ChIP and reporter gene assays indicated that KLF4 interacted with MFN2 promoter region and activated the transcription of MFN2 in HepG2 cells.

In conclusion, KLF4 could alleviate high-fat-induced insulin resistance by up-regulating MFN2 expression directly in hepatic cells.

**INSULIN ACTION—SIGNAL TRANSDUCTION, INSULIN, and OTHER HORMONES**

**208-LB Fatty Liver and Insulin Resistance in the Liver Specific Knockout Mice of Mitogen Inducible Gene (Mig-6)**

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Mitogen inducible gene 6 (Mig-6) is a feedback inhibitor of ERG signaling pathway. Thus, deletion of the Mig-6 gene leads to activation of ERG signaling pathway. The liver-specific knock-out mice of the Mig-6 gene showed hepatomegaly and increased plasma concentration of cholesterol, indicating the roles of Mig-6 gene in the metabolic syndrome. In this study, we analyzed the biomarkers of insulin resistance and the effects of high fat diets in the wild (Mig-6fl/fl) and liver specific K.O. (Mig-6mice of Mig-6. The fasting plasma concentrations of glucose, triglyceride, cholesterol, free fatty acids and HOMA-IR were measured and the glucose tolerance and insulin resistance tests were performed in the 25-week-old Mig-6fl/fl and the Mig-6mice. The protein levels of active components of insulin signaling pathway and gluconeogenesis were analyzed in the liver and fat. The fasting plasma cholesterol and glucose concentration were higher in the Mig-6mice than the wild mice with increased fat deposition in the liver. But the Mig-6mice had the improved glucose intolerance and insulin resistance without increased amount of p-IR after insulin infusion in the liver. The hepatic concentration of Pgc-1α, a key enzyme in the gluconeogenesis was increased in fasting Mig-6mice. The feeding of high-fat diet accelerated the plasma lipids profiles and HOMA-IR in the Mig-6mice but had no differential effects in oral glucose tolerance and insulin tolerance in both genotypes. These results suggest that the activated ERG signaling might mainly increase the fasting plasma glucose concentration through inducing the hepatic steatosis and the improved whole body insulin resistance in the K.O. mice might be caused by decreased fat deposition in fat tissues.

**209-LB Obestatin Stimulates Insulin Secretion Under Glucose-stimulated Condition**

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Obestatin, a 23 amino acid peptide derived from the ghrelin gene, is expressed in various tissues including stomach and pancreas. Obestatin is known to reduce food intake and body weight, improve memory and regulate sleep, but has no effect on secretion of growth hormone and corticosterone. Obestatin is also known to increase mass and survival of pancreatic β cells but it’s effect on insulin secretion remains unclear.

We studied the effect of obestatin on insulin secretion under glucose-stimulated conditions both in vitro and ex vivo using rat insulinoma INS-1 cells and mouse pancreatic islets. To determine whether the effect of obestatin on insulin secretion is mediated through the ghrelin receptor, growth hormone secretagogue receptor (GHS-R), we transiently knocked down GHS-R in INS-1 cells and generated pancreatic β cell-specific GHS-R knockout mouse model (MIP-Cre GHSIR). Our results indicate that obestatin has profound stimulator effect on insulin secretion in both INS-1 cells and mouse pancreatic islets. Moreover, treatment of obestatin in GHS-R knockdown INS-1 cells also showed significant increase of insulin secretion under glucose-stimulated condition. Similarly, incubation of pancreatic islets from β cell GHS-R deficient MIP-Cre GHSIRmice with obestatin produced almost doubled the amount of insulin secretion compared to controls.

In conclusion, our studies indicate that obestatin is a potent insulin secretagogue under glucose-stimulated condition. This effect of obestatin is not likely mediated via its receptor GHS-R in pancreatic β cells, which is in agreement with the binding studies that obestatin doesn’t activate GHS-R. Obestatin’s stimulatory effect on insulin secretion and promoting effect on β cell survival together make obestatin a powerful therapeutic candidate for Type 2 diabetes.

**210-LB Stem Cell Factor Stimulates Glucose Uptake and GLUT4 Expression In Vitro and In Vivo**

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The glucose transporter 4 (GLUT4) mediates insulin-stimulated glucose uptake and accounts for 80% of glucose transporters in skeletal muscle and adipose tissue. AMP-activated protein kinase (AMPK) is involved in GLUT4 expression and cellular glucose uptake. Considering the structural and functional homology between insulin and c-Kit tyrosine kinase receptors, we asked whether c-Kit and its ligand, stem cell factor (SCF), are involved in glucose homeostasis. We demonstrated that c-Kit and SCF proteins are expressed in adipose tissue and skeletal muscle in mice and humans. In mice, adipose c-Kit expression correlated directly with adipose GLUT4 expression and inversely with blood glucose concentration. Intraperitoneal administration of recombinant SCF resulted in an acute and dose-dependent decline in blood glucose concentration in mice. Similarly, recombinant SCF treatment stimulated glucose uptake into cultured 3T3-L1 adipocytes and C2C12 myotubes. Recombinant SCF treatment resulted in activating phosphorylation of AMPK, but not the insulin receptor, and increased GLUT4 protein expression in cultured adipocytes and myotubes. In line with these findings, c-Kit knockout mice demonstrated greater fasting blood glucose and serum insulin levels than congenic wild-type mice did. Moreover, insulin-stimulated glucose disposal was attenuated in c-Kit knockout mice. In addition, c-Kit knockout mice demonstrated diminished GLUT4 protein expression in adipose tissue and skeletal muscle. In conclusion, recombinant SCF stimulates glucose uptake and GLUT4 expression in vitro and in vivo. These effects are independent of the insulin receptor and may involve AMPK. The salutary effects of recombinant SCF on glucose homeostasis may be used in the treatment of hyperglycemic states including diabetes mellitus.

**211-LB The Effects of Vaspin on NF-κB and PI3K/Akt Signaling Pathway in HUVEC**

SHIBEI LIU, YUANBIN LI, YI FEI, MINGMING WANG, JUN WANG, LI ZHANG, LINLIN GAO, YUJIE HE, Taiyuan, China

Aims: In this study, we investigated the effects of visceral adipose tissue-derived serpin (vaspin) on nuclear factor-kappa B (NF-κB) and Phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway in human umbilical vein endothelial cells (HUVECs) stimulated by tumor necrosis factor-α (TNF-α) to elucidate the role of vaspin in human endothelial cells of inflammation and insulin resistance.

Methods: Human umbilical vein endothelial cells were isolated and cultured in vitro. A NF-κB luciferase reporter system was constructed and transients transfected into human umbilical vein endothelial cells. Following transfection, HUVECs were pretreated with various concentrations of vaspin (0-330 ng/ml) before 10μg/ml TNF-α stimulation. The transcription activity of NF-κB was determined using luciferase reporter assay. The level of Akt phosphorylation was checked by western blot. Expression levels of NF-κB downstream inflammatory cytokines IL-1 and IL-6 were measured by enzyme-linked immunosorbent assay (ELISA). mRNA and protein expression levels of intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 (MCP-1) were determined by quantitative real-time PCR (qRT-PCR) and western blotting irrespectively.

Results: Showed that vaspin inhibited TNF-α mediated activation of NF-κB and its downstream molecules in a concentration-dependent manner (P<0.05). Vaspin significantly increased Akt phosphorylation in TNF-α stimulated endothelial cells in a concentration-dependent manner (P<0.05), which effects were abolished by pretreatment with the PI3-kinase inhibitor, Wortmannin (P<0.05).
Conclusions: Our results suggest that vagus protected endothelial cells from TNF-α induced inflammation and insulin resistance by combination the inhibition of NF-κB, its downstream molecules and the upregulation of the PI3-kinase/Akt signaling pathway.

Supported By: NSFC (81202091)

**INTEGRATED PHYSIOLOGY—INSULIN SECRETION IN VIVO**

**212-LB**

**Disturbed Glucose Homeostasis after Sleep Restriction Is Independent of the Chronobiological Time-Point of Sleep**

**FELIX MACHELDEIT, DENISA TROST, NELLI FORCÉ, MAREN WALTJ, HENDRIK LEHNERT, SEBASTIAN M. SCHMID, Luebeck, Germany**

Short sleep duration has been shown to detrimentally affect glucose metabolism in humans. However, little is known about the influence of chronobiological time-points of sleep on insulin sensitivity, i.e. in which part of the night sleep takes place. Against this background we investigated effects of sleep duration and sleep time-points on glucose metabolism in men. In a balanced cross-over design 15 healthy normal weight men underwent 3 different sleep conditions. We conducted two conditions of sleep restriction with only 4 hours of sleep per night: early (sleep between 11 p.m. - 3 a.m.) vs. late (sleep between 3 a.m. - 7 a.m.) condition. In the control condition participants were allowed to sleep for 8 hours (11 p.m. - 7 a.m.). After each condition we performed a Botnia clamp combining an intravenous glucose tolerance test with a subsequent hyperinsulinemic-euglycemic clamp. Thus it is possibly to assess acute β-cell secretory performance (first phase insulin response) as well as insulin sensitivity. Insulin sensitivity was defined as the ratio of glucose infusion rate and mean insulin plasma levels during steady state during the last 60 minutes of the clamp (M-Value). Baseline parameters were comparable between all conditions. Both, after the early and late condition of sleep restriction M-Values were significantly lower compared to controls reflecting reduced insulin sensitivity (p<0.05). Furthermore, first phase insulin response tended to be diminished after both conditions of sleep restriction as compared to regular sleep (p=0.089). There were no differences in glucose metabolism between the early and late sleep restriction. Taken together, we could demonstrate that acute sleep restriction impairs glucose homeostasis independent of chronobiological time-points of sleep. The detrimental effects of sleep restriction are mainly due to reduction of insulin sensitivity and—to a lower extent—to disturbed acute secretory performance of the β-cell.

**213-LB**

**Patients with Long-Duration Type 2 Diabetes Have Blunted Glycemic and β-Cell Function Improvements after Bariatric Surgery**


Although bariatric surgery improves glycemic control, it is unclear if glucose regulation is improved comparably in short vs. long duration type 2 diabetes (T2D). Therefore, we evaluated the effect of T2D duration on glycemic control and glucose regulation (insulin secretion and sensitivity) in subjects who were randomized to bariatric surgery (RYGB n=12 and SG n=15). Twenty-seven adults (18f/9m, age: 51.0y [41.5,57.3], BMI: 34.6kg/m² [34.5,37.9], HbA1c: 9.1% [8.6,10.5]) with short (n=14; α=0.0, 46% SG) and long-duration (n=13; α=0.9, 52% SG) T2D received a mixed-meal tolerance test at baseline and 24 months (m) post-surgery. Body composition (BMI, body fat via DXA), insulin sensitivity (Matsuda Index), 1st and 2nd phase meal-stimulated insulin secretion (MSIS, C-peptide iAUC/glucose iAUC (1st, 0-30min) and (2nd, 60-120min), disposition index (DI or β-cell function), MSIS x Matsuda Index) and incretin (GLP-1, GIP) responses were examined. Before surgery, while both early and long-duration T2D had similar BMI, HbA1c, and % insulin use (all p>0.2), long-duration T2D required more oral medications and had lower fasting C-peptide compared with early T2D (p<0.02). At 24 m, both early and long-duration T2D had similar improvements in BMI, body fat, insulin sensitivity and meal-stimulated incretin responses (all p>0.10). However, early T2Ds had better HbA1c (-3.0[-4.9,-2.1] vs. -1.8[-3.1,-1.0]; p<0.003) and greater 1st (0.140[0.1, 0.3] vs. 0.02[0.01-0.01,0.1]) and 2nd phase DI (0.4[0.2,0.6] vs. 0.03[0.02,0.2], both p<0.02), compared with long-duration T2D. Indeed, baseline T2D duration correlated with smaller reductions in HbA1c (r=0.39, p=0.04) and 1st (r=-0.53, p=0.003) and 2nd DI (r=-0.51, p=0.001). Therefore, despite equal weight loss and changes in incretin hormones to short duration T2D, inadequate β-cell function in people with long duration T2D appears key in explaining better glycemic control responses to bariatric surgery.

Supported By: ADA (1-11-CT26)

**214-LB**

**Most People with Long-Duration Type 1 Diabetes Are Insulin Microsecretors and Produce Their Own Endogenous Insulin**

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Ultrasonisitive assays that can detect C-peptide under 5 pmol/L allow detection of very low levels of c-peptide. We aimed to use urine c-peptide creatinine ratio (UCPCR) to assess endogenous insulin in a large cross-sectional population-based study of patients with Type 1 diabetes (T1D). We recruited 944 patients from primary and secondary care in 2 UK centres. All diagnosed under 30 years, duration >5 years, clinical diagnosis of T1D. Median(IR) age of diagnosis 116-17y, duration 181-21y, HbA1c 7.7[7.9-9.8], insulin dose 0.780[0.60-0.97]kg/24hr, and BMI 25.6[23.3-28.6]Kgm-2. All provided a home post-meal UCPCR. 81% (790/944) had detectable endogenous production (median(IR) UCPCR 0.012 [0.004-0.038]nmol/mmol). Most had very low, historically undetectable, levels (492/944, 53%, UCPCR <0.001-0.03 mmol/mmol). 8% had C-peptide levels above the DCCT cut off of significant endogenous insulin. Absolute UCPCR levels fell with duration but the proportion with detectable UCPCR never fell below 73% (maximum duration 47 years). Age of diagnosis and duration independent predictors of C-peptide in multivariate modelling. The majority of people with long duration T1D are insulin microsecretors and have detectable urine c-peptide. Some rare individuals with T1D maintain higher levels of endogenous insulin for many years after diagnosis of diabetes. The fact that some beta cells remain in most with longstanding T1D may reflect escape from immune attack, or beta cell regeneration. Understanding this may lead to a better understanding of pathogenesis in T1D and open new possibilities for treatment.

Supported By: HICF Health Innovation Challenge Fund (091885/HICF1009-041)

**215-LB**

**A Potent, Efficacious, and Selective GPR40 (FFAR1) Agonist Provides Immediate and Durable Glucose Control in Rodent Models of Insulin Resistance and Type 2 Diabetes**

**YANYUN CHEN, MIN SONG, XIANTAO JIANG, CHARLIE HU, DONALYN SCHEUER, KRISTEN B. BOVIST, PRANAB MAITI, CHARIF HAMDOUCHI, ANNE REIFEL MILLER, INDIANAPOLIS, IN, Bangalore, India**

LY2881835 (LY) is a high affinity, potent and efficacious GPR40 agonist when examined in hGPR40 binding and FLIPR assays. A statistically significant (SS) increase in insulin secretion was demonstrated when LY was examined in primary islets from mice, rats and humans; although, insulin secretion was not seen when LY was tested in primary islets from GPR40 KO mice. G0TGs performed in wild type (WT), GPR40 KO and GPR120 KO mice following oral administration of LY at 30 mg/kg demonstrated SS reductions in glucose AUCs in WT and GPR120 KO mice but not in GPR40 KO mice. These findings demonstrate that LY induces specific GPR40-mediated anti-diabetic activity when examined ex vivo and in vivo. LY was administered orally at 30 mg/kg to diet-induced obese (DIO) mice, an early model of T2D due to insulin resistance, for 14 days with G0TGs performed on days 1 and 14. SS reductions in glucose AUCs were seen on days 1 and 14. Interestingly, glucose levels were also SS reduced at time 0 of the G0TGs (60 minutes after LY was administered); although, glucose levels never fell below 100 mg/dl in any mouse during the study. A similar study was performed in streptozotocin (STZ)-treated DIO mice to explore glucose control in a model of type 2 diabetes (T2D). In this model, pancreatic insulin content was reduced ~80% due to STZ-treatment plus the mice were insulin resistant due to the high fat content of their diet. Glucose AUCs were SS reduced during G0TGs performed on days 1, 7 and 14 compared to control mice. In conclusion, these results demonstrate that LY functions as a GPR40-specific insulin secretagogue mediating immediate and durable glucose control in rodent models of insulin resistance and T2D. The findings suggest that a GPR40 agonist could benefit glucose control in individuals with insulin resistance and substantially reduced beta-cell function.

**216-LB**

**Mosapride, a Serotonin 5-HT4 Receptor agonist and Alogliptin, a Selective Dipeptidyl Peptidase-4 Inhibitor, Exert Additive Effects on Plasma Active GLP-1 Levels in Mice**

**KATSUNORI NONODAKI, Sendai, Japan**

Aims: Mosapride citrate, a selective serotonin 5-HT4 receptor, typically activates gastrointestinal tract motility. The aim of the present study was to determine the effects of mosapride with or without alogliptin, a selective dipeptidyl peptidase-4 inhibitor, on feeding-dependent and -independent glucagon-like peptide-1 (GLP-1) secretion in mice.

For author disclosure information, see page LB91.
Methods: In the first experiment, mosapride citrate was administered intraperitoneally to C57BL/6J mice treated over 4 days with or without alogliptin (0.05%). The mice were food-deprived after mosapride citrate administration. One hour later, the mice were decapitated and blood was obtained to determine the plasma active GLP-1 levels. In the second experiment, mosapride citrate was administered intraperitoneally after 24-h food deprivation to C57BL/6J mice treated over 4 days with or without alogliptin (0.05%). The mice were then provided food pellets and 1 h later the mice were decapitated and blood was obtained to determine the plasma active GLP-1 and insulin levels.

Results: Despite food deprivation, systemic administration of mosapride citrate significantly increased plasma active GLP-1 levels in mice. In addition, mosapride citrate significantly increased plasma active GLP-1 and insulin levels after refueling following 24 h of fasting. Moreover, alogliptin treatment enhanced the feed-dependent and independent increases in the plasma active GLP-1 levels induced by mosapride citrate, as well as the refueling-induced insulin secretion compared with saline controls.

Conclusions: 5-HT4 receptors upregulate active GLP-1 secretion independent of feeding. Pharmacologic stimulation of 5-HT4 receptors and the pharmacologic inhibition of DPP-4 exert additive effects on plasma active GLP-1 levels in mice.

217-LB
Across Glucose Tolerance (GT) Spectrum, Men (M) Display Greater Decreases in Insulin Secretion (IS) Than Women (W): A Cross-Sectional Analysis
RALPH RAYMOND, DANNY CHEN, CLAUDIO COBELLI, CHIARA DALLA MAN, DOUGLAS S. LEE, R. PAUL ROBERTSON, SUDHA S. SHANKAR, DARKO STEFANOVSKI, MARIA T. VASSILEVA, DAVID FRYBURG, FOR BETA CELL TEAM OF FNIH BIOSCIENCES CONSORTIUM, Skillman, NJ; Grooten, CT, Padova, Italy; Cambridge, MA; Seattle, WA; Indianapolis, IN; Los Angeles, CA; Bethesda, MD; East Lyme, CT

Little is known about gender differences in IS. As part of a project examining the utility of Beta cell function tests, we studied effect of gender on IS response to arginine (ARG) stimulation test (AST) and the mixed meal tolerance test (MMTT). ANOVA WITHIN women OR men for IS and across populations. ANCOVA showed that in both AST and MMTT, decline in IS for M > W.

N=11 T2Dm (m)
N=11 PDm (m)
N=11 PDm (W)
N=11 ngT (m)
N=11 ngT (W)

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219-LB
The Serine Protease Prostasin Regulates Hepatic Insulin Sensitivity by Modulating TLR4 Signaling
KOHEI UCHIMURA, MANABU HAYATA, TERUHIKO MIIZUMOTO, YOSHIKAZU MIYASATO, YUTAKA KAKIZOE, KAZUKI FUKUDA, TATSUYA KONO, TAKESHI MATSUMURA, EIICHI ARAKI, KIMIO TOMITA, KENCHEI KITAMURA, KAMAMOTO, Japan

Although the effects of a high fat diet (HFD) and postprandial endotoxemia in the development of type 2 diabetes have been extensively studied, the precise mechanisms are not fully understood. Here we show that a serine protease prostasin (PRSS8) regulates hepatic insulin sensitivity by modulating Toll-like receptor 4 (TLR4)-mediated signaling. We demonstrate that HFD triggers the suppression of PRSS8 expression by inducing endoplasmic reticulum (ER) stress and increases TLR4 levels in the liver. PRSS8 released the endowment of TLR4 by cleaving at the Lys560/Lys561 residues, which resulted in a reduction in the full-length form at the plasma membrane and reduced activation of TLR4 by its ligands. Liver-specific PRSS8 knockout (LKO) mice developed hepatic insulin resistance associated with an increase in hepatic TLR4. Restoration of PRSS8 expression in the liver of HFD, LKO, and db/db mice decreased TLR4 levels and ameliorated hepatic insulin resistance. Furthermore, we demonstrated that a major component of serum PRSS8 may originate from the liver and that the serum PRSS8 levels were negatively correlated with body mass index (BMI) and homeostasis model assessment-insulin resistance (HOMA-IR) in healthy human subjects. Our results identify a novel role for PRSS8 and provide a new insight into the development of diabetes resulting from HFD or metabolic endotoxemia.
**220-LB**

Selectivity Silencing of Nfkb in Kupffer Cells Improves Systemic Insulin Sensitivity in Obese Mice

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Obesity is often accompanied by liver inflammation that could promote fatty liver disease and insulin resistance. Activation of liver macrophages, known as Kupffer cells (KC), may contribute to hepatic lipid accumulation and impairment of insulin signaling. However, this hypothesis has not been directly tested due to lack of technology to manipulate gene expression specifically in KCs without targeting other cell types or organs. To address this question, we developed a system based on glucan-encapsulated siRNA particles (GeRPs) to selectively deliver siRNA to KCs in vivo. Following intravenous administration in obese mice, GeRPs were internalized by KCs in liver but were not detected in hepatocytes or macrophages within other tissues. Importantly, GeRPs loaded with an siRNA targeting Nfkb, a major regulator of inflammation, selectively silenced its expression in KCs, while hepatocytes were unaffected. GeRP-mediated silencing of Nfkb resulted in a decreased expression of downstream cytokines, including IL-1β. Strikingly, silencing Nfkb in KCs, over a 14-day period, improved glucose tolerance in genetically obese mice. Taken together, these results demonstrate a major contribution of KCs in the development of insulin resistance. Furthermore, the GeRP technology provides a unique method to validate novel therapeutic targets expressed by KCs involved in hepatic inflammation and insulin resistance.

Supported By: ADA (7-12-MN-14), DK085753, AO48629, 17-2009-546, DK25220

**221-LB**

The b-ZIP Transcription Factor E4BP4 as a Novel Regulator of Hepatic Glucose Metabolism in Obesity

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Increased gluconeogenesis in the liver is one of the key pathological changes in diabetic patients. FOXO1-dependent activation of gluconeogenic enzymes such as PEPCK and G6Pase has been shown to contribute significantly to increase gluconeogenesis of diabetic mouse models. However, it remains unclear how liver FOXO1 activities are persistently enhanced in diabetic conditions. A previous study suggests an interaction between FOXO1 and E4BP4, a b-ZIP transcription factor, in cancer cells. In our lab, we observed that the mRNA and protein levels of E4BP4 were elevated in the liver tissues of both ob/ob mice and high-fat diet-fed mice. Whether E4BP4 can influence FOXO1 expression and gluconeogenesis in diabetic condition has not been investigated yet. Here we reported that E4BP4 controls hepatic gluconeogenesis by regulating FOXO1 protein expression and activity in a diet-induced mouse model. Genetic deletion of E4BP4 protects mice from high-fat-diet-induced hyperglycemia and insulin resistance. Compared with wild-type mice, E4bp4-/- mice displayed about 50% lower glucose levels and 50% lower gluconeogenesis in liver tissues under fasting condition. Moreover, we reported that E4BP4 controls hepatic gluconeogenesis by regulating FOXO1 and G6Pase expression and glucose production, whereas acute over-expression of E4BP4 increases G6Pase expression and glucose production, supporting a cell-autonomous role of E4BP4 in regulating gluconeogenesis in hepatocytes. E4BP4 deletion suppresses the FOXO1-induced G6Pase-luc activity while reducing the protein abundance of FOXO1. Further analysis showed acute E4BP4 knockdown promotes FOXO1 protein polyubiquitination and subsequent proteasome-dependent degradation in cultured hepatocytes. In summary, our results highlight a critical role for E4BP4 in regulating FOXO1 expression and hepatic gluconeogenesis in diabetic conditions, indicating that inhibition of E4BP4 expression or function might be a novel target to treat hyperglycemia in diabetes.

Supported By: P000K020572

**222-LB**

Elevated Systemic Soluble Amyloid Precursor Protein β as a Risk Factor for the Development of Type 2 Diabetes Mellitus

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Epidemiological studies have shown that type 2 diabetes mellitus (T2D) is highly correlated with Alzheimer’s disease (AD). T2D doubles the risk of developing AD and, conversely, individuals with AD are more likely to exhibit impaired fasting glucose levels. It has been suggested that insulin resistance may represent a common pathological link connecting these two chronic diseases. To date, the most reliable marker for AD progression remains the proteolysis of amyloid precursor protein (APP) into amyloid peptide Aβ and soluble APPβ (sAPPβ). To determine if there is a mechanistic connection between these circulating factors and peripheral insulin resistance, we collected plasma samples from a subset of participants in the Pfizer/Massachusetts General Hospital-sponsored Cardiology and Metabolic Patient Cohort (CAMP Cohort). The CAMP Cohort consists of 4,000 phenotyped subjects that include lean and obese individuals with and without insulin resistance and T2D. Analysis of the plasma samples revealed a positive correlation between Aβ and glucose levels (r>=0.0, p< 1.0e-08) and a negative correlation between Aβ and total plasma cholesterol, HDL and LDL after adjusting for T2D status and BMI (p = 0.001 for HDL, p < 0.05 for all 3). We then explored diet induced insulin resistance in APP over expressing mice (tg2576). When placed on a high-fat diet, APP overexpressing mice (tg2576) had greater body weight gain, impaired glucose and insulin tolerance, and increased hepatic insulin resistance compared to wild type animals. Treatment of human or murine primary hepatocytes with recombinant human sAPPβ strongly impaired insulin signaling. Comparison of the copper-binding domain of human sAPPβ with human insulin revealed striking structural similarities suggesting sAPPβ could directly interfere with insulin action. These observations provide a potential molecular explanation for the peripheral and central insulin resistance observed in T2DM and AD patients.

**223-LB**

PLA2G5 Regulates Glucose and Fatty Acid Metabolism in Human Hepatocytes

Christian Pehmiller, Jessica Calloway-Jones, Lanba Adhiraj, Nicholas B. Vera, Cécile Vernichet, Olivier Bezy, Cambridge, MA

Cardiac metabolism in obesity and type 2 diabetes (T2D) has been proposed to regulate whole body energy homeostasis. Several observations provide a potential molecular explanation for the peripheral and central insulin resistance observed in T2DM and AD patients. In vitro treatment of human hepatocytes with hPLA2G5 resulted in rapid (in 10 minutes) internalization of hPLA2G5 and a similar FDA profile exclusively released into the cytoplasm of hepatocytes. To further assess the effect of PLA2G5 on hepatic insulin metabolism, we performed metabolic flux analysis ( Seahorse Flux Analyzer) following overnight hPLA2G5 exposure. Our data revealed an increase in both oxygen consumption rate (OCR) and fatty acid oxidation (FAOx). Consistent with the increase in FAOx, extracellular acidification rates (ECAR) indicated glycolysis was significantly reduced following hPLA2G5 incubation. Gene expression analysis indicated a down regulation of genes involved in glycolysis and TCA cycle in response to hPLA2G5. These data further establish PLA2G5 as a key regulator of energy homeostasis and suggest PLA2G5 is an important regulator of hepatic metabolism.

**224-LB**

The Relationship between Sarcopenia and Non-alcoholic Fatty Liver Disease: The Korean Sarcopenic Obesity Study

Hye Jin Yoo, Ho Cheol Hong, Sin Gun kim, Nan Hee kim, Sei Hyun Baik, Dong seop Choi, Kyung Mook Choi, Nam Hoong Kim, Sun Hwa Kim, Seoul, Republic of Korea

Previous studies have shown that non-alcoholic fatty liver disease (NAFLD) and sarcopenia may share pathological mechanisms, such as insulin resistance, inflammation, vitamin D deficiency, and decreased physical activity. However, their direct relationship has not been investigated. The association between NAFLD and sarcopenia may share pathophysiological mechanisms, such as insulin resistance, inflammation, vitamin D deficiency, and decreased physical activity. In this study, we examined the association between NAFLD and sarcopenia in overweight and obese adults enrolled in the Korean Sarcopenic Obesity Study (KSOs), an ongoing prospective observational cohort study. As the liver attenuation index (LAI), which measured using abdominal computed tomography (CT), was used as a parameter for the diagnosis of NAFLD. Sarcopenia was defined using a skeletal muscle mass index (SMI) (SMI (%) = total skeletal muscle mass (kg) / weight (kg) x 100) that was measured by dual energy X-ray absorptiometry (DXA). After adjusting for age and sex, both SMI and LAI were negatively correlated with the homeostasis model assessment of insulin resistance (HOMA-IR) (r<0.001) and high sensitivity C-reactive protein (hsCRP) (r<0.001) as well as brachial ankle pulse wave velocity (baPWV), an indicator of arterial stiffness. Furthermore, SMI and LAI had positive relationships with HDL-cholesterol, but negative relationships with TG, total body fat, and total body water. In a multiple logistic regression analysis, the odds ratio for NAFLD risk was 5.16 (95% CI = 1.63-16.33) in the lowest quartile of SMI and 2.16 (95% CI = 1.0-4.67) of LAI. This study suggests the importance of screening for sarcopenia in NAFLD patients.
SMI compared to the highest after adjusting for potential confounding factors. Conclusion. Individuals with lower muscle mass exhibited increased risk of NAFLD. This result may provide a novel insight into the mechanism linking between sarcopenia and NAFLD.

225-LB

AAV8-mediated SIRT1 Gene Transfer to the Liver Prevents High Carbohydrate Diet-induced Non-alcoholic Fatty Liver Disease

IVET ELIAS, LAIA VILA, CARLES ROCA, ALBERT RIBERA, TURA FERRE, ALBA CAVELLAS, RICARDO LAGE, SYLVIE FRANCKHAUSER, PATIMA BOSCH, Barcelona, Spain

Non-alcoholic Fatty Liver Disease (NAFLD) is the most common hepatic disease worldwide, and evidence suggests that it promotes insulin resistance and type 2 diabetes. To date, the only treatment capable of counteracting or ameliorating liver steatosis is based on lifestyle intervention by means of caloric restriction (CR). The protein deacetylase Sirtuin1 (SIRT1), which is activated by CR, increases cataleptic metabolism and decreases lipogenesis and inflammation, both involved in the development of NAFLD. Here we show that adipose-associated viral vectors of serotype 8 (AAV8)-mediated liver-specific Sirt1 gene transfer prevents the development of NAFLD induced by a high carbohydrate (HC) diet. Long-term hepatic SIRT1 overexpression led to up-regulation of key hepatic genes involved in beta-oxidation such as Peroxisome proliferative activated receptor gamma, coactivator 1 alpha (Pparg-1a), Long-chain acyl-CoA dehydrogenase (Acad), Very long chain acyl-CoA dehydrogenase (Acadvl), Sirtuin 6 (Sirt6) and Sirtuin 3 (Sirt3), prevented HC diet-induced lipid accumulation, reduced macrophages infiltration and liver inflammation. AAV8-Sirt1-treated mice showed improved insulin sensitivity, increased oxidative capacity in skeletal muscle and reduced white adipose tissue inflammation. Moreover, HC feeding induced leptin resistance, which was also attenuated in AAV8-Sirt1-treated mice. Therefore, AAV-mediated gene transfer to overexpress SIRT1 specifically in the liver may represent a new gene therapy strategy to counteract NAFLD and related diseases such as type 2 diabetes.

226-LB

Argininosuccinate Synthetase Regulates Hepatic AMPK Activity Linking Protein Catabolism and Ureagenesis to Hepatic Glucose Metabolism

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AMP-activated protein kinase (AMPK) is a key sensor of cellular energy status and plays a critical role in the regulation of major metabolic processes. AMPK activation relies on allosteric interaction with AMP via binding at the y subunit, which increases Thr172 phosphorylation. The urea cycle enzyme argininosuccinate synthetase (ASS1) produces a single AMP per turn of the urea cycle, a predominant pathway in the liver. Here we show that ASS1 activity regulates hepatic AMPK activity, revealing a central role for urea cycle flux in the regulation of cellular metabolism via AMPK. Antisense oligonucleotide (ASO) knockdown of hepatic ASS1 gene expression in awake rats reduced liver AMPK activation by 57±6% (P<0.05), and decreased phosphorylation of the downstream AMPK target acetyl-coA carboxylase (ACC) by 27±7% (P<0.05). ASS1 ASO treatment increased plasma glucose concentrations (106±5 mg/dL vs. 141±6 mg/dL, P<0.01), without altering rates of endogenous glucose production. Acute intravenous treatment of rats with L-citrulline [30 mg/kg], the substrate of ASS1, increased hepatic AMPK activation by 41±3% (P<0.01) and increased ACC phosphorylation. 35±10% (P<0.05). L-citrulline treatment decreased fasting plasma glucose concentrations (120±2 mg/dL vs. 141±6 mg/dL, P<0.01), inducing the inverse effect of hepatic ASS1 ablation. Significantly, immunoprecipitation (IP) of hepatic AMPK protein pulled down AMPK, and IP of AMPK brought down ASS1, providing evidence for a direct physical interaction between AMPK and ASS1. Taken together these findings demonstrate that the urea cycle enzyme ASS1 resides in a complex with liver AMPK and can lead to direct activation of AMPK via increased production of AMP. This interaction may link increased protein catabolism and ureagenesis with hepatic AMPK activation and alterations in hepatic glucose metabolism.

227-LB

Treatment with a Monoclonal Antibody Blocking the Glucagon Receptor Is Not Associated with Perturbations in Liver Function or Lipid Metabolism

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Blockade of the elevated glucagon signaling in patients with type 2 diabetes mellitus (T2DM) is emerging as an attractive potential treatment for this condition. Clinical trial data have shown robust HbA1c lowering in T2DM patients receiving glucagon receptor (GCCR) antagonists, however, this has been accompanied by elevations in liver function tests (LFT), and in some cases, increases in LDL-cholesterol (LDL-C), leading to the notion that blockade of glucagon signaling in humans may be obligated connected to these deleterious changes. As these changes have been observed upon treatment with small molecule antagonist drugs only, an alternate explanation may be hepatic pharmacophore accumulation and toxicity. Hence to address the role of mechanism versus drug modality, a monoclonal antibody (mAb), shown to fully block the GCCR, was administered to non-human primates (NHP). Clinical chemistry parameters were measured. No significant changes in LFT’s, LDL-C, and triglycerides (TG) were observed following repeat dosing of this mAb, comparing to both the treated animals’ baseline values and to a cohort receiving only vehicle. These data are in agreement with the observation that a human patient, who carries a homozygous GCCR PBBS mutation resulting in significantly reduced glucagon signaling, presents normal LFT’s, LDL-C and TG’s. Additional experiments were conducted in lean and disease mouse models using another mAb with cross-reactivity to murine GCCR. No treatment-related changes in LFT’s were observed in any model following chronic dosing. LDL-C showed variable and inconsistent responses to treatment, in contrast to the NHP and human data described, and likely reflects well known species differences in lipid metabolism. In summary, these data suggest glucagon signaling can be fully blocked without undesirable effects on LFT’s and LDL-C, and point to monoclonal antibodies as the potential modality of choice in the treatment of T2DM.

228-LB

Prevention of Diet-induced Hepatic Insulin Resistance by Antisense Oligonucleotides Targeted to mNDY

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mNDY as part of the SLC13 protein family is a high-affinity di- and tricarboxylate plasma membrane transporter involved in citrate import. In Drosophila, genetic depletion of INDY alters energy metabolism and extends lifespan. Mice lacking INDY are protected from both diet-induced and age-associated hepatic insulin resistance. Here, we examined the impact of selective hepatic knockdown of mammalian Indy protein (mNDY) expression using anti-sense oligonucleotides (ASOs) on hepatic glucose metabolism in 4 week high fat fed rats (n=5 per group) assessed by hyperinsulinemic-euglycemic (HEC) clamp studies. After 4 weeks of ASO treatment, mNDY mRNA expression was reduced by 91% (P<0.001) in the treatment group. The mNDY ASO treated rats showed a 34% reduction in fasting plasma insulin concentrations compared to the control group (14.5 vs. 3.6 µU/ml, P<0.05) and was associated with ~30% reduction in basal rates of endogenous glucose production (5.9 ± 0.6 vs. 4.8 ± 0.8 mg/kg/min). Furthermore, hepatic insulin responsiveness was increased in the mNDY ASO rats as reflected by increased suppression of hepatic glucose production during the HEC [19.7 ± 6.1%, P<0.05]. Taken together these data suggest that hepatic mNDY may be a novel therapeutic target for the treatment of hepatic insulin resistance and type 2 diabetes.

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229-LB

The ER Stress Sensor IRE1alpha Controls Fasting-induced Metabolic Adaptation Response in the Liver

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In eukaryotes, accumulation of unfolded/misfolded proteins in the endoplasmic reticulum (ER) activates the cellular unfolded protein response (UPR). The ER-localized transmembrane signal transducer IRE1 (insoluble-requiring enzyme 1) is an ancient ER stress sensor that possesses both protein Ser/Thr kinase and endoribonuclease (RNase) activities. Activated through trans-autophosphorylation and dimerization/oligomerization upon ER stress, IRE1 initiates a key branch of the UPR by non-conventional splicing regulation of the transcription factor XBP1 (X-box binding protein 1). Despite that
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the mammalian IRE1alpha-XBP1 branch has been implicated in metabolic processes; the exact metabolic role of IRE1alpha remains largely elusive. We found that hepatic IRE1alpha is a catalytic sensor that regulates the metabolic adaptation response to prolonged fasting. Deprivation of food or consumption of a ketogenic diet could activate the IRE1alpha-XBP1 pathway in mouse livers. Hepatocyte-specific ablation of IRE1alpha resulted in impaired of fatty acid oxidation and ketogenesis under chronic fasting or ketogenic conditions. Liver-specific ablation of XBP1 reversed the defects in IRE1alpha knockout mice. Furthermore, XBP1’s could directly bind to and activate the promoter of PPARalpha, the master regulator of starvation responses. These findings suggest that hepatic IRE1alpha promotes starvation-induced adaptive shift of fuel utilization through the XBP1s-PPARalpha pathway.

230-LB
An Immuno-affinity Method to Separate Chylomicrons from VLDL and to Ascertain the Conversion of Sugar to Fat by De Novo Lipogenesis in the Human Intestine

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The kinetics of VLDL and chylomicron lipoprotein particles as well as their respective triglyceride (TG) content are important to understand the mechanisms by which dietary or pharmacological interventions modify particle size, lipid profile and cardiovascular risk. Ultrafiltration cannot separate remnant chylomicrons from large VLDL particles. Therefore, we devised an immuno-affinity method to separate VLDL (apoB100) from chylomicron (apoB48) particles in triglyceride-rich lipoproteins (TRL) using an apoB100 antibody. The separated lipoproteins were analyzed by Silver Stain and revealed a depletion of apoB100 in the sequential flow-through fraction and the elution of apoB48 contained no apoB48. To further validate the separation process we examined the TG content of the separated VLDL and chylomicrons by GC-MS and LC-MS/MS analysis. Traditional GC-MS analysis was used to validate a triglyceride-specific palmitate enrichment LC-MS/MS technique. The incorporation of 1-13C-acetate into palmitate, using mass isotopomer distribution analysis, was used to calculate fractional de novo lipogenesis (DNL) in VLDL and chylomicrons.

Both LC-MS-MS and GC-MS analysis revealed a difference in the incorporation of 13C-acetate into palmitate between TG in the VLDL versus chylomicron particles. Additionally, LC/MS-MS analysis revealed a difference in the types of fatty acids incorporated in palmitate-containing TG in VLDL as compared to chylomicrons.

Together, these results demonstrate that we have developed and validated methods that: 1) allow for the isolation of apoB48 particles from apoB100 particles in human TRL samples; 2) permit the discernment of the fatty acid composition in palmitate containing TG and 3) support intestinal conversion of sugar to fatty acids by DNL. The physiological significance of enterocyte DNL on postprandial lipid profiles remains to be explored.

231-LB
Effect of Antisense Oligonucleotide Knockdown of Hepatic AMP Deaminase 2 Expression on AMPK Activity and Hepatic Fat and Glucose Metabolism


AMP activated kinase (AMPK) is a key regulator of hepatic fat oxidation and lipid synthesis through its regulation of acetyl CoA carboxylase (ACC) 1 and 2 activity. Furthermore, inhibition of AMPK activity is associated with hepatic fat accumulation through increasing hepatic lipogenesis and decreasing fat oxidation. Given that AMPK is a key activator of AMPK, it has been suggested that inhibition of hepatic AMP deaminase 2 (AMPD2), a key enzyme that converts AMP to IMP, would be a potential therapeutic target for nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2D). To test this hypothesis, we examined the effects of knockdown of hepatic AMPD2 expression using antisense oligonucleotides (ASO) in four week high-fed fat rats. AMPD2 ASO treatment did not alter fasting plasma glucose concentrations but surprisingly led to an increase in basal rates of endogenous glucose production (7.5±0.4 vs. 5.8±0.3 μmol/kg/min; P<0.05). Furthermore AMPD2 ASO treatment did not lead to activation of AMPK or downstream targets (JACC1 and 2 phosphorylation, hepatic malonyl-CoA concentrations (0.41 ± 0.05 control-ASO, 0.6 ± 0.1 pmol/mg AMPD2-ASO; P>0.05), suggesting that fatty acid oxidation in the liver was unaltered. Consequently AMPD2 ASO treatment did not impact hepatic insulin responsiveness, as reflected by similar suppression rates of endogenous glucose production during the hyperinsulinemic-euglycemic clamp, as well as hepatic triglyceride content (25±2 mg/g Control ASO vs. 21±2 mg/g AMPD2-ASO, P>0.05). Conclusion: These findings demonstrate that AMPK activity is not regulated by AMPD2 and that AMPD2 may not have therapeutic potential for NAFLD and T2D. Supported By: T32

232-LB
Endoplasmic Reticulum Stress and mirRNA-122/370 Expression in Mice Offspring: Effects of Maternal Consumption of High-Fat Diet

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Maternal consumption of high fat diet (HFD) has been associated to changes in lipid metabolism, hepatic steatosis and impairment in insulin signaling in hypothalamus and liver in the offspring. HFD also can modify the expression of miRNAs related to fatty metabolism and to active cellular response to endoplasmic reticulum stress (ERS) leading to obesity and insulin resistance. Our objective was to evaluate the miRNAs and ERS in the offspring before the development of obesity. To answer this question we used male offspring mice recently weaned (d28) from dams fed with HFD (HFD-0) or standard chow (SC-0) during pregnancy and lactation. We evaluated unfolded proteins response (UPR) activation, as an indicator of ERS, the expression of miR-122/370 and enzymes related to lipid metabolism in liver. Body weight, mass of white adipose tissue, food intake, and hepatic triglycerides were increased in HFD-0 compared to SC-0 mice (1.3, 3.0, 1.1, 1.5-fold, respectively). Furthermore, hypothalamic and hepatic level of p-ERK and p-eIF2alpha were increased in HFD-0 (1.4, 2.1, 1.8, 3.8-fold, respectively), as well as hypothalamic GRP78, GRP78 and XBP1s proteins compared to SC-0 (3.3, 2.7, 2.2-fold, respectively). In addition HFD-0 mice showed reduced hypothalamic p-AKT stimulated by insulin (2.1-fold), increased level of p-JNK1 (2.2-fold), and immunostaining to CD11c cells and Tnfalpha. Liver SC01 was increased in HFD-0 mice (3-fold), indicating an increase in phospholipid synthesis that contributes to ERS and liver triglycerides storage. Furthermore liver aGPAT expression increased (1.7-fold) while CPT1 and ACOAVL expression was reduced in HFD-0 compared to SC-0 (40% and 30%, respectively). In addition, liver from HFD-0 showed reduced expression of miR-122 (25%) and increased in miR-370 (3-fold) compared to SC-0. Taken together these results suggesting that recently weaned mice present metabolic and epigenetic changes before the development of obesity. Supported By: FAPESP (2009/50809-5)

233-LB
Clinical Utility of Serum Fibroblast Activation Protein in the Risk Assessment of Severe Liver Fibrosis Secondary to Non-alcoholic Fatty Liver Disease in Diabetes and Obesity

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Non-alcoholic fatty liver disease (NAFLD) is common in diabetes and obesity, sometimes occurring as non-alcoholic steatohepatitis (NASH) with fibrosis. Non-invasive methods to assess for NASH risk are needed. Objectives: To determine whether blood levels of the pro-fibrotic enzyme, fibroblast activation protein (FAP), have clinical value in assessing for significant fibrosis, particularly when combined with the established NAFLD Fibrosis Score (NFS). Research Design: Two adult cohorts were examined: 106 with type 2 diabetes who had transient elastography (Cohort 1) and 146 with morbid obesity who had liver biopsy (Cohort 2). Results: In Cohort 1, FAP level was an independent risk factor for median liver stiffness (MLS) ≥10.3 kPa (consistent with severe fibrosis) with odds ratio (per SD increase) of 2.0 (95% CI 1.2-3.4), p=0.006. There was 8.1 fold (95% CI 1.6-39.7) odds ratio of MLS ≥10.3kPa for those in the highest compared with the lowest FAP tertile (p=0.010). FAP levels below 730pmol AMCl/min/mL had 95% negative predictive value for significant fibrosis. Low FAP reclassified 41% of 64 patients from “intermediate risk” of severe fibrosis by NFS, to “low risk.” In Cohort 2, per SD increase in FAP, there was 1.7 fold (95% CI 1.1-2.8) increased odds of significant fibrosis (F ≥3) and 8.1 fold (95% CI 1.6-39.7) odds ratio of MLS ≥10.3kPa for those in the highest compared with the lowest FAP tertile (p=0.010). FAP levels below 730pmol AMCl/min/mL had 95% negative predictive value for significant fibrosis. Low FAP correctly reclassified 49% of 73 patients from “intermediate risk” of severe fibrosis by NFS, to “low risk.” Conclusions: Circulating FAP co-ordinates with liver fibrosis in NAFLD and lower FAP combined with NFS has clinical utility in excluding severe fibrosis in populations with type 2 diabetes and obesity. Supported By: NHMRC

For author disclosure information, see page LB91.
INTEGRATED PHYSIOLOGY—MACRONUTRIENT METABOLISM AND FOOD INTAKE

234-LB
Dysregulation of Intestinal Glucose Transporters to Systemic and Luminal Glucose Cues in Type 2 Diabetes
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Expression of intestinal sweet taste receptors (STRs) is dysregulated in type 2 diabetes (T2D), in association with augmented glucose absorption. [1] Here, we determined whether expression of the glucose transporters (GTs) sodium-glucose co-transporter-1 (SGLT-1) and glucose transporter-2 (GLUT2) were (i) regulated acutely by changes in luminal and blood glucose in non-diabetic subjects (ND), (ii) modified in T2D, and (iii) associated with changes in expression of the STR, T1R2. Eleven ND subjects (BMI: 31 ± 3y, BMI: 25 ± 2 kg/m²) and 12 T2D patients (BMI: 28 ± 1 kg/m²) were studied during euglycemia (6 mmol/L) and hyperglycemia (12 mmol/L) on 2 study days. Duodenal biopsies were collected at baseline and after a 30 min duodenal glucose infusion (30g/150ml water + absorption marker 3-O-methylglucose (3-OMG)) to assess transcript levels. Patients with T2D showed dysregulated transcription of intestinal GTs following luminal glucose, and a lack of suppression in response to elevated blood glucose (see table). Responses of SGLT-1 to luminal glucose indicate a specific contribution of this GT to augmented glucose absorption during hyperglycemia in T2D patients. Changes in T1R2 transcript levels were related to glucose absorption in T2D patients (3-OMG, P ≤ 0.05), however, GT transcription is regulated acutely by factors other than STRs in humans.

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235-LB
A Moderate Calorie Restriction and Intermittent Fasting Prevent Type 2 Diabetes in a Diabetic Mouse Model by Increasing Fatty Acid Oxidation in Glycolytic Muscles
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Calorie restriction (CR) and intermittent fasting (IF) are known to have several beneficial effects on health, longevity and prevention of type 2 diabetes (T2D). The aim of the present study was to clarify detailed mechanisms on the suppression of T2D by these dietary interventions.

We tested if moderate CR and elongated fasting periods prevent the onset of T2D in New Zealand Obese (NZO) mice, a model for polygenic obesity and diabetes. At 4 weeks of age male NZO mice were fed a high-fat diet (HFD; 35% calories from fat) ad libitum (AL) or were subjected to 10% CR. Additionally, one group had access to HFD AL but was fasted every other day (IF). Body weight development, as well as blood glucose, body composition and diabetes prevalence (73 g glucose + 2 g [U13C] glucose) were studied during euglycemia (6 mmol/L) and hyperglycemia (12 mmol/L) on 2 study days. Duodenal biopsies were collected at baseline and after a 30 min duodenal glucose infusion (30g/150ml water + absorption marker 3-O-methylglucose (3-OMG)) to assess transcript levels. Patients with T2D showed dysregulated transcription of intestinal GTs following luminal glucose, and a lack of suppression in response to elevated blood glucose (see table). Responses of SGLT-1 to luminal glucose indicate a specific contribution of this GT to augmented glucose absorption during hyperglycemia in T2D patients. Changes in T1R2 transcript levels were related to glucose absorption in T2D patients (3-OMG, P ≤ 0.05), however, GT transcription is regulated acutely by factors other than STRs in humans.

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236-LB
A Human Model of Oral Saturated Fatty Acid Induced Insulin Resistance
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Models of lipid-induced insulin resistance (IR) in humans often rely on acute infusion of fatty acid (FA) mixtures to study mechanisms of IR and its potential treatments. Limitations of this method of inducing IR include bypassing the gastrointestinal tract, non-physiological acute plasma FA elevation and use of a more soluble unsaturated FA, rather than the saturated FA (SFA) present in the typical Western diet. We therefore developed an oral human model of a SFA-enriched diet induced IR.

In a series of cross-over studies, subjects with normal or impaired glucose tolerance (NGT or IGT) consumed a SFA-rich, high-calorie diet compared to a standard American Heart Association (AHA) diet for 24 hours (breakfast to bedtime) or daylong (breakfast to bedtime). IR was determined 4, 10 and 24 hours after completion of each diet from steady state plasma glucose (SSPG) levels during the final 30 minutes of a 3-hour insulin suppression test (IST). SSPG was increased 61% 4 hours (Figure) after 24-hour of a SFA diet. IR increased in both NGT and IGT, and persisted 10 (overnight) or 24 hours after the last SFA meal (Figure insert).

In summary, we developed a human model of diet-induced IR by use of short-term oral administration of SFA. The SFA diet induced IR in both NGT and IGT subjects and persisted for at least 24 hours. This model offers unique opportunities for identifying mechanisms and potential treatments of diet induced IR.

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237-LB
Six Days High-Fat Overfeeding Does Not Alter Whole-Body Insulin Sensitivity in Young, Healthy Males
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We aimed to investigate the whole body mechanisms associated with consumption of high fat, high energy diets in healthy males using dual stable isotopic tracer methodology. A secondary aim was to consider whether increased fish oil (FO) intake could protect against diet induced insulin resistance and to examine the mechanisms for this effect. Twenty healthy males (22 ± 1y; 71 ± 2 kg) were matched to 1 of 2 groups; all underwent 6 of high fat overfeeding (150% of total kcal, 10% FAT, 25% CHO, 15% PTO). One group received 10% of fats from FO (FO, n = 10) while the other consumed mostly SFAs and MUFA (HF, n = 10). The overfeeding was bokced by two trial days; identical in all respects. Following an overnight fast participants underwent a primed continuous [6,6-2H2]glucose infusion followed 1h later by an OGTT (73 g glucose + 2 g [U13C] glucose) with blood samples drawn at 10 min intervals. Whole blood fatty acid profiling by GCMS revealed a significant elevation of basal EPA and DHA in the FO group only (p < 0.05) confirming dietary compliance. Insulin sensitivity, indicated by Matsuda and HOMA-IR, and plasma glucose kinetics (CI-GCMS) were unaltered by high fat overfeeding (Image 1). Despite changes in plasma fatty acid composition, six days of high fat overfeeding does not alter whole body insulin action in healthy males. However, changes in tissue specific mechanisms may precipate whole body insulin resistance development.
Standardizing Diet Significantly Reduces Inter-subject Variability in Metabolomic Profiles

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Metabolomic profiling is used in clinical trials to detect treatment effects of anti-diabetic compounds, but inter-subject variability may reduce precision and reproducibility. The purpose of this study was to assess the value of standardizing diet prior to metabolomic profiling. Plasma was collected from 64 healthy fasting subjects on admission (street diet) and after consuming 6 identical meals over 48 h in a research unit (standard diet). Metabolomic analyses were performed by liquid chromatography - mass spectrometry (LC-MS) on Thermo Exactive systems. Raw mass spectral data were processed with in-house metabolomic software. Peak areas reported for 192 individual metabolites were analyzed for inter-subject variability and outliers by open source R scripts. Inter-subject variability (CV) for the 192 metabolites was significantly higher on street diet than on standard diet, 59% CV vs. 40% CV (p <0.001). Feeding an identical diet for 48h prior to sample collection caused 19% absolute reduction and 32% relative reduction in inter-subject variability and 50% reduction in outliers (p<0.002). Standardizing the diet of study subjects in phase I-II drug trials may enhance precision and reproducibility of metabolomic profiling by reducing inter-subject variability.

Supported By: Diabetes Research and Wellness Foundation

INTEGRATED PHYSIOLOGY—MUSCLE

239-LB

Mutation of Leucine-rich Pentatricopeptide Repeat Containing Protein (LRPPRC) Leads to Impaired AMPK Regulation in Leigh Syndrome French Canadian Type (LSFC) Fibroblasts

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LSFC is a rare mitochondrial disease caused by mutations in the LRPPRC gene. This leads to a decrease in both the LRPPRC protein and mitochondrial respiratory chain enzyme cytochrome c oxidase (COX) activity. Mitochondrial diseases increase the risk of developing diabetes. We hypothesized that mitochondrial dysfunction in LSFC patients would induce alterations in energy- and nutrient-signaling pathways similar to those found in insulin-resistant and diabetic patients. Using skin fibroblasts as a model, we examined AMPK activation, a key pathway involved in energy homeostasis, under various stress conditions. Basal AMPK activity was similar in control and LSFC cells. In control cells, acute nutrient overload (1 mM palmitate + 10 mM lactate for 4 h) increased AMPK and acetyl CoA carboxylase (ACC) phosphorylation by 90% (p<0.01) and 154% (p<0.05), respectively. Palmitate/lactate also increased the expression of SIRT1, a downstream target of AMPK, by 57% (p<0.05), LRPPRC by 23% (p<0.05) and COXIV by 19% (p<0.05). All these effects were absent in LSFC cells. We also evaluated the effect of chronic AMPK activation on these signaling pathways using a specific activator of AMPK, ZMP (0.5 mM, 48 h). The effects of ZMP pretreatment were additive to those of palmitate/lactate, leading to further increases in the phosphorylation of ACC (364%, p<0.001) and SIRT1 (120%, p<0.05), as well as increased expression of SIRT1 (79%, p<0.01), LRPPRC (63%, p<0.001) and COXIV (38%, p<0.001). Overall, all these effects were absent in LSFC cells. However, AMPK could be activated in response to chemical hypoxia induced by dinitrophenol (0.1 mM, 10 min) in LSFC fibroblasts. In conclusion, LSFC cells showed impaired AMPK activation in response to nutrient overload. The reduction in nutrient-induced AMPK activation may contribute to the development of insulin resistance in these patients and ultimately predispose them to diabetes.

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INTEGRATED PHYSIOLOGY—MUSCLE

240-LB

Sixteen Weeks of Caloric Restriction in Abdominally Obese Adults Improves Skeletal Muscle Insulin Sensitivity and Preserves Mitochondrial Function

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Reductions in body weight and abdominal fat by caloric restriction (CR) improve insulin action, which is often impaired in overweight and obese individuals. Improvements in the efficiency of fuel utilization by mitochondria are hypothesized to be an underlying mechanism responsible for CR’s insulin sensitizing effects. The purpose of this study was to investigate the effect of CR on insulin sensitivity and mitochondrial function in abdominally obese men and women (45-65 years) before and after a 16-week CR program or Control (CON) period. Fifteen (15) abdominally obese participants undertook a CR program (9) managed by the dietician staff at the Mayo Clinic Clinical Research Unit that resulted in weight loss averaging 9.5% ± 1.4% of total bodyweight or CON (6). Percent body fat declined from 45.4 ± 1.9% to 41.8 ± 1.5% (P<0.05) with reductions in total fat mass (42.0 ± 2.9 kg vs. 35.0 ± 2.5 kg; P<0.05) and no change in lean body mass (50.7 ± 3.9 kg vs. 49.0 ± 3.8 kg) in CR, while the CON group did not change. These changes resulted in body mass index declining from 33.8 to 30.8 (P<0.05) in CR with no change 34.1 to 34.7 in CON. The CR program lowered post absorptive overnight fasting blood glucose levels from 107.4 ± 3.1 mg/dL to 102.6 ± 3.0 mg/dL (P<0.05) and increased the glucose infusion rate required to maintain glycemia during a 6-hour two-stage hyperinsulenic-euglycemic clamp (P<0.05), while the CON showed no change. No change in response to the 16-week intervention for state 3 skeletal muscle mitochondrial capacity (452.8 ± 34.5 vs. 393.8 ± 52.1 in CR and 505 ± 42.1 vs. 510.3 ± 44.6 in CON pmol/s/mg tissue) or respiratory control ratio (0.8 ± 0.6 vs. 5.8 ± 0.4, CR and 6.2 ± 0.6 vs. 7.3 ± 0.6 in CON) was found as measured by high-resolution respirometry of mitochondria isolated from vastus lateralis muscle biopsies. In conclusion a 16-week CR program in abdominally obese individuals increases skeletal muscle insulin sensitivity and preserves mitochondrial function.

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Enhanced mTOR Signaling Attenuates Cardiac Injury in OVE26 Diabetic Mice

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Diabetic cardiomyopathy is an important causative factor for the heightened risk of heart failure in diabetic patients. Yet, our understanding of the underlying mechanism has been limited, making it difficult to design effective strategies for preventing diabetic heart failure and reducing the high mortality in diabetic patients. The serine/threonine protein kinase mammalian target of rapamycin (mTOR) has been implicated in the pathogenesis of several types of heart disease. mTOR signaling is activated in diabetic heart. However, the functional significance of mTOR in the diabetic heart remains unclear. We addressed this question by crossing the OVE26 type 1 diabetic mice with transgenic mice expressing a constitutively active (CA) mTOR or dominant negative (DN) mTOR in the heart. Diabetes-induced cardiac damage was substantially attenuated in CA-mTOR mice as shown by improved cardiac function as well as reduced levels of oxidative stress, interstitial fibrosis and myocyte apoptosis. Conversely, diabetic cardiac damage was markedly exacerbated in DN-mTOR mice, suggesting that the increased mTOR signaling is an adaptive response that limits cardiac dysfunction in type 1 diabetes. CA-mTOR expression inhibited autophagic flux in the heart, while DN-mTOR accelerated this process, consistent with the regulatory role of mTOR in autophagy. Since autophagy is detrimental in type 1 diabetic heart, mTOR-induced cardioprotection may be mediated, at least in part, by its inhibitory effect on autophagy. Together, these findings demonstrate that the enhanced mTOR signaling protects from cardiac injury in type 1 diabetes likely through the inhibition of autophagy.

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Mitochondrial Metabolism of Diabetic Skeletal Muscle Measured by Hyperpolarized 13C MR Spectroscopy

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We have performed in vivo experiments using hyperpolarized [1-13C] lactate (Lac), [2-13C]pyruvate (Pyr), and diluciferolactate (DCA) to examine in control (CRL) and T2DM skeletal muscle the pyruvate dehydrogenase (PDH) and tricarboxylic acid cycle, which reflects oxidative metabolism activity. Sprague-Dawley (SD) rats with UCD-T2DM and CRL SD rats were scanned using a 3T MR scanner. Immediately after a 40-mM hyperpolarized [1-13C]Lac bolus injection, [13C] MR signal was acquired from CRL (n=6) and T2DM rats (n=5), and 3 of the T2DM rats were additionally scanned following another 40-mM Lac injection 1h after a DCA infusion. A separate group of animals were scanned after injecting 80-mM hyperpolarized [2-13C]Pyr (n=3 for each group). Bicarbonate (Bic), which reflects the PDH activity, was significantly lower (P<0.02) in T2DM than in CRL when [1-13C]Lac was injected. However, DCA remarkably increased Bic, indicating that PDH in T2DM muscle can be activated. When [2-13C]Pyr was injected [1-13C]lactoyl-carnitine (ALC), [1-13C] acetate (ACC), [5-13C]glutamate (Glu) appeared in CRL and T2DM muscle after DCA infusion. Surprisingly, PDH was more activated by DCA in diabetic models than in CRL. While Glu was comparable between T2DM and CRL, ALC and ACC in T2DM muscle were about twice higher than in CRL, indicating that PDH activity and oxidative metabolism differ in T2DM vs. CRL skeletal muscles.

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Activation of 4E-BP1 in Skeletal Muscle Protects against High Fat Diet- and Age-induced Metabolic Dysfunctions

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Obesity is a major risk factor driving the global type II diabetes pandemic. Yet, the molecular factors linking obesity to disease remain to be fully elucidated. It is unclear why only a subset of obese individual progress to metabolic syndromes and the others do not. Gender differences are also apparent in humans and in murine models. For instance, male and female mice fed a high fat diet (HFD) similarly become obese, but males are prevalence to develop insulin resistance and glucose intolerance, the hall marks of type II diabetes. Here we report gender differences in expression of eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) upon HFD feeding. 4E-BP1 expression is significant reduced in skeletal muscle and adipose tissue of male mice, but not female mice. Strikingly, transgenic whole body 4E-BP1 expression protects male but not female mice against HFD-induced obesity and insulin resistance suggesting that 4E-BP1 is a gender-specific suppressor of metabolic dysfunctions. 4E-BP1 represses cap-dependent mRNA translation initiation by sequestering elf4E and is a master effector on protein translation controlled by mTOR. We explore possible mechanisms that underlie the health benefits of reduced mTOR signaling with altered activity of 4E-BP1. We found that the selective activation of 4E-BP1, which is resistant to mTOR regulation, in mouse skeletal muscles, instead of adipose tissue, is protective against high fat diet-induced type II diabetes in both genders. These mice has increased energy expenditure, altered adipose tissue distribution including reduced white adipose accumulation and preserved brown adipose mass, and protected from hepatic steatosis. The results presented here suggest that (1) 4E-BP1 may be the critical target of downstream of mTOR that relates to metabolic diseases and (2) interventions activating 4E-BP1 may have therapeutic potential on diet or aging induced metabolic diseases.

Supported By: Ellison Medical Foundation

Galbladder Emptying and Single-Dose Metformin Elicit Robust and Additive Glucagon-like Peptide-1 Responses

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Preclinical studies suggest that gallbladder emptying and subsequent activation of the bile acid receptor TGR5 on enteroendocrine L cells leads to glucagon-like peptide-1 (GLP-1) secretion. Drugs affecting bile acid binding (colesevelam (COL)) or reabsorption (metformin (MET)) seem to increase postprandial GLP-1 secretion in humans. We hypothesized that gallbladder emptying stimulates human GLP-1 secretion and that COL and MET, respectively, would potentiate any GLP-1 secretion induced by gallbladder emptying.

For author disclosure information, see page LB1.
Ten subjects (age [mean±SD]: 23.4±3.8 years; BMI: 21.9±1.8 kg/m²; HbA1c: 5.1±0.3%) were studied on 6 randomized days. In a double-blind fashion the subjects received 1) COL (3.75 g); 2) MET (1.5 g); or 3) placebo (PLA) in 50 ml water admixed 1.5 g acetylaminoephiln (for evaluation of gastric emptying) administered via nasogastric tube, with a concomitant 60-minute iv infusion of saline and cholecystokinin-8 (CCK), respectively. Blood was sampled for 4 hours for measurements of plasma GLP-1, glucose, insulin, C-peptide and glucagon. Gastric emptying was measured by ultrasound. Food intake was assessed at the end of each day.

CCK infusion during PLA induced complete gallbladder emptying and a significant GLP-1 response (incremental area under curve) compared to saline infusion (392±173 (mean±SEM) vs. 277±94 pmol/min, P<0.02). MET without CCK elicited a significant GLP-1 response (215±87 vs. 273±94 pmol/min (saline+PLA), P=0.002), which was potentiated by CCK-induced gallbladder emptying (963±220 pmol/min (CCK+MET), P<0.003). COL did not elicit significant GLP-1 responses. Plasma glucose was not affected by the interventions, nor was insulin, C-peptide, glucagon or food intake.

CCK-induced gallbladder emptying and single dose MET, respectively, elicited robust and additive GLP-1 responses in humans. We, therefore, speculate that MET’s mode of action includes stimulation of GLP-1 secretion by both bile acid-dependent and independent mechanisms.

**246-LB**

**Ghrelin Antagonizes GLP-1 as well as Glucose-stimulated Insulin Secretion in Healthy Humans**

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The gastric hormone ghrelin suppresses insulin secretion and causes glucose intolerance in humans. Paradoxically these effects occur during meal absorption when ghrelin also increases GLP-1 secretion. We hypothesized that blocking GLP-1 action by Exendin-9 (Ex-9) would magnify the effect of ghrelin to impair glucose tolerance in healthy individuals. Eight healthy non-obese subjects (5 females, 3 males, aged 25±3 [mean±SD]) were randomly assigned to receive acyl ghrelin (2 μg/kg/h), Ex-9 (0.15 mg/kg/h), the combination of ghrelin and Ex-9, or saline on 4 different days. Ghrelin and Ex-9 were given as primed, continuous iv infusions for a total of 4.5 hours before and after consumption of a standardized mixed meal. Glucose and insulin were sampled continuous throughout the 240 min of the meal tolerance test (MTT). Ghrelin (p<0.01) and ghrelin plus Ex-9 administration (p=0.001) impaired glucose tolerance (AUcGluucose 0-240 min) (Ghrelin: 9092±4030; Ex-9: 9092±4030; ghrelin+ex-9: 41.0±19, p=0.037). No difference was found between ghrelin and Ex-9 plus ghrelin treatment. Ex-9 infusion alone did not alter glucose tolerance or insulin secretion. In conclusion, blocking endogenous GLP-1 action by Ex-9 did not further impair postprandial glucose tolerance or insulin secretion induced by ghrelin administration. These findings indicate that the effects of ghrelin to suppress insulin secretion are not modulated by its action to increase plasma GLP-1. This suggests that ghrelin antagonizes GLP-1- as well as glucose-stimulated insulin secretion.

Supported By: 1R01DK097550

**247-LB**

**Effects of Sitagliptin on Blood Pressure and Heart Rate in Response to Intraduodenal Glucose Infusion in Type 2 Diabetes: A Potential Role for GIP**


Meal ingestion induces secretion of GLP-1 and GIP, which may play a role in the regulation of blood pressure (BP) and heart rate (HR). We evaluated, in type 2 patients, the effects of the DPP-4 inhibitor, sitagliptin, on BP and HR during intraduodenal (ID) glucose infusion at 2kcal/min - a rate where GIP is the major incretin in the circulation.

10 type 2 patients were studied on two occasions 30min after oral ingestion of sitagliptin (100mg) or placebo. ID glucose was infused at 2kcal/min (80g over 120min). BP, HR, plasma inctreins, glucose and glucagon, and serum insulin were evaluated.

Sitagliptin increased HR (treatment effect: P=0.001) and serum insulin (treatment × time interaction: P=0.041), without affecting BP, plasma glucose or glucagon. During ID glucose infusion, there was a substantial increase in plasma total GIP on both days (P<0.001), but no increase in total GLP-1. After sitagliptin, plasma intact GLP-1 increased slightly (treatment × time interaction: P=0.044) and GIP substantially (P=0.003). The HR response to ID glucose was directly related to plasma intact GIP concentrations (r=0.75, P=0.008).

Sitagliptin increased the HR response to ID glucose at 2kcal/min in type 2 patients, associated with augmentation of plasma intact GIP concentrations. These observations suggest a potential role for GIP in the control of the “gut-heart” axis.
250-LB

 Estradiol Suppresses Liver Fat Accumulation and Reduces Diabetes Prevalence in New Zealand Obese Mice

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In humans, as well as in rodents, prevalence to type 2 diabetes (T2D) is sexually dimorphic with an increased incidence in males. However, after menopause in women and ovarectomy in mice the T2D incidence matches that of male counterparts. Thus, we hypothesized that estrogen has protective effects and examined the influence of estradiol (E2) on onset of T2D in female New Zealand Obese (NZO) mice.

NZO females exhibited a diabetes prevalence of 43% in week 22 on a high-fat diet (HFD, 60% fat). Liver fat content, as determined by computed tomography (CT), at week 10 was used as a predictor marker for diabetes-prone (DP) and diabetes-resistant (DR) NZO females. For subsequent 10 weeks, both DP and DR mice were either supplemented with E2 (800 µg/kg HFD, DP-E and DR-E groups) or not (DP-C and DR-C groups). At week 20, an additional CT scan of the liver was conducted. Transcriptome analysis of DP and DR livers was performed in week 11.

E2 treatment reduced diabetes prevalence in both DP-E and DR-E groups by 73%. Moreover, E2 prevented an increase in liver fat content and β-cell loss under HFD and improved insulin signalling in pancreatic β-cells. Transcriptome analysis and Western blotting revealed an increased abundance of the MOGAT1 enzyme and the CD36 fat transporter in livers of DP mice, accompanied by increased hepatic diacylglycerol concentration. Furthermore, E2 treatment reduced the expression of CD36 and MOGAT1 in both DP-E and DR-E groups. In silico analysis indicated a high abundance of putative estrogen responsive elements in the promoter region of Mogat1.

Early elevation of CD36 and MOGAT1 in the liver caused an enhanced production and accumulation of triglycerides and diacylglycerols, presumably resulting in reduced hepatic insulin sensitivity. Continuous administration of E2 could prevent this effect. Moreover, E2 improved insulin signalling in pancreatic β-cells and reduced the diabetes prevalence in NZO females.

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251-LB

 Insulin Suppresses Fatty Acid Binding Protein and Omentin Levels

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Circulating omentin (OM) and Fatty Acid Binding Protein (FABP) have been reported to be altered in insulin resistance (IR). However, the role of insulin (I) in the regulation of these adipokines remains unclear. We tested the hypothesis that I alters circulating OM and FABP, by measuring OM and FABP responses to elevating I in a group of healthy volunteers.

Our results show for the first time that hyperinsulinemia reproducibly suppresses omentin and FABP in healthy humans, suggesting a potential role for I in regulating omentin and FABP. This may have implications for the regulation of these adipokines in IR.

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Eradication of Methane on Breath Testing and Reduction in Intestinal M. smithii Results in Improved Insulin Sensitivity and Lipid Profiles in Prediabetic Obese Subjects

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The methanogenic archaea are important colonizers of the gastrointestinal tract, and produce methane which can be detected on breath analysis. Methanobrevibacter smithii is the most common methanogen in the human gut. Studies suggest that methane positive (M+) subjects have a greater BMI. Here, we examine metabolic parameters before and after antibiotic treatment in subjects with M. smithii colonization and methane on breath test.

Using ADA criteria, we identified 11 pre-diabetic (9 FM, 2 MF) obese (BMI 35.2±7.7kg/m2) M+ subjects aged 47±9 yrs. Subjects underwent breath testing, OGTT, lipid profile and gastric transit analysis. They then received a 10 day course of antibiotics (neomycin 500mg bid/ rifaximin 550 mg tid), shown to eradicate methane on breath test in up to 85% of patients. Testing was repeated post intervention.

Baseline M. smithii levels measured by qPCR of stool correlated with breath methane (R=0.7, P=0.03). Eight subjects (73%) eradicated breath methane and showed reduced stool M. smithii (P=0.09). After therapy, cholesterol (P=0.03) and LDL (P=0.08) were lower, with more pronounced reductions in methane-eradicated subjects (P=0.01 and P=0.028, respectively). Insulin sensitivity (SI), estimated using Modified Minimal Model for OGTT analysis, showed significant improvement pre vs. post-treatment (0.62 ± 0.21 vs. 0.95 ± 0.17, P=0.05). Further, unit change in methane tended towards being inversely proportional to SI change (P=0.08). Gastric emptying was unchanged.

Eradication of methane on breath testing and reduction of M. smithii in stool is associated with improved glucose metabolism and SI improvement up to 50%. Lipid profiles also improved significantly with eradication. The mechanisms linking reductions in methanogens to improvements in insulin sensitivity need further elucidation.

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Role of Kinin B1 Receptor in Streptozotocin-induced Insulitides

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Kinins are pro-inflammatory peptides whose effects are mediated by two GPCR, B1R and B2R. While B1R is virtually absent in sane tissues, it is highly inducible in diabetes and after exposure to pro-inflammatory cytokines. This study aims at investigating the mechanism by which kinin B1R partakes to insulitis. It is hypothesized that kinin B1R can either initiate the trafficking/ infiltration of immune cells into the pancreas or activate primary sensory C-fibers.

Insulitis. It is hypothesized that kinin B1R can either initiate the trafficking/ infiltration of immune cells into the pancreas or activate primary sensory C-fibers (CGRP and TRPV1) to cause neurogenic inflammation. Male rats were made diabetic with streptozotocin (STZ, 65 mg/kg/ ip) and treated with B1R antagonist (SSZ240612, 10 mg/kg/day for 7 days by gavage) or its vehicle. After sacrifice, the pancreas was harvested for studying insulitis. The expression of B1R, INOS, TNF-α,macrophages,TCDF4- CGRP and TRPV1 was measured in the pancreas by Western blot analysis, qRT-PCR and immunofluorescence. Number and size of Langerhans islets were measured by immunostaining with insulin antibody to evaluate the severity of damaged β-cells. Macrophages and TCD4+-lymphocytes were present abundantly throughout the pancreas of STZ-diabetic rats but absent in control. Importantly, B1R was expressed and upregulated on these immune cells infiltrating the diabetic pancreas. B1R was not expressed on primary sensory C-fibers even if the expression of TRPV1 and CGRP was significantly enhanced in the diabetic pancreas. This finding is not supporting a primary neurogenic inflammatory component mediated by B1R. SSZ240612 treatment prevented the infiltration of macrophages and TCD4+-lymphocytes in addition to normalizing the upregulation of B1R, INOS and TNF-α. Concomitantly, SSZ240612 reduced significantly hypoglycemia and partially restored plasma insulin levels by preventing the loss of Langerhans islets. Data suggest that kinin B1 receptor is a key player in insulitis and its antagonism may offer a new strategy to prevent destruction of Langerhans islets by immune cells assault in this model of type 1 diabetes.

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OBESITY—ANIMAL

256-LB

Hypomorphism for Rpgrip1l, a Ciliary Gene Vincinal to the FTO Locus Associated with Increased Body Weight in Humans, Causes Increased Adiposity in Mice

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Common polymorphisms in the first intron of FTO are highly associated with ~1.5 kg per-risk-allele increased body weight in adults. Previous studies have suggested that CUX1 regulatory elements in intron 1 of FTO control the expression of FTO and the nearby gene, Rpgrip1l. Given the implication of Rpgrip1l in the biology of the primary cilium, and the established role of ciliary genes in energy homeostasis, we explored the possibility that mice heterozygous for an Rpgrip1l null allele (Rpgrip1l+/−) would display obesity susceptibility comparable to the dose-dependent effect that the FTO intrinsic polymorphisms have on adiposity in humans. Rpgrip1l+/− mice are hypophagic, have more fat than +/+ littermates, and display diminished suppression of food intake in response to exogenous leptin. Moreover, mice deleted for Rpgrip1l in specific hypothalamic neuronal subpopulations displayed a similar phenotype, suggesting that Rpgrip1l hypomorphism in the hypothalamus may be the main cause of the apparent hyperphagia and increased adiposity of Rpgrip1l+/− mice. Supporting these in vivo observations, we find that in the hypothalami of Rpgrip1l+/− mice, and fibroblasts derived from humans segregating for hypomorphic mutations in Rpgrip1l, localization of ciliary marker ACII is diminished, accompanied by impaired localization of the leptin receptor in the vicinity of the cilium, and diminished pSTAT3 levels in response to leptin administration. These findings suggest a mechanism by which apparently functional polymorphisms in intron 1 of FTO affects Rpgrip1l expression and influences energy homeostasis.

257-LB

Integrin Ligand Mfge8 Is a Key Regulator of Fatty Acid Uptake, Obesity, and Insulin Resistance

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Obesity and insulin resistance are key risk factors in the development of coronary artery disease, stroke, and adult-onset diabetes mellitus. Fatty acid uptake by cells is critical for fat storage and the development of obesity which then promotes insulin resistance. Dietary triglycerides are broken down into free fatty acids prior to uptake for both storage and consumption in peripheral tissues. The mechanisms by which fatty acids are taken up by cells remain incompletely understood. Inhibition of fatty acid uptake by cells is one approach to prevent the development of obesity and insulin resistance. Expression of the integrin ligand Mfge8 is increased in human obesity and in mice on a high-fat diet (HFD). The role of Mfge8 in obesity is unknown. We found that Mfge8 promotes the development of obesity by facilitating cellular uptake of fatty acids. Mfge8 deficient (Mfge8−/−) mice absorb less dietary triglycerides and are protected from weight gain, steatohepatitis and obesity-associated insulin resistance on a HFD. Mfge8−/− cells have impaired fatty acid uptake in vitro and in vivo. Mfge8 coordinates fatty acid uptake through alpha v beta 3 and alpha v beta 5 integrin-dependent phosphorylation of Akt by PI3 kinase and mTOR complex 2 (Rictor) leading to translocation of CD36 and FadT1 from cytoplasmic vesicles to the cell surface. From the therapeutic viewpoint, delivery of Mfge8 to the small intestine may aid in the treatment of malabsorption syndromes. Alternatively, inhibition of the Mfge8-dependent pathway provides a novel therapeutic target for the treatment of obesity that directly inhibits the molecular pathways of fatty acid uptake by cells. Collectively, our results implicate a central role for Mfge8 in regulating fatty acid uptake and insulin resistance in multi organ systems.

258-LB

Xbp1s in Pomc Neurons Connects ER Stress with Energy Balance and Glucose Homeostasis

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The molecular mechanisms underlying neuronal leptin and insulin resistance in obesity and diabetes remain unclear. Here we show that induction of the unfolded protein response transcription factor “spliced X-box binding protein 1” (Xbp1s) in pro-opiomelanocortin (Pomc) neurons alone is sufficient to protect against diet-induced obesity as well as improve leptin and insulin sensitivity—even in the presence of strong activators of ER stress. The improved body weight was accompanied by increased energy expenditure and heat production. We also demonstrate that constitutive expression of Xbp1s in Pomc neurons contributes to improved hepatic insulin sensitivity and suppression of endogenous glucose production. Together our results identify critical molecular mechanisms linking ER stress in arcuate Pomc neurons to acute leptin and insulin resistance as well as liver metabolism in diet-induced obesity and diabetes.

Supported By: NIH

259-LB

Chronic Postnatal Overfeeding in Female Mice Predisposes Development of Obesity in Their Offspring via an Altered Central Leptin Signaling

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The prevalence of obesity among child-bearing female has increased significantly. Adverse consequences of maternal obesity on the descendants have been well accepted, but few studies have examined the underlying mechanisms. We investigated whether neonatal overfeeding in female mice alters metabolic phenotypes in their offspring and whether the hypothalamic leptin signaling is involved. The chronic postnatal overfeeding was induced by reducing the litter size to 3 pups/litter, in contrast with normal litter size of 10 pups/litter. Normal and neonatally-overfed female mice were bred with normal male mice, and offspring of chronic postnatal overfeeding mothers (OOM) and the control mothers (OCM) were generated. We examined body weight, daily food intake, leptin responsiveness, and the number of positive neurons for phospho-signal transducer and activator of transcription-3 (pSTAT3) and neuropetidyl Y (NPY) in the arcuate nucleus of the hypothalamus (ARH) and NPY in the nucleus tractus solitarius (NTS) of the brain stem. The body weight and daily food intake of OOM were significantly higher than those of OCM. Leptin significantly reduced food intake and increased the number of pSTAT3 positive neurons in the ARH of OOM mice, whereas no significant changes in food intake and pSTAT3 neurons were found in the leptin-treated OOM mice. The number of NPY neurons in the ARH and NTS of the OOM mice was significantly higher than that of the OCM mice. Our studies indicated that maternal obesity can be pass into the subsequent generation which is possibly associated with hypothalamic leptin resistance.

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260-LB

Lipid Storage by Adipose Tissue Macrophages Regulates Systemic Glucose Tolerance

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Adipose tissue (AT) inflammation and infiltration by macrophages is associated with insulin resistance and type 2 diabetes in obese humans. Using an siRNA delivery method to silence genes expressed by macrophages specifically localized within AT depots, while leaving macrophages in other tissues unaffected, we showed that AT macrophages (ATMs) directly contribute to systemic glucose intolerance in obese mice. We reported that intra-peritoneal administration of siRNA encapsulated by glucan shells (GelRPs), to selectively silence inflammatory genes in ATMs, caused significant improvement in glucose tolerance in obese mice. Here we show that ATMs may also be beneficial as repositories for excess lipid that adipocytes are unable to store. Selective silencing of AT lipoprotein lipase (LPL) decreased foam cell formation in AT of obese mice, consistent with a reduced supply of fatty acids from lipoprotein hydrolysis. Unexpectedly, silencing LPL also decreased the expression of genes involved in fatty acid uptake (FFAs) and esterification in ATMs. This resulted in increased circulating serum FFAs. AT LPL silencing also caused a marked increase in circulating fatty acid binding protein 4 (fabp4/ap2), an adipocyte-derived lipid chaperon previously reported to induce liver insulin resistance and glucose intolerance. Consistent with this concept, obese mice with LPL-depleted ATMs exhibited higher hepatic glucose production from pyruvate and glucose intolerance. Thus, lipid storage by ATMs promotes systemic glucose tolerance. Using the GelRP technology we showed that ATMs can express both beneficial and deleterious factors, and the overall effect under a given physiological condition is the integration of the effects of these multiple factors in real time.

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For author disclosure information, see page LB1.
Bone Marrow Adiposity: Lineage Origin and Differentiation Potential of Bone Marrow Resident Adipocyte Progenitor Cells

THOMAS AMBROSI, CARLA BOCIAN, TIM J. SCHULZ, Nutheral, Germany

Aging results in increased bone marrow adiposity, i.e. the replacement of hematopoietic cells by adipocytes in the cavities of long bones. Current evidence suggests that increased marrow adiposity negatively affects the regenerative potential of osteogenic progenitors, hematopoietic stem cells, and metabolic homeostasis locally and systemically. While it has been demonstrated that marrow adipocytes arise from a population of presumably bi-potential, osteo-adipogenic progenitors, the developmental origin and the effects of aging on these cells remain poorly understood.

Developmental lineage tracing in the mouse reveals a mesenchymal, but non-hematopoietic and non-endothelial origin of the osteo-adipogenic cells that is consistent with corresponding adipogenic cells derived from adipose tissue. Interestingly, cells expressing common markers of bi-potential progenitors, such as platelet-derived growth factor receptor (PDGFR-α), reside in two distinct anatomical locations, the endosteme and in proximity to sinuosids. Conversely, expression of zinc-finger protein (Zfp)-α, which exclusively marks adipogenic cells, is observed only in the sinusoidal location, suggesting that two distinct populations with either adipogenic or osteogenic potential exist within bone. Prospective, flow-cytometric isolation and culture reveals an age-related impairment of osteogenic potential whereas adipogenesis is unchanged or even increased. Microarray analysis further suggests that changes in extracellular matrix production play a role in this pro-adipogenic switch. These findings taken together suggest the presence of distinct sub-populations with either osteogenic or adipogenic potential that arise from a common population of stem cells. Aging-related changes in the microenvironment favor adipogenesis over an osteogenic regeneration phenotype. This process could in turn impair hematopoiesis and metabolic health on a systemic level.

Supported By: German Research Foundation; European Research Council; German Center for Diabetes Research

The Gut Microbiota Induces Obesity, Reduces Leptin Sensitivity, and Decreases the Expression of the Obesity-Suppressing Neuropeptide Brain-derived Neurotrophic Factor (BDNF) in the Central Nervous System

JOHN-OLIV JANSSEN, Gothenburg, Sweden

The gut microbiota contributes to fat mass and the susceptibility to obesity, but the underlying mechanisms are not completely understood. The brain-derived neurotrophic factor (BDNF), regulates mood and memory. In addition, it has recently been found to be a potent anti-obesity substance in both humans and experimental animals, probably exerting these effects at the level of the hypothalamus, especially the ventromedial nucleus (VMN) and the brainstem. Interestingly, recent findings indicate that a BDNF mRNA variant with a long 3’ untranslated region (long 3’ UTR) is targeted toward dendrites of the neuron, and that this variant of BDNF mRNA is essential for energy balance and responsiveness to leptin. We found that conventional mice on normal chow had decreased expression of the (anti-obesity form of) long 3’ UTR Bdnf mRNA in the hypothalamus and the brainstem, compared to germ free mice. Moreover, conventional mice on high fat diet had decreased expression of the long 3’ UTR Bdnf mRNA in the hypothalamus, compared to germ free mice on high fat diet. Leptin treatment caused less weight reduction in conventional mice compared with germ free mice.

In conclusion, the gut microbiota reduces the expression of the anti-obesity dendritic targeting form of long 3’ UTR Bdnf mRNA in the hypothalamus and the brainstem. This may contribute to gut microbiota induced leptin resistance and fat mass in mice.

Supported By: Vetenskapsrådet (Sweden)

CCL2 Deficiency Leads to Eosinophilia, Alternative Macrophage Activation, and TH2 Polarization in Adipose Tissue

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Adipose tissue (AT) inflammation during obesity is mediated by inflammatory immune cells and closely correlates with systemic insulin resistance and type 2 diabetes. In AT, inflammatory status is tightly associated with the number and type of infiltrating leukocytes. In lean AT, eosinophils are relatively abundant and are capable of promoting macrophage alternative activation via their production of IL-4. In wild type (CCR2+/-) mice, obesity causes the proportion of eosinophils in AT to decline, potentially contributing to the classical activation of inflammatory AT macrophages. In the current study we show that CCR2 deficiency leads to eosinophilia in AT and the peritoneal cavity. In contrast to CCR2+/- mice, eosinophils in CCR2-/- AT is sustained and even amplified during high fat diet feeding. Interestingly, the majority of eosinophils in the AT of CCR2-/- mice are localized within crown-like structures. The accumulation of these immune cells was found to be independent of the ability of CCR2-/- precursor cells to differentiate into eosinophils. Rather, the proportion of eosinophils in AT was positively correlated with the expression of IL-4, a potent eosinophil chemokine. The eosinophils in CCR2-/- mice was detected in all fat pads, but was not found in bone marrow, blood, spleen, or liver. In CCR2-/- mice, AT eosinophils coincided with macrophage alternative activation and increased TH2 gene expression. This is the first study to provide a link between CCR2 function and eosinophils in AT.

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Macrophage and Preadipocyte Interactions in Adipose Tissue Fibrosis

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Extracellular matrix (ECM) accumulation in adipose tissue is a feature of chronic obesity and adipose tissue fibrosis is associated with insulin resistance. The adipocyte ECM in adipose tissue is a critical regulator of adipocyte function and metabolism and the loss of ECM remodeling flexibility promotes metabolic dysfunction. The mechanisms by which adipose tissue fibrosis is initiated and maintained with obesity are not completely understood. The goal of our studies is to assess the source of ECM production and how ATMs might regulate ECM remodeling in adipose tissue. Microarray analysis identified preadipocytes (CD31-CD45-Sca1-+PDGFRα+) as enriched for ECM genes compared to ATMs in lean and obese mice. ECM genes were further induced in preadipocytes but not ATMs with diet-induced obesity (DIO). To identify the source of the collagen production in adipose tissue, intracellular flow-cytometry was used to identify preadipocytes as the primary Collagen Type 1 and Elastin expressing cells in adipose tissue in lean and obese mice. Collagen+ preadipocytes increased in number in obese visceral adipose tissue

Supported By: ADA-Funded Research

The Gut Microbiota Induces Obesity, Reduces Leptin Sensitivity, and Decreases the Expression of the Obesity-Suppressing Neuropeptide Brain-derived Neurotrophic Factor (BDNF) in the Central Nervous System

JOHN-OLIV JANSSEN, Gothenburg, Sweden

The gut microbiota contributes to fat mass and the susceptibility to obesity, but the underlying mechanisms are not completely understood. The brain-derived neurotrophic factor (BDNF), regulates mood and memory. In addition, it has recently been found to be a potent anti-obesity substance in both humans and experimental animals, probably exerting these effects at the level of the hypothalamus, especially the ventromedial nucleus (VMN) and the brainstem. Interestingly, recent findings indicate that a BDNF mRNA variant with a long 3’ untranslated region (long 3’ UTR) is targeted toward dendrites of the neuron, and that this variant of BDNF mRNA is essential for energy balance and responsiveness to leptin. We found that conventional mice on normal chow had decreased expression of the (anti-obesity form of) long 3’ UTR Bdnf mRNA in the hypothalamus and the brainstem, compared to germ free mice. Moreover, conventional mice on high fat diet had decreased expression of the long 3’ UTR Bdnf mRNA in the hypothalamus, compared to germ free mice on high fat diet. Leptin treatment caused less weight reduction in conventional mice compared with germ free mice.

In conclusion, the gut microbiota reduces the expression of the anti-obesity dendritic targeting form of long 3’ UTR Bdnf mRNA in the hypothalamus and the brainstem. This may contribute to gut microbiota induced leptin resistance and fat mass in mice.

Supported By: Vetenskapsrådet (Sweden)
and were identified in omental fat samples from obese patients. Weight loss by caloric restriction of obese mice was found to increase visceral adipose tissue fibrosis. This was associated with a sustained increase in Collagen-
preadipocytes and CD11c+ ATMs. To assess the contribution of AT-derived signals to preadipocyte ECM production, in vitro studies demonstrated that TNFα induced Collagen I protein expression in 3T3-L1 preadipocytes. M1 macrophage conditioned media had no effect or decreased preadipocyte ECM gene expression. M2 macrophage conditioned media increased ECM gene expression. Overall, our studies support a model by which preadipocytes are the primary regulated source of adipose tissue ECM production and that ATMs have to capacity to provide signals that enhance or suppress this function.

Supported By: ADA (1-12-CD-08); NIH (DK088262)

266-LB

A Diabetic Monkey Model Can Be Used for Diabetes Therapy Evaluation

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We have proved that the diabetes symptoms may appear in rhesus monkeys fed with high-calorie diet. To study the application of diabetic monkeys in drug evaluation, three anti-diabetic drugs of different mechanisms were tested in these models. The results were compared with what were found in clinical trials. TAK-875 (GPR40 agonist). Before administration, NGT was conducted in four diabetic monkeys and the plasma glucose curve (AUC0-120 min) was calculated as baseline. A week later, the same test was conducted after 20mg/kg TAK-875 was injected respectively. With baseline, AUC0-120 min of monkeys injected with TAK-875 decreased by 17.51% in average. In a clinical OGTT, AUC0-3h of the 120m patients injected with 400mg/day TAK-875 for 2 weeks decreased by 12.98%. Bydureon (a long acting formulation of exenatide). Five diabetic monkeys were injected with Bydureon at a dosage of 40 µg/kg/week and another 5 were injected with saline for 4 weeks. Body weight, FPG, 2h postprandial glucose (2hPPG) and HbA1c were measured. Comparing with saline group, body weight of the Bydureon group decreased by 6.98%, FPG decreased by 17.70%, the 2hPPG decreased by 22.78%, HbA1c decreased by 0.20%. A stronger action of Bydureon on the postprandial glucose than that on FPG was found. These results are consistent with reports in clinical literature. Pioglitazone. Five diabetic monkeys were orally dosed with pioglitazone at a dosage of 1 mg/kg/day and another 5 were dosed with placebo for 4 weeks. FPG, 2hPPG, HbA1c and Lipid levels were measured. Comparing with saline group, the FPG of pioglitazone group decreased by 20.09%, 2hPPG decreased by 11.75%, HbA1c decreased by 0.24% and LDL-c decreased by 7.90%. In a clinical report, after a 40 mg/day pioglitazone treatment for 12 weeks, FPG was found. These results are consistent with reports in clinical literature.

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267-LB

Validation of Schad (Medium- and Short-Chain 3-I-Hydroxacyl-coa Dehydrogenase) as a Target for Treatment of Obesity and Insulin Resistance

NADJA SCHULZ, STEPHAN SCHERNECK, OLIVER KLUTH, TIMOD KANZELEITER, MICHAELA RATH, HANS-GEORG JOOST, ANNETTE SCHÜRMANN, Nuthetal, Germany

We have recently shown that SCHAD (gene name Hadh), which catalyzes the third reaction of the mitochondrial beta-oxidation is involved in thermogenesis, maintenance of body weight, and in the regulation of nutrient-stimulated insulin secretion (Endocrinology 152: 4641-4651, 2011). In order to assess SCHAD as a target for treatment of obesity, Hadh−/− mice on the B6.V- mice background were characterized on a ketogenic diet. At 10 weeks of age, Hadh−/−/− mice exhibited 7.8 g lower body weight and 6.8 g lower fat mass than Hadh+/+/+ mice. Lean body mass was not affected. This effect associated with a significant reduction of blood glucose and fasted plasma insulin concentrations in Hadh−/−/− mice. SCHAD generates NADH which is a substrate for complex I of the respiratory chain through its enzymatic conversion of 3-I-hydroxyacyl CoAs to 3-I-ketoacyl CoAs. Mitochondria of livers from Hadh−/− mice contained lower amount of complex 1 enzymes (e.g. NDUFB8) than mitochondria from Hadh+/+ mice. Furthermore, oxygen consumption rate (OCR) of hepatic mitochondria of Hadh−/− mice as measured with an extracellular flux analyzer was significantly reduced when respiration was stimulated with hexanoylcamitine (Hadh−/−: 30.1 ± 2.9 pmol/min vs. Hadh+/+: 85.3 ± 3.1 pmol/min). OCR was not affected after stimulation with succinate-rotenone. Thus, our data indicate that SCHAD is a potential target for pharmacological interventions in obesity and diabetes, because its inhibition—in particular under conditions of fat overload—imparts fuel efficiency.

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268-LB

Obesity Changed the Expression Pattern of Extracellular RNAs in Circulation and in Adipose Tissue Niche

RICHARD CHENG-AN CHANG, HUI SONG, WEI YING, HAIQING WANG, SRIKANTH KANAMENI, TAYLOR SPLAWN, BEIJIAN ZHOU,College Station, TX

Compelling evidence demonstrated that adipose-tissue-resident macrophages (ATMs) are critical coordinators in adipose tissue niche by regulating adipocyte functions, immune cell compartment, and subsequently metabolic homeostasis. As the major cell compartment in adipose stroma, ATMs exert profound regulatory effects by secreting large amount of molecules such as various cytokines and chemokines upon environmental cues. Recent study suggested that, in addition to protein and peptide molecules, RNAs can also be detected in the extracellular fluid and may function as a new type of cell communicating molecules. However, why these extracellular RNAs (exRNAs) are produced and how they function has not been investigated. In the context of obesity induced chronic adipose tissue inflammation and insulin resistance, the exRNA profile has not been generated.

To better understand the regulatory mechanism of ATMs on adipose tissue function, we generated exRNA profiles from 1) the plasma from obese and lean mice, 2) the conditioned medium from classically (macrophage type 1, M1) and alternatively (macrophage type 2, M2) activated murine bone-marrow-derived macrophage and from sorted lean and obese mice ATMs. Interestingly, our results revealed that exRNA profiles in the adipose tissue niche are distinct from circulation; the significant concentration difference of exRNAs from local and circulation is a cue that ATM-secreted exRNA might serve as cell-to-cell communicator in adipose tissue niche. Moreover, a group non-coding RNAs are differentially released by ATMs at polarized activation status; this evidence also supports that exRNAs secretion is a novel marker of macrophage secretion. In conclusion, our study provides the first set of evidence to support that ATMs can regulate adipose tissue function by actively releasing exRNAs acting in a paracrine manner in the context of obesity-associated metabolic syndromes.

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269-LB

Chronic Effects of Exenatide vs. Metformin Treatment on Body Weight and Endogenous GLP-1 Secretion in High-Fat-Diet-induced Obese Rats

XIAO JUAN ZHANG, FANGJI ZHOU, YANG LONG, WEIYI WANG, SHANGFU ZHANG, XIANGXUN ZHANG, HONGLING YU, XIANG CHEN, ZHONG CHENG, HAIMING TIAN, Chengdu, China

The anorexic role of glucagon-like peptide-1 (GLP-1) may contribute to the weight loss effect of both exenatide and metformin. In the current study, we targeted to compare the chronic effects of exenatide vs. metformin on body weight loss and intragastic glucose induced endogenous GLP-1 secretion in high-fat diet induced obese rats and to investigate the mechanisms involved in regulating circulating GLP-1 levels. Forty eight male adult wistar rats were randomly divided into high-fat diet fed and normal chow fed groups. Four months later, diet induced obese rats were submitted to exenatide treated (EX, 3μg/kg, twice a day), metformin treated (M, 300mg/kg/d) and high-fat diet fed control groups (HF-C). After 1 month, endogenous GLP-1 secretion was measured by intragastic glucose tolerance test. Blood samples were also collected for the detection of insulin, leptin levels and DPP 4 activity. Intestinal tissues were harvested for the measurement of L cell numbers, the expressions of sweet taste molecules and leptin receptor. Our results showed that similar weight losses and food reduction were found after both treatments. Besides, they all exhibited a positive role in stimulating endogenous GLP-1 secretion. Intestinal L cell numbers were increased in EX rats but stayed unchanged in M rats. The changes of sweet taste molecule expressions were not the same in the two treatment groups. Insulin and leptin sensitivity augmented after both treatments. DPP 4 activity decreased in M rats, while stayed almost the same in EX rats. In summary, chronic treatment with exenatide or metformin could lead to similar reductions in body weight and food intake in high-fat diet induced obese rats, meanwhile, intragastic glucose induced endogenous GLP-1 secretion are elevated after both treatments and the underlying mechanisms are not identical. The unraveling of the story may provide new agents targeting on incretin effects.

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For author disclosure information, see page LB91.
Evidence that Hypothalamic gliosis in Humans is Associated with Normal weight, never obese and 19 obese subjects. Using a nested design, would be associated with obesity in humans. We measured T2 relaxation resonance imaging (MRI) could detect radiologic evidence of MBH gliosis that astrocyte populations) in the ARC. We hypothesized that quantitative magnetic high-fat diet induces obesity and a ‘reactive gliosis’ (expansion of microglia and activity. Thus, PPAR

mice model through increasing of PPAR

Supported By: NIH/NIDDK

Our data show that Pioglitazone is able to minimize PAH effects in obese animals and determine if PPAR

Supported By: FAPESP

The preliminary report demonstrates rapid improvement of C fiber function in hands and feet after bariatric surgery that correlates with reduced % body fat will enable us to investigate how IS and IR patients respond to bariatric surgery. This is a preliminary report on the first 25 patients who have completed 12-week cold and warm perception thresholds and sural nerve conduction studies. This study was designed to examine whether effects in rodents translate to human obesity. Further studies are needed to determine the site/s and mechanism/s of action, and whether effects in rodents translate to human obesity.

The Calcinus Sensing Receptor (CaSR), widely expressed in the gastrointestinal (GI) tract, senses Ca2+ and other substances, including amino acids. It is considered part of the GI chemosensory system, and may play a role in metabolic regulation. We evaluated the effects of GS3K004774, a luminal-bound, potent, CaSR agonist in rodent models of obesity and diabetes. In obese C57BL/6 mice fed a 45% high-fat diet, a 15-day treatment of GS3K004774 resulted in a weight loss of 7.5 ± 1.8% compared to vehicle (p<0.05). The weight loss, predominantly from fat (-1.85 ± 0.5 g vs. vehicle 0.5 ± 0.3 g, p<0.05) was associated with a 12% reduction in cumulative food intake (p<0.05 vs. vehicle). It was not associated with increased plasma GIP, GLP-1 and PYY concentrations, an observation confirmed in normal Sprague Dawley rats during a food challenge test. GS3K004774 did, however, increase CCK/ gastrin secretion. GS3K004774 had no effects on body weight and glycemic control in Zucker Diabetic Fatty rats. In conclusion, activation of the CaSR in the GI tract could be a potential approach to treatment of obesity. Further studies are needed to determine the site/s and mechanism/s of action, and whether effects in rodents translate to human obesity.

Pulmonary artery hypertension (PAH) is a progressive disease of poor prognosis characterized by vasoconstriction of pulmonary arteries (PA) and proliferation of pulmonary vascular endothelial and smooth muscle cells. There is emerging evidence that many key genes involved in PAH development are targets of the insulin-sensing transcription factor PPARγ, and that pharmacological PPARγ activation would lead to their beneficial induction or repression and subsequent antiproliferative, anti-inflammatory, proapoptotic, and direct vasodilatory effects in the vasculature.

Based on previous data, the aims of this study were:
- Establish an animal model of insulin resistance induced by high fat diet and explore the development of PAH;
- Evaluate PPARγ expression in pulmonary artery of obese animals and determine if PPARγ agonist drugs pioglitazone can reverse PAH.

Male C57BL/6 mice were further randomized to receive Pioglitazone (20 mg/kg/day) in a reversal protocol (after 8 months of HF diet and PAH induction and treatment for 4 weeks) by gavage. After 4 weeks, the tissue expression and phosphorylation levels of PPARγ, JNK and ERK1/2 in artery, lung and right ventricle of control, obese and treated mice were measured.

Our data show that Pioglitazone is able to minimize PAH effects in obese mice model through increasing of PPARγ and decreasing ERK1/2 activity. Thus, PPARγ and ERK1/2 are important mediators of PAH and obesity, since they are related to imbalance on vascular proliferation and can be potential target for the therapy of these medical conditions.

This preliminary report demonstrates rapid improvement of C fiber function in hands and feet after bariatric surgery that correlates with reduced % body fat. This is the first study to demonstrate the utility of Sudoscan™ as a measure of C fiber function responses to intervention.

For author disclosure information, see page LB91.
**275-LB**

**Increased Adipocyte Mitochondrial Respiration in Insulin-Resistant vs. Insulin-Sensitive Obese Subjects**

ANJA BOEHM, ANDRAS FRANKO, HANS-URICH HAERING, MARTIN HRABE DE ANGELIS, HARALD STAIGER, Tübingen, Germany, Neuherberg, Germany

Among obese subjects, metabolically healthy and unhealthy obesity (MHO/MUHO) exists, but underlying pathomechanisms are not well understood yet. Mitochondrial dysfunction in obesity and diabetes is known, potential therapies augmenting energy expenditure are promising ideas currently under discussion. Aim of this study was to characterize the mitochondrial respiration capacity in subcutaneous (sc) human adipocytes from insulin-resistant (IR) vs. comparatively insulin-sensitive (IS) metabolically obese subjects; thus, providing hints for novel pathomechanisms. Primary sc preadipocytes from 4 IR vs. 4 IS non-diabetic Caucasians (BMI >40kg/m2), matched for gender, age, BMI, and percentage of body fat were in vitro differentiated to adipocytes. Mitochondrial respiration was measured (day 0 and 21 of differentiation) by an XF24 Seahorse Analyzer. Data were protein-normalized. Stimulation of lipolysis was done by forskolin (FSK)-treatment. Statistics was done with a two-sided t-test.

Mitochondrial respiration was 4-fold higher in adipocytes vs. preadipocytes, p=0.01. No difference regarding the respiration between IR and IS was found in preadipocytes. In adipocytes, several differences were detected. I) Basal respiration was higher in IR vs. IS (5.12±0.79 vs. 3.18±0.81, p=0.0002). II) Maximal respiration and spare respiratory capacity was not different among the groups. III) Proton leak was higher in IR vs. IS (2.08±0.75 vs. 0.97±0.04, p=0.0396), while non-mitochondrial respiration was not affected. IV) ATP production was higher in IR vs. IS (2.04±0.84 vs. 0.84±0.22; p=0.0124; n=4). V) There was no difference in mitochondrial coupling between the groups. VI) Stimulation of lipolysis with FSK showed a significant increase (2.12-fold; p=0.0002) in basal respiration in IR as well as in IS. In conclusion, our results point to an increased mitochondrial respiration in adipocytes from IR vs. IS, perhaps reflecting a compensatory state in MUHO.

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**276-LB**

**Circulating Branched-Chain Amino Acids and Irisin Level in Morbid Obese Individual with Type 2 Diabetes (T2DM) after Roux-en-Y Gastric Bypass (RYGB)**

PRAPIPMORN CHATTANUKULCHA SHANTAVASINGL, ALESSANDRO MOR, LEO-NOR CORSINO, ALFONSO TORUJATI, Durham, NC

Elevation of circulating branched-chain amino acids (BCAA) has been associated with insulin resistance. RYGB has been shown to improve insulin sensitivity which partly mediated by decreasing of BCAA level. Irisin, recently identified myokine, causes browning white adipose tissue and increasing thermogenesis. The benefit of irisin has been proposed to be a potential novel thermogenic factor. Thus, the aim of this study was to investigate the association of circulating irisin and BCAA level in morbid obese individuals with T2DM but also the association of the reduction of irisin and the change of BCAA level after RYGB. Further studies are needed to explore whether irisin is associated with BCAA metabolism and its role in improvement of glucose homeostasis in morbidly obese individual after RYGB.

**277-LB**

**Effect of Acyl Ghrelin infusion on Glucose Disposal and Production in Obese and Lean Humans**

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Ghrelin is an orexigenic peptide produced primarily in stomach. Fasting ghrelin levels are lower in the obese. Ghrelin infusion was shown to worsen insulin sensitivity in lean humans, but there is no information about is effect in the obese, which is the objective of this study.

Eight obese and nine lean participants underwent an infusion of acyl ghrelin (1 pmol kg⁻¹ min⁻¹) or saline in random order on consecutive days. Hyperinsulinemic-euglycemic clamps with glucose tracer infusions were performed each day with ghrelin or saline infusions. Data are presented as median (interquartile range), and comparisons were made with nonparametric tests.

Fasting acyl ghrelin levels (ng/ml) were lower in the obese than the lean [360 (194-581) vs. 770 (569-988), P = 0.006]. Acyl ghrelin infusion resulted in similar basal plasma acyl ghrelin in the obese and lean [3498 (2886-4483) vs. 2955 (2558-3666), P=0.2]. During the clamp, the obese had higher plasma acyl ghrelin than the lean [4249 (3138-4872) vs. 2586 (1804-3062), P=0.002]. This translated into significantly higher clearance rates of plasma acyl ghrelin (~50%) in the obese compared to lean, which did not change with insulin infusion. Peripheral glucose uptake was significantly reduced with ghrelin infusion in both the lean and obese; however, the obese had a greater percent reduction compared to the lean [44% vs. 24%, P=0.001]. Hepatic glucose production was not altered by ghrelin infusion in either group (P=0.35).

The lower plasma acyl ghrelin in the obese appear to be due to increased clearance from the plasma compartment. At high physiological levels, acyl ghrelin worsens peripheral insulin sensitivity in both lean and obese; however, this effect appears to be more exaggerated in the obese. Hence, we hypothesize that lower plasma ghrelin levels in the obese might be protective against further worsening of the peripheral insulin resistance commonly present in the obese.

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**278-LB**

**WITHDRAWN**

**279-LB**

**WITHDRAWN**

**280-LB**

**Short-term Changes in Lipopolysaccharide and Lipopolysaccharide-Binding Protein Levels after Two Different Bariatric Surgery Procedures in Normoglycemic and Diabetic Morbidly Obese Patients**

MERCEDES CLEMENTE-POSTIGO, MARIA DEL MAR ROCCA-RODRIGUEZ, FERNANDO CARDONA, FRANCISCO J. TINAHONES, Madrid, Spain

Bariatric surgery mostly improves metabolic improvements within few days from intervention, but the underlying mechanism is not understood and may differ depending on the bariatric procedure. Lipopolysaccharides (LPS) from the gut microbiota have been proposed as a triggering factor for the inflammatory state in obesity related with the development of insulin resistance and diabetes. Roux-en-Y gastric bypass leads to LPS decrease in the medium-term. Thus, the aim of this study was to analyze LPS and LPS-binding protein (LBP) in both normoglycemic (NG) and prediabetic-diabetic (PD) morbidly obese patients in the short-term after two different bariatric surgery procedures.

50 morbidly obese patients after bariatric surgery: 24 sleeve gastrectomy (SG) and 26 biliopancreatic diversion of Scopinaro (BPD). Patients
were classified according their glycomic status in NG and P/D patients. LPS and LBP levels and biochemical and anthropometric variables were determined before and at days 15 and 90 after bariatric surgery. A significant LPS reduction was only seen in P/D patients at 90d after SG. LBP levels rose at 15d after BPD but at 90d returned to baseline in NG and P/D patients. At 90d after SG, LBP levels significantly decreased compared to baseline in NG and P/D patients. LBP levels correlated significantly and positively with anthropometric variables and with baseline triglycerides, insulin, HOMA-IR and CRP levels, and negatively with adiponectin levels. Short-term LPS decrease after bariatric surgery depends on the surgery procedure as well as on the previous glycomic status of the patients. LBP is closely related to anthropometrical and biochemical parameters in morbidity obese patients undergoing bariatric surgery.

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Dopaminergic Effects on Brown Adipose Tissue
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Bromocriptine is a centrally acting dopamine receptor agonist that improves insulin sensitivity in obese subjects. Yet, no explanation has been found for this effect of bromocriptine. Brown adipose tissue (BAT), a tissue that converts calories into heat, might be involved in this process. Since the central sympathetic nervous system is the primary activator of BAT, we hypothesized that dopamine plays a role in the activation of BAT. Therefore, the aim of this study was to investigate the influence of bromocriptine on BAT activity in 8 lean (BMI 23.2±1.5 kg/m2), healthy Caucasian males (20.9±17.23 years).

All subjects were studied before and after using bromocriptine (1st week 1.25mg/day, 2nd week 2.5mg/day) in a climate room at 21°C after an overnight fast. On these 2 study visits we measured metabolic BAT activity, defined as maximal standardized uptake value (SUVMax), using 18F-Fluorodeoxyglucose Positron Emission Tomography (PET) scans. Furthermore we investigated glucose metabolism with a 7 point oral glucose tolerance test, energy expenditure (EE) using indirect calorimetry, weight and body temperature. Subjects recorded their eating behavior in the 4 days before the study visits.

The use of bromocriptine did not significantly alter metabolic BAT activity (SUVMax before 11.9±4.3-3.18) after 10.3±2.7±18.2), EE (before 2103 Kcal/day [1340-2488], after 1915 [1704-2437]), body temperature (before 36.0 °C [35.6-36.4], after 36.2 [35.3-36.7]) or weight (80 kg [72.1-82.2]), after 80 [72.1-81.8]). Unexpectedly, subjects became significantly less insulin sensitive after bromocriptine use. The area under the curve for glucose increased (before 652 [35.6-36.4]; after 36.2 [36.0-36.7]) or weight (80 kg[72.1-82.2]; after 80 [72.1-81.8]).

We conclude that bromocriptine does not activate BAT and does not increase EE in lean, healthy males.

3 months was also greatest for the CB group with a median 5.3% loss (IQR: 2.6 to 7.9%, p=0.016). The ACC group lost 4.9% (IQR: 2.1 to 8.9%, p=0.063) and the MDI group lost 1.4% (2.9% loss to 1.0% gain, p=0.69).

Practical Implications: Having interventions that help in decreasing weight and obesity would help aide in diabetes management, decrease disease risk and increase quality of life.

Disclaimer. The views expressed in this presentation are those of the author and do not reflect the official policy of the Department of Defense or U.S. Government.

The aimed study was to examine the association eating disorders and gene polymorphism of the serotonin system. Seven hundred six-five people of both genders (320 men and 445 women) were included in the study with overweight, grade 1 and 2 obesity (BMI ≥ 25 kg/m² and ≥ 29.9 kg/m²). Women in their turn were divided into genod (n = 131) and android (n = 314) fat depots. Three genes polymorphisms of the serotonin system - the serotonin transporter (SERT (SHITPLR)), serotonin receptor type 2A (HTR2A -1438G / A) and serotonin receptor type 2C (HTR2C (Ser235Gln)) were exam polymorphism of the serotonin transporter (SERT (SHITPLR)), serotonin receptor type 2A (HTR2A -1438G / A) and serotonin receptor type 2C (HTR2C (Ser235Gln)) were examined by RT-PCR. All patients were assessed for eating disorder psychopathology (externalities, restrictive and emotiogenic) by DEBQ questionnaire.

We concluded that the association between the polymorphism of the serotonin system and types of eating disorders in the group of men and women with gender type of obesity, was revealed. AG and GG genotypes HTR2A gene in women with android fat depots were associated with high scores on a scale of restrictive type of eating disorder (19.7 and 19.8 vs. 16.1 genotype AA), Anova, p < 0.01, and genotype AA - with high scores on a scale emotiogenic type of eating disorder (18.1 vs. 13.6 genotype AG), Anova, p = 0.04. Genotype Ser/ Cys HTR2C gene was associated with high scores in a scale of externalities type of eating disorder (21.2 vs. 17.4 genotype Cys/Cys), Anova, p = 0.02. Scores of emotiogenic type scale of eating disorder was also associated with the group of women with SS genotype SERT gene compared with LG genotypes (17.1 vs. 13.7), but these results were not statistically significant (p = 0.06).

Regression analysis revealed a negative correlation of expression of restrictive eating disorder which BMI for genotypes GG gene HTR2A A (k = -0.33, p < 0.05).

The study of serotonin system genes' polymorphisms showed a correlation of eating disorders only in the subgroup of women with android fat depots - the lowest possible score in a restrictive eating disorder scale was significantly associated with a high BMI for genotypes GG HTR2A A gene.

A Calcium-dependent Protease As a Potential Therapeutic Target for Wolfram Syndrome, a Prototype of Endolipemic Reticulum-associated Diabetes
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Endo-lipemic reticulum (ER) is an emerging target for human chronic diseases, and Wolfram syndrome characterized by diabetes and neurodegeneration is a prototype of human ER disease. Here we show that the calpain protease is a link between the two Wolfram syndrome genes and death of neurons and β cells. Calpain activation is mediated by calcium leakage from the ER, which is enhanced by the loss of function of the Wolfram syndrome 1 gene. We show that the Wolfram syndrome 2 gene product (WFS2) associates with and regulates calpain 2. Elevated activation of calpain 2, seen with WFS2 knockdown, correlates with increased death in neurons and β cells; whereas suppression of calpain 2, seen with over-expression of WFS2, protects these cells from death. Evidence of calpain hyperactivity is observed in a mouse model of Wolfram syndrome as well as in neural progenitor cells derived from plipotent stem cells of patients with Wolfram syndrome. Our results demonstrate that the pathway leading to calpain 2 activation provides potential therapeutic targets for Wolfram syndrome and other ER-associated diseases.

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Neurog3 expression in the endocrine progenitor cells. Loss of Sox4 in the model, we demonstrated that Sox4 cooperated with Neurog3 to amplify led to a significant 70% reduction of endocrine cells at embryonic day (E)18.5.

In order to assess how Sox4 regulates development, a role for these factors in establishment of that many other Sox family members are expressed during pancreas formation in the mouse Sox4flox/flox) specific Sox4 null mice. Loss of Sox4 in the pancreatic anlage to be determined. In order to determine the basis for developing new therapeutic interventions to enhance apoptosis and Neurog3 expression. Expression profiling and cell culture models, demonstrated that Sox4 cooperates with Neurog3 to directly transactivate both Pax4 and Neurod1 in the nascent endocrine cell progenitors. Finally, we demonstrate that loss of Sox4 in endocrine progenitors does not lead to differentiation down an alternate cell fate but a dramatic appearance of chromogranin positive, hormone negative cells. In summary, Sox4 is essential for normal pancreatic endocrine cell differentiation both concomitant with, and downstream of Neurog3. These studies may allow refinement of stem cell differentiation protocols in order to generate large numbers of beta cells that could be used to treat those with diabetes.

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285-LB
Glucagon-like Peptide-1 Specifically Ablates Functionally Deficient Insulin Cells in Mouse Islets in Vivo
GLADYS TEITELMAN, YELENA GUM, MAMDOUNI KEDEES, Brooklyn, NY

In the present study, we sought to determine whether Glucagon-like peptide-1 (GLP-1) modified the beta cell composition of islets. Two lines of bogenic mice were generated by crossing mice containing a transgene comprised of the rat insulin promoter (RIP) linked to Cre recombinase-estrogen receptor (RIP-CreER) mice with strains containing a floxed reporter gene encoding for either human Placental Alkaline Phosphatase (PLAP, RIP-CreER-ZAP mice) or Enhanced Yellow Fluorescent Protein (EYP, RIP-CreER-EYP mice). Injection of Tamoxifen (TM) induces Cre activity, resulting in the expression of the reporter gene (EYP or PLAP). Only cells that contain an active RIP-CreER transgene at the time of TM injection will express the reporter protein and transmit it to their progeny.

Injection of TM to normoglycemic 2 month old RIP-CreER- EYP mice resulted in expression of EYPF in 46.5 ± 2.1% (3 mice and a total of 8881N+ cells scored) in 6 month bogenic mice. To prevent the cleavage of GLP-1 in vivo, an inhibitor (MK6626, Merck) of the enzyme dipeptidyl-peptidase (DPP4i) was administered to 5 month old RIP-CreER-EYP mice for two months. The DPP4i therapy induced a decrease in the percentage of N+ EYPF+ to 17.5 ± 1.73 (3 mice and a total of 9474 N+ cells scored). GLP-1 mediates this action of the DPP4i since daily injection (10 nmol/kg daily) of the GLP-1 agonist exendin 4 (ex-4) to 4 month RIP-CreER-PLAP mice for two weeks dramatically reduced the percentage of N+PLAP+ cells (2.83 ± 0.7; 5232 N+ cells scored). Administration of GLP-1, but not of the DPP4i, resulted in a significant decrease in the beta cell mass in bogenic mice but not in similarly treated CD-1 mice. Neither ex-4 nor the DPP4i affected the rate of beta cell proliferation. Expression of the RIP-Cre transgene can induce glucose intolerance (J. Biol.Chem. 281:2649-2653) due to toxic effects of Cre expression. Taken together, these results reveal a novel function of GLP-1, which is to ablate functionally deficient beta cells in islets in vivo.

286-LB
Cannabinoids Regulate Bcl-2 and Cyclin D2 Expression in Pancreatic Beta Cells
JINH-JE KIM, DA EUN JEONG, WOOK KIM, Suwon, Republic of Korea

We previously reported that cannabinoid 1 receptors (CB1Rs) are expressed in pancreatic β-cells, where they induce cell death by directly inhibiting insulin receptor activation. Here we report that anti-apoptotic protein Bcl-2 and cell cycle regulator Cyclin D2 are involved in cannabinoid-induced pancreatic β-cell death and growth arrest. Treatment of MIN6 pancreatic β-cells with a synthetic CB1R agonist WIN55,212-2 leads to decreases in the expression of Bcl-2 and Cyclin D2, in turn inducing caspase-3-dependent apoptosis and an arrest of the cells in the G0/G1 phase of the cell cycle. Consistently, pharmacological and genetic blockade of CB1Rs leads to reduced blood glucose and increased β-cell survival and proliferation after injury due to increased levels of Bcl-2 and Cyclin D2. These findings provide evidence for involvement of Bcl-2 and Cyclin D2 in the regulation of β-cell survival and growth and will serve as a basis for developing new therapeutic interventions to enhance β-cell function in diabetes.

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ISLET BIOLOGY—BETA CELL—DEVELOPMENT AND POSTNATAL GROWTH

287-LB
Sox4 Cooperates with Neurogenin3 to Regulate Endocrine Pancreas Formation in the Mouse
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The Sry/HMG box (Sox) family of transcription factors is essential for normal endocrine cell formation and Sox9, the best-studied member of this family, is required for endocrine cell specification. Despite the longstanding knowledge that many other Sox family members are expressed during pancreas development, a role for these factors in establishment of β-cell fate remains to be determined. In order to assess how Sox4 regulates β-cell formation, we utilized pancreas (Pdx1-Cre; Sox4flox/flox) and endocrine (Neurog3-Cre; Sox4flox/flox) specific Sox4 null mice. Loss of Sox4 in the pancreatic anlage led to a significant 70% reduction of endocrine cells at embryonic day (E18.5). Further analyses of this mutant at E15.5 demonstrated that Neurogenin3 (Neurog3)-expressing cells were 50% reduced in number. Using a new cell model, we demonstrated that Sox4 cooperated with Neurog3 to amplify Neurog3 expression in the endocrine progenitor cells. Loss of Sox4 in the Neurog2-Cre-expressing endocrine progenitors also resulted in significant 60-75% reductions in mature endocrine cells without differences in proliferation, apoptosis or Neurog3 expression. Expression profiling and cell culture models, demonstrated that Sox4 cooperates with Neurog3 to directly transactivate both Pax4 and Neurod1 in the nascent endocrine cell progenitors. Finally, we demonstrate that loss of Sox4 in endocrine progenitors does not lead to differentiation down an alternate cell fate but a dramatic appearance of chromogranin positive, hormone negative cells. In summary, Sox4 is essential for normal pancreatic endocrine cell differentiation both concomitant with, and downstream of Neurog3. These studies may allow refinement of stem cell differentiation protocols in order to generate large numbers of beta cells that could be used to treat those with diabetes.

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288-LB
Pdx1-mediated Islet Cell Replication Is Enhanced Through Regulation of the miR17-92 microRNA Cluster
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A major goal of diabetes research is to uncover pathways that increase pancreatic islet β-cell mass by promoting β-cell replication while preserving function. Our lab has discovered that overexpression of the β-cell transcription factors Pdx1 or Nkx6.1 in rat islets is sufficient to drive β-cell replication while maintaining function. Furthermore, only Pdx1-mediated replication is blocked by Cdk4 inhibition, demonstrating that these factors act through separate pathways. In this study, we sought to determine whether miRNAs differentially regulated by Pdx1 or Nkx6.1 contribute to the ability of either factor to drive β-cell replication.

Here we show that Pdx1, but not Nkx6.1, overexpression in rat islets causes a 2-fold increase in the miR-17-92 miRNA cluster, which has previously implicated in promoting replication in other cell types. Chemical inhibition of the canonical miR-17-92 regulator, Myc, does not blunt Pdx1-induced miR-17-92 expression suggesting that the observed effect of Pdx1 on miR-17-92 expression is not mediated through Myc. Analysis of published Pdx1 ChIP-seq data from both mouse and human islets reveal Pdx1 binding to the miR-17-92 promoter, which indicates that Pdx1 may be a direct trans-activator of the miR-17-92 locus. We performed computational simulations to predict the regulatory impact of each known miRNA on the Pdx1 gene network in both mouse and human. Interestingly, we found that miR-17, miR-19, and miR-92 from this cluster represent 3 of the 10 miRNAs with the highest Pdx1 network interaction scores. In support of this prediction, we found that inhibition of miR-17-92 cluster members significantly diminishes Pdx1-mediated rat islet cell replication by ~35%. In summary, these findings implicate a novel β-cell specific Pdx1/miR-17-17 circuit in the regulation of β-cell proliferation.

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289-LB
Parturbation Events Terminate Endocrine Neogenesis
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Pancreatic endocrine cell neogenesis can be evaluated by quantifying the number of cells expressing the endocrine progenitor marker neurogenin 3 (Neurog3). Neurog3-expressing cells are observed adjacent to the ductal structure in the embryonic pancreas, but few cells staining for Neurog3 persist after birth. Although this perinatal decline in Neurog3 is well recognized, its exact timing and mechanism are uncertain. Using Neurog3-Timer mice that express the green/red florescent “Timer” protein specifically in endocrine progenitors, we quantified the number of endocrine progenitors by flow cytometry, and found that endocrine neogenesis abruptly declined over the 24 period between embryonic day 18.5 (E18.5) and postnatal day 0.5 (P0.5). We hypothesized that signals associated with parturition control endocrine neogenesis, and tested whether inducing delayed delivery by progesterone administration in pregnant mice impacts the timing of the extinction of Neurog3 expression. Comparing E19.5 embryos with P0.5 newborn pups born at E19 revealed a preservation of Neurog3 expressing cells in the E19.5 embryos (80% compared to E19.5 pups (21%) even though both groups were 19.5 days post coitus (dpc)). Quantitative RT-PCR revealed that the P0.5 newborn pups also expressed much lower Neurog3 mRNA than E19.5 embryos. In contrast, pancreata from pups delivered one day early at 18.5 dpc due to induction with RU486, a progesterone receptor antagonist, had significantly smaller numbers of endocrine progenitors than normal E18.5 dpc. Moreover, Insulin-Timer mice that express the ”Timer” protein specifically in insulin-producing cells revealed significant reduction of beta cell neogenesis for normal pancreatic endocrine cell differentiation both concomitant with, and downstream of Neurog3. These studies may allow refinement of stem cell differentiation protocols in order to generate large numbers of beta cells that could be used to treat those with diabetes.

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For author disclosure information, see page LB91.
in P0.5 pups compared to E19.5 embryos. These data demonstrate that signals associated with parturition tightly control pancreatic endocrine neogenesis including β-cell neogenesis.

290-LB

Beta-Cell Expansion Is Governed by Intrinsic Replication “Speed Limit,” Even in Response to Extreme Metabolic Stimuli
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β-cell expansion and represents a powerful tool to examine the capacity for β-cell regeneration. However, current models are unreliable or alter fetal β-cell development. We created a model of acute extreme obesity in young mice via whole body inducible gene deletion of the leptin receptor. Our goal was to determine if acute extreme obesity stimulates β-cell expansion, and to resolve the lineage mechanism and kinetics of β-cell regeneration.

We induced whole body leptin receptor (UCB-CreERT2, LepRcreERT2) gene deletion and sacrificed 3 or 5 weeks later for quantification of β-cell mass. A subgroup of mice received Canagliflozin, an inhibitor of renal glucose reabsorption, or PD 0332991, an inhibitor of the cell cycle regulator, Cdk4.

Whole body inducible LepR deficiency resulted in massive obesity. Surprisingly, acute LepR deficient mice only exhibited mild glucose intolerance and never developed frank diabetes, in sharp contrast with the phenotype of db/db mice. Acute LepR knockout mice compensate for insulin resistance by massively expanding β-cells (3-fold within 5 weeks). We carried out sequential labeling with thymidine analogs and observed that LepR deficiency only stimulated β-cells to expand by self-renewal, with no evidence of contribution by highly replicative β-cell progenitors. Notably, this extreme stimulus for β-cell proliferation was unable to bypass the replication refractory period of β-cells. Further, acute LepR deficiency induced β-cell proliferation occurs in a glucose independent manner (largely unaltered by Canagliflozin-mediated lowering of ambient blood glucose) that requires Cdk4 activation (sensitive to PD 0332991).

In conclusion, acute disruption of LepR signaling results in massive obesity and a remarkable increase in β-cell mass. However, even extreme β-cell expansion is governed by an intrinsic replication “speed limit” that restricts the generation of new β-cells.

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291-LB

Reprogramming of Adult Pancreatic Exocrine Cells to Beta-like Cells
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The current cell replacement therapy as a means of treating patients suffering from type I diabetes is severely hampered by a lack of available donor material. Cellular reprogramming of pancreatic non-endocrine cells may provide an attractive approach and could potentially advance development in regenerative medicine.

We recently provided proof of concept by showing that chronic hyperglycemia in adult mice can be alleviated through the administration of Epidermal Growth Factor (EGF) and Ciliary Neurotrophic Factor (CNTF), by the conversion of terminally differentiated acinar cells to beta cells. The regenerative process requires Stat3 activation and depends on the expression of Neurogenin 3 (Ngn3) in acinar cells.

As rodent acinar cells exhibit a remarkable plasticity in vitro as they can transdifferentiate to duct-like cells, hepatocyte-like cells and, following growth factor-induced activation of MAPK and STAT3 signaling, to beta-like cells, we evaluated whether exocrine cells isolated from adult human pancreas are similarly responsive to pro-endocrine stimuli.

Human exocrine cells were transduced directly after isolation with lentiviruses expressing MAPKα and STAT3β and cultured as monolayers or as 3D structures in matrix, with or without free-floating pre-culture.

Simultaneous expression of activated STAT3 and MAPK in human exocrine cells activated the expression of the embryonic master switch for endocrine differentiation Ngn3 in transduced exocrine cells. When the exocrine cells were kept in suspension followed by 3D culture a significant increase in the number of beta-like cells was observed. Genetic lineage tracing identified human acinar cells as the source of Ngn3- and insulin-expressing cells.

Our data provide evidence that exocrine cells from human pancreas can be reprogrammed to beta-like cells. Given the large number of exocrine cells, this approach may present a novel strategy to improve cell therapy in type 1 diabetes.

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292-LB

In Vitro Direct Reprogramming of Sox9 Positive Progenitor Cells of the Human Bile Duct towards a Beta-Cell Fate: Progress towards Making Beta Cells for Autologous Cell Therapy in Type 1 Diabetic Patients
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We have previously demonstrated that the gene combination Pdx1, Ngn3 and Mafa separated by 2A peptide sequences using an adenovaliral vector (Ad-PNMI), can uniquely reprogram a cell population in the mouse liver into insulin secreting ducts and persistently restore glucose homeostasis in diabetic mice. We identified the reprogrammed progenitor population as Sox9- expressing cells residing in the mouse intra-hepatic biliary tract. Sox9 positive cells serve as progenitor cells in mammalian intestine, pancreas and liver and in our rodent reprogramming experiments suggest they may provide a suitable target cell population for in-vitro reprogramming to generate beta cells for human cell replacement therapy.

Immunohistochemistry analysis of human liver sections revealed Sox9 expressing cells in the bile duct form a distinct population of epithelial cells, also expressing Ecad and EpCam, a putative marker for liver stem cells. When isolated from digested patient liver samples, the bile duct epithelial cells formed aggregated clusters upon culturing in low attachment plates. Subsequent plating on adherent dishes with or without collagen allowed the bile duct cell aggregates to attach and be separated from residual hepatocytes and red blood cells. When infected with Ad-PNMI in vitro, the aggregates were seen to express insulin three days after infection along with expression of other beta cell markers. We are now investigating the extent of reprogramming towards a beta cell phenotype with respect to normal human islets.

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293-LB

Exposure to Exendin-4 In Utero Results in Diabetes
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The long-acting glucagon-like peptide 1 (GLP-1) agonist, exendin-4, has insulinotropic properties in vitro. Previous studies have demonstrated that postnatal injections of exendin-4 into mice with intrauterine growth retardation, a model of type 2 diabetes, prevented the development of adult adipocyte insulin resistance. There is, however, little data concerning the effects of gestational exendin-4 exposure on the postnatal mouse. Our preliminary data show that exendin-4 injection in utero at gestational day 12 or gestational day 15 using an ultrasound guided micro injection system induced precocious endocrine development evidenced by increased beta-cell proliferation and islet hyperplasia in the neonatal pancreas of wild type mice. However, exposure of endocrine cells was severely impaired in exendin-4 treated embryos by postnatal day 8. Subsequently, embryos exposed excretively in utero developed diabetes by 8 weeks of age. We found that the severity of the diabetic phenotype is dependent on the age of in utero exposure to exendin-4. Despite an enhanced early endocrine differentiation in the neonatal pancreas following in utero exendin-4 treatment, we saw a blunted postnatal endocrine proliferation and the development of insulin resistance which resulted in diabetes in the adult mice.

294-LB

A New Method to Mark Live Proliferating Human Pancreatic β-Cells
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A critical goal in the treatment of diabetes is to find ways to induce proliferation in adult human β-cells. Understanding the molecular differences between proliferating β-cells and their senescent counterparts is essential to finding ways to stimulate β-cell proliferation. To do this, it is key to be able to mark, sort and isolate live proliferating β-cells for molecular analysis. Toward this end, we have developed an adenovaliral vector (Ad-Prllf) that can simultaneously mark live cells that are insulin-producing and dividing. The vector contains fluorescent reporters, ZsGreen1 downstream of a rat insulin promoter, and mCherry downstream of a cell division cycle protein 2 (cdc2) homolog promoter. Live cell imaging in mouse and human islet cells transduced with Ad-Prllf shows ZsGreen1 expression 48h post-infection. Containing of fixed cells for insulin and ZsGreen1 demonstrates that ZsGreen1 expression is specific to insulin-producing cells. We have previously shown that Ad-CyclinE/cdk2 induces human β-cell proliferation. Human islet cell cultures co-infected
with Ad-Prlf and Ad-CyclinE/cdk2 have increased number of mCherry-positive cells by 72h relative to Ad-Prlf and Ad-LaZc-co-infected cells. Together, this suggests that the cdck2 promoter driving mCherry in Ad-Prlf, is activated in dividing human islet cells. This is confirmed by colocalization of mCherry and phospho-histone H3, an endogenous proliferation marker, by immunostaining of fixed cells. Thus, Ad-Prlf vector can mark live proliferating human pancreatic β-cells in vitro. This vector is currently being modified by adding a Cre-lox component in order to permanently mark proliferating β-cells, allowing us to determine if an increase in human β-cell proliferation actually results in an increase in cell number.

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295-LB

Exendin-4 Enhances Endocrine Differentiation and Redirects Acinar Progenitors to an Endocrine Fate

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While insulinotropic peptides, such as glucagon-like peptide-1 (GLP-1), have been shown to increase POX1 expression in pancreas cells in mice and enhance endocrine differentiation of human islet-like cell clusters in vitro, little is known about its effect in vivo on the developing pancreas. To further investigate the effect of GLP-1 in the mouse embryo, we injected exendin-4 (Ex-4), a long-acting GLP1-1 analog, into the amniotic fluid at embryonic day 12 or 15 using an ultrasound-guided microinjection system. On embryonic day 17 or on the day of birth, the pancreas was harvested. Immunohistochemical staining revealed a significantly increased endocrine cell area in the treated embryos. Proliferation studies using BrdU showed an increase in the number of dividing cells in treated embryos. Next, to identify the genes associated with the expansion of endocrine cells, we isolated and sorted lineage tagged insulin-positive cells from embryonic day 18 MIP-GFP embryos after receiving exendin-4 in utero. Real-time PCR analysis showed an enhanced expression of several genes including cyclin D1, cyclin D2, SMAD7 and GCG.

Concurrent with the expansion of endocrine cells, we saw a decrease in the acinar cell population in Ex-4 treated embryos. To determine if the increase in endocrine cells was at the expense of acinar progenitors, we lineage tagged early acinar progenitor cells using a tomato reporter mouse crossed with a cre-recombinase driven by the Mist1 promoter mouse. Based on our labeling efficiency, up to 24% of endocrine cells may have derived from progenitor cells previously directed towards an acinar fate.

In this study, we demonstrate the expansion of endocrine cells in the embryonic mouse pancreas after in utero Ex-4 treatment. While some of the expansion is due to increased proliferation of endocrine cells and their progenitors, a proportion of endocrine cells are derived from progenitors previously directed towards acinar differentiation.

296-LB

Determining whether Rodent β Cell Mitogens Also Stimulate Human β Cell Proliferation

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Numerous compounds stimulate rodent β cell proliferation; however, translating these findings to human β cells remains a challenge. To examine human β cell proliferation in response to such compounds, we developed an in vitro medium-throughput, semi-automated method of quantifying human β cell proliferation using normal human islets. Dispersed human islets were plated onto collagen-coated 384-well plates, treated for 72 hours with compounds reported to stimulate rodent β cell proliferation, then fixed and labeled directly in the well for the β cell markers insulin and POX4, and the proliferation marker Ki67. Imaging was automated using a Leica fluorescence microscope and the LAS AF MATRX M3 Developer Suite to obtain a single image for each well encompassing the entire area of the well at 20x magnification. Images were analyzed using ImageJ 1.4.6j software with pots and surfaces functions, and this quantification procedure was validated by comparing it against results from manual cell counts (CV-10%). Transduction of dispersed human islet cells with a combination of adenoviruses encoding cyclin D3 or cdck2 was used as a positive control to ensure that the human β cells were capable of entering the cell cycle. All human islets had a robust insulin secretory response to glucose using a dynamic cell perfusion system. Human islet cells from three donors (average age = 50 years, range 40-57; average BMI = 32.9, range 25.4-37.9) were treated with prolactin, platelet-derived growth factor A, GABA, or serotonin at physiologic (5nM) and high (11nM) glucose levels and 1300-11,000 β cells were counted per treatment per donor. Cells transduced with cyclin D3 and K6B+ cells (14%) compared to baseline levels (0.2%). However, none of these compounds increased K6B+ β cells at either physiologic or high glucose. This method will allow testing of potential mitogens on human β cell proliferation.

297-LB

Endocrine Progenitor Cells in the Adult Human Pancreas

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Type 1 diabetes (T1D) is caused by autoimmune destruction of pancreatic β cells. Understanding the regulatory mechanisms controlling pancreatic endocrine differentiation has far reaching implications for treatment of T1D. A balance between endocrine progenitor cell recruitment, maintenance and differentiation result in islet mass sufficient to maintain normal glucose levels. One approach to cell-based treatment of T1D is to expand the endocrine progenitor cell pool in the pancreas then direct their development towards β cell fate. This requires a thorough understanding of the mechanisms that restrict progenitor cells from adopting a mature endocrine cell fate under normal circumstances, yet allow recruitment and islet regeneration when necessary. The identification and characterization of endocrine progenitor cells in the adult pancreas is essential.

Neurogenin 3 (NGN3) is necessary and sufficient for endocrine differentiation during murine pancreatic development. Approximately 2-10% of cells in normal adult pancreas express NGN3. Expression of NGN3 and NEUROD1, a proximal target of NGN3, increases following culture. The percentage of NGN3+ cells can be increased by pharmacologic inhibition of Notch signaling and inhibition of proteasome degradation. Viable NGN3+ cells can be isolated from human exocrine tissue using the cell surface marker CD133. In suspension culture, a subpopulation of CD133+ cells undergoes clonal proliferation and forms spherical aggregates containing cells that coexpress insulin C-peptide (CPEP), chromogranin A (CgA) and pancreatic and duodenal homebox 1 (PDX1), as well as cells expressing glucagon and CgA. When CD133+ cells are cultured in hydrogel and nanofiber scaffolds, the percentage of CPEP-expressing cells increases >100-fold, compared to suspension culture, and release CPEP in a glucose-responsive manner. CD133+/NGN3+ cells from adult human pancreatic tissue recapitulate aspects of endocrine development and may offer an innovative therapy for the treatment of T1D.

298-LB

Type 1 and 2 Diabetes: Long-Lost Relatives

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It is widely, though not universally, believed that type 2 diabetes (T2D) results from failure to compensate adequately for insulin resistance. We have previously developed a mathematical model for a rodent model of diet-induced diabetes, the female Zucker Diabetic Fatty rat, in which compensation depends on glucose- and secretion work-dependent increases in β-cell mass and function (Ha et al, Diabetes 62(Suppl. 1):A55 2013). We now explore whether the same model structure, with quantitative adjustments, applies to human diabetes. We find that accounting for increased body mass and blood volume (30%-fold) and slowed responses of mass (100-fold for adults) and function (25-fold) is sufficient to reproduce human trajectories of T2D. As for the rodents, the model shows that insulin resistance leads to T2D only if there are pre-existing, otherwise silent, defects in mass or function. We also incorporate data from the literature on rates of beta-cell replication in children from infancy to age 20 and apply it to the onset of type 1 diabetes (T1D). We find that progression to T1D is slow with age in parallel with the slowing of replication rate, implying that slow replication is actually protective. This is possible because cell death rate also slows. The model also naturally simulates the honeymoon period of 6 - 9 months when insulin therapy is introduced shortly after crossing the diabetic threshold. The improvement is due to enhanced beta-cell function but is short-lived because mass continues to decline. Honeymoon duration also increases with age due to slowing of replication. In summary we find that T1D and T2D, despite many critical differences in the environment faced by the β cells, share a common core of beta-cell biology.

299-LB

Uncovering Conserved Beta-Cell Transcriptome of Type 2 Diabetes by Meta-analysis of Microarray Data

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Pancreatic β cell dysfunction is an early manifestation during progression to diabetes. Transcriptional profiling studies of betal cells from subjects with diabetes have revealed various genes or pathways of beta cell dysfunction. We aimed to uncover conserved beta cell transcriptional signature in diabetes by meta-analyzing microarray datasets and find regulators.

Microarray data of three independent transcriptional profiling studies of beta cells from diabetes patients (GSE20966, GSE26724, and GSE38842)
ISLET BIOLOGY—BETA CELL—STIMULUS-SECRETION COUPLING AND METABOLISM

301-LB

The Furany Fatty Acid Metabolite CMPF Is Elevated in Diabetes and Induces β-Cell Dysfunction
KAREY J. PRENTICE, LEMIEUX LUUL, EMMA M. ALISTER, YING LIU, LUCY S. JUN, KYLE W. SLOOP, ALEXANDRE B. HARDY, LI WEI, WEIPING JIA, I. GEORGE FANTUS, KACEY J. PRENTICE, LEMIEUX LUUL, EMMA M. ALISTER, YING LIU, LUCY S. JUN, KYLE W. SLOOP, ALEXANDRE B. HARDY, LI WEI, WEIPING JIA, I. GEORGE FANTUS, KACEY J. PRENTICE, LEMIEUX LUUL, EMMA M. ALISTER, YING LIU, LUCY S. JUN, KYLE W. SLOOP, ALEXANDRE B. HARDY, LI WEI, WEIPING JIA, I. GEORGE FANTUS, KACEY J. PRENTICE, LEMIEUX LUUL, EMMA M. ALISTER, YING LIU, LUCY S. JUN, KYLE W. SLOOP, ALEXANDRE B. HARDY, LI WEI, WEIPING JIA, I. GEORGE FANTUS, KACEY J. PRENTICE

In diabetes, increased lipolysis is associated with increased plasma free fatty acids (FFAs) that can be metabo-lized into a furan fatty acid metabolite, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF). The activating and inhibitory effects of FFAs can be mediated through G-protein-coupled FFAR2 and FFAR3 receptors. CMPF has also been shown to have effects on glucose metabolism in diabetic cells. However, the involvement of CMPF in diabetes-induced β-cell dysfunction remains unclear. In this study, we measured CMPF levels in human islets and in β-cells of diabetic mice (db/db and db/db-somatostatin−/−). We also measured the effect of CMPF on β-cell metabolism and function using human β-cells and the INS-1 832/13 cell line. Our results showed that CMPF levels were significantly increased in both human islets and β-cells of db/db mice, and that treatment of β-cells with CMPF resulted in increased glucose uptake, ATP synthesis, and glucose-stimulated insulin secretion. These effects were associated with the activation of AMPK and increased expression of the glucose transporter GLUT2. In contrast, CMPF treatment of β-cells resulted in reduced insulin secretion, increased cell death, and decreased β-cell survival. These findings suggest that CMPF is a novel mediator of β-cell dysfunction in diabetes, and that targeting CMPF may be a potential therapeutic strategy for the treatment of diabetes.

302-LB

Loss of Responsive Beta Cells Is the Major Secretory Deficit in the db/db Animal Model of Type 2 Diabetes
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It is now understood that a reduction in insulin secretion is an important characteristic of type 2 diabetes. However, the nature of this secretory defect remains unclear. Here, we have developed a 2-photon assay to measure individual insulin granule fusion events from cells within intact islets. In response to 15mmol/l glucose, db/db islets secreted ~77% less insulin compared to +/+ islets. Consistent with previous findings, the db/db islets had slower and smaller calcium responses to glucose and a decrease in syntaxin 1A expression. Finally, the calcium ionophore, ionomycin induced insulin secretion in +/+ islets but not in db/db, showing there is a defect in granule fusion. Consistent with the reduced glucose-induced insulin secretion, our 2-photon assay showed an ~80% reduction in exocytic fusion events. Image analysis determined that this overall loss of insulin granule fusion was described by a 73% loss of responding cells and a 50% decline in exocytic events in the remaining, responsive cells. Our assay also measured granule lifetime and post-fusion fluorescence intensity, and found no significant differences in responses between db/db and +/+ islets. However, in a modification of the assay, pH sensitive dye was used to identify kiss-and-run exocytic events and showed that in the remaining db/db exocytic responses, there was a higher proportion of kiss-and-run exocytosis compared to +/+ islets. This change in the behavior of a very small number of granule fusion events is interesting but the predominant characteristic of the db/db islets is the loss of full fusion. We conclude that the major cause of the reduction in insulin secretion in db/db islets is the loss of responding beta cells.

303-LB

Investigating Intra-islet Interactions between Pancreatic Cells
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Secretion of the pancreatic hormones insulin, glucagon, and somatostatin depends on both intrinsic responses from each cell type and modulation by paracrine secretion from the other cell types. We have combined existing mathematical models for electrical activity in response to glucose of alpha- and beta-cells with a new model for delta-cells to create an islet model with secretion of the three hormones into the interstitial space. As a first test, we reproduce the pulsatile secretion of glucagon, which is anti-synchronous with the secretion of the other two hormones. However, we find that, in order to synchronize beta- and delta-cells, somatostatin secretion must be stimulated by beta-cells, possibly mediated by GABA. The paracrine effects of insulin, direct through somatostatin, play a key role in taming the heterogeneity of the alpha- and delta-cells, notably suppressing any alpha-cells that inappropriately secrete glucagon. The model reproduces the glucose dependence of glucagon and somatostatin secretion, with or without inhibitors of K(ATP) channels and SERCA pumps (Vieira et al. Diabetes 50(2):370). It is consistent with the effects of somatostatin knock-out mice (Cheng-Xue et al. Diabetes 52(5):1523) and confirms that, while somatostatin lowers the tone of insulin and glucagon secretion, it does not determine the response to glucose.

For author disclosure information, see page LB91.

ADA-Funded Research
Finally, if insulin secretion is reduced to simulate diabetic islets, then glucose oscillations are lost and glucagon secretion is increased. This may explain the hyperglucagonemia that exacerbates the hyperglycemia of diabetes.

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304-LB
Identification of Zinc Transporters Responsible for Zinc Influx into β Cells
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Zinc ions play an essential role in the regulation of pancreatic cellular functions, which include insulin synthesis, insulin secretion, anti-oxidative and anti-apoptotic processes, linked to both T1D and T2D. Our lab has previously shown the critical role of Zn1B, a member of zinc efflux transporter family, in insulin synthesis and secretion. However, very little is known regarding how zinc enters beta cells. We are the first to examine the zinc influx transporter (ZIP) transcriptome in pancreatic islets and show consistent high expression levels of ZIP6 and ZIP7 genes (Slc39a6 & Slc39a7) across human islets, mouse islets and MIN6 mouse pancreatic β cells. We also show that the cytosolic zinc content in pancreatic β cells is tightly associated with the expression levels of ZIP6 and ZIP7 under both basal and glucose stimulated conditions, confirming their important role in regulating cellular zinc homeostasis. Disrupted cellular zinc homeostasis, caused by down-regulation of ZIP6 and ZIP7 expression, impairs insulin secretion in response to both glucose and membrane depolarization, with no changes in total insulin content in MIN6 cells. More importantly, we also show interactions between ZIP6, ZIP7 and the GLP-1 receptor in MIN6 cells, and the disruption of this interaction diminishes GLP-1 enhanced glucose stimulated insulin secretion. And this insulin secretion impairment is related to zinc related oxidative stress and apoptosis. Our data suggests that ZIP6 and ZIP7 are two important zinc influx transporters in pancreatic β cells, and alterations in their expression levels may contribute to β cell dysfunction (insulin secretion) in diabetes via cellular zinc homeostasis and GLP-1’s insulin secretagogue action.

A Novel Pathway for Regulation of Insulin Secretion by Fractalkine and CX3CR1 System
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Fractalkine (FKN) (CX3CL1) and its receptor CX3CR1 mediate cell-to-cell interactions in different tissues. Here, we demonstrate that the FKN/CX3CR1 system represents a novel regulatory mechanism for pancreatic islet beta cell function and insulin secretion. CX3CR1 KO mice exhibit glucose intolerance with normal insulin sensitivity, due to a marked beta cell defect in glucose and GLP1-stimulated insulin secretion. The defect in insulin secretion was also observed in vitro in isolated islets from CX3CR1 KO mice. In vivo administration of FKN improved glucose tolerance with an increase in insulin secretion. In vitro treatment of islets with FKN increased intracellular Ca2+ level and potentiated insulin secretion. The KO islets exhibited reduced expression of a set of genes which are necessary for the fully functional, differentiated beta cell state, whereas, treatment of WT islets with FKN leads to increased expression of these genes. Lastly, expression of FKN in islets was decreased by aging and HFD/obesity, suggesting that decreased fractalkine/CX3CR1 signaling could be a mechanism underlying beta cell dysfunction in type 2 diabetes.

Beta Cells Respond to Hyperglycermia by Altering the Surface Expression of K(ATP) Channels
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An open question in the etiology of type 2 diabetes is the relative importance of beta-cell mass and function in compensating for insulin resistance. In previous work (Ha et al, Diabetes 62(Suppl. 1):A557 2013) we proposed that functional adaptation precedes mass adaptation and predicted reduced K(ATP) channel surface expression in response to hyperglycermia. The purpose of this study was to test this hypothesis. We exposed mouse islets to high (11.1 mM) or low (2.8 mM) glucose overnight in vitro and then assayed glucose-dependent oscillations of cytosolic free calcium using fura-2 fluorescence and islet patch clamp to measure membrane potential. Chronic high glucose left-shifted the glucose thresholds of both calcium and electrical oscillations, whereas low glucose caused a right shift. To test whether these shifts were caused by reduced gKATP, we applied voltage ramps to beta cells within intact islets. The conductance changes observed reflected changes in K(ATP) surface expression, not ATP sensitivity, suggesting that adaptation is mediated by alterations in channel trafficking to the plasma membrane. Insulin secretion measured using static incubation was shifted in parallel with changes in calcium, electrical activity and gKATP. To test the involvement of insulin in the control of gKATP we co-applied the K(ATP) channel opener diazoxide (Dz) with 11 mM glucose overnight. Dz, which inhibits insulin secretion, caused a monophasic left shift vs. 11 mM glucose alone, consistent with an ability of Dz to increase gKATP. This suggests that insulin inhibits its own secretion unless overruled by rises in glucose. To test whether AMPK might link channel trafficking and metabolism, we included the AMPK activator AICAR with high glucose/Dz overnight, and found this reduced the size of the left shift in islet glucose sensitivity. We conclude that beta cells have a novel mechanism for adaptation to varying metabolic challenges by altering the number of K(ATP) channels on the cell surface.

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307-LB
A Novel Pathway of Glucodetoxification in Pancreatic β-Cells Linked to Glycerol Release
YVES MUGABO, SHANGANG ZHAO, JOSE IGLESIAS, SARI GEZZAR, ANFAL AL-MASS, DONGXIE ZHANG, JULIEN LAMONTAGNE, MARCO PINEDA, ERIK JOLY, MURTHY MADIRA-JU, MARC PRENTKI, Montreal, QC, Canada
Chronic excess supply of glucose and fatty acids to β-cells can be toxic and lead to apoptosis and depletion of insulin stores. It is important to identify the detoxification mechanisms inherent in β-cells to escape fuel surfeit toxicity. We found that fuel detoxification in β-cells involves glycerolipid fatty acid cycle, storage of glucose and fatty acid carbons as triglycerides and glycogen and release of glucose-derived metabolites. During glucose metabolism, glycerol and fatty acids are thought to be released from the β-cell exclusively due to accelerated triglyceride lipolysis that is linked with insulin secretion as orlistat completely inhibits glucose stimulated insulin secretion (GSIS) and fatty acid release. We find that while GSIS response and glucose oxidation in rat islets reach plateau by 15 mmol/l glucose, glycerol release increases up to 25 mmol/l glucose and, paradoxically, is unaffected by orlistat above 10 mmol/l glucose. This revealed that at high glucose, glycerol is produced by mechanisms other than lipolysis. We found that β-cells harbor a specific glycerol-3-phosphatase (G3Pase) that participates in glycerol release from glucose-derived carbons, bypassing oxidation. The presence of a specific G2Pase in mammalian tissues was not known and our results show that this enzyme helps in glucodetoxification, protects β-cells from metabolic stress and influence GSIS. Thus overexpression of this enzyme increases glycerol release, reduces GSIS and protects INS832/13 β-cells from glucotoxicity while RNAi knockdown has opposite effects. In conclusion, we have identified a novel enzyme that participates in β-cell glucodetoxification and GSIS by facilitating direct production of glycerol from glucose-derived glycerol-3-phosphate.

Supported By: CHR

308-LB
A Reliable and Sensitive Chemiluminiscent Enzyme Immunoassay to Accurately Measure C-peptide Levels in Human Samples
CHRIS WISHER, JULIE MELITO, TIM MICKENDY, COLLIN SHAW, STACY DION, JULIE DONALDSON, TAI MURTHY, MARTIN BLANKFARD, Salem, NH
Measurement of C-peptide, a 31 amino acid peptide, is being used to further understand diabetes mellitus, hypoglycemia and insulinoma. C-peptide is a byproduct formed in the process involving a series of enzymatic cleavages of proinsulin and proinsulin, with proinsulin being the immediate precursor of insulin and C-peptide. C-peptide and insulin are known to be released in equimolar amounts from beta cells of pancreas. Since the half-life of C-peptide is about 30 minutes compared to insulin which is only about 3 minutes, measuring C-peptide may be more attractive for indirectly estimating glucose stimulated insulin secretion, understanding beta cell function and/or identifying beta cell functional mass. Furthermore, measurement of insulin and C-peptide together may provide valuable information for the evaluation of hypoglycemia and insulinoma. Lastly, the ability to quantify exogenous or xenotransplanted human C-peptide without cross-reaction with endogenous c-peptide is of interest to many. Evidently, measuring C-peptide in serum samples requires human C-peptide without cross-reaction with endogenous c-peptide is of interest to many. Evidently, measuring C-peptide in serum samples requires
mid and high spikes, respectively. Furthermore, we observed an inter and intra assay variation of <10% and minimal or no cross-reactivity with human insulin/ proinsulin and C-peptide from other species. The assay eliminates need for sample dilution, saves time and reagent cost, leads to potential savings for screening labs.

309-LB
Comparing Effects of Circulating Nonesterified Fatty Acids on Alpha and Beta Cell Responses Following Carbohydrate-rich, Mixed, and Fat-Rich Liquid Meals between Normal Glucose Tolerant South Asians and Caucasians
RUPA AHLUWALIA, FILIP K. KNOP, TINA VILSBØLL, LAKSHMINARAYAN RANGANATH, JURIS J. MEIER, JITEN VORA, Liverpool, United Kingdom; Helleupen, Denmark, Bochum, Germany

Non-esterified fatty acids (NEFAs) stimulate endogenous insulin secretion along with mediating insulin resistance and pancreatic beta cell dysfunction. They are also known to influence postprandial incretin hormone responses. We aimed to compare fasting and postprandial levels of circulating NEFAs following meals of varying compositions, respectively, in normal glucose tolerant (NGT) South Asians and Caucasians.

Eleven NGT South Asian (5 women; means±SEM age: 35±4 years; BMI: 24.7±1.0 kg/m²; fasting plasma glucose (FG): 4.7±0.2 mM) and 15 age, gender and BMI-matched Caucasians (8 women; age: 32±3 years; BMI: 25.1±1.0 kg/m²; FG: 4.6±0.1 mM) underwent three isocaloric liquid meals (~500 kcal) of varying compositions (carbohydrate-rich (CHO), mixed (MIX) and fat-rich (FAT)).

Both fasting NEFA and insulin levels were higher in South Asians vs. Caucasians (NEFA: 0.60±0.02 vs. 0.47±0.02 mM, p<0.003; Insulin: 11.02±0.9 vs.7.3±0.4 mM/l, p<0.0001) insulin responses (area under the curve (AUC)) were higher in South Asians vs. Caucasians (CHO: 27.63±5.901 vs. 10.35±2.000 mM/l/min, P<0.02; MIX: 15.54±5.295 vs. 8.064±2.73 mM/l/min, P=0.02; FAT: 7.22±1.092 vs. 4.027±4.383 mM/l/min, P=0.06). Postprandial NEFA responses (AUC) were lower in South Asians vs. Caucasians (CHO: 3.8±1.5 vs. 5.5±2.1 mMxmin, P=0.02; MIX: 7.5±1.5 vs. 10.0±1.5 mMxmin, P=0.02; FAT: 18.6±4.2 vs. 20.5±2.2 mMxmin, P<0.5).

NGT South Asians compared to Caucasians, demonstrate altered postprandial NEFA levels in the presence of higher insulin responses. Role of incretin hormones in NEFA dynamics coupled with insulin resistance in South Asians, is less well understood.

We aimed to compare fasting and postprandial levels of circulating NEFAs following meals of varying compositions, respectively, in normal glucose tolerant (NGT) South Asians and Caucasians.

310-LB
Isolation and Identification of Mesenchymal Stem Cell–derived Adult Human Pancreases
SONG LEE, SEONGHEE JEONG, HANA PARK, SONG-CHEOL KIM, Seoul, Republic of Korea

Mesenchymal stem cells (MSCs), derived from bone marrow, adipose tissue and most connective tissues have been recognized as a promising source for cell therapy. MSCs have been detected in human pancreatic endocrine and exocrine tissue cultures, have resided in the pancreas and have been derived from chronic diabetes patients expressing c-peptide and insulin. These cells have generated a great deal of interest because of their potential uses in regenerative medicine and tissue engineering.

In this study, we isolated MSCs from adult human pancreata of partially pancreaticectomized patients, whether isolated MSC-like cells from discarded pancreata after pancreatectomy may be able to use in stem cell based therapy. The pancreata was digested by collagenase using Ricordi chamber circulation system and obtained enriched exocrine fraction after COBE gradient. To remove the endocrine cells, enriched exocrine cell fraction incubated with microbead conjugated PSA-NCAM, endocrine cell surface marker, antibody for 1h at 4°C and sorted out using magnetic-activated cell sorting. Pancreatic duct cells also sorted with CA19-9 antibody in enriched exocrine fraction. Purified exocrine cells are cultured 6 days in RPMI 1640 media supplemented with 10% FBS. We observed growing cells morphological changes and analysis MSCs classic surface markers such as CD73, CD90, CD105 by Fluorescence-activated cell sorting. The MSCs-like morphological changes were detected in culture 4 day and all surface markers positive cells > 80% detected in culture 6 day.

These results indicate human adult pancreata is a new source of MSC might be affects therapy of patient with type 1 diabetes in clinical, because isolated MSCs from living donor is expected to extensive capacity to proliferation, self-renewal and differentiation into insulin producing cells.

311-LB
Activation of mTOR is Essential for Pancreatic Islet a Cell Hyperplasia Induced by Glucagon Receptor Blockade
DAVID TSAO, TERRENCE PARK, MITSUKO LYNN YAMAMOTO, ERICK KINDT, TIM NICHOLS, ALISON FORSIE, JOHN LIN, South San Francisco, CA, La Jolla, CA

Glucagon signaling increases hepatic glucose output and leads to a rise in circulating glucose. Thus blocking the glucagon receptor (Gcgr) is beneficial in regulating glucose homeostasis in animal models of diabetes and also shows promising results in clinical trials for patients with type 2 diabetes. However, a consequence of blocking the glucagon receptor is islet α cell hyperplasia, which is a potential safety concern for the development of future treatments targeting this receptor pathway. The molecular and cellular mechanisms leading to this induced α cell phenotype remain unknown. Using mice on a high fat diet to induce diabetes, we show that a novel monoclonal antibody, which blocks the glucagon receptor, improves glucose homeostasis and leads to α cell hyperplasia. We also found that antibody treatment leads to a decrease in liver amino acid catabolism genes and an increase in plasma amino acid levels, similar to that seen in other models deficient in glucagon signaling. Since mTOR signaling is dependent on the availability of nutrients such as amino acids, and is involved in growth and proliferation, we measured mTOR activation in mice. We found that mTOR is hyper-activated in pancreatic islets compared to other tissues in antibody-treated mice. We then co-treated mice with the glucagon receptor blocking antibody and the mTOR inhibitor, rapamycin. Antibody-treated mice that were also dosed with rapamycin showed a significant decrease in α cell number compared to mice treated with antibody alone, and similar levels to non-treated mice, demonstrating α cell hyperplasia resulting from blocking glucagon signaling is dependent on mTOR activation.

312-LB
Beta-arrestin 2 Recruitment and Biased Agonism at the Free Fatty Acid Receptor GPR40
ARTURO MANDINI, GYSLAINE BERTRAND, ÉRIC CARPENTIER, JULIEN GHISLAIN, KEVIN VIVOT, MICHEL BOUVIER, VINCENT POUJOT, Montreal, QC, Canada, Montpellier, France

GPR40 is a G protein-coupled receptor (GPCR) for free fatty acids primarily expressed in pancreatic beta cells. Pharmacological activation of GPR40 is a potential strategy to increase insulin secretion in type 2 diabetes (T2D). Yet, current knowledge of GPR40 pharmacology remains limited. GPR40 mainly signals via the heterotrimeric G protein Gα11. However, it is now evident that GPR40s can also engage functionally distinct G protein-independent signaling via beta-arrestins (bArr) 1 and 2. Further, G protein- and bArr-based signaling can be differentially modulated by different ligands, thus eliciting ligand-specific responses (“biased agonism”). Whether GPR40 i) engages bArr-dependent signaling, and ii) is subject to biased agonism is not known. Using BRET-based biosensors for real-time monitoring of cell signaling in living cells, we detected a direct, ligand-induced GPR40-bArr2 interaction, with the synthetic GPR40 agonist TAK875 being 2.01 ± 0.18-fold more effective than palmitate (PA) in recruiting bArr2 (p<0.001, n=4-7). Conversely, TAK875 acts as a partial agonist with respect to Gα11-dependent GPR40 signaling (0.56 ± 0.06- vs. 0.47 ± 0.05-fold vs. PA for Gg11 activation (p=0.012, n=3-4) and cytosolic [Ca2+]) (p=0.0012, n=12-14), respectively). Importantly, TAK875, but not PA-induced insulin secretion is attenuated in bArr2 -/− mouse islets (31.3 ± 6.1% vs. WT, p<0.001, n=5-8), thus providing functional validation of our bArr2 biosensor data and establishing bArr2 as a novel mediator of GPR40 insulinotropic signaling. Taken together, these data reveal for the first time that in addition to coupling to Gα11, GPR40 is functionally linked to a bArr2-mediated signaling axis. Further, our findings identify ligand-specific signaling signatures downstream of GPR40. These observations expose a level of previously unrecognized complexity for GPR40 signal transduction and may guide the development of pathway-selective GPR40 agonists showing improved clinical efficacy and safety in T2D.

Supported By: CDA, CIHR
Effect of High Glucose and High Fat on β Cell Proliferation and Cell Death in Type 2 Diabetes

RICA AGARWAL, NI ZENG, BANGYAN STILES, Los Angeles, CA

Pancreatic β cells, which are insulin producing cells localized in the islets of Langerhans, are responsible for maintaining glucose homeostasis and thus play an important role in diabetes therapy. Type II diabetic patients usually have high levels of glucose and free fatty acids (FFAs) and exhibit reduced β cell mass. Previous studies have indicated that both glucose and FFAs can have either pro- or anti-proliferative effects on β cells depending upon the exposure time, but the underlying molecular mechanisms remain unclear. The objective of my study is to understand how FFAs influence islet mass under both short-term and long-term exposure. My preliminary data indicates increased islet mass and β cell proliferation in mice fed with high fat diet (HFD) for 14 days (short-term treatment). Islets isolated from these mice show increased expression of cell cycle regulator Cyclin D1 and decreased protein levels of cell cycle inhibitor p16, suggesting that these two factors may mediate the pro-proliferating effects of short-term high fat diet. Consistent with that, in a β cell line cultured in vitro, Cyclin D1 was up-regulated and p16 down-regulated upon short-term palmitic acid exposure, further confirming the important roles of cell cycle regulators in β cells’ response to FFA. Together, my results show that short-term exposure of β cells to FFAs causes increase in both islet mass and β cell proliferation through cell cycle regulatory protein such as Cyclin D1 and p16.

For author disclosure information, see page LB91.
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Cree-Green, Melanie Disclosed no conflict of interest.
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Dai, Ge Disclosed no conflict of interest.
D’Alessio, David Disclosed no conflict of interest.
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Dallongeville, Jean Disclosed no conflict of interest.
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Deboer, Mark Disclosed no conflict of interest.
Debuschere, Xavier Disclosed no conflict of interest.
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AUTHOR RELATIONSHIP/COMPANY

D’Eon, Stephanie A. Disclosed no conflict of interest.
Des Rosiers, Christine Disclosed no conflict of interest.
Desai, Tejal Disclosed no conflict of interest.
Despa, Florin Disclosed no conflict of interest.
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Di Bartolo, Belinda Disclosed no conflict of interest.
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DiMeglio, Linda Disclosed no conflict of interest.
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Gauton, Kyle J. Disclosed no conflict of interest.
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Keders, Mamlouk Disclosed no conflict of interest.
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Kelsey, Megan M. Research Support: Daiichi-Sankyo, Inc.
Kemph, Christian Disclosed no conflict of interest.
Kenny, Brian Disclosed no conflict of interest.
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Kizion, Rich E. Employee: Oramed Pharmaceuticals; Stock/Shareholder: Oramed Pharmaceuticals.
Kim, Dong-jo Disclosed no conflict of interest.
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Kirid, Erick Employee: Pfizer, Inc.
King, George L. Research Support: Boehringer Ingelheim Pharmaceuticals, Inc., Sanofi.
King, Kourtney Disclosed no conflict of interest.
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Disclosed no conflict of interest.

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Disclosed no conflict of interest.

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Disclosed no conflict of interest.

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Zhang, Yurong
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Zhao, Shao-Ke
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Zhuang, Xuan
Disclosed no conflict of interest.

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<th>AUTHOR</th>
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<td>Ziegler</td>
<td>Consultant, Roche Diagnostics.</td>
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<td>Zoughbie</td>
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