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SEE YOU IN SAN DIEGO!
**1-LB**

### Decreased Glycemic Variability during Insulin Therapy Improves Patient-Centered Outcomes in Type 1 and 2 Diabetes

**MARCIA A. TESTA, LAWRENCE BLOINDE, JASVINDER GILL, RALPH R. TURNER, DONALD C. SIMONSON, Boston, MA, New Orleans, LA, Bridgewater, NJ, Wellesley Hills, MA**

While managing intra-day glucose fluctuations using continuous glucose monitoring (CGM) has been studied extensively, the association between glycemic variability and patient-centered outcomes has not been investigated. We conducted a randomized clinical trial in 52 US centers using blinded CGM to determine whether changes in intra-day sensor glucose standard deviations (CGM-SD) impact patient satisfaction (PS: glycemic effectiveness, advocacy, preference, general satisfaction) and perceived health (PH: vitality, general health, sleep) controlling for CGM daily glucose (CGM-G), type of diabetes, age, sex, BMI and HbA1c. 306 insulin-treated T2DM and 62 T1DM (47% male, age 54±11 yrs, HbA1c 7.8±0.7%) were randomized to either basal-bolus (BB; n=192) or premix (PM; n=116) insulin for 12 wks and crossed to alternate treatment for 12 wks while being titrated to HbA1c < 7.0%. Three-day CGM (288 glucose/day) and HbA1c were obtained at Wks 0, 12, and 24. Data were analyzed longitudinally using generalized linear mixed models. 12-wk means±SD CGM-G was 154±37 mg/dl, CGM-SD 45.5±17.6 mg/dl (range 12–111 mg/dl), PS (scaled 0–100) 57.2±23.9 and PH (scaled 100–600) 421±82. Final model estimates (means±SE) indicated that for each 10 mg/dl decrease in CGM-G and CGM-SD, baseline-adjusted 12-wk PS improved by 1.1±0.3 and 1.5±0.6, and for each % decrease in HbA1c, PS improved by 4.7±1.2 (all p<0.01). Each 10 mg/dl decrease in CGM-G and CGM-SD resulted in improvements in PH of 1.2±0.6 (p=0.05) and 3.1±1.3 (p=0.01), while HbA1c was not a significant predictor. Age, gender, duration of diabetes and BMI were not statistically significant predictors in either model. CGM-SD was lower for the BB than PM by 5.4±1.2 mg/dl (p<0.001), and exhibited more favorable PS (p<0.001) and PH (p<0.01). Decreased glycemic variability was associated with improvements in patient satisfaction and perceived health independent of concurrent lowering of daily glucose and HbA1c. Glycemic variability estimated by CGM mediated improvements in patient-centered outcomes and should be used for evaluating the comparative effectiveness of insulin regimens.

Supported by sanofi-aventis, US.

**2-LB**

### Personalized Decision Support in Routine Diabetes Care—A Two Years Update

**ECKHARD SALZSIEDER, PETRA AUGSTEIN, Karlsruhe, Germany**

It was the aim of this study to evaluate acceptance and medical outcome of personalized decision support in conventional diabetes care after two years of running a patient focused decision support system.

The German health insurance fund BBK Gesundheit launched in April 2007 the Diabetiva® program. Diabetiva® offers continuous glucose monitoring (CGM) and personalized decision support generated by the Karlsburg diabetes management system KADIS®. The Diabetiva® timeline included an annual CGM followed by generating KADIS®-based decision support for therapy optimisation and quarterly check-up of HbA1c. Patients with two CGM readings were retrospectively analysed for acceptance of the KADIS®-based decision support using a questionnaire and the outcome was evaluated according to HbA1c, as primary outcome parameter. After running Diabetiva® for 24 months 580 insured diabetics (95.4 % Type 2) were enrolled and had received 950 CGMs. Patients were cared for by 299 general practitioners (GP) and 44 diabetes specialists (DSP). Approximately 74 % of physicians accepted KADIS® as personalized decision support to optimise diabetes therapy of their patients. Logistic regression revealed that KADIS® acceptance and outcome were dependent on HbA1c at baseline (p<0.01). GP or DSP, age, onset of diabetes, and type of diabetes or of diabetes therapy had no significant influence whether on acceptance nor on outcome parameters. If KADIS®-based decision support was accepted HbA1c could significantly be decreased overall by -0.33% (p<0.01), whereas HbA1c at baseline < 6.5 HbA1c decreased by -0.1%, 6.5 – 7.5 by -0.3%, and > 7.5% by -1.0 %. If KADIS® was declined the impact of Diabetiva® was completely diminished: overall increase of HbA1c by +0.40% (HbA1c at baseline < 6.5: +0.5%: 6.5 – 7.5: +0.3%: > 7.5%: +0.1%: +0.3%: +0.5%: > 7.5%: +0.1%: +0.3%: +0.5%).

Personalized decision support is well accepted by physicians. The acceptance rate is strongly related to HbA1c at baseline. Personalized decision support in combination with CGM improves significantly outcome in routine diabetes care.

**3-LB**

### Should Blinded Continuous Glucose Monitoring Be Used to Improve the AIC?

**ELIZABETH E. DURAN-VALDEZ, MARK R. BURGE, PAULA BRODERICK, LYnda SHEY, VirGINIA VALENTINE, DAVID S. SCHADE, Albuquerque, NM, Los Lunas, NM**

Five day blinded (no visible patient feedback) Continuous Glucose Monitoring (blinded-CGM) is frequently used by physicians to determine why the A1C is elevated and to make adjustments to the patient’s treatment regimen. Two FDA approved commercial devices are currently available to specify performance of this function. However, the AIC value is determined by the glucose concentration during the two months prior to its measurement and prior to the blinded-CGM analysis. No data are available in the medical literature to validate the use of blinded-CGM to improve the A1C.

In order to address this deficiency, 73 blinded-CGM studies were performed in individuals with Type 1 Diabetes Mellitus. Twenty subjects were restested in order to assess intra-subject reproducibility. Blinded-CGM was performed immediately after the A1C was measured. Various glycemic parameters obtained from the blinded-CGM analysis were correlated with the A1C. The figure below depicts a lack of important correlations between the A1C and the blinded-CGM data. The parameters that were examined included: 1) A1C, 2) fasting glucose, 3) mean glucose for the entire 5 days of blinded-CGM, 4) postprandial glycemia, 5) % time hyperglycemic (> 180 mg/dl), 6) % time euglycemic (70-180 mg/dl), and 7) % time hypoglycemic (<70 mg/dl).

Our results unexpectedly demonstrate that the principal glycemic parameters that are assessed with blinded-CGM do not clinically correlate with concurrent A1C concentration. One plausible reason for this is the difference in the time frame over which the blinded-CGM and the A1C were obtained. Another explanation may relate to A1C being a blood measurement in contrast to blinded-CGM data being an interstitial fluid measurement. Physicians must use extreme caution before altering a patient’s treatment regimen to improve the A1C using blinded-CGM data.

Supported by the University of New Mexico Clinical Translational Science Center 5M01 RR000997.

**4-LB**

### Structured SMBG Significantly Reduces A1c Levels in Poorly Controlled, Non-Inulin Treated Type 2 Diabetes: Results from the Step Study

**WILLIAM POLONSKY, LAWRENCE FISHER, CHARLES SCHIKMAN, DEBORAH HINNEN, CHRISTOPHER PARKIN, ZHIHONG JELSOVSKY, MATTHIAS SCHWEITZER, ROBIN WAGNER, San Diego, CA, San Francisco, CA, Skokie, IL, Wichita, KS, Carmel, IN, Tampa, FL, Indianapolis, IN**

The value and utility of SMBG in insulin-naive type 2 diabetes (T2DM) remains controversial. The Structured Testing Protocol (STEP) study, a 1-year, prospective, cluster-randomized, multi-center, clinical trial, assessed the effectiveness of structured SMBG use in poorly-controlled (A1c≥7.5%) insulin-naive T2DM patients. 522 patients from 35 US primary care practices were randomized to a structured testing protocol (STG) or active control (ACG). STG patients used the Accu-Chek® 360° View Blood Glucose Analysis System, an easy-to-use paper tool that facilitates

For author disclosure information, see page LB41.
collection and interpretation of 7-point glucose profiles over 3 consecutive days. STG patients completed the tool quarterly and brought it to medical visits. STG physicians and patients received standardized training in SMBG pattern recognition/interpretation. STG physicians received an algorithm for suggested medication strategies in response to observed SMBG patterns. STG and ACG patients received free blood glucose meters and test strips. The 12-month intent-to-treat analysis showed significantly greater reductions in A1c over time in the STG compared with the ACG (Δ -1.2% vs. -0.9%; ΔΔ -0.3%; p<0.04). Per protocol analysis showed even greater reduction in the STG compared with the ACG (Δ -1.3% vs. -0.8%; ΔΔ -0.5%; p<0.003). SMBG frequency was positively associated with A1c improvement in both groups. STG patients achieved significantly greater A1c improvement without requiring more frequent SMBG than ACG patients. 94% of STG patients received at least one treatment change recommendation during the 12 months vs. only 63% of ACG patients (p<0.0001). Over time, both groups showed a significant decrease in diabetes-related distress and depression (p<0.05) and a significant increase in positive well-being (p<0.01); well-being in the per protocol STG increased significantly more than in the ACG (p<0.04). Use of structured SMBG significantly improves glycemic control in non-insulin-treated T2DM, without increasing emotional distress, being in the per protocol STG increased significantly more than in the ACG (p<0.05) and a significant increase in positive well-being (p<0.01); well-being in the per protocol STG increased significantly more than in the ACG (p<0.04). Use of structured SMBG significantly improves glycemic control in non-insulin-treated T2DM, without increasing emotional distress, in adults with poorly controlled T1D whatever the age.

After the study, 2/3 of patients in G2 and 3/4 in G3 wished to continue with teleconsultation. There was no difference in the frequency of hypoglycemic episodes or in medical time spent for hospital or telephone consultations during the study. SMBG frequency was positively associated with A1c improvement in both groups. STG patients received at least one treatment change recommendation during the 12 months vs. only 63% of ACG patients (p<0.0001). Over time, both groups showed a significant decrease in diabetes-related distress and depression (p<0.05) and a significant increase in positive well-being (p<0.01); well-being in the per protocol STG increased significantly more than in the ACG (p<0.04). Use of structured SMBG significantly improves glycemic control in non-insulin-treated T2DM, without increasing emotional distress, in adults with poorly controlled T1D whatever the age.

Supported by sanofi­aventis, unrestricted grant, and Voluntis and Orange, technical support.

6-LB
A Single-Blind, Two-Period Study to Assess the Safety and Pharmacodynamics of an Orally Delivered GLP-1 Analog (Exenatide) in Healthy Subjects
RODY ELDOR, MIRIAM KIDRON, EHUD ARBIT, Jerusalem, Israel

GLP-1 analogs are incretin mimetics with proven antihyperglycemic capacity and effectiveness in reducing weight. As such, this drug family is attracting increasing attention as a potential pharmaceutical alternative to management of diabetes. However to date, these agents are available in parenteral dosage forms only. In addition, approximately 40% of diabetics who could benefit from their use, abstain from such treatment due to significant side effects, cost or irresponsiveness. Oramed Pharmaceuticals, developer of a novel proprietary drug delivery technology providing for oral delivery of polypeptides and proteins, has previously demonstrated effective oral delivery of exenatide (Byetta), a biologically active GLP-1 analog, in canine and porcine animal models. Here, we describe the results of a first-in-human, single-blind, two-period study focusing on the safety of and induced insulogenic responses to orally administered exenatide. Four fasting, healthy, male volunteers (mean age/BMI: 19/21.5) were administered a placebo and exenatide-based capsule (150 mg), formulated with Oramed’s absorption enhancing excipients, on visits 1 and 2, respectively. Subjects were challenged with a 75 g oral glucose load 60 minutes after capsule administration. The oral formulation of exenatide was well tolerated by all subjects and no adverse events of any degree were reported. As expected of healthy individuals, glucose responses were similar at both visits, demonstrating robust glycemnic control. In contrast, mean insulin AUC values were 17.6% higher in exenatide-treated subjects, when compared to their counterpart placebo sessions, reflecting exenatide absorption and bioactivity. In this first-in-human study, Oramed’s proprietary technology demonstrated safe and effectual oral exenatide delivery with proven retention of its biological functionality. These encouraging results provide a strong impetus for us to continue the development of this promising drug.

Comparing ITCA 650, Continuous Subcutaneous Delivery of Exenatide Via DUROS® Device, vs. Twice Daily Exenatide Injections in Metformin-Treated Type 2 Diabetes
ROBERT CUDDIHY, JULIO ROSENSTOCK, ROBERT HENRY, TOM ALESSI, KEN LUSKEY, Minneapolis, MN, Dallas, TX, San Diego, CA, Hayward, CA

ITCA 650 provides for the continuous and consistent delivery of exenatide using the DUROS technology, a subcutaneous osmotic delivery system that can deliver exenatide for up to 12 months with a single administration. This provides the opportunity to deliver optimal doses of exenatide with good tolerability and ensure 100% patient compliance. A phase 2 study to evaluate the efficacy, safety and tolerability of ITCA 650 treatment with an introductory treatment dose is being conducted in subjects with inadequately controlled, metformin-treated type 2 diabetes (T2DM). In this study, subjects (n=50 / group) were initially randomized to receive either 20 or 40 mcg/day of exenatide delivered by ITCA 650 for 12 weeks or twice daily exenatide injections at 5 mcg BID for 4 weeks followed by 10 mcg BID for 8 weeks. Subsequently, subjects were randomized to receive ITCA 650 at 20, 40, 60 or 80 mcg/day for an additional 12 weeks. Treatment with ITCA 650 was well tolerated with less reported nausea at 20 mcg/day relative to exenatide injections. As indicated, HbA1c was significantly lower after 12 weeks and weight was also reduced in all treatment groups.
In conclusion, ITCA 650 represents a novel treatment to deliver exenatide in a well-tolerated fashion that ensures 100% compliance for the long-term treatment of T2DM, resulting in substantial changes in HbA1c and weight, without the need for repeated self injections.

**8-LB**

**DURATION-5: Exenatide Once Weekly Resulted in Significantly Greater Improvement in Glycemic Control with Less Nausea Than Exenatide Twice Daily in Patients with Type 2 Diabetes**

**THOMAS BLEVINS, JOHN PULLMAN, JARET MALLOY, PING YAN, KRISTIN TAYLOR, CHRISTINE SCHULTEIS, MICHAEL TRAUTMANN, LISA PORTER, CATHLEEN CARTER, Austin, TX, Butte, MT, San Diego, CA, Indianapolis, IN**

The GLP-1 receptor agonist exenatide produces improved glycemic control and weight loss in patients with type 2 diabetes. This 24-week, randomized, open-label, comparator-controlled study compared treatment with exenatide once weekly (Ex OW; 2 mg) to exenatide twice daily (Ex BID; 10 mcg). The study was conducted in 252 intent-to-treat patients with type 2 diabetes (baseline [means±SD]: A1C 8.4±1.2%, fasting plasma glucose [FPG] 171±47 mg/dL, weight 96±20 kg). Patients were drug-naive (18.7%) or treated with one (46.8%) or a combination (34.5%) of oral antidiabetic medications. Over 24 weeks, Ex OW resulted in significantly greater decreases from baseline (LS means±SE) vs. Ex BID in A1C (-1.6±0.1% vs. -0.9±0.1%; P<0.001) and FPG (-35±5 mg/dL vs. -12±5 mg/dL; adjusted P=0.0008). Improvement in A1C were consistent across different background antidiabetic therapies. A significantly greater percentage of Ex OW patients (58.1%) achieved the A1C target of ≤7% compared to Ex BID patients (30.1%; adjusted P<0.0001). 41.1% Ex OW and 16.3% Ex BID patients achieved A1C of ≤5.5% (P<0.0001). Progressive reductions in mean body weight were observed in both treatment groups (change from baseline to Week 24 [LS means±SE]: -2.3±0.4 kg [Ex OW]; -1.4±0.4 kg [Ex BID]). Ex OW and Ex BID were well tolerated. Nausea, the most frequent AE, occurred less frequently with Ex OW (14%) than with Ex BID (35%) and was predominantly transient and mild or moderate in intensity. Injection-site reactions were infrequent, but more common with Ex OW. No major hypoglycemia occurred. Minor hypoglycemia was frequent and occurred only in patients using a concomitant sulfonylurea. No change in mean calcitonin concentrations was observed during the study. Pancreatic amylase and lipase concentrations were variable, both pre- and postbaseline; changes in these enzymes were not predictive of gastrointestinal AEs. In conclusion, continuous exenatide exposure with Ex OW therapy resulted in superior glycemic control with fewer gastrointestinal AEs compared to Ex BID in patients with type 2 diabetes. Both groups lost weight.

**9-LB**

**Effect of Sitagliptin on Glycemic Control in Patients with Type 1 Diabetes—A Pilot Study**

**SAMUEL L. ELLIS, EMILY G. MOSIR, JANET K. SNELL-BERGEON, PETER A. GOTTLIB, ANASTASIA S. RODIONOVA, SATISH K. GARG, Aurora, CO**

Despite new therapies and technology, the average A1c in patients with type 1 diabetes (T1DM) remains well above ADA recommended targets. This investigator-initiated pilot study was designed to evaluate the effects of sitagliptin in poorly controlled (A1c >8.5%) patients with T1DM. The study outcomes included area-under-the-curve for glucose excursions, A1c, and mean glucose values and other glycemic indices from CGM. 20 patients were enrolled in this pilot randomized, double-blind, cross-over study to receive sitagliptin 100 mg daily or placebo for 1 month and then crossed over for 1 month. All patients used a blinded DexCom continuous glucose monitor (CGM) throughout the study period. There were no differences in baseline demographics between the two groups. Mean ± SD age and duration of diabetes were 32.5 ± 12.3 and 17.3 ± 7.5 years respectively. 1 patient was discontinued while on placebo for severe hypoglycemia. There was a significant reduction in insulin dose in subjects while on sitagliptin (p=0.02). Sitagliptin use reduced A1c values during both periods within groups. After controlling for period, treatment and insulin dose, there was a significant reduction in A1c in patients receiving sitagliptin (LSM = -0.27±0.11%; p=0.025; Figure). The CGM downloads showed a decrease in mean (± SE) blood glucose (-10.9 ± 3.6, p=0.012), J Index (-9.0 ± 3.1, p=0.007) and HOMA IR (2.2 ± 0.70, p=0.007) when patients were receiving sitagliptin. Time spent in euglycemic range (80-140 mg/dL) was also significantly increased during sitagliptin use. We conclude that sitagliptin reduced total daily insulin dose, A1c and mean blood glucose values in patients with T1DM. Further research involving larger sample size for a longer period is needed to determine efficacy and safety of sitagliptin in patients with type 1 diabetes.

**10-LB**

**Exenatide Added to Insulin Glargine-Treated Patients with Type 2 Diabetes Achieved Glycemic Targets and Weight Loss with No Increased Risk of Hypoglycemia**

**JOHN BUSE, RICHARD M. BERGENSTAL, LEONARD C. GLASS, CORY R. HEILMANN, MICHELLE S. LEWIS, ANITA Y. M. KWAN, BYRON J. HODGWERF, JULIO RIGENSTOCK, Chaper Hill, NC, Minneapolis, MN, Indianapolis, IN, Dallas, TX**

Basal insulin replacement in long-standing type 2 diabetes (T2D) often requires additional prandial intervention to reach A1c targets. This first double-blind, placebo-controlled study of exenatide (EXE) added to insulin glargine (IG) oral agents in T2D (A1C 7.1-10.5%); randomized 259 patients (mean age 59y, weight 94kg, A1C 8.4%; diabetes duration 12y, insulin dose 48U [0.51U/kg] to EXE 10ug BID [n=137] or placebo [n=122]). Groups were generally comparable at baseline. Insulin dose was decreased by 20% if A1C≥8% or maintained if A1C<8% for 5 wks, then titrated to achieve a target fasting glucose of <100mg/dl. Primary endpoint was change in A1C. At 30 wks, A1C decreased by -1.7% to 6.7% in EXE, -1.0% to 7.4% in PBO; endpoint fasting plasma glucose (FPG) was similar between treatment groups (Table). Continuous glucose monitoring (n=23) and 7-point glucose profiles demonstrated statistically significant postprandial effects with EXE. Weight decreased in EXE (-1.8±0.3kg) and increased in PBO (+1.0±0.3kg). Insulin dose increased more in PBO (20±2%) than in EXE (13±2%). Hypoglycemia was similar for both groups; major hypoglycemia occurred twice in one PBO patient. Adverse events were more frequent for EXE vs PBO: nausea 41 vs 8%; diarrhea 18 vs 8%; vomiting 18 vs 4%; headache 14 vs 4%; constipation 10 vs 2%. This randomized study demonstrated complimentary action of EXE and basal insulin in T2D, allowing glycemic targets to be achieved in difficult to control insulin-treated patients with long-standing T2D without increasing risk of hypoglycemia or weight gain.

**Supported by Merck through a grant to the University of Colorado.**

For author disclosure information, see page LB41.
Exenatide Is Not Associated with Increased Acute Renal Failure

MERRI PENDERGRASS, WILLIAM CHEN, Franklin Lakes, NJ

Post marketing surveillance has revealed cases of acute renal failure (ARF) with exenatide use and this has led to an FDA warning. To investigate whether exenatide is associated with increased incidence of ARF (ICD-9, 584.), we analyzed a de-identified dataset of medical and pharmacy claims. We evaluated 8,910 patients started on exenatide, 18,155 patients started on a sulfonylurea, metformin or thiazolidinedione (DM control), and 395,844 non-diabetic patients (non-DM control). Patients with previous ARF, end-stage renal disease, or dialysis were excluded. Subjects were followed for at least 6 months. Baseline characteristics are shown below. Incidence of ARF was 0.33% in the non-DM control group, 1.12% in exenatide group and 1.25% in the DM control group. Cox analysis adjusted for baseline differences demonstrated diabetic subjects had a higher risk of ARF (HR 1.7, CI 1.1-1.9, p=0.001) than the non-DM control group. Subjects started on exenatide did not have an increased risk of ARF (HR 1.04, CI 0.71-1.53, p=0.94) compared with the DM control group. We also examined the association between ARF and sitagliptin. In the adjusted Cox analysis sitagliptin (N=15,738) was not associated with an increased risk of ARF (HR 1.25, CI 0.93-1.67, p=0.13). In conclusion, this study does not suggest exenatide is associated with an increased risk of ARF.

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GFT505 Improves Glucose and Lipid Homeostasis in Patients with Impaired Fasting Glucose and Impaired Glucose Tolerance

RÉMY HANF, ERIC BRUCKERT, BERTRAND CARIOU, RAPHAEL DARTEIL, DEAN W. HUM, BART STAELS, Leos, France, Paris, France, Nantes, France, Lille, France

GFT505 is a dual PPAR α/β agonist. In animals, GFT505 simultaneously ameliorates lipid and glucose homeostasis. In healthy volunteers, it is well tolerated with no clinically relevant adverse effect identified. In a double blind, randomized, placebo controlled trial included 47 patients with impaired fasting plasma glucose (>100 and <126 mg/dL), impaired glucose tolerance (2-hour glucose >140 and <200 mg/dL during OGTT) and waist circumference >94 cm for men and >80 cm for women. The objective was to assess the safety and efficacy of 35 days oral treatment with GFT505 at 80mg/d. Efficacy was assessed at Day-28 by comparing changes in fasting plasma glucose (FPG), insulin, C-peptide, HOMA-IR, plasma lipids and hsCRP and IL6 were not affected. No clinically relevant emergent adverse effect was reported while body weight, red blood cell count, hemoglobin or hematocrit, plasma homocysteine and adiponectin did not change. This demonstrates the potential of GFT505 for the management of prediabetes and associated cardiometabolic risk factors.

11-LB

CLINICAL THERAPEUTICS/NEW TECHNOLOGY

GS-9667, a Partial A, Adenosine Receptor Agonist, Lowers Free Fatty Acids (FFA) in Overweight Healthy Volunteers without Decrease in Renal Excretion

PETER STAEBH, ARVINDER K. DHALLA, XUEGONG WANG, YU HO, JUSTUS BINGHAM, RAJNEESH NATH, LUIZ BELARDINELLI, Foster City, CA

Chronically elevated circulating FFA levels play a significant role in the pathogenesis of insulin resistance and T2DM. Lowering FFA levels by reducing lipolysis may improve insulin sensitivity and glucose homeostasis. GS-9667 (formerly CVT-3619) is a partial agonist of the A1 adenosine receptors that has previously been shown to reduce plasma FFA levels in a Single Ascending Dose study. We carried out a Multiple Ascending Dose placebo-controlled study in 60 obese (BMI 30-45 kg/m2, waist circumference ≥40” males/≥35” females) but otherwise healthy volunteers. Up to 10 subjects (7 active, 3 placebo) in each group were dosed with 800 mg QD, 1200 mg QD, 1800 mg QD, 2400 mg QD as well as 1200 mg BID and 800 mg QID for 14 consecutive days. Overall, GS-9667 was safe and well-tolerated with no significant changes in heart rate, blood pressure, ECG, and clinical laboratory tests. Headache and nausea were the most common adverse events at all doses. Plasma concentrations of GS-9667 (Cmax) reached maximum (Tmax) between 30-60 minutes after dosing and were dose proportional but highly variable. The initial half-life (<17) of GS-9667 was ~1 hour, and the terminal t1/2~10 hours. Unlike placebo, consistent reductions (up to ~70%) in plasma FFA levels were seen with GS-9667, occurring at ~1.5-2 hours after dosing. Dosing at night showed similar FFA lowering effects. Reductions in FFA were comparable between Day 1 to Day 14 for all doses suggesting no desensitization of the FFA lowering effect of GS-9667.

13-LB

Human Regular Insulin U-500 Shows Prolonged Time-to-Peak Effect and Longer Duration of Action vs Human Regular Insulin U-100 in Healthy Obese Subjects

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Few pharmacokinetic (PK) and pharmacodynamic studies of U-500 human regular insulin (U-500R) have been conducted. The primary aim of this study was to evaluate the relative exposure after two clinically relevant doses of U-500R vs U-100 human regular insulin (U-100R) in healthy obese subjects. Fourteen male and 10 female subjects (age [mean±SD] 39.6±12.1 yrs; body weight 98.1±12.9 kg; body mass index 34.4±2.6 kg/m2) participated in a single-site, 4-period, 4-sequence, crossover, randomized, double-blind study. Following subcutaneous administration of 50-U and 100-U doses of U-500R to each subject in each period. Although U-500R had significantly longer duration of action compared to U-100R, with no rebound increase in FFA levels was observed during daily dosing or the 2 day washout period following dosing cessation. The FFA lowering effect after an individual dose was similar for the QD, BID and QID dose regimens. In summary, GS-9667 safely lowers FFA in a dose-dependent manner with no desensitization or rebound.

14-LB
Liraglutide Protects Against Traumatic Brain Injury in a Mouse Model

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Glucagon-like peptide-1 (GLP-1) analogs are emerging as an important drug class for the treatment of diabetes because they not only lower blood glucose but also body weight. Recently, several articles have pointed to a protective effect of GLP-1 or analogs on the brain and potential efficacy in animal models of stroke, Alzheimer’s, Huntington’s and Parkinson’s disease. However, in some cases the compounds were dosed by the intracerebroventricular route. We here report the effect of liraglutide treatment during traumatic brain injury (TBI) in a mouse model. Liraglutide is a once-daily human GLP-1 analog, the first to give 24-hour coverage in animals. Liraglutide was injected s.c. (0.4 mg/kg) twice daily before and after mice received a cryo-induced cortical lesion until 7 days post-lesion. Control mice received a match volume of s.c. saline. Mice were processed for immunohistochemistry. When compared to the saline-treated controls, liraglutide-treated mice had less inflammation as reflected by a decrease in lectin + microglial activation. In liraglutide-treated mice 8-oxoguanine DNA adduct formation was reduced and largely specific to endothelial cells, whereas in saline-treated mice adducts were widespread through the lesion and present primarily in neurons, microglia and endothelial cells. Liraglutide improved tissue healing response in TBI mice through concerted, lesion-directed astrogliosis, neurons, microglia and endothelial cells. Liraglutide improved tissue healing and blood-brain barrier reconstitution through reduced albumin leakage and increased perivascular astroglia. In conclusion, our data demonstrate that the GLP-1 analog liraglutide promotes an anti-inflammatory, antioxidant state in a murine traumatic brain injury model and improves tissue healing and blood–brain barrier reconstitution. These findings warrant further investigation into the mechanisms of action and future studies will explore the potential of liraglutide in protecting against neuropathology.

Supported by Lilly USA, LLC.

Liraglutide Treatment for 1 Year Offers Sustained and More Effective Glycemic Control and Weight Reduction Compared with Sitagliptin, Both in Combination with Metformin, in Patients with Type 2 Diabetes

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In adults with T2D taking metformin (mean age 55 years, BMI 33 kg/m², diabetes duration 6.2 years), the efficacy and safety of once-daily (OD) liraglutide (1.2mg or 1.8mg) were compared with OD sitagliptin 100mg in a 26-week randomized trial. A total of 487/554 (90%) completers continued the same treatments for another 26 weeks with 436/497 (88%) completing 1 year. The 3 groups were well balanced for demographics. After 1 year, both liraglutide 1.2mg and 1.8mg produced greater reductions in A1c, FPG, and bodyweight compared with sitagliptin, with more individuals reaching the A1c ADA target (Table). Overall scores on the Diabetes Treatment Satisfaction Questionnaire were better for liraglutide 1.8mg than sitagliptin (+1.35 point; p<0.05, LOCF). The proportions of patients reporting serious adverse events and adverse events (including hypoglycemia) were generally comparable among all 3 groups. One exception was nausea that initially occurred in more patients with liraglutide than sitagliptin but which was <2% in all groups in weeks 27–52 (Table). The overall adverse events from these 1-year data confirm the previous safety profiles of liraglutide and sitagliptin. Liraglutide offers superior and sustained glycemic control and greater bodyweight reduction, and 1.8mg offers greater overall treatment satisfaction than sitagliptin over 1 year.

Supported by Novo Nordisk.

16-LB

LX4211, a DUAL SGLT2/SGLT1 Inhibitor, Shows Rapid and Significant Improvement in Glycemic Control over 28 Days in Patients with Type 2 Diabetes (T2DM)

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Inhibiting the sodium glucose cotransporters SGLT2 and SGLT1 is a novel approach for achieving glycemic control in T2DM patients. LX4211 is a once-daily, orally-administered small molecule that inhibits SGLT2 and to a lesser extent SGLT1, with the objective of improving glycemic parameters by reducing glucose transport. LX4211 functions by inhibiting both glucose reabsorption by the kidney, resulting in urinary glucose loss, and glucose absorption by the small intestine, likely triggering the normal homeostatic incretin signaling response.

In this Phase 2a study, patients (N=36) with T2DM received 150 mg or 300 mg of LX4211 monotherapy, or placebo, administered orally once daily for 28 days. Preliminary data show significant and sustained glucosuria, with rapid and significant improvement in multiple parameters of glycemic control over
Once-Daily Basal Insulin Added to Oral Antihyperglycemic Medications (OAMs) and Exenatide (Ex) Improves Glycemic Control in Patients (Pts) with Type 2 Diabetes (T2D)

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This is the first open-label, multicenter, randomized, 24-week clinical trial conducted to evaluate efficacy and safety of once-daily basal insulin added to 1 or 2 OAMs plus Ex in adults with suboptimally controlled T2D. The primary aim was to determine if insulin lispro protamine suspension (ILPS), a basal analog available outside the US, is noninferior to glargine (G) in change in hemoglobin A1C (A1C).

Pts with A1C ≥7% and ≤5.0%, body mass index (BMI) ≤45 kg/m², and ≥2 months treatment with Ex (≥10 µg twice daily) and metforminsulfonfonylurea/pioglitazone were randomized to ILPS (n=171) vs G (n=168). Insulin was titrated to fasting plasma glucose (FPG) targets: ILPS 80-99 mg/dL, G 73-99 mg/dL. Statistical analysis was performed on intent-to-treat population. Prespecified noninferiority margin was 0.4%.

Mean baseline characteristics were similar across treatment groups (age 56 yrs; T2D duration 9.9 yrs; weight [wt] 102 kg, BMI 34.9 kg/m², and A1C [ILPS 8.2%, G 8.2%]). Leastsquares mean difference (ILPS minus G) in A1C change was 0.22% (95% confidence interval: 0.06 to 0.38). Although ILPS was noninferior to G, mean A1C reduction was significantly less for ILPS (1.18±0.84% vs G (1.46±0.97%; p=0.008). Target A1C ≤7.0% was achieved by 53.7% of ILPS-treated pts and 61.7% of G-treated pts (p=0.177). Endpoint FPG was similar (ILPS: 130±32, G: 127±29 mg/dL; p=0.179). Insulin dose was lower for ILPS (0.30±0.17 U/kg) vs G (0.37±0.17 U/kg; p<0.001).

Overall glycemia rates were similar (ILPS: 15.9±4.2 episodes/pt yr vs G: 18.1±24.6 episodes/pt yr; p=0.576). Nocturnal glycemia rate was higher for ILPS (4.3±4.8 episodes/pt yr) vs G (3.0±7.2 episodes/pt yr; p=0.004). Noninferiority of ILPS vs G was shown for A1C change. This study demonstrates that adding treat-to-target basal insulin is an effective option to improve glycemic control with minimal wt gain in pts with suboptimally controlled T2D treated with OAM(s) and Ex.

Supported by Eli Lilly and Company.

Positron Emission Tomography (PET) Imaging with [18F]FP-DTBZ Clearly Discerns Loss of Pancreatic Beta Cell Mass in Patients with Type 1 Diabetes Mellitus

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Adding basal insulin to oral therapy controls fasting plasma glucose (FPG) in T2DM, but many patients do not reach A1c goals because postprandial hyperglycemia persists. Weight-gain and hypoglycemia limit intensification of insulin therapy in such patients. Exenatide attenuates postprandial hyperglycemia and favors weight-loss. Hence, combining these agents is logical. We compared metformin+exenatide+insulin glargine, used in controlled T2D treated with OAM(s) and Ex.

Mean baseline characteristics were similar across treatment groups (mean age 55±10 yrs; mean ±SD BMI: 33.4±6.6 kg/m², mean ±SD duration of T2DM 8.5±5.7 years, mean ±SD BM after run-in 33.4±6.6 kg/m², median ±95%CI A1c after run-in 7.8% [6.9-9.2]). Were randomized to duration of T2DM 8.5±5.7 years, mean ±SD BMI after run-in 33.4±6.6 kg/m². 34 patients metformin±one other oral agent or <0.4 units/kg twice daily). Ctl subjects had a mean age of 27yrs, BMI of 26 kg/m², HbA1c of 5.6%.

Of BCM in 5 (3M, 2F) healthy control subjects (Ctl), and 6 (2M, 4F) long-standing T1DM subjects (T1DM) at baseline. Two groups were randomized to replace open-label exenatide with [18F]FP-DTBZ for VMAT2-directed imaging and quantification of BCM in 5 (3M, 2F) healthy control subjects (Ctl), and 6 (2M, 4F) long-standing T1DM subjects (mean duration: 19yrs). T1DM subjects had a mean age of 42yrs, BMI of 23 kg/m², HbA1c of 7.1%, and insulin dose of 48 units/kg/d. Ctl subjects had a mean age of 27yrs, BMI of 26 kg/m², HbA1c of 5.6%.

Functional β-cell capacity was assessed from C-peptide release following arginine stimulation test (AST). C-peptide AUC post-AST was reduced ≥50% in both groups (Ctl: 8.6±7.3 pmol/L, T1DM: 0.17 pmol/mL; p<0.001). Dynamic PET images were obtained after a bolus injection of −5.5 mCi [18F]FP-DTBZ. PET image data and metabolite-corrected arterial blood radioactivity were used to calculate distribution volume (Vd) in pancreas, liver, and kidney. Vd was a measure of the equilibrium concentration of radioligands in tissues relative to plasma. Panncreas Vd was −45% lower in T1DM subjects vs Ctls (Ctl: 230±44, T1DM: 127±20, p=0.086), whereas renal cortex Vd was comparable in both groups (Ctl: 22±4, T1DM: 25±3). Using the renal cortex as a reference tissue, the binding potential (BPVd) was reduced −55% in the subjects with diabetes (Ctl: 9.6±0.7, T1DM: 4.1±0.7; p=0.001).

In comparison to previous studies using [18F]FP-DTBZ, these data indicate that [18F]FP-DTBZ substantially improves, both qualitatively and quantitatively, the ability to non-invasively image pancreatic BCM in humans.
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Recently, the NAVIGATOR trial, a long-term intervention study performed in normotensive subjects with impaired glucose metabolism (IGM), showed that treatment with the angiotensin-receptor blocker valsartan (VAL) for 5 years, resulted in a relative reduction of 14% in the incidence of T2DM. The underlying mechanisms are incompletely understood and may, besides improvement in insulin sensitivity include a delay in beta-cell function decline.

We assessed the effect of 26-weeks VAL (320mg QD) vs. placebo (PLB) on beta-cell function and insulin sensitivity in normotensive subjects with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). In this randomised, controlled, double-blind, two-center study, 43 IFG (51% males; mean±SE age 58±1.1 yrs; BMI 28.8±0.7 kg/m²; BP 128/82±2/1 mmHg) and 36 IGT (53% males; age 58±1.1 yrs; BMI 29.5±0.7 kg/m²; BP 131/82±2/1 mmHg) subjects, received VAL (n=40) or PLB (n=39). Beta-cell function and insulin sensitivity were assessed at baseline and after 26 weeks using a combined euglycemic-hyperinsulimic and hyperglycemic clamp with subsequent arginine-stimulation and a 2-hour 75 g oral glucose tolerance test (OGTT). Treatment effects were analyzed using ANCOVA, adjusting for center, glucometabolic state and gender.

At week 26, VAL vs PLB, increased 1st-phase (P=0.06), and 2nd-phase glucose-stimulated insulin secretion (P=0.028), however, the enhanced arginine-stimulated insulin secretion did not differ (P=0.25). VAL vs PLB improved the disposition index (P=0.067) and insulin sensitivity (P=0.045). VAL but not PLB increased the OGTT derived insulinoergic index, representing 1st-phase insulin secretion after an oral glucose load (P=0.035). At 26 weeks, VAL compared to PLB treatment resulted in a greater reduction in systolic and diastolic blood pressure (P<0.001). BMI remained unchanged in both groups.

Twenty-six week VAL exposure showed an improvement in glucose-stimulated insulin release and insulin sensitivity in normotensive subjects with IGM. These findings may partly unveil the mechanisms underlying the observed effects of VAL in the prevention or delay of onset of T2DM.

Supported by an investigator-initiated study grant from Novartis Pharma.

The Effect of Valsartan on Beta-Cell Function and Insulin Sensitivity in Normotensive Subjects with Impaired Glucose Metabolism

The GLP-1 Analogue Liraglutide does not Induce Pancreatitis in Mice, Rats or Monkeys

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GLP-1 receptor agonists may be associated with an increased risk of pancreatitis in patients with type 2 diabetes. However, the low overall incidence of pancreatitis and a 3-fold increase in pancreatitis rates in diabetes patients makes it difficult to assess if the association is true. Phase 3 studies with liraglutide in 4400 type 2 diabetes patients did not demonstrate a clear association between treatment and pancreatopathy.

A mechanism linking GLP-1R activation to pancreatic inflammation has not been forthcoming. We report pancreas safety data in mice, rats and cynomolgus monkeys (NHP) during the non-clinical development programme for liraglutide. Pancreases from mice, rats and NHPs were macro- and microscopically examined using state-of-the-art diagnostic criteria. Mice and rats were dosed for 2 years, NHPs for 87 weeks (n=66-79/sex/group for mice, n=50 for rats, n=5 for NHPs; doses up to 3, 0.75 and 5 mg/kg/day, in mice, rat and NHP). Proliferation was assessed using PCNA staining in rats after 26 weeks. The evaluation in NHPs included detection of signs of pancreatic intraepithelial neoplasia in the ductal epithelium. There were no macroscopic observations of pancreatitis in mice, rats or NHPs.

After 2 years treatment, 8/358 male (3 in the control group, and 2, 1, 1, 1 in the different liraglutide groups) and 12/354 female mice (0 in the control group and 3, 3, 3, 3 in the liraglutide groups) were microscopically diagnosed with pancreatitis based on histological criteria of inflammatory infiltrates with or without fibrosis and/or loss of exocrine tissue. Pancreatitis was not the cause of death in any of these animals and pancreatitis is seen spontaneously in mice. There were no cases of pancreatitis in 405 male and female rats after 2 years dosing. Cell proliferation in the exocrine pancreas was not increased in rats dosed with liraglutide for 26 weeks. Neither pancreatitis nor pre-neoplastic proliferative lesions were found in NHPs dosed for 87 weeks, resulting in plasma liraglutide exposure levels 80-fold higher than that in humans at the maximal clinical dose. In conclusion, liraglutide does not induce pancreatitis in mice and rats dosed for 2 years or in NHPs dosed for 87 weeks.

Supported by Novo Nordisk A/S.

For author disclosure information, see page LB41.
ZGN-201, a Methionine Aminopeptidase 2 Inhibitor, Normalizes Glucose Tolerance in Overweight Dogs

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Methionine aminopeptidase 2 (MetAP2) inhibitor treatment reduces body weight, food intake (FI), increases fat oxidation and improves insulin sensitivity in obese mice; however efficacy has not been established in non-rodent species. Feeding with a high-fat-high-fructose (HFF) diet in dogs has been observed to induce a selective hepatic insulin resistance, reducing liver glucose extraction and causing glucose intolerance. We evaluated the effects of an 8 week treatment with ZGN-201 (ZGN) on body weight and metabolic parameters in 6 overweight dogs (3 HFF and 3 Chow-fed (OC)). The study was comprised of three phases – pretreatment (PT; 4 weeks), treatment (ZGNx; 8 weeks), and washout (WO; 4 weeks). FI, BW, blood chemistry and hematology, beta-hydroxybutyrate (BHB), and glycerol were assessed weekly. Oral glucose tolerance tests (OGTT; 0.9 g/kg initial BW) were performed at the end of PT, ZGNx, and WO. In HFF dogs, daily oral treatment with ZGN promoted loss of 81% of excess BW, whereas treatment in OWG dogs inhibited weight gain. ZGN reduced FI in HFF dogs by 29%, but had minimal effect (9% reduction) on FI in OWG dogs. Glycerol levels increased, reflecting enhanced lipolysis, while BHB levels, reflecting fat oxidation, were increased in HFF only.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>High-fat-high-fructose</th>
<th>Overweight Chow</th>
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<tbody>
<tr>
<td>PT</td>
<td>ZGNx</td>
<td>WO</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>32.5 ± 2.6</td>
<td>30.8 ± 3.4*</td>
</tr>
<tr>
<td>FI (g/d)</td>
<td>1677 ± 262</td>
<td>1187 ± 258*</td>
</tr>
<tr>
<td>BHB (µM)</td>
<td>51 ± 13</td>
<td>76 ± 8*</td>
</tr>
<tr>
<td>*p&lt;0.05 vs. PT</td>
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</table>

ZGN treatment reversed 75% of the abnormality in the plasma glucose excursion during the OGTT, despite a 32% reduction in insulin secretion (C-peptide AUC). After the drug was washed out, the improvement in plasma glucose was completely reversed. These results suggest that MetAP2 inhibitor treatment appears to correct the defect in hepatic glucose uptake seen in this model, and thereby decreases the demand for beta cell insulin secretion.

COMPLICATIONS—HYPOGLYCEMIA

Increased Brain Monocarboxylic Transport and Metabolism in T1DM Patients with Hypoglycemia Unawareness

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We have previously reported that the brain transport of the alternative fuel, acetate, is increased in intensively treated T1DM. To test the hypothesis that T1DM patients with hypoglycemia unawareness not only exhibit increased brain acetate transport, but also more efficient metabolism of acetate when challenged by acute hypoglycemia, we used magnetic resonance spectroscopy to measure the transport of infused [2-13C]acetate into the brain via MCA transporters and its incorporation into glutamine within glia under hypoglycemic conditions. For this purpose, brain acetate utilization was measured in 7 T1DM participants with hypoglycemia unawareness (mean age 42.7±11.9yrs, mean BMI 24.3±3.7, mean HbA1c 6.6±1.7%, 4 male/3 female, 4/7 islet cell transplant candidates) and 10 healthy non-diabetic controls (mean age 34.7±11.7yrs, mean BMI 26.6±3.0, mean HbA1c 5.2±0.4%, 4 male/6 female) during a 2 h hypoglycemic clamp (plasma glucose ~55mg/dl, insulin infusion 2mU/kg/min). As expected, glycogen and epinephrine levels did not significantly increase during hypoglycemia in the hypoglycemia unaware T1DM group, whereas modest increases were seen in the control group. There was no significant difference in circulating lactate levels between the two groups (at 120min post hypoglycemia, T1DM group 1.7mmol/l versus controls 1.6mmol/l, p=0.660). In contrast, brain C4 glucose fractional enrichments were significantly higher among T1DM group as compared to controls (T1DM 8.9±2.3% versus 5.9±2.0% among controls, p=0.011). These data demonstrate that not only is acetate transport increased, but that fractional glial metabolism of acetate is increased as well in T1DM patients with hypoglycemia unawareness compared to healthy nondiabetic controls. These results are consistent with the hypothesis that increased transport and metabolism of MCAs may be a protective adaptation among T1DM individuals with hypoglycemia unawareness by providing alternative non-glucose fuels during episodes of acute glucose deprivation.

Supported by Grant Number R01 DK02409 from the NIDDK and by Grant Number ULI RR024139 from the NCRR.

COMPLICATIONS—MACROVASCULAR—ATHEROSCLEROTIC CVD AND HUMAN DIABETES

Dietary Salt Intake and Mortality in Patients with Type 2 Diabetes

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High dietary salt intake is associated with an increase in blood pressure (BP) but it is unclear if it is also associated with an increase in mortality. In this study we explored the association between dietary salt intake, as measured by 24h urinary sodium excretion (U Na), and all cause mortality in patients with type 2 diabetes attending a single diabetes clinic during 2000-1 who were followed up until 2008. Baseline U Na was measured in 638 subjects. Mean baseline characteristics were as follows; age 64 years, 56% male, duration of diabetes 11 years, HbA1c 7.8%, LDL 2.6 mmol/L, BP 145/80mmHg, 47%, BMI ≥ 30kg/m2, 45% had previous history of cardiovascular (CV) disease, 33% had retinopathy, 30% had at least CKD stage 3, and 45% had increased albuminuria. During the study, 19 patients were lost to follow up and 156 died, with 46% of deaths CV related, 48% non-CV and 6% of unknown cause. U Na was inversely associated with all-cause mortality (Figure). For every 100 mmol rise in U Na, all-cause mortality was decreased by 29% (HR 0.71, CI 0.52-0.97, p=0.03). The relationship between U Na and all cause mortality remained significant after adjusting for age, gender, duration of diabetes, atrial fibrillation, the presence of renal and cardiovascular disease and systolic BP. The proportion of all-cause mortality explained by salt intake was greater than that conferred by conventional modifiable risk factors, including HbA1c, lipid and blood pressure levels. Furthermore, for cardiovascular mortality a similar relationship was observed with U Na.

The pathophysiological mechanisms underlying the relationship between U Na and mortality in this population remain to be established.

For author disclosure information, see page LB41.

ADA-Funded Research

LB8
Cumulative survival in individuals with Type 2 diabetes, stratified for tertiles of UAER (mmol/24h) at baseline.

Supported by National Health and Medical Research Council post graduate scholarship and Cardiovascular Lipid Grant, Pfizer.

**COMPLICATIONS**

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**COMPLICATIONS—MACROVASCULAR—CELLULAR MECHANISMS OF AtherosGENESIS IN DIABETES**

27-LB

Endoplasmic Reticulum Stress Inhibition Prevents ABCA-1 Reduction and Improves the Apo-Al-Mediated Cholesterol Efflux in Glycoxidized Macrophages

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Advanced glycation end products (AGE) are independently associated with atherosclerosis in diabetes mellitus (DM). In vivo and in vitro glycated albumin (alb) decreases ABCA-1 compromising the HDL-mediated cholesterol (chol) efflux. ABCA-1 reduction is not related to alterations in the gene transcription or mRNA levels. We tested in macrophages the effect of in vitro (AGE-alb) and in vivo (DM-alb) glycated-aldol on the endoplasmic reticulum (ER) stress and on its adaptive response — unfolded protein response (UPR). AGE-alb was made by incubating browning alb with 10 mM glycolaldehyde (4 days, 37°C) and control alb (C-alb) with PBS alone. DM-alb was isolated from uncontrolled DM patients serum by FPLC (HbA1c>10%) and alcoholic extraction. Mouse peritoneal macrophages (MPM) were incubated with DM-alb or AGE-alb along time and ER stress markers were assessed by immunoblot. ABCA-1 content was determined in MPM submitted to glycoxidation in the absence or presence of proteinosomal inhibitor (MG132, 1 mM) and ER stress inhibitor 4-phenylbutyric acid (PBA). Briefly, cells were overloaded with acetylated LDL (50 mg/mL) together with 14C-chol and evaluated in MPM incubated with AGE-alb in the presence of PBA. Briefly, cells were overloaded with acetylated LDL (50 mg/mL) together with 14C-chol (48h), treated for 18h with AGE-alb or C-alb and incubated for 8h with apol-Al (30 mg/mL). As compared to C-alb, AGE-alb induced a time-dependent increase in Grp78, Grp94, elf2a and ATF6. Both AGE-alb and DM-alb increased ubiquitin expression, suggesting greater proteinosomal activity. However, inhibition of ER stress with PBA, but not with MG132, was able to recover the ABCA-1 content in AGE-alb-treated MPM. chol efflux was 47% reduced in AGE-alb-treated MPM in comparison to C-alb-treated cells (p <0.0001; n=6). PBA was able to completely restore the chol efflux in agreement to the recovery of ABCA-1 protein content. In conclusion, ER stress elicited by in vivo and in vitro alb glycated impair the macrophage reverse cholesterol transport, contributing to the atherosclerosis in diabetes mellitus.

Supported by FAPESP (Brazil).

28-LB

PDE3A Deletion Suppresses Mouse Vascular Smooth Muscle Cell Proliferation Via Inhibition of MAPK Signaling and Differential Regulation of Cell Cycle Regulatory Proteins

N AJMA BEGUM, STEVEN HOCKMAN, VINCENT MANGANIELLO, Bethesda, MD

Phosphodiesterase type 3 (PDE3) is an important regulator of cAMP-mediated responses within the cardiovascular system. However, the function and role of individual PDE3A and PDE3B isoforms in VSMC growth regulation is largely unclear, primarily due to the lack of isoform-selective PDE3 inhibitors. In this study, we examined the role of PDE3A and B isoforms in VSMC growth regulation, and the mechanisms by which PDE3 isoforms may affect signaling pathways that mediate PDGF-induced VSMC proliferation. Cultured VSMCs expanded from the aortas of PDE3A deficient mice (PDE3A−/−) exhibited >90% inhibition of serum- and PDGF-induced DNA synthesis and on total protein from kidneys of Sprague Dawley rat showed that our purified SGLT3a protein reacted with single protein with the expected MW size of 72 kDa. In the 5 weeks old ZDF rats, glucose excretion was only 0.02 g/day but it sharply increased to 9, 16, and 20 g/day in 8, 12, and 15 weeks old diabetic rats, respectively. SGLT3a protein was expressed in the kidneys of ZDF rats at all ages. And, its levels doubled from 5 to 19 weeks of age. Our study showed for the first time that SGLT3a protein is expressed in the kidneys of mouse and rat, and that its level is increased in response to diabetes. We propose that SGLT3 may mediate increased sodium reabsorption in diabetes.

Supported by NIH/NIDDK funded Pilot and Feasibility Grant.

29-LB

Expression of SGLT3a in the Kidneys of Zucker Diabetic Fatty (ZDF) Rats

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Diabetes can change normal expressions of sodium-dependent glucose transporters, SGLT1 and SGLT2, in the kidney, however, effect of diabetes on renal expression of SGLT3 has not previously been investigated and is the focus of this study. At the amino acid sequence level, human SGLT3 (hSGLT3) is most homologous to hSGLT1; however, it may function as a glucose stimulated sodium channel. While human has one gene encoding SGLT3, mouse and rat each has two genes encoding SGLT3a and SGLT3b. We have previously shown that the mRNA of SGLT3a and SGLT3b are expressed in the kidneys of mouse. Here, we investigated whether SGLT3a is expressed in the kidneys of rat and if its expression is modulated in response to diabetes. Our BLAST analysis showed that the amino acid sequence of rSGLT3a is 80% and 92% identical to hSGLT3 and mSGLT3a, respectively. Next, amino acid sequences of SGLT3a from mouse and rat were aligned, a common peptide sequence was selected, and was used as antigen to raise polyclonal antibody in rabbit. To examine expression of SGLT3a in diabetes, we used Zucker Diabetic Fatty (ZDF) rat, Leprfa+/+ Cri, a model of non-insulin dependent diabetes. Three male fa/fa animals at each 5, 8, 12, 15, and 19 weeks of age were used. Overnight urine was collected to measure glucose excretion. Then, kidneys were isolated and total protein was prepared. Western blot was performed with our SGLT3a antibody and actin was used for normalization. The result of Western blot analysis on cortical brush border membrane proteins from mouse kidney and on total protein from kidneys of Sprague Dawley rat showed that our purified SGLT3a antibody reacted with single protein with the expected MW size of 72 kDa. In the 5 weeks old ZDF rats, glucose excretion was only 0.02 g/day but it sharply increased to 9, 16, and 20 g/day in 8, 12, and 15 weeks old diabetic rats, respectively. SGLT3a protein was expressed in the kidneys of ZDF rats at all ages. And, its levels doubled from 5 to 19 weeks of age. Our study showed for the first time that SGLT3a protein is expressed in the kidneys of mouse and rat, and that its level is increased in response to diabetes. We propose that SGLT3 may mediate increased sodium reabsorption in diabetes.

Supported by NIH/NIDDK funded Pilot and Feasibility Grant.

30-LB

WITHDRAWN

31-LB

USF1 Transcription Factor Is Critical to the Development of Albuminuria in Early and Late Stage Diabetes

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Diabetes is the leading cause of end stage renal disease, but the factors that lead to diabetic nephropathy (DN) in some individuals and not others are not clearly understood. The transcription factor Upstream Stimulatory Factor 1 (USF1) binds the glucose responsive element in the promoter region of key genes involved in DN in response to high glucose. To determine the role of USF1 in vivo in progression of DN, we evaluated male USF1 knockout mice that were intercrossed with Akita mice (Ins2/ Akita), an autosomal dominant model of type 1 diabetes that develop diabetes at approximately 4 weeks of age. Akita mice were found to have significantly elevated albuminuria compared to wild type controls (WT, C57Bl/6) as early as 6 weeks of age (2 weeks of diabetes, mean albuminuria in Akita: 369.8 +/- 117.2 mcg/mgCr compared to WT: 19.6 +/- 4.7 mcg/mgCr, p<0.0001) and this pattern persisted after 20 weeks (p<0.05) and 28 weeks (p<0.05) of sustained hyperglycemia (blood glucose -600mg/dl). However, USF1 deficient Akita mice did not exhibit significantly elevated albuminuria when compared to WT in the early stage.

For author disclosure information, see page LB41.
of diabetes (6 week mean albuminurias: 72.4 +/− 20 mcg/24h, p=NS vs. WT) or in later stages of diabetes at 20 and 28 weeks (p=NS vs. WT). 24 hour urinary hydrogen peroxide excretion, a marker for oxidative stress/inflammation, was significantly elevated in Akita mice after 2 weeks of diabetes (p<0.0001), but not in USF1 deficient Akita mice. Levels of mRNA of the major podocyte protein nephrin in kidney cortex were significantly reduced in Akita mice (p<0.05), but were not significantly different from WT in the USF1 deficient Akita mice. There was no significant difference in blood glucose, body weight, or kidney weight between the different diabetic groups. Together this data indicates that the transcription factor USF1 plays a central role in development of albuminuria in both early and late stages of diabetes, and this action is likely mediated via USF1’s role on podocyte dysfunction, as USF1 deficient Akita diabetic mice have similar amounts of albuminuria, urinary hydrogen peroxide excretion, and mRNA expression of nephrin compared to wild type controls.

ADA-Funded Research

32-LB
Wnt/β-catenin Pathway in Podocytes Modulates Albuminuria and the GBM in Diabetic Kidney Disease
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The Wnt/β-catenin signaling plays key role in development, cell growth, cell differentiation. In the canonical pathway, binding of the soluble ligand Wnt to its receptor leads to dephosphorylation, stabilization and nuclear translocation of β-catenin (Ctnnb1).

By performing gene expression analysis, we found that genes belonging to the Wnt/β-catenin signaling pathway are regulated in glomeruli and podocytes of patients with diabetic kidney disease (DKD) and mouse models of DKD.

To understand the role of β-catenin in podocytes, we generated mice in which stabilized β-catenin is expressed in podocyte (NPHP2Cre/Ctnnb1E34H WT). These animals presented with severe glomerular basement membrane thickening at 4 weeks of age and albuminuria around 10 weeks of age. The morphologic and phenotype changes were similar to human DKD. Animals homozygous for stabilized β-catenin (NPHP2Cre/Ctnnb1E34HA/ A) exhibited high grade albuminuria and severe glomerulosclerosis. Mice with podocyte specific deletion of β-catenin (NPHP2Cre/Ctnnb1Ex3/ Ex3) presented with occasional GBM splitting and increased susceptibility for albuminuria. In vitro studies performed on isolated podocytes indicate that wnt/β-catenin might play important role in cell differentiation. In vivo studies performed on isolated podocytes indicate that wnt/β-catenin pathway is important for cell motility and adhesion.

In summary, we show that the Wnt/β-catenin signaling plays key role in podocyte differentiation and the appropriate level and activity of Wnt/β-catenin is critical for maintaining the glomerular filtration barrier. Dysregulation of the Wnt/β-catenin plays role in DKD development.

Supported by NephCure Foundation.

ADA-Funded Research

33-LB
Loss of Intraepidermal Neural Endocannabinoid Receptors is Reflected in Reduced Thermal Perception and Sympathetic Function
GARY L. PITTENGER, HENRI PARSON, MICHAEL ORCIGA, KATHRYN SIMMONS, LUCY GEE, PHIL ALBRECHT, FRANK RICE, AARON I. VINIK, GARY L. PITTENGER, HENRI PARSON, MICHAEL ORCIGA, KATHRYN SIMMONS, LUCY GEE, PHIL ALBRECHT, FRANK RICE, AARON I. VINIK, NORFOLK, VA, Albany, NY

Diabetic peripheral neuropathy (DPN) is a common complication of type 1 and type 2 diabetes; with loss of small intraepideral nerve fibers (IENF) and causing impaired sensory and autonomic function. However, measures of IENF density do not allow for evaluation of specific subpopulations of small nerve fibers subserving different sensory modalities. Nerve fibers positive for transient receptor potential vaniloid type 1 (TRPV1), an endocannabinoid/vaniloid receptor, are fibers responsible for thermal perception. We hypothesized that TRPV1-positive fibers are lost in diabetes and will correlate with specific sensory dysfunctions. We studied 12 patients from 4 groups: controls, metabolic syndrome, newly diagnosed diabetes (>5 yr) and established diabetes (>5 yr) patients. Contact heat-evoked potential analysis, quantitative sensory tests, autonomic function tests and neuropathy symptom scores were determined, and a 3mm punch skin biopsy was taken 10 cm proximal to the lateral malleolus. Immunofluorescence for TRPV1 was performed on 14 μm sections of the biopsies and evaluated by 2 independent observers for prevalence of TRPV1-positive fibers using a qualitative scale from 1 (no fibers) to 4 (numerous fibers). There was a clear progressive loss of TRPV1 fibers from controls (mean score 2.8) to metabolic syndrome (2.0) to newly diagnosed patients (1.7) to established diabetes patients (1.6). There was an inverse correlation between TRPV1 scores and both warm (p<0.05) and cold (p<0.04) thermal perception. Surprisingly, there was a strong positive correlation between TRPV1 score and low frequency (LF)/high frequency (HF) responses to autonomic challenge, a measure of autonomic balance between sympathetic (LF) and parasympathetic (HF) function (p<0.006). In addition, there was a trend toward impaired sympathetic function with reduced TRPV1 score (p=0.056). This is the first demonstration that there is a progressive loss of a specific subset of IENF, TRPV1-positive, with progressing metabolic disease. Exploring means of preventing or correcting this fiber loss could result in novel therapies for DPN.

ADA-Funded Research

34-LB
WITHDRAWN

35-LB
Prevalent Diabetes, Peripheral Vascular Disease and Cystatin-C Are Related to Peripheral Nerve Declines in the Oldest Old Adults
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Type 2 diabetes (DM) is the major risk factor for peripheral nerve impairments that occur with aging but does not entirely explain age-related decline. Among older adults with prevalent DM (N=188), incident DM (N=105) and no DM (N=724), we evaluated the association of traditional (age, height, alcohol use, smoking) and non-traditional (subclinical vascular disease, high lipids, renal function) factors with greater sensory and motor nerve declines in the Health ABC Study (N=3075 well-functioning persons, 70-79 years in 1997-98). Sensory vibration threshold (VT; range 0-130 μ; VSA-3000, Medoc) and peroneal motor nerve conduction velocity (NCV, Neuromax, Xilete) and amplitude (CMAP) were assessed in 2000-01 (52.9% women, 32.5% black, 76.0±2.7 years; 19.5% DM) and 2007-08 (28.8% DM). Those with maximum VT (N=5), clinically low NCV (<40 m/s; N=67) and CMAP (<1 mV; N=36) at both time points were excluded from respective analyses of decline due to previous clinical impairment. Prevalent and incident DM had higher A1C in 2000-01 (7.0±1.4 and 5.7±0.5 vs. 5.3±0.4%; p<0.001) and 2007-08 (6.9±1.0 and 6.4±0.8 vs. 5.7±0.4%; p<0.001) vs. no DM. VT worsening was 15.4±40.1 μ for prevalent DM, 14.4±40.2 μ for incident DM, and 13.5±36.3 μ for no DM (REF; ns). NCV decline was -2.68±1.28 m/s for prevalent DM, -2.66±3.89 m/s for incident DM, and -2.06±4.93 m/s for no DM (REF). Multivariable regression was performed separately for each peripheral nerve decline, adjusting for variables described above and baseline nerve function. Prevalent DM, taller height, cystatin-C >1 mg/L and ankle/arm index <0.9 (CMAP only) predicted greater motor nerve decline. Older age and taller height predicted greater VT change but DM was not significant. Incident DM was not related to decline though a small sample size for incident DM may have limited our findings. The results suggest that in addition to DM, comorbidities common in DM - renal function and peripheral vascular disease - are markers for peripheral nerve declines in the oldest adults.

36-LB
Angiotensin (1-7) Peptide Protects Retinal Vascular Dysfunction in Animal Model of Diabetic Retinopathy
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Diabetic retinopathy (DR) is the most common form of diabetic vascular complications, and the leading cause of severe vision loss in people under age of sixty. Increasing evidence indicates that the non-angiotensin-system (RAS) in the eye plays a significant local role in retinal vascular dysfunction in DR. This coupled with our previous observations that enhanced ACE2/Ang (1-7) axis of RAS produces beneficial effects on hypertension-induced cardiovascular remodeling, we hypothesize that the Ang 1-7 peptide would protect diabetic retina by counteracting the deleterious effect of Angiotensin II (AngII). Our objective

For author disclosure information, see page LB41.
in this study was to test this hypothesis and identify underlying mechanisms. We constructed an AAV vector that constitutively express the Angl-7 peptide. The vector was injected into intravitreal cavity of one eye of streptozocin (STZ)-induced diabetic animals (both mice and rats). The other eye served as a control. The progression of the retinopathy was followed by fluorescein angiography, retinal vasculature was evaluated by albumin extravasations to quantitatively measure the vascular permeability, fluorescein-isothiocyanate (FITC)-conjugated Concanavalin A lectin (ConA) perfusion to quantify the adhesive leucocytes and the pericyte/endothelial cell loss was evaluated by trypsin-digested retinal vascular preparation. Cytokine expression profiles and NOS enzymes were examined by real-time PCR, ELISA, and western blotting. We found that treatment with Angl-7 expressing vector resulted in a significant reduction in retinal vascular permeability and attenuation of pericyte/endothelial cell loss. Treated eyes also showed significant reduction of VEGF, ICAM1 and iNOS activation. The protective role of Angl-7 is likely mediated by blocking the inflammatory responses and iNOS activation, leading to reduced oxidative stress induced by hyperglycemia.

ADA-Funded Research

DIA-BETES EDUCATION

WITHDRAWN

37-LB

Knowledge, Comfort Level and the Perceived Role of Nurses in Promoting Nutritional Management of Diabetes

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Diabetes Mellitus (DM) affects 18 million people in the United States. While the responsibility of DM education lies primarily with registered dietitians (RDs) and certified diabetes educators (CDEs) these professionals may not be available to patients as often as nurses. Ninety percent of nurses report receiving requests for nutrition advice from patients. A review of the literature suggests the current knowledge of nutritional management of DM is low among nurses. The objective of this study was to investigate the knowledge, comfort level and perceived role among nursing students regarding nutritional facts and the nurse’s role in promoting nutritional management of DM. A survey was developed based on the American Diabetes Association’s nutrition guidelines. Reviewers’ comments and results of previously conducted focus groups were used to modify the survey for content validity. Survey reviewers included a focus group expert, 2 doctoral nursing professionals and 2 CDEs/RDs. Greater than 80% of nursing students reported that when caring for patients with DM nurses have a responsibility to treat high and low blood glucose levels and provide and reinforce basic nutrition education. When asked to select CHO foods, 29% chose cheese and 15% chose eggs. Fifty-six percent and 45% (respectively) did not consider milk or bran cereal to be CHO foods and 43% percent were not able to identify CHO content of 4oz orange juice. Thirty-one percent selected one or more macronutrients to be excluded from diabetic meal plans and 50% did not know where to locate CHO content of foods on a food label. When asked to select the best treatment for symptomatic hypoglycemia 30% selected ice cream, cake or candy bar instead of glucose tablets. Nursing students perceive nurses to have an important role in promoting nutrition management of DM to patients however nursing students are not able to consistently identify CHO content of many foods. Approximately 1/3rd of nursing students may not be aware of the appropriate treatment of hypoglycemia. This study emphasizes the need for collaboration between dietitians and nurses to improve nutrition knowledge for DM among nursing students.

ADA-Funded Research

39-LB

Chokeberry Extract Regulates the Postprandial Overproduction of Apolipoprotein B48-Containing Lipoproteins by Modulating the Gene and Protein Expression of Multiple Pathways in Small Intestine of Rats Fed a Fructose Rich Diet

BOLIN QIN, RICHARD A. Anderson, Beltsville, MD

Antioxidants have been shown to exert benefits on dyslipidemia in animal and human studies. Triglyceride-rich lipoprotein–apolipoprotein (apo)B48 (TRL-apoB48) plays an important role in postprandial lipoprotein metabolism. The aim of present study was to determine if an extract from chokeberries, which are a rich source of anthocyanins, improves the postprandial TRL-apoB48, in rats fed a fructose-rich diet (FRD, 60%), and explore the mechanisms related to insulin signaling, lipoprotein metabolism and inflammatory related pathways in the small intestine. Chokeberry extract (CBE, CellBerry®, Integrity, 200 mg/kg/d) was added to the drinking water for 6 wks in rats made insulin resistant by feeding a FRD. Chronic CBE intake lowered body weight gain, blood glucose, triglycerides, cholesterol, and LDL-C in the insulin resistant rats. Glucose intolerance and elevated plasma adiponectin levels were also improved. In an olive oil loading study, CBE inhibited postprandial triglycerides and the overproduction of total- and TRL-apoB48. In ex vivo ES labeling experiments, significant decreases were also observed in apoB48 secretion into the media, in the CBE treated primary enterocytes of FRD fed hamsters. mRNA of the small intestine for Irs1, Irs2and P38k were increased by the CBE supplement. Sterol regulatory element binding protein-1c (Srep1p1ca), microsomal triglyceride transfer protein (Mttp) mRNA and protein levels were decreased by CBE, and Cebpa mRNA and protein and mRNA levels of Niammann-Pick C1-like 1 (Apc11f) and peroxisome proliferator-activated receptor (Pparg) were increased by the CBE and are consistent with improved lipid metabolism. The levels of gene expression of inflammatory genes such as Il-1α, Il-8 and Tnfα were lowered by CBE. In summary, these results suggest that the chokeberry extract (CellBerry®) improves the metabolic profiles and the postprandial TRL-apoB48 production in insulin resistance by modulating multiple pathways associated with insulin signaling, lipoprotein metabolism and inflammation. Supported by USDA/ARS CRADA No.58-3K95-7-1184 with Integrity.

Supported by USDA/ARS CRADA No.58-3K95-7-1184 with Integrity.

DIABETIC DYSLIPIDEMIA

38-LB

Glucagon-Like Peptide-2 Stimulates Intestinal Fat Absorption and Chylomicron Overproduction Via Activation of Nitric Oxide Synthase

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With diabetic dyslipidemia increasingly acknowledged as a postprandial phenomenon, it is important to understand hormonal regulation of dietary lipid absorption. We have recently demonstrated that the intestinotrophic gut-derived peptide glucagon-like peptide-2 (GLP-2) can rapidly accelerate intestinal fatty acid uptake and apolipoprotein B48 (apoB48)-containing triglyceride-rich lipoprotein (TRL) secretion in both hamsters and mice in a CD36-dependent mechanism. However, the GLP-2 receptor is not directly expressed on the absorptive enterocyte and the intermediary molecule(s) required for GLP-2’s postprandial hyperlipidemic effect is currently unidentified. GLP-2 has been shown to stimulate mesenteric blood flow in a nitric oxide synthase (NOS)-dependent pathway. We investigated the role of NOS in GLP-2-stimulated fat absorption in Syrian golden hamsters. Conscious hamsters were pre-treated with a NOS inhibitor L-Nω-nitroarginine methyl ester (L-NAME, 75 mmol/kg) and challenged with an oral fat load containing [3H]triolein. Inhibition of NOS prevented the GLP-2-exacerbated dietary fatty acid uptake at 60, 90, and 120 min post gavage. However, L-NAME alone did not change fat absorption relative to control. To monitor apoB48 secretion rates, hamsters were also injected with Poloxamer 407 to block peripheral TRL catabolism. L-NAME pretreatment completely abrogated GLP-2-enhanced TRL-liquid secretion in response to a fat load. TRL-apoB48 levels were depressed, but to a lesser extent, in hamsters pre-treated with L-NAME. Intestinal NOS activity was assessed by NADPH diaphorase histochemistry. Enterocyte proteins susceptible to S-nitrosylation were labelled by the biotin-switch assay and identified by mass spectrometric methods. Primary hamster enterocytes were also exposed to the NO-donor S-nitroso-L-glutathione (GSNO, 100 mmol/L) to examine apoB48 synthesis. In conclusion, GLP-2 stimulates the absorption of dietary fatty acids in a mechanism dependent on NOS activation. It appears the role of NOS extends beyond stimulating blood flow to increase circulating free fatty acid delivery to the small intestine.

ADA-funded research accompanies this study.

40-LB

Cholecalciferol Regulates the Expression of Multiple Pathways in Small Intestine of Rats Fed a Fructose Rich Diet

BOLIN QIN, RICHARD A. ANDERSON, Beltsville, MD

Antioxidants have been shown to exert benefits on dyslipidemia in animal and human studies. Triglyceride-rich lipoprotein–apolipoprotein (apo)B48 (TRL-apoB48) plays an important role in postprandial lipoprotein metabolism. The aim of present study was to determine if an extract from chokeberries, which are a rich source of anthocyanins, improves the postprandial TRL-apoB48, in rats fed a fructose-rich diet (FRD, 60%), and explore the mechanisms related to insulin signaling, lipoprotein metabolism and inflammatory related pathways in the small intestine. Chokeberry extract (CBE, CellBerry®, Integrity, 200 mg/kg/d) was added to the drinking water for 6 wks in rats made insulin resistant by feeding a FRD. Chronic CBE intake lowered body weight gain, blood glucose, triglycerides, cholesterol, and LDL-C in the insulin resistant rats. Glucose intolerance and elevated plasma adiponectin levels were also improved. In an olive oil loading study, CBE inhibited postprandial triglycerides and the overproduction of total- and TRL-apoB48. In ex vivo ES labeling experiments, significant decreases were also observed in apoB48 secretion into the media, in the CBE treated primary enterocytes of FRD fed hamsters. mRNA of the small intestine for Irs1, Irs2and P38k were increased by the CBE supplement. Sterol regulatory element binding protein-1c (Srep1p1ca), microsomal triglyceride transfer protein (Mttp) mRNA and protein levels were decreased by CBE, and Cebpa mRNA and protein and mRNA levels of Niammann-Pick C1-like 1 (Apc11f) and peroxisome proliferator-activated receptor (Pparg) were increased by the CBE and are consistent with improved lipid metabolism. The levels of gene expression of inflammatory genes such as Il-1α, Il-8 and Tnfα were lowered by CBE. In summary, these results suggest that the chokeberry extract (CellBerry®) improves the metabolic profiles and the postprandial TRL-apoB48 production in insulin resistance by modulating multiple pathways associated with insulin signaling, lipoprotein metabolism and inflammation. Supported by USDA/ARS CRADA No.58-3K95-7-1184 with Integrity.

Supported by USDA/ARS CRADA No.58-3K95-7-1184 with Integrity.
Novel Insight into the Function of C-Terminal Domain of Human ApoC-III in Promoting Hepatic VLDL Production under Lipid-Rich Conditions

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Triacylglycerol (TAG)-rich VLDL overproduction plays an important role in the progression of diabetic dyslipidemia. Our previous study showed that hepatic expression of apoC-III enhances VLDL assembly/secretion under lipid-rich conditions, and that the N-terminal region of apoC-III, particularly Ala23, is critical for its stimulatory role in the final stage of VLDL maturation. In this study, we determined whether the putative lipid-binding domain within the C-terminal region of apoC-III is involved in promoting oleate-induced VLDL production. Metabolic labeling experiments in McA-RH7777 cells stably expressing wildtype apoC-III (C3wt cells) or a naturally-occurring apoC-III missense mutant Lys58Glu (C3KE cells) showed that Lys58Glu effectively abolished the role of apoC-III in promoting the secretion of VLDL-associated TAG. The impaired VLDL secretion in C3KE cells was not due to a decreased secretion of the apoC-III mutant protein. Moreover, subcellular fractionation experiment showed no apparent difference in the distribution of Lys58Glu between the ER and Golgi, indicating normal protein trafficking of the apoC-III mutant through the secretory pathway. In contrast, the mobilization of newly synthesized TAG into the microsomal lumen, which is considered a prerequisite step for the formation of luminal lipid droplets and subsequent VLDL maturation, was dramatically reduced in C3KE cells, as compared with that of C3wt cells. The decreased luminal lipid accumulation in C3KE cells was phenotypically similar to what was observed in cells treated with an inhibitor of microsomal triglyceride transfer protein (MTP). However, unlike MTP inhibitor treatment, cytosolic TAG pool was not increased in C3KE cells. In conclusion, our study suggested that the C-terminal structural element of apoC-III, particularly Lys58, may also be critical for MTP-mediated luminal lipid accumulation for VLDL production. Lys58Glu-mediated suppression in VLDL production, without accompanying hepatosteatosis, may provide a new therapeutic approach for controlling diabetic dyslipidemia in comparison to MTP antagonists.

Supported by CIHR (China-Canada joint health research initiative program)

The Reduction of LXR Activity by Ezetimibe Improves the Metabolic Diseases

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Ezetimibe is the inhibitor of cholesterol absorption and used in treating hyperlipidemia. It has been reported that ezetimibe not only decreases serum total cholesterol, but also reduces serum triglyceride and improves hepatic steatosis and insulin resistance in mice and human. However, that mechanism is not fully understood. Here we report the mechanism for that. We used KK-A mice, which is the animal model of the diabetes and obesity, fed a high fat diet with or without ezetimibe. In ezetimibe-treated mice, serum and hepatic lipids, serum glucose and insulin were decreased and the hepatic steatosis was ameliorated. OGTT and IPITT indicated improved glucose tolerance and insulin sensitivity. Administration of ezetimibe decreased serum total cholesterol, but also reduces serum triglyceride and improves hepatic steatosis and insulin resistance. It has been reported that ezetimibe not only decreases serum total cholesterol, but also reduces serum triglyceride and improves hepatic steatosis and insulin resistance. However, ezetimibe was not increased in C3KE cells. In conclusion, our study suggested that the C-terminal structural element of apoC-III, particularly Lys58, may also be critical for MTP-mediated luminal lipid accumulation for VLDL production. Lys58Glu-mediated suppression in VLDL production, without accompanying hepatosteatosis, may provide a new therapeutic approach for controlling diabetic dyslipidemia in comparison to MTP antagonists.

Supported by CIHR (China-Canada joint health research initiative program)

Very Low Density Lipoprotein (VLDL)-ApoB100 Overproduction Is Associated with the Systemic and Hepatic Inflammation in Otsuka Long-Evans Tokushima Fatty (OLETF) Rats, an Animal Model of Type 2 Diabetes

BOLIN QIN, YOSHIHARU SHIMOMURA, Beltsville, MD, Nagoya, Japan

Overproduction of hepatic VLDL1 particles is the main abnormality of lipoprotein metabolism in type 2 diabetes (T2D). Molecular mechanisms linking systemic, hepatic inflammatory and insulin resistance and apoB100-containing VLDL1 production are not clear. In this study, compared with the control rats, OLETF rats at 19 weeks showed obesity and systemic inflammation (plasma TNF-α and IL-6 levels increased); insulin resistance (plasma glucose, insulin, retinol binding protein 4 (RBP4), and soluble CD36 levels were higher); dyslipidemia (plasma triglyceride, cholesterol, and FFAs levels were higher; as well as VLDL1-apoB100, VLDL-triglyceride were overproduced). In livers of OLETF rats, the mRNA levels of the inflammatory factors, TNf alpha and Il6 were increased, but the anti-inflammatory, zinc finger protein (ZFP36), and its mRNA expression were decreased; the mRNA expression of insulin receptor substrate 2 (Irs2), glucose transporter 1 and 2 (Glu1 and Glu2) were decreased, but phosphatase and tensin homolog deleted on chromosome ten (Pten) and cyclophilin synthase kinase 3β (Cskβ3) mRNAs were overexpressed, compared with the control rats. We further found that sterol regulatory element binding protein-1c (Srebp1c) mRNA, triglyceride transfer protein (Mtp) mRNA and protein, and Cdelta3 mRNA and protein levels were increased and that peroxisome proliferator-activated receptor (Pparα) and lipoprotein lipase (Lpl) mRNA levels were decreased, which are all genes involved in lipoprotein metabolism, than in the control rats. Together, these abnormal gene and protein expression of multiple pathways related to inflammatory, insulin signaling and lipoprotein metabolism may be important underlying factors in VLDL1-apoB100 overproduction observed in T2D. Our data contributes to the understanding of association between systemic metabolic environment, abnormal multiple pathways in the liver and dyslipoproteinemia.

Supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (20300216 to Y.S.)

American Diabetes Association (ADA) Cut-Points for Glycated Hemoglobin (A1c) and the Risk of Diabetes, Kidney, and Cardiovascular Disease

ELIZABETH SELVIN, MICHAEL W. STEFFES, RICHARD M. BERGENSTAL, JOSEF CORESH, FREDERICK L. BRANCATI, Baltimore, MD, Minneapolis, MN

In a major change to clinical practice, the ADA now recommends the use of A1c for the diagnosis of diabetes and identification of persons at increased risk of diabetes. We investigated the association of new ADA cut-points (A1c<5.0, 5.0–<5.7, 5.7–<6.5, >6.5%) with risk of various clinical outcomes. We analyzed data from 11092 participants in the Atherosclerosis Risk in Communities (ARIC) Study without cardiovascular disease or diabetes. During 15 years of follow-up, there were 2251 incident cases of diabetes, 785 cases of kidney disease, 55 ESRD cases, 1198 coronary disease cases, 385 strokes, and 1447 deaths. The adjusted HRs by A1c category are shown in the Table. The 10 and 15-year cumulative incidence rates (Kaplan-Meier estimate) of diagnosed diabetes were 11% and 23%, respectively. The 10-year cumulative incidence of diagnosed diabetes by A1c categories are shown in the Figure. New A1c categories strongly predict the subsequent diagnosis of diabetes and are a marker of kidney and cardiovascular risk. Persons with A1c 5.7–6.4% are at high risk for diabetes and other outcomes. These data support the use of glycated hemoglobin for the diagnosis of diabetes.

| HR (95% CI) for Clinical Outcomes by ADA 2010 HbA1c Category |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| A1c <5.0                        | 1.0             | 1.0             | 1.0             | 1.0             |
| A1c 5.0–<5.7                    | 1.0             | 1.0             | 1.0             | 1.0             |
| A1c 5.7–<6.5                    | 1.0             | 1.0             | 1.0             | 1.0             |
| A1c >6.5                        | 1.0             | 1.0             | 1.0             | 1.0             |

Adj for age, sex, race, lipids, adiposity, hypertension, family hx of diabetes, education, alcohol, physical activity, smoking
Gamma-Glutamyltransferase (GGT) is Strongly Associated with Metabolic Status in the MESA Cohort

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Gamma-glutamyltransferase (GGT) functions in glutathione metabolism and recycling and has been proposed as a biomarker of oxidative stress relevant to cardiometabolic disease.

The Multi-Ethnic Study of Atherosclerosis (MESA) includes 6,814 participants aged 45-84 years representing four major ethnic groups: Chinese-American, Black, Hispanic, and Caucasian. We performed cross-sectional analyses to: 1. Assess metabolic status in MESA participants at baseline, including metabolic syndrome (MetS) and type 2 diabetes (DM2); 2. Evaluate adjusted associations between GGT and established metabolic risk factors including fasting glucose (FG), fasting insulin (FI), hemoglobin A1c (HbA1c) and HOMA-IR, and 3. Test trend in adjusted odds ratios for prevalent MetS and DM2 across GGT quintiles. Linear regression models were adjusted for age, race, gender, study site (Model 1); model 1 plus alcohol, exercise, smoking (Model 2); and model 2 plus blood pressure, lipids and medications (Model 3). Metabolic disease was prevalent in 2,895 (42.7%) participants, including 229 (3.3%) with isolated impaired fasting glucose (IFG), 1,935 (28.4%) with MetS and 731 (11%) with DM2. The distribution of GGT was skewed with median = 31.8 U/l; (IQR: 25.8-41.8); 10.4% (male) and 20.8% (female) of values were outside of the laboratory reference range. GGT was strongly associated (model 3) with each metabolic variable (FG, FI, HbA1c and HOMA-IR).

Adjusted odds ratios (OR) for MetS across GGT quintiles (Q) relative to Q1 were: Q2: 1.6 (1.3-2.1), Q3: 2.3 (2.2-3.7), Q4: 3.8 (3.0-5.0) and Q5: 4.2 (3.2-5.5); p<0.0001 for trend. Corresponding adjusted ORs for DM2 across GGT quintiles were: Q2: 1.7 (1.1-2.6), Q3: 1.9 (1.2-3.0), Q4: 2.9 (1.8-4.6) and Q5: 3.3 (2.1-4.5); p<0.0001 for trend. Increased odds of metabolic disease across GGT quintiles persisted in each ethnic group. Significant interaction with ethnicity was not evident. This study provides first-time evidence that GGT was strongly associated (model 3) with each metabolic variable (FG, FI, HbA1c and HOMA-IR).

Supported by Contracts N01-HC-95159 through N01-HC-95166 from the NHLBI and 1K22RR025015-01 from the NCRR.

ADA-Funded Research

HbA1c and Glucose Relationships with Retinopathy Assessed by Retinal Photography: Implications for Diagnostic Criteria

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We previously reported fasting plasma glucose (FPG), 2-hour post-load plasma glucose (2hFG) and HbA1c to be equivalently related to diabetic retinopathy (DR). These results influenced the recent adoption of HbA1c as a diabetes diagnostic criterion, but were limited by the subjectivity of DR assessment by direct ophthalmoscopy. We now report relationships of HbA1c, FPG and 2hPG with DR assessed by retinal photography in 2735 adults with or without diagnosed diabetes examined independently of medical history or laboratory findings. DR was defined as Airlie House grade ≥20 in either eye. Prevalence of DR was 6.5% in the entire population. Among 1547 participants without DR at baseline, 136 new cases developed during follow-up (median 6.0 years).

To account for differences in units and scales, DR prevalence (Figure 1) and incidence (Figure 2) are plotted by deciles of each glycemic measure and their associations with DR expressed by area under the receiver operating characteristic (ROC) curve.

Prevalence of DR was very low at lower glucose or HbA1c levels and rose sharply after the eighth decile. Areas under the ROC curve were 0.849, 0.851, and 0.849 for HbA1c, FPG, and 2hPG, not significantly different from each other. Each glycemic measure was also equally predictive of incident DR. In conclusion, HbA1c is strongly related to prevalence and incidence of DR assessed objectively; given that retinopathy is a specific complication of diabetes, these findings confirm the value of HbA1c in diagnosing diabetes.

Supported by Grants from the NIH/NIDDK (R21 DK080294 and K01 DK076595, to E. Selvin; and K24 DK82222 and P60 DK079637, to F. Brancati).

ADA-Funded Research

Low-Carbohydrate Diets and the Risk of Type 2 Diabetes among Men

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The purpose of this study is to compare the associations of several low-carbohydrate diet scores with T2D risk. Men from the Health Professionals Follow-up Study free of T2D, cardiovascular disease or cancer (n=41,212) in 1986 were followed for ≤ 20 years. Three low-carbohydrate diet scores were derived from food frequency questionnaires every 4 years: (1) a low-carbohydrate high-total protein/fat score, (2) a low-carbohydrate high-animal protein/fat score, and (3) a low-carbohydrate high-vegetable protein/fat score. Scores were calculated by summing decile ranks (1 to 10 for increasing intake) of protein and fat with inverted decile ranks of carbohydrate (10 to 1 for increasing intake). Each score had a maximum value of 30. Cox proportional hazard models were used to determine T2D risk across score quintiles.

During follow-up, 2781 cases of T2D were ascertained. After adjustment for age, smoking, physical activity, BMI, coffee intake, alcohol intake, family history of diabetes and total energy, the hazard ratio for T2D (top vs bottom score quintile) was 1.32 (95% CI: 1.16-1.50, p for linear trend < 0.0001) for the high-protein/fat score, 1.41 (95% CI: 1.23-1.62, p < 0.0001) for the high-animal protein/fat score, and 1.28 (95% CI: 1.15-1.42, p < 0.0001) for the low-carbohydrate high-vegetable protein/fat score.
high-animal protein/fat score, and 0.93 (95% CI: 0.82-1.05, p = 0.44) for the high-vegetable protein/fat score. The high-total protein/fat score was not significantly associated with T2D after adjusting for animal protein and fat.

The high-animal protein/fat score was attenuated after adjusting for red and processed meat (HR: 1.19, 95% CI: 1.02-1.39, p = 0.01) but no changes were observed after adjusting for poultry (HR: 1.40, 95% CI: 1.22-1.61, p < 0.0001) or fish (HR: 1.42, 95% CI: 1.24-1.63, p < 0.0001). Associations were slightly stronger after adjusting for dairy (HR: 1.49, 95% CI: 1.30-1.72, p < 0.0001).

In conclusion, low-carbohydrate diets that are high in animal protein and fat may increase the risk of T2D, particularly if they contain large quantities of red and processed meat. Low-carbohydrate diets high in vegetable protein and fat are not associated with changes in T2D risk.

Supported by CHFR and the CDA.

**EXERCISE**

### 48-LB

Prudent Diet Scores and Incident Type 2 Diabetes among Men

LAURENCE DE KONDING, TERESA T. FUNG, STEPHANIE CHIUVE, ERIC B. RIMM, WALTER C. WILLETT, FRANK B. HU, Boston, MA

Prudent diets are recommended for preventing type 2 diabetes (T2D). However, numerous definitions exist and it is unclear which should be used for optimal risk reduction.

The purpose of this study is to compare the associations of several prudent diet scores with the risk of T2D. Men from the Health Professionals follow-up study without a history of T2D, cardiovascular disease or cancer (n=41,212) in 1986 were followed for ≤20 years. Seven prudent diet scores were calculated from food frequency questionnaires given every 4 years: (1) the Healthy Eating Index (HEI), (2) the 2005 revision of the HEI (HEI-2005), (3) an alternative HEI (aHEI), (4) the Healthy Diet Score (HDS-2010), (5) the Recommended Food Score (RFS), (6) a Dietary Approaches to Stop Hypertension score (DASH), and (7) an alternative Mediterranean diet score (aMED).

Scores were calculated by summing values assigned to the intakes of protective and harmful dietary components. Cox proportional hazard models were used to determine T2D risk across score quintiles, and Akaike’s information criteria (AIC) was used to assess model fit.

There were 27,671 cases of T2D during follow-up. After adjustment for age, smoking, physical activity, body mass index, coffee intake, family history of diabetes and total energy, the HEI, aHEI, AMED, DASH, and HDS-2010, in order of lowest to highest risk reduction, scores were significantly associated with a decreased risk of T2D. Hazard ratios for T2D, representing a comparison of the top vs the bottom score quintiles, ranged from 0.71 for the HDS-2010 (95% CI: 0.62-0.81, p for trend < 0.0001) to 0.80 for the HEI (95% CI: 0.70-0.91, p for trend = 0.01). While associations were not significantly different, the HDS-2010 fit the data best according to the AIC.

The HDS-2010 was also significantly associated with a reduced risk of T2D after adjusting for the other scores. The protective association of prudent diet with T2D was primarily due to low intakes of red and processed meat, high intakes of whole grains, and moderate intakes of alcohol.

In conclusion, prudent diets that contain low intakes of red and processed meat, high intakes of whole grains, and moderate intakes of alcohol have similar protective associations with T2D.

Supported by CHFR and the CDA.

### 49-LB

Characterization of the AMP-Activated Protein Kinase Kinase (AMPKK) Complex in Adult Skeletal Muscle

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The LKB1-AMPK axis of metabolic signaling is a prime target of current pharmaceutical research to develop treatments for type II diabetes mellitus (T2DM). In liver tissue, the AMPKK complex was identified as the trimeric protein complex in adult skeletal muscle composed of LKB1, MO25α, and STRADα. A clearer understanding of this AMPKK complex in adult skeletal muscle may facilitate the development of better treatments to prevent or delay the onset of T2DM.

Supported by NIH grant RO1AR051298 from the NIAAMS.

### 50-LB

The Persistent Effect of Prior Exercise on Insulin-Stimulated Glucose Transport in Rat Skeletal Muscle Is Accompanied by Sustained and Simultaneously Increased Phosphorylation of AS160 on Two Key Regulatory Sites

GEORGE G. SCHWEITZER, GREGORY D. CARTEE, Ann Arbor, MI

A single bout of exercise can induce a persistent increase in insulin-stimulated glucose transport (GT) by rat skeletal muscle secondary to greater GLUT4 translocation with unaltered proximal insulin signaling (e.g., insulin receptor, IRS-1, Akt) and a sustained elevation in phosphorylation of Akt Substrate of 160 kDa (AS160) on T642. Published evidence suggests that simultaneous phosphorylation of AS160 on both T642 and S588 is required for insulin’s full effect on GLUT4 translocation. Therefore, we hypothesized that in rat skeletal muscles: 1) a sustained post-exercise (PEX) increase in AS160 phosphorylation on T642 would be accompanied by a sustained increase in phosphorylation on S588, and 2) AS160 would be simultaneously phosphorylated on both sites. Rats were sedentary (SED) or performed a 2h swim exercise protocol. At 2h PEX, both epididymal muscles from each rat were removed and incubated in 50mM glucose. AS160 phosphorylation was determined by immunoblotting with phospho-site specific antibodies (pT642 and pS588). Muscle lysates were also immunoprecipitated using anti-pS588 followed by immunoblotting with anti-pT642 (IP:pS588, IB:pT642) to assess if AS160 was simultaneously phosphorylated on both sites. At 3h PEX, GT without insulin was not different between the groups, but insulin-stimulated GT was greater for 3hPEX vs SED rats indicated increasing insulin action. AS160 phosphorylation on T642 and on S588 determined by separate immunoblotting was greater for 3hPEX vs SED rats, in both the absence and presence of insulin. A similar pattern occurred with IP:pS588, IB:pT642, i.e., 3hPEX was greater than SED values in both the absence and presence of insulin. GT was significantly and positively correlated with pT642 (R=0.80), pS588 (R=0.84) and IP:pS588, IB:pT642 (R=0.80). Our working hypothesis is that, in rat skeletal muscle, sustained and simultaneously increased phosphorylation of AS160 on these two key regulatory sites is important for the persistent PEX elevation in insulin sensitivity.

### 51-LB

Seven Days of Aerobic Exercise Augments Skeletal Muscle Blood Flow Responses to a Glucose Load in Patients with Type 2 Diabetes

CATHERINE R. MIKUS, JESSICA L. LIBLA, SETH T. FAIRFAIX, LERYN J. BOYLE, LAURUO VIANNA, DOUGLAS J. OBERLIN, GRACE M. UPTERGROVE, SHEKHAR H. DED, AREUL KIM, JILL A. KANALEY, PAUL J. FADEL, JOHN P. THYFAULT, Columbia, MO

Type 2 diabetes (T2D) is associated with vascular complications characterized by endothelial dysfunction. In the endothelium, insulin stimulates production of the vasodilator, nitric oxide, as well as the vas-
constrictor, endothelin-1. The vasodilatory effects of insulin account for up to 40% of insulin-mediated glucose disposal; however, insulin-stimulated vasodilation is impaired in T2D, contributing to diminished vascular perfusion and delivery of glucose to target tissues. It is not clear whether aerobic exercise improves BF responses to a glucose load in T2D. We assessed femoral BF (FFB, Doppler ultrasound) responses to an oral glucose tolerance test (OGTT, 75g glucose) in ten obese (BMI: 34±2 kg/m²), sedentary (aerobic capacity, VO2peak: 22±2 ml/kg/min) individuals (5±2±2) with non-insulin-dependent T2D (HbA1c, 6.7±2.0%) before and 12-24 h after 7 d of supervised treadmill/cycling exercise (60 min/d, 60-75% VO2peak).

Fasting glucose, insulin, and FBF were not different after 7 d of exercise (122±8 vs. 118±8 mg/dl, 11±2 vs. 11±2 μU/ml, and 314±40 vs. 307±29 ml/min), nor were glucose or insulin responses to the OGTT (270±5±1844 vs. 268±2±2089 μg/ml, P=0.82 and 236±1867 vs. 402±826, P=0.34). Prior to exercise, FBF did not change in response to the OGTT (% of fasting FBF, -4±5, -9±5, and -2±5% at 60, 90, and 120 min, respectively). In contrast, after 7 d of exercise, FBF increased by 18±7, 42±15, and 48±19% at 60, 90, and 120 min following the OGTT, respectively (P=0.05 vs. corresponding pre-exercise time-points). In summary, these data are the first to demonstrate that 7 d of aerobic exercise enhances BF responses to a glucose load in obese individuals with T2D and support previous work demonstrating that acute exercise improves endothelial function in this population. These early adaptations likely contribute to favorable metabolic and cardiovascular outcomes associated with chronic exercise training in T2D.

Results were consistent when podiatric care was defined as at least 3 pre-ulcer podiatry visits. In a population of adults with diabetes and foot ulcer, care by podiatrists appears to prevent or delay lower extremity amputation and hospitalization.

Supported by the American Pediatric Management Association.

FOOT CARE—LOWER EXTREMITIES

52-LB Podiatrist Care and Outcomes for Patients with Diabetes and Foot Ulcer

TERESA B. GIBSON, VICKIE R. DRIVER, JAMES WROBEL, JAMES R. CHRISTINA, JILL E. BAGALMAN, ROY DE FRANCIS, MATTHEW G. GAROUFALIS, GINGER S. CARLS, SARA WANG, Ann Arbor, Mt, Boston, MA, Chicago, IL, Bethesda, MD, Washington, DC, Buffalo, NY, Chicago, IL, Bentonville, AR, Cambridge, MA

The purpose of this study was to examine outcomes of care (amputation and hospitalization) differ between patients with diabetes who received care from podiatrists and those who did not receive care from podiatrists.

Adult patients with diabetes (ICD-9-CM: 250.xx) and a diagnosis of foot ulcer (ICD-9-CM: 707.00, 707.06, 707.07, 707.09, 707.10, 707.12, 707.13, 707.14, 707.15) were found in the MarketScan Research Databases, 2005-2008. The date of the first claim with evidence of foot ulcer was the index date. Patients with previous evidence of foot ulcer or amputation were excluded. Propensity score matching (PSM) was used to create a matched sample of patients with diabetes and podiatry visits and patients with diabetes and no podiatry visits, based on sociodemographic variables, plan type, general health status, adherence to diabetes medications, and risk factors for amputation (patient-level and foot-level). The sample comprised 20,330 patients aged 65+ (Medicare-eligible with employer-sponsored supplemental insurance) and 11,766 patients aged <65 (non-Medicare eligible commercially insured). Patient experience was available for up to 60 months. Cox proportional hazard models estimated the hazard of inpatient hospitalization, lower extremity amputation, and major amputation (i.e., below the knee or higher), controlling for the covariates in the PSM.

Care by podiatrists, defined as at least 1 pre-ulcer podiatry visit, was associated with a lower hazard of hospitalization, amputation, and major amputation in the Medicare population. Results were similar in the non-Medicare population, where the difference in major amputation was not statistically significant (P=0.1).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>HR</th>
<th>95% CI</th>
</tr>
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<tbody>
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<td>Hospitalization</td>
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<tr>
<td>Medicare</td>
<td>0.910</td>
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<td>Non-Medicare</td>
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<td>Amputation</td>
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<tr>
<td>Medicare</td>
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<tr>
<td>Non-Medicare</td>
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<td>0.725-1.002</td>
</tr>
<tr>
<td>Major Amputation</td>
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<td></td>
</tr>
<tr>
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<tr>
<td>Non-Medicare</td>
<td>0.771</td>
<td>0.547-1.088</td>
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</table>

| ADA Funded Research      |        |           |

Results were consistent when podiatric care was defined as at least 3 pre-ulcer podiatry visits.

GENETICS—TYPE 1 DIABETES

53-LB Genetic Variation at Adenylate Cyclase 5 (ADCY5) Is Associated with Glycemic Control in Type 1 Diabetes

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Hyperglycemia, a major risk factor for diabetic complications, is known to have a genetic component in type 1 diabetes (T1D). Evidence from twins and genetic association studies point to common genetic determinants for glycemia in both T1D and non-diabetics. A meta-genome-wide association study of glucose (fasting or 2 hr post challenge) in individuals without diabetes has recently identified 17 loci for either trait, some also associated with type 2 diabetes. We sought evidence for association of these loci with glycemic traits and diabetic complications in two T1D cohorts: DCCT (Diabetes Control and Complications Trial: intensive (INT) vs conventional (CON) therapy) and GoKinD (Genetics of Kidneys in Diabetes: nephropathy vs no nephropathy controls). Genotyping was performed on SNP arrays with imputation using HapMap (Hap) data.

Of 17 loci tested by linear regression, two intronic SNPs of ADCY5 (rs11708067, rs2877716) were associated with mean A1C (rs11708067: p=0.00024) and mean 7-point daily glucose measures (p=0.0049) in DCCT/INT (n=637) in the same direction as non-diabetics. These 2 SNPs also showed borderline association with A1C in the same direction (p=0.057) in GoKinD cases (n=531). Consistent with their direction of effect on glycemia, these SNPs were also associated with time-to-event for diabetes complications in INT (p=0.0393, 0.034, 0.063 for time to clinically significant macular edema, severe retinopathy and severe nephropathy respectively). Adjusting for glycemic control attenuated these associations (p=0.225, 0.24, 0.21). No association with glycemic traits was observed in DCCT/CON (n=837) or GoKinD controls (n=831). Detection of association in INT may be explained by the A1C level most similar to non-diabetics, smaller between-individual variation of A1C (SD=0.89) compared to the other cohorts (SD≥1.6), the availability of repeated measures, or chance. With correction for the Winner’s curse, >1600 individuals would be needed to detect comparable genetic effects with 80% power (α=0.05).

ADCY5 is the second example of a locus (after BNC3 associated with glycemia in both T1D and those without diabetes).

54-LB Scanning of Epigenetic Histone Post-Translational Modifications (PTMs) at Type 1 Diabetes Susceptible Genes

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Although the role of genetic susceptibility genes in Type 1 diabetes (T1DM) is undisputable, less than 10% of susceptible individuals progress to T1DM. This suggests substantial involvement of environmental factors and potential epigenetics. Nuclear chromatin is the interface between genetics and environment and affected by environmental stimuli. The basic unit of chromatin, the nucleosome, consists of histones H2A, H2B, H3, and H4 wrapped by DNA. Posttranslational modifications of histones (PTMs) can lead to epigenetic changes that affect gene transcription.

Our objective is to determine whether patients with T1DM depict significant changes in their epigenome. We used the chromatin immunoprecipitation-linked to microarray (ChIP-array) approach to profile histone PTM variations genome-wide. Our recent study provided evidence of associations between T1DM and altered histone H3K9me2 of key genes. We now extended this to compare other histone marks, H3K4me3, H3K9me3, H3K27me3, H3K9Ac, H4K16Ac patterns in blood cells from T1DM patients versus controls using the chromatin immunoprecipitation-linked to microarray (ChIP-array) approach to profile histone PTM variations genome-wide. Our recent study provided evidence of associations between T1DM and altered histone H3K9me2 of key genes. We now extended this to compare other histone marks, H3K4me3, H3K9me3, H3K27me3, H3K9Ac, H4K16Ac patterns in blood cells from T1DM patients versus controls using promoter tiling arrays. We have uncovered variations in key histone marks between these two subject groups.

Genome-wide association studies have revealed 41 T1DM susceptible regions containing about 300 genes. To explore potential new roles for...
these regions, we hypothesized that T1DM susceptible genes could have
histone PTM variations. We therefore map and compare histone PTMs of
these T1DM susceptible genes in lymphocytes from T1DM patients and
non-diabetic controls using human promoter tiling arrays, and histone
PTMs of the entire MHC locus (containing IDDM1) in monocytes from T1DM
patients and no-diabetes controls using human Chromosome 6 tiling arrays.
Importantly, we observed significant PTM variations at key genes located in
the IDDM1 region. These results provide new insights into the pathogenesis
of T1DM.

Supported by JDRF.

GENETICS

GENETICS—TYPE 2 DIABETES

55-LB

Common Genetic Risk Variants Influence Different Phases during the Progression from Normal Fasting Glucose to Type 2 Diabetes

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Whether common genetic variants associated with type 2 diabetes (T2D) exert effects at the early and/or late phases in the progression from normal fasting glucose (NFG) to diabetes is unknown.

We selected 14 T2D-associated variants from the IBC array genotyped in 26,753 Candidate-gene Association Resource participants. Participants were grouped into NFG, impaired fasting glucose (IFG), or Diabetes categories at baseline and follow-up. A regression model was used to test whether alleles associated with T2D were over-represented in the IFG vs NFG (early phase), diabetes vs IFG (late phase), or both (equal influence) comparisons at baseline. We then examined time from NFG to IFG or IFG to diabetes based on the presence of risk alleles by Cox analysis.

**Table:**

<table>
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<tr>
<th>Gene</th>
<th>CArE SNP</th>
<th>P-value Early Phase</th>
<th>P-value Late Phase</th>
<th>NFG-&gt;IFG</th>
<th>IFG-&gt;diabetes</th>
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<td>0.57</td>
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<td>KCN2J1</td>
<td>rs231362</td>
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<td>0.24</td>
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<td>SLC30A8</td>
<td>rs1266834</td>
<td>1 X 10^-7</td>
<td>0.63</td>
<td>4 X 10^-3</td>
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<tr>
<td>MTN1B</td>
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<td>5 X 10^-4</td>
<td>6 X 10^-14</td>
<td>0.90</td>
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<td>CDKN2A/2B</td>
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<td>5 X 10^-3</td>
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<td>0.14</td>
</tr>
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All results adjusted for age and gender

Supported by Harvard CTSC (Award #UL1 RR025758) and NIH training grant DK007028 to G.A.W.

ADA-Funded Research

56-LB

WITHDRAWN

57-LB

Finding Meta-Analysis of Type 2 Diabetes in Mexicans and Mexican Americans

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To identify loci influencing T2D risk in populations of Mexican descent, we performed a meta-analysis on three large-scale genome-wide studies comprised of 400,000 cases and controls: 837 MA cases and 781 random controls from Starr County, Texas (SC), 1202 Mexican American participants from 40 families in the San Antonio Family Heart Study (SA), and 954 cases with relatively early T2D onset (most of them earlier than 50 years) and 350 controls from Mexico City, Mexico.

Test statistics at more than 320,000 directly interrogated and imputed loci from genome-wide association analyses of SC and MC, and the joint test of linkage/association in SA were combined using METAL. We identified several SNPs with suggestive evidence for replicated association with T2D. These include SNPs in previously identified T2D genes discovered through GWAS – KCN2J1 (rs39277617 with combined P-value 1.4e-6), or other types of studies, including CACNA1E (rs13048942, 5.0e-6), INS (rs9259807, 1.2e-4), and PIK3R1 (rs1044066, 7.8e-6). We found novel GWAS support for genes influencing insulin regulation, including MYO5A (rs1724625, 8.7e-5), an actin-based molecular motor present in pancreatic islet cells, which has been implicated in the regulation of glucose-induced insulin secretion, and PRKCE (rs4953268, 1.3e-5), a kinase, which among its many roles, is a regulator of insulin exocytosis.

An additional 1.4 million SNPs were evaluated in two of the three studies, and in these we found additional support for KCN2J1 (rs2074197, 1.2e-5). The top SNPs in this category fell in CSM01 (rs9743386, 2.6e-7), which in the Framingham Heart Study is associated with Metabolic Syndrome, and DEPDC (rs5998144, 2.3e-6). We also found support for AKAP6 (rs1535541, 1.4e-5), IFG2BP2 (rs1374910, 2.3e-5), and HNF1A (rs1699286, 2.5e-5).

ADA-Funded Research

58-LB

Identification of Highly Sensitive C-Reactive Protein (hsCRP) as a Biomarker To Aid Diagnosis of Maturity Onset Diabetes of the Young (MODY) Due to HNF1A Mutations

GAYA THANABALASINGHAM, KATHARINE R. OWEN, TIMOTHY J. JAMES, FREDIK KARPE, ANDREW J. FARMER, MARK I. MCCARTHY, ANNA L. GLOYN, OXFORD, UNITED KINGDOM

An accurate molecular diagnosis in monogenic diabetes is important but many individuals remain undiagnosed due to restricted access to genetic testing. There is a need for biomarkers to prioritise patients for genetic testing. Recent genome-wide association studies demonstrated that common variants near the Hepatocyte Nuclear Factor 1-alpha (HNF1A) gene influence serum C-reactive protein (CRP) levels in healthy adults. We hypothesised that large-effect inactivating HNF1A mutations causal for MODY would be characterised by markedly reduced levels of hsCRP and assessed the value of hsCRP as a diagnostic biomarker for HNF1A-MODY.

Serum hsCRP levels were analysed in subjects with: HNF1A-MODY (n=31), glucokinase (GCK)-MODY (n=24), autoimmune diabetes (n=316), young adult-onset type 2 diabetes (T2DM, n=290) and non-diabetic subjects (n=190). hsCRP values >10mg/l were excluded to reduce the impact of concurrent infection. Receiver operating characteristic (ROC) curve analysis was used to assess the discriminatory accuracy of hsCRP with respect to diabetes etiology. Serum hsCRP levels in HNF1A-MODY subjects were substantially lower than all other groups (p<0.009). Geometric mean [95%CI] hsCRP values

ADA-Funded Research

For author disclosure information, see page LB41.
Circulating triglyceride levels in the etiology of insulin resistance
with T2D was different from that expected (odds ratio 1.52, p=6.35x10^-21).

triglyceride-raising alleles had triglyceride levels that were 0.43 S.D (95%CI 0.36-0.50) higher than the 20% with the fewest (p<4x10^-31), but no increased risk of T2D (odds ratio 0.93 (0.82-1.08, p=0.27). This lack of association with T2D was different from that expected (odds ratio 1.52, p=3.35x10^-21 in instrumental variable test) given the alleles-triglyceride and triglyceride-T2D associations.

In the insulin resistance study, the 20% of individuals with the greatest number of triglyceride-raising alleles had triglyceride levels that were >0.3 S.D (95%CI 0.36-0.50) higher than the 20% with the fewest (p<4x10^-31), but no increased risk of T2D (odds ratio 0.93 (0.82-1.08, p=0.27). This lack of association with T2D was different from that expected (odds ratio 1.52, p=3.35x10^-21 in instrumental variable test) given the alleles-triglyceride and triglyceride-T2D associations.

In conclusion, we provide the first evidence that serum hsCRP provides a promising and readily-available biomarker for HFHA-MODY. By improving the ability to identify diabetic subjects most likely to have HFHA-MODY, we have the potential to enable more efficient use of molecular diagnostics and more effective personalisation of diabetes treatment.

Supported by NIH/NIHR Oxford Biomedical Research Centre.

Mendelian Randomization Studies Do Not Support a Role for Raised Circulating Triglyceride Levels in the Etiology of Insulin Resistance or Type 2 Diabetes

MANÉKA G. DE SILVA, RACHEL M. FREATHEY, LOUISE DONNELLY, TOM PALMER, JIANAN LUAN, CLAUDIA LANGENBERG, MICHAEL N. WOODEN, BEVERLEY SHIELDS, BETTINA A. KNIGHT, ANDREW T. HATTERSLEY, MARK I. MCCARTHY, NICHOLAS WAREHAM, ANDREW D. MORRIS, COLIN PALMER, TIMOTHY M. FRAYLING, Exeter, United Kingdom, Dundee, United Kingdom, Bristol, United Kingdom, Cambridge, United Kingdom, Oxford, United Kingdom

Circulating triglyceride levels are strongly correlated with insulin resistance and type 2 diabetes (T2D), but the causal nature of these correlations is unclear. Prospective epidemiological studies and animal studies suggest raised triglyceride levels may causally influence insulin resistance and T2D. This collection of evidence has led to the proposal that lowering lipid could reduce the risk of T2D.

We used Mendelian randomization to provide further evidence for or against a causal role of raised triglyceride levels in insulin resistance and T2D. We used 10 polymorphisms that are robustly associated with circulating triglyceride levels. We analyzed 5637 patients with T2D and 6680 controls from the Diabetes And Research in Tayside (DARTs) project, Scotland, and 3740 non-diabetic individuals with the HOMA-R measure of insulin resistance from the DARTs, Fenest and Exeter Family Studies.

In the T2D study, the 20% of individuals with the greatest number of triglyceride-raising alleles had triglyceride levels that were 0.43 S.D (95%CI 0.36-0.50) higher than the 20% with the fewest (p<4x10^-31), but no increased risk of T2D (odds ratio 0.93 (0.82-1.08, p=0.27). This lack of association with T2D was different from that expected (odds ratio 1.52, p=3.35x10^-21 in instrumental variable test) given the alleles-triglyceride and triglyceride-T2D associations.

In the insulin resistance study, the 20% of individuals with the greatest number of triglyceride-raising alleles had triglyceride levels that were >0.3 S.D (95%CI 0.36-0.50) higher than the 20% with the fewest, but no increase in insulin resistance (-0.017 (95%CI -0.048, 0.013) to 0.028 (95%CI -0.002, 0.057) S.D (depending on study). This lack of association with HOMA-R was significantly different from that expected given the alleles-triglyceride and triglyceride-T2D associations.

Genotypes are unlikely to be confounded or be influenced by disease processes. Using this Mendelian randomization principle we provide strong evidence that raised triglyceride levels do not contribute to the onset of T2D or the HOMA-R measure of insulin resistance.

Meta-Analysis of 70 Publicly-Available Microarray Experiments of Type 2 Diabetes Links CD44 in Adipose Tissue with Diabetes Susceptibility

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Type 2 diabetes (T2D) is a polygenic disease, and phenotypically characterized by insulin resistance. Recent studies have shown that chronic inflammation in adipose tissue is causally linked with the development of insulin resistance. A number of GWAS have revealed dozens of genes consistently associated with T2D. However, these robust T2D-associated genes explain approximately 6% of the heritability and pathogenesis of T2D to date. To find additional T2D susceptibility genes, we meta-analyzed T2D-related publicly-available genome-wide functional studies (518 microarrays), based on our hypothesis that genes repeatedly differentially expressed in T2D-related microarray experiments are strong candidates for T2D susceptibility genes. We then extracted the immune-cell receptor CD44 as our top-most candidate (Figure 1: P = 6.3 x 10^-18). We found that CD44 deficiency ameliorates insulin resistance (Figure 2), and suppresses adipose tissue inflammation in a diabetic mouse model. We confirmed the genetic association of a CD44 coding SNP with T2D in two separate Japanese cohorts totaling 4,758 individuals (OR = 1.43 [1.17-1.74], P = 3.9 x 10^-4). The risk allele of CD44 increased its mRNA expression in human adipose tissue, and serum CD44 levels were positively correlated with insulin resistance in human. Our findings demonstrate that CD44 in adipose tissue is implicated in the pathogenesis of T2D, and large-scale meta-analysis of genome-wide functional experiments may yield novel susceptible genes for T2D.
a locus near AR15 (ADP-ribosylation factor-like 15) on chromosome 5p15 (Tao et al. 2015; meta-analysis p=3.2 x 10^{-6}, OR=1.10 [0.92-1.32]). These data are consistent with a long tail of common variant association signals of modest effect contributing to T2D-susceptibility. Metabolism-genotyping underway in >30,000 T2D cases and 50,000 controls should add considerably to the tally of proven T2D-susceptibility loci. Even in this modestly scaled initial effort, we identified one novel T2D-susceptibility signal mapping to chromosome 5p15.

62-LB
Sex-Differentiated Meta-Analysis of Genome-Wide Association Studies of Type 2 Diabetes
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Despite the recent success of genome-wide association studies (GWAS) of type 2 diabetes (T2D), much of the genetic component of disease risk is still unexplained. One potential source of genetic variation contributing to this “missing heritability” is that with effects that differ in magnitude and/or direction in males and females which may not be readily identified through traditional analysis of both sexes, combined. We have therefore undertaken the first large-scale meta-analysis of male- and female-specific T2D GWAS with the aim of identifying novel sex-differentiated signals of association with the disease for follow-up.

We performed GWAS of T2D in six cohorts of European ancestry (total effective sample size of 3332 males and 7744 females). Genotype data were imputed in each cohort for up to 2.5 million SNPs, genome-wide, including chromosome X. Allelic odds ratios (OR) at each SNP were obtained for males and females, separately, and then combined across cohorts through fixed-effects inverse-variance weighted meta-analysis. Subsequently, undirected z-scores from these male- and female-specific meta-analyses were combined, for each SNP, to provide sex-differentiated tests of association. The two strongest novel signals of T2D association from our sex-differentiated analysis were male-specific and occurred with SNPs flanking SLC35D3 on 1.07. The signal at DGKB previously reported at this locus (p=1.1 x 10^{-7}, male OR = 1.10 [1.07-1.12]) was observed at an additional eight loci. Of these, the region flanking SMARCA1 on chromosome X, was unique in demonstrating clearly opposing effects between the sexes (p=6.1 x 10^{-4}, male OR = 1.10 [1.04-1.17], female OR = 0.87 [0.81-0.94]).

Lead SNPs at all ten loci demonstrating at least suggestive evidence of sex-differentiated association are currently being followed up in additional cohorts through in silico replication and/or de novo genotyping.

63-LB
Variants Associated with Young-Onset Type 2 Diabetes in American Indians among 454,194 Single Nucleotide Polymorphisms (SNPs)
ROBERT L. HANSON, WILLIAM C. KNOWLER, CLIFTON BORGADURS, LI BIAN, SAYUKO KOBES, LESLIE J. BAIER, Phoenix, AZ

Most diabetes-associated variants identified through genome-wide association studies (GWAS) have been identified in European populations. We now report a GWAS for young-onset type 2 diabetes in an American Indian population. 303 cases with type 2 diabetes with onset < age 25 yrs and 334 nondiabetic controls who were > age 45 yrs were genotyped on the Affymetrix 1M array, resulting in 454,194 SNPs with highly reproducible genotypes and minor allele frequency > 0.05. SNPs were analyzed for association in cases and controls using a regressive logistic model to account for family membership. The figure shows the p-values for association after genomic control. 244 SNPs were associated at p < 0.0005 (corresponding to a false discovery rate of 0.88). Among these, 165 were selected as tag SNPs (with r2 > 0.95 indicative of redundancy), and analyzed for association with diabetes in 2855 individuals (43% with diabetes) from the population who were not included in the GWAS. Among the 83 SNPs examined to date, 22% replicate (one-tailed p<0.05 in the same direction as the GWAS), significantly more than the 5% expected by chance. To date 7 SNPs have been genotyped in an additional 2723 American Indians (20% with diabetes) who were not in the GWAS, and 3 (rs523656, rs569964, rs548838) were significant in the combined sample. Rs523656, located near STARD13, is also associated with diabetes in Caucasians in the publicly available DIAGRAM database (odds ratio=1.12 per copy of the G allele, p=8.3 x 10^{-6}). When data from all American Indian samples are combined with DIAGRAM, the odds ratio is 1.14 (p=7.4 x 10^{-7}). These data suggest several regions where marker alleles are potentially in linkage disequilibrium with variants that confer susceptibility to young-onset type 2 diabetes in American Indians. ADA-Funded Research

HEALTH CARE DELIVERY—ECONOMICS

64-LB
Variants in CDKAL1 and MTNR1B Differentially Affect Absolute Versus Temporal Change in Type 2 Diabetes-Related Quantitative Traits (T2DQT)
JIE REN, ZHANGHUA CHEN, YU-HSIANG SHU, JAANA HARTIALA, JEAN M. LAWRENCE, JOYCE M. RICHEY, ENRIQUE TRIGO, HOODMAN ALLAYEE, ANNY H. XIA, THOMAS A. BUCHANAN, RICHARD M. WATANABE, Los Angeles, CA, Pasadena, CA

Genome-wide association studies have shown that CDKAL1 and MTNR1B are associated with type 2 diabetes. These have been replicated in many populations, with some also showing association with T2DQT. Only one study in northern Europeans has examined the association of these variants with temporal changes in T2DQT. We examined whether variation in CDKAL1 (rs754840) and MTNR1B (rs10830963) were associated with T2DQT in the BetaGene Study: 2038 individuals from 507 Mexican American families of probands with or without previous gestational diabetes. Subjects were genotyped by DEXA scan, 75g oral glucose tolerance test (OGTT) and insulin-modified intravenous glucose tolerance test. We also examined whether these variants were associated with temporal changes in T2DQT in a subset of 131 BetaGene subjects recalled (median: 4.2 yrs) after baseline for repeat phenotyping. Association between SNPs and T2DQT or temporal changes in T2DQT was assessed using variance components analysis. We report p-values corrected for multiple tests. At baseline, rs754840 was associated with acute insulin response (AIRg; p=0.00017), disposition index (DI; p=0.010) and OGTT 30-minute insulin (p=0.0021) rs10830963 was associated with AIRg (p=0.0045), DI (p=0.019) and fasting glucose (p=0.0058). Percent body fat (PBF) modified association between rs754840 and OGTT 30-minute insulin (p=0.0052) and between rs10830963 and insulin sensitivity (S; p=0.024). rs754840 was not associated with temporal changes in T2DQT, whereas rs10830963 was significantly and marginally associated with temporal changes in DI (p=0.017) and S (p=0.076), respectively. PBF modified association between rs10830963 and temporal changes in S (CC: higher slope in low vs. high PBF; GC+GG: similar slopes in low vs. high PBF; GC-GG: similar slopes in low vs. high PBF; p=0.0057). We conclude that CDKAL1 variants are associated with cross-sectional difference, but not temporal change in T2DQT, while MTNR1B variants determine both cross-sectional and temporal change.

HEALTH CARE DELIVERY—ECONOMICS

65-LB
Screening for Diabetes and Prediabetes Should Be Cost-Saving in High-Risk Patients
RANEE CHATTERJEE, K.M. VENKAT NARAYAN, JOSEPH LIPSCHOM, PAUL KOLM, LAWRENCE S. PHILLIPS, Baltimore, MD, Atlanta, GA

Screening for diabetes and prediabetes is infrequent, in part because we do not know how best or whom to screen. To address these issues, we compared costs for different screens in subjects with graded risk. 1,573 adults without known diabetes had 4 screening tests – random plasma/capillary glucose, and plasma/capillary glucose 1 hr after 50g oral glucose for author disclosure information, see page LB41.
IMMUNOLOGY

66-LB
Administration of a Naturally Occurring Peptide Kinase Inhibitor Increases β Cell Mass and Reduces Pancreatic Inflammation in Animal Models of Type 1 and Type 2 Diabetes

JOHN R. SIMMS, SOREN MOGELSVANG, ANGELA CHUNG, ODED FOREMAN, DONEL A. SEQUEA, GREGORY D. CARTEE, Ann Arbor, MI

Previously we reported that OPL1 (a naturally occurring 38-mer peptide isolated from the serum of the Cohen rat model) functions in vitro as a potent inhibitor of human kinases. Also, studies have shown that OPL1 treatment of STZ-induced mice delays the onset of diabetes and reduces the incidence of overt disease.

To assess the efficacy of OPL1 in T1D, NOD mice were treated IP bi-weekly at the pre-diabetic stage (BG <200 mg/dL) or after the onset of overt disease (BG >300 mg/dL). After 28 days, 40% of mice remained disease-free when treatment was initiated early, compared to 20% in the therapeutic group, and 0% in untreated controls. In a second experiment, OPL1 was given daily to NODs at the pre-diabetic stage and efficacy was improved to 90% in treated animals. Serum profiling revealed a reduction in IL-6 levels and an increase in adiponectin levels compared to untreated controls (p<0.05). No differences in pro-inflammatory cytokine levels in the pancreas. The precise mechanism of action of OPL1 in these models is still under investigation.

INSULIN ACTION

67-LB
Fiber Type Heterogeneity for Glucose Uptake by Single Ila, Iib and Ibx Fibers from Rat Epididymal Muscle

JAMES G. MACKRELL, GREGORY D. CARTEE, Ann Arbor, MI

Skeletal muscle is a heterogeneous tissue composed of fibers with variable contractile and metabolic profiles. Our purpose was to determine the effect of fiber type on glucose uptake (GU) by single fibers from the same muscle by combining a novel single fiber GU method that we recently developed with a standard method for classifying fiber type (myosin heavy chain, MHC, by SDS-PAGE). Paired rat epididymal muscles were isolated and incubated (80min) in Krebs-Henseleit Buffer (KHB) and [3H]-2-Deoxy-D-glucose (2DG) with 0 or 12nM insulin. Muscles were rinsed (cold KHB) followed by enzymatic digestion (collagenase). Single fibers were dissected out and incubated in KHB-Trypan Blue. Undamaged fibers were analyzed for fiber volume (microscopy and Image J software), MHC and GU. After fibers were homogenized, separate aliquots were used for SDS-PAGE and [3H]2DG accumulation by scintillation counting relative to fiber volume, nmol/mm².

In whole epididymal muscle, we found MHC distribution of 51% type Iib, 13% type Iia, 28% type Ibx and 8% type I. We found a roughly similar distribution of MHC isoforms in single fibers used for GU (86% Iib, 9% Iia and 25% Ibx/a - a hybrid expressing 2 MHC isoforms). Basal GU was not different among fiber types (Iib=0.28±0.05, Iia=0.28±0.2, and Ibx/a=0.32±0.05). ANOVA revealed a significant difference for GU among single fibers from insulin-stimulated muscle; Iib (3.44±0.27), Iia (5.65±1.84) and Ibx/a (4.95±0.80). The ~40% lower value for Iib vs. Iia compares with published values for different muscles composed of mainly Iibs vs. Iias (~35-60% decrease). The current data extend insights from whole muscle experiments which are limited because they cannot unambiguously identify differences attributable to fiber type per se from other differences between 2 different muscles. Whole muscle analysis also cannot assess GU by hybrid fibers. Our ability to measure GU and MHC in a single fiber will allow analysis of: 1) effects of in vivo interventions (diet, drugs, exercise) on each fiber type, and 2) results from muscle electroporation (a variably efficient process) enabling comparisons between fibers that were or were not transfected.

68-LB
Relationship between Phosphorylation of Akt and AS160 on Key Regulatory Sites and Insulin-Stimulated Glucose Uptake in Rat Skeletal Muscle

DONEL A. SEQUEA, GREGORY D. CARTEE, Ann Arbor, MI

Studies using cultured cells have found that insulin's full effects on GLUT-4 translocation and glucose uptake (GU) require phosphorylation of Akt on T308 and S473 and Akt Substrate of 160 kDa (AS160) on T642 and S688. Because cells are imperfect models for adult skeletal muscle, the purpose of this study was to elucidate the relationship between insulin-stimulated GU and site-specific phosphorylation of Akt and AS160 in isolated rat soleus muscle. Wistar rats were anesthetized and each soleus was isolated and split into 2 longitudinal strips for incubations with a range of concentrations of a specific Akt inhibitor (AI, Calbiochem #124017; 0, 0.1µM, 1.0µM) and insulin (0, 1.2, 12nM) along with radiolabeled 2–deoxyglucose.

Phosphorylation of Akt (pT308 and pS473) and AS160 (pT642 and pS688) were assessed by immunoblotting with phosphosite specific antibodies. Insulin caused dose-dependent increases in GU, pT308, pS473, pS688 and pT642. The lower AI dose (0.1µM) decreased insulin's effects as follows: GU (48% at 1.2nM; 50% at 12nM insulin), pT308 (48% at 1.2nM; 49% at 12nM insulin), pS473 (17% at 1.2nM; 45% at 12nM insulin), pT642 (6% at 1.2nM; 32% at 12nM insulin), and pS688 (10% at 1.2nM; 94% at 12nM insulin). The higher AI dose (1.0µM) decreased insulin's effects as follows: GU (82% at 1.2nM; 75% at 12nM insulin), pT308 (74% at 1.2nM; 66% at 12nM insulin), pS473 (70% at 1.2nM; 58% at 12nM insulin), pT642 (15% at 1.2nM; 24% at 12nM insulin) and pS688 (63% at 1.2nM; 70% at 12nM insulin). GU was significantly correlated with insulin signaling as follows: GU vs. pT308 (R=0.732), GU vs. pS473 (R=0.503), GU vs. pS688 (R=0.305) and GU vs. pT642 (R=0.269). Akt phosphorylation on each site was a stronger predictor of GU than either AS160 phosphorylation site. These results are consistent with Akt-mediated regulation of insulin-stimulated GU being dependent on other aspects of AS160 regulation (e.g., AS160 subcellular localization; phosphorylation on sites other than pS688 and T642; 14-3-3 binding) and/or AS160--independent processes (e.g., other Akt substrates).
Defective Hepatic Autophagy in Obesity Causes Insulin Resistance

LING YANG, PING LI, SUNENG FU, EDIZ S. CALAY, GOKHAN S. HOTAMISLIEGLI, Boston, MA

Autophagy is an evolutionarily conserved cellular homeostatic process involved in the bulk degradation of cytoplasmic components including damaged organelles and proteins. Since this adaptive catabolic process can respond to metabolic stress and nutrient fluctuations, we postulated that its regulation would be critical in obesity and insulin resistance, characterized by nutrient surplus and dysfunctional organelles such as the endoplasmic reticulum (ER). In both genetic and dietary models of obesity, we observed a severe downregulation of hepatic autophagy, particularly in Atg7 expression levels. We did not observe significant alteration of autophagic markers in the liver of lean mice following lipid infusion, or in ob/ob mice treated with STZ, or of aged db/db mice. Interestingly, we identified a dramatic increase in Calpain 2 expression in liver of ob/ob mice compared to lean control. Inhibition of Calpain 2 in vivo by Calpain inhibitors resulted in enhanced Atg7 protein expression level, indicating that the calpain-mediated depletion of Atg7 may be a critical contributing mechanism to obesity-related defects in autophagic responses. Moreover, suppression of Atg7 both in vitro and in vivo resulted in defective insulin signaling, elevated ER stress and impaired glycogen breakdown in liver of lean mice. In contrast, restoration of the defective liver Atg7 expression improved insulin signaling, elevated ER stress and impaired glycogen breakdown in liver

Improved Glucose Homeostasis in Mice with Liver-Specific Deletion of Sre Homology Phosphatase 2

KOSUKE MATSUD, SIMING LIU, MIRELA DELBIGEOVIC, IZUMI MATSUO, NADTO NAGATA, AHMED BETTAEIB, YANNAN XI, SUSAN GRAY, DA EUNG JUNG, KAZUSHI ARAKI, WENTIAN YANG, JASON K. KIM, BARBARA B. KAHN, BENJAMIN G. NEIL, FAIZAW G. HAII, DAVIS, CA, BOSTON, MA, WORCESTER, MA, TORONTO, ON, Canada

The SH2 domain-containing protein-tyrosine phosphatase (Shp2) has been implicated in a variety of growth factor signaling pathways, but its role in insulin signaling has remained unresolved. In vitro studies suggest roles for Shp2 both as a negative and positive regulator of insulin signaling, while its physiological role in a number of peripheral insulin-responsive tissues remains unknown. To address the metabolic role of Shp2 in the liver, we generated mice with either chronic or acute hepatic Shp2 deletion using tissue-specific Cre-LoxP and adenoviral Cre approaches, respectively. We then analyzed insulin sensitivity and glucose tolerance, and insulin signaling in liver-specific Shp2-deficient and control mice. Mice with chronic Shp2 deletion exhibited enhanced insulin-induced suppression of hepatic glucose production, improved insulin sensitivity and enhanced glucose tolerance compared with controls. Hepatic Shp2 deficiency also prevented development of insulin resistance following high fat feeding. Acute Shp2 deletion yielded comparable results, indicating that the observed metabolic effects are caused by the lack of Shp2 in the liver. These findings correlated with, and were most likely caused by, increased IRS1/IRS2 tyrosyl phosphorylation in the liver, accompanied by increased PI3K/Akt signaling. In contrast, insulin-induced Erk activation was dramatically attenuated, yet there was no effect on the putative Erk site on IRS1 (Ser812) or on S6K1 activity. These studies show that Shp2 is a negative regulator of hepatic insulin action, and most likely acts by dephosphorylating the PI3K binding sites on IRS1/2. Specific targeting of Shp2 in the liver could be a useful approach for treatment and prevention of insulin resistance and metabolic syndrome.

Role of Brain Insulin Signaling on Tissue-Specific Glucose Disposal

CLAUDIA P. DIOMANS, NIENKE P. BIERMANS, LOUIS M. HAVEKES, JOHANNES A. ROMIJN, LEIDEN, THE NETHERLANDS

Circulating insulin inhibits hepatic glucose production and stimulates glucose uptake in peripheral tissues. It has been shown that hypothalamic insulin signaling is required for the inhibitory effects of circulating insulin on endogenous glucose production. In this study, we examined the central effects of circulating insulin on tissue-specific glucose uptake. Tolbutamide, an inhibitor of ATP-sensitive potassium channels, was infused in the lateral ventricle (i.c.v.) in hyperinsulinemic euglycemic clamp conditions in chow-fed and in diet-induced obese C57Bl/6J mice. Whole body glucose uptake was measured by D-[14C]glucose kinetics and tissue-specific glucose uptake.

The Reactive Nitrogen Derivative Peroxynitrite Acts as a Feed-forward Modulator of the Hepatic NFκB/INOS Inflammatory Pathway in Mice Models of Endotoxin and Lipid-Induced Insulin Resistance

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Recent studies suggest that reactive nitrogen species (RNS) are key modulators of energy metabolism and may play a role in the development of insulin resistance. We have recently reported that RNS derivative peroxynitrite (ONOO-) is implicated in the inhibition of hepatic insulin/glucagon action (LB20). For author disclosure information, see page LB41.
and KO mice received an iv injection (30 mg/kg) of the ONOO- decomposition catalyst FETPPS prior to the administration of LPS or Intralipid. In WT mice, LPS and lipid treatments reduced glucose infusion rate (GIR), elevated basal glucose production (GP) and caused marked insulin resistance as revealed by impaired suppression of liver GP and reduced peripheral glucose disposal (RD) during the clamp. Liver insulin resistance was linked to impaired phosphorylation of Akt (Ser473) and a robust induction of NFB signaling and increased expression of iNOS protein levels. In contrast to their WT counterparts, KO mice were protected from endotoxin or lipid-induced hepatic and peripheral insulin resistance. Unexpectedly, the activation of the NFκB pathway was also reduced in the liver of KO mice challenged with LPS and lipids. Interestingly, FETPPS-treated animals were also protected against LPS and Intralipid-dependent inhibition of hepatic insulin signaling and induction of the NKB/iNOS pathways. These findings further support the growing body of evidence that RNS are implicated in the development of insulin resistance. Our data further suggest that ONOO- is a key player in a feedforward loop whereby iNOS-dependent production of ONOO- is key to the activation of the NFκB pathway, possibly for maintaining iNOS induction during pro-inflammatory and nutrient stresses.

**INSULIN ACTION—SIGNAL TRANSDUCTION**

**74-LB**

**AS160 Ser711 Phosphorylation Is Impaired in Skeletal Muscle from Insulin Resistant Subjects**

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Individuals with type 2 diabetes (T2DM) have reduced insulin-stimulated GLUT4 translocation and glucose uptake in skeletal muscle. Animal studies show that the distal signals for insulin-stimulated GLUT4 translocation involve a complex pattern of phosphorylation of the Rab-GTPase activating proteins AS160 and TBC1D1. Insulin-stimulated AS160 phosphorylation occurs at multiple sites including phospho-Akt substrate (PAS) motifs and at Ser711, a site phosphorylated by both Akt and AMPK. TBC1D1 phosphorylation includes stimulation at PAS sites and Thr590, an Akt consensus sequence. We determined whether insulin increases AS160 and TBC1D1 phosphorylation at these sites in human skeletal muscle, and whether this effect was altered in subjects with obesity and/or T2DM. Age-matched (n=8-10/group) lean control [BMI=25.4±0.5 kg/m², Fasting insulin 0.53 ± 0.02, Tg 8.2 ± 0.06, p<0.01] and obese (n=10/group) subjects [BMI=34.1±0.8, FPG=161±24, Fasting insulin 175±14, p<0.01]. Insulin similarly increased AS160 Ser711 phosphorylation at 30 and 180 min in all groups (P<0.05 vs. baseline), but the increase was significantly lower in the obese and T2DM subjects (P<0.05). In contrast, insulin similarly increased AS160/TBC1D1 PAS phosphorylation in all 3 groups (P<0.05 vs. baseline). Insulin increased AS160 Ser711 phosphorylation by 2-fold in lean controls (P<0.05), an effect completely abolished in the obese and T2DM groups. Insulin had minimal effects on TBC1D1 Thr590 phosphorylation in all groups, and interestingly, was only significantly increased in the T2DM subjects at 30 min (1.5 fold, P<0.05). In summary, insulin increases AS160 Ser711 in human muscle and this effect is impaired in obese and T2DM subjects. There is site-specific regulation of AS160 and TBC1D1 phosphorylation in human muscle, and insulin resistance is associated with altered regulation of some, but not all of these phosphorylation sites.

**75-LB**

**Lever-Specific iNOS Expression Causes Hepatic Insulin Resistance and Hyperglycemia in Mice**

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Inducible nitric oxide synthase (iNOS), a major mediator of inflammation, plays an important role in the development of insulin resistance. Inhibition of iNOS reverses or ameliorates obesity-induced insulin resistance in skeletal muscle and liver. However, it is unknown whether increased expression of iNOS per se is sufficient to induce insulin resistance. To address this issue, we generated liver-specific iNOS transgenic (Tg) mice, where expression of the transgene, iNOS, is regulated under the control of the mouse albumin promoter. Two lines of liver-specific iNOS Tg mice exhibited fasting and fed hyperglycemia, hyperinsulinemia, glucose intolerance, and insulin resistance, as compared with wild-type (WT) littermates (Fasted US [mg/dl]: WT: 120 ± 9, Tg: 175 ± 14, p<0.01; Fasted Insulin [mg/ml]: WT: 0.53 ± 0.02, Tg: 8.2 ± 0.06, p<0.01). These effects are independent of adiposity. Euglycemic hyperinsulinemic clamp study revealed that insulin-stimulated suppression of hepatic glucose output was attenuated in Tg mice relative to WT mice (Clamp HGO [mg/kg/min]: WT: 5.4 ± 2.1, Tg: 11.7 ± 1.6, p<0.05), while basal hepatic glucose output did not differ between WT and Tg mice. No significant difference was found in glucose infusion rate during the clamp. Immunoblot analysis confirmed increased iNOS expression in liver, but not skeletal muscle, of Tg mice relative to WT mice. Insulin-stimulated phosphorylation of IRS-1, IRS-2, and Akt was significantly impaired in liver, but not in skeletal muscle, of Tg mice, while insulin receptor phosphorylation was not affected by iNOS transgene. Insulin failed to increase phosphorylation of GSK-3β and FoxO1 in liver of Tg mice, whereas insulin induced robust phosphorylation of GSK-3β and Fox01 in WT mice. However, liver-specific iNOS expression had no effects on the protein expression of insulin receptor, IRS-1, IRS-2, Akt, GSK-3β, Fox01, PTP-1B, PTEN, and P13K. These results clearly indicate that increased iNOS expression is sufficient to induce insulin resistance and impaired insulin signaling in the liver. Furthermore, our data highlight a pivotal role for iNOS in the development of hepatic insulin resistance and hyperglycemia.

**Supported by NIH (DK585127).**

**ADA-Funded Research**
77-LB

Rac1-Activation Is a Novel Convergence-Point between Insulin and Exercise-Signaling in Human Skeletal Muscle

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The cortical actin-cytoskeleton and the actin-regulatory GTPase Rac1 are required for insulin-stimulated GLUT4 translocation and glucose uptake in cell culture and mature muscle, and failure of insulin to cue actin-remodelling has been proposed as a major defective step in insulin resistant states. In humans, exercise can stimulate glucose uptake in insulin-resistant muscle and acutely reverse insulin resistance but it is not known whether (i) contraction-stimulated glucose uptake requires an intact actin-cytoskeleton in mature muscle and (ii) whether insulin or exercise in humans activates Rac1. To investigate this, 2-deoxyglucose uptake was measured in incubated mouse soleus and EDL muscles pretreated with or without 20 µM latrunculin B, an actin-depolymerising agent. While basal glucose uptake in soleus and EDL was unaffected, latrunculin B inhibited glucose uptake by ~ 50% and 60%, respectively in response to insulin, by 100% and ~ 70%, respectively by electrically-stimulated contraction; and by ~ 60% in EDL muscles incubated by the AMPK-activating glucose uptake-stimulator AICAR (P<0.01). In humans, Rac1 activity was significantly increased by 1.3-fold in vastus lateralis after 2h hyperinsulinemic euglycemic clamp (P=0.01). Interestingly, also 45 min of inclined (15%) walking exercise at 70% VO2 max significantly increased Rac1 activity by 1.4- and 1.3-fold in human soleus (P<0.01) and gastrocnemius (P<0.05), respectively. In contrast, serine-3 phosphorylation of Cofilin, another actin-regulatory protein previously shown to be dephosphorylated with insulin in cultured muscle cells, was decreased by the exercise bout but did not change with either 30 min or 2 h insulin (P>0.05). These data show that both insulin- and contraction-stimulated glucose uptake require an intact actin-cytoskeleton and that activation of Rac1-regulated processes like actin remodelling may underlie the ability of insulin, as well as muscle contraction to increase glucose uptake in human skeletal muscle.

78-LB

Specific Inhibition of Insulin Signaling in the Exocrine Pancreas Reduces Pancreas Size and Pancreatic Digestive Enzymes Synthesis

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Patients with type-1 diabetes present pancreatic inefficiency and potential nutrient malabsorption due to a reduction of duodenal digestive enzymes. Experimentally-induced streptozotocin diabetes in mice and rats inhibits digestive enzyme synthesis and secretion. Our aim was to demonstrate that direct insulin action on acinar cells is required for the synthesis of pancreatic digestive enzymes without systemic diabetes. We generated exocrine pancreas insulin receptor knockout mouse (EPIR-KO) mice by crossing mice with a floxed insulin receptor to mice with tamoxifen regulated elastase Cre that expresses only in acinar cells. To examine the effect of insulin on acinar cells, we removed basal insulin and treated EPIR-KO mice with a complete diet (Re-fed-C); EPIR-KO mice (Fasted-EPIR-KO) and re-fed-EPIR-KO mice. Protein levels were assessed by western blotting. We show that the lack of insulin results in severe pancreatic insulin deficiency and severe pancreatic insufficiency. Insulin receptor KO mice showed a 25-30% decrease in pancreatic insulin levels compared to control. Insulin receptor deficient mice also showed a 25-30% decrease in exocrine enzyme levels compared to control. These results may help in the development of strategic therapies for the disease

80-LB

Extracellular Conversion of Adiponectin Hexamers to Trimmers

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Adiponectin is an insulin-sensitizing hormone secreted mainly from adipocytes. In vivo, it exists as trimers, hexamers, and several larger species collectively referred to as higher molecular weight (HMW) adiponectin. While circulating HMW adiponectin concentration more closely matches insulin sensitivity in vivo, trimeric forms of adiponectin have multiple insulin-sensitizing effects in vitro or following injection into animals. Lack of correlation between circulating trimer concentration and insulin action could not be explained by conversion of one oligomer to another. Prior studies have not observed such phenomenon in circulation. To better understand the relationship between adiponectin production and insulin sensitivity, we examined the biogenesis of trimeric adiponectin from adipocytes. Accumulation of adiponectin trimers from cycloheximide-treated 3T3-L1 adipocytes persisted despite complete absence of intracellular trimers, suggesting the trimers in conditioned media were derived from larger oligomers. To confirm this result, 3T3-L1 adipocyte-conditioned media were incubated with purified FLAG-epitope tagged individual oligomers. In conditioned media without cells, FLAG epitope-tagged hexamers underwent conversion to trimers. No conversion was observed with epitope-tagged HMW and trimeric adiponectin. Conversion of hexamers to trimers was inhibited by diamide or N-ethylmaleimide, suggesting the process is thiol-dependent. Consistent with this observation, purified hexamers were significantly more sensitive to reduction than HMW isoforms. Conversion of hexamers to trimers was accelerated by 100 µM NADPH, but not NADP+, providing further evidence for a redox-based mechanism. In addition to cultured adipocytes, hexamers could be converted to trimers in conditioned media from differentiated C2C12 myotubes. Significantly, adiponectin secreted from isolated rat primary adipocytes also underwent conversion to trimers, suggesting in vivo relevance for our observations from cultured cell. Our data indicate that adiponectin trimers can be generated near cell surface from hexamers after exiting from circulation, providing a potential mechanism to activate trimer-mediated adiponectin signaling.  

Supported by NIH-R01 DK 20572 (W. Herman, PI) 2008, Michigan Diabetes Research and Training Center (MDRTC), Pilot Feasibility Grant 12/01/07 – 02/28/09, Eli Lilly Research grant.

INTEGRATED PHYSIOLOGY—ADIPOCYTE BIOLOGY

79-LB

The Effect of Tribbles-Related Protein 3 (TRB3) on ER Stress-Suppressed Insulin Gene Expression in INS-1 Cells

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The highly developed endoplasmic reticulum (ER) structure in pancreatic beta cells is related to its heavy engagement in insulin biosynthesis. Thus, any perturbation of ER function inevitably impacts insulin biosynthesis. Recent studies showed that the expression of trimbles-related protein 3 (TRB3), mammalian homolog of Drosophilia tribbles, in various cell types is induced by ER stress. Here we examined whether ER stress decreases insulin gene expression and this process is mediated by TRB3. To determine whether increased ER stress influences insulin and TRB3 expression in beta cells, INS-1 cells were treated with tunicamycin and thapsigargin, well known ER stress inducers. The treatment of INS-1 cells with tunicamycin and thapsigargin decreased insulin gene expression but increased TRB3 gene expression. To determine the effect of TRB3 on the expression of the insulin gene, INS-1 cells were infected with an adenovirus encoding TRB3. Adenovirus-mediated overexpression of TRB3 decreased insulin gene expression in a dose dependent manner. A transient transfection study showed that TRB3 inhibits insulin promoter activity, suggesting that TRB3 inhibits insulin gene expression at transcriptional level. The transcriptional activity of insulin gene is primarily regulated by transcription factors that are enriched in expression in beta cells including PDX-1 and MafA. To determine the mechanism of impairment of insulin gene expression by TRB3, we examined the effect of TRB3 on the expression of PDX-1 and MafA. Adenovirus-mediated overexpression of TRB3 decreased PDX-1 mRNA expression but did not influence MafA mRNA expression. Collectively, this study showed that ER stress-induced TRB3 expression decreased insulin and PDX-1 gene expression in INS-1 cells. Our data suggests that TRB3 plays an important role in ER stress-induced beta cell dysfunction.

INTEGRATED PHYSIOLOGY

ADA-Funded Research

For authors disclosure information, see page LB41.
Fructose Metabolism and Its Influence on Glucose Metabolism in Human Adipocytes

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Fructose consumption has dramatically increased in the last 3 decades and fructose is now implicated in the pathogenesis of obesity. Studies in rodents show that fructose stimulates lipogenesis and causes hepatic and extra-hepatic insulin resistance due to ectopic lipid accumulation and dyslipidemia. Studies in humans also demonstrate an increase in de novo lipogenesis, increased plasma fasting and post prandial triglycerides, and decreased lipidolysis. Not many studies to our knowledge have examined the effects of fructose on adipose tissue metabolism, as the prevailing concept is that fructose is cleared rapidly by the liver with insignificant amounts in systemic circulation. However, recent studies have demonstrated higher serum fructose levels, which could be coming from excess consumption, and fructose is not endogenously synthesized. Fructose can thus affect extra-hepatic tissues such as adipose tissue and skeletal muscle. In this study, we examined the influence of fructose on adipocyte glucose metabolism using stable isotope dynamic metabolic profiling (SIDMAP) assay that uses [1, 2-13C2] glucose to measure the flow of molecules through the metabolic network. SGBS preadipocytes in triplicates were induced to differentiate in the presence of 20 mM fructose or glucose respectively, added to differentiation medium containing 5 mM glucose and compared to a control set that lacked the added glucose or fructose. Media and cell lysates were subjected to metabolic assays and analyzed. Our study demonstrates that a) Fructose is not oxidized in adipocytes but used for triglyceride synthesis. b) Fructose drives more glucose through the oxidative branch of the pentose phosphate pathway, a pathway generating energy via NADPH production for lipid biosynthesis. c) The presence of fructose makes the TCA cycle more anaplerotic leading to increased lipid biosynthesis in adipocytes. Thus, fructose exerts its lipogenic action in part by directing more glucose into lipogenic pathways, leading to increased lipid biosynthesis and accumulation in the adipocytes. This can consequently alter the adipocytokine profile of the adipocyte and result in a proinflammatory state that is seen in obesity.

Inverse Correlation of Adipocyte Cell Size with Insulin Sensitivity in Lean, Healthy Individuals

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Large adipocytes are associated with insulin resistance, but one must account for the confounding effect of obesity, which is associated with both insulin resistance and large adipocytes. A recent study comparing moderately obese, sensitive and resistant subjects, with comparable body mass index (BMI) did not detect any significant difference in the size of the large cells, but rather a larger proportion of large cells in the sensitive subjects, suggesting impaired adipogenesis in the insulin resistant state. Here we extend the investigation to leaner resistant and sensitive subjects who were the first degree relatives of type 2 diabetics. The recruited subjects were healthy individuals with BMI 18-34. The degree of insulin sensitivity was measured by euglycemic hyperinsulinemic clamp and expressed as glucose infusion rate (GIR) normalized by total body weight or lean body mass. Needle biopsies of abdominal subcutaneous fat (20-30 mg) were obtained, fixed with osmium tetroxide, and assayed for cell weight or lean body mass. Needle biopsies of abdominal subcutaneous fat of insulin-resistant subjects (N=4) with either impaired glucose tolerance or newly diagnosed type 2 diabetes (2 hour OGTT plasma glucose 141-236 mg/dl) and 2 months of pioglitazone (P) treatment. Global adipocyte cell protein abundance was determined by a combination of one-dimensional SDS-PAGE and High Performance Liquid Chromatography-Electron Spray Ionization-tandem Mass Spectrometry (HPLC-ESI-MS/MS). As expected, PIO increased insulin sensitivity, determined by hyperinsulinemic euglycemic clamps, ~25% (p=0.01). Proteomics analysis by HPLC-ESI-MS/MS identified a total of 1701 proteins, of which at least 707 were common before and after PIO treatment. Analysis of proteins sets revealed that PIO consistently increased the abundance of key enzymes in oxidative metabolism and mitochondrial function including proteins involved in beta-oxidation, the citric acid cycle as well as the electron transport chain, and consistently decreased the abundance of proteins involved in lipolysis. Furthermore, PIO significantly decreased the abundance of cytoskeletal proteins including actin (ACTB, ACTA1), tubulin (TUBB, TUBB2A, TUBB2C, TUBA1B), septin (SEPT2, SEPT7, SEPT11), and laminin (LAM). These results suggest that the increased lipid disposal in adipose tissue by TZDs in humans may be due to increased machinery for fatty acid oxidation and energy production and decreased machinery for lipolysis in subcutaneous adipocytes. The lowered abundance of cytoskeletal proteins is consistent with increased adipocyte differentiation.

Supported by Takeda Pharmaceuticals.

TNF-α Induction of Adipocyte Lipolysis Is Mediated through Downregulation of GOS2 Expression

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Surplus fuel in the form of fatty acids and glycerol is made available through stimulated lipolysis in adipocytes. This process is physiologically stimulated by β-adrenergic hormones that activate adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) through PKA-dependent phosphorylation of the lipid droplet coat protein perilipin. In pathological states of nutritional overload such as obesity, TNF-α also potently stimulates lipolysis and this may contribute to hyperlipidemia and insulin resistance. Recent work in our laboratory has identified GOS2 as a novel inhibitory protein in ATGL-mediated lipolysis. Here we have investigated whether the lipolytic action of TNF-α is mediated by altering GOS2 protein level and have studied the mechanisms by which it occurs. Time course analysis showed that TNF-α treatment decreased the GOS2 protein content in 3T3-L1 adipocytes preceding the increase in lipolysis. By real time RT-PCR we demonstrated that TNF-α drastically decreased the GOS2 mRNA content. Moreover, treatment of cells with protein synthesis inhibitor cycloheximide revealed the protein half-life of GOS2 to be < 1 h. Protein degradation of GOS2 in TNF-α-treated adipocytes could be prevented by the proteasome inhibitor MG-132 but not by the lysosomal inhibitor leupeptin. Furthermore, both basal and TNF-α-stimulated lipolysis was blunted when either ATGL or the ATGL-coactivator CGI-58 was knocked down in adipocytes. In parallel, adenosinergic-mediated overexpression of ATGL or CGI-58 enhanced lipolysis under the same conditions. Interestingly, overexpression of GOS2 was able to significantly decrease TNF-α-stimulated lipolysis both in the absence and in the presence of ectopic ATGL or CGI-58. Based upon these observations, we propose that TNF-α-stimulated lipolysis by decreasing GOS2 protein level through the combined effects of
downregulation of GOS2 transcription and acute proteasomal degradation of the existing protein.

ADA-Funded Research

INTEGRATED PHYSIOLOGY

86-LB

Delineation of the Central Melanocortin Circuitry Controlling the Pancreas
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The central melanocortin system plays an important role in the regulation of pancreatic endocrine functions and glucose metabolism. However, the exact underlying neurosubstrate remains elusive. To delineate the central melanocortin circuits connecting pancreas tissues via autonomic nervous system, we applied a specific trans-synaptic retrograde tracer, the attenuated pseudorabies virus (PRV-614) and the retrograde neuronal tracer cholera toxin B subunit (CTb) into the pancreas of melanocortin 4 receptor (MC4R)-green fluorescent protein (GFP)-transgenic mice and pro-opiomelanocortin (POMC)-EGFP transgenic mice, respectively. The fluorescence immunocytochemistry (ICC) was used to examine distribution feature of dual-labeled MC4R-GFP-immunoactivity (IR)/PRV-614-bearing neurons and POMC-EGFP/PRV-614-bearing neurons in the CNS at various time points after PRV inoculation, as well as dual-labeled MC4R-GFP-IR/CTb-IR neurons in the brainstem. Specific subsets of dual-labeled MC4R-GFP-IR/PRV-614-bearing neurons were found in multiple nuclei of the CNS, including the intermediolateral column (IML) of the spinal cord, the dorsal motor nucleus of the vagus (DMV), the raphe pallidus nucleus (RPa), the gigantocellular nucleus parax (Gia) of brainstem, and the paraventricular nucleus of the hypothalamus (PVH). A subset of POMC-EGFP-expressing neurons in both the arcuate nucleus of the hypothalamus (ARC) and the nucleus of the solitary tract (NTS) were infected by the PRV-614 after 6-7 days survival of animals. A subset of MC4R-GFP-IR neurons in both rostral and caudal of the DMV contain CTb-IR, suggesting that a specific subset of MC4R neurons in the DMV directly innervate the pancreas. In addition, bilaterally vagotomized animals showed absence of PRV in the DMV after 4 days survival, whereas dual-labeled MC4R-GFP-IR/PRV614-bearing neurons were detected in the RPas, indicating that the RPas MC4R neurons involve sympathetical control of the pancreas. These results reveal a hierarchical organization of the descending MC4R neuronal circuits controlling the pancreas, thus providing neuroanatomical substrates for the central melanocortin system to regulate pancreatic endocrine functions.

ADA-Funded Research

Insulin Resistance and Glucose Intolerance Independently Reduce the Incretin Effect Via a β-Cell Defect
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The incretin effect is impaired in type 2 diabetes. We wanted to evaluate the separate impact of insulin resistance and glucose intolerance on the incretin effect and whether changes in the incretin effect were associated with β-cell defects.

21 normal glucose tolerant 1st degree relatives to type 2 diabetes patients underwent a 75g GTT and an isoglycemic i.v. glucose test before and after 5 days treatment with 2mg dexamethasone (dex) bid. Insulin, C-peptide, GIP and GLP1 were measured. The incretin effect was calculated by relating the incremental insulin response during OGTT to the incremental insulin response during iv glucose. We calculated β-cell glucose sensitivity during the i.v. glucose infusion and a disposition index by multiplying β-cell glucose sensitivity with 1/HOMA-

Post-dex insulin resistance increased in all 21 subjects, and 11 subjects in addition to insulin resistance also developed IGT. The incretin effect was in the NGT and IGT group 71±3.2 and 67±4.8% (NS) pre-dex and 58±5.2 and 32±8.8% (P < 0.05) post-dex, respectively. A multiple regression analysis of pooled data related the changes in incretin effect (Δincretin effect) to changes in insulin resistance (ΔHOMA-

The disposition index did not differ between the NGT and IGT groups at baseline (NS). Post-dex this index was not reduced in the NGT group (NS) but in the IGT group (P < 0.05). Responses of GLP-1 and GIP did not differ between groups pre-og post-dex during the OGTT.

ADA-Funded Research

87-LB

In conclusion insulin resistance and glucose intolerance contribute independently to the reduced incretin effect seen in type 2 diabetes. The incretin effect is reduced very early in NGT insulin resistant people, before the β-cell sensitivity to i.v. glucose is affected, and deteriorates further when β-cell function declines in people with IGT. This points to an early, specific β-cell defect in the action of the incretin hormones before the development of type 2 diabetes.

Supported by the Novo Nordisk Foundation.

INTEGRATED PHYSIOLOGY

88-LB

Mechanisms by Which Two Kinds of PPAR Gamma Agonists Improve Insulin Sensitivity and Increases Body Weight in Type 2 Diabetic Patients
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Pioglitazone (PIO), full PPAR gamma agonist, improves the insulin sensitivity by the increase circulating adiponectin levels, despite the increase in body weight. On the other hands, telmisartan (Tel), partial PPAR gamma agonist, also improves the insulin sensitivity without the increase in body weight. However, the mechanisms of these different action are not fully understood.

We investigated the effect of these two kinds of PPAR gamma agonists on circulating adiponectin multimers levels to elucidate the mechanism by which these agonists improve insulin resistance and increase body weight in type 2 diabetes mellitus (T2DM). Fourteen subjects with T2DM were added on PIO (15mg/day), and 7 subjects combined with T2DM and equivalent hypoglycemia were added on Tel (40mg/day), for 6 months. Before and after treatment, subjects received euglycemic hyperinsulinemic clamp studies (at 1.5 mU/kg/min insulin infusion rate) to assess insulin sensitivity. There were no change in diet and other medications.

After 6 months with the treatment, glucose infusion rate (GIR) was significantly increased by 56% in PIO group and by 35% in Tel group. However, body weight was significantly increased by 4.7% without edema in PIO group, but no significant change in Tel group. Total adiponectin levels was significantly increased by 130% in PIO group, but not significant change in Tel group. Moreover, plasma adiponectin multimers (high (HMW), middle (MMW), and low molecular weight (LMWW)) were measured by ELISA kit. PIO group significantly increased HMW, MMW, and LMW adiponectin by 230%, 97%, and 72%, respectively, whereas Tel group significantly increased MMW adiponectin by 56% and significantly decreased LMW adiponectin by 34%. In all subjects, before and after treatment, the increase in GIR was correlated with the increase in HMW and MMW adiponectin levels. Interestingly, the increased in body weight was significantly correlated with the increased in LMW adiponectin levels. These results support the hypothesis that HMW and MMW adiponectin can play an important role to improve insulin sensitivity, whereas LMW adiponectin may exacerbate obesity in patients with T2DM.

INTEGRATED PHYSIOLOGY—LIVER

89-LB

Hepatocyte-Specific SHP-1 Disruption Reveals a Key Role for the PTPase in the Regulation of Glucose Homeostasis
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We have previously reported that SH2-domain-containing protein tyrosine phosphatase 1 (SHP-1) modulates glucose homeostasis in the mouse. Using the Ptpn6me-v/me-v viable motheaten mice and liver-specific mouse models of SHP-1 deficiency we have demonstrated that SHP-1 is a key regulator of hepatic insulin clearance and metabolic signaling in liver (Nat Med 2006 May;12(5):549-56). However, given that SHP-1 is expressed in several cell types in liver tissue, notably hepatocytes, Kupffer cells, macrophages, and endothelial cells, the precise role of hepatocyte SHP-1 in the regulation of glucose metabolism, and its potential contribution to the development of insulin resistance and type 2 diabetes, remains obscure.

To clarify its hepatocyte function, we have generated hepatocyte-specific SHP-1 knockout mice (Hesp1KO) using the Cre/LoxP system and compared them to their SHP-1 LoxP-containing littermates (WT). On standard chow diet, male Hesp1KO mice exhibited lower fasting glycemia and improved glucose tolerance. Hyperinsulinemic-euglycemic clamp studies also revealed higher hepatic insulin sensitivity in Hesp1KO mice as compared to their littermate WT controls, even after feeding a high fat diet (HFD) for 8 weeks. Furthermore, the HFD-induced impairment in insulin activation of...
90-LB

Induction of Extra-Hepatic Gluconeogenesis in the Absence of Glucose Production by the Liver

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The liver is considered the major source of endogenous glucose production (EGP) in any postabsorptive situation. Nevertheless, the kidney and intestine can also produce glucose in blood, particularly during fasting and under protein feeding. To better define the importance of the liver in glucose homeostasis, we generated a mouse model of inducible liver-specific deletion of the glucose-6-phosphatase gene (G6Pase), encoding an essential enzyme for EGP. Male adult B6.G6pclox/loxSACreERT2/ and C57Bl/6J (wt) mice were treated with tamoxifen to obtain Lg6pc-/- and control mice, respectively.

Blood concentration was monitored during fasting (6-45h) and EGP was determined using [3-14C]glucose at steady-state. G6Pase and PEPCK c-mRNA activity were determined on frozen-tissue homogenates. As expected, hepatic G6Pase activity was undetectable 5 weeks after initiation of G6pc deletion (0.3±0.5 U/g protein in Lg6pc-/- vs control 4.2±1 U/g protein in wt), whilst the expression of hepatic PEPCK-C was not modified. The loss of hepatic G6Pase led to the accumulation of hepatic glycogen and triglycerides, resulting in hepaticomegaly and steatosis. This liver-specific picture ascertained that Lg6pc-/- mice were incapable of hepatic glucose release. Consistent with the role of hepatic glycogenolysis in the early post-absorptive state, EGP levels were found to be lower in 6 h fasted Lg6pc-/- than in wt mice; this was correlated with a lower blood glucose concentration in Lg6pc-/- mice (25±4 mg/dl in Lg6pc-/- vs 15±5 mg/dl in wt).

However, there was a progressive re-increase of glycemia in Lg6pc-/- mice, to reach the same level as control mice at 30 h of fasting. Interestingly, this could be explained by an induction of all gluconeogenic enzymes, in the kidneys and the intestine of Lg6pc-/- mice. In conclusion, we show here that the loss of hepatic G6pc activity was sufficient to normalize serum glucose. However, this could be explained by an induction of all gluconeogenic enzymes, in the kidneys and the intestine of Lg6pc-/- mice. In conclusion, we show here that the loss of hepatic G6pc activity was sufficient to normalize serum glucose. This result challenges the current dogma relating to the role of the liver in glucose homeostasis requires re-examination.

Supported by CIHR and ADA.

91-LB

alpha-Hydroxybutyrate Reduces Mitochondrial ATP Synthesis in Human Muscle

SANGEETA A. GHOSH, RALPH DEFRONZO, MUHAMMAD A. ABDUL-GHANI, WALTER E. GALL, NICOLAS MUSI, San Antonio, TX, Durham, NC

Mitochondrial dysfunction has been postulated to play an important role in the pathogenesis of insulin resistance. Through biochemical profiling of plasma from 399 nondiabetic subjects using a mass spectrometric metabolomic platform, previously we found that alpha-hydroxybutyrate (alpha-HB) is the most significant metabolite associated with insulin resistance. Because of the relationship between insulin resistance and mitochondrial dysfunction, in this study we tested whether alpha-HB impairs mitochondrial ATP Synthesis in human muscle. We isolated mitochondria from vastus lateralis muscle biopsies performed in healthy non-diabetic subjects and under protein feeding. To better define the importance of the liver in glucose homeostasis, we generated a mouse model of inducible liver-specific deletion of the glucose-6-phosphatase gene (G6Pase), encoding an essential enzyme for EGP. Male adult B6.G6pclox/loxSACreERT2/ and C57Bl/6J (wt) mice were treated with tamoxifen to obtain Lg6pc-/- and control mice, respectively.

Glycogen Storage Disease.

INTEGRATED PHYSIOLOGY—MusCLE

91-LB

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Glycogen Storage Disease.
the basal glucose production in Rko mice by 61% (41±2 vs. 16±1 mg/kg/min, p<0.05). Glucose infusion rate (GIR) under hypoglycemic-euglycemic clamp was significantly lower in BAC-Tg than Rko (68±4 vs. 98±10 mg/kg/min, p<0.05), hepatic glucose production (HGP) was higher (18±3 vs. 8±1 mg/kg/min, p<0.05), and glucose uptake in adipose tissue was lower (14±1 vs. 26±6 mmol/g/min, p<0.05). Therefore, resistin modulates the response to LPS by inducing insulin resistance in liver and adipose tissue. We propose that resistin may have an important role in counteracting the hypoglycemic effect of acute endotoxemia.

Supported by NIDDK P01 DK49210 to M.A. Lazar.

ADA-Funded Research

ISLET BIOLOGY—APOTOPSIS

96-LB

Protection Against Glucose-Induced Apoptosis and Maintenance of INS-1E Cell Proliferation in Cells Harbouring the Naturally Occurring Activating Glucokinase Gene Mutation GK-E442K

NADIA COBO-VILLEUMIER, RAQUEL ARAUJO, PABLO RODRIGUEZ-BADA, ANA GAVITO, ELENA BAIXERAS, RICARDO GOMEZ-HUELGA, FRANZ MARTIN, FRANCISCO BEDOYA, ANTONIO BERNAD, BENITO GAUTHIER, ANTONIO CUESTA-MUNOZ, Malaga, Spain, Sevilla, Spain, Madrid, Spain

In addition to its glucose sensor function in islet β-cells, glucokinase (GK) has been proposed to participate in cell proliferation and survival. In humans with congenital hypoglycaemia, we have previously described the naturally activating glucokinase gene mutation GK-E442K. In order to establish a potential link between GK activity and β-cell survival as well as expansion, we constitutively expressed this mutant variant in the insulinoma INS-1E cell line. Using lentiviral technology, we created stable clones of INS-1E cells constitutively expressing GK (INS-1E-cmv.GCK-WT, INS-1E-cmv.GCK-E442K, INS-1E-cmv.GCK-WT.ires.GFP), the mutant INS-1E-cmv.GCK-E442K.ires.GFP, and the control GFP lentivector. Transcript levels for GK-WT, GK-E442K, GFP, BAD and IRS2 were determined by quantitative real time RT-PCR. Acute insulin secretion in response to glucose (3 and 15 mM) was also measured. In addition, the impact of increasing concentration of glucose (3, 11 and 20 mM) on proliferation and survival was estimated at 72 hours. Overexpression of GK-WT or GK-E442K in INS-1E did not alter glucose-induced insulin secretion as compared to control GFP cells. Both the GK-WT and GK-E442K transcript were expressed at similar levels but lower than GFP transcript levels. Interestingly, BAD and IRS2 expression levels remained unaltered in all clones. After 72 hours at either 3 mM or 20 mM glucose, the clone expressing the GK-E442K variant displayed significantly reduced apoptosis (12- and 6-fold, respectively), while GK-WT had no protective effect. In contrast all clones exhibited protection at 11 mM glucose (6-fold as compared to GFP), corresponding to normal culture conditions. Proliferation at 11 or 20 mM glucose was identical for all clones. Astoundingly, the GK-E442K clone was also able to sustain proliferation even at 3 mM glucose. These results indicate that the naturally occurring activating GK mutation GK-E442K may convey protection against apoptosis under altered glycaemic conditions and that, in spite of being in a low glucose environment, these cells maintain proliferation of β-cells.

Supported by Fundacion Progreso Y Salud (B. Gauthier) and MICINN (SAF2006-12663) and Junta de Andalucía (SAS/PI-024/2007; SAS/PI-0236/2009) to A. Cuesta.

97-LB

Unacylated Ghrelin Fragment Promotes Survival of Pancreatic β-Cells and Human Pancreatic Islets and Improves Diabetes in Streptozotocin-Treated Neonatal Rats

RICCARDA GRANATA, MARIA FELICE BRIZZI, FABIO SETTANNI, GABRIELE TGULIATTI, ALESSANDRA BARAGLI, THIERRY ABRIBAT, AART-JAN VAN DER LEVY, LEZIO GHIGO, Twin, Italy, Ecully, France, Rotterdam, The Netherlands

The 28-aminoacid peptide unacylated ghrelin (UAG), although unable to bind the acylated ghrelin (AG) receptor GHS-R1a, exerts many biological effects. UAG, similarly to AG, protects in vitro β-cells and human pancreatic islets against apoptosis, stimulates their proliferation and enhances glucose-induced insulin secretion at physiological concentrations. In vivo, UAG prevents diabetes at adult age in streptozotocin (STZ)-treated neonatal rats by reducing blood glucose, and increasing β-cell mass and insulin secretion. Based on these evidence, we investigated the potential survival effects of UAG fragments in HIT-T15 β-cells and human pancreatic islet cells. These fragments either included or excluded serine-3, the site for ghrelin octanoylation essential for GHS-R1a binding. UAG fragment comprising aminoacids 6-13 displayed survival and antiapoptotic actions.
equal to or even greater than full length UAG (1-28). Moreover, although to a lesser extent, fragments 1-14 and 1-18 retained all the effects of UAG 1-28, including stimulation of glucose-induced insulin secretion. Conversely, fragments 8-13, 8-12 and, particularly, 1-5 and 17-28, showed reduced or no activity. Notably, UAG(6-13), which showed the best protective effects in vitro, also prevented diabetes in adult age in STZ-treated neonatal rats, by decreasing blood glucose and increasing blood and pancreatic insulin levels, similarly to UAG. The pH findings indicate that UAG fragments, similarly to the full length peptide, exert protective effects in β-cells and human islets. The strong UAG(6-13)-induced survival action suggests that this fragment may be sufficient per se to display the protective effects of UAG, without involvement of serine 3. Recently, we have also shown that UAG mobilizes endothelial progenitor cells (EPCs), protects them from oxidative stress and senescence and causes de-novo vessel formation. Herein we show that the fragment UAG(6-13) similarly protects EPCs from oxidative stress, further supporting the view that this peptide exhibits the same pharmacological profile and therapeutic potential as its parent molecule.

Supported by University of Turin (Ex-60% 2007); Azienda Farmaceutica Compagnia di San Paolo 2008.

ISLET BIOLOGY — BETA CELL — DEVELOPMENT

98-LB
Human Reg3α Gene Protein as a Novel Islet Neogenesis Therapy for Reversal of Type 1 and 2 Diabetes

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HIP was previously shown to significantly 1) increase insulin levels in human pancreatic ductal tissue and 2) lower glucose levels and increase islet numbers in STZ-rendered diabetic mice. The half-life of native HIP is 9.9 minutes, and the close developmental similarity of the liver and the pancreas, in the in vivo transition of hepatocytes towards a β-cell phenotype in vivo is a key pathway for the β-cell response to insulin resistance. To avoid neonatal effects on β-cell mass, we employed a tamoxifen inducible β-cell specific Cre recombinase (Ins2Cre-ERT) to delete floxed b-caten (GSK3β) in the liver for molecular marker characteristic of β-cells were indistinguishable from the hepatocytes in morphology. Analysis for molecular marker characteristic of β-cells showed the expression of genes essential for β-cell endocrine function including glucose transporter 2 (Glut 2), Kir6.2 and SUR1 and the key β-cell transcription factors NeuroD and Pax4 in the mice liver cells. Further analysis of the liver also showed activation of the endogenous Pdx1 that is essential to convert a non β-cell into stable new β-like phenotype. This was also evident by the very strong immunostaining of Pdx1 in the liver sections compared to Ngn3 and Mafa. Investigation of the origin of the new β-like cells, by co staining of the induced insulin+ cells with albumin (hepatocyte marker) and NeuroD (key β-cell transcription factor), revealed an overlap of most of the of the insulin+ cells with albumin (81.6%) and NeuroD (85.3%) staining. Together these data shows that the induced insulin producing cells, originated from in vivo transition of the hepatocytes into β-like cells induced by the three transcription factors. However further evidence is required to determine the long term stability of the new β-like state.

ISLET BIOLOGY — BETA CELL — POSTNATAL GROWTH

100-LB
Beta Cell Specific Knockout of Beta-Catenin in Adult Mice Increases Glucose Intolerance and Decreases Insulin Secretion on a High Fat/High Sucrose Diet

ERICA V. GONZALEZ, LAYLA ABU-SHAMAT, RENUKA MENON, VIJAY YEHOOR, LAWRENCE CHAN, SUSAN L. SAMSON, Houston, TX

The increased prevalence obesity is paralleled by a similar increase in diabetes (β-cells) and failure of β-cell cells to secrete enough insulin to compensate for insulin resistance. However, not all obese patients become diabetic so additional β-cell specific factors may enable some compensation. In mouse models of diet induced insulin resistance, the islet responds with β-cell hyperplasia (1). The canonical Wnt signaling pathway has become an important focus because of its role in post-natal β-cell expansion (2,3). Also, genetic variations in the Wnt transcription factor 7-like 2 (TCF7L2) are associated with type 2 diabetes risk. Therefore, Wnt signaling may play a role in the β-cell response to insulin resistance in the adult, beyond neonatal β-cell expansion. Our hypothesis is that Wnt signaling through β-catenin (β-cat) is a key pathway for the β-cell response to insulin resistance. To avoid neonatal effects on β-cell mass, we employed a tamoxifen inducible β-cell specific Cre recombinase (Ins2Cre-ERT) to delete floxed b-caten (GSK3β) in the liver (gavage 4 mg q2days for 5 doses). Males were treated at 6 weeks with tamoxifen (Tam; n=5) or vehicle (n=5) (gagevage 4 mg q2days for 5 doses). This leads to absence of β-caten in the majority of β-cells by immunohistochemistry. At 10 weeks, both groups were started on a Surwit diet (80% fat, high sucrose; HFHS). Over 8 weeks on the diet, fat mass increased by 3-fold in both groups and there was no difference in insulin tolerance testing. However, an intraperitoneal glucose challenge (1.5 g /kg) at 8 weeks revealed that the Tam group had a higher glucose excursion, with an increased area-under-the-curve (p=0.04). At the same time, insulin levels were significantly lower for the Tam group (0.71 ± 0.16 versus 1.37 ± 0.59 mg/L, p=0.04 at 60 min) with a 5.3-fold lower incremental insulin response (AUC ΔG0-60, p=0.02). The combination of worsened glucose tolerance with decreased insulin secretion with a β-cell specific β-caten knock out suggests that Wnt signaling has a role in β-cell compensation for increased insulin demands on a HFHS diet. The signals which activate the Wnt pathway require further elucidation.

Endocrine and Exocrine Pancreatic Regeneration Is Stimulated by In Vivo GSK3β Knockdown in 90 % Pancreactomized Rats

JAMIEH MOVAASSAT, FLORENCE FIGEAC, ANISSA ILIAS, MONIQUE FARO, BERNARD PORTHIA, Paris, France.

The Wnt signaling pathway has been recently implicated in pancreas development as well as in beta cell biology. Glycogen synthase kinase 3 β (GSK3 β), a pivotal partner of the Wnt signaling is a multifunctional enzyme that negatively regulates the growth and function of the beta cells. The aim of our study was to assess the impact of GSK3 β downregulation on β-cell proliferation and function.
on the stimulation of exocrine and endocrine regeneration after subtotal pancreatectomy in rat.

Adult Wistar rats underwent 90% pancreatectomy. In groups of pancreatectomized rats, either antisense oligonucleotides directed against GS3k β-AS-GS3k β or LiCl were injected directly within the remnant pancreatic tissue immediately after pancreatectomy. Two additional groups were administered with non specific standard oligonucleotides (Std group) or with saline and were used as control for the AS-GS3k β and LiCl treated groups respectively. Beta cell mass was assessed by morphometry 7 days and 4 weeks after pancreatectomy.

Beta cell, ductal cells and acinar cell proliferation was measured by BrdU incorporation method 8h and 48h after surgery. Apoptosis was assessed in beta cells, ductal cells and acinar cells by the TUNEL method.

GS3kb down regulation via administration of LiCl or AS-GS3k β greatly improved the beta cell regeneration 7 days after surgery (p<0.01). Moreover the use of AS-GS3k β had sustained effect on the beta cell mass, since 4 weeks after surgery the beta cell mass in the remnant pancreas was found to be higher (p<0.05) in AS-GS3k β treated group compared to the Std treated group. The effect of GS3k β inactivation on the beta cell mass seemed to be mediated by the stimulation of beta cell proliferation. Regarding the exocrine pancreas, GS3k β knockdown significantly stimulated acinar cell proliferation. Interestingly, GS3k β inactivation reduced the number of apoptotic acinar cells compared to that found in the control Std group.

Here we show that intra pancreatic in vivo knockdown of GS3k β promotes both exocrine and endocrine regeneration within the remnant pancreas and could have potential application in the treatment of diabetes and other pancreatic diseases such as pancreatitis.

102-LB
Serotonin Increases β-Cell Proliferation through Htr2b Receptor during Pregnancy
HAIL KIM, TAKESHI MIYATSUKA, KATHERINE YANG, JUEHU WANG, MICHAEL S. GERMAN, San Francisco, CA

During pregnancy, the energy requirements of the fetus impose changes in maternal metabolism. Increasing insulin resistance in the mother maintains nutrient flow to the growing fetus, while prolactin and placental lactogen hyperglycemia due to driving expansion of the maternal population of insulin-producing β-cells. However, the exact mechanisms by which the lactogen hormones drive β-cell expansion remain uncertain. Here, we show that serotonin acts downstream of lactogenic signaling to drive β-cell proliferation. Serotonin synthetic enzyme Tph1 and serotonin production increased sharply in β-cells during pregnancy or after treatment with lactogens in vitro. Pancreatic β-cells expressed the G-protein linked serotonin receptor Htr2b and its expression increased during pregnancy. Pharmacological blocking of Htr2b signaling in pregnant mice blocked β-cell expansion and caused glucose intolerance. Mice with genetic ablation of Htr2b did not increase the β-cell mass during pregnancy and had null β-cells did not increase their proliferation rate when transplanted into wild type mice. These studies reveal an integrated signaling pathway linking β-cell mass to anticipated insulin need during pregnancy.

Supported by Grants from the Larry L. Hillblom Foundation, the JDRF and NIH/NIDDK. ADA-Funded Research

ISLET BIOLOGY—BETA CELL—STIMULUS-SECRETION COUPLING & METABOLISM
103-LB
Characterizations of Transgenic Mice Wherein Diabetes Results from Primary Mitochondrial Malfunction of β-Cells by Ectopic HIM1P High-Expression
XIANYING ZHANG, LINDA DEGENSTEIN, JEFFREY STEIN, YUN CHAO, KWAME OSEI, JIE WANG, Columbus, OH, Chicago, Il.

Pancreatic β-cells are stress-susceptible and lack a recently described nuclear-encoded stress-response hypoglycemia/hypoxia inducible mitochondrial protein 1 (HIM1P). To explore the possible linkage of β-cell stress-susceptible features to lack of HIM1P, we have generated transgenic mice in which the mouse HIM1P gene is ectopically expressed in β-cells under the control of the mouse Ins1 gene promoter.

Transgenic mice of 3 lines denoted as HIM1P-Tg L1, L2 and L3 were produced by breeding of transgenic founders with C57BL/6J mice. Immunoblot and histological studies demonstrated a steady increase of mitochondrial HIM1P protein level in β-cells from L1 to L3 heterozygotes.

The glycemic level of L1 heterozygous mice declined somewhat while the blood insulin level rose slightly from 4 to 22 weeks of age and the resistance of islets to hypoxia/hypoglycemia assaults was enhanced. Intriguingly, hyperglycemia appeared around 5 weeks of age in the L2 and L3 heterozygous whereas no obesity or insulin resistance appeared in these mice until 42-weeks of age. The L3 line was difficult to maintain due to severe diabetic symptoms. β-cell HIM1P protein levels and phenotypes correlated well in the 3 lines, with hyperglycemia occurring in L2 and L3 heterozygous mice due to the elevated HIM1P level in β-cells compared to those in their non-diabetic, heterozygous parents. The L1 homozygous and L2 heterozygous mice show a significant decrease of blood insulin level and impaired glucose tolerance at diabetes onset, and a reduced β-cell mass by 35-weeks of age. Surprisingly, insulin resistance appeared in all three lines at 4, 5, 8, and 35-weeks of age (both heterozygous/homozygous). This early-onset diabetes is dominantly inherited without significant sex differences in this model.

This study shows that ectopic HIM1P expression over a range of levels somewhat enhances β-cell function at lower levels while causing progressive β-cell failure at higher levels, resulting in diabetes. The mechanisms by which HIM1P induces primary mitochondrial dysfunction in β-cells thus deserve further study.

104-LB
Functional Roles of Diacylglycerol Kinases on Insulin Secretion from Pancreatic β-Cells
YUKIKO KANEKO, YOSUKE KOBAYASHI, KESUKE MOTOKI, TAKAHIRO FUJINUKI, KUNIHIKO NAKATA, TOSHIHISA SHIKAWA, Shouka, Japan

Diacylglycerol (DAG) acts as a lipid signal messenger in pancreatic β-cells. One of the important roles of DAG is the activation of PKC, which has been shown to regulate insulin secretion. Phosphatidic acid, another bioactive lipid, has also been reported to stimulate insulin secretion. The intracellular levels of these lipid messengers are strictly regulated by diacylglycerol kinases (DGKs). Nine mammalian DGKs have been identified to date. However, little study has been reported on DGKs in β-cells. The present study thus investigated the expression of DGKs in β-cells and their effects on insulin secretion. Moreover, the mechanism of DGK activation in β-cells was investigated. The mRNA expression of type I DGKs (α, β, γ) was detected in mouse pancreatic islets. DGKγ protein expression was also detected in islets and the β-cell line MIN6. R59949, a specific type I DGK inhibitor, concentration-dependently inhibited glucose- and high K+-induced insulin secretion from MIN6 cells. Real-time imaging of intracellular Ca2+ concentration ([Ca2+]i) revealed that R59949 and DiC8, an membrane permeable DAG analog, lowered glucose- and high K+-induced [Ca2+]i elevation in MIN6 cells. In contrast, a high concentration of propranolol (300 μmol/l), which has been shown to inhibit phosphatidic acid phosphatase, increased glucose-induced [Ca2+]i elevation. The suppression of insulin secretion by R59949 is therefore likely to be due to intracellular accumulation of DAG. We have also investigated mechanisms for the activation of DGKs by real-time imaging in MIN6 cells transfected with GFP-conjugated DGKγ. Phorbol 12-myristate 13-acetate, a DGK activator, quickly and irreversibly induced translocation of DGKγ to the plasma membrane (PM), which is an index of DGK activation, whereas high K+ slowly and reversibly translocated it to PM. However, the translocation was not observed when stimulated with high glucose. These results suggest that type I DGKs are present in β-cells and contribute to insulin secretion, possibly by changing intracellular lipid balance. Since DGKγ is activated by high K+, but not by glucose, type I DGKs other than DGKγ may be involved in glucose-induced insulin secretion.

Supported by KAKENHI (No.21070154).

105-LB
Type 2 Diabetic Subjects Are Highly Responsive to GLP-1 Induced Insulin Biosynthesis In Vivo
DANIEL T. STEIN, HARSHA JAYATILLAKE, BING NA ZHENG, GREG CRUIKSHANK, ROMAN HOVORKA, EDUARD ROGATSKY, BRONX, NY, Cambridge, United Kingdom

Insulin Biosynthesis is known to be upregulated in vitro and in vivo by glucose and GLP-1. It has been suggested that insulin biosynthesis may be defective in Type 2 Diabetes, but at present there are no data from free living human diabetic subjects. We have recently validated the use of deuterated leucine combined with Mass Isotopomer Distribution Analysis (MIDA) for the measurement of fractional insulin biosynthetic rate (FSR) by measuring the incorporation rate of d-leucine into newly synthesized pro-insulin C-peptide. Fractional Insulin biosynthetic rate was monitored in vivo using a primed constant infusion of 3H-leucine (1.2 mg/kg; 1.2 mg/kg/hr)

For author disclosure information, see page LB41.

ADA-Funded Research
into Type 2 Diabetic (DM) and age/BMI matched NGT controls (C) for 6-7 hrs under conditions of fasting glycemia (F), fasting glycemia + GLP-1 infusion (FGLP, glucose clamped at basal levels), or during hyperglycemia (HG, 250 mg/dl). DM subjects were admitted overnight for an insulin infusion to normalize fasting glucose prior to study start.

Incorporation of d-leucine into M1 and M2 C-peptide species was monitored by high resolution 2D LC-MS between 120 and 420 minutes (plasma), and peak enrichment (urine). Fractional insulin biosynthesis rate (FSR) was calculated by two isotopomer analysis of C-peptide (M1, M2).

Results: M1 and M2 leucine appearance lagged after initial d-leucine infusion by 60-120 minutes consistent with the known delay due to leucine tRNA synthesis, translation and processing within ER/golgi, proinsulin processing and vesicle transport for exocytosis. Hourly FSR, measured in plasma, was increased by hyperglycemia but not by GLP-1 infusion in control subjects (0.602 ± 0.077, 0.821 ± 0.080, 0.955 ± 0.109 pools/day; F vs FGLP vs HG, p<0.02 F vs HG; p<0.01 F vs FGLP; p<0.01 F vs HG; p=0.1 FGLP vs HG). In contrast, insulin biosynthesis in diabetic subjects was highly responsive to both hyperglycemia and basal GLP-1 infusion (0.696 ± 0.059, 1.656 ± 0.305, 1.110 ± 0.151; F vs FGLP vs HG; p<0.001 F vs FGLP; p<0.01 F vs HG; p=0.1 FGLP vs HG). We document for the first time that diabetic subjects strongly respond to GLP-1 as well as hyperglycemia to increase insulin biosynthesis rate.

**ISLET BIOLOGY—SIGNAL TRANSDUCTION**

**106-LB**

### The Autonomic Innervation of the Human Islet

**RAYNER RODRIGUEZ-DIAZ, DAMARIS MOLANO, CAMILLO RICORDI, ANTONELLO PILEGGI, PER-OLOF BERGSQUEIN, ALEJANDRO CACERES, Miami, FL**

The islets of Langerhans are innervated by the autonomic nervous system, but the abundance and organization of this innervation is highly variable between species. Whereas different fiber systems have been described for islets of different species using immunohistochemistry, it is not possible to obtain a coherent picture. Comparisons between species are not possible because common techniques or markers have not been used. There are few if any studies on the innervation of human pancreatic islets and since its cell composition and cytoarchitecture are distinct, it is very likely that its innervation patterns also differ. We have examined islets for the presence of parasympathetic markers (ChAT, vAChT), sympathetic markers (TH, vMAT), sensory fiber markers (CGRP, substance P), and axonal markers (Neurofilament 200, PGP 9.5, synapsin). Mouse islets were densely innervated by sympathetic fibers in the periphery and by parasympathetic fibers in the core. In human islets, by contrast, autonomic fibers did not penetrate the islet parenchyma but ran along blood vessels. Very few sympathetic varicosities were seen closely apposed to human alpha cells. Instead, sympathetic fibers innervated vascular myocytes of blood vessels inside the human islet. Our results indicate that endocrine cells of the mouse islet are directly innervated by the parasympathetic and sympathetic components of the autonomic nervous system. In human islets, the autonomic fibers may exert its effects on islet function indirectly by acting on vascular cells and altering blood flow.

**107-LB**

### Effects of Oral Magnesium Supplementation on Metabolic Biomarkers and Global Gene Expression and Proteomic Profiling among Overweight Individuals: A Randomized, Controlled, Cross-over Trial

**SARA CHACKO*, JAMES SUL*, YIQING SONG, XINMIN LI, JAMES LEBLANC, YUKO YOU, SIMIN LIU, Los Angeles, CA, Boston, MA**

Dietary magnesium (Mg) intake has been favorably associated with reduced risk of metabolic outcomes in observational settings; however, few randomized trials have introduced systems-biology approaches to explore the molecular mechanisms of placitropic metabolic actions of Mg supplementation. We conducted a randomized crossover pilot trial to examine the effects of oral Mg treatment on metabolic biomarkers and global genomic and proteomic profiling among overweight individuals.

Fourteen healthy and overweight volunteers (BMI ≥ 25kg/m2) were randomly assigned to receive Mg citrate (500 mg elemental Mg daily) or placebo for 4 weeks. After a 1 month washout period, participants were assigned to the opposite treatment for 4 weeks. Fasting blood and urine specimens were collected at each visit according to a standardized protocol. Biochemical assays were conducted on all blood specimens. RNA was extracted and subsequently hybridized using HumanGene ST 1.0 array. Urine proteomic profiling was analyzed using the CM10 ProteinChip array. Differences in biomarkers were compared using the Wilcox signed-rank test.

We observed that Mg treatment appeared to decrease fasting levels of insulin (Change: -2.1 mcU/mL after Mg vs. +0.9 mcU/mL after placebo; p=0.26) and C-peptide (-0.4 ng/mL vs. +0.1 ng/mL; p=0.004). Our analyses of gene expression profiling revealed down regulation of genes related to metabolic and inflammatory pathways including C1q and tumor necrosis factor related protein 9 (C1qtnf9) and pro-platelet basic protein (PPBP) but up regulation of genes related to active transport channels (TRPM6 and TRPM7). Results of urine proteomic profiling showed a number of peptides/proteins significantly differentially expressed in response to Mg.

In conclusion, our preliminary data suggest that Mg supplementation for 4 weeks among overweight individuals led to distinct changes in gene expression and proteomic profiling consistent with metabolic pathways. Future long-term, large-scale randomized trials are warranted to confirm these metabolic effects of Mg supplementation.

*Authors Sara Chacko and James Sul contributed equally

Supports by General Mills Health and Nutrition (Grant No. 20060222) and Burroughs Wellcome Fund Inter-school Training Program in Metabolic Diseases.

**109-LB**

### Nutrition—Clinical

#### Effect of High Protein or High Carbohydrate Diet on Markers of Oxidative Stress and Proinflammatory Cytokines in Obese Non-Diabetic Premenopausal Women

**ABBAS E. KITABCHI, MERCHANT L. HUTSON, FRANCES A. TYLAVSKY, FRANKIE B. STENTZ, JIM Y. WAN, EBENEZER A. NYENWE, CHRISTOPHER W. SANDS, Memphis, TN**

The health hazard of obesity and its increased prevalence are well established. Diet and exercise are important venues of therapy for obesity. A palatable diet which can provide satiety and essential nutrients capable of long term adherence are important aspects of diet therapy.

**NUTRITION—CLINICAL**

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The health hazard of obesity and its increased prevalence are well established. Diet and exercise are important venues of therapy for obesity. A palatable diet which can provide satiety and essential nutrients capable of long term adherence are important aspects of diet therapy.

We hypothesized that moderately high protein (30% protein, 40% CHO, 30% fat) diet may cause less excursion of glucose, decrease in oxidative stress, lipid peroxidation and inflammatory cytokines than high CHO diet (15% protein, 55% CHO, 30% fat). This could occur with similar degrees of weight loss in a group of obese non-diabetic premenopausal women on a six month diet therapy. Foods (prepared with reduced intake of 500 Kcal/day) were supplied to the subjects on a weekly basis for enhanced diet compliance. The glucose area under curve for OGGT (AUC), proinflammatory cytokines (TNFα, IL6), marker of oxidative stress (dichlorofluorescin – DCF), lipid peroxidation (malondialdehyde – MDA), CRP and BP were measured at baseline and 6 months of diet therapy.

Parameters | HP | HC | p-value after treatment HP vs HC
--- | --- | --- | ---
Weightloss (lbs) | 2.14 ± 4.6 | 21.1 ± 3.7 | 0.16
BP (sys) change | -10 mm | -8 mm | NS
CRP (mg/L) change | -17.7 ± 2.1 | -13.6 ± 3.8 | 1.0
TNFa (pg/ml) change | -0.92 ± 2.4 | -0.12 ± 0.36 | 0.69
IL-6 (pg/ml) change | -0.42 ± 0.80 | -0.43 ± 0.96 | 0.52
DCF (uM) change | -0.68 P=0.01 | -0.34 P=0.02 | 0.04
MDA (uM) change | -0.40 P=0.02 | -0.22 P=0.03 | 0.038
AUC % Reduction | 12.1 ± 1.8 | 7.8 ± 2 | 0.03

Although HP and HC diets resulted in weight loss and improvement of CRP and TNFα, these changes were not significantly different in the two diets. However, changes in DCF, MDA and reduction in glycemic excursions showed significant improvement in HP versus HC. **ADA-Funded Research**
**110-LB**

**Efficacy and Safety of a Dietary Supplement (SAI) in a Randomized, Double-Blind Placebo Controlled Studies in 3 Centers**

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The primary objective of this study is to evaluate the efficacy and safety of SAI compared to that of a placebo with Type 2 diabetic subjects using two randomized double-blind, placebo controlled multicenter studies.

SAI is a dietary supplement formulated with bioactive peptides from soy and whey specifically developed to lower Hba1c levels of Type 2 diabetic patients. These bioactive peptides are fortified with synergistic minerals, antioxidants, vitamins, cinnamon extracts, insulinotropic amino acids/peptides, soluble and insoluble fiber and then formulated with milk protein and organic soy milk.

Subjects were screened using the study design protocol approved by IRB. The Type 2 diabetic subjects were treated for 12 weeks with two doses of either SAI or placebo which were taken every day with morning breakfast and evening meals.

The analysis of covariance (ANCOVA) model on the change from baseline values of Hba1c, fasting blood glucose levels, body weight, BMI, lipid panel, blood pressure and kidney function tests with baseline value and age as covariates was used for assessing statistically significant difference between SAI and placebo. Statistically significant reduction in Hba1c (+1.62) and fasting blood glucose levels (+38.9) were observed in the patients treated with SAI dietary supplement compared to placebo. Also, reduction is seen in body weight, BMI, microalbumin and increase in HDL in patients treated with SAI.

**Analysis of Change From Baseline of Hba1c and Fasting Blood Sugar with 95% Confidence Interval**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Estimate of Placebo Mean (N=30)</th>
<th>Estimate of SAI Mean (N=29)</th>
<th>Placebo vs SAI Difference</th>
<th>95% Confidence Interval</th>
<th>SEpValue</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hba1c</td>
<td>-0.733</td>
<td>-1.621</td>
<td>-0.89</td>
<td>0.037</td>
<td>-0.20</td>
<td>-1.632 -0.144</td>
</tr>
<tr>
<td>Fasting Blood Glucose</td>
<td>-5.389</td>
<td>-38.956</td>
<td>-33.57</td>
<td>15.81</td>
<td>0.039</td>
<td>45.381 -1.753</td>
</tr>
</tbody>
</table>

# P-values based on the t tests given by ancova model analysis.

**OBSERVATION—ANIMAL**

**111-LB**

**Altered Cross-Talk between Perivascular Adipose Tissue and Resistance Arteries in Muscle Blunts Insulin-Mediated Vasodilation in db/db Mice**


Obesity is associated with insulin resistance and vascular dysfunction. We have previously hypothesized that accumulation of Perivascular Adipose Tissue in muscle (mPAT) causes insulin resistance in obesity by reducing insulin-mediated vasodilation. In the present study, we studied whether mPAT controls insulin-mediated vasodilation in muscle and the role of adiponectin (Adn) in this interaction.

mPAT morphology and function were studied in gracilis muscles of lean C57/B16 (control) and 10 week-old db/db mice, a mouse model of obesity and insulin resistance. In db/db mice, we found a massive increase of mPAT (0.021±0.006 mg vs. 0.723±0.06 mg, P<0.01). To study functional properties of mPAT, responses to insulin of arterioles isolated from control mice were studied in the pressure myograph. In the presence of mPAT isolated from control mice, insulin induced vasodilation (66±29 percent at 2 nM of insulin). Pretreatment with a soluble fragment of the adiponectin R1 receptor abolished this vasodilator effect (to -7±4 percent at 2 nM, P=0.05 vs. control mPAT).

In contrast to mPAT of control mice, mPAT from db/db mice failed to induce insulin-mediated vasodilation (-4±3 percent at 2 nM, P=0.05 vs. control mPAT).

This impairment was associated with a reduced insulin-mediated activation of Akt, a critical mediator of insulin’s vasodilator effects. To study whether the blunted vasodilator effect of insulin by db/db mPAT is caused by reduced Adn secretion, we measured Adn concentrations by ELISA in mPAT-conditioned medium. Indeed, secretion of Adn was markedly decreased in db/db mPAT (178±33 vs. 52±151 ng/mL, P=0.02).

In conclusion, perivascular adipose tissue in muscle controls insulin-mediated vasodilatation in muscle arteries by secreting adiponectin. This effect is impaired in obese db/db mice, leading to impaired insulin-mediated vasodilatation. Our findings provide a novel mechanism underlying obesity-related vascular dysfunction and insulin resistance.

Supported by grants from the Netherlands Organisation for Scientific Research (E.C.E), grant number: 460026633, and the Netherlands Heart Foundation (E.H.S.), grant number: 2009BO98.

**112-LB**

**Epac1⁻/⁻ Mice Are More Susceptible to High-Fat Diet Induced Obesity and Insulin Resistance**

ALAIN K. L. KAI, ANDREW C. P. TAI, XINMEI ZHANG, ANGELA K. W. LAI, AIMIN XI, KAREN S. LAM, PAUL M. VANHOUTTE, STEPHEN S. CHUNG, SOOKJA K. CHUNG, Hong Kong, Hong Kong

Signaling pathways mediated via the second messenger cAMP play a critical role in potentiating glucose-stimulated insulin secretion in β-cells and development of obesity. In addition to PKA, two isoforms of cAMP-binding proteins designated as exchange protein directly activated by cAMP (Epac1 and Epac2) were identified to be direct effectors of cAMP. Previously, we reported that deletion of the Epac1 gene in vivo impairs glucose tolerance and also reduces the glucose-stimulated insulin levels in mice under regular diet (RD) condition. To investigate the roles of Epac1 in development of obesity and insulin resistance, Epac1⁻/⁻ mice were treated with high-fat diet (HFD) either 4 weeks of age or 12 weeks. During the high-fat diet feeding, obesity was evident 8 weeks after and Epac1⁻/⁻ mice gained significant more weight than age-matched Epac1⁺/⁺ mice. During the OGTT challenge, severe impairment of glucose tolerance was observed in HFD-fed Epac1⁻/⁻ mice. Furthermore, we identified that HFD-fed Epac1⁻/⁻ mice are not responsive to exogenous insulin during the IPITT test, suggesting that the severe impairment of glucose tolerance in these mice is due to the insulin resistance. Indirect calorimetry analysis revealed that the Epac1⁻/⁻ mice displayed significant increases in respiration exchange rates even under RD feeding, implicating an impairment of lipid utilization in the absence of Epac1. Histological examination of interscapular brown adipose tissue (BAT) in the RD-fed Epac1⁻/⁻ and Epac1⁻/⁻ mice revealed no obvious difference. In comparison with HFD-fed Epac1⁻/⁻ mice, BAT in the HFD-fed Epac1⁻/⁻ mice lost the typical morphology and demonstrated a markedly increased size of lipid droplets. Taken together, our results indicate that Epac1 deletion enhances susceptibility to high-fat diet-induced obesity and its associated metabolic dysregulation, implicating a functional role of Epac1 as a causative factor of metabolic disease.

**113-LB**

**Leptin Action Via NOS1/Leprb-Expressing Neurons Controls Food Intake and Energy Balance**

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Leptin acts via its receptor (Leprb) on CNS neurons to signal the repletion of long-term energy stores and control energy balance and endocrine function. While leptin action via the hypothalamic arcuate nucleus (ARC) has garnered much attention, the majority of Leprb neurons lie elsewhere in the CNS and the disruption of Lepreb expression in major ARC populations alters energy balance only modestly. The number of Leprb neurons in the sexually dimorphic hypothalamic ventral premammillary nucleus (PMV) is comparable to that in the ARC (~15% of total CNS Leprb neurons). We found that most PMV Leprb neurons express neuronal nitric oxide synthase (NOS1), although few Leprb neurons outside of the PMV contain NOS1. We thus generated Nos1⁻/⁻ mice for use in combination with Lepr to produce KOxOS1 animals, deleting Leprb specifically in Leprb/NOS1 cells. To understand the role for leptin action via Leprb/NOS1 neurons, we compared the phenotype of KOxOS1 animals to control and KOxTotal animals (devoid of Leprb throughout the body). While both male and female KOxOS1 animals displayed hypophagia, decreased energy expenditure, and obesity, the phenotype in females was intermediate between control and KOxTotal animals, while KOxOS1 males were not different from KOxTotal mice. Additionally, KOxTotal females maintained normal glucose homeostasis (unlike KOxTotal females), while the hyperglycemia of KOxOS1 and KOxTotal males was similar. KOxOS1 animals also demonstrate substantial impairments in neuroendocrine function. Thus, NOS1/Leprb neurons represent major mediators of leptin action.

For author disclosure information, see page LB41.
Loss of Akt1 Increases Energy Expenditure and Prevents Diet-Induced Obesity in Mice
MIN WAN, RACHAEL EASTON, MORRIS BIRNBAUM, Philadelphia, PA

The serine-threonine kinase Akt, also known as PKB, mediates growth and metabolic signals downstream of the insulin signaling pathway. Three Akt isoforms, Akt1, Akt2 and Akt3, are encoded by distinct genes, and single deletions of these Akt isoforms in mice have shown distinct phenotypes by our laboratory. Loss of Akt1 in mice leads to growth retardation in a cell autonomous manner without altering the serum glucose and insulin levels; loss of Akt2 in mice causes diabetic phenotypes; while Akt3 knockout mice have reduced brain size. Here we show that mice without Akt1 are smaller, have enhanced O2 consumption and CO2 production, but unaltered food intake or activity compared to wildtype controls. When challenged with high fat diet feeding, Akt1 knockout mice were protected from diet-induced obesity, and had improved insulin sensitivity. Akt1 knockout mice also showed increased energy expenditure when comparing with weight-matched wildtype controls. To exclude the possibility of another locus tightly linked to Akt1 contributes to the phenotypes, we generated an independent Akt1 knockout line, and showed similar phenotypes. Our data also suggested that deletion of Akt1 results in an upregulation of UCP3 and PDK4 in skeletal muscle, which likely leads to increase mitochondrial uncoupling and fatty-acid oxidation. Together these findings reveal a novel role of Akt1 in energy homeostasis.

Fig. 1. Akt1 -/- showed increased energy expenditure
(A) Young Akt1 -/- mice have decreased body weight. (B) Young Akt1 -/- mice increased VO2 consumption and VC02 production, without altering RER and Activity. N=10 for each group. (C) Weight matched Akt1 -/- mice show slightly increased fat mass percentage. (D) Weight matched Akt1 -/- mice have increased energy expenditure during dark cycle. N=5-6 for each group (All mice are males in C57BL6 background, data are expressed as mean±sem, *, P<0.05; **, P<0.01 by one-way ANOVA or student t test.)

Fig. 2. Akt1 -/- are protected from diet-induced obesity
(A) Young Akt1 -/- have smaller body weight after 22-week normal chow or high-fat-diet feeding, however, on high-fat-diet, Akt1 -/- has significantly reduced fat percentage comparing with wild type controls. (B) Akt1 -/- show increased energy expenditure on both diets. N=8-10 for each group. (C) Akt1 -/- have improved glucose tolerance. N=4 for each group, 1g/kg BW glucose IP injected after overnight fasting, mice were on high-fat-diet for 18 weeks. (D) Akt1 -/- mice show improved insulin sensitivity by euglycemic-hyperinsulinemic clamp. N=3 for each group, 22 weeks on high-fat-diet. (E) Gene expression of skeletal muscle from young Akt1 -/- mice. Mice were 8-10 weeks old, n=10 for each group. (All mice are males in C57BL6 background, data are expressed as mean±sem, *, P<0.05; **, P<0.01 by one-way ANOVA or student t test.)

TRPV1 Mediates Discordant Effects on the Regulation of Fat Mass and Glucose Metabolism
GARY A. WITTERT, PAUL CAVUOTO, GEORGE HATZINIKOLAS, L. ASHLEY BLACKSHAW, Adelaide, Australia

In addition to a central mechanism of action, endocannabinoids regulate fat mass and glucose metabolism via peripheral effects considered to be mediated predominantly by the cannabinoid receptor type 1 (CB1). Endocannabinoids, for example, 2-arachidonoylglycerol (2-AG), are also ligands for the transient receptor potential vanilloid sub-family member 1 (TRPV1). TRPV1-/- mice are resistant to high fat (HF) diet-induced obesity. We have previously shown that TRPV1 is expressed in skeletal muscle, and have also found that this occurs to a greater extent in soleus than extensor digitorum longus muscle. We aimed to determine TRPV1 mediated effects on peripheral glucose metabolism.

In rodent (LB) skeletal muscle myotubes, chronic (24 h), but not acute (30 min) exposure to 2-AG (6 mM and 15 mM, biphasic dose response curve) increased insulin-stimulated glucose uptake by ~25%, an effect partially reversed by the TRPV1 specific antagonist, SB366791 [0.5 mM and 5 mM; P<0.01]. In TRPV1-/- mice, intraperitoneally injected glucose (2 mg/g body weight) was cleared more rapidly than in wild-type (WT) mice (P=0.023), although body weights were similar. After 18 weeks HF feeding, cumulative weight gain was 18 ± 2 g and 11 ± 1 g (P<0.001) in the WT and TRPV1-/- mice, respectively, and was similar in HF fed TRPV1-/- and standard chow fed WT mice. In contrast, glucose tolerance was similarly impaired in HF fed TRPV1-/- and WT mice in comparison to standard chow fed WT mice (P<0.05). TRPV1 plays a role in endocannabinoid modulation of insulin stimulated glucose uptake in skeletal muscle. In the absence of TRPV1, there is protection from HF diet induced obesity, but not impaired glucose tolerance, an observation that has implications for the potential therapeutic use of TRPV1 antagonists.

For author disclosure information, see page LB41.
A Catechin-Rich Beverage with No Caffeine Ameliorates Body Fat and Circulating High-Molecular Weight Adiponectin (HMW-Ad) in Overweight/Obese Men

MASAO TAKESHITA, HIDETO TAKASE, KAZUHIRO OTSUKA, MITSUHIRO KATASHIMA, KOICHI YASUNAGA, TAKESHI YASUMASU, Tokyo, Japan

Adiponectin, an adipokine secreted exclusively by adipocytes, plays a key role in the regulation of fat and glucose metabolism. Increasing attention has focused on HMW-Ad, which is the most active form of this hormone. The HMW-Ad to total adiponectin ratio (HMWR) is also the best predictor of the development of insulin resistance and type-2 diabetes. Accumulating evidence in humans indicates that tea, especially green tea extract (GTE) of the development of insulin resistance and type-2 diabetes. Accumulating evidence in humans indicates that tea, especially green tea extract (GTE) influenced by MBG-independent factors measured by HGI. Skin fluorescence associated with HbA1c, we hypothesized that skin AGE levels might also be influenced by MBG-independent factors measured by HGI. Skin fluorescence is a known surrogate of skin AGE levels. In this experiment, we examined relationships between skin fluorescence, HbA1c, HGI and MBG.

We recruited 54 children (27 males) with type 1 diabetes, age 14±4 years, duration of diabetes 7±4 years, race (35 Caucasian, 19 African American) from the diabetes clinic at Children’s Hospital of New Orleans. Skin AGEs were non-invasively assessed on the volar surface of the left forearm using a SCOUT DS system (Veralight, Albuquerque, NM) that measures skin fluorescence corrected for skin optical properties (skin intrinsic fluorescence=SIF). MBG was derived from patient self monitored blood glucose meter data. HbA1c was measured at the same clinic visit by a National Glycohemoglobin Standardization Program certified immunoassay. HGI was calculated from HbA1c and MBG for each patient as previously described. The associations of HGI, HbA1c and MBG adjusted for patient age, gender, race, and duration of diabetes were analyzed in various linear regression models with SIF as the dependent variable.

Multivariate analyses showed that HbA1c, HGI, gender and age were significantly (p<0.01) associated with SIF. Although MBG had a significant positive correlation with HbA1c (r=0.44, p<0.001), it was not a statistically important independent variable associated with SIF. These results suggest that MBG-independent factors measured by HGI have an important influence on glycation of both hemoglobin and skin proteins associated with risk for diabetes complications.

ADA-Funded Research

The Feasibility of Detecting Neurocognitive and Neuroanatomical Effects of Type 1 Diabetes Mellitus in Young Children

TANDY AVE, SHELLI KESLER, SHERRY HIBANG, DARRELL M. WILSON, ALLAN L. REISS, BRUCE A. BUCKINGHAM, Stanford, CA

Type 1 diabetes mellitus (T1DM) impacts the developing brain and both hypoglycemia and hyperglycemia have been associated with neurocognitive and neuroanatomical changes. Young children with T1DM have wide excursions in blood glucose during a period when the brain is undergoing dynamic changes including myelination and repair of synapses. Therefore, we hypothesized that frequent exposure to hypoglycemia and hyperglycemia during early childhood may potentially lead to neurocognitive deficits and changes in brain anatomy. Young children, ages 3 to less than 10 years, with T1DM and age, gender and socioeconomic matched healthy controls (HC) completed age appropriate batteries of neuropsychological (NP) tests and non-sedated MRI scans of the brain. Ninety-eight and 93% of the children successfully completed NP testing and MRI scanning respectively. In our cohort of 28 children with T1DM and 17 HC, we found no differences in the mean full scale IQ score between subjects with T1DM (109.4 ± 13.1) and HC (115.8 ± 14.1). There were no statistically significant differences in general intellect, executive function, memory/attention, motor, and visual-spatial domains between subjects with T1DM and HC. The MRI findings showed similar grey matter (GM) (662 ± 101 mm³ vs 838 ± 95 mm³), white matter (WM: 377 ± 63 mm³ vs 370 ± 57 mm³) and hippocampal (HP) (6 ± 0.8 mm³ vs 6.1 ± 0.8 mm³) volumes in T1DM and HC respectively. However, after controlling for age and gender, we detected a significant diagnosis by age interaction (p=0.008) such that WM volume was progressively more reduced in older children with T1DM, in contrast to HC who showed the expected normal increase in WM volume with age. A similar trend was detected for HP volume (diagnosis x age: p=0.07). We also noted that those T1DM children who had experienced seizures showed significantly reduced (p=0.049) GM and WM volumes relative to children with T1DM who had not experienced seizures. We show that it is feasible to perform MRI and NP testing in young children with T1DM and that early signs of neuroanatomical variation may be present in this population.

PEDIATRICS

Hemoglobin Glycation Index Is Positively Associated with Skin Intrinsic Fluorescence

DANIA L. FELIPE, SHUDIAN LIU, JAMES M. HEMPE, NATHANIEL I. MATTER, JOHN D. MAYNARD, STUART A. CHALEW, New Orleans, LA, Albuquerque, NM

Our group previously reported that HbA1c levels are a function of mean blood glucose (MBG) and MBG-independent factors. The effect of MBG-independent factors on HbA1c can be quantified by the Hemoglobin Glycation Index (HGI) which is positively associated with risk for diabetes complications. As skin advanced glycation end products (AGE) are positively influenced by MBG-independent factors measured by HGI. Skin fluorescence is a known surrogate of skin AGE levels. In this experiment, we examined relationships between skin fluorescence, HbA1c, HGI and MBG.

We recruited 54 children (27 males) with type 1 diabetes, age 14±4 years, duration of diabetes 7±4 years, race (35 Caucasian, 19 African American) from the diabetes clinic at Children’s Hospital of New Orleans. Skin AGEs were non-invasively assessed on the volar surface of the left forearm using a SCOUT DS system (Veralight, Albuquerque, NM) that measures skin fluorescence corrected for skin optical properties (skin intrinsic fluorescence=SIF). MBG was derived from patient self monitored blood glucose meter data. HbA1c was measured at the same clinic visit by a National Glycohemoglobin Standardization Program certified immunoassay. HGI was calculated from HbA1c and MBG for each patient as previously described. The associations of HGI, HbA1c and MBG adjusted for patient age, gender, race, and duration of diabetes were analyzed in various linear regression models with SIF as the dependent variable.

Multivariate analyses showed that HbA1c, HGI, gender and age were significantly (p<0.01) associated with SIF. Although MBG had a significant positive correlation with HbA1c (r=0.44, p<0.001), it was not a statistically important independent variable associated with SIF. These results suggest that MBG-independent factors measured by HGI have an important influence on glycation of both hemoglobin and skin proteins associated with risk for diabetes complications.

ADA-Funded Research

Adiponectin Independently Associated with Atherogenic Lipoprotein Pattern in Adolescents

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The study objectives were to determine: 1. the relationship between adiponectin, and lipoprotein subclass concentration and particle size in obese and lean adolescents, and 2. whether this relationship is dependent on obesity and/or insulin resistance.

In this cross-sectional study of pubertal (Tanner >1) adolescents (12-17 yrs), obese (BMI 25±6%) nondiabetic subjects (N=74) were compared to lean controls (N=40). Lipoprotein subclass analysis was by NMR spectroscopy (N=43 Ob, N=32 L).

Obese and lean groups were similar in demographics and pubertal stage. Obese adolescents had decreased adiponectin and an atherogenic lipid pattern compared to lean subjects.
Continuous Subcutaneous Insulin Infusion Versus Multiple Daily Injections of Insulin in Pregnant Women with Type 1 Diabetes

SAADIA SKALLI, EMA FERREIRA, CAROLINE MORIN, KARINE TOUZIN, EVELYNE REY, NICOLE MICHON, Montreal, QC, Canada

Objective: To compare glycemic control and perinatal outcomes in type 1 diabetes pregnant women treated with two types of intensified insulin therapy, continuous subcutaneous insulin infusion (CSII) or multiple daily insulin injections (MDII).

Patients and methods: Retrospective and observational study including all pregnant women with type 1 diabetes treated at Centre Hospitalier Ste-Justine, Montreal, who delivered at ≥ 16 weeks between 2000 and 2009. Maternal characteristics, glycemic control, and perinatal outcomes were recorded.

Results: A total of 108 pregnancies in 79 pregnant women were evaluated: 44 were treated with CSII and 44 with MDII. Duration of diabetes was longer in CSII group than in MDII group (21.2±5.3 vs. 13.9±6.9 years) (p<0.0004) and diabetic complications were similar in both groups. There were no differences between the two groups in glycated haemoglobin level in the 1st, 2nd and 3rd trimester of pregnancy (CSII group: 6.9±0.2, 6.3±0.5, and 6.0±0.8; MDII group: 7.6±1.1, 6.7±1.1 and 6.6±0.9 respectively), as well as in the rate of caesarean section between studied groups. The rate of macrosomia was 71.4% and 42.6% in the CSII and MDII groups, respectively (p<0.05) with a mean birth weight percentile of 92±11 for the CSII group and 77±26 (p<0.05) in MDII group. Rate of gestational age, APGAR score, and stillbirth, were similar in both groups.

Conclusion: Continuous subcutaneous insulin infusion was associated with higher rate of macrosomia despite similar diabetic control at conception and diabetic complications.

Continuous Subcutaneous Insulin Infusion Versus Multiple Daily Insulin Injections in Pregnant Women with Type 1 Diabetes: A Randomized Controlled Trial

SAADIA SKALLI, EMA FERREIRA, CAROLINE MORIN, KARINE TOUZIN, EVELYNE REY, NICOLE MICHON, Montreal, QC, Canada

Objective: To compare glycemic control and perinatal outcomes in type 1 diabetes pregnant women treated with two types of intensified insulin therapy, continuous subcutaneous insulin infusion (CSII) or multiple daily insulin injections (MDII).

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Conclusion: Continuous subcutaneous insulin infusion was associated with higher rate of macrosomia despite similar diabetic control at conception and diabetic complications.

Toward a New Marker Assessing Fetal Metabolic Programming: The Fetal Glycated Hemoglobin

FELIX DUPONT, RENE GAGNON, CHRISTIANE AURAY-BLAIS, JEAN-LUC ARDILouce, Sherbrooke, QC, Canada

The concept of fetal metabolic programming (FMP), seen as playing an etiologic role in the development of cardiometabolic diseases in offspring of women with diabetes during pregnancy, is now accepted. However, no tool is available to evaluate its intensity. We hypothesized that glycated fetal hemoglobin (FHb) is a marker for FMP. FHb comprises 2 α- and 2 β-chains in adults versus 2 α- and 2 γ-chains (γ2 and Aγ variants) in fetuses. Routine determination of Ac measured at the NH2-terminal of the β-chain but does not allow determination of FHb. Our aim was to develop a method to measure glycation of FHb in adults and fetuses (GHS & GHb). We used electrospray ionization time-of-flight mass spectrometry (ESI-TOFMS-Waters): a) to identify α, β- and γ-chains by their molecular weight (MW); b) to determine glycation of the α-chain in AHb and FHb. Whole adult or cord blood were diluted in denaturing solvent, desalted and introduced in the ESI-TOFMS system. Mass spectra were thereafter processed to obtain MW for each chain and the percentage of glycation of the α-chains. In AHb, mean MW of the α- and β-chains were 15126.29±0.20 Da and 15867.11±0.21 Da, respectively. Correlation between Ac measured by approved NGSP/DCC method and glycation of α- and β-chains measured by our new method was nearly perfect (r=0.9997). Intra-assay glycation coefficients of variation (CVs) (n=5) for low Ac levels (<6%) and high Ac levels (≥8%) in adult blood were 2.7%/1.6% and 2.0%/1.0% for α-β-chains, respectively. Inter-assay glycation CVs (n=5 days) with low and high Ac, in adult blood were 2.9%/2.0% and 1.2%/2.0% for α-β chains, respectively. In cord blood obtained from normal pregnancy, the MW of γ-chains was 15895.06±0.10 Da for the γ2 variant and 15009.97±0.07 Da for the Aγ variant. α-chain glycation was 1.98±0.04%, with intra-assay CVs (n=5) of 2.1% and inter-assay CVs of 3.7% over 5 days. Our preliminary results show a strong Spearman correlation (r=0.97) between α-chain glycation in 9 neonates and mothers. To conclude, measurement of glycation of the Hb α-chain is now feasible using ESI-TOFMS. These data are promising and should allow further research on fetal physiology and FMP.

Supported by Fondation des Étoiles.

For author disclosure information, see page LB41.
Psycosocial—Behavioral Medicine

Diabetes, Depression, and Inflammation in Older Adults: Results from the Health, Aging, and Body Composition Study (Health ABC Study)

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Up-regulated levels of interleukin-6 (IL6), TNF-α (TNF), and C-reactive protein (CRP) are common to both type 2 diabetes (T2DM) and depression, yet inflammation as a possible biological link between these disorders has gone unexamined. This study tested the interaction between T2DM and depressed mood on IL6, TNF, and CRP. It was hypothesized that inflammation would be greatest among those with T2DM and depressed mood (DM+DEP), followed by T2DM (DM) or depressed mood (DEP) alone, followed by healthy controls (HC).

Baseline data were analyzed from 3014 adults, 70-79 years, participating in the HABC Study. Presence of T2DM was assessed per self-report, medication use, fasting glucose and/or glucose tolerance test results. Depressed mood was categorized using the Center for Epidemiologic Studies Depression scale (CES-D) with a cut score of ≥20.

Log-transformed IL6, TNF, and CRP were analyzed using analysis of covariance. After adjustment for race, gender, study site, percent body fat, smoking status, and heart and lung disease, the interaction between T2DM and depressed mood status on levels of IL6 (pg/mL) was significant, F(1, 2761)=5.3, p<.05. IL6 was significantly higher (p<.05) among those with DM+DEP compared to all other groups (4.4±2.9 versus 2.7±2.0 [DM], 2.6±2.2 [DEP], & 2.3±1.9 [HC]). The interaction between T2DM and depressed mood status on levels of CRP (mg/L) was also significant after adjustment for percent body fat, triglycerides, race, gender, smoking status, and lung disease, F(1, 2875)=5.5, p<.05. CRP was significantly higher among those with DM+DEP (5.3±4.8) compared to DEP (2.9±4.2, p<.05) or HC (2.8±4.5, p<.05) groups.

Difference in levels of CRP between those with DM+DEP (5.3±4.8) and DM (3.6±5.5, p=.07) approached significance. A graded relationship was not observed for TNF after adjustment for covariates.

These findings support an additive model of inflammation linking T2DM and depressed mood, with a stringent cut score of self-reported depressive symptoms. Further investigation into these relationships could aid in understanding the biological pathways underlying the relationship between T2DM and depression.