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69TH SCIENTIFIC SESSIONS
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CLINICAL THERAPEUTICS/NEW TECHNOLOGY— GLUCOSE MONITORING AND SENSING

1-LB

Accuracy of a Novel Intravascular Fluorescent Continuous Glucose Sensor

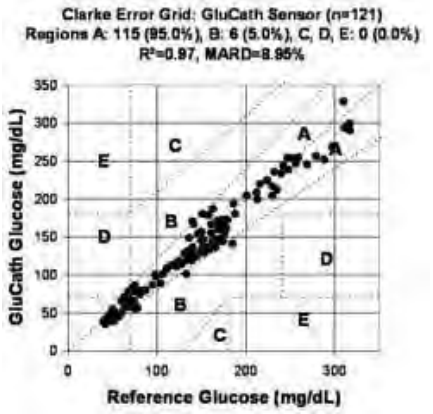
HOWARD ZISSER, LOIS JOVANOVIC, UZMA KHAN, TOM PEYSER, SOYA GAMSEY, MATT ROMEY, HELENE SPENCER, *Santa Barbara, CA, Irvine, CA*

The results of the Nice-Sugar study have cast doubt on the optimal target range for tight glycemic control (TGC) in ICU patients. A continuous glucose sensor (CGM), accurate in the hypoglycemic range, has been cited as a mitigation for future studies of TGC in the critically-ill. A novel fluorescent intravascular CGM, the GluCath(GluMetrics, Irvine, CA) was evaluated in an 8-hour outpatient feasibility study. The sensor was placed in a peripheral vein at the antecubital fossa in five subjects with type 1 diabetes. One sensor failed during insertion due to mechanical damage. Data from the other four sensors were taken at one-minute intervals and compared with hospital and laboratory blood glucose measurements from venous samples in the contralateral arm every 15 minutes.

The sensor is based on quenched-fluorescence using a modified boronic-acid glucose receptor. In contrast to electrochemical enzymatic sensors, the fluorescent sensor exhibits a nonlinear modulation response function giving the highest level of accuracy in the hypoglycemic range. This type of sensor is oxygen independent.

The sensor response was in compliance with the performance standard in ISO-15197: 100% (30/30) of all values < 75 mg/dL were within ±15 mg/dL of the reference values and 94.5% (86/91) of all values > 75 mg/dL were within ±20% of the reference values. The mean absolute relative difference (MARD) was 8.95%. Clarke Error Grid analysis gave 95.0% (115/121) in the A zone & 5.0% (6/121) in the B zone.

Sensor insertion was performed using a 22 Ga needle and a retractable cannula. Data was analyzed retrospectively using a temperature-corrected simulated factory calibration with a one-point in vivo adjustment made within thirty minutes of the sensor insertion. There were no adverse events in the study.



2-LB

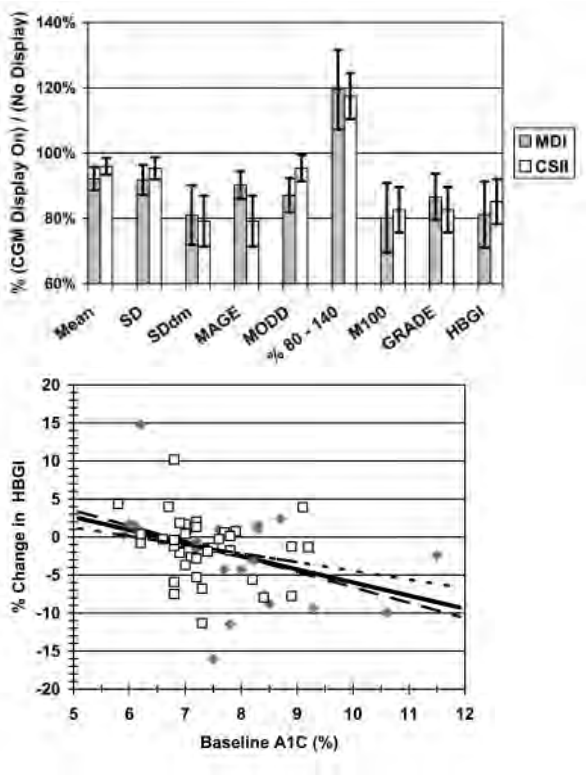
Impact of Continuous Glucose Monitoring (CGM) on Glycemic Variability and Control in Subjects with Type 1 Diabetes Using Multiple Daily Injections (MDI) vs Insulin Pump

DAVID RODBARD, LOIS JOVANOVIC, SATISH GARG, *Potomac, MD, Santa Barbara, CA, Aurora, CO*

CGM using DexCom SEVEN was studied in 64 patients with type 1 diabetes using MDI (26 patients) or continuous subcutaneous insulin infusion (CSII; 38 patients). CGM data was withheld during Week 1 and displayed during Weeks 2 and 3. Fifteen parameters improved in response to CGM display ($P<0.05$). The relative percentage of glucose values within the target range (80 – 140 mg/dL) increased by 19% and 17% compared to control for MDI and CSII, respectively. There were significant reductions in the mean glucose, overall SD, SD between daily means (SDdm), Mean Amplitude of Glycemic Excursion (MAGE), and Mean of Daily Differences (MODD), and improvements in quality of glycemic control (Schlichtkrull's M100, Hill's GRADE, and Hyperglycemia Index or Kovatchev's High Blood Glucose Index (HBGI) (Fig. 1). Changes were not significantly different between the MDI and CSII groups for any of the 15 criteria ($P > 0.05$), but were usually linearly related to baseline A1C (e.g. Fig. 2). We conclude that use of CGM with continuous display in subjects with type 1 diabetes results in rapid and significant improvements in 15 measures of quality of glucose control and glycemic variability; these responses are indistinguishable in subjects using MDI and CSII.

Fig. 1. Responses to display of CGM (± 1 sem) as % of control for 9 measures of Glycemic Variability and Quality of Control. MDI (shaded bars), CSII (open bars). % 80 – 140: glucose (mg/dL).

Fig. 2. % Change in HBGI in response to display of CGM vs baseline A1C. MDI = closed diamonds, dashed line; CSII = open squares, dotted line; MDI and CSII combined: solid line.



CLINICAL THERAPEUTICS/NEW TECHNOLOGY— INSULIN DELIVERY SYSTEMS

3-LB

Automated Insulin Delivery System Demonstrates Safe and Efficacious Control of Glycemia

MATTHEW W. PERCIVAL, BENYAMIN GROSMAN, EYAL DASSAU, HOWARD ZISSER, LOIS JOVANOVIC, FRANCIS J. DOYLE III, *Santa Barbara, CA*

We present clinical results of an automated subcutaneous insulin delivery system for subjects with diabetes that demonstrates safety, therapeutic efficacy, and minimal user intervention.

The insulin delivery system is comprised of a CSII pump (OmniPod, Insulet Corp) and a CGM (DexCom Seven) linked with artificial pancreas software. The insulin delivery algorithm is personalized and includes a novel formulation of an insulin-on-board (IOB) safety constraint based on clinical parameters; the control algorithm is implemented as a simple lookup table suitable for the next generation of CSII pumps. Closed-loop trials were performed on four subjects at the Schneider Children's Medical Center of Israel; the controller was initiated during a period of hyperglycemia and was challenged with unannounced meals.

The mean closed-loop trial duration was 5 hours (range 2-7 hours) and included a meal of 30 g carbohydrate (CHO). Considering all trials, the mean Low Blood Glucose Index was 0.02 (range 0-0.06), the mean High Blood Glucose Index was 9 (range 4.2-15), and the median Daily Risk Range was 'low' (range 'low' to 'moderate').

The figure below shows a typical trial result. The system automatically delivers insulin to correct for the induced hyperglycemia; the insulin infusion rate is reduced to avoid hypoglycemia and thus ensuring a smooth return to normoglycemia. After a meal is consumed, the sensor readings increase and a series of insulin deliveries above basal are made; glycemia normalizes several hours later, without any hypoglycemia.

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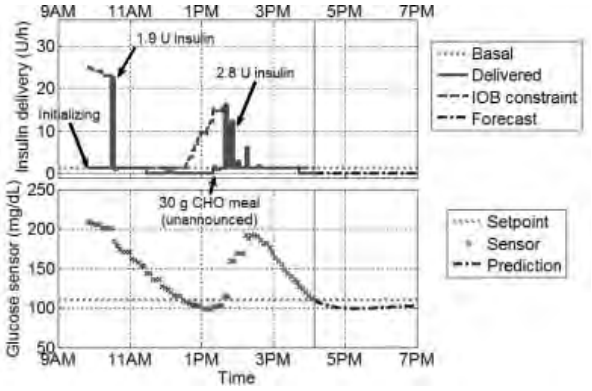
The proposed system restored normoglycemia without any outside intervention from both induced hyperglycemia and unannounced meals. The algorithm prevented excessive insulin dosing during hyperglycemia, and forced pump shut-off to prevent hypoglycemia.



Supported by JDRF 22-2007-1801

CLINICAL THERAPEUTICS/NEW TECHNOLOGY—OTHER DRUG DELIVERY SYSTEMS

4-LB

A Phase 1b Study of ITCA 650: Continuous Subcutaneous Delivery of Exenatide Via DUROS® Device Lowers Fasting and Postprandial Plasma Glucose

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Exenatide is a proven effective treatment for type 2 diabetes that improves glucose control and induces weight loss. However, use of exenatide has been limited due to the twice-daily self-injection schedule and frequent nausea which may be associated with peak concentrations of drug. The DUROS® technology is a subcutaneous continuous delivery system which has been utilized in the Viadur® device, an FDA-approved therapy for prostate cancer. It is an osmotic delivery device consisting of a small sterile titanium cylinder (4mm x 45 mm) that is placed subcutaneously for extended periods of time. ITCA 650 is a DUROS device engineered to deliver exenatide at a continuous and consistent rate for treatment durations of 3 to 12 months and over a broad range of dose levels. Preclinical studies have shown that ITCA 650 delivers exenatide at a consistent rate for >6 months with preserved stability of the drug product.

A phase 1b study to evaluate the safety and tolerability of ITCA 650 treatment is being conducted in subjects with inadequately controlled type 2 diabetes for 28 days. In this study, subjects were randomized to receive either 10 mcg/day or 20 mcg/day of exenatide.

Changes in fasting plasma glucose (FPG) in both groups are shown in the following table.

Changes in Fasting Plasma Glucose (FPG)

Dose Arm	Baseline FPG	FPG at 24 hours	FPG at 1 week	FPG at 2 weeks
10 mcg/day (n = 12)	161±34 mg/dL	146±30 mg/dL	143±33 mg/dL	139±36 mg/dL
20 mcg/day (n = 11)	170±23 mg/dL	145±33 mg/dL	139±28 mg/dL	127±15 mg/dL

In addition to changes in FPG, a decrease in the 2-hour postprandial glucose was also observed at two weeks. ITCA 650 was well tolerated primarily with observations of anticipated mild bruising, itching and local pain at the insertion site, as well as transient mild nausea and vomiting in a small number of the subjects. Based on the favorable tolerability observed at the studied doses, higher doses of ITCA 650 are currently under clinical evaluation.

ITCA 650 represents a novel method to deliver exenatide for extended periods of time with 100% compliance for the long-term treatment of type 2 diabetes.

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

5-LB

Analytical Characterization, Safety and Clinical Bioequivalence of Recombinant Human Insulin from Transgenic Plants

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This work demonstrates the quality, safety and pharmacological equivalence of recombinant human insulin produced from transgenic safflower plants. A cGMP process was developed for the purification of insulin expressed in transgenic seed. Physical-chemical, structural, and biological analysis confirmed the identity and functionality of the resulting product. The plant-made insulin (SBS-1000) was also shown to meet all compendial specifications and to be free of host-specific impurities. Repeat dose (28-day) toxicity studies comparing the effects of SBS-1000 with Humulin®R were performed in Fischer 344 rats and Cynomolgus monkeys. Parameters evaluated included clinical signs, injection site changes, tissue/organ pathology and toxicokinetics. Data from these studies indicated that SBS-1000 had a safety profile indistinguishable from Humulin®R. A 3-way crossover study in healthy volunteers (n=23) using a euglycemic clamp was conducted to compare the pharmacokinetics and pharmacodynamics of SBS-1000 with two reference insulins, Humulin®R and Humulin®S. Results from this study showed that the three insulins displayed similar adverse event profiles. Although an analytical error resulted in a reduced concentration in the formulated SBS-1000 (accounting for lower values for the point estimate ratios), the 90% CI of all primary endpoints fell within the 80-125% range required for bioequivalence to Humulin®R. Similar results were obtained with Humulin®S for all endpoints except GIR_{max}. This difference between SBS-1000 and Humulin®S was also observed between Humulin®R and Humulin®S and was exacerbated by the greater variability observed for this parameter. Together these results demonstrate the feasibility of our transgenic plant manufacturing platform for the production of pharmaceutical-quality human insulin.

Bioequivalence Data

	SBS-1000: Humulin R		SBS-1000: Humulin S		Humulin R: Humulin S	
Parameter	Ratio (%)	90% CI	Ratio (%)	90% CI	Ratio (%)	90% CI
Insulin AUC 0-8 hrs	91	87-95	90	85-95	99	95-103
GIR AUC 0-8 hrs	92	84-100	92	85-100	101	92-110
Insulin C max	94	87-102	86	80-93	92	84-100
GIR max	93	81-108	81	68-97	87	74-103

6-LB

DURATION-2: Exenatide Once Weekly Demonstrated Superior Glycemic Control and Weight Reduction Compared to Sitagliptin or Pioglitazone after 26 Weeks of Treatment

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The once weekly GLP-1 receptor agonist exenatide (EQW) has demonstrated sustained improvements in glycemic control and body weight through 2 years of treatment. DURATION-2, a randomized, double-blind, double-dummy, 26-week study, compared the efficacy, safety, and tolerability of weekly treatment with EQW (2 mg; n=160) to maximum daily doses of a DPP-4 inhibitor (sitagliptin 100 mg; n=166) or a TZD (pioglitazone 45 mg; n=165) in patients with type 2 diabetes on a stable metformin background (ITT N=491; A1C 8.5±1.1%, FPG 164±47 mg/dL, weight 88.0±20.1 kg). EQW produced a clinically and statistically superior reduction in A1C compared to both sitagliptin and pioglitazone that was first evident at Week 6. A significantly greater proportion of EQW patients achieved A1C targets of ≤7.0% and ≤6.5%. EQW resulted in significantly greater weight loss versus sitagliptin from Week 4 through Week 26; patients on pioglitazone gained weight (+5.1 kg weight difference vs. EQW at Week 26). Significant improvements from baseline in systolic BP, albumin:creatinine, hsCRP, BNP, and adiponectin were observed with EQW; improvements in hsCRP and adiponectin also occurred with sitagliptin and pioglitazone. All treatments were generally well tolerated, with over 80% of

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patients completing the trial. Nausea was transient and predominantly mild (24% EQW; 10% sitagliptin; 5% pioglitazone; 1 withdrawal due to nausea in each treatment arm). No major hypoglycemia was observed. Pancreatitis, including one case of necrotizing pancreatitis, occurred in 2 patients treated with pioglitazone; no EQW patients experienced pancreatitis. Once weekly treatment with exenatide was well tolerated and elicited superior improvements in glucose control and body weight compared to maximum daily doses of sitagliptin and pioglitazone on a background of metformin.

Week 26	Exenatide QW	Sitagliptin	Pioglitazone
Δ A1C (%)	-1.55±0.10	-0.92±0.10*	-1.23±0.10*
% to A1C ≤7.0 / ≤6.5%	66/43	42*/18*	56*/33*
Δ FPG (mg/dL)	-32±4	-16±4*	-27±4
Δ Body Weight (%)	-2.7±0.4	-0.9±0.4*	+3.2±0.4*

ITT LS mean±SE for A1C, FPG, weight; Evaluable (n=387) for % to A1C target; *P<0.05 vs EQW.

7-LB

Efficacy and Safety of the 11-β-HSD1 Inhibitor, INCB13739, Added to Metformin Therapy in Patients with Type 2 Diabetes

JULIO ROSENSTOCK, SALOMON BANARER, VIVIAN FONSECA, SILVIO INZUCCHI, GREGORY F. HOLLIS, ROBERT FLORES, RICHARD LEVY, BILL WILLIAMS, REID HUBER, *Dallas, TX, New Orleans, LA, New Haven, CT, Wilmington, DE*

INCB13739 (13739) is a selective inhibitor of 11-beta-hydroxysteroid dehydrogenase type 1 (11-β-HSD1) being developed for the treatment of type 2 diabetes (T2DM). This randomized, double-blind, placebo (PBO)-controlled trial assessed the efficacy and safety of 13739 in combination with metformin (MET) in patients with T2DM inadequately controlled by MET monotherapy. The primary endpoint was the change from baseline in HbA1c at week 12. After a 2 week PBO run-in period, patients (n=159; mean BMI=32.7. kg/m2; mean HbA1c=8.3%; mean duration of T2DM=6.8 yr) were assigned to receive 13739 at doses of 5 mg (n=27), 15 mg (26), 50 mg (24), 100 mg (27), 200 mg (27) or PBO (28) once-daily in addition to a stable MET regimen for 12 weeks. Mean changes from baseline in HbA1c were significantly ($p<0.01$) greater for 13739 100 mg (-0.6%) and 200 mg (-0.5%) vs. PBO (0.0%) at week 12. Although plasma lipids were well controlled at baseline, both the 100 mg and 200 mg doses trended downward with a significant ($p=0.03$) treatment effect observed for the 100 mg dose in LDL-cholesterol (LDL-C) (-16 and +3 mg/dL for 100 mg vs. PBO, respectively). The frequency and severity of adverse events following 13739 treatment were similar to those observed in subjects randomized to PBO. 13739 did not affect sex hormone levels or the aldosterone-renin axis. A dose dependent increase in morning plasma ACTH was reached by week 4 and then plateaued (mean = 48 pg/mL in the 200 mg group at week 12; normal range <63.3 pg/mL). Plasma DHEA-s increased in a dose-dependent manner in concert with ACTH, reaching a maximum at week 4 in the 200 mg group (mean = 198 ug/dL) which was within age and gender-based normal ranges. Plasma morning cortisol, late-night salivary cortisol, and response to Cortrosyn challenge were unchanged from baseline, indicating that the changes in ACTH likely reflect compensatory HPA axis activity to maintain normal circulating cortisol homeostasis. In summary, 13739 was very well tolerated and is the first 11-β-HSD1 inhibitor to demonstrate improvements in HbA1c and LDL-C in patients with T2DM.

8-LB

Evaluation of CV Risk in the Saxagliptin Clinical Trials

ROBERT WOLF, ROBERT FREDERICH, FRED FIEDOREK, MARK DONOVAN, ZHIYING XU, SUSAN HARRIS, ROLAND CHEN, *Princeton, NJ, Wilmington, DE*

Several recent diabetes, obesity, and lipid drug trials have had unexpected and unfavorable cardiovascular (CV) results and deaths. The saxagliptin (SAXA) phase 2b/3 program was designed to enroll a broad range of patients with diabetes, include a controlled, long-term safety extension phase, and allow glycemic rescue therapy to enhance patient retention. SAXA is a potent, selective dipeptidyl peptidase-4 (DPP-4) inhibitor, specifically designed for extended inhibition of the DPP-4 enzyme. For this analysis of the SAXA clinical data, the primary endpoint, major adverse cardiovascular events (MACE; stroke, myocardial infarction or CV death, analyzed post hoc) and acute cardiovascular events (ACE; acute, clinically significant events, including cardiac revascularization procedures) were identified using selected MedDRA Preferred Terms. CV events were analyzed in the most comprehensive available dataset: 8 randomized, double-blind, phase 2b/3 trials, which included 4607 patients (3206 randomized to SAXA 2.5, 5, or 10 mg; 150 randomized to SAXA 20, 40, or 100 mg; and 1251 randomized to placebo, metformin, or up-titrated glyburide). Overall exposure was 3758 patient-years on SAXA and 1293 patient-years on comparators. Within the SAXA population, 81% had at least 1 CV risk factor in addition to diabetes, with hypertension (52%), dyslipidemia (44%), or history of smoking (39%) the most common; 12% had known prior CV disease. Similar proportions were observed in the comparator group. Numbers of patients with events are shown in Table. The Cox proportional hazard ratio for MACE was 0.44 (95% CI: 0.24–0.82) and for ACE was 0.59 (95% CI: 0.35–1.00). A series of sensitivity analyses using related endpoints and alternative analytic methods produced consistent results. Based on a >5000 patient-year clinical trial experience, there was no evidence of increased CV risk with SAXA treatment–as monotherapy or in combination with other oral antidiabetic agents. These data raise the hypothesis of a cardioprotective effect of SAXA, which merits further study in a prospectively designed trial.

Table

Event Type, n (%)	SAXA, N=3356	Control, N=1251
ACE	38 (1.1)	23 (1.8)
MACE	23 (0.7)	18 (1.4)
All Death	10 (0.3)	12 (1.0)
CV Death	7 (0.2)	10 (0.8)

9-LB

GLP-1 Receptor Activation Modulates Pancreatic Mass and Gene Expression but Does Not Modify the Susceptibility to Experimental Pancreatitis in Mice

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Objective: Recent reports describe a putative association between use of the GLP-1R agonists exenatide and liraglutide and pancreatitis, however the effects, if any, of these agents on the exocrine pancreas, are poorly understood.

Research Design & Methods: We assessed the effects of exendin-4, sitagliptin or liraglutide on genes associated with the development of experimental pancreatitis in the murine pancreas. The consequences of exendin-4, either administered prior to or following the initiation of caerulein-induced experimental pancreatitis was determined. The importance of endogenous GLP-1R signaling for the development of pancreatitis was assessed in Glp1r-/- mice.

Results: Acute administration of exendin-4 increased the expression of egr-1 and c-fos in the exocrine pancreas. Treatment of mice with exendin-4 or liraglutide for 1 week increased pancreas weight, and induced the expression of mRNA transcripts encoding the anti-inflammatory proteins PAP (RegIIIb) and RegIIIa. These actions required a functional GLP-1 receptor as they were abolished in Glp1r-/- mice. Chronic exendin-4 treatment of high fat fed mice increased expression of PAP and reduced pancreatic expression of mRNA transcripts for proinflammatory proteins such as MCP-1, TNFa and STAT3. Exendin-4 administration did not modify the expression of genes associated with pancreatitis. Repeated GLP-1R activation with exendin-4, or complete loss of GLP-1R signaling in Glp1r-/- mice, did not modify the severity of caerulein-induced experimental pancreatitis.

Conclusions: These findings demonstrate that GLP-1 receptor activation increases pancreatic mass and modulates the expression of genes associated with the development of pancreatitis, however activation or genetic elimination of GLP-1R signaling does not modify the severity of experimental pancreatitis in mice

10-LB

Pancreatitis in Patients Treated with Exenatide or Sitagliptin

VIVIAN HERRERA, RON AUBERT, LORRAINE TULLY, ZHULIANG TAO, GLEN STETTIN, INDERPAL BHANDARI, MERRI PENDERGRASS, *Franklin Lakes, NJ*

Although cases of pancreatitis have been reported in association with exenatide treatment, it is unknown whether exenatide plays a causative role in the association. It is difficult to assess this question statistically, even with a very large database, because pancreatitis is such a rare event. We performed a descriptive study to provide information that may be helpful to clinicians assessing potential risks and benefits of incretin-based antihyperglycemic therapy. We report on the incidence of pancreatitis in diabetic patients treated with an oral hypoglycemic agent regimen that included exenatide, sitagliptin, or no incretin-based antihyperglycemic therapy. No demographics or chronic conditions were controlled for due to a small number of cases of acute pancreatitis.

We analyzed a research database of integrated pharmacy and medical claims that is comprised of de-identified medical and pharmacy claims data

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for 13 million individuals. The study included non-insulin users aged 18-63 yrs with continuous enrollment with a pharmacy benefit manager from 3/1/06–8/31/2008 and at least 1 ICD-9 code 250. Subjects with at least 2 pharmacy claims for exenatide (EXN), sitagliptin (SIT) or an oral hypoglycemic regimen that did not include exenatide or sitagliptin (control) were identified. Subjects were excluded if they had any claims suggesting pancreatic disease, alcohol abuse, or hepatitis (5% EXN, 5% SIT, 4% control) or fibrate treatment (18% EXN, 21% SIT, 13% control) in the 6 months prior to cohort entry. Following these exclusions, there were 9,260 patients in the EXN group, (mean age 53±7 yrs, 59% female), 2,143 patients in the SIT group (mean age 54±7 yrs, 44% female), and 112,218 patients in the control group (age 54±7 yrs, 47% female). All patients were followed for 540 days. An initial episode of acute pancreatitis was identified in 41 (0.44%) EXN, 6 (0.28%) SIT, and 438 (0.39%) of control patients.

Although these data are only descriptive, they suggest the incidence of pancreatitis in all groups is extremely rare. Furthermore, these data do not suggest the risk is increased in exenatide or sitagliptin users compared to control subjects.

11-LB

Pronounced Glucose (G) Reduction in Poorly Controlled T2DM with MB07803, a Novel Fructose-1,6-Bisphosphatase Inhibitor (FBPasel) with Reduced Potential for Acid-Base Disturbance vs the 1st Generation FBPasel CS-917

BARRY GUMBINER, PAUL VAN POELJE, DAVID BULLOUGH, SHARON WATLING, MARK MILAD, THERESA STERN, HOWARD FOYT, MARK ERION, *La Jolla, CA*

MB07803 and CS-917 are oral prodrugs of 2 structurally similar FBPasels. Both undergo metabolism to N-acetylated (N-ac) metabolites that do not inhibit FBPase but impair mitochondrial function at low uM concentrations. MB07803 was designed to improve PK and lessen N-ac vs CS-917. To assess MB07803's safety, tolerability, and effects on G and acid-base balance, a randomized, double-blind, placebo (PBO)-controlled, 14-day domiciled study was conducted in 42 T2DM patients (pts) (mean FPG=221 mg/dL, HbA1c=8.8%; T2DMx7y). G, fasting LAC and bicarbonate, and MB07982 (N-ac metabolite) were assessed. Dose-related G lowering was observed.

MB07803 mg Q12h		PBO-adjusted LSM	PBO-adjusted 95%CI
FPG mg/dL Day 15			
50	-16	-63, 30	0.4791
200	-58	-104, -12	0.0142
400	-55	-105, -6	0.0302
24h Glucose AUC mg x h/dL Day 13-14			
50	-764	-1504, -24	0.0435
200	-1186	-1927, -445	0.0026
400	-1508	-2279, -737	0.0004

400mg group: 5/10 pts had emesis; 4 down-dosed to 200mg Q12h and completed the trial. 200mg group: 4/12 pts had mild nausea but all completed the study. 50mg group: no pts had nausea or emesis; their overall adverse event profile was similar to PBO. Mean fasting bicarbonate remained normal and similar across all groups. No lactic acidosis or consecutive LAC >4.5mM occurred. Nonconsecutive, asymptomatic LAC >4.5mM occurred in 1 pt when G <60 mg/dL and symptoms of hypoG at the end of an 18h fast. 4 others had asymptomatic G <60 mg/dL, most after 2 doses during an 18h fast. MB07982 Cavg,ss±SD in 50, 200 and 400mg groups: 0.0074 ± 0.0036; 0.026 ±0.0125; 0.0187±0.0041uM. Conclusion: MB07803 was safe at all doses and well-tolerated to a dose of 200mg Q12h. G lowering was statistically and clinically significant. MB07982 concentrations were >100 fold below those observed with CS-917 and well below levels that impair mitochondrial function and alter LAC homeostasis. Thus, MB07803 may lessen LAC metabolism and acid-base balance disturbances. MB07803 is a potential oral drug therapy capable of pronounced reduction of fasting and postprandial glycemia in pts with well-established and poorly-controlled T2DM.

12-LB

Titration Effect of LY2189265 (GLP-1 Analog) Once Weekly on Metabolic Outcomes and GI Events in Uncontrolled Type 2 Diabetes Mellitus (T2DM): The EGO Study

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LY2189265, a novel, long-acting glucagon-like peptide 1 (GLP-1) analog, consists of a dipeptidyl peptidase-IV (DPP-IV)-protected GLP-1 analog covalently linked to a human IgG4-Fc heavy chain by a small peptide linker. This phase 2, randomized, placebo-controlled, double-blind clinical trial investigated the effect of dose titration of LY2189265 in overweight/obese T2DM patients.

Patients (n=262; 49% female) with elevated BMI (mean 33.9 kg/m^2) and T2DM (mean 8.3 years) suboptimally controlled despite therapeutic doses of 2 oral antihyperglycemic agents (OAH; mean HbA1c 8.2%) were randomized to once-weekly subcutaneous injections of placebo, LY2189265 1.0 mg (16 weeks), or 1 of 2 titrated doses of LY2189265 (0.5 mg, 4 weeks then 1.0 mg, 12 weeks; or 1.0 mg, 4 weeks then 2.0 mg, 12 weeks). The primary metabolic measure was glycemic control, as measured by HbA1c change from baseline at 16 weeks. Secondary measures were fasting serum glucose (FSG), solid mixed meal test glucose excursion, changes in body weight, and treatment emergent adverse events (TEAEs).

Statistically significant decreases were observed in all metabolic outcomes in response to LY2189265 treatment, as shown in the following table:

Metabolic Outcome (units)	Change from Baseline (Least-Squares Mean)**			
	Placebo	LY2189265		
		0.5/1.0 mg	1.0/1.0 mg	1.0/2.0 mg
HbA1c (%)	-0.27	-1.28*	-1.29*	-1.52*
FSG (mmol/L)	-0.49	-2.09*	-2.04*	-2.64*
Test Meal AUC	36.36	30.71*	32.21*	28.24*
Weight (kg)	-0.07	-1.58*	-1.40*	-2.51*

*p<0.05 vs placebo
**Except Test Meal AUC, reported as Mean for test meal at endpoint.

LY2189265 was generally well-tolerated. The incidence of hypoglycemic episodes was not statistically significantly different across treatment groups. Nausea (13%), diarrhea (8.8%) and abdominal distension (8.0%) were the most frequently recorded TEAEs. No titration effect was demonstrated for glycemic or TEAE outcomes.

In conclusion, in patients with T2DM sub-optimally controlled by OAHs, adjunctive administration of once-weekly LY2189265 (titrated or not titrated) resulted in significant reductions in HbA1c, fasting and postprandial glucose, and body weight.

CLINICAL THERAPEUTICS/NEW TECHNOLOGY—TREATMENT OF INSULIN RESISTANCE

13-LB

Farnesoid-X Receptor Agonists: A New Therapeutic Class for Diabetes and Fatty Liver Disease? The First FXR Therapeutic Study in Diabetes

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6-ethyl chenodeoxycholic acid (CDCA), INT-747, is a novel derivative of the primary human bile acid CDCA, (the natural ligand for the farnesoid-X receptor (FXR), a nuclear hormone receptor). INT-747 is ~100x more potent an FXR agonist than CDCA and in vitro increases insulin secretion by human pancreatic islets, enhances adipocyte lipid storage and secretion of adiponectin and leptin. This double blind, placebo (Pbo) controlled, study evaluated the effects of INT-747 on insulin sensitivity by means of a two stage euglycemic insulin clamp. Patients with Type 2 diabetes and non-alcoholic fatty liver disease (NAFLD), diagnosed by elevated aminotransferases (20%), imaging (84%) and/or histology (3%), were randomized to receive placebo, INT-747 25mg or 50mg once daily for 6 weeks. 64 patients enrolled (Pbo: n=23; INT-747 25mg: n=20, INT-747 50mg: n=21). Glucose disposal rate (GDR) (mg . kg-1 . min-1) was determined (pre and post treatment) after steady state was achieved with low and high dose insulin (Ins) infusions (60 and 120mU x m2 body surface area/min).

For author disclosure information, see page LB32.

Mean + s.d.	Δ GDR Low dose Ins	Δ GDR High dose Ins	%Δ GDR Low dose Ins	%Δ GDR High dose Ins	%Δ Weight Change
Pbo (n=17)	-0.51±1.88	-0.61±1.88	-5.55±35.9	-5.54±24.3	-0.03±1.96
INT-747 25mg (n=15)	0.69±1.12*	0.73±1.53*	28.0±40.2*	18.3±36.3*	-1.22±1.75†
INT-747 50mg (n=12)	0.24±1.62	0.42±1.42	20.1±32.6†	10.8±21.8†	-1.70±2.37*
Both doses (n=27)	0.49±1.36*	0.59±1.46*			

*:p<0.05; †p=0.05 to 0.10 (trend compared to placebo)

Fasting plasma insulin concentrations tended to be higher after treatment with 25mg vs. Pbo. The greatest responses appeared to occur in patients with BMI <40kg/m2 and HbA1c >7%. Adverse experiences (AEs) were generally mild/moderate and not clearly different across groups (placebo 61%, 25mg 45%, 50mg 76%). Constipation (19% in 50mg group) was the most common. Minor increases in LDL (25 and 50mg) and decreases in HDL and triglycerides (50mg) were seen. ALT (25mg) and GGT (both doses) decreased by ~25% and 50% (p<0.001), respectively. Thus, in this study INT-747 was well tolerated and improved GDR and weight loss in Type 2 diabetics with NAFLD.

COMPLICATIONS—MACROVASCULAR—ATHEROSCLEROTIC CVD AND HUMAN DIABETES

14-LB

Cardiovascular Function in Type 1 Diabetes

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Background: The EDIC observational follow-up of the Diabetes Control and Complications Trial (DCCT) has reported benefit of prior intensive therapy on retinopathy, nephropathy, neuropathy and cardiovascular disease events (CVD). Using Cardiac MRI (CMRI), we evaluated cardiac function in former DCCT intensive (INT) and conventional (CON) therapy subjects 14 to 15 years after DCCT closeout.

Methods: CMRI was performed in 28 clinics and read centrally using MASS software. Six functional outcomes were evaluated: end diastolic volume (EDV) and systolic volume (ESV), stroke volume (SV), cardiac output (CAROUT) and left ventricular end diastolic mass (LVDM), adjusting for body surface area, and ejection fraction (EF).

Results: Of 1211 active EDIC subjects asked to participate, 90% agreed. A total of 850 scans (~75% of the consented participants) have been performed. Fifty-one patients with CVD were excluded from this analysis. On a quality control scale of 0,1,2, the mean score was 1.77 with only 2% unacceptable (0 score) examinations. Reproducibility was high for all measures (intra-class correlations > 0.91). Normal EF (50-70%) was prevalent (>85%) in both INT and CON treatment group.

Least Square Means by Gender (adjusting for basic covariates (BC)—reader, machine type and attained age) and by Group (adjusting for BC and gender)

Outcome	Females	Males	INT	CON
EDV (ml/m^2)	66.4	74.0	70.3	70.1
ESV (ml/m^2)	24.2	28.8	26.4	26.6
SV (ml/m^2)	42.2	45.2	43.9	43.5
EF (%)	63.7	61.4	62.7	62.4
CAROUT (L/min/m^2)	3.1	3.2	3.1	3.1
LVDM (g/m^2)	66.5	80.2	73.4	73.2

*All p-values < 0.01 between genders. No significant differences between INT and CON

Conclusion: A large multicenter study of CVD in type 1 diabetes is feasible, attractive to participants and yields new information. After excluding patients with clinical CVD events, 89% of the subjects had normal EF; least square mean differences between men and women were statistically significant on all the functional outcomes; no differences were detected between the INT and CON treatment groups.

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15-LB

Pioglitazone Reduces Long-Term Progression of Carotid Atherosclerosis in IGT

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As part of the ACT NOW diabetes prevention study, we performed serial measurements of carotid intima-media thickness (CIMT) to determine long-term effects of pioglitazone on pre-clinical atherosclerosis in those with impaired glucose tolerance (IGT). 602 IGT subjects from 8 ACT NOW study sites were randomized to pioglitazone (PIO, 45 mg/d) or placebo. 490 participants from 7 sites also underwent high-resolution B-mode ultrasound carotid imaging at baseline, and, 393 persons (PIO:194, placebo:199; age, 53 ± 12 yr; female, 54%; non-Hispanic White, 55%; BMI, 33.2±5.3; HbA_{1c}, 5.4 ± 0.4%), completed 2-3 repeat CIMT scans (total of 1115 scans) during the 39 month study. Baseline characteristics and cardiovascular disease (CVD) risk factors did not significantly differ between treatment groups. Annual rates of change in CIMT were determined from mixed models fitted to CIMT data for persons who completed two or more scans. During a median follow-up of 2.7 yr (range: 0.4- 4.1 yr) and with a median of 3 CIMT measurements per subject, the annual rate of change in CIMT was 38% lower in the PIO group (mean ± SE 0.0055 ± 0.0011 vs. 0.0089 ± 0.0011 mm/yr, difference= .0034 mm/yr, p = 0.025). The results were unchanged when individuals that developed diabetes during the study were excluded, indicating differential rates of CIMT progression were not simply a consequence of elevated glucose in those converting to diabetes while receiving placebo. The annual rates of CIMT change were linear, indicating the relative benefits of PIO were persistent over the study duration, and were unaltered after adjustment for baseline standard CVD risks factors. The beneficial effects of PIO were not significantly different in subgroups defined by higher age, BMI, systolic or diastolic blood pressure, total or LDL cholesterol, triglyceride, and lower HDL cholesterol. In summary, PIO slowed the rate of CIMT progression in pre-diabetic individuals, for whom glucose lowering effects are more modest, and this anti-atherogenic effect persisted over nearly 3 yr. These data support the concept that use of PIO therapy early in the evolution of diabetes may have beneficial effects on CVD.

16-LB

WITHDRAWN

17-LB

WITHDRAWN

18-LB

The Role of Aortic Arch Stiffening in Cardiac and Cerebral Damage in Type 1 Diabetes Mellitus Patients, Assessed by Magnetic Resonance Imaging

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Type 1 diabetes mellitus (DM) patients develop stiffer arteries than normal subjects. As aortic arch function may directly affect physiology of both the heart and the brain, we hypothesized that aortic arch stiffening may be directly associated to left ventricular (LV) heart function as well as to manifestations of cerebral small vessel disease. Therefore, the purpose of this study aimed to find a possible association between aortic arch stiffness and cardiac LV function as well as cerebral damage in type 1 DM patients using magnetic resonance imaging (MRI).

In a cross-sectional observational design 86 consecutive type 1 DM patients (49 male, mean age 46.9±11.7 years, mean DM duration 23.8±11.0 years) were included from the local outpatient clinic. MRI of the aortic arch, heart and brain was performed in all study-participants for the assessment of aortic arch pulse wave velocity (PWV), as a marker of aortic arch stiffness, parameters of diastolic and systolic LV function, as well as the presence of cerebral white matter hyperintensities (WMHs), microbleeds and lacunar infarcts.

Mean aortic arch PWV was 6.39±2.14m/s. Six patients showed impaired diastolic LV function. Twenty-seven patients showed impaired systolic LV

For author disclosure information, see page LB32.

function. Fourty patients had abnormal WMHs, 7 patients had microbleeds and in 2 patients lacunar infarcts were found.

Aortic arch PWV showed to be an independent predictor of parameters of systolic LV function, including LV ejection fraction (Beta=-0.285, p=0.020) and LV stroke volume (Beta=-0.293, p=0.010) after statistical correction for age, DM disease duration and hypertension. No significant independent relation was found between aortic arch PWV and diastolic LV function or MRI parameters of cerebral small vessel disease.

In conclusion, aortic arch stiffness is independently related to systolic LV function, but not to diastolic LV function or manifestations of cerebral small vessel disease in a population of relatively young type 1 DM patients.

19-LB

Urinary Adiponectin Excretion Is a Novel Biomarker of Atherosclerotic Burden in Type 2 Diabetes Patients

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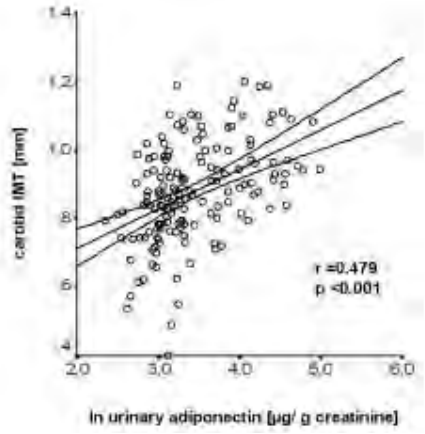
In contrast to previous studies on serum adiponectin, this study assesses whether urinary adiponectin excretion represents a more consistent vascular damage marker in type 2 diabetes mellitus (T2DM).

Urinary adiponectin isoforms were characterized by western blot analysis. The excretion rate of total adiponectin was detected in 156 T2DM patients with a history of diabetic nephropathy as well as 40 healthy controls by ELISA. Atherosclerotic burden was assessed by means of carotid artery intima-media-thickness (IMT).

Low-molecular adiponectin isoforms (~30-70kDA) were detected in urine by western blot analysis. Urinary adiponectin was significantly increased in T2DM (7.68±14.26 vs. controls: 2.91±3.85µg/g creatinine, p=0.008) and adiponectinuria was significantly associated with IMT (r=0.479, p<0.001).



Adiponectinuria proved to be a powerful and independent predictor of IMT (ß=0.360, p<0.001) in multivariable regression analyses:

Independent predictors of IMT in patients with type 2 diabetes

Dependent variable:	Common Carotid Artery IMT		
	ß	t	p
Age	0.239	2.562	0.012
Sex	-0.118	-1.114	0.268
WHR	0.142	1.274	0.206
LDL	0.231	2.649	0.009
AER*	0.034	0.385	0.701
Diabetes duration*	0.197	2.269	0.026
Serum adiponectin*	-0.128	-1.362	0.177
Urinary adiponectin*	0.360	4.005	<0.001
r	0.665		
^{2}	0.442		

*: log-transformed variables; AER: albumin excretion rate; IMT: intima-media-thickness; WHR: waist-to-hip ratio; Model additionally adjusted for: HDL, hsCRP, HbA1c, smoking status, mean arterial pressure (data not shown)

Furthermore, urinary adiponectin, but not the albumin excretion rate, added significant value for the prediction of increased IMT in a model including variables of the 'UKPDS CHD risk engine' (p=0.007).

Quantification of urinary adiponectin seems to be a marker of atherosclerotic burden in T2DM exceeding the predictive value of urinary albumin excretion in this study.

COMPLICATIONS—MACROVASCULAR—CELLULAR MECHANISMS OF ATHEROGENESIS IN DIABETES

20-LB

Chronic Hepatic Inflammation Severely Aggravates Atherosclerosis Development in APOE*3-Leiden Mice

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In addition to atherogenic dyslipidemia, characterized by high plasma levels of (V)LDL-cholesterol and low levels of HDL-cholesterol, inflammatory processes are considered as major contributors of atherogenesis. The liver is the key organ involved in inflammatory responses, but the relation between hepatic inflammation and atherogenesis is poorly understood. Since nuclear factor-kappaB (NF-κB) is the central regulator of inflammatory processes, we studied whether chronic selective activation of hepatic NF-kB aggravates atherosclerosis development in APOE*3-Leiden (E3L) mice. E3L mice were crossbred with Liver-specific IKK-beta (LIKK) transgenic mice, which have a continuous activation of the NF-kB pathway in the hepatocytes, to generate E3L.LIKK (experimental group) and E3L (control group) littermates. Female mice were fed a Western-type diet containing 0.25% cholesterol (t=0) and plasma was collected every 4 weeks for determination of plasma lipid levels and plasma parameters. Atherosclerosis was assessed after 24 weeks in the aortic root. E3L.LIKK mice had increased plasma levels of total cholesterol (TC) only at week 8 (+43%; P<0.001) and week 12 (+22%; P<0.01), and not at the other time points during the study. As a result, LIKK expression enhances the total TC exposure during the study by 16% (P<0.05). LIKK did not affect plasma Serum Amyloid A and a panel of cytokines (e.g. IL-1β, IL-6, IL-10). Importantly, E3L.LIKK mice showed highly increased atherosclerotic lesion area (+131%; P<0.05) and more advanced atherosclerosis, as indicated by a reduced number of lesion-free segments (11 vs 44%; P<0.01) and an increased number of severe lesions (25 vs 14%; P<0.05). We can conclude that continuous selective activation of hepatic NF-kB in E3L mice severely aggravates atherosclerosis development, which highlights the significance of inflammation at nonvascular sites in the development of atherosclerosis.

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21-LB

WITHDRAWN

COMPLICATIONS—NEPHROPATHY

22-LB

Early Change of Innate and Adaptive Immune Responses in Diabetic Nephropathy in Autoimmune Diabetes

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Results: Diabetic nephropathy is the leading cause of end-stage renal failure in young people and affects about 40% of individuals with long-standing type 1 diabetes .We used non-obese diabetic (NOD) to examine immunopathology of diabetic nephropathy and to investigate the involvement of cellular and humoral immunity at various time points after diabetes onset. We found that the glomeruli of diabetic NOD mice were infiltrated with T and B cells, as well as CD11c+ dendritic cells, which had close contact with CD4+ and CD8+ T cells in the infiltrates. We observed elevated perforin and granzyme B mRNA expression in the kidney of diabetic mice by real-time PCR, which was further confirmed by co-localization of CD8+ T cells and perforin staining. We also found that IgG deposition in diabetic NOD mice was particularly located in the glomeruli, related to CD31 and laminin, and accompanied by the presence of complement C3.

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Meanwhile, IgA and IgM were deposited in the glomerular mesangial areas and capillary walls in diabetic mice 1 to 3 months after the disease onset. Moreover, the serum from diabetic mice contained autoantibodies directed towards components of the normal glomeruli of non-diabetic mice and these antibodies were not present in non-diabetic NOD mice.

Immunostaining also demonstrated that Toll-like receptors (TLRs), such as TLR4, were expressed by intra-kidney leukocytes in diabetic mice. We are currently investigating the change of TLR4 endogenous ligands, such as high-mobility group box 1 (HMGB1), heat shock proteins, hyaluronan, and biglycan, to support whether one or more of these ligands may be the source for TLR4 activation. We are also using TLR4$^{-/-}$ and MyD88$^{-/-}$ NOD mice to confirm that engagement of TLR4 by the endogenous ligands and extracellular matrix components may be a major trigger of inflammation infiltrate in the kidney of diabetic mice.

We believe that understanding the role that the immune system plays in the pathogenesis of diabetic nephropathy could lead to identification of new strategies and/or additional therapeutic targets for prevention and treatment of diabetic nephropathy.

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COMPLICATIONS—NEUROPATHY

23-LB

Risk of Microvascular Events Following Initiation of Insulin Glargine or NPH Insulin in Type 2 Diabetes in the US

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It is unknown if glycemic control with different insulins is associated with differential risk for subsequent microvascular events (ME). A database of 47 US managed care plans was analyzed to determine the incidence of ME following initiation of glargine (GLAR,n=10,667) or NPH (n=2,009) in patients treated with oral antidiabetic drugs between 2001 and early 2008. MEs were defined based on ICD9-CM codes for diabetes specific eye, kidney or nerve complications. Patients with MEs or no HbA1c value available during the 1 year period (baseline) prior to insulin initiation were excluded. Patients initiating GLAR vs NPH were older (53 vs 48 years), predominantly male (57 vs 36%), had a higher mean HbA1c (9.3 vs 8.3%), fewer specialist visits (18 vs 29%), and lower Charlson comorbidity score (0.86 vs 1.0). Initial crude, unadjusted ME incidence estimates were 225 vs 201 events/1,000 patient-years (Rate Ratio=1.12,p=0.02) with mean follow up of 15 vs 16 months from initiation of GLAR vs NPH, respectively. Unadjusted 1-year HbA1c change was similar between the groups (-1.0 vs -0.96%). Analysis adjusted for multiple confounders showed an increasing risk for ME with baseline HbA1c (Hazard ratio (HR)=1.07,p<0.001) and the risk trended slightly lower in patients achieved HbA1c <7% vs those who did not during first year follow-up (HR=0.95,p=0.32). After accounting for substantial baseline group differences, 1:1 propensity matched comparisons (Table) showed a 15% lower risk of ME in patients who initiated GLAR (n=1,589) instead of NPH (n=1,589). In conclusion, these provocative data suggest a lower incidence of ME following initiation of GLAR vs NPH after adjusting for important baseline confounders. This effect of GLAR appears incompletely explained by the differences in HbA1c lowering. Further well controlled studies are necessary to evaluate this hypothesis.

Comparisons on incidence of microvascular events in propensity matched (1:1) GLAR vs NPH initiator groups

Agent	n	Baseline A1C %	% Patients with ME	OR (95% CI)	Wilcoxon S (P)
Match criterion: difference in estimated propensities between glargine and NPH < 0.1					
Glargine	1589	8.64 (p=0.49)	27.5	0.85 (0.73, 0.99)	4.12 (0.04)
NPH	1589	8.70	30.9		
Match criterion: difference in estimated propensities between glargine and NPH < 0.05					
Glargine	1523	8.69 (p=0.16)	27.5	0.83 (0.71, 0.97)	5.28 (0.02)
NPH	1523	8.77	31.5		
Match criterion: difference in estimated propensities between glargine and NPH < 0.01					
Glargine	1449	8.70 (p=0.02)	28.2	0.82 (0.70, 0.96)	6.06 (0.01)
NPH	1449	8.96	32.4		

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COMPLICATIONS—OCULAR

24-LB

12-Lipoxygenase: A New Target for Therapeutic Intervention in Diabetic (Ischemic) Retinopathy

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Diabetic retinopathy (DR) is a vision threatening complication of diabetes. Vascular injury in diabetic retina involves leukostasis, and vascular leakage and growth. Disruption of the normal balance between the pro-and antiangiogenic factors vascular endothelial growth factor (VEGF) and pigment epithelium derived factor (PEDF) has been reported to be critical in DR. Arachidonic acid is metabolized by 12-lipoxygenase (12-LO) to 12- hydroxyeicosatetraenoic acid (12-HETE) which has been reported to beinvolved in angiogenesis, and promotes mitogenic and migration properties of microvascular endothelial cells. 12-HETE has also been shown to induce leukostasis. The goal of this study is to investigate the changes in 12-LO expression and activity and to correlate these changes with VEGF and PEDF balance and the vascular injury associated with DR.

The experiments have been performed on vitreous from diabetic and non-diabetic patients and retinas from eye donors, mouse model of diabetes and oxygen-induced retinopathy (OIR) treated with or without 12-LO inhibitor, baicalein (10mg/kg). Western blotting and ELISA were used to evaluate the changes in 12-LO, PEDF, ICAM-1 and VEGF expression. LC/MS was used to assess the activity of 12-LO. Vascular density determination on flat mount retinas from OIR labeled with Isolectin B4 was used to evaluate the effect of 12-LO inhibition on retinal neovascularization. We also tested the effect of 12-HETE (0.5-1mM) on Müller cell expression of VEGF and PEDF. Diabetes increased retinal 12-LO expression in human and mice; this was associated with a dramatic increase in the retinal level of ICAM-1. Both expression and activity of 12-LO were significantly increased in OIR and baicalein significantly decreased neovascularization, and restored the balance in VEGF and PEDF expression. 12-HETE significantly increased VEGF and decreased PEDF production by Müller cells. Analysis of patient vitreous confirmed the clear inverse relationship between 12-LO activity and PEDF expression. This indicates that 12-LO activity is key to the development of DR by disrupting the balance between PEDF and VEGF. Inhibition of 12-LO could be a new target to treat DR.

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25-LB

Markers of Oxidative Stress and Haptoglobin Genotype in Adult T1D

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Oxidative stress (OS)-induced cellular damage may explain the relationship between glycemia and diabetes complications. Iron and iron compounds such as hemoglobin (Hb) can enhance OS. Haptoglobin (Hp) is a plasma protein that binds to free Hb and thereby provides protection against Hb-mediated OS, but the polymorphic allele 2 of the Hp gene provides inferior protection compared with the Hp1 allele. Notably, the Hp 2-2 genotype has been associated with higher rates of micro- and macrovascular complications. We hypothesized that T1D patients carrying the high risk Hp 2 allele would have higher levels of OS and more diabetes complications. We studied 40 adults with T1D receiving continuous subcutaneous insulin infusion +/- real-time continuous glucose monitoring with HbA1c<7.0%, age 44±12 yrs, and diabetes duration 24±13 yrs (mean±SD). OS was assessed by measuring urinary isoprostane, TAOS, TBARS, GSH, GSSG, and oxLDL. Hp levels, and Hp genotype were also analyzed. Results showed that subjects with the protective Hp 1-1 genotype had significantly lower oxLDL, isoprostane, and TBARS and significantly higher Hp and glutathione levels, compared with subjects with one or more Hp 2 risk allele (p=0.002, 0.039, 0.009, <0.001, 0.033, respectively). Interestingly, TAOC was significantly lower in subjects with Hp1-1 (p=0.001). In subjects with >10 years diabetes duration, each copy of the Hp 2 allele was associated with an increased frequency of diabetes micro- and/or macrovascular complications at baseline (20% vs. 53% vs. 73% for genotypes 1-1, 2-1, and 2-2, respectively, p=0.005). However, each copy of the Hp 2 allele was also associated with increased age and diabetes duration (p=0.017, 0.032, respectively). Thus, as expected, the Hp 1-1 genotype was associated with less OS. Furthermore, we observed an enrichment for diabetes complications in subjects with >10 years diabetes duration with one or more Hp risk alleles. In conclusion, Hp genotypes may have a prognostic value in identifying individuals susceptible to developing diabetes complications. More comprehensive studies are needed to explore the provocative link between Hp risk alleles and diabetes complications.

Supported by the JDRF.

For author disclosure information, see page LB32.

26-LB

The Prevalence of Metabolic Abnormalities in Patients with Arcus Senilis

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Until now, it has been assumed that arcus senilis (arcus) primarily reflected increased cardiac risk and hyperlipidemia. While there is no question that some relationship exists among these factors, whether this is a primary relationship is unclear, partly due to the probable association of arcus with increasing age and cardiac risk, which, in turn, would confer increasing likelihood of greater occurrence of hyperlipidemia and other metabolic abnormalities in any study population. We studied the associations between arcus and age, BMI, blood pressure, fasting blood sugar, hemoglobin A1c and various components of fasting lipid profiles in 284 subjects (Ss) who were found to have arcus on routine eye exam. ATP III criteria were used to define normal ranges for lipid fractions and blood pressure observations. WHO criteria were used to define normalcy of blood sugars, and patients with hemoglobin A1c (Hgb A1c) > 6% were considered abnormal for the purposes of this evaluation. Patients were 20-88 years of age (mean= 55.3 ±3.8 yrs), all voluntarily participated in a vision care program where services were provided at no charge. Each of these metabolic abnormalities was quite prevalent in these Ss. Hyperglycemia in 88%; elevated BMI in 66%, elevated blood pressure in 68.5%; but elevated LDL was present in only 21%. 53% of Ss did have an HDL cholesterol < 40 mg/dl and 24% had a serum triglyceride level > 150 mg/dl. Multivariate regression demonstrated that the primary variables, associated with arcus in this population were age ($p<0.0001$), fasting blood sugar ($p=0.0037$), systolic blood pressure ($p=0.0068$), diastolic blood pressure ($p=0.02$), and Hgb A1c ($p=0.01$). The data does not support a significant association with abnormality of lipid fractions, including LDL-cholesterol ($p=0.3455$), HDL ($p=0.2311$), serum triglycerides (0.3436) or weight ($p=0.642$). Results suggest that the occurrence of arcus is related to increasing age, increasing blood sugar and increasing blood pressure. Apparent relationships of arcus with other metabolic abnormalities may be related to their dependent increase in prevalence with increasing age, blood sugars or blood pressure. This may be especially true for LDL cholesterol levels.

DIABETIC DYSLIPIDEMIA

27-LB

Glucose-Mediated Very Low Density Lipoprotein (VLDL) Production Requires Impaired Insulin Action

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The overproduction of triglyceride (TG) in the form of very low density lipoprotein (VLDL) is an independent risk factor for coronary heart disease (CHD) in patients with diabetes. Insulin resistance and hyperglycemia both substantially contribute to CHD risk, but studies of how hyperglycemia impacts TG metabolism *in vivo* have been limited by difficulties in separating glucose-signaling from insulin-signaling. The molecular basis that links hyperglycemia to VLDL overproduction remains poorly understood.

We performed hyperglycemic-*hyper*insulinemic (4 mU/kg/min) clamp studies in rats in which blood glucose was clamped at 300 mg/dl for 2 h. Surprisingly, hyperglycemia did not promote VLDL secretion compared to baseline (VLDL AUC 0.52 ± 0.20 µg after hyperglycemia *vs.* 0.38 ± 0.16 µg baseline, p=ns). We speculated that hyperinsulinemia (47 ± 4mU/L) impaired glucose-mediated VLDL production, and next performed hyperglycemic-*hypo*insulinemic clamp studies in which insulin was clamped at fasting levels with somatostatin (BG 300 mg/dl, Insulin 0.25 mU/kg/min). Under low insulin conditions, glucose potently augmented VLDL production (VLDL AUC 2.69 ± 0.81 µg after hyperglycemia vs. 0.90 ± 0.57 µg baseline $p<0.05$). We hypothesized that molecular disruption of insulin signaling to VLDL would be sufficient to allow glucose-stimulated VLDL production, even under hyperinsulinemic conditions. In the liver, forkhead box O1 (FoxO1) coordinates glucose and lipid metabolism. We used adenovirus-mediated delivery of constitutively active FoxO1 gene (CA-FoxO1, $1x10^9$ PFU) to rats. We found that when the ability of insulin to inactivate FoxO1 is blocked with a CA-FoxO1, intraperitoneal glucose potently augmented VLDL-TG production (VLDL AUC 15.85 ± 3.84 µg) compared to no glucose treatment (VLDL AUC 5.23 ± 2.08 µg). These animals were hyperinsulinemic secondary to endogenous insulin secretion (21 ± 2 mU/L).

These data suggest that glucose itself is a prominent driver of VLDL production in the liver, but this requires either insulin deficiency (such as in type 1 diabetes), or impaired insulin action (such as in type 2 diabetes).

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EPIDEMIOLOGY

28-LB

Diabetes and Auditoryneuropathy

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There is a dramatic rise in the worldwide prevalence in Diabetes Mellitus (DM) over the past two decades It is a progressive multisystem disorder with well known microvascular complications affecting retina,peripheral nerves and other organs. But sensorineural hearing loss (SNHL) is often an under recognized complication. We conducted a study in a group of 50 diagnosed cases of DM with an equal number of age and sex matched controls to find out the incidence of SNHL by pure tone audiometry (PTA). We tried to find out any correlation between duration of diabetes and SNHL and also between the glycemic status of diabetic patients and the severity of hearing loss .We excluded patients with history of ototoxic drugs consumption,occupational exposure to noise,smoking,ear discharge and head injury.

Sensorineural hearing loss was significantly higher in cases (94%) compared to controls(18%) ($p = .001$). SNHL was found in all cases with HbA1C >7 (mild to moderate 55.5%, moderate to severe 35%, severe to profound 9%) whereas those with HbA1c <7, only 40% had mild to moderate SNHL and the difference was statistically significant ($p=0.001$)

Cases with duration of diabetes > 8 years had 8.33%, 75%, and 16.67% of mild to moderate, moderate to severe and severe to profound SNHL respectively. The corresponding figures for those with duration <8 years were 68.4%, 18.4% and 5.2% respectively. However 87.5% of cases with age less than 50 years had high frequency SNHL, whereas 100% of those above 50 years had hearing loss. Main limitation of study is that age might be a confounding factor which cannot be ruled out and it is a small pilot study which requires validation after a study on a larger population.

In conclusion high frequency SNHL is a very common but underdiagnosed complication of diabetes.With more and more elderly people living longer with diabetes it carries more significance. It makes sense treating diabetes intensively with early insulin initiation which probably would prevent this complication.SNHL increases in severity with longer duration of DM and poor glycemic status.

29-LB

Trends in Type 2 Diabetes Mellitus and Body-Mass Index in Asian Americans and Whites, United States, 1997-2007

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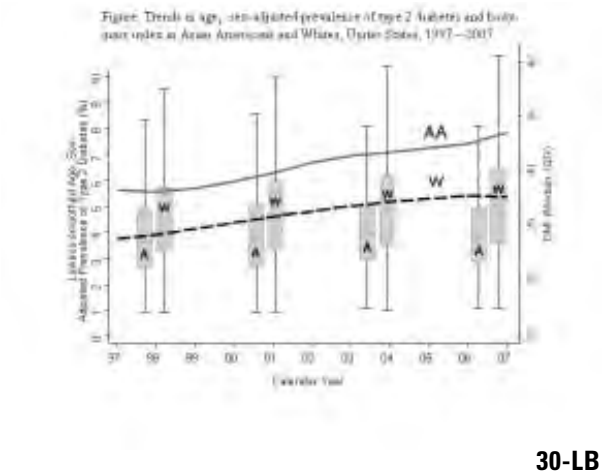
Asians are more susceptible to type 2 diabetes than whites (W). Despite their growing numbers in the US population, few studies have focused specifically on Asian Americans (AA). We therefore examined trends in the prevalence of type 2 diabetes and related conditions in AA (n=11,471) vs. W (n=208,663) in a representative sample of 319,705 US adults aged ≥18 who participated in the National Health Interview Survey (NHIS) from 1997 through 2007. Compared to their white counterparts, AA had lower mean body-mass index (BMI; 24.1 vs. 27 kg/m^2, *$p<0.001$)* and higher educational attainment (69 vs. 59% with some college, $p<0.001$), but slightly less physical activity (31 vs. 34% met AHA guidelines, $p=0.023$) in 2006. Over 11 years, 20,275 adults reported having been told by a physician that they had diabetes after age 25 (641 AA; 11,454 W). Age-, sex-adjusted type 2 diabetes prevalence was significantly higher in AA (6-8%) than in their white counterparts (4-6%) throughout the entire period and there was a significant upward linear trend in both groups ($p<0.05$; see Figure). The AA-W gap appeared to grow after 2006. These trends were not explained by shifts in underlying risk diabetes factors. Most notably, over the 11 years, BMI increased steadily in W, but remained lower and more stable in AA (see Figure). In multiple logistic regression models that adjusted simultaneously for age, sex, BMI, physical activity, and education, the relative odds [95% CI] of prevalent type 2 diabetes in AA vs. W rose from 2.26 [1.62-3.15] in 1997 to 2.98 [2.14-4.15] in 2007. Compared to their white counterparts, AA are at increasingly high risk for type 2 diabetes, despite substantially lower BMI. Future research should determine the explanation for this health disparity with the aim of tailoring optimal prevention strategies for AA.

For author disclosure information, see page LB32.



30-LB

Validation of the Diabetes Risk Score, a Multi-Marker Panel That Assesses the Risk of Type 2 Diabetes: Combined Results of the Inter99 and Botnia Studies

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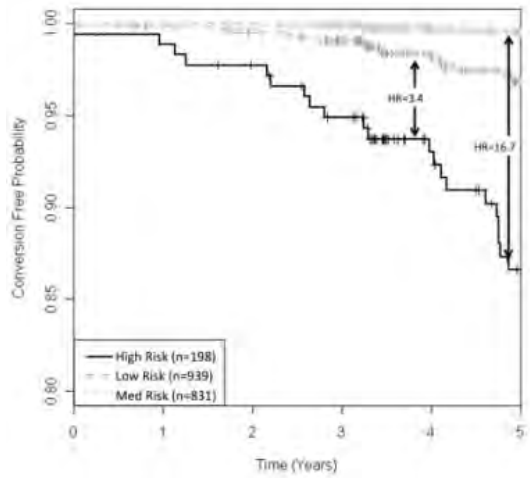
The aim of this study was to validate a Diabetes Risk Score (DRS) based on a multi-marker panel that predicts 5-year risk of T2D on an independent population. The DRS was based on a 7-biomarker panel developed from the Inter99 cohort, a Danish longitudinal population-based study of middle-aged participants. The test was performed on an independent population, the Botnia cohort, a Finnish family-based study designed to identify genetic factors associated with the development of T2D. A total of 1968 Botnia participants had 5-year outcomes available for this study.

The area under the receiver-operating characteristic curve (AUC) was 0.85 on the Botnia validation cohort, confirming that the score generalizes beyond the population in which it was developed. The performance of the DRS in Botnia was superior to other tests including fasting plasma glucose, oral glucose tolerance, HbA1c, HOMA-IR, and clinical models based on the San Antonio Heart Study and the Framingham Offspring Study. The difference in AUC between the training and validation studies was not statistically significant (AUCInter99=0.812, AUCBotnia=0.850), indicating that the model is robust across populations. Stratifying Botnia participants by DRS into low (DRS<4), medium (DRS≥4–7.3) and high (DRS≥7.3) risk groups, five year T2D conversion rates were 0.5% for the low-risk, 3.7% for medium-risk and 15.3% for the high-risk groups, with 68%, 25% and 7% of the population in each respective category. Kaplan-Meier curves comparing conversion-free survival in the 3 groups are plotted.



The hazard ratios (HR) were highly significant, with high to low HR of 16.74 (95% CI: 10.36-27.03; p<0.0001) and high to medium HR of 3.43 (95% CI: 2.27-5.18; p<0.0001). Ongoing studies will assess risk stratification in additional cohorts and ethnicities.

EXERCISE—ANIMAL

31-LB

WITHDRAWN

EXERCISE—HUMAN

32-LB

Cardiovascular and Pulmonary Response to Exercise in Trained Type-I Diabetics; Influence of Glycemic Control

NICHOLAS A. CASSUTO, COURTNEY M. WHEATLEY, WILLIAM TRAVIS FOXX-LUPO, ALLISON R. DENNEY, BRITTANY D. STOAKES, JAMES C. BALDI, ERIC M. SNYDER, *Tucson, AZ, Flagstaff, AZ*

Type-I diabetes can lead to decrements in cardiovascular and pulmonary function in patients who are not exercise-trained. Few studies have examined the influence of type-I diabetes on the cardiopulmonary response to exercise in exercise-trained subjects. To determine the influence of diabetes and glycemic control on the cardiopulmonary response to exercise in trained patients we recruited 10 type-I diabetic patients who were training for an Ironman triathlon (female=20%, age=37±7yrs., ht.=181±9cm., wt=75±9kg., BMI=23±2kg/m^2, VO_{2peak}=130±20%predicted, HbA1c=7±0.7%, glucose=200±52mg/dL mean±SD) along with 10 healthy subjects (female=20%, age=28±6yrs., ht.=180±10cm., wt=72±2kg., BMI=22±4kg/m^2, VO_{2peak}= 115±30% predicted, HbA1c=4±0.2%, glucose=90±4mg/dL) and measured cardiac output (Q), stroke volume (SV), systolic and diastolic blood pressures (SBP and DBP) and calculated systemic vascular resistance (SVR), mean arterial blood pressure (MAP), and airway function (FVC, FEV1, FEF50) in these two groups. The diabetic subjects were also stratified into low HbA1c (<7%, n=5) and high HbA1c (>7%, n=5) groups for statistical comparison. There were no differences in cardiopulmonary parameters at rest. At peak exercise, the diabetic group had a higher workload, Q, and SV, but there were no differences in SBP, DBP, MAP, SVR or airway function (watts=265±44 vs 211±47w, Q=21±3.5 vs 18±3.6 l/min, SV=79±10 vs 65±11ml for diabetic and healthy, respectively). Within the diabetic group, subjects with optimal glycemic control had better airway function and cardiac function, but these results did not reach statistical significance (FVC= 5.4±0.9 vs 4.7±0.2 liters, FEV1=4.8±0.6 vs 4.3±0.2 liters, FEF50= 6.0±0.6 vs 5.3±0.9 liters, Q=22.5±3.4 vs 19.5±2.5 l/min, SV=83±12 vs 76±9 ml, for low HbA1C and high HbA1C, respectively). These results suggest trained diabetic subjects have a normal cardiopulmonary response to exercise, highlighting the importance of exercise training in this population. Furthermore, those with optimal glycemic control demonstrated slightly greater cardiopulmonary function at peak exercise, highlighting the importance of optimal glycemic control in this population.

33-LB

Glycemic Control and Alveolar-Capillary Membrane Conductance at Rest and during Exercise in Trained Type-I Diabetic Patients

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Optimal glycemic control is imperative in patients with type-I diabetes, particularly in those patients who are physically active. Limitations in lung diffusing capacity can result in impairment of O_2 transfer from the lungs into the blood stream which may have important implications in O_2 delivery during exercise. The diffusing capacity of the lungs for carbon monoxide (DLCO) represents the movement of alveolar air to capillary blood and is affected by alveolar-capillary membrane conductance (D_M) and pulmonary capillary blood volume (Vc). We sought to determine the influence of glycemic control (through the measurement of glycosylated hemoglobin, HbA1c) on cardiac output (Q), DLCO, D_M, and Vc at rest and during exercise in trained patients with type-I diabetes. To determine this we recruited 12 type-I diabetic patients who were training for an Ironman triathlon (female = 25%, age=40±8yrs., ht.= 181±9cm., wt= 74±9kg., BMI= 23±2kg/m^2, VO_{2peak}= 140±25% predicted, HbA1c=7±0.8%, mean±SD). The subjects were stratified into low HbA1c (<7%, n=5) and high HbA1c (>7%, n=7) groups for statistical comparison. D_M and Vc were determined using the DLCO and diffusing capacity of the lungs for nitric oxide (DLNO) technique. The low HbA1c group had higher Q, DLCO, and D_M at rest, peak exercise, and into recovery when compared to the high HbA1c group but there were no differences in Vc (low HbA1c vs. high HbA1c: Q=4.2±0.4 vs. 3.3±1.1, 14.5±1.2 vs. 12.1±2.3 and 6.1±0.6 vs. 3.9±1.8 l/min., DLCO=27±4 vs. 19±5, 38±9 vs. 29±7, and 29±5 vs. 22±7 ml/min/mmHg,

For author disclosure information, see page LB32.

D_M=36±5 vs. 26±6, 48±10 vs. 35±9, and 39±7 vs. 29±9 ml/min/mmHg, Vc=76±26 vs. 54±32, 135±39 vs. 133±43, and 103±53 vs. 63±38 ml for rest, peak exercise, and recovery, respectively). These results suggest glycemic control is imperative for optimal gas transfer in the lungs in trained type-I diabetics at rest and during maximal exercise.

EXERCISE—REGULATION OF MUSCLE METABOLISM

34-LB

TBC1D1 Phosphorylation and Insulin-independent Glucose Transport Are Increased Immediately Post-Exercise in Rat Skeletal Muscle, but TBC1D1 Phosphorylation Is Reversed at 3h Post-Exercise When Insulin Sensitivity Is Increased

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A single exercise bout can increase: 1) insulin-independent glucose transport (GT) by rat skeletal muscle immediately post-exercise (PEX), and 2) insulin-stimulated GT at 3 or 27hPEX. TBC1D1, a RabGAP protein that can modulate GT, is phosphorylated with in vitro contraction or insulin, but the effect of in vivo exercise on TBC1D1 and its role(s) in PEX-stimulated GT are unknown. We hypothesized that exercise would: 1) increase insulin-independent TBC1D1 phosphorylation (PAS-TBC1D1) concomitant with elevated GT immediately PEX; and 2) increase insulin-stimulated GT without elevated PAS-TBC1D1 at 3 or 27hPEX. Rats were assigned to PEX (2h swim) or time-matched sedentary (SED) groups, with muscles sampled at: 0hPEX, 3hPEX, or 27hPEX. One epitrochlearis per rat was incubated without insulin. The contralateral muscle was incubated with 50μU/ml insulin. 3-O-[^{3}H]Methyl-D-glucose transport (3MGT), PAS-TBC1D1 (immunoprecipitated with anti-TBC1D1; immunoblotted with anti-phospho Akt substrate, PAS) and Akt activity were determined. 3MGT and PAS-TBC1D1 were greater for 0hPEX vs 0hSED. 3MGT in insulin-stimulated muscles was enhanced for 3hPEX vs 3hSED and 27hPEX vs 27hSED, but at neither time did PEX vs SED values differ for PAS-TBC1D1, with or without insulin. Akt activity did not differ for 3hPEX vs 3hSED. These results: 1) support the idea that PAS-TBC1D1 may be important for the insulin-independent GT immediately PEX; 2) indicate enhanced PAS-TBC1D1 is not essential for the insulin-stimulated GT at 3 or 27hPEX; and 3) demonstrate the increased insulin-stimulated GT at 3hPEX is not attributable to increased Akt activity. Previous research suggested that phosphorylation of another RabGAP protein (TBC1D4, also called AS160) may be important for the persistent increase in insulin-stimulated GT post-exercise, but it may not play a major role in insulin-independent GT immediately PEX. Taking these earlier results together with data from the current study, we hypothesize that TBC1D1 and AS160 play complementary roles in modulating exercise effects on GT.

Supported by NIH R01 AG-010026 and DK-071771 to (G. D. Cartee).

FOOT CARE—LOWER EXTREMITIES

35-LB

Enhanced Healing of Diabetic Foot Ulcers Using Local Dry Heat for 30 Minutes 3 Times Per Week: A Pilot Study

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Electrical stimulation (ES) has been used as an adjunct to wound care with questionable results. In previous studies, it has been shown that the use of ES with either global heating of the whole body or local heating of the wound (with an infrared lamp), was effective in healing chronic non healing wounds. Question arose as to the reason for the healing. Is it the heat, ES or both? A few previous studies show that local heating of a wound enhances the healing process but little research has been done with chronic wounds and especially chronic diabetic ulcers. In the present investigation, twenty subjects with chronic non healing diabetic foot ulcers participated in a longitudinal randomized study and received local dry heat (10 subjects) or local dry heat plus electrical stimulation (10 subjects) three times a week over a 4 week period. Average age was 48.4 +/- 14.6 years, average height was 173.7+/-8.4 cm, average weight was 91.4+/-28.1 kg, and the average duration of the wounds was 38.9 +/-23.7 months. A heat lamp was used for 30 minutes to keep the wound warm (37 deg C). For half the subjects, the lamp was used with ES with biphasic sine wave stimulation, a frequency of 30 Hertz, pulse width 250 microseconds, and a current of about 20 mA. Skin blood flow (BF) in and around the wound was measured with a Laser Doppler Flow Imager. In the ES group, average wound area significantly decreased by 68.4+/-28.6% ($p<0.05$) and wound volume decreased by 69.3+/-27.1% ($p<0.05$) over the one month period. Blood flow increased from rest 102.3+/-25.3% with local heat to 152.3+/-23.4% with ES plus local dry heat during the average session. For the local dry heat only group, wounds which would not heal for at least 2 months, showed 30.1+/-22.6% healing after 1 month. This healing was significant but was significantly less than the ES group ($p<0.05$). In conclusion, ES and local dry heat work well together for the healing of chronic diabetic foot wounds, however, local heat would appear to be a relevant part of the equation since ES results alone showed little healing in previous studies.

36-LB

Inhibition of Prostaglandin Transporter Accelerates Wound Healing

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Prostaglandins (PGs), such as PGE_2, are autocrine/paracrine lipid mediators that maintain vasodilation and stimulate cell growth. PGE_2 induces vascular endothelial growth factor (VEGF) and thereby angiogenesis, suggesting that upregulation of PGE_2 could be beneficial for wound healing. Since PGE_2 signaling termination requires its uptake by the prostaglandin transporter (PGT), we hypothesized that PGT inhibition would accelerate wound healing by raising local [PGE_2]. We developed a potent PGT inhibitor, T26A (K_i of 370 nM) by high throughput screening of a small molecule library and structure-activity relationship studies. We previously validated T26A by showing that it raised blood and urinary PGE_2 when given orally to mice. Here, we made paired wounds on the dorsum of wild type C57BL6 mice and applied T26A topically. Compared to vehicle, T26A significantly shortened wound closure time by 3 days. Exogenous PGE_2 also accelerated healing, but not as well as T26A alone. By histological examination, granulation tissue was 650% of control on day 3 and 142% on day 7, which was accompanied by increased epithelialization. Wounds labeled for BrdU revealed T26A-induced increases in proliferating cells (600% and 200% on days 3 and 7, respectively). Judged by CD31 positive cells, vascularization was significantly increased by T26A on day 3. On day 7, the presence of large vessels was 350% that of vehicle treated wounds, and more vessels were close to the wound edge after T26A. To understand the cellular and molecular mechanisms of this vascularization, we created "wounds" in human umbilical vein endothelial cell (HUVEC) monolayers and treated them with PGE_2 ± T26A. Either PGE_2 or T26A significantly enhanced "wound" closure, and the combination was additive. T26A and PGE_2 also caused 3-fold and 6-fold increases in HUVEC migration, respectively, and the combination caused an 8-fold increase. Importantly, T26A caused a 2-3 fold increase in tube formation, an essential of angiogenesis. This enhanced HUVEC angiogenesis by T26A was accompanied by increased VEGF mRNA and protein expression. In summary, these studies add new insights into the pathophysiology of wound healing and offer a novel therapeutic approach.

GENE EXPRESSION—CHIPS AND MICROARRAYS

37-LB

Lysophosphatidylcholine Acyltransferase 3 Is a Direct Target Gene of Peroxisome Proliferator-Activated Receptor α

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Recent evidence suggests the usefulness of peroxisome proliferator-activated receptor (PPAR) ligands in treating type II diabetes and metabolic syndrome including obesity and insulin resistance. PPARα is believed to participate in fatty acid oxidation mainly in the liver and heart. Synthetic ligands for PPARα such as fenofibrate have been used in patients with type 2 diabetes. Identification of direct target genes of PPARα will help us to understand how PPARα ligands improve type II diabetes and metabolic syndrome. In this study, C57B6 mice are treated with vehicle, fenofibrate and rosiglitazone. Lysophosphatidylcholine acyltransferase 3 (LPCAT3) is found to be a hepatic target gene of PPARα. LPCAT3 is a major liver phosphatidylcholine (PC) remodeling enzyme. The LPCAT3 regulation by fenofibrate is lost in PPARα knockout mice. The regulation of LPCAT3 by PPARα is conserved among human, mouse and rat since the regulation is also observed in HepG2 cells and primary rat hepatocytes. We identified an identical DR-1 PPARα response element (PPRE) within -200/+1 region of the LPCAT3 promoters of all three species. Both deletion and point mutagenesis confirm that the conserved PPRE is necessary for the PPARα ligand response of LPCAT3. Electrophoresis mobility shift assay (EMSA) suggests that the PPARα/RXRα heterodimer directly binds to this PPRE. Chromatin immunoprecipitation experiments in Huh7 cells, C57B6 mice and primary rat hepatocytes suggest that LPCAT3 is a direct target gene of PPARα in vivo. These data imply that hepatic phosphatidylcholine remodeling may be important in the pathophysiology of type II diabetes and metabolic syndrome.

For author disclosure information, see page LB32.

GENETICS—TYPE 1 DIABETES

38-LB

Sel1l Is Essential for the Differentiation of Pancreatic Epithelial Cells

SHUAI LI, ADAM FRANCISCO, ANISH K. VANI, ROBERT J. MUNROE, JOHN C. SCHIMENTI, QIAOMING LONG, *Ithaca, NY*

The vertebrate pancreas contains multiple cell types that are derived from a common pool of pancreatic progenitors. Pancreatic development is a highly concerted process controlled by a complex network of transcription factors and signaling molecules. The gene *Suppressor enhancer lin12/Notch 1 like* (*Sel1l*) encodes a cytoplasmic protein and has previously been shown to be highly expressed in the pancreatic epithelial cells. However, the precise morphological or cellular events regulated by *Sel1l* remain unclear. Using a genetic approach in mice, we have revealed a key role of *Sel1l* in the differentiation of endocrine cells of the pancreas. We show that *Sel1l* expression coincides with pancreatic cell differentiation. *Sel1l* is initially expressed in the precocious endocrine cells and is later restricted to the pancreatic epithelial cells that are exiting or exited the cell cycle (post-mitotic). Mice homozygous for a hypomorphic *Sel1l* allele (Sel1l$^{\beta gal}$) die prenatally and display an impaired pancreatic epithelial morphology and endocrine cell differentiation. We demonstrate that the pancreatic epithelial cells of *Sel1l* mutant embryos are trapped in a progenitor cell state. Taken together, our results suggest that *Sel1l* is essential for the differentiation of endodermal-derived endocrine cells. This is a previously unknown function for *Sel1l* and our finding may have important implications for the development of cell-based therapies for type 1 diabetes.

GENETICS—TYPE 2 DIABETES

39-LB

Disruption of Mouse _Sel1l_ Results in Endoplasmic Reticulum Stress and Altered Organismic and Cellular Phenotypes

RAJNI SINGH, ADAM FRANCISCO, SHUAI LI, ANISH K. VANI, ROBERT J. MUNROE, JOHN C. SCHIMENTI, QIAOMING LONG, *Ithaca, NY*

The accumulation of terminally misfolded proteins in the endoplasmic reticulum (ER) will result in "ER stress" if the folding capacity and degradation within the ER is insufficient. In this condition of disequilibrium, the unfolded protein response (UPR) is activated, and consists of several distinct pathways which operate in parallel. The mouse and human *suppressor-enhancer-lin12-1-like* (*Sel1l*) gene encodes a structurally complex protein with high expression in the adult pancreas. Evidence from *in vitro* studies in lower organisms implicates a role for SEL1L in endoplasmic reticulum-associated degredation (ERAD) of unfolded or misfolded proteins, a pathway of the UPR. However, the physiological role of SEL1L in the whole organism remains elusive. We have generated mice carrying a gene trap insertion in intron 14 of the *Sel1l* gene. Mice homozygous for this hypomorphic allele (*Sel1l*$^{-/-}$, mutant) are early embryonic lethal with decreased proliferation in the liver and central nervous system. Mouse embryonic fibroblasts (MEFs) derived from embryonic day 12.5 were used to further characterize the cellular phenotypes of the *Sel1l* mutants. The proliferation of *Sel1l*$^{-/-}$ MEFs was significantly reduced as compared to the *Sel1l*$^{+/+}$ (wild type) and *Sel1l*$^{+/-}$ (heterozygous) MEF counterparts. Challenge of the MEFs with tunicamycin, an *N*-glycosylation inhibitor, shows that *Sel1l*$^{-/-}$ MEFs are more sensitive to ER stress, and exhibit decreased cell viability in comparison to *Sel1l*$^{+/+}$ MEFs. Immunoblot analysis following the transfection of expression plasmids encoding misfolded proteins indicates *Sel1l*$^{-/-}$ MEFs are impaired in their ability to properly degrade such proteins. Electron microscopy reveals distention and fragmentation of the ER in *Sel1l*$^{-/-}$ MEFs. Finally, RT-PCR, immunoblot, and immunohistochemical analysis confirm up-regulation of key ER stress markers in the *Sel1l*$^{-/-}$ MEFs versus *Sel1l*$^{+/+}$ MEFs. Together, these results suggest that the disruption of the mouse *Sel1l* gene results in impaired ERAD, which in turn causes ER stress in embryonic cells. We will discuss the implications of these findings in the context of pancreatic beta cells.

40-LB

Genetic and Chemical Knockdown of Retinol-Binding Protein 4 (RBP4) Does Not Improve Insulin Resistance in High-Fat-Fed Mice

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Retinol-binding protein 4 (RBP4) is secreted by adipocytes and its role in insulin resistance remains controversial. To further understand the involvement of RBP4 in insulin resistance, we have identified A1120, a non-retinoid small molecule which binds with high affinity to RBP4 and displaces its binding partner transthyretin (TTR) from the RBP4-TTR complex. We then conducted a comparative efficacy study to evaluate A1120 (30mg/kg) vs. a synthetic retinoid fenretinide (30mg/kg), the PPARg agonist rosiglitazone (10mg/kg), or vehicle ad libitum as a high fat diet (Bioserv S1850) admixture in RBP4 knock-out (Rpb4$^{-/-}$, n=36) and wild-type (Rpb4$^{+/+}$, n=48) mice. After 2 and 9 weeks of compound administration, the mice were subjected to an insulin suppression test (IST). After 5 and 10 weeks compound administration, an oral glucose tolerance test (OGTT) was performed and serum RBP4 was measured by ELISA. Serum RBP4 levels were significantly decreased in Rpb4$^{+/+}$ mice receiving A1120 and fenretinide compared to vehicle-treated mice after 5 weeks (6.7±0.5 and 20.1±1.6 vs. 70.6±5.0, p<0.001), and 10 weeks (7.8±0.6 and 20.2±1.9 vs. 77.6±4.0, p<0.001) of compound treatment. However, neither compound improved glucose tolerance and insulin sensitivity in Rpb4$^{+/+}$ nor Rpb4$^{-/-}$ mice. In contrast, rosiglitazone remarkably lowered plasma glucose and increased insulin sensitivity in both Rpb4$^{+/+}$ and Rpb4$^{-/-}$ mice without lowering their serum RBP4 and there was no difference in the magnitude of the response between Rpb4$^{+/+}$ and Rpb4$^{-/-}$ mice. In addition, Rpb4$^{-/-}$ mice were indistinguishable from Rpb4$^{+/+}$ in glucose tolerance and insulin secretion following IST and OGTT after high-fat feeding, suggesting that Rpb4$^{-/-}$ mice are not protected from high-fat diet induced insulin resistance. Collectively, our data indicate that RBP4 lowering is not involved in the improvement of glucose tolerance and insulin sensitivity in mice.

41-LB

Genetic Variation in _KCNQ1_ and Type 2 Diabetes: A Population-Based Study

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Recently, genome-wide association studies identified common variants in KCNQ1 to be consistently associated with type 2 diabetes. We aimed to examine whether the two most strongly associated variants (rs2237892 and rs2237897, r^2=0.61) were also associated with type 2 diabetes in a population-based cohort of 3,210 Chinese Hans and to explore the underlying mechanisms. Both SNPs were significantly associated with type 2 diabetes ($OR_{rs2237892}$ 1.36[1.15-1.62], P=0.0004 and $OR_{rs2237897}$ 1.33[1.12-1.57], P=0.0009) and the combined phenotype of IFG/type 2 diabetes ($OR_{rs2237892}$ 1.23[1.10-1.38], P=0.0004 and $OR_{rs2237897}$ 1.24[1.11-1.38], P=0.0002), adjusted for age, sex, region and BMI under an additive model. The corresponding population attributable risks of type 2 diabetes were 32.5% and 35.8%, respectively. The risk C-alleles of the SNP rs2237892 were also significantly associated with lower HOMA-B values (P=0.0175) while the rs22378897 C-alleles showed significant association with lower HOMA-B values (P=0.0005) and higher fasting glucose level (P=0.0115). Notably, the associations with type 2 diabetes were markedly attenuated ($OR_{rs2237892}$ 1.33[1.05-1.68], P=0.018; $OR_{rs2237897}$ 1.22[0.98-1.53], P=0.081) after adjusting for HOMA-B. In the haplotype-based association studies, CC haplotypes showed similar association with type 2 diabetes (OR 1.41[1.18-1.70], P=0.00018), combined IFG/type 2 diabetes (OR 1.27[1.12-1.43], P=0.00012), and with lower HOMA-B values (b=-3.68 ±1.26, P=0.0034). These results suggest that KCNQ1 is a major type 2 diabetes gene in the Chinese Hans and it may confer type 2 diabetes risk by impaired ß-cell function.

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42-LB

Genome-Wide Association (GWA) Meta-Analysis and Replication Identifies Novel Loci Influencing Type 2 Diabetes Risk

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Genome wide association studies (GWAS) have provided novel insights into the biology of type 2 diabetes (T2D). We aimed to identify additional loci influencing T2D susceptibility by meta-analysis of GWAS case control and replication data. In stage 1 we performed a meta-analysis of 8 T2D GWAS studies including 8,130 cases and 38,987 controls (effective sample size 22,570). This approximately doubles the sample size from previous meta-analyses. Excluding 17 previously-proven associations, 25 SNPs ($p<10^{-5}$) were followed-up in 15 stage 2 studies (sample size upto 76,161). A total of 23 signals showed consistent direction of effect in the follow-up samples (binomial $p=2x10^{-6}$), and 18 stage 2 association p values were directionally-consistent at p<0.05. When stage 1 and stage 2 data were combined, 9 SNPs reached genome-wide significance (p-values $2x10^{-8}$ to $7x10^{-17}$). Three of these represent signals near *MTNR1B, IRS1 and KCNQ1* for which there is already strong evidence of T2D-susceptibility effects. One (rs243021 in *BCL11A*) has been reported previously but now reaches genome-wide significance ($1x10^{-13}$). The remaining five signals (on chromosomes 5, 7, 8, 11, and 12) represent novel T2D loci (odds ratios ranging from 1.07-1.13). These 5 novel regions include genes

For author disclosure information, see page LB32.

encoding enzymes in the phosphodiesterase and carboxypeptidase families and other proteins of potential relevance to diabetes pathogenesis. Our findings provide compelling evidence for six novel T2D-susceptibility loci and bring the number of regions known to confer risk for T2D in European populations to 26. The genes located in these 26 loci are involved in a wide range of molecular processes including gene transcription (e.g. *TCF7L2, HHEX, JAZF1),* basic cell cycling (e.g. *CDKAL1, CDKN family, CDC123),* ion transport (e.g. *KCNJ11, KCNQ1, SLC30A8),* circadian clock *(MTNR1B)* and now phosphodiesterase and carboxypeptidase enzymes.

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44-LB

Novel Genetic Loci Implicated in Fasting Glucose Homeostasis and Their Impact on Related Metabolic Traits

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Genetic analyses of continuous glycemic traits can offer insights into the regulation of physiological processes, and have previously led to the discovery of *MTNR1B*, a novel locus influencing type 2 diabetes risk. To identify additional glycemic trait loci and investigate their metabolic impact, we performed meta-analyses of 21 genome-wide associations studies informative for fasting glucose (N=46,263), fasting insulin, and indices of β-cell function (HOMA-B) and insulin resistance (HOMA-IR) (N=38,413). Follow-up of 25 loci in 61,219 independent samples discovered nine new loci for fasting glucose (in or near *ADCY5, MADD, ADRA2A, CRY2, FADS1, GLIS3, SLC2A2, PROX1 and FAM148B*) and a novel genome-wide significant ($P<5x10^{-8}$) association with fasting insulin and HOMA-IR (*IGF1*). Known type 2 diabetes loci *TCF7L2 and SLC30A8* and previously reported fasting glucose loci *GCK, GCKR, G6PC2, MTNR1B* and *DGKB/TMEM195* were also associated with fasting glucose. *GCKR* also achieved genome-wide significant associations with fasting insulin and HOMA-IR. The impact of fasting glucose loci on type 2 diabetes and related metabolic traits suggests shared genetic determinants: *DGKB/TMEM195, ADCY5 and PROX1* with type 2 diabetes and *FADS1/FADS2 and MADD* with lipid levels. Of all associated loci, the most likely biological candidate genes are expressed in islets as determined by qRT-PCR and influence signal transduction, development, glucose-sensing and circadian regulation. The wealth of novel fasting glucose loci and their association with HOMA-B contrasts with the sole fasting insulin/HOMA-IR novel finding, and suggests a different genetic architecture for β-cell function and insulin resistance. **ADA-Funded Research**

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45-LB

Novel Roles of alpha-Synuclein in Energy and Glucose Homeostasis

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Alpha-Synuclein (α-Syn), a 14.5kD protein, was originally isolated from Alzheimer's disease (AD) plaques and was thought to be a pre-synaptic nerve terminal protein. Recent reports suggest that α-Syn is also widely expressed peripherally, including the macrophages. The expression of α-Syn is enhanced in activated macrophages, suggesting that α-Syn may modulate macrophage function and thereby inflammatory processes. It has been well established that adipose tissues in obesity are associated with inflammation. We explored whether the presence or absence of α-Syn may affect macrophage infiltration and activation into adipose tissues, thereby affecting diet-induced obesity and/or glucose homeostasis. Using α-Syn deficient mice, we showed that the lack of α-Syn resulted in reduced body weight, improved glucose tolerance and lower fasting glucose levels. Remarkably, when challenged with high-fat diet (HFD), α-Syn-deficient mice were partially resistant to weight gain and impaired glucose metabolism. Consistent with peripheral site of action of α-Syn, these metabolic effects were reversed upon peripheral over-expression of α-Syn *in vivo*. Furthermore, the number of classically activated adipose tissue macrophages (ATMs) isolated from these mice was reduced, as were major pro-inflammatory cytokines and chemokines in the circulation. In contrast, the alternatively activated macrophages were greatly increased in the visceral adipose tissues of α-Syn-deficient mice, compared to those of the wild type controls. In summary, we show for the first time that α-Syn's function is not exclusive to the central nervous system, but it is also involved in modulating inflammatory responses associated with obesity and glucose intolerance. Finally, our data suggest α-Syn as a novel, promising therapeutic target for the treatment of type II diabetes and obesity.

46-LB

Variants in *GIPR* Are Associated with Insulin Response to Oral Glucose Load: Results from the First Genome-Wide Meta-Analysis of 2h Glucose Levels

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Genome-wide association studies (GWAS) have identified loci underlying type 2 diabetes and related traits. Oral glucose tolerance test (OGTT) can be used to evaluate glucose tolerance status and to assess insulin response to oral glucose load. MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium) recently completed a GWAS-based meta-analysis of glucose levels at 2h during OGTT in individuals without diabetes (24 studies, N=40,442) and identified 5 loci achieving genome-wide significance. Among these loci was *GIPR* (meta $P=1.46x10^{-10}$), which encodes for the glucose-dependent insulinotropic peptide (GIP) receptor. GIP is involved in the incretin effect; the phenomenon of oral glucose eliciting a greater insulin response than intravenous (IV) glucose stimulation. We tested if *GIPR* variant was associated with measures of insulin response during OGTT in up to 16 studies (plus two studies with IV glucose testing). An additive genetic model with age, sex, and study-specific covariates was used to test for genetic association. The *GIPR* variant allele associated with higher 2h glucose levels was associated with lower 2h insulin levels (adjusted for 2h glucose $P=3.12x10^{-14}$; n=27,886), lower insulinogenic index ($P=4.61x10^{-12}$; n=14,970) and lower ratio insulin-to-glucose area under the curve (AUC) during the OGTT ($P=1.00x10^{-14}$; n=14,691). Variation in *GIPR* was not associated with acute insulin response from the intravenous glucose tolerance test (IVGTT; P=0.28, n=562), consistent with its presumed role in the incretin effect. Finally, *GIPR* was associated with the incretin effect (estimated as 100% x ($AUC_{ins\ OGTT}$-$AUC_{ins\ IVGTT}$)/$AUC_{ins\ OGTT}$) in 351 Botnia Study participants with paired OGTT and IVGTT (P=0.003). Further adjustment for BMI did not change the results significantly. In conclusion, genetic variation in *GIPR* is associated with higher 2h glucose levels and lower insulin secretion indices during OGTT, consistent with a receptor defect in mediating the incretin effect of GIP.

47-LB

Variation in *KCNQ1* Is Associated with Reduced Insulin Secretion and Increased Risk of Type 2 Diabetes, but Lower Body Mass Index in Pima Indians

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Variants in *KCNQ1* are associated with type 2 diabetes in Japanese and other populations. To assess the role of *KNCQ1* in diabetes susceptibility in Native Americans, 6 variants strongly associated with diabetes in Japanese were genotyped in a population-based sample of 3501 Pimas. In addition, coding and promoter regions of *KCNQ1* were sequenced in 24 Pimas, and tag SNPs ($r^2>0.8$) that captured all variants identified by sequencing (N=30) and 5 additional database intronic variants were also genotyped. Four of the variants associated with diabetes in Japanese (rs2237892, rs2283228, rs7480855 and rs163170) were highly concordant ($r^2>0.79$) in Pimas and were associated with diabetes (e.g. rs2237892 had an odds ratio (OR) per copy of the C allele = 1.24 (95% CI 1.10-1.40); p = 0.0006 adjusted for age, sex, birth year and family membership). The risk allele frequency in Pimas (0.50) was less common than in Japanese (0.62) and Caucasians (0.92). These variants were also associated with BMI in Pimas ($p=3.5 \times 10^{-6}$ adjusted for age, sex, birth year and family membership), where subjects homozygous for the diabetes risk allele had a mean BMI 2.5 kg/m^2 lower than those homozygous for the non-risk allele. The OR for diabetes increased to 1.32 (95% CI:1.16-1.50, $p=2.7\times10^{-5}$) when adjusted for BMI. Among 278 Pimas with normal glucose tolerance, subjects carrying the diabetes risk allele were not more insulin resistant as measured by the hyperinsulinemic euglycemic clamp, but did have a lower acute insulin response to an IV bolus of glucose (p= 0.0007, adjusted for age, sex, percentage of body fat and insulin action), indicating that this variant increases risk for diabetes via impaired insulin secretion.

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The Population Attributable Fraction of the rs2237892 C allele was 10%, and it explains ~1% of the variance in liability to diabetes.

HEALTH CARE DELIVERY—ECONOMICS

48-LB

Health Disparities in Diabetes Care in the VA

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Eliminating health disparities is a national priority. However, progress has been difficult, due in part to limitations from racial/ethnic differences in socioeconomic status, insurance coverage, and access to care. We asked whether there were differences in diabetes care in blacks vs. whites in the VA– a setting where access would be expected to be relatively uniform. We evaluated care at the times of confirmed evidence of hyperglycemia, initial diagnosis, and first diabetes drug Rx. Initial diagnosis was assigned by either first use of diabetes ICD-9 code 250.xx at a primary care visit, or VA Diabetes Epidemiology Cohort criteria – any use of the code twice, or diabetes drug Rx. In South Carolina, Georgia, and Alabama, 1,456 black and 2,624 white patients had consistent primary care follow-up (at least 3 visits over 24 months prior to initial diagnosis, and at least 4 visits over at least 36 months after initial diagnosis), and met glycemic criteria for diagnosis of diabetes (any two or any value twice of fasting glucose ≥126 mg/dl, random glucose ≥200 mg/dl, or A1c ≥6.5% [<1% of nondiabetics have A1c this high]). At diagnosis, blacks were younger (58 vs. 65 yr, p<0.001) and included more females (4.9% vs 1.6%, p<0.001), but had comparable BMI (30.2 vs 30.0 kg/m^2); blacks and whites also had comparable intervals between hyperglycemia and initial diagnosis (16 vs. 17 months), and between diagnosis and first drug Rx (10 vs. 11 months). However, A1c was higher in blacks vs. whites when hyperglycemia was first confirmed (7.6% vs 7.2%), at initial diagnosis (7.8% vs. 7.1%), and at initiation of drug Rx (8.5% vs. 7.8%), all p<0.001. Multivariate analysis showed that differences in A1c were too large to be explained by differences in age, gender, BMI, and specific effects of race on A1c at comparable glucose levels.

Conclusions: Blacks have higher A1c levels than whites when hyperglycemia is confirmed, the diagnosis is made, and drug Rx is initiated. Such racial disparities in care are present even in the VA healthcare system – a setting where access to care should be uniform. Understanding the basis for such a health disparity will likely be important to improve the health of racial/ethnic minorities in the U.S.

49-LB

Will Wal-Mart Help Patients with Diabetes Get to Target?

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Objective: Increased out-of-pocket expenses for medications are associated with poor compliance and more frequent ER visits. With new generic drug plans available from discount stores, we sought to determine the price differential among pharmacies and project potential effects on medication adherence in patients with diabetes.

Methods: Based on a database covering 52 million insured patients from 91 health plans across the United States, a list of the ten most-prescribed medications in patients with diabetes was compiled (Table 1). Prescription drug pricing was then obtained from the New York and New Jersey State Attorney General offices and verified with the pharmacies themselves.

The Ten Most Widely Used Chronic Medications among Patients with Diabetes

Use Rank	Medication	Number of individual diabetic patients taking this medication over 18 month period 1/1/05-6/30/06
1	Metformin	160,208
2	Atorvastatin	135,450
3	Lisinopril	96,244
4	Rosiglitazone*	68,260*
5	Furosemide	66,999
6	Pioglitazone	66,314
7	Simvastatin	59,814
8	Hydrochlorothiazide	58,827
9	Insulin glargine	56,347
10	Amlodipine	51,008
11	Atenolol	47,070

* Excluded: less commonly prescribed today

Results: Discount stores and mail-order companies have far lower medication prices than neighborhood retailers and convenience chain stores (Figure 1). Price-conscious shopping can save a patient with diabetes several thousand dollars per year.

Figure 1. Cost of a 1-month Supply of Medications*

Conclusions: To patients with diabetes, the new plans offered by discount stores present lower-cost alternatives to neighborhood pharmacies for out-of-pocket medication expenses. As the number of uninsured and underinsured patients grows in difficult economic times, and disposable incomes are set to decline, low-cost alternatives may help improve outcomes and decrease overall costs for the healthcare system.

IMMUNOLOGY

50-LB

WITHDRAWN

51-LB

WITHDRAWN

52-LB

Ectopic Pancreas in the NOD liver

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The NOD mouse is a well characterized model of Type 1 diabetes. In most colonies, approximately 80% of females develop diabetes by 35 weeks of age. However a subset of female mice older than 40 weeks does not develop diabetes despite extensive destruction of beta cells. To investigate potential ectopic insulin expression in these mice (n= 6), their livers were examined by preparing multiple H&E sections. In addition livers from 4 younger non diabetic NOD females (12 weeks old) were also examined in the same manner. Four 48 weeks SCID-NOD female and four 43 weeks Balb/c female mice were utilized as negative controls.

In 3/6 female non-diabetic aged NOD mice the liver contained well formed pancreatic tissue with acinar, ductal and endocrine cells (with multi-hormonal islets). The ectopic pancreatic tissue was centered on the intrahepatic portion of the hepatic duct. Consistent with the autoimmune destruction of their orthotopic pancreatic islets, many ectopic endocrine pancreata showed significant lymphocytic infiltration. Ectopic pancreas was also observed in 2/4 aged SCID-NOD mice, but was not observed in the livers of young female NOD or aged Balb/c mice.

In one of four 12 weeks old NOD mice immunofluorescence staining with a Pdx-1 antibody of liver sections revealed clusters of strongly positive Pdx-1 cells budding from the biliary epithelium of large branches of the hepatic duct.

These data show that the liver of older NOD and SCID-NOD mice contains ectopic pancreas. Although the ectopic pancreas was first detected in long term non diabetic NOD with beta cell destruction, its presence in the liver of two SCID NOD mice indicates that this phenomenon it is not associated with beta cell loss but is characteristic of aging in the NOD strain.

Furthermore the presence of Pdx-1 positive clusters of cells associated with the biliary epithelium, in the absence of ectopic pancreas, suggests that in the NOD mouse there is post-natal pancreatogenesis from the biliary epithelium. Experiments to investigate this phenomenon in additional mouse strains are currently ongoing.

For author disclosure information, see page LB32.

53-LB

Human B Cell Toll-Like Receptor Expression in Insulin Resistance

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Obesity-related insulin resistance is a chronic inflammatory state in which inflammation severity correlates with insulin resistance. The intermediaries of this association are unclear. Recently, the role of Toll-like receptors (TLRs) in causing insulin resistance via cytokines has become appreciated. TLR2 activation is associated with insulin resistance in adipocytes, monocytes, macrophages, and muscle. Novel preliminary work suggests that circulating B lymphocytes expressing TLR2 may have a unique role in modulating systemic inflammation in type 2 diabetes (T2D). We measured B cell TLR2 expression in insulin-resistant subjects with normoglycemia or well-controlled T2D. We hypothesized that TLR2 expression on peripheral B cells would predict lower insulin resistance (IR) and lower levels of inflammatory markers.

We assayed TLR2 expression on circulating B cells in fresh whole blood samples in 33 subjects (Table 1). The median BMI was 39.1kg/m2 and median A1c was 6%. The percentage of TLR2+ B cells was inversely associated with HOMA ($r=-0.50$, $p<0.01$) and triglycerides (TG) (-0.41, $p<0.02$) and positively associated with HDL ($r=0.55$, $p<0.01$). TLR2 expression was lower among subjects with the metabolic syndrome (MetX) than subjects without MetX (1.9% vs 5.6%, $p=0.04$). These relationships persisted when diabetic subjects were excluded from analysis. Furthermore, among non-diabetic subjects HOMA correlated with hsCRP ($r=0.52$, $p<0.03$).

These results suggest that B cell TLR2 expression correlates with a protective phenotype (i.e., lower IR) in overweight and obese subjects at risk for developing T2D. TLR expression on B lymphocytes may reflect modulation of inflammation in obesity, and the failure of B cells to upregulate these receptors in response to systemic inflammation may be a clinical predictor of the development of T2D. Functional studies to support these findings are pending.

Sample characteristics

	Median	Range
Age	46	20-70
BMI (kg/m2)	39.1	28.2-69.8
LDL (mg/dL)	102	39-204
HDL (mg/dL)	45	20-93
TG (mg/dL)	107	34-858
A1c (%)	6.0	4.8-10.2
Fasting Glucose (mg/dL)	98	57-375
HOMA	2.4	0.4-70.6
TLR2 (%)	3	0-54
	n	%
Males	11	33
T2D	15	44
MetX	22	65

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54-LB

Impaired Innate Immunity to *Staphylococcus aureus* in the Diabetic NOD Mouse

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Diabetic patients show a higher incidence of *Staphylococcus aureus* infections than nondiabetic individuals. We developed a *S. aureus* hindpaw infection model and showed that diabetic NOD mice exhibited a delayed inflammatory response and were unable to resolve the infection compared to age-matched nondiabetic NOD mice. To explore defects in innate immunity associated with type 1 diabetes, we utilized a mouse model of systemic staphylococcal infection. When age-matched NOD mice were challenged with *S. aureus* by the intraperitoneal route, the diabetic mice showed impaired clearance of staphylococci from the peritoneal cavity and the blood compared to nondiabetic mice. Blood from nondiabetic NOD mice incubated ex vivo with *S. aureus* showed elevated levels of TNF-a and IL-1b, as well as bactericidal activity. In contrast, *S. aureus* was not killed in diabetic mouse blood, and this correlated with diminished blood levels of TNF-a and IL-1b. Likewise, the respiratory burst of blood neutrophils from diabetic animals incubated in vitro with *S. aureus* was blunted compared to that of nondiabetic mice. Naïve peritoneal macrophages from nondiabetic NOD mice produced higher levels of TNF-a and IL-1b in response to in vitro stimulation with UV-killed *S. aureus* than macrophages from diabetic NOD mice. We examined whether the reduced cytokine production by macrophages from diabetic mice is a result of defects in signaling by Toll-like receptors (TLRs). Up-regulation of TLR-2 expression in response to *S. aureus* was diminished in macrophages from diabetic mice compared with that of nondiabetic mice. A better understanding of how diabetes impacts the innate immune response to infection will aid in the design of treatment or prophylactic modalities to prevent frequent bacterial infections associated with diabetes.

55-LB

Insulin Expression in Thymic Epithelial Cells Is Essential for Insulin Tolerance

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Insulin is one of major targets for type 1 diabetes, and insulin expression in thymus plays a crucial role for tolerance to islet autoimmunity. NOD mice transgenic for insulin 2 driven by I-E MHC class II promoter (I-E: ins2 mice) is strongly protected from diabetes development, and it has been shown that bone marrow derived cells from this strain suppress diabetes but not perfectly. In this study, we demonstrated the critical role of insulin expression in thymus and identified the cell population that is essential for the protection from diabetes. We first investigated the relationship between levels of insulin expression in thymus and diabetes incidence using multiple strains of double insulin knockout NOD mice transgenic for the native proinsulin 2 gene driven by the rat insulin promoter 7. The insulin mRNA expression in thymus was relative to the copy number of the insulin transgene, and the higher copy number of the insulin transgene mice carried, more efficiently diabetes was suppressed ($R^2=0.98$). The question was what thymic cell populations were responsible for insulin tolerance. To investigate whether eliminating insulin expression in thymic epithelial cells abrogate tolerance to insulin, we transplanted insulin 2-knockout thymic epithelial cells [thymus treated with 2-deoxyguanosine] into I-E: ins 2 mice followed by irradiation and bone marrow transplant from I-E: ins2 donor mice. I-E: ins2 mice whose thymic epithelial cells were replaced with that of insulin 2-knockout mice developed insulin autoantibodies and diabetes (n=4), whereas none of non-manipulated I-E: ins2 mice did (n=5, P=0.02). I-E: ins2 mice that were transplanted with insulin 2-knockout bone marrow cells were still protected from diabetes development, thus eliminating insulin expression in bone marrow derived cells was not sufficient to restore islet autoimmunity. Taken together, we conclude that insulin expression by thymic epithelial cells is essential for insulin tolerance and diabetes prevention, and bone marrow derived cells cannot substitute for "stromal" cells provided by insulin expressing thymic epithelial cells.

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56-LB

MicroRNA Expression Profiling in Type 1 Diabetes

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MicroRNAs are non-coding RNAs which regulate gene expression by selective binding to complementary mRNA sequences and inhibition of translation. Unique microRNA profiles are reported in selected cell types and are associated with key physiological processes and diseases. The high frequency of polymorphisms and epigenetic modifications in microRNA genes is consistent with their association with complex diseases. Our ongoing study aims at identifying microRNAs that may be dysregulated in CD4 T cells in type 1 diabetes (T1D). We are testing the hypothesis that selected miRNAs may be over/under expressed in CD4 T cells and that specific microRNA expression patterns may be associated with T1D. We purified naive CD4 T cells and compared microRNA expression in the resting and activated states following *in vitro* stimulation with anti-CD3/CD28 antibodies. MicroRNA expression was quantitatively assessed using Applied Biosystems Taqman Low Density Arrays. We used a<5% false positive rate to identify differentially expressed microRNAs with a $\geq$2 fold-change using the statistical analysis of microarray (SAM) method. We studied 5 T1D patients, 5 autoantibody-negative siblings and 5 autoantibody-positive siblings belonging to 5 families, of which 4 are trios, and 10 healthy subjects. The microRNA miR-565 was upregulated in 4/5 T1D patients, and 5/5 autoantibody-positive siblings compared to 0/5 autoantibody-negative and 0/10 healthy subjects (T1D vs healthy subjects, $p=0.0003$; T1D vs autoantibody-negative siblings, $p=0.007$; autoantibody-positive siblings vs autoantibody-negative siblings, $p=0.047$; autoantibody-positive siblings vs healthy subjects, $p=0.0037$). The mean increase fold change after activation was $18.1 \pm$ SEM 7.7 in T1D patients and $13.1 \pm$ SEM 5 in autoantibody-positive relatives. miR-565 was undetectable

For author disclosure information, see page LB32.

in resting and activated states in autoantibody-negative siblings and healthy subjects. While limited to a few families, our preliminary findings suggest that miRNAs may be powerful markers and large differences could be detected even with limited sample sizes. If confirmed by further and eventually prospective studies, miR-565 could become a biomarker and may aid in predicting T1D.

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57-LB

Modulation of Toll-Like Receptors in Type 2 Diabetes

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Toll-like receptors (TLRs) are a variety of innate immune receptors that recognize bacterial/viral products. TLR4 recognises lipopolysaccharide; TLR2 (with either TLR1 or TLR6) recognizes lipid moieties from gram-positive bacteria. Studies show increased TLR2/4 expression in type 2 diabetic (T2D) patients thus TLR2/4 may mediate diet-induced obesity/insulin resistance and may be involved in the pathogenesis of T2D. We sought to investigate mRNA expression levels of TLR2, TLR6 and TLR4 in non diabetics (n=10, HbA1c 5.3±0.2%) and compare to T2D patients with good (n=9, HbA1c 6.7±0.7%*) and poor glucose control (n=8, HbA1c 10.9±2.2%*) by qPCR (*p<0.05 vs. control). Serum levels of IL-6, IL-1b and TNFa were measured by ELISA. Our data clearly show for the first time that TLR2, TLR4 and TLR6 expression are suppressed in T2D patients with good glucose control (Figure 1). In contrast, TLR expression levels are not suppressed in T2D patients with poor glucose control. Also, whilst IL-1b mRNA and protein are normal in all T2D patients, significant increases in serum IL-6 are evident in all patients with T2D (Median IL-6 values: C, 1.23; GC, 13.33**; PC, 9.23** pg/ml where **p<0.001 vs. C). Whilst TNFa levels in T2D patients with good glucose control are comparable to normal individuals, elevated TNFa is evident in T2D patients with poor glucose control (Median TNFa values: C, 1194; GC, 1147; PC, 2062* pg/ml; *p<0.05 vs. C). These data demonstrate that T2D patients express reduced levels of TLR2, -4 and -6 and support a role for TLRs in T2D. Whether suppression of TLR expression is a causative factor or a consequence of T2D remains to be investigated.

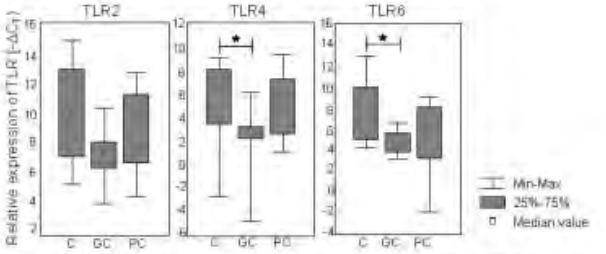

Fig 1: Expression of TLRs in blood mononuclear cells from non diabetic (C) & T2D patients with good (GC) & T2D with poor glucose control (PC)

Supported by Health Research Board (Ireland) and Science Foundation Ireland.

58-LB

Normalization of Obesity-Associated Insulin Resistance through Immunotherapy: Th2 and CD4$^+$Foxp3$^+$ T Cells Control Glucose Homeostasis

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Obesity and its associated metabolic syndromes represent a globally growing challenge, yet mechanistic understanding and current therapeutics are unsatisfactory. Here we report the discovery of unsuspected immune-regulators of obesity-associated insulin resistance: Th2 and CD4+Foxp3+ T-lymphocytes. Visceral adipose tissue (VAT) from diet-induced obese mice and humans (BMI>30) progressively accumulates pathogenic IFNγ secreting Th1 cells that overwhelm static numbers of regulatory Th2 and Foxp3+ T-cells. In obese B6.OT2 T-cell receptor-transgenic mice, there is an accumulation of T-cells in VAT displaying secondary TCR-α rearrangements with TCR-Vα bias, implicating clonal T cell accumulations in adipose tissue. TCR-α bias was also present in CD4+ T cells isolated from VAT of WT mice. Therapeutic increases of Foxp3+ T-cells in VAT, following brief systemic treatment with αCD3 antibody or its $F(ab')_2$ fragment, reverses insulin resistance for months (>3), despite continuing high-fat diet. In contrast, rescue of glucose abnormalities following transfer of purified CD4+ T cells into obese lymphocyte-free RAG^{null} mice proceeded through Th2 cells. Strategies designed to increase Th2 or CD4+Foxp3+ T cells in VAT represents a novel and effective treatment strategy for obesity-associated insulin resistance, whose etiology is unexpectedly under the control of CD4+ T cells and possesses characteristics of tissue-selective autoimmunity.

Supported by Canadian Institutes of Health Research (CIHR).

59-LB

Pre-Proinsulin Specific T Cells Can Be Isolated from the Islets of a T1D Pancreas

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Histological studies of the pancreas in T1D have shown infiltration of islets with T cells. In addition, several studies provide evidence for the presence of autoantigen-specific T cells in the peripheral blood and pancreatic lymph nodes of T1D patients. Immune therapies aimed at preventing lymphocyte destruction of the beta cells require an understanding of the islet-infiltrating cells. However, data on islet infiltrating T cells from T1D subjects are scarce as pancreata from recent onset T1D are rarely available for study. We isolated islets from the pancreas of a 19 year-old patient that was diagnosed 3 years prior with T1D. Flow cytometry performed on islets 12 hours post isolation showed increased expression of MHC class I HLA-A2 relative to non-diabetic islets. Lymphocytes were obtained from the T1D islets by culturing with IL-2 and IL-15 for 7 days. Flow cytometry revealed 38.8% of these cells were CD3$^+$ T cells, of which 62.2% were CD4$^+$ and 30% CD8$^+$ T cells. Histology of the pancreas revealed CD8$^+$ and CD4$^+$ T cells infiltrating islets in situ. Recently, it was shown that processed epitopes from the human preproinsulin (PPI) signal peptide were major targets for circulating effector CD8$^+$ T cells from HLA-A2$^+$ patients with T1D. Using $PPI_{15\text{-}24}$ HLA-A2 tetramer we demonstrated that <1% of the CD8$^+$ T cells from the islets were specific for the $PPI_{15\text{-}24}$ peptide. Five of these clones were stimulated with peptide and expanded using anti-CD3 antibody. This is the first study showing it is possible to culture lymphocytes of this specificity from the islets of a T1D pancreas.

Supported by Australian Federal Department of Health and Aging and JDRF International.

60-LB

WITHDRAWN

INSULIN ACTION—INSULIN RESISTANCE IN VITRO

61-LB

Identification of the Functions of the C-Terminal Fragment of Adiponectin Receptor 1 (CTF1) during the Diabetic Process

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Adiponectin is a hormone secreted by adipocytes that regulates energy metabolism and inflammation. Adiponectin levels are low in obesity, which suggests a function in the suppression of metabolic derangement. Adiponectin also antagonizes the inflammatory effects of TNF-α, which in turn suppresses adiponectin production. Three adiponectin receptors have been identified: AdipoR1 in skeletal muscle, AdipoR2 in liver, and T-cadherin as a novel receptor for high-molecular-weight (HMW) adiponectin multimers. In normal humans, the ectodomain C-terminal fragment of AdipoR1 (CTF1) has been identified, using immunoaffinity SELDI-TOF mass spectrometry, as a 32-amino-acid peptide with 75% hydrophobic residues released in the plasma. Surprisingly, the CTF1 was missing from most diabetic patients tested. Synthetic CTF1 improved insulin resistance (HOMO-IR) by ~30% from one injection in obese diabetic rats. A sharp dose- and time-dependent inhibition of ADAM17/TACE (A disintegrin and metalloproteinases 17 or TNF-α–converting enzyme) activity was observed with CTF1 but not CTF1 truncates. The activity of ADAM17 resumed and remained at 50% after 60 minutes' incubation with 50 mg/L CTF1. The CTF1 also led to the inhibition of insulin-degrading enzyme (IDE), for a reduction of enzyme activity to 60% after 90 minutes' incubation. The ability to produce an acute decrease of ADAM17/TACE activity and a moderately slow decrease of IDE activity suggests the possible cleavage of AdipoR1 by the sheddase ADAM17/TACE to generate CTF1. The highly hydrophobic CTF1 would

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then enter the endosome and inhibit IDE to decrease the degradation of intracellular insulin, which in turn would lead to a rise in insulin sensitivity. The inhibition of ADAM17/TACE by CTF1 may also be a clue for the mechanism of the opposite effects of adiponectin and TNF-α.

INSULIN ACTION—METABOLISM

62-LB

An SSRI Antidepressant Restores Hippocampo-Hypothalamic Corticosteroid Feedback and Recovers Insulin Action in Low Birth Weight Rats—A Novel Path-Finding Pharmacological Strategy

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Objective: Low birth weight (LBW) is associated with type 2 diabetes and depression, which may be related to prenatal stress and chronic hypothalamic-pituitary-adrenal (HPA)-axis hyperactivity leading to insulin resistance. Therefore, we studied whether treatment with a selective serotonin reuptake inhibitor (SSRI, Escitalopram) could down-regulate HPA-axis activity and restore insulin sensitivity in LBW rats.

Research Design and Methods: After 4-5 weeks of SSRI-treatment prenatally stressed LBW (N=75) and saline treated control rats (Cx; N=43) underwent an oral glucose tolerance test or a hyperinsulinemic euglycemic clamp to assess whole-body insulin sensitivity. Hepatic phosphoenol-pyruvate-carboxy-kinase (PEPCK) mRNA expression and red skeletal muscle PkB Ser 473 phosphorylation were used to assess tissue specific insulin sensitivity.

Results: mRNA expression of the hypothalamic mineralo-corticoid-receptor was 5-fold up-regulated in LBW ($P<0.05$ vs. Cx) and accompanied by increased corticosterone release during restraint stress and total 24-hour urinary excretion ($P<0.05$ vs. Cx). Insulin stimulated whole-body glucose uptake was reduced by 26% in LBW ($P<0.001$ vs. Cx), and were associated with impaired suppression of hepatic PEPCK mRNA expression ($P<0.05$ vs. Cx) and a tendency for reduced red muscle PkB 473 phosphorylation. The SSRI-treatment normalized corticosterone secretion ($P<0.05$ vs. LBW), and reversed whole-body insulin sensitivity, postprandial suppression of hepatic mRNA PEPCK expression, and red muscle PkB 473 phosphorylation ($P<0.05$ vs. LBW).

Conclusion: These data support the hypothesis that insulin resistance in LBW is due to chronic HPA-axis hyperactivity and show that treatment with an SSRI down-regulates HPA-axis activity and restores insulin sensitivity in LBW rats.

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63-LB

Circulating Insulin Promotes CD36-Dependent Fatty Acid Storage in White Adipose Tissue for a Considerable Part Indirectly through K_{ATP} Channels in the Central Nervous System

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Central insulin administration increases mass and cell size of white adipose tissue (WAT). The aim of this study was to determine to what extent the stimulating effects of circulating insulin on fatty acid (FA) uptake in WAT are mediated through the central nervous system.

First, we studied the effect of icv administration of insulin in C57Bl/6 mice on tissue-specific partitioning of plasma TG and free FA using glycerol tri[^{3}H]oleate labeled VLDL-like emulsion particles and albumin-bound [^{14}C]oleate as tracers. Icv administration of insulin selectively increased the uptake of both TG-derived FA (+69%; $P<0.05$) and albumin-bound FA (+86%; $P<0.05$) by WAT, which was completely blocked by co-administration of tolbutamide, an inhibitor of ATP-dependent K-channels, required for central insulin action. In these experiments there were no differences in relevant plasma parameters (insulin, glucose, FA and TG levels) between different conditions. The fact that central insulin increased the uptake of TG-derived FA as well as albumin-derived FA indicates a mechanism in WAT distal to lipoprotein lipase. These effects of icv insulin on FA uptake by WAT were completely abrogated in CD36-/- mice, indicating that the long chain FA transporter CD36 plays a crucial role in the effects of central insulin on FA uptake by WAT.

Second, we investigated to what extent insulin signaling in the central nervous system is involved in the stimulation of tissue-specific partitioning of plasma TG and FA by circulating insulin. Iv infusion of insulin during a glucose clamp, stimulated the uptake of TG-derived FA (+72%; $P<0.01$) and albumin-bound FA (+265%; $P<0.01$) by WAT. These stimulatory effects of circulating insulin were abrogated by concomitant icv administration of tolbutamide.

In conclusion, circulating insulin stimulates CD36-dependent FA uptake by WAT for a considerable extent indirectly through activation of K_{ATP} channels in the brain. These observations highlight a paradigm that circulating hormones can act on target tissues directly as well as indirectly through the central nervous system.

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64-LB

CREBL2, the Homolog of a Nutrient-Egulated Fly Gene (CG18619), Is Involved in Glucose Homeostasis and Associated with Diabetes and BMI in Humans

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The Drosophila gene, CG18619, is a nutrient-responsive gene that is rapidly and dramatically down-regulated following protein refeeding in flies. Expression is reduced by 75% within 3 hours of refeeding and remains suppressed for 12 hours. Conversely, expression is increased in larvae on a diet of sugar only. The protein encoded by CG18619 was found to be 73% homologous to the product of the mammalian gene, Cre-binding protein-like 2 (CREBL2). CREBL2 contains a 48 amino acid region that exhibits 41% identity with the bZip domain of the DNA binding protein CREB, suggesting that it functions as a DNA-binding transcription factor. The human CREBL2 gene is localized on Chromosome 12, and nominal associations of CREBL2 with HOMA, BMI and waist circumference were observed in two populations in a whole genome scan for genes associated with metabolic disease. A followup candidate gene study looking at CREBL2 genetic variation in a population of 884 Caucasians revealed strong associations with BMI, fasting plasma glucose, HbA1c and diabetes. Little is known about the function of the CREBL2 gene product. However, it has been reported to localize to the nucleus, consistent with its putative transcription factor function (Saydam et al, Vet. Micobiol. 113: 185, 2006) and to be a target of the HNF1α and HNF4α transcription factors in hepatocytes and pancreatic islets (Odom et al., Science, 303:1378, 2004). In order to gain more insight into CREBL2 function, mice lacking CREBL2 were generated. CREBL2 KO mice are viable and fertile, however they exhibit fasting hyperglycemia and glucose intolerance, without significant differences in plasma insulin levels. Together, these data suggest that CREBL2 has an important, evolutionarily-conserved function in glucose homeostasis.

INSULIN ACTION—SIGNAL TRANSDUCTION

65-LB

Inactivation of Glycogen Synthase Kinase-3α Improves Insulin Sensitivity in Lep ob/ob Mice and Prevents High Fat Diet-Induced Glucose Intolerance

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Glycogen Synthase Kinase-3α/β (GSK-3) are related serine/threonine protein kinases which act to inhibit glycogen synthase, the primary regulator of glycogen synthesis. GSK-3 expression and activity have been found to be upregulated in tissues of diabetic humans and rodents. We have recently shown that GSK-3α knockout (GSK-3α KO) mice exhibit improved glucose tolerance and insulin sensitivity compared to wild type (WT) littermate controls. This was accompanied by enhanced hepatic insulin signaling and glycogen deposition. We hypothesized that GSK-3α KO animals might rescue, in part, the diabetic phenotype associated with the Lep ob/ob mouse model. In addition, we predicted that GSK-3α KO mice would exhibit partial protection against diet induced insulin resistance. Knockout of GSK-3α in Lep ob/ob animals does not significantly alter body weight or fat/lean mass. Glycaemic excursion following an i.p. glucose load was significantly reduced in GSK-3α KO/Lep ob/ob compared to WT/Lep ob/ob control animals. The GSK-3α KO/Lep ob/ob mice also display significantly improved insulin sensitivity compared to Lep ob/ob alone. WT and GSK-3α KO animals were fed ad libitum a regular chow or high fat diet with 5 % or 45 % kcal from fat, respectively for 20 weeks. GSK-3α KO and WT animals displayed comparable weight gain on a high-fat diet. However, glucose tolerance was markedly improved in high fat fed GSK-3α KO animals compared to WT high fat fed mice. Food intake and energy expenditure was unchanged in GSK-3α KO animals on either the Lep ob/ob background or in animals fed a high fat diet. In summary, genetic inactivation of GSK-3α partially rescues the severe insulin resistance associated with the Lep ob/ob model and prevents high fat diet-induced

For author disclosure information, see page LB32.

glucose intolerance. These data suggest GSK-3α as a potential therapeutic target for the alleviation of insulin resistance and Type II diabetes.

66-LB

Role of PKCδ in High Fat Diet-Induced Insulin Resistance

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Insulin resistance is a hallmark of type 2 diabetes. It has been postulated that insulin resistance arises when pathological levels of free fatty acids (FFA) and proinflammatory cytokines disrupt insulin signaling in responsive tissues. Recently, the protein kinase C delta isoform (PKCδ) has been implicated as a FFA- and a proinflammatory cytokine-regulated protein kinase that is associated with inhibition of insulin signaling and action. To gain insight into the physiological role of PKCδ in diet-induced obesity, glucose intolerance, insulin resistance, and inhibition of insulin signaling, PKCδ null mice and their wild-type littermates were fed a high-fat or control diet for 10 weeks. Weight gain was monitored every five days, and, at the end of the 10 weeks, *intraperitoneal* glucose and insulin tolerance tests were performed. No difference was observed in weight gain, insulin sensitivity or glucose tolerance between PKCδ null mice and their wildtype littermates on the control diet. In contrast, PKCδ null mice on a high fat diet were protected from overt weight gain compared to wild type littermates; the deleterious effect of a high fat diet on glucose tolerance in wildtype mice was completely blocked in PKCδ null mice; and the PKCδ null mice on a high fat diet had improved insulin sensitivity compared to wild type littermates. To directly test the role of PKCδ in induced cellular insulin resistance, primary hepatocytes and adipocytes from the high-fat diet mice were isolated and stimulated with insulin. Primary hepatocytes from PKCδ null mice had improved insulin-stimulated Akt and FOXO phosphorylation compared to hepatocytes from wildtype littermates. Consistent with this result, the ability of insulin to inhibit lipolysis and stimulate Akt phosphorylation was improved in primary adipocytes isolated from PKCδ null mice. These results indicate that PKCδ plays a role in high-fat diet induced insulin resistance and are consistent with the hypothesis that PKCδ is a negative regulator of insulin signaling and thus may be a therapeutic target for the treatment of type 2 diabetes.

INTEGRATED PHYSIOLOGY—ADIPOCYTE BIOLOGY

67-LB

CD36 Plays an Essential Role in Thermogenesis in Mice

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The scavenger receptor CD36 (CD36) is found on the surface of brown adipocytes (BA), skeletal muscle cells and others. In muscle, CD36 level has been shown to correlate with maximal fatty acids (FA) transport capacity and it has been suggested to be required for mitochondrial transfer of FA. Nevertheless, CD36 role in brown adipose tissue (BAT) biology remains unexplored.

By housing CD36KO mice at 4°C for 5 hrs, a sharp drop in body temperature (BT) was observed (WT=30.3°C±1.23;KO=21.4 ±0.9).

Plasma FA levels were increased in the CD36KO prior and during cold exposure (CE) (time 0: WT 0.36±0.05mmol/L vs KO 0.64±0.018; 2.5hrs WT 0.46±0.04 vs KO 0.82±0.05; 5hrs: WT 0.52±0.06 vs KO 0.83±0.05) indicating that FA fuel levels were not limiting for thermogenesis. A FA uptake assay with WT and CD36KO BA showed no changes (WT 100% vs KO 129%±35). Macroscopic observation was suggestive of KO BAT hypertrophy; when expressed as % body weight, KO BAT was enlarged (WT 0.33±0.09; KO 0.44±0.17). The BAT triglycerides (TG) stores were stained with $BODIPY_{493/503}$ and microscopy revealed that both WT and KO BAT were engorged with fat but the KO BAT failed to deplete its fat stores (WT 1.44% fat droplet/cell volume; KO 5.19%) after the CE.

We speculated that the main BAT defect in CD36KO is due to impaired mitochondrial utilization of FA. We indeed detected CD36 in purified mitochondria from BAT. The ability to shift fuel utilization from carbohydrate to fat in response to a β_3 adrenergic agonist was reduced by 38% in the KO. The production of $^{14}CO_2$ from ^{14}C-Palmitate was reduced by 66% in BA from the KO, confirming they have a defect in the utilization of FA. The failure to oxidize FA leads to an enhanced utilization of glucose (GLU). CD36KO experience hypoglycemia during CE (WT 189±15.6mg/dl;KO 90±1.85mg/dl). We confirmed this by rescuing CD36KO mice from hypothermia with an IP injection of GLU at 1.5, 3 and 4.5 hrs of CE, resulting in normalized BT (WT 33.5°C ±0.8; KO 26.8±2.7; GLU-injected KO 32.4±0.9).

We conclude that CD36 plays a role in FA oxidation for heat production in BAT. Absence of CD36 limits the ability of rodents to fight cold. It decreases fat consumption and might lead to obesity. **ADA-Funded Research**

68-LB

Fat-Specific Protein 27 Protects Against TNFa Mediated Lipolysis in 3T3-L1 Adipocytes

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Fat-specific Protein 27 (FSP27)/Cidec is a lipid droplet protein whose expression is differentiation-dependent in adipocytes. Expressing FSP27-GFP in preadipocytes and COS cells increases triglyceride accumulation, whereas siRNA mediated depletion of FSP27 in 3T3-L1 adipocytes causes lipid droplet fragmentation, and enhanced basal and stimulated lipolysis. In the present study, we have examined the function of FSP27 in relation to TNFa, a cytokine known to cause lipolysis. We have shown that TNFa decreases FSP27 both at mRNA and protein levels which is accompanied by increases in lipolysis. Under these conditions, further depletion of FSP27 using siRNA accentuates TNFa-mediated lipolysis. Overexpression of FSP27 using adenovirus protects against TNFa mediated lipolysis, and against formation of small lipid droplets. We also found that decreases in FSP27 after TNFa treatment coincided with lipid droplet fragmentation but not with lipolysis, suggesting that TNFa mediated lipolysis and lipid droplet fragmentation are temporally different processes. As expected, we observed that adipose triglyceride lipase (ATGL) is required for the increased lipolysis caused by FSP27 depletion and/or TNFa treatment. These data also implicate FSP27 in maintaining lipid droplet dynamics, perhaps indirectly effecting lipolytic rates. Together these data show that FSP27 has an important role in TNFa mediated lipid droplet fragmentation and lipolysis in cultured adipocytes.

69-LB

Metabolic Role of Atrial Natriuretic Peptide in Human Adipocytes

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The natriuretic peptides (NPs) play an important role in sodium and blood pressure homeostasis by regulating natriuresis and vasomotor tone. Recently a metabolic role for atrial natriuretic pepetide (ANP) has been proposed based on the observation that ANP stimulates lipolysis in primate adipocytes by activating the natriutretic peptide receptor-A (NPR-A) and increasing intracellular cGMP. ANP-induced lipolysis increases circulating fatty acid levels which likely contribute to skeletal muscle energy utilization during exercise. The effects of ANP on human adipocytes have not been well characterized.

We hypothesized that ANP plays an important role in regulating energy homeostasis in human adipocytes by inducing AMP-activated protein kinase (AMPK), triggering the switch from carbohydrate to lipid oxidation and increasing oxygen consumption. To test this hypothesis, human subcutaneous preadipocytes obtained from healthy subjects (Cell Applications, Inc) were differentiated to adipocytes in media containing 5mM glucose, 10% fetal bovine serum, insulin (5µg/ml), IBMX (250 µM) dexamethasone (1.25 µM) and indomethacin (100 µM). After overnight serum depletion, adipocytes were treated with 100 nM ANP for 1h. Concurrent with a 4-fold increase in lipolysis, ANP activated phosphorylation of AMPK and resulted in a 50% increase in maximum respiration capacity (measured by O_2 consumption in the presence of FCCP). A similar increase in maximum O_2 consumption (~45%) was also observed with 100 nM of the β-adrenergic agonist, isoproterenol. No further increase was observed in adipocytes treated with both lipolytic agents. These results demonstrate for the first time that ANP-induced lipolysis in human adipocytes is associated with activation of AMPK and increased O_2 consumption. The metabolic response to ANP was not altered when adipocytes were loaded with 1 mM oleate or palmitate for 48 h indicating that increased triglyceride accumulation does not dysregulate adipocyte energy utilization in response to ANP, in normal human adipocytes. These data are consistent with a metabolic role for ANP in human adipocytes through activation of AMPK to regulate cellular energy homeostasis.

70-LB

Niacin Decreases Serum Retinol Binding Protein 4 (RBP4) Concentrations in Humans with Metabolic Syndrome

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Serum retinol binding protein 4 (RBP4) concentrations are primarily derived from liver and adipose tissue and are positively associated with insulin resistance. Niacin is one of the most effective pharmacological strategies to

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reduce serum triglycerides. However, recent studies from our laboratory demonstrate that niacin also increases serum adiponectin concentrations and the secretion of adiponectin following GPR109A activation. Since serum adiponectin concentrations are inversely associated with serum RBP4, our purpose was to characterize the effects of niacin on serum RBP4 concentrations and to explore the direct effects of niacin on RBP4 secretion in primary adipocyte and hepatocyte culture. Fifteen male participants with metabolic syndrome were treated for 6 weeks with extended-release niacin. Serum RBP4 concentrations were reduced by 14.8% from 36.6 ± 3.2 to 30.8 ± 2.8 µg/mL. The reduction in RBP4 was associated with initial RBP4 concentrations ($r = -0.49$, $P < 0.05$) and the HMW/LMW adiponectin ratio ($r = -0.59$, $P < 0.05$). To further address these findings, male Sprague-Dawley rats were treated with a single dose of niacin (n=6; 30 mg/kg, p.o.) or placebo (n=6; 0.9% saline). Niacin reduced serum RBP4 concentrations by 15.5% within 1 hour and the reduction was maintained for up to 24 hours. In addition, wild-type (n=6) and GPR109A knockout (n=8) mice were randomly assigned to receive a single dose of niacin (30 mg/kg, i.p.) or placebo (0.9% saline). Niacin administration decreased serum RBP4 by 17.5% within one hour in wild-type mice but did not affect serum RBP4 in GPR109A knockout mice. We then tested the direct effects of niacin on RBP4 secretion in isolated primary rat adipocytes and hepatocytes. Niacin (10 µM - 1 mM) had no effect on RBP4 secretion over the course of 24 hours in either primary adipocyte or hepatocyte cultures. These results suggest that GPR109A activation is required to reduce serum RBP4 concentrations following niacin administration and that the reduction in RBP4 is not mediated by direct effects of niacin on adipocyte or hepatocyte secretion.

71-LB

Prediction of Insulin Resistance by Large Visceral Adipocyte Cell Size in the Fat Fed Dog: Reversal with CB1 Receptor Antagonist

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Diet-induced obesity is associated with increased adipocyte cell size. Hence, independent of cell quantity in fat depots, cell size is known to play a key role in the development of insulin resistance. We have previously shown that six weeks of fat feeding (6g/kg/day) in the canine model increases both subcutaneous (SQ) and visceral (VIS) fat depots with a concomitant reduction in insulin sensitivity (SI). We also demonstrated that rimonabant reduced both body fat and body weight along with an improvement in SI. The current study examines the longitudinal changes in adipocyte size and distribution in VIS and SQ fat depots during the development of obesity-induced insulin resistance and following treatment with rimonabant. In addition, we examined whether adipocyte cell size and distribution could predict insulin resistance. Animals were fed a high fat diet fat (52% fat) diet for a period of 6 weeks to increase fat deposition in both VIS and SQ adipose depots and then treated for an additional 16 weeks with either 1) placebo (PL; n=9) or with the CB-1 receptor antagonist rimonabant (RIM; 1.25 mg/kg per day; n=11). Utilizing univariate normal mixture decomposition, we demonstrated that fat feeding during the initial 6 weeks increased mean adipocyte size and induced a bimodal distribution pattern only in the VIS depot, SQ distribution was unchanged but mean cell size was increased. An additional 16 weeks of fat feeding revealed 4 normal distribution patterns in the VIS depot while SQ adipocytes became larger and exhibited a bimodal distribution pattern. In both VIS and SQ depots, RIM completely prevented the formation of large cells and essentially normalized cell size to pre-fat conditions. Moreover, multilevel mixed modeling linear regression method revealed that visceral adipocytes > 75 mm were predictors of whole body and hepatic insulin sensitivity. In contrast, none of the traits of the SQ adipocytes and RIM treatments had any predictive power. These data are the first to provide direct evidence that large adipocytes and their distribution in the visceral fat depot are critical predictors of insulin resistance.

INTEGRATED PHYSIOLOGY—NUTRIENT METABOLISM (AMINO ACID AND FATTY ACID COMBINED)

72-LB

CVT-3619, a Partial A_1 Adenosine Receptor Agonist, Safely Lowered Free Fatty Acids (FFA) in Normal and Overweight Healthy Volunteers

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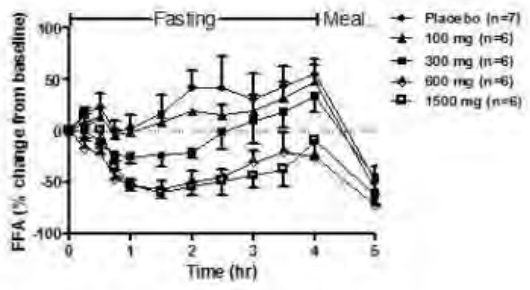
Elevated FFA levels play a significant role in the pathogenesis of insulin resistance and diabetes; lowering them by reducing lipolysis may improve insulin sensitivity and serve as a potential treatment of type II diabetes. CVT-3619 is a selective novel partial A_1 adenosine receptor agonist that reduced circulating FFA and triglyceride (TG) levels in animal models, resulting in improved insulin sensitivity. We carried out a First in Human, placebo-controlled study in normal (Part A, N=55) and obese/overweight (Part B, N=23, BMI ≥ 25 and ≤ 45 kg/m^2 with serum TG > 150mg/dL) volunteers. Single ascending doses, given to sequential dose groups, were 10, 30, 100, 300, 600, 1200, 1500 and 1800 mg in Part A and 300, 600, and 1500 mg in Part B. In parts A and B subjects fasted overnight through 4 hours post dose to maximize baseline FFA levels. Dose-dependent reductions (maximum ~65%) in circulating FFA levels were seen in parts A and B after a single dose of CVT-3619. No significant changes in TG, insulin or glucose were observed. Overall, CVT-3619 was safe and well tolerated up to 1800 mg in normal and up to 1500 mg in obese/overweight subjects. Headache was the most common adverse event (AE) across dose groups in both Parts A (6/47) and B (4/17). All adverse events (AE's) were mild to moderate and no serious AE's were observed.

No significant effects were observed on heart rate, systolic or diastolic blood pressure even at the highest dose; anti-lipolytic effects were observed at doses ≥300 mg. Highly variable but dose proportional increases in maximum plasma concentrations of CVT-3619 (C_{max}) and area under the plasma concentration curve (AUC) were observed. C_{max} was reached 30-45 minutes (T_{max}) after dosing. The plasma concentration half-life ($t_{1/2}$) of CVT-3619 was ~1 hour. Pharmacokinetics in Part A and Part B were similar.

Figure: Dose- and Time-dependent Effect on FFA Levels in Healthy Volunteers Part A



73-LB

Fatty Aldehyde Dehydrogenase Is Upregulated by PUFA Via PPARα and Ameliorates PUFA-Induced ER Stress

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Fatty aldehyde dehydrogenase (FALDH, also known as ALDH3A2 or ALDH10) is a 54 kDa protein that oxidizes medium or long chain aliphatic aldehydes. FALDH deficiency in humans is known to be the cause of Sjögren-Larsson syndrome (SLS) that displays neurological symptoms and cutaneous abnormality. Our previous study showed that FALDH-V, a splice isoform of FALDH, is localized in the peroxisome and contributes to the oxidization of pristanal, an intermediate of the α-oxidation pathway. FALDH-N, another splice isoform of FALDH, is induced by peroxisomal proliferator activated receptor α (PPARα) ligands but its activation mechanism has not been clarified. Here, we show that transcriptional activation of *FALDH* is directly regulated by PPARα through a direct repeat-1 (DR-1) site located in the *FALDH* promoter. In addition, FALDH is efficiently induced by palmitic acid and linoleic acid in rat hepatoma Fao cells through transcriptional activation by PPARα. Furthermore, ectopic expression of ER-localizing FALDH-N, but not peroxisome-localizing FALDH-V, suppresses ER stress caused by linoleic acid in HEK293 cells. It has been proposed that ER stress and the cell toxicity caused by free fatty acids in plasma, called lipotoxicity, is associated with the development of metabolic syndrome and diabetes mellitus. It was shown that the ER stress response plays crucial roles in the mechanisms of metabolic syndrome, diabetes mellitus, obesity and neurodegenerative disorders such as amyotrophic lateral sclerosis and Alzheimer's disease. The ER stress response is a biological defense system for resisting environments that induce the accumulation of misfolded proteins. Excess ER stress might induce apoptosis as the ultimate response. These results suggest the autocatalytic nature of the FALDH-N system against lipid peroxidation; oxygen-sensitive linoleic acid binds to PPARα to activate expression of FALDH-N, which then catalyzes oxidative detoxification of linoleic acid-derived fatty aldehydes and protects cells from linoleic acid toxicity.

For author disclosure information, see page LB32.

74-LB

Metabolomic Analysis of Muscle Insulin Resistance in Humans Suggests Defects in Lipid Oxidation and TCA Cycle Oxidative Capacity

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It is of high importance from a scientific and public health perspective to identify reliable biomarkers for diabetes prevention. To this aim, we used microarray and MS-based metabolic profiling of skeletal muscle biopsies from 23 nondiabetic humans with a spectrum of insulin sensitivity (S_I). Among the 186 named compounds identified, 19 metabolites differed ($p<0.05$) in insulin resistant (IR) vs. insulin sensitive (IS) subjects. Strikingly, 9 of the 19 metabolites belonged to pathways positively correlated with S_I in parallel microarray analysis: fatty acid metabolism and tricarboxylic acid (TCA) cycle substrate delivery pathways, including branched-chain amino acid (BCAA) metabolism.

We observed distinct patterns of lipid metabolites in IR subjects. While long-chain acylcarnitines (C14-18) tended to be decreased, medium/short-chain acylcarnitines (octanoylcarnitine, hexanoylcarnitine and valerylcarnitine) were significantly increased (162%, 96%, & 183%, respectively), suggesting higher, but incomplete, fatty acid oxidation. We hypothesize that impaired fatty acid oxidation is mediated, at least in part, by reduced substrate delivery to the TCA cycle. Consistent with this hypothesis, TCA intermediates α-ketoglutarate, fumarate and citrate were decreased (56%, 38% & 37%) in IR; oxalacetate and succinate tended to be similarly reduced. Finally, the 3 branched-chain α-ketoacids (3-methyl-2-oxobutyrate, 4-methyl-2-oxopentanoate and 3-methyl-2-oxovalerate) were decreased in IR (37%, 47% & 41%), suggesting altered BCAA oxidation.

Together, these data suggest that IR is characterized by increases in overall fatty acid oxidation, but accumulation of short- and medium-chain intermediates resulting from incomplete oxidation. This pattern may result from reduced TCA oxidative capacity, as suggested by reductions in multiple TCA intermediates. We further hypothesize that decreased gene expression within anaplerotic pathways may contribute to reduced TCA substrate delivery and thus impair TCA oxidative capacity in IR. These patterns may be useful for metabolic staging and identification of early defects in IR, prior to the onset of dysglycemia.

INTEGRATED PHYSIOLOGY—OTHER HORMONES

75-LB

Ghrelin Suppresses Glucose-Stimulated Insulin Secretion in Healthy Humans

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The orexigenic gut hormone ghrelin and its receptor, the growth hormone secretagogue receptor 1a, are present in pancreatic islets and ghrelin reduces insulin secretion in rodents. However, the effect of ghrelin on insulin secretion in humans has not been established. We tested the hypothesis that peripheral administration of ghrelin suppresses glucose-stimulated insulin secretion in healthy subjects. Methods: Ghrelin (1, 3 and 5 mcg/kg/hr) or saline was infused over 75 min in 9 healthy subjects (7M/2F; age 26±11.4 years [mean ± SD]; BMI 24.1±4.2 kg/m^2) with normal fasting glucose levels (4.7±0.5 mM) on 4 separate occasions in a counterbalanced fashion. An IV glucose tolerance test was performed after ghrelin or saline had been infused for 55 min. An IV bolus of 50% Dextrose (11.4g/m^2 body surface area) was given at time 0 and frequent blood sampling performed between 2 and 20 min. The acute (first phase) insulin response to IV glucose (AIRg) was calculated from plasma insulin between 3 and 6 min. Total ghrelin was measured using a radioimmunoassay. Results: Ghrelin infusions raised plasma total ghrelin levels to 2.4-, 7.8-, and 13.6-fold above fasting concentrations (1390.8±579.6 vs. 3365.7±1197; 1384.8±387.7 vs. 10765.7±4372.1; 1573.1±729.6 vs. 21387.2±7164.1 pg/ml, respectively), and steady-state levels were reached after approximately 50 minutes. Baseline plasma insulin and glucose levels were not affected by ghrelin infusion (p=0.09 and 0.38, respectively). The 1, 3, and 5 mcg/kg/hr infusions of ghrelin significantly decreased AIRg compared to the saline control (333.6±178.7, 332.0±200.8 and 297.7±104.5 vs. 488.4±271.0 pM/l, p=0.028, 0.009 and 0.004, respectively). Conclusions: Exogenous ghrelin reduces the first-phase insulin response to IV glucose in healthy humans. These findings raise the possibility that endogenous ghrelin has a role in physiologic insulin secretion, and that ghrelin antagonists could improve β-cell function.

Supported by NIH/NIDDK (DK080081-01).

76-LB

Inhibition of Transthyretin Expression by Antisense Oligonucleotides Lowers RBP4 Levels, Improves Insulin Sensitivity and Increases Heart Glucose Uptake in Diet-Induced Obese Mice

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Retinol Binding Protein 4 (RBP4) is secreted by liver and adipose tissue and increased circulating RBP4 levels have been implicated in insulin resistance in rodents and humans. RBP4 binds to transthyretin (TTR) to form a protein complex that reduces renal clearance of RBP4. Circulating TTR levels are also elevated in obese, insulin resistant humans in conjunction with increased RBP4 levels. In the present study, we evaluated the effects of reducing serum RBP4 levels by inhibiting circulating TTR levels in vivo. Obesity and insulin resistance were induced in normal 6 week old C57BL/6J mice by feeding a 58% high-fat diet for 4 months. Subsequently, the animals were treated with saline, control ASO or TTR ASO (25 mg/kg twice weekly for 4 weeks). TTR ASO reduced hepatic expression by 98% and serum TTR levels were undetectable by western blot analysis. This reduction in TTR was accompanied by a 95% reduction in circulating RBP4 levels suggesting increased RBP4 renal clearance. In addition, TTR ASO reduced plasma insulin levels by 32% compared to control groups indicating an improvement in insulin sensitivity. To assess peripheral and hepatic insulin sensitivity, hyperinsulinemic-euglycemic clamps were conducted in these mice. Glucose infusion rate was 50% higher in TTR ASO treated mice (p=0.02) compared to control group. The ability of insulin to suppress hepatic glucose production was improved by 20% (p=0.04) in TTR ASO treated group suggesting improved hepatic insulin action. Peripheral glucose disposal and whole body glycolysis were also improved after TTR ASO treatment. Furthermore, TTR ASO increased glucose uptake in gastrocnemius muscle by 44% (p=0.002) and heart by 59% (p=0.02). These data indicate that reduction of hepatic and circulating TTR levels resulted in significant improvements in insulin sensitivity, indicating that TTR may be a potential therapeutic target for the treatment of type 2 diabetes and associated cardiomyopathy.

INTEGRATED PHYSIOLOGY—LIVER

77-LB

A Novel Network by Which Glutamine/Glutamate Controls Phosphoenolpyruvate Carboxykinase Expression: Potential Role in Development oEf Type 2 Diabetes

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The majority type 2 diabetic patients are obese, yet many insulin-resistant obese individuals do not develop diabetes. To understand this dichotomy, we previously integrated genomic, transcriptomic and metabolomic analysis of an F2 intercross between diabetes-resistant C57BL/6 ob/ob and diabetes-susceptible BTBR ob/ob mouse strains to identify clinical quantitative trait loci (QTL), gene expression QTL, and metabolite QTL. This led to identification of a causal network by which the metabolite glutamine/glutamate (Glx) can induce expression of cytosolic Phosphoenolpyruvate carboxykinase (Pck1), a key gluconeogenic enzyme. Confirming the existence of this network, treatment of hepatocytes from lean C57BL/6 or BTBR mice with 10 mM glutamine increased Pck1 expression, coupled with increased expression of Alanine-glyoxylate aminotransferase (Agxt) and Arginase 1 (Arg1), the two intermediate nodes between Glx and Pck1. Here we report that treatment of lean Wistar rat hepatocytes with 10 mM glutamine also increases Agxt (209% ± 30), Arg1 (429% ± 107), and Pck1 (432% ± 57) expression. These in vitro studies validated our method for identification of QTL-directed causal networks and indicate that the Glx network is not species specific.

To address whether the Glx network contributes to development of diabetes, we studied two rodent models of obesity-induced diabetes. We found that the 10-week-old diabetes-susceptible BTBR ob/ob mouse has significantly increased hepatic Pck1 expression and Glx abundance compared to the 10-week-old diabetes-resistant C57BL/6 ob/ob mice. Similarly, diabetic ZDF fa/fa rats show increased expression of liver Pck1 mRNA and Glx metabolite concentrations relative to age-matched obese, non-diabetic Zucker fa/fa rats. Our findings to date suggest that hepatic Glx levels are increased in two independent models of obesity-associated diabetes in rats and mice relative to non-diabetic obese controls. These increases in Glx may be related to increased expression of Pck1 in both animal models that predisposes to poorly controlled hepatic glucose production and development of type 2 diabetes.

For author disclosure information, see page LB32.

78-LB

Hepatic Mitochondrial Fluxes Are Not Impaired in Diet Induced Obese Mice

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Skeletal muscle insulin resistance and other metabolic abnormalities have been linked to mitochondrial dysfunction, but it is unclear whether a similar etiology is active in the insulin resistant liver. Recently, we demonstrated that mitochondrial fat oxidation is impaired, but other mitochondrial fluxes related to glucose production are elevated in the liver of the Zucker Diabetic Fatty rat. The purpose of the current study is to determine whether these abnormal hepatic mitochondrial fluxes are a required feature of hepatic insulin resistance in a diet induced model of obesity. To test this, male C57/Bl6J mice were made obese and insulin resistant by feeding them a 60% high fat diet (HFD) for 16 weeks and the effect on in vivo hepatic metabolic fluxes was measured by stable isotope tracers, NMR and LC/MS-MS. Mice on the HFD were nearly double the weight of low fat (10%) fed (LFD) controls (46.9 vs 24.5 g), had fasting hyperglycemia (208 vs 113 mg/dl), hyperinsulinemia (2.02 vs 0.145 ng/ml) and were glucose intolerant. Fasting endogenous glucose production was slightly elevated (26.4 vs 21.1 umol/min/g liver protein) in HFD mice, and the hepatic mitochondrial fluxes of pyruvate carboxylase (114 vs 69.0 umol/min/g liver protein), Kreb's cycle flux (66.7 vs 33.3 umol/min/g liver protein) and overall b-oxidation (124 vs 75.6 umol/min/g liver protein) were also significantly elevated compared to LFD mice. These results demonstrate that mitochondrial fat oxidation is not impaired in the liver during mild diabetes, but rather induced, and suggest that impaired mitochondrial fat oxidation is not a required feature of hepatic insulin resistance. **ADA-Funded Research**

79-LB

Lysophsphatidylcholine as a Mediator of Free Fatty Acid-Induced Insulin Resistance in Hepatocytes

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It's well established free fatty acids (FFAs) cause insulin resistance, however, it is still obscure which metabolites of FFA are the effector molecules directly inducing insulin resistance. We have reported an important role for lysophosphatidylcholine (LPC) in lipoapoptosis of hepatocytes by saturated FFAs. Here, we studied a potential for LPC in insulin resistance of hepatocytes because both lipid injury and insulin resistance by lipids are components of metabolic syndrome and could share a common biochemical pathway. Palmitic acid (PA) induced insulin resistance in hepa1c1c7 cells characterized by JNK activation, IRS-1 (Ser307) phosphorylation and decreased insulin-induced Akt phosphorylation. PA-induced insulin resistance was partially reversed by $iPLA_2$ inhibitors such as bromoenol lactone (BEL) or palmitoyl trifluoromethyl ketone (PACOCF3) but not by Fumonicin B1 or Myriocin, suggesting involvement of iPLA2-mediated LPC pathway rather than ceramide pathway. Insulin resistance by PA was also inhibited by Pertussis toxin (PTX), an inhibitor for G protein-coupled receptor (GPCR)/Gαi and a dominant-negative mutant of Gα2i, suggesting a possible role of GPCR/Gαi pathway in the signaling downstream of LPC. These results suggest a potential role for endogenous LPC produced by iPLA2 and GPCR/Gi2α in insulin resistance by FFAs.

INTEGRATED PHYSIOLOGY—REGULATION OF FOOD INTAKE

80-LB

Differential Brain Responses to Ingestion of Glucose and Fructose

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Increases in fructose consumption have parallelled the rise in obesity, and high-fructose diets promote weight gain and insulin resistance in animals. In addition, central administration of fructose provokes feeding in rodents, whereas centrally administered glucose produces satiety. Neuroimaging studies in humans demonstrate a reduction in activation of hypothalamic-limbic regions in response to food stimuli. To test the hypothesis that fructose and glucose intake would produce different responses in brain regions that regulate feeding, we used functional magnetic resonance imaging (fMRI) to compare regional brain activation after acute ingestion of equal quantities of fructose and glucose. Ten healthy, non-diabetic volunteers underwent two fMRI sessions together with ingestion of either a fructose or glucose drink in a blinded, random-order crossover design. Subjects underwent baseline fMRI acquisitions including pulsed arterial spin labeling (PASL) and BOLD sequences to determine regional cerebral blood flow (CBF), a marker of neural activation, and functional connectivity. Subsequently, they drank 75 g of either sugar followed by a 60-min post-drink acquisition and blood sampling period. CBF within the hypothalamus, amygala, hippocampus, and striatum was greater after fructose compared to glucose intake ($p<0.05$). Moreover, fructose ingestion produced an increase in functional connectivity between the hypothalamus (the seed region) and the bilateral insula, amygdala, and hippocampus. In contrast, glucose ingestion resulted in a reduction in functional connectivity between these regions ($p<0.05$). Fructose ingestion caused a much smaller rise in PG, insulin, and GLP-1 than glucose ($p<0.01$), but no difference in plasma leptin levels. Our results suggest that fructose ingestion has a markedly different effect on the activation of the hypothalamic-limbic system than glucose. This disparate response was associated with reduced systemic levels of insulin and GLP-1 and might play a role in promoting feeding behavior.

INTEGRATED PHYSIOLOGY—REGULATION OF GLUCOSE KINETICS

43-LB

Glucose Sensing by Lateral Hypothalamic MCH Neurons Regulates Glucose Homeostasis and Is Modulated by UCP2

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Discrete groups of neurons in the brain are excited by physiological concentrations of glucose. While the mechanism for this effect has been established (↑glucose→↑ATP→closure of K_{ATP} channels), its physiological relevance remains largely unknown. To address this issue, we are using two approaches to perturb glucose-sensing pathways in mice: 1) neuron-specific expression of a mutant Kir6.2 subunit which renders K_{ATP} channels resistant to closure by ATP, and 2) neuron-specific deletion of uncoupling protein 2 (UCP2) which increases glucose-stimulated ATP levels. We have previously established that glucose sensing by pro-opiomelanocortin (POMC) neurons contributes to the regulation of glucose homeostasis. However, left unknown is the role of other glucose-excited neurons. If other glucose excited neurons also possess physiological functions, then their combined effects on glucose regulation are expected to be very large. If true, this would indicate that glucose sensing by the brain is an extremely important component of the system to control global glucose homeostasis.

In the present study, we have investigated the role of melanin-concentrating hormone (MCH) neurons in the lateral hypothalamus, which have previously been shown to be excited by glucose. Of interest, we found that MCH neurons, but not other lateral hypothalamic neurons, express high levels of the K_{ATP} channel subunit, Sur1, and UCP2. Using mice expressing Cre in MCH neurons and mice bearing an allele that confers Cre-dependent expression of mutKir6.2, we specifically expressed ATP-insensitive K_{ATP} channels in MCH neurons. This blocked excitation of MCH neurons by glucose and produced glucose intolerance in mice. Furthermore, we deleted UCP2 specifically in MCH neurons. This manipulation, which is expected to increase intracellular ATP levels, increased the sensitivity of MCH neurons to glucose. Of importance, knockout of UCP2 in MCH neurons dramatically improved glucose tolerance. These studies indicate that glucose-sensing by MCH neurons plays an important role in regulating whole-body glucose homeostasis and could have significant implications for the pathogenesis of T2DM.

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ISLET BIOLOGY—APOPTOSIS

81-LB

Targeted Disruption of Exchange Protein Directly Activated by Cyclic-AMP 1 in Mice Leads to Altered Islet Architecture and Reduced β-Cell Distribution of GLUT-2

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Signaling pathways mediated via the second messenger cAMP play a critical role in regulating glucose-stimulated insulin secretion in β-cells. Recently, two isoforms of cAMP-binding proteins designated as exchange protein directly activated by cAMP (Epac1 and Epac2) were identified to mediate diverse actions of cAMP. Previously, *in vitro* studies using Epac activator 007 highlighted the importance of Epac in insulin secretion. Here,

For author disclosure information, see page LB32.

we demonstrated that Epac1 is expressed in mouse pancreas by Western blot analysis and specifically in the pancreatic islets by immunocytochemistry. To determine the significance of Epac1 in β-cells cells *in vivo*, we have generated Epac1 knockout (Epac1$^{-/-}$) mice. Under normal condition, the Epac1$^{-/-}$ mice maintained similar random and fasting blood glucose levels compared to those of the Epac1$^{+/+}$ mice. However, Epac1$^{-/-}$ mice exhibited impaired glucose tolerance and reduced plasma insulin levels during the IPGTT. Interestingly, although the total pancreatic insulin content and expression level of PDX-1 remained unchanged, the expression level of GLUT2 was remarkedly reduced in Epac1$^{-/-}$ β-cells. Furthermore, Epac1$^{-/-}$ mice displayed abnormal islet cytoarchitecture with an increased number of α-cells in central regions of islets. To gain further insights into the *in vivo* role of Epac1 in β-cell functions, we administrated single injection of streptozocoin (200mg/kg) into both groups of mice and found that the STZ treatment induced comparable levels of diabetes in all mice. However, when exposed to multiple-low-dose streptozocoin (MLDS, 40mg/kg) treatments, Epac1$^{-/-}$ mice rapidly developed more severe hyperglycemia than Epac1$^{+/+}$ mice. Preliminary examination of MLDS-treated pancreatic islets by TUNEL staining revealed a tendency for a higher number of apoptotic cells in Epac1$^{-/-}$ islets than in Epac1$^{+/+}$ islets. All of these data suggest that Epac1 plays an important *in vivo* role in β-cell functions and glucose homeostasis.

ISLET BIOLOGY—BETA CELL GROWTH AND DIFFERENTIATION

82-LB

Novel microRNAs Are Over Expressed in Benign Insulinomas Compared to Benign Non-Functional Pancreatic Endocrine Tumors (PET)

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Pancreatic endocrine cells arise from a common precursor and differentiate into islets comprised of α, β, δ and PP cells. We reasoned that microRNAs involved in proliferation, maintenance, and regulation of insulin secretion in islet β cells differ from those in the other cell types. To examine this, we performed microRNA analysis of benign insulinomas with PETs as the control group. Snap frozen surgical specimens were obtained from patients with benign insulinomas (n = 19) resected for relief from severe hypoglycemia and PETs (n = 10) detected incidentally and resected subsequently. Patients in the insulinoma group were 52.9 ± 13 years old and 53% were male. Patients in the PET group were 55.3 ± 16 years old and 70% male. There was no significant difference in age and gender between groups. We performed microRNA analysis using the HumanMI_V2 chip with ~1145 microRNAs. Analysis performed included normalization using Loess and quality assessment. We removed unexpressed microRNA transcripts and used an ANOVA model to detect differentially expressed microRNAs between the two groups. At a p value of 0.05, there were 105 microRNAs significantly different between the two groups. MicroRNA expressed at log2 fold change greater than mean ± 1, 2, and 3 standard deviation and significant at p value < 0.05 were 72, 31 and 13 respectively between insulinoma and PETs. Among 13 microRNAs identified using the more stringent criteria, five miRNAs were over expressed in insulinomas and 8 others in PETs. MicroRNAs over-expressed in insulinomas were miR-9*, 19a, 19b, 18a and 140-5p. MicroRNA's over-expressed in PETs were miR-211, 130b*, 190b, 433, 129*, 1179, 204 and 184. In contrast, a previous report using less number of patients and a smaller probe set identified over expression of miR-204 in insulinomas. In conclusion, insulinomas and PETs overexpress different microRNAs. The biologic significance of each of these microRNAs remains to be elucidated.

ISLET BIOLOGY—BETA CELL TRANSCRIPTION REGULATION

83-LB

Heterologous Expression of OASIS/CREB3L1 in Pancreatic β-Cells Induces Apoptosis and Genes with CRE and C/EBP Promoter Elements

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OASIS has previously been shown to be a putative endoplasmic reticulum (ER) stress sensor in astrocytes whose mechanism of activation is similar to ATF6. In this study we investigated the expression and activation of endogenous and over-expressed OASIS in pancreatic β-cells. By RT-PCR OASIS mRNA expression was detected in pancreatic β-cell lines and rat islets and the expression level was up-regulated by ER stress-inducing compounds. However, endogenous OASIS protein was not detectable in pancreatic β-cell lines or islets by either western blotting or immunocytochemistry. Over-expressed full-length rat and human OASIS in pancreatic β-cells was targeted to the ER and was cleaved by regulated proteolysis. Over-expression of an active, nuclear-localized version of OASIS failed to induce GRP78 promoter activity in pancreatic β-cells, but did induce promoter activity in C6 glial cells. This indicates that β-cells lack endogenous co-factors required for GRP78 promoter activation. Over-expression of an active, nuclear-localized version of OASIS in an inducible INS-1 β-cell line caused apoptosis and the up-regulation of several genes with CRE or C/EBP promoter elements, but no up-regulation of classical ER stress-inducible genes. These results suggest that OASIS requires additional factors to induce ER stress-inducible genes in cells that naturally express this putative ER stress sensor.

84-LB

Post-Transcriptional Regulation of the beta-Cell-Specific Factor Nkx6.1

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Embryonic development of the pancreas requires the expression of a well-orchestrated network of transcription factors. Within this network, the homeodomain transcription factor Nkx6.1 acts downstream of another homeodomain factor, Nkx2.2, in regulating the differentiation of beta-cells. Interestingly, the Nkx6.1 mRNA is expressed more broadly than the protein it encodes, and in the absence of Nkx2.2, Nkx6.1 protein levels are reduced far more than the mRNA levels, suggesting that Nkx2.2 promotes Nkx6.1 expression post-transcriptionally. Therefore, we explored the role of translation in regulating Nkx6.1 expression and beta-cell differentiation.

Multiple mechanisms can control protein translation, including cis-acting elements of mRNAs such as Internal Ribosome Entry Sites (IRES) and trans-acting factors such as microRNAs. The Nkx6.1 mRNA includes a 1-kb long 5'UTR that we previously showed acts as an IRES. Using sequential deletion analysis, we mapped multiple sequences with independent IRES activity within the Nkx6.1 5'UTR, implicating their involvement in the translation of Nkx6.1. Nkx2.2 strongly enhanced the activities of several of these IRES elements, as well as the intact 5'UTR. Although much shorter, the 3'UTR of the Nkx6.1 mRNA contains several microRNA recognition sites. The microRNAs miR-148a, miR-152 and miR-190 are expressed in the developing pancreas, and their recognition sites are evolutionarily conserved within the 3'UTR of Nkx6.1. Furthermore, we have found that both individually and in combination these three microRNAs strongly down-regulate the activity of the Nkx6.1 3'UTR.

These data demonstrate that the control of Nkx6.1 expression involves complex mechanisms of post-transcriptional regulation. By demonstrating the involvement of translational regulation, these studies extend our overall understanding of the interactions among the network of genes that control pancreas development and beta-cell differentiation.

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ADA-Funded Research

85-LB

Quantification of Glucose-Induced Premature Preproinsulin mRNA Expression from a Single Human Islet for an Assessment of Islet Quality

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The development of a method to assess the β cell function using a small amount of islets allows the transplantation of more islets and facilitates transplantation success in Type 1 diabetes. Although glucose-induced insulin synthesis is mostly regulated at the translational level, transcriptional changes play a role by regulating translation efficacy. In this study, we established an assay to measure changes in human insulin gene (*INS*) expression and assess the biosynthetic capacity of insulin in β cells. Human islets isolated from brain dead donors for research use were hand picked and cultured with either low-glucose (3.3mM) or high-glucose (17mM) media for up to 16 hours. Poly (A)+ mRNA was purified from a set of single handpicked human islets using a 96-well Filterplate and oligo(dT)-immobilized microplate. Glucose induced mature (post-spliced) and premature (pre-spliced) insulin mRNA was quantified by RT-PCR using several insulin mRNA primers designed at different locations in intron, exon, and exon-intron junction. The synthesis of premature *INS* mRNA was significantly increased after 16-hour incubation in high glucose medium as compared to 4-hour incubation ($p<0.05$), while mature *INS* mRNA failed to show any difference. Attenuation of glucose induced premature *INS* mRNA synthesis was observed in the heat-damaged islets. Stimulation index (SI) of the premature *INS* mRNA normalized by the mature *INS* mRNA (SI_mRNA) positively correlated

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with SI of insulin release over 16 hours (r=0.66, p=0.02) and SI determined by perifusion assay (r=0.67, p=0.02). SI_mRNA also correlated negatively with β cell apoptosis (r=0.75, p=0.005) (n=12). Multiple regression analysis of 16 hours premature *INS* mRNA synthesis along with insulin release, reliably predicted blood glucose level (mg/dl) in streptozotocin induced diabetic NOD*scid* mice 21 days after islet transplantation (p=0.005, Adjusted R^2=0.44) (18 mice from 8 donors). The measurement of glucose induced premature *INS* mRNA normalized by the mature *INS* mRNA can be used to assess functional quality of human islets and may predict islet function after transplantation in patients with type 1 diabetes.

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ISLET BIOLOGY—HORMONE SECRETION AND EXOCYTOSIS

86-LB

Glucose Effects on alpha-Cells within Intact Pancreatic Islets

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Glucagon is secreted by pancreatic islet α-cells at low glucose levels. While glucose stimulation of insulin secreting β-cells is well-understood, the mechanisms underlying glucagon release and suppression are still unclear. One hurdle is the lack of reliable method to distinguish α-cells from other islet cell-types. Here we describe the use of new transgenic mouse lines that specifically express fluorescent proteins within α-cells. Islets from these animals allow us to measure glucose-stimulated biophysical properties of α-cells present in intact islets.

Two-photon excitation measurements of NAD(P)H autofluorescence show that glucose stimulates α-cell metabolism in a dose-dependent fashion (signal increase of 46% from 1 to 12mmol/l), although to lesser extent than in β-cells (increase of 81% over the same glucose range). Mitochondrial membrane hyperpolarization, measured by TMRE fluorescence, confirms that α-cells metabolize glucose. Also, application of D-mannoheptulose, an inhibitor of hexokinases, reduces glucose-mediated changes in NAD(P)H and TMRE signals.

As is well-known, glucagon secretion from islets is inhibited at glucose levels higher than 5mmol/l. However, the α-cell plasma membrane continues to depolarize at these concentrations (measured using $DiBAC_2(3)$ fluorescence). Likewise, the average intracellular [Ca^{2+}] (measured using Fura-Red or Fluo-4 fluorescence) increases as well, although the total increase of 45% from 1 to 12mmol/l is less that the 90% increase seen in β-cells over the same glucose range. In summary, our study on α-cells in intact islets reveals many similarities between α- and β-cell responses to glucose: activation of metabolism, membrane depolarization and increase in average [Ca^{2+}].

Further, our results from pure flow-sorted α-cell population show that glucose does not inhibit glucagon secretion when α-cells are separated from their intra-islet environment.

Overall, these observations suggest a paracrine inhibition model where the glucose-mediated suppressive effect of glucagon secretion acts downstream from α-cell Ca^{2+} signals, likely at the exocytotic machinery level.

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ISLET BIOLOGY—METABOLIC REGULATION

87-LB

Lipid Droplet Formation in the Pancreatic β-Cell Is Regulated by mTOR

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Type 2 diabetes mellitus (T2DM) has become an epidemic, proportional to ever increasing obese population in the developed countries around the world. Pancreatic β-cell defect associated with ectopic lipid accumulation within the β-cell has been proposed, in part, to cause the development of obesity-mediated T2DM. Specific molecular mechanisms involved in lipid droplet formation in β-cells, however, are unknown. We elected to study lipid droplet formation and its regulation in rat islets and dispersed single islet cells. Treatment of Islets with excess nutrients, 25 mM glucose and 500 μM FFA (oleate:palmitate=1:1) complexed with BSA at 3:1 ratio, for 4 days stimulated the expression of adipocyte differentiation related protein (ADRP), a lipid droplet-associated protein. Rapamycin (25 nM), a potent and selective inhibitor of mammalian target of rapamycin (mTOR), blocked the expression of ADRP, suggesting that mTOR is a regulator of ADRP expression. Triacylglyceride (TAG) levels accordingly were elevated in islets treated with excess nutrients as compared to those in control and rapamycin-treated islets. We then studied lipid droplet formation in dispersed single islet cells by immunohistochemistry. β- and α-cells were determined by insulin and glucagon staining, respectively, and lipid droplets were detected by Nile Red staining. Surprisingly, a large amount of lipid droplet formation was observed in β-cells but not in α-cells. Rapamycin significantly blocked lipid droplet formation in β-cells, correlating with its inhibition of ADRP expression and TAG accumulation. The ectopic lipid accumulation in β-cells in response to excess nutrients was reversible. Removing excess nutrients by replacing the culture media followed by additional 1 or 2 days of culture significantly reduced ADRP expression by islets. Transparent appearance of islets due to excess lipid accumulation also has reversed to opaque appearance. In conclusion, mTOR is a key enzyme that regulates lipid droplet formation in β-cells by regulating ADRP expression. It is postulated that mTOR plays a role as a nutrient sensor which regulates ectopic lipid accumulation or lipolysis in β-cells based on nutrient availability.

88-LB

Regulation of Pancreatic Beta-Cell Function by Protein-Tyrosine Phosphatase 1B

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Type 2 diabetes mellitus is characterized by insulin resistance and gradual loss of pancreatic β-cell function. The detailed mechanism underlying insulin signaling remains incompletely understood, but tyrosine phosphorylation plays an important role. Tyrosine phosphorylation is tightly controlled by the opposing actions of protein-tyrosine kinases and protein-tyrosine phosphatases (PTPs). Protein-tyrosine phophatase 1B (PTP1B) has been implicated as a major physiological regulator of insulin signaling and metabolism. Insights into the role of PTP1B, using tissue-specific deletion, revealed a diverse and complex function in various insulin-responsive tissues. However, the role of PTP1B in regulating pancreatic function remains largely unexplored. To address the role of PTP1B in β-cells, we created pancreas-specific PTP1B knockout (panc-PTP1B KO) mice using Cre-loxP strategy. Efficient deletion of PTP1B protein was confirmed in isolated islets and acinar tissues. In addition, PTP1B protein levels were not altered in the liver, skeletal muscle, adipose tissue or hypothalamus confirming specificity of deletion. Wild type and KO mice were placed on high fat diet and monitored for four months. No significant difference in body weight was detected; however panc-PTP1B KO mice exhibited impaired glucose tolerance using glucose tolerance tests. Interestingly, no differences were detected using insulin tolerance tests indicating defects in islets without alteration of insulin sensitivity in peripheral tissues. Indeed, in vivo glucose stimulated insulin secretion (GSIS) was blunted in panc-PTP1B KO mice compared to controls. C-peptide levels in the plasma collected during GSIS were also lower in KOs compared to controls thus ruling out potential differences in insulin clearance. Moreover, GSIS in isolated islets ex vivo demonstrated significant decrease in insulin secretion in KOs under basal and glucose-stimulated conditions. Collectively, our data suggest that PTP1B plays a significant role in regulating pancreatic β-cell function and glucose homeostasis and might be a potential target for therapeutic intervention.

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ISLET BIOLOGY—SIGNAL TRANSDUCTION

89-LB

Cross-Talk between Osteopontin and Angiotensin II Signaling Pathways in the Pancreatic Islets and Beta Cells

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Monocyte chemoattractant protein-1 (MCP-1) is a peptide chemokine that is negatively correlated with successful engraftment and long-lasting insulin independence after islet transplantation. We have recently shown that in the pancreatic islets and beta cells, angiotensin II (AngII), the major peptide of the renin angiotensin system, induces MCP-1 gene expression and protein secretion. Osteopontin (OPN), a phosphorylated glycoprotein that binds to an integrin-binding motif that we showed to be constitutively expressed in the islets and to have a protective role against cytokine-mediated cytotoxicity. Here, we explored the presence of a cross talk between the AngII and OPN signaling pathways in the pancreatic islets and beta cells. In vivo, in NOD mice, islet inflammation and progression to hyperglycemia correlated with progressive reduction of OPN expression and increased AngII generation. In vitro, addition of OPN prior to AngII in freshly isolated rat islets and beta cells, dose-dependently improved their glucose stimulated insulin secretion and inhibited AngII-induced MCP-1 production in an RGD-independent manner. Transient transfection of OPN gene in RINm5F

For author disclosure information, see page LB32.

beta cells fully prevented the AngII-mediated MCP-1 gene transcription and promoter activation. In OPN transfected cells, the AngII-induced NF-κB activity was significantly reduced. Islets exposed to AngII revealed a naturally occurring early up-regulated OPN transcription. OPN promoter activity was increased dose-dependently in the presence of AngII. Our data suggest the presence of a unique trans-regulatory mechanism in which AngII-induced OPN feedback to regulate MCP-1 transcription through deactivation of NF-κB. Our data also suggest that influencing OPN expression represents an approach to prevent or reduce activation of the cascade of downstream devastating effects after islet transplantation. Exhaustion of this local OPN system is associated with progression of the destructive insulitis and diabetes severity.

Supported by Diabetes Transplant Fund.

90-LB

In Vivo Interactions between GLP-1 and Insulin Signaling in Pancreatic β-Cell

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Mice with β-cell-specific knockout of the insulin receptor (βIRKO) exhibit glucose intolerance and age-dependent decrease in β-cell mass, and a susceptibility to develop overt diabetes. Glucagon-like peptide-1 (GLP-1), an incretin hormone secreted from intestinal L-cells, regulates β-cell function and proliferation likely by acting on proteins in the insulin/IGF-I signaling pathway including insulin receptor substrate-2 (IRS-2) and phosphatidyl inositol 3-kinase (PI3K). The aim of this study was to explore *in vivo* interactions between GLP-1 and insulin signaling pathways in the regulation of β-cell function and proliferation. Acute GLP-1 treatment (500 μM/kg b.wt) to control and βIRKO mice exhibited significant glucose lowering effects 30 min after intraperitoneal injection. Interestingly, βIRKOs were significantly more responsive to GLP-1-mediated blood glucose lowering effects compared to controls (Δ change, mg/dl: βIRKO; GLP-1-treated, -11.16±0.55 versus saline-treated +9.20±0.92; control: GLP-1-treated, -6.06±0.64, versus saline-treated +5.97±0.62). However, chronic GLP-1 treatment (500 μM/kg b.wt. I.P., twice a day for 20 days) failed to significantly alter either glycemia or body weights. Morphological analyses of pancreas sections revealed an increase in β-cell proliferation in GLP-1-treated βIRKOs. Next, to prolong the effects of GLP-1 *in vivo*, we treated mice with the DPP-4 inhibitor vildagliptin (~1 mg/day in drinking water for 42 days). A significant suppression of plasma DPP-4 activity after treatment was evident in both control and βIRKO groups; however, plasma GLP-1 levels, five min after oral glucose load, were significantly enhanced only in the βIRKO group treated with vildagliptin. Vildagliptin-treated βIRKOs exhibited significantly improved glucose tolerance and plasma insulin after oral glucose. Furthermore, we observed an increase in islet number, size, and proliferation of β-cells in the vildagliptin-treated βIRKOs. These data suggest that prolonging the action of GLP-1 in the βIRKOs improves β-cell proliferation despite impaired insulin signaling, and have implications for GLP-1 therapy in patients with type 2 diabetes exhibiting insulin resistance in β-cells.

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NUTRITION—CLINICAL

91-LB

A Low-Fat Supplemented with EPA and DHA Reduces the Risk of Metabolic Syndrome

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Metabolic syndrome (MetS) is characterised by hyperglycemia, dyslipidemia, abdominal obesity and hypertension. The syndrome affects most developed countries and confers increased risk of type 2 diabetes and cardiovascular disease. To investigate the effects of four diets with different fat quantity and quality for the prevention of the MetS (The LIPGENE proyects). This study included 337 volunteers with at least 3 components of National Cholesterol Education Program MetS criteria from eight European centers. They were randonly assigned to one of four diets: two high fat diets rich in saturated fat (HFSFA) or monounsaturated fat (HFMUFA) and two low-fat, high complex carbohydrate diets without (LFHC-control) and with (LFHC n-3) 1,24 g/day of very long chain n-3 polyunsaturated fatty acid (VLC n-3 PUFA) supplementation for 12 weeks. All diets were isocaloric, and there was no changes in physical activity during any of intervention. The type and number of MetS criteria were recorded before and after each intervention. At baseline, 100% of participants met the criteria for the MetS (3=40,4%, 4=38,6% AND 5=21%), The abdominal circumference (>102 for men and > 88 cm for women) was meet by 94,4%, blood pressure by 88,4%, fasting glucose (≥ 5,6 mmol/L) 51,3%, triacilglycerols (≥ 1,7 mmol/L) by 51,3% and HDL cholesterol (< 1,0 mmol/L for men and < 1,3 mmol/L for women) by 72,7% of volunteers. Each factor was randonly distributed and was not significantly different between diets at baseline. After intervention, MetS was decreased by 12,3% following SFA rich diet, 12,0% with MUFA rich diet, 10,3% with LFCHO rich diet and 25,0% with LFHC n3 diet ($p < 0,05$), In conclusion, an isocaloric low-fat diet supplemented with VLC n-3 PUFA rewduced the risk of metabolic syndrome compared with high fat and LFHC diets. **ADA-Funded Research**

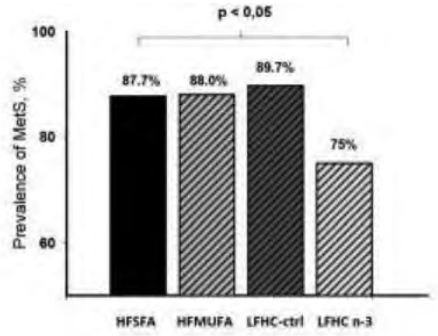

Figure 1. Prevalence of the metabolic syndrome (MetS) after 12-weeks of diet assignment. HFSFA is high fat diet rich in saturated fat and HFMUFA is high fat diet rich in monounsaturated fat. LFHC-control is a low-fat, high complex carbohydrate diet and LFHC n-3 is a low-fat, high complex carbohydrate diet supplemented with 1.24 g/day of very long chain n-3 polyunsaturated fatty acid (VLC n-3 PUFA). Chi-square test, $p<0,05$)

OBESITY—ANIMAL MODELS

92-LB

A New Selective Glucocorticoid Antagonist Suppresses Body Weight Gain as Well as Improves Insulin Sensitivity

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One of the most common symptoms of Cushing's syndrome is progressive obesity caused by chronically elevated glucocorticoid. Mifepristone is the only glucocorticoid receptor antagonist that is developed for the pharmacological treatment for Cushing's syndrome. In some reports, Mifepristone successfully ameliorated obesity and other symptoms of Cushing's syndrome. But Mifepristone is also a potent progesterone receptor antagonist which significantly complicates its use as a glucocorticoid antagonist.

We have successfully developed a selective glucocorticoid antagonist, ADS108297, which has no activity at the progesterone receptor. We tested the efficacy of the ADS108297 on diet induced obesity mice to determine if the body weight gain was suppressed.

Ten week old, C57BL/6J, male mice were used. Mice were fed a diet containing 60% fat and 20% sucrose supplemented water ad libitum. Mice were divided into 3 groups, 8 mice per group. Each group received the following drugs twice daily by oral administration: ADS108297 (80mg/kg/day), Rosiglitazone (10mg/kg/day), and vehicle control. All drugs were dispersed in 10%DMSO in 0.5% CMC. Insulin sensitivity was evaluated every week by insulin suppression test: a mixture of insulin, glucose and somatostatin was injected intraperitoneally, and steady state plasma glucose (SSPG) determined by the average of the glucose values at 60, 70 and 80 minutes after injection.

Body weight gain was observed in Rosiglitazone and Vehicle compared to the baseline body weight. On the other hand, no significant body weight gain was observed in the ADS108297 group suggesting that our selective glucocorticoid antagonist is capable of controlling diet-induced body weight gain.

Insulin resistance, determined by SSPG, was highest in Vehicle group. No SSPG differences were noted between ADS108297 and Rosiglitazone.

We found that administration of a high fat and high sucrose diet for 4 weeks induced insulin resistance in mice. A selective glucocorticoid antagonist, ADS108297, suppressed diet-induced body weight gain as well as maintained insulin sensitivity.

For author disclosure information, see page LB32.

93-LB

Leptin-Dependent Control of Glucose Balance and Locomotor Activity by POMC Neurons

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Leptin plays a pivotal role in regulation of energy balance. Via unknown central pathways leptin affects peripheral glucose homeostasis and locomotor activity.

We hypothesized that specifically Pro-opiomelanocortin (POMC) neurons mediate those actions. To examine this possibility we applied Cre-Lox technology to express leptin receptors (ObRb) exclusively in POMC neurons of the morbidly obese, profoundly diabetic, and severely hypoactive leptin receptor deficient db/db mice. We here show that expression of ObRb only in POMC neurons leads to a marked decrease in energy intake and a modest reduction in body weight in db/db mice. Remarkably, blood glucose levels are entirely normalized. This normalization occurs independently of changes in food intake and body weight. In addition, physical activity is greatly increased despite profound obesity. Our results suggest that leptin signaling exclusively in POMC neurons is sufficient to stimulate locomotion and prevent diabetes in severely obese db/db mice. Combined, the data reveal a remarkable novel capacity of POMC neurons to control serum glucose and physical activity in morbidly obese and severely diabetic rodents.

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94-LB

Prenatal Protein Deficiency Alters Circadian Rhythms in Clock and Metabolism in the Offspring

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The mechanisms linking intrauterine growth retardation (IUGR) with adulthood obesity and diabetes are unknown. These studies investigated energy metabolism in 8 wk and 5 month old male and female mice subjected to protein malnutrition in utero. We investigated the metabolic phenotype of IUGR offspring prior to the onset of obesity. We also investigated weather B6 mice subjected to prenatal protein deficiency also develop signs of Type II Diabetes. Undernourished pups from pregnant C57BL/6J dams fed a protein deficient diet (6 % protein, UO) were cross-fostered to lactating dams fed normal breeder chow. UO exhibited lower birth weight (~60% of normal weight), but displayed rapid catch-up growth. 8 wk old chow-fed UO mice exhibit improved glucose clearance relative to CO. However, UO exhibited marked abnormalities in circadian rhythms of wheel running, feeding behavior and metabolic activity. Specifically, food intake, energy expenditure and the respiratory exchange ratio (RER) were increased in UO during the lights-on period. Expression of genes involved in hepatic lipid and glucose metabolism revealed that expression of Rev-erbα in liver was dramatically reduced in UO at 2 mo of age. Rev-erbα repressed genes involved in circadian regulation (Bmal1, Per2) and inflammation (plasminogen-activator inhibitor-1) were increased in UO mice. The same profile of gene expression was observed in male UO at 5 mo of age. UO mice exhibit a metabolic disorder involving abnormal circadian patterns of feeding behavior, increased lipogenesis and inflammation prior to obesity. Loss of Rev-erbα expression and function may be a key factor in metabolic dysregulation associated with IUGR.

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95-LB

Rosiglitazone Intervention Induces Prompt and Reversible Amelioration of Inflammation and Insulin Resistance Elicited by High Fat Diet

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Obesity is causally linked to type 2 diabetes through two critical mechanisms—inflammation and insulin resistance. Rosiglitazone (ROSI), a PPARγ agonist, has anti-inflammatory and insulin-sensitizing functions. However, the time-dependent signals through which ROSI triggers its actions are unclear.

We investigated the effects of ROSI on inflammation and insulin sensitivity in high fat diet (HFD)-fed C57Bl/6 mice. Mice were fed HFD for 10wk, then fed ROSI+HFD for 5wk, then fed only HFD for 8wk. Control mice were fed HFD alone for the entire 23wk time course. ROSI+HFD treated mice showed greater increases in body mass (52.9 ± 1.3 vs 45.2 ± 0.7g, P = 0.02) and epididymal white adipose tissue mass (eWAT) (1.5 ± 0.1 vs 1.2 ± 0.1g, P = 0.018) compared to control HFD-fed mice. Infiltration of pro-inflammatory, F4/80$^+$/CD11b$^+$/CD11c$^+$ macrophages into eWAT (assayed by FACS) was reduced after only 1wk and sustained during the 5wk of ROSI+HFD treatment compared to HFD-fed mice (P = 0.036). ROSI treatment resulted in improved insulin sensitivity as measured by glucose tolerance test (GTT) and insulin tolerance test. GTT showed ROSI improved glucose clearance by 48 and 77 percent after 1wk and 5wk, respectively. Interestingly, ROSI-induced weight gains and improvement in insulin function were reversed only 2wk after ROSI removal. Gene expression of inflammatory cytokines in eWAT demonstrated that ROSI administration during HFD down-regulated IL-1β, IL-6, IL-12, MCP-1, TNFα, iNOS2, MAPK8, VCAM1, MMP9, Cxcl10 and Cxcl1 mRNAs by 1-2wk. Within 2wk of ROSI removal, these inflammatory markers were up-regulated to levels similar to those in constitutively HFD-fed control mice. ROSI treatment lowered plasma insulin and PAI-1 and increased adiponectin, effects which also reversed after 2wk of ROSI removal. We conclude ROSI treatment ameliorates insulin resistance in HFD-fed mice within only 1wk by decreasing inflammation (measured by diminished levels of macrophage infiltration and inflammatory cytokines) and improving insulin sensitivity. Furthermore, we observed that these ROSI-mediated effects are reversible only 2wk after ROSI removal from the HFD.

OBESITY—CLINICAL TREATMENT

96-LB

Lorcaserin Reduces Body Weight in Obese and Overweight Subjects: Behavioral Modification and Lorcaserin for Overweight and Obesity Management, the BLOOM Trial

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Lorcaserin (LOR) is a selective 5-HT2C receptor agonist, an important modulator of food intake. BLOOM was a 2-year, randomized, double-blind, placebo-controlled trial that evaluated the safety and efficacy of LOR. Subjects received LOR 10 mg BID orally or placebo (PBO), and standardized diet and exercise counseling. After 1 yr, LOR subjects were re-randomized to continue LOR or to switch to PBO, and all PBO subjects remained on PBO. Echocardiograms were performed at screening and months 6, 12, 18, and 24.

The trial enrolled 3182 subjects: 84% women; mean age 44.1±0.2 yr, weight 100±0.4 kg, and BMI 36.2±0.1 kg/m^2. Completion rates were 55.4% (LOR) and 45.1% (PBO) at yr 1, and 74.3% and 72.7% at yr 2, respectively. At yr 1, 47.5% of subjects on LOR and 20.3% on PBO lost ≥5% of baseline body weight ($p<0.0001$); 22.6% on LOR lost ≥10% of baseline body weight vs. 7.7% on PBO ($p<0.0001$) by ITT-LOCF analysis. Mean weight loss at yr 1 was 5.8±0.2 kg with LOR vs. 2.2±0.1 kg with PBO ($p<0.0001$; ITT-LOCF), and 8.2±0.3 kg vs. 3.4 ±0.3 kg, respectively by per protocol analysis ($p<0.0001$). Less weight regain occurred during yr 2 in patients on LOR than in those re-randomized to PBO. Cholesterol decreased 3.2±0.6 mg/dL at yr 1 in the LOR group vs. 0.3±0.7 mg/dL in PBO ($p=0.0002$); triglycerides decreased 19.0±1.5 mg/dL vs. 9.2±1.6 mg/dL, respectively ($p<0.0001$). Blood pressure decreased significantly in LOR vs. PBO. Insulin, glucose, HOMA-IR, acute phase reactants and other measures are pending and will be presented. The proportion of patients who developed FDA-defined valvulopathy did not differ between LOR and PBO at any time point. The most frequent AEs were headache, upper respiratory infection, and nasopharyngitis.

In conclusion, lorcaserin was associated with significant weight loss and improvement in plasma lipids as compared to placebo, with no evidence of cardiac valve toxicity. Lorcaserin is a promising novel investigational agent for treating obesity.

97-LB

Mifepristone Prevents Risperidone Induced Weight Gain in Healthy Men

COLEMAN GROSS, CHRISTINE BLASEY, ROBERT ROE, JOE BELANOFF, *Menlo Park, CA*

Atypical antipsychotics are associated with weight gain, dyslipidemia, insulin resistance, and diabetes mellitus. This double-blind controlled study examined whether the glucorticoid receptor antagonist mifepristone would prevent weight gain associated with risperidone treatment in healthy,non-diabetic, normal weight (BMI 18-23) men . The objective was to determine if subjects receiving risperidone (R, 1.5 – 2 mg BID) gained a different amount of weight than those receiving risperidone + mifepristone (R+M, 600 mg qday) over a 28 day period. Waist circumference, lipid fractions, as well as fasting insulin and glucose levels were also assessed. 75 subjects, age 19-39, were randomized to R (N=30), R+M (N=30), or M only (N=15). Subjects were inpatients from 7 days before randomization until Day 30, 2 days after dosing was completed. Food was provided ad libitum and weight was measured daily. Mean baseline weight was 58.8 kg. The mean increase in weight at Day 28 in the R group was greater than in the R+M group (4.2±.3 vs 2.3±.3 kg, p=.0001); significant differences in weight gain appeared by Day 6 (p=.002). Subjects in the M group had weight gain (2.9±.4 kg) similar to the R+M group. Waist circumference increased less in the R+M group than the R group (2.0 cm vs 3.6 cm increase, p=.035). Fasting insulin and triglyceride levels increased by

For author disclosure information, see page LB32.

11.0 mU/L (169%) and 30.6 mg/dL (35.6%), respectively in the R group and these levels remained stable in the R+M group (1.8 mU/L and 3.1 mg/dL); group differences were significant for both measures p =.0001, p = 0.001. HOMA-IR increased in the R group (1.76 at baseline vs 4.73 at Day 28) and remained stable in the R+M group (1.75 at baseline vs 2.22 at Day 28); p = .04 for group differences. In conclusion, mifepristone prevented weight gain and metabolic abnormalities associated with risperidone treatment.

PEDIATRICS—TYPE 2 DIABETES

98-LB

High Normal Fasting Glucose Level in Obese Youth: a Marker for Insulin Resistance and Beta Cell Dysregulation

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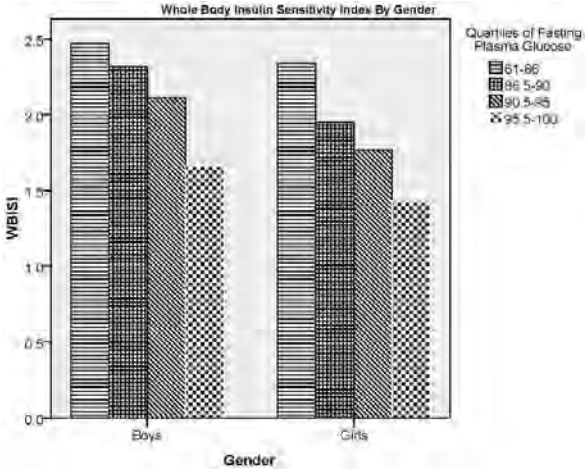
High but normal fasting glucose (NFG) level in adults is a risk factor for future development of Type 2 diabetes (T2DM). Thus, before fasting glucose reaches the diagnostic range for impaired glucose tolerance (IGT), derangements in insulin sensitivity and secretion might be present. We investigated whether NFG levels, i.e. fasting glucose levels less than 100mg/dl, could be used to identify children with insulin resistance (IR) and reduced beta cell function and thus at risk for IGT. One thousand and ninety-three children and adolescents (boys=39%) with NFG underwent a standard oral glucose tolerance test (age 13±3 years, BMI Z 2.35±.5) with calculation of indices of insulin resistance (IR) and beta cell function. In addition, anthropometric indices were measured. Of them, 150 had IGT, while 6 had T2DM. Quartiles of NFG were calculated (I 61-86 $mgdl^{-1}$; II 86.5-90 $mgdl^{-1}$; III 90.5-95 $mgdl^{-1}$; IV 95.5-99.9 $mgdl^{-1}$). Interestingly, in children with normal glucose tolerance, we observed a significant increase across quartiles for IR ($p<0.0001$), 2-hour glucose ($p<0.0001$), and HbA1C ($p<0.0001$) and a significant decrease in the disposition index ($p<0.0001$) and whole body insulin sensitivity ($p<0.0001$). Moreover, as FPG increased, the odds ratio of presenting with IGT was 1.655 (95% CI 1.2-2.3). These data suggest that in children and adolescents, independent of age, BMI, gender and ethnicity, insulin sensitivity and secretion decline progressively when moving from low to high NFG. The simple measure of FPG may assist clinicians in identifying children for targeted diabetes screening and subsequent lifestyle management.



Supported by Fulbright Award 2008/09.

PREGNANCY

99-LB

The High Rate of Gestational Diabetes in Urban Well-Educated Scandinavian Women Is Unchanged in Spite of Lower BMI. A Longitudinal Study (2002–2005 and 2005–2008)

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The aims of this study was to monitor anthropometry, glucose and insulin metabolism prospectively, in a large cohort of pregnant women, to elucidate mechanisms that influence glycemic control.

1032 pregnant Scandinavian women were investigated prospectively with 75g-OGTT at week 14-16 and 30-32, and with anthropometry of mother and infant. Insulin sensitivity (IS_{ogtt} and HOMA-IR) and beta-cell function (insulinogenic index (II) and insulin secretion sensitivity index (ISSI)) were calculated from OGTT. Comparisons between the cohorts were done with t-test or chi square test.

Cohort characteristics

	2002-2005 (n=553)	2005-2008 (n=479)	p<
Maternal age (years)	31.2 (4)	31.3 ± 4	ns
Maternal weight (kg, week 14-16)	70.8 (12.1)	69.0 (11.9)	0,02
BMI (week 14-16)	24.9 (4.1)	24.1(3.7)	0,001
Weight gain during pregnancy (kg)	10.6 (3.8)	10.4 (3.3)	ns
Gestational age at birth (weeks)	40.0 (1.8)	40.1 (1.8)	ns
Primipara (%)	53	53	ns
Birth weight (g)	3619 (570)	3556 (578)	0,08
Birth weight ≥ 4200g (%)	15.2	11.4	0,08
GDM (%) week 14-16	1.9	1.2	ns
GDM (%) week 30-32	10.6	12.5	ns

Data are mean (SD) or %.

Cohort characteristics: for both periods the rate of gestational diabetes (GDM) is higher than previously reported in this region. We found significant reductions in maternal body weight and BMI at inclusion in the latter cohort, but no difference in GDM rate. Neither HOMA-IR nor IS_{ogtt} changed significantly between the cohorts, when subdivided by glucose tolerance (NGT or GDM), insulin sensitivity remained unaltered, except for a 11% reduction in HOMA-IR at week 30-32 in the 2005-2008 cohort, $p<0.04$. II and ISSI fell significantly in the latter cohort, for the whole group (23-30%), in NGT subjects (23-30%) and most in the GDM subgroup (30-39%, $p<0.006$ for all vs the earlier cohort).

In spite of rising BMI and GDM rate in many Western countries, the high GDM rate remained unchanged in our cohort, although BMI in early pregnancy decreased significantly in the latter part of the STORK study. Differing levels of insulin sensitivity and beta-cell function could be an indication of greater beta cell dysfunction in the leaner cohort.

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PSYCHOSOCIAL—BEHAVIORAL MEDICINE

100-LB

A Brief Intervention Using a Computerized Patient Self Management Assessment Tool Improves Blood Glucose Control (HbA1c)

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Computerized patient self management assessment tools have shown promise in office-based diabetes education research and offer the potential benefit of wide translation. We evaluated the Diabetes Self Care Profile (DSCP) that briefly assesses diet, exercise, medication, and physical activity behaviors, identifies one behavior selected by the patient for discussion, documents practical and psychosocial barriers to optimal self management, including attitudes and barriers to insulin therapy, and provides visual feedback on HbA1c control. The DSCP cost $15,000 for web development and was applied within a larger clinical trial evaluating novel patient centered approaches to diabetes education. Fifty eight type 2 diabetes pts were recruited from several sources (hospital Endocrine Clinic, a hospital lab database search, a local primary care clinic, using community fliers) and took part in the intervention. Patient characteristics: mean age 57.2 ± 10.9; 63.8% female, 81.0% White, 12% Hispanic; 72.3% married; mean diabetes duration 7.0±6.5 yrs; 49.4% some college education or higher; 63.6% had received diabetes education before; 84.5% were oral agent treated and 22.4% insulin treated; mean baseline HbA1c 8.9 ± 1.2; mean CES-D depression score 18.6 ±10.9; patients attended 3.5 ± 0.8 out of 4 scheduled intervention sessions over 6 months with a Certified Diabetes Educator who used the DSCP report to guide education sessions. Results showed that HbA1c improved by -1.0 ± 1.3% (effect size=0.8) over the 6 month intervention ($p<0.01$). Univariate analyses showed significant improvements in BMI (-0.8 ± 1.7), self care behaviors (10.8 ± 11.1), diabetes distress (-10.6 ± 15.8), diabetes treatment satisfaction (10.9 ± 5.0), and social support (13.5 ± 27.8) during the intervention (all $p<0.01$). A final regression model examining mediators of HbA1c change included diabetes self care, diabetes distress, and treatment satisfaction and explained 40% of HbA1c variance ($F=8.13$, $p=0.0003$).

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For author disclosure information, see page LB32.

101-LB

Increased Rates of Clinically Significant Cognitive Impairment in Older Primary Care Practice Patients with Diabetes

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Cognitive dysfunction is a well known complication of diabetes. Older diabetic adults perform more poorly on tests of attention, memory and mental flexibility but little is known about the 'clinical significance' of those findings, particularly for patients seeing a physician for a routine medical care. To address this, 535 patients were recruited from 11 primary care practices (PCP). Each PCP was asked to refer patients at least 65 years old without a medical chart diagnosis of dementia. A battery of cognitive tests were administered that assessed memory, executive function, spatial skills, attention, and language. Clinical diagnoses were made at consensus meetings with 3 licensed neuropsychologists. 'Dementia' was defined as scores > 2 standard deviations (SD) below age-corrected norms on 2 cognitive domains, 1 being memory; 'mild cognitive impairment' (MCI) was at least 2 scores 1 to 2 SDs below norms; 'Questionable' was only 1 score below 2 SDs or 2 tests ~ 1 SD below norms; 'Normal' was all scores within the normal range.

Results showed a higher proportion of diabetic patients in the 'impaired' range ('dementia,' 'MCI' or 'questionable') compared to those without diabetes ($\chi^2 = 12.2$; p = .007); when 'questionable' diagnoses were excluded, the same pattern was obtained ($\chi^2 = 9.4$; p = .002; odd ratio = 2.03 (95% CI: 1.28, 3.21)). Logistic regression analyses showed the best predictors of MCI diagnoses were demographic data, and a diagnosis of diabetes. Hypertension, coronary artery disease, or hypercholesterolemia were unrelated to MCI diagnosis.

	No Diabetes	Diabetes
Number	393	142
Age (yrs)	72.9	73.2
Normal	41.7%	25.4%
Questionable	18.3%	25.4%
MCI	36.4%	45.1%
Demented	3.6%	4.2%

This is the first medical practice-based study to use clinical criteria to demonstrate elevated rates of MCI in patients with diabetes. MCI is of great concern because it may be a transitional state between normal cognition and dementia. Moreover, people with MCI have more difficulty performing instrumental activities of daily living (e.g., driving, handling finances, cooking). We will discuss possible pathophysiological mechanisms and long-term implications.

SIGNAL TRANSDUCTION (NOT INSULIN ACTION)—CYTOKINES AND APOPTOSIS

102-LB

Increased Plasma Levels of Macrophage Specific sCD163 in Type 2 Diabetes and Obesity

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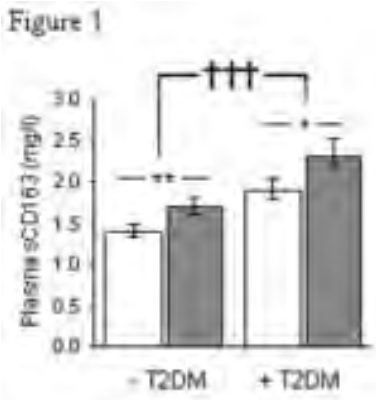
Activation of adipose tissue macrophages with concomitant low-grade inflammation is believed to play a central role in the development of type 2 diabetes (T2DM). CD163 is strongly and specifically expressed on tissue macrophages. However, in the case of macrophage Toll Like Receptor activation the molecule is shed to plasma. We therefore hypothesized that plasma sCD163 may be increased in T2DM and obesity, and may be related to adipose tissue low-grade inflammation.

In a cross-sectional, case-control design we investigated the plasma levels of sCD163 (ELISA) in 199 subjects. They were divided into 4 groups depending on the presence or absence of T2DM (positive oral glucose tolerance test), and further sub-grouped into obese (BMI > 30 kg/m^2) and non-obese (BMI < 30 kg/m^2).

Figure 1 illustrates geometric means±SEM of plasma sCD163. Patients with T2DM had higher plasma sCD163 levels than subjects without T2DM (†††: 2.32 vs. 1.61 mg/L, p<0.001). In both of these groups the obese sub-groups (shaded in Figure 1) showed significantly increased levels compared to non-obese (*P<0.05,**P>0.01).

Figure 1



In multivariate analysis (adjusted for age, sex, smoking and CRP) plasma sCD163 was strongly positively associated with plasma triglycerides, insulin, glucose, IL6 and TNF-α, respectively (all p<0.01). Furthermore, sCD163 was strongly negatively associated with plasma HDL and cardiorespiratory fitness (VO_{2max}) (both p<0.001). Total and trunk fat-mass were not found associated with sCD163.

In conclusion, the macrophage specific plasma protein sCD163 is significantly increased in T2DM and obesity. The observed increase in obesity seems not to be directly associated to the amount of fat tissue, but to metabolic and inflammatory processes.

SIGNAL TRANSDUCTION (NOT INSULIN ACTION)—HORMONES

103-LB

A Soluble 150 kDa Protein Containing Leucine Zipper, Atg16 and Rab5-Binding Motifs Interacts with APPL1 under Adiponectin Exposure

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Type II diabetes is associated with high glucose production. Obesity increases glucose production by lowering circulating levels of the hormone adiponectin. Therefore, type II diabetes can be treated by stimulating the adiponectin signaling pathway.

Adiponectin lowers circulating glucose by inhibiting glucose production from the liver. Adiponectin inhibits glucose production by activating AMP-activated kinase (AMPK). AMPK stimulates fatty acid (FA) oxidation. The inhibition of glucose production by a signaling intermediate that increases FA oxidation is counter-intuitive because ATP generated from FA oxidation fuels glucose production. Thus, we speculated that adiponectin inhibits glucose production by decreasing the hydrolysis proteins and glycogen through autophagy. Autophagy is initiated by the formation of a membrane around a targeted region of the cell separating the contents from the rest of the cytoplasm. The resultant vesicle, called an autophagosome, fuses with a lysosome.Insulin inhibition of glucose production in the liver is mediated by the suppression of lysosome activity. We treated rat hepatoma cells with full-length recombinant adiponectin and identified the proteins that were bound to APPL1 in a co-immunoprecipitation assay using proteomics analysis. APPL1 was previously identified in a yeast 2-hybrid screen using the intracellular region of the adiponectin receptor. Proteomics analysis revealed a gene we are calling TOA (target of adiponectin) that encodes a 150 kDa protein containing (1) a leucine zipper motif that enables binding to the leucine zipper motif of APPL1 and (2) Atg16 and Rab5-binding motifs that enable participation in membrane assembly for autophagy. The hydrolysis of proteins and glycogen by autophagy increases glucose production by producing biochemical intermediates for gluconeogenesis and glycogenolysis. Northern blot analysis revealed that TOA is ubiquitously expressed as a 3.0 and a 4.5 kb mRNA. TOA overexpression in hepatoma cells increased lysosomal activity, proteolysis, and glucose production. Our current hypothesis is that adiponectin inhibition of TOA can suppress glucose production.

For author disclosure information, see page LB32.

SIGNAL TRANSDUCTION (NOT INSULIN ACTION)—PHOSPHATASES–KINASES

104-LB

Map4k4 Negatively Regulates PPARγ Protein Translation by Suppressing the mTOR Signaling Pathway in Cultured Adipocytes

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The receptor peroxisome proliferator-activated receptor γ (PPARγ) is considered a master regulator of adipocyte differentiation and promotes glucose and lipid metabolism in mature adipocytes. We recently identified the yeast Sterile 20 (Ste20) protein kinase ortholog, Map4k4, in an RNAi-based screen in cultured adipocytes as an inhibitor of PPARγ expression. Here we show that RNAi-mediated silencing of Map4k4 elevated the levels of both PPARγ1 and PPARγ2 proteins 2-3 fold in 3T3-L1 adipocytes without affecting PPARγ mRNA levels, suggesting that Map4k4 regulates PPARγ at a post-transcriptional step. PPARγ degradation rates are remarkably rapid as measured in the presence of cylcoheximide (t1/2=2 hours), and silencing Map4k4 had no effect on PPARγ degradation. However, depletion of Map4k4 significantly enhances [S-35]-incorporation into proteins, suggesting that silencing of Map4k4 increases protein translation. Our results indicate this is due to elevated rapamycin-sensitive mTOR activity, resulting in enhanced 4E-BP1 phosphorylation. In addition, we show that 4E-BP1 is required for the increase in PPARγ protein expression upon Map4k4 knockdown. Interestingly, Map4k4 was found to be required for the TNF-α stimulated expression of the AP-1 transcription factors FRA1 and Fosb. Depletion of these transcription factors mimicked Map4k4 knockdown in enhancing PPARγ protein level, mTOR activity and 4E-BP1 phosphorylation. These data show that Map4k4 regulates PPARγ post-transcriptionally, through mTOR signaling and a 4E-BP1 dependent mechanism.

SIGNAL TRANSDUCTION (NOT INSULIN ACTION)—TRANSCRIPTIONAL REGULATION

105-LB

A Newly Identified Role of Nuclear Receptor Corepressor, SMRT (Silencing Mediator of Retinoid and Thyroid) in Improving Hyperglycemia and Insulin Sensitivity in Obese and Diabetic Mice

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The nuclear receptor corepressor SMRT is utilized by a variety transcription factors to mediate transcriptional repression. We earlier reported an improvement in lipid metabolism in hypothyroid and high-fat diet (HFD)-fed mice after SMRT inhibition (Pandey SK et al., ADA, 2008, 1936-P). In the current study, we investigated if reduction of SMRT expression would ameliorate hyperglycemia and insulin resistance in diabetic mice. Male (C57BL/6) mice were fed 58kCal% HFD for 3 months to induce insulin resistance and then treated with control antisense oligonucleotides (ASO) or SMRT ASO at 25 mg/kg body weight (BW) twice a week for 9 weeks. SMRT ASO treatment relative to control ASO, reduced SMRT expression in liver and WAT by ~70% and ~40% respectively. This resulted in a significant lowering of fed (15-20%) and fasted (~25%) plasma glucose and insulin (30-40%) levels and improvement in insulin tolerance. SMRT ASO treatment increased circulating levels of plasma adiponectin (30%) and reduced leptin levels (55-60%) compared to control ASO suggesting improved insulin and leptin sensitivity. In ob/ob mice, SMRT ASO treatment (25 mg/kg twice a week) reduced SMRT expression in liver and WAT by 80% and 50% respectively. This was accompanied by lowered fed plasma glucose levels (control ASO: 587±16 vs SMRT ASO: 287± 20 mg/dl, P<.0001), reduced plasma insulin levels (30-35%) and improved insulin tolerance. In addition, SMRT ASO treatment decreased the expression of gluconeogenic genes, PEPCK (~25%) and G6P (~50%). Treatment of db/db mice with SMRT ASO reduced mRNA expression in liver and WAT by ~85% and ~35% respectively and lowered plasma glucose by ~ 90 mg/dl. A mild increase in transaminase levels was noticed which was not associated with increase in serum bilirubin or any overt toxicity. A similar phenotype was seen with a second SMRT ASO which targets a different region of SMRT gene. Thus, these data demonstrate a novel role of SMRT in regulation of glucose homeostasis and suggest that antisense reduction of SMRT may be a therapeutic strategy for the treatment of type 2 diabetes.

106-LB

Evidence that the Human SNP rs387083 (G/A) Is Associated with Leptin Levels through the Modulation of G-protein Coupled Receptor 41

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Single nucleotide polymorphisms (SNPs) are naturally occurring genetic variations in a single nucleotide which can be positioned both inside and / or outside the coding sequences of a gene and may alter the synthesis or function of proteins in that region. Identifying SNPs that associate with higher risk of disease promises to significantly advance our ability to better understand and treat these conditions. In a recent statistical association study of type 2 diabetes offspring, we discovered a strong correlation (p=0.0016) between the human SNP rs387083 (G/A) and increased plasma leptin levels along with suggestive correlations with several body weight parameters. rs387083 is located within the intergenic region between G-protein coupled receptors 40 and 41. However, previous studies have shown that of the two receptors, only GPR41 is expressed in adipocytes. Furthermore, it has been demonstrated that GPR41 mediates leptin production in response to short chain fatty acid stimulation in adipocytes. Taken together, these data suggest that the association of SNP rs387083 with leptin levels may be due to modulation of GPR41 expression. In line with the regulatory role of rs387083, here we show computational as well as functional evidence for a putative consensus binding site for the sequence-specific transcriptional repressor protein, RP58 with the "G" allele but not the "A" allele. Therefore, we provide the first evidence suggesting a novel molecular mechanism by which RP58 decreases leptin production in adipocytes through transcriptional repression of GPR41 when both alleles are "G". In contrast, the transcriptional repressor protein cannot bind as efficiently when one or both alleles are "A", leading to a higher expression of GPR41 and, hence, increased leptin production.

107-LB

Hypoxic Regulation of Adipocyte Gene Expression

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Recent studies have demonstrated the importance of adipose tissue hypoxia in the pathogenesis of obesity-related insulin resistance. Data suggest that white adipocytes become avascular with obesity due to their inability to mount a compensatory angiogenic response to hypoxic stress. Brown adipocytes, however, are more highly vascularized, likely due to a greater production of angiogenic factors. In the present study, we investigated the role of PGC1α and PGC1β in the angiogenic response of brown adipocytes to hypoxia, based on previous evidence linking Pgc1α to upregulation of VEGF expression. In addition, we analyzed the role of the NAD+-dependent deacetylase, SIRT1, that has been shown to regulate PGC1α and whose activity is regulated by the redox state of the cell.

When exposed to 1% hypoxia, PGC1α knockout adipocytes showed similar repression of adipocyte genes and activation of hypoxia target genes as observed in control brown adipocytes. However, when both PGC1α and PGC1β were knocked out of brown adipocytes exposure to hypoxia caused a reduction in the activation of hypoxia target genes. Examination of the angiogenic VEGF response revealed that a distinctly smaller isoform of VEGF is produced in the PGC1α/PGC1β knockout, compared to the control brown adipocytes, with both forms being produced in the PGC1α knockout. It is likely that these two forms have distinctly different capacities for angiogenesis.

Knockdown of Sirt1 in white preadipocytes enhances their differentiation and potentiates the ability of agonist-activated PPARγ to induce a subset of hypoxia-target genes including VEGF, EGLN3, and ERO1-Lα. These data highlight a possible switch from a white fat phenotype to a more vascularized brown fat phenotype in response to SIRT1 and PPARg activity.

These results suggest that the PGC1s and SIRT1 play vital roles in the response of the adipocyte to hypoxia, as well as the "browning" of white cells to better respond to a hypoxic challenge with an appropriate level of angiogenesis. These proteins have been introduced as potential targets for the development of therapeutics to enhance the brown phenotype within white fat, to combat the progression of obesity-related disorders.

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For author disclosure information, see page LB32.

SIGNAL TRANSDUCTION (NOT INSULIN ACTION)—TRANSGENIC MODELS

108-LB

Altered Metabolism in Salt-Inducible Kinase 2 (SIK2) Deficient Mice

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The Salt-inducible kinase family (SIK) is represented by three mammalian genes, SIK1/SNF1LK, SIK2/SNF1LK2, and SIK3/QSK. Sequence conservation of the SIK kinase domain places the SIKs in a subfamily with AMPK (AMP-activated kinase) and MARKs (Microtubule Affinity Regulating Kinase). AMPK, SIK and MARK are all activated by the LKB1 kinase and all three have been implicated in the overlapping regulation of at least one common downstream target (CRTC2/TORC2). Recent work in cell-based and in vivo models has implicated SIK kinases in the regulation of both CRTC2/TORC2- and SREPB1-mediated transcription with effects on gluconeogenesis and lipogenesis, respectively. The relative in vivo and tissue-specific roles of each of the SIKs is currently unknown. Here we describe the generation and characterization of mice deficient for SIK2. Both protein and RNA expression analyses indicate that SIK2 is highly expressed in white and brown adipose tissue, with relatively low levels in other tissues, including the liver. SIK2 KO mice exhibit increased adiposity at eight weeks of age on regular chow diet. This increase in adiposity is observed concomitant with modest but significant fasting hyperglycemia. In addition we find that SIK2 deficient mice exhibit elevated levels of SCD1, FAS and ACC gene expression. These data support the models that SIK2 acts as a negative regulator of both gluconeogenesis and lipogenesis. Curiously, SIK2 deficient mice fed a high fat diet are indistinguishable from wild-type littermates, in terms of glycemia, adiposity and food intake. The finding that SIK2 deficiency does not exacerbate the effects of diet-induced obesity suggests that other SIK family members may compensate for the loss of SIK2 in this paradigm. Alternatively it is possible that the relevant inputs to SIK2 signaling which drive the observed effects on adiposity and hyperglycemia are independent of dietary fat consumption. We discuss these and additional data aimed at further clarifying the physiological functions of SIK2.

109-LB

PIKE Is Essential for Diet-Induced Obesity and Insulin Resistance

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Phosphoinositide-3 kinase enhancers (PIKE) are a family of GTPases that directly interact with phosphoinositide-3 kinase and Akt and enhance their kinase activity. It contains three isoforms: PIKE-L, PIKE-S and PIKE-A. Both PIKE-L and PIKE-S are involved in protecting the cells from apoptosis. However, the functional role of PIKE-A in peripheral tissues remains unknown. To address this issue, we generated the PIKE knockout (PIKE -/-) mice using loxP/Cre recombination. PIKE -/- mice exhibit a prominent phenotype of lipoatrophy with strong protection from high fat diet induced obesity and diabetes. Ablation of PIKE prevents adipocyte differentiation *in vitro and in vivo*. Moreover, PIKE -/- mice preferentially utilize lipid oxidation as the energy source. AMPK and Akt phosphorylation in PIKE -/- muscle are significantly elevated, thus protects the mice from lipid-induced insulin resistance. Collectively, these results demonstrate the PIKE is an essential factor for obesity and associated diabetes development.

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TRANSPLANTATION

110-LB

Islet Cell Aggregates Are Superior to Islets for Transplantation in Microcapsules

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Small islet-cell aggregates were studied to determine if their survival and function were superior to intact islets within microcapsules during low oxygen culture and transplantation.

Islet-cell aggregates were generated by dispersing rat islets into single cells and reaggregating them. Islets and islet-cell aggregates were encapsulated in barium alginate capsules and cultured in low (0.5% or 2%) or normal (20%) oxygen, or transplanted. Following culture, tissue was assessed with measurements of oxygen consumption rate (OCR), nuclei counts, insulin to DNA content ratio, glucose-stimulated insulin secretion (GSIS), and gene expression.

Culture in low oxygen (2%) resulted in maintenance of pre-culture levels of viable tissue (determined by OCR) in aggregates, but only 61% of that for islets, $p < 0.01$. There was a substantial loss of tissue (by nuclei counts) in the islets cultured at 0.5% oxygen but only modest loss of tissue in the aggregate capsules (59% vs 87% of pre-culture values, $p < 0.05$). The ratio of insulin to DNA content decreased markedly in islets cultured in 0.5% oxygen for 2 days, in contrast to no decline in aggregates. After 2 days of culture in 2% oxygen islet cell aggregates showed differential increased secretion to high glucose whereas islets did not. Pro-inflammatory genes MCP-1 and tissue factor were expressed at significantly lower levels in islet cell aggregates than in whole islets (36 ± 9% and 59% ± 11%, $p < 0.01$ and 0.05, respectively) after overnight culture in 2% oxygen. Two weeks after transplant into syngeneic (Lewis rat) recipients, large areas of central necrosis were apparent in many of the islets, but not in the aggregates.

Encapsulated aggregates cured a greater proportion of diabetic xenogeneic transplant recipients compared to islets (83% vs 30%) ($p < 0.03$) and the overall average blood glucose concentration was substantially lower. IPGTTs performed on the cured transplant recipients showed aggregates were as responsive as islets to a glucose load and similar to the normoglycemic controls.

Encapsulated islet-cell aggregates were superior to intact islets in terms of survival and function in low oxygen culture and under transplantation conditions.

Supported by RO1 DK 50657; JDRF; Diabetes Research and Wellness Foundation, National University of Ireland.

111-LB

Targeting Uncoupling Protein-2 Improves Islets Graft Primary Function and Reduces the Amount of Islets Required in Islets Transplantation to Achieve Normoglycemia

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Islet transplantation has great potential to normalize glucose metabolism in diabetic patients. However, the success of islet transplants need higher mean islet mass prepared from 2 to 4 donor pancreases. Mitochondrial uncoupling protein-2(UCP2) negatively regulates insulin secretion of β cells. Here we investigate if targeting UCP2 can enhance insulin secretion and reduce the number of islets required in transplantation to restore the normoglycemia.

In vitro, the deficiency of UCP2 activity led to increased insulin secretion in response to high glucose. Islets harvested from UCP2 gene knock out (C57BL/6J background) secrete one fold higher insulin in glucose-stimulated insulin secretion than C57BL/6J mice (33.30±5.44 vs. 16.70±2.19 ng of insulin/islet/ug DNA/ 30 min, respectively; $P<0.05$ [Figure 1b]).

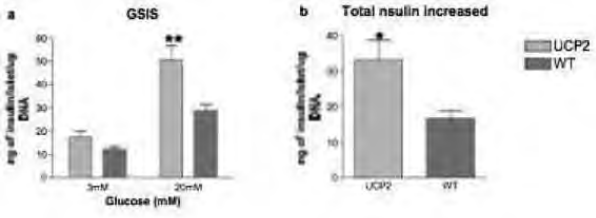

Figure 1. Glucose-stimulated insulin secretion from isolated pancreatic islets. Insulin content corrected for DNA content. Following overnight culture, islets were exposed to 3mM glucose and then 20mM glucose for 30 min each. (a) insulin released in respose to varing glucose was measured. (b) insulin increased inresponse to high glucose stimuation. n per group: KO=13 and WT=9. Results are expressed as mean ± SEM. *=P<0.05 and **=P<0.01 versus WT, unpaired, two-tailed t test.

In a syngeneic islet transplantation model, recipients were rendered diabetic by single injection of streptozotocin five days prior to transplantation. Two hundred islets from UCP2-KO donors were capable of completely restoring normal glycemia in all recipients (n=6). In contrast, wilt-type donors (n=6) had delayed primary graft function up to 16 days post-transplantation [Figure 2].

For author disclosure information, see page LB32.

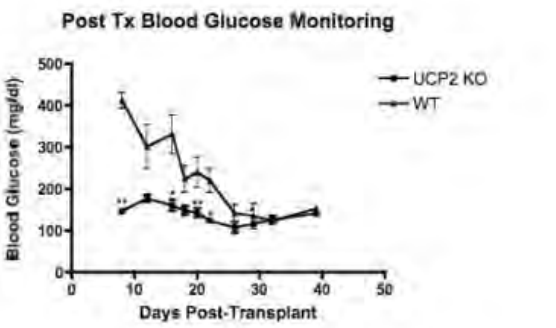

Figure2. Blood glucose levels post islets transplantation. Blood glucose values were assessed every 4 days post islets transplantation. n per group: KO=6 and WT=6. Results are expressed as mean ± SEM. *=P<0.05 and **=P<0.01 versus WT unpaired, two-tailed t test.

Quantitative real-time PCR reveals that UCP2 mRNA expression was decreased 2.5 fold after acute high glucose (25mM) challenge in vitro ($P<0.05$). It seems that transplanted islets respond to acute glucose challenge by down regulating UCP2 to increasing insulin secretion, a process like β cell compensation in the develop of type 2 diabetes. Our results highlight the role for UCP2 in regulating insulin secretion in β cell. And these findings suggest that pancreatic β cell UCP2 is a useful target for improving transplanted islets function.

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Notes: 1) The number following the name refers to the abstract number, not the page number.

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