Late Breaking Abstracts Handout

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CLINICAL THERAPEUTICS/NEW TECHNOLOGY—GLUCOSE MONITORING AND SENSING

1-LB

Disagreement Between HbA1c Derived Average Glucose (ADAG) and Patient Monitored Average Glucose (PMAG) in Two Populations: Implications for Clinical Management

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HbA1c is highly correlated with average glucose levels (AG). Thus, it has been recently proposed to report HbA1c as an estimated AG calculated from the HbA1c (ADAG) in hopes of improving patient management. Pertinent to the clinical utility and safety of ADAG would be the degree of agreement in practice between ADAG and PMAG. Ideally, observed divergence between ADAG and PMAG should be small and not clinically relevant, otherwise clinical decision making may be jeopardized.

Therefore, we examined agreement between ADAG and PMAG by Bland-Altman analysis (Lancet i:307, 1986) from two different populations of type 1 diabetes patients: 150 children at our clinic in New Orleans, and publicly available data from 1439 participants in the DCCT. In New Orleans, PMAG was derived from the mean of each patient's self-monitored glucose records over the 3 months before the HbA1c was obtained. HbA1c was traceable to the DCCT. In DCCT participants, at each quarterly visit a mean blood glucose (MBG) was calculated from the patient's 7-sample glucose profile set. HbA1c and PMAG were calculated as the means of each participant's HbA1c and MBG respectively from each quarterly visit during study participation.

ADAG was calculated from each individual's HbA1c using a previously reported regression equation of AG vs HbA1c, ADAG=(HbA1c*31.5)-68.58, derived from a continuous glucose monitoring protocol over a 12 week period (Diabetologia 50:2239, 2007). The difference of the glucose averages (DiffGlu)=PMAG-ADAG. The upper and lower limits of agreement (LOA) = DiffGlu± 2 standard deviations. Variables are reported as mean±1SD.

Study	HbA1c (%)	ADAG (mg/dL)	PMAG (mg/dL)	DiffGlu (mg/dL)	Upper LOA (mg/dL)	Lower LOA (mg/dL)
New Orleans	8.3±1.5	194.0±46.1	187.7±42.4	-6.1±38.7	71.3	-83.5
DCCT	8.2±1.4	188.9±45.0	194.8±53.1	6.0±30.8	67.6	-55.6

The analysis indicates that there is frequent and clinically significant disagreement between ADAG and PMAG. Approximately 1/3 of patients had discordance of PMAG from ADAG of ±31 mg/dL or greater. As various methods to ascertain PMAG are now readily available and widely used, we believe that frequent discordance between ADAG and PMAG may be extremely confusing to patients and clinicians. In patients where ADAG overestimates the patient's actual mean blood glucose, absence of, or disregard for PMAG may lead the clinician to incorrectly prescribe more aggressive glucose lowering therapy which could provoke serious hypoglycemic episodes.

ADA-Funded Research

CLINICAL THERAPEUTICS/ NEW TECHNOLOGY— INSULIN DELIVERY SYSTEMS

2-LB

Pharmacokinetics and Glucodynamics of an Insulin Analog Injected with Recombinant Human Hyaluronidase: Fast-Acting Insulin Analog Made Faster

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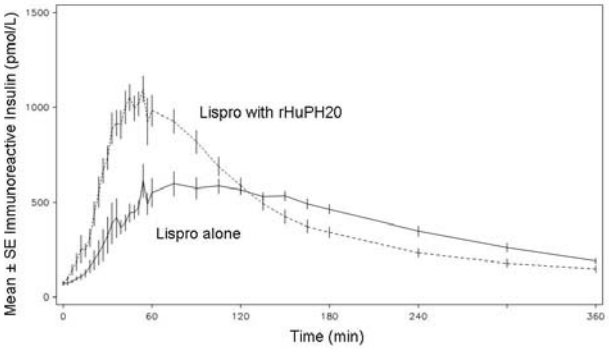
This prospective, double-blind, placebo controlled, Phase I trial compared pharmacokinetics (PK) and glucodynamics (GD) for insulin lispro injected subcutaneously with and without recombinant human hyaluronidase (rHuPH20). A drug product with the rHuPH20 enzyme is FDA-approved to increase dispersion and absorption of other injected drugs. A faster, more physiologic insulin delivery may improve glycemic control and diabetic patient management.

Twelve fasting, healthy, male volunteer subjects (mean age 38 yrs (range 25-47); BMI 26.3 kg/m^2 (22.6-28.5)) were randomized to a crossover sequence of the two injections of 20 U lispro alone (L) and with (LH) 1,364 U/mL rHuPH20 in the abdomen. Insulin and glucose samples were collected over 6 hours during euglycemic clamp (target plasma glucose 90-110 mg/dL).

Insulin PK responses to L and LH (figure) showed a 54% reduction in median T_{max} from 105 (L) to 48 (LH) min (p=0.0006), an effect seen in all 12 subjects. Geometric mean C_{max} increased 87% from 697 (L) to 1,300 (LH) pmol/L (p=0.0003). Mean $AUC_{0\text{-}360min}$ increased 13% from 139,000 (L) to 157,000 (LH) pmol*min/L (p=0.076), $AUC_{0\text{-}30min}$ increased 154% (p=0.0006), $AUC_{30\text{-}360min}$ increased 7% (p=0.29), and at 360 min L and LH were comparable. Inter-subject variability (CV/mean) in T_{max} improved from 44% (L) to 19% (LH).



GD response to L and LH reinforced PK findings, with median time to maximal effect ($tGIR_{max}$) shortened 36% from 210 (L) to 135 (LH) min (p=0.063), and maximal metabolic effect (GIR_{max}) increased 13% from a mean of 181 (L) to 205 (LH) mg/kg*min (p=0.35). The median time to early half-maximal effect ($tGIR_{Early50\%}$) decreased 38% from 68 (L) to 42 (LH) min (p=0.0006).

All injections were well tolerated, without serious, severe, or moderate adverse events.

In conclusion, this first study of co-injection of these drugs showed that rHuPH20 consistently produced statistically significantly faster and more complete absorption and metabolic effects of lispro. Additional studies are planned.

CLINICAL THERAPEUTICS/ NEW TECHNOLOGY—OTHER DRUG DELIVERY SYSTEMS

3-LB

Autoantigen Specific Regulatory T cells Induced in Patients with Type 1 Diabetes Mellitus

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Type 1 diabetes mellitus (T1DM) is the most common chronic childhood disease, a serious burden to the patients, to their families and to the society. The underlying autoimmune process is the result of the imbalance between the self-antigen specific autoagressive and regulatory T cells.

We report the results of a double blind, placebo controlled phase I clinical trial (sponsored by the Immune Tolerance Network) of a novel drug in patients with recently diagnosed T1DM. This new antigen based therapeutic approach uses human insulin B-chain in an incomplete Freund adjuvant (IFA) as a single intramuscular injection. We enrolled 12 patients (6 in each arm), who received either active or the placebo vaccinations within 3 months of their diagnoses and were followed for 2 years. Safety monitoring revealed excellent safety profile in both arms. Mixed meal stimulated C-peptide responses were tested every 6 months and we found no statistical differences, however there was a better trend in the stimulated C-peptide decline in the insulin B-chain vaccinated group after three months of the vaccination. All patients vaccinated with the autoantigen and none clinical trial (sponsored by the Immune Tolerance Network) of a novel drug in patients with recently diagnosed T1DM. This new antigen based therapeutic approach uses human insulin B-chain in an incomplete Freund adjuvant (IFA) as a single intramuscular injection. We enrolled 12 patients (6 in each arm), who received either active or the placebo vaccinations within 3 months of their diagnoses and were followed for 2 years. Safety monitoring revealed excellent safety profile in both arms. Mixed meal stimulated C-peptide responses were tested every 6 months and we found no statistical differences, however there was a better trend in the stimulated C-peptide decline in the insulin B-chain vaccinated group after three months of the vaccination. All patients vaccinated with the autoantigen and none who received placebo developed robust insulin specific humoral and T cells responses. The insulin B-chain specific CD4+ T cells isolated and cloned from the peripheral blood showed phenotypic and functional characteristics of regulatory T cells. There was no successful insulin B-chain specific T cell cloning attempt in any of the placebo treated subjects.

Human insulin B-chain based novel drug therapy showed excellent safety profile in patients recently diagnosed with T1DM and induced a robust immune response generating autoantigen specific regulatory T cells.

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

4-LB

WITHDRAWN

5-LB

Exenatide Achieved Tighter Glycemic Control (A1C ≤6.5%) Compared to Insulin and had a More Favorable Accompanying Metabolic Profile

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Exenatide (Ex) and insulin (Ins) are injectable therapies used in the treatment of type 2 diabetes. In pooled datasets from two studies comparing 10 µg exenatide BID to either Ins glargine or biphasic Ins aspart in patients treated with background therapy of metformin and sulfonylurea for approximately 6 months, we examined metabolic endpoints in those patients who achieved A1C of ≤6.5%. In this post-hoc analysis, 1015 patients comprised the pooled ITT population with entry A1C >6.5% and at least one post-baseline A1C value (547 M, 468 F; age 58.8±9.1; BMI 30.8±4.3 kg/m^2 [mean±SD]; baseline A1C 8.4±0.03%; baseline FSG 193±2 mg/dL [mean±SE]). A1C threshold, body weight change, and blood pressure data were as follows:

	Exenatide		Insulin	
	A1C ≤6.5%	**A1C >6.5%**	**A1C ≤6.5%**	**A1C >6.5%**
Dose	10 µg	10 µg	24.2±2.1 U[¥]	25.4±1.2 U[¥]
N=	133	383	85	414
% of patients	26*	74	17	83
Δ Body weight (kg)	-3.7±0.3**	-1.6±0.1**	+0.19±0.3	+2.0±0.1
Δ Systolic blood pressure (mm Hg)	-7.2±1.5*	-3.6±0.8[†]	-0.3±1.4	-0.4±0.8
Δ Diastolic blood pressure (mm Hg)	-2.1±0.9[§]	-1.1±0.5[§]	-0.9±1.0	-0.5±0.5

(mean±SE); [¥]Ins dose at approximately 6 months, *P<0.005, **P<0.0001, [†]P<0.05, [§]P=NS; Ex vs. Ins

In the patients who achieved A1C of ≤6.5%, Ex induced favorable reductions from baseline compared to Ins in total cholesterol (Ex: -8.53±1.94 mg/dL, P<0.0001; Ins: +0.08±3.59 mg/dL, P=NS; ΔP<0.005) and LDL-cholesterol (Ex: -6.25±1.73 mg/dL, P<0.0005; Ins: +5.02±2.91 mg/dL, P=NS; ΔP<0.0005). Both Ex and Ins induced favorable changes compared to baseline in HDL-cholesterol (Ex: +2.24±0.53 mg/dL, P<0.0001; Ins: +3.44±0.71 mg/dL, P<0.0001; ΔP=NS) and triglycerides (Ex: -16.4±8.0 mg/dL, P<0.05; Ins: -37.3±14.5 mg/dL, P<0.05; ΔP=NS). In patients achieving tight glycemic control (A1C of ≤6.5%), hypoglycemia occurred more frequently with Ins treatment compared to Ex [overall: Ins 60%, Ex 52% (ΔP=NS); nocturnal: Ins 36%, Ex 20% (ΔP<0.01)]. In summary, patients are more likely to achieve tight glycemic control (A1C of ≤6.5%) with Ex than with basal or biphasic aspart Ins. The exenatide-treated patients able to achieve tight glycemic control manifest with a more favorable overall metabolic profile than those treated with insulin. The implications of the differential effects observed with these two therapeutic approaches deserve further study.

6-LB

Renoprotective Effects of the Direct Renin Inhibitor Aliskiren, Irbesartan and the Combination in Patients With Type 2 Diabetes, Hypertension and Albuminuria

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Aliskiren (ALI) and the angiotensin receptor blocker irbesartan (IRB) have antiproteinuric and antihypertensive effects in patients with type 2 diabetes. We compared the antiproteinuric effects of ALI, IRB and the combination (ALI+IRB) in a double-blind, randomized, cross-over trial. After 1 month washout, 24 patients with type 2 diabetes, hypertension and albuminuria (>30 mg/day) were randomized to four 2-month treatment periods with once-daily placebo, ALI 300 mg, IRB 300 mg or ALI+IRB.

Patients received furosemide to control sodium retention. Primary endpoint was change in urinary albumin excretion rate (UAER). Secondary measures included change in blood pressure (BP) and renin system biomarkers. Results are geometric mean change vs placebo.

ALI reduced UAER (baseline 258 mg/day) by 48% (95% CI 27, 62), not different from IRB (58% reduction [42, 70]; both p<0.001). ALI+IRB reduced UAER by 71% (59, 79), significantly more than either monotherapy (p≤0.028). Seated office BP (baseline 135/78 mmHg) was reduced 7/4 mmHg by ALI, 6/4 mmHg by IRB and 12/8 mmHg by ALI+IRB, all significant (p<0.05) except IRB for diastolic BP. GFR (baseline 89 mL/min/1.73m^2) was reduced 4.6 (-8.8, -0.3) mL/min by ALI, 8.0 (-12.3, -3.6) mL/min by IRB and 11.7 (-15.9, -7.4) mL/min by ALI+IRB. ALI+IRB increased plasma potassium by 0.2 mmol/L (p=0.036). ALI reduced high sensitivity plasma renin activity (hsPRA), angiotensin (Ang) I and Ang II by 87%, 75% and 52%; IRB increased these biomarkers by 321%, 207% and 237%, respectively (all p<0.001). Although ALI+IRB caused a 12-fold increase in plasma renin concentration (PRC), the stimulatory effect of IRB on hsPRA, Ang I and Ang II was inhibited by ALI. Active treatment-related reductions in UAER correlated significantly with increases in PRC (r=-0.339, p=0.005). In conclusion, ALI and IRB each reduced albuminuria to the same degree, with different effects on renin system biomarkers. ALI+IRB provided larger reductions in albuminuria that correlated with more complete intra-renal renin system blockade (increases in PRC), suggesting the potential for improved renoprotection.

7-LB

Significantly Better Glycemic Control and Weight Reduction with Liraglutide, a Once-daily Human GLP-1 Analog, Compared with Glimepiride: All as Monotherapy in Type 2 Diabetes

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This 52-week randomized trial compared the efficacy and safety of two doses of liraglutide (1.2 and 1.8 mg, QD) to glimepiride (8 mg QD). Subjects were previously treated with diet and exercise (D/E) or previous OAD monotherapy (mono). In total, 746 subjects were randomized (mean age 53.0±10.9); mean body mass index 33.1±5.8 kg/m^2, mean HbA_{1c} 8.3±1.1%). Liraglutide 1.2 and 1.8 mg reduced HbA_{1c} more than glimepiride (ANCOVA, p=0.0014 and p<0.0001) and more of the subjects in the liraglutide groups reached HbA_{1c} ≤6.5 and <7.0% (p<0.01 vs. glimepiride). In addition, the decrease in HbA_{1c} with liraglutide 1.8 mg was significantly greater than the decrease with liraglutide 1.2 mg (p=0.0046). At the end of the study, there was significant weight decrease in the liraglutide groups, as compared to weight gain in the glimepiride group. The most common adverse events in the liraglutide groups were gastrointestinal disorders (mainly nausea). Nausea occurred in approximately 29% of subjects in the liraglutide groups, but was transient. The rates of minor hypoglycemic episodes (<56 mg/dL) were significantly lower for the liraglutide groups, vs. glimepiride. No subjects reported major hypoglycemic events. In conclusion, liraglutide monotherapy significantly lowered HbA_{1c} versus glimepiride and, at the same time, resulted in weight loss and lower rates of hypoglycemia.

	Liraglutide 1.2 mg N=251	**Liraglutide 1.8 mg N=247**	**Glimepiride N=248**
Final HbA_{1c}, % (SD) Change HbA_{1c}, all % Change HbA_{1c}, from D/E % Change HbA_{1c}, mono %	7.5 (1.3) -0.84* -1.19 -0.47*	7.2 (1.2) -1.14*† -1.60*† -0.71*†	7.8 (1.2) -0.51 -0.88 -0.17
% HbA_{1c} <7.0%	43*	51*	28
% HbA_{1c} ≤6.5%	28*	38*	16
Weight change, kg (SE)	-2.05 (0.28)*	-2.45 (0.28)*	1.12 (0.27)
Final FPG, mg/dL (SD) Change FPG, mg/dL	155 (56) -14*	150 (49) -26*†	167 (54) -5.4
Change PPG, mg/dL	-31	-38*	-25
% reporting minor hypo events/subject/year	12 0.30*	8 0.25*	24 1.96
% reporting nausea	27.5	29.3	8.5

*p<0.05 vs. glimepiride; †p<0.05 1.8 mg vs. 1.2 mg
FPG, fasting plasma glucose; PPG, post-prandial glucose

8-LB

When Is a Unit of Insulin not a Unit of Insulin? Detemir Dosing In Type 2 Diabetes

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Insulin detemir (Levemir) is a new long-acting insulin analogue. Detemir has two unique pharmacologic properties: first, it has a much lower potency than other insulins on a molar basis; second, it is highly bound to albumin in blood. These unique properties may offer some benefits when compared to other insulins, including less variability in serum concentration, therefore resulting in less hypoglycemia. However, detemir may also pose difficulties for dosing.

It appears patients with type 2 diabetes previously treated with NPH insulin require much more than a unit per unit dose conversion. In patients with body mass indices (BMI) greater than 35, as much as double the dose of detemir may be necessary in order to achieve basal glucose control. Substantially increased dose requirements greatly increase the cost of the insulin treatment.

Published studies comparing detemir to other insulins were reviewed and the average dose in units/kg was determined.

An analysis of five trials and 2491 patients showed an average detemir dose of 0.71 units/kg compared with an average dose of other insulins of 0.49 U/kg.

In the four studies which released the data, average BMI was 30, with exclusion criteria for BMI being greater than 35. However, the average BMI of a person with type 2 diabetes in the United States is closer to 35 than 30. A cost comparison of detemir to glargine using an individual of 100kg (corresponding to a height of 169 cm and BMI of 35) is displayed in the second table. Detemir and its chief competitor glargine (Lantus) are priced similarly, approximately $163 for a box of five pen cartridges (total 1500 units). Using the results from the pooled analysis the average weekly cost of a detemir regimen pen would be $54.01 while the average glargine cost would be $37.27. Glargine dosing is 70-80% of total NPH dosing. In the one study comparing detemir with glargine, there was a greater disparity in dosing than in previous studies comparing detemir with NPH. A glargine regimen reflecting the ratio found in this study would translate to an average weekly cost of $30.43.

The unique structure of detemir may make it less potent in type 2 diabetics. Studies examining the efficacy of detemir in very obese type 2 diabetics need to be done. It is important for providers and patients to know that type 2 diabetics will usually require substantially higher doses of detemir than other insulins. This should be considered when titrating the dose as well as in cost-benefit analyses of detemir versus other insulins.

Studies comparing detemir to other basal insulins in type 2 diabetes

Trial	N	Detemir dose (U/kg)	Comparison Basal Insulin	Other Basal Dose (U/kg)
A	394	0.58	NPH	0.46
B	505	0.42	NPH	0.40
C	715	0.86	Biphasic Aspart	0.63
D	475	0.77	NPH	0.52
E	582	0.78	Glargine	0.44
Total	**2671**	**0.71**		**0.49**

Estimated average cost of basal insulin in a 100kg individual

	Detemir cost (0.49 U/kg)	Glargine cost (0.49 U/kg)	Glargine cost (0.40 U/kg)
Daily	$7.72	$5.32	$4.35
Weekly	$54.01	$37.27	$30.43

COMPLICATIONS—HYPOGLYCEMIA

9-LB

Antecedent Hypoglycemia Attenuates Baroreflex Sensitivity - Implications for Rigorous Glycemic Control.

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Background: Rigorous glycemic control decreases the incidence and progression of diabetic complications. It is therefore concerning that a recent multi-center trial reported an increase in mortality in subjects with diabetes randomized to highly intensive therapy (targeting HbA1c values of <6%). The increased mortality did not appear directly related to hypoglycemia. Intensive glycemic therapy may lead to an increased incidence of hypoglycemia and repeated exposure to hypoglycemia impairs the hormonal and autonomic responses to subsequent hypoglycemia. We hypothesized that prior exposure to hypoglycemia would impair the autonomic responses to stressors other than hypoglycemia, specifically cardiovascular stressors.

Methods: Healthy subjects participated in two 3-day admissions, separated by1-3 months. During each admission, autonomic testing was performed on Days 1 and 3 with a 2 hour hyperinsulinemic [hypoglycemic(50 mg/dL) or euglycemic (90 mg/dL)] performed in the morning and repeated in the afternoon of Day 2. Autonomic testing included: (1) measures of parasympathetic function (time and frequency assessment of heart rate variability); (2) baroreflex function (the heart rate-blood pressure relation to transient, short-term hemodynamic fluctuations induced by sequential boluses of nitroprusside and phenylephrine); and (3) sympathetic function (the hemodynamic and norepinephrine response to a lower body negative pressure - a graded, stepwise hemodynamic stress).

Results: 20 healthy subjects (age 28 ± 2 years; 10 males, 10 females) were studied. There were no differences in autonomic testing results between the pre-euglycemic and pre-hypoglycemia Day 1 sessions. The slope of the linear segment of the baroreflex function curve (the baroreflex gain) was significantly reduced on the post-hypoglycemia compared to the post-euglycemia Day 3 session (15.6±7.5ms/mmHg vs. 11.9±4.5ms/mmHg, $p<0.05$). Blood pressure and heart rate measured at baseline and over lower body negative pressures ranging from 0 to -40 mmHg were similar on both Day 3 sessions. The plasma norepinephrine level at -40mmHg of lower body negative pressure was significantly lower on the post-hypoglycemia compared to the post-euglycemia Day 3 session (500±47pg/ml and 341±32pg/ml, $p<0.05$). Measures of heart rate variability in the time and frequency domain showed no differences between the post-euglycemic to the post hypoglycemic Day 3 session.

Conclusion: These data suggest that cardiovascular autonomic function - specifically, baroreflex sensitivity and the sympathetic response to a hypotensive stress - is attenuated following antecedent hypoglycemia. Attenuation of baroreflex sensitivity is an independent predictor of mortality in post-myocardial infarction patients, and, by inference, may contribute to the increased mortality observed in some studies of rigorous glycemic control.

10-LB

Medial Amygdalar Nucleus: A New Site for Glucosensing Neurons

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Specialized glucosensing neurons within the hypothalamus and brainstem alter their firing rate when ambient glucose levels change. Glucose excited (GE) neurons increase and glucose inhibited (GI) neurons decrease their activity as glucose levels rise. Glucokinase (GK) is a gatekeeper for glucosensing in a majority of these neurons; when GK is expressed in a given neuron, it is highly likely that that neuron can sense glucose. We previously found that GK mRNA was expressed in the medial amygdalar nucleus (MAN) and that MAN neurons express urocortin III (UCNIII) and project to the ventromedial hypothalamic nucleus (VMN). The VMN, in turn, is critical for the regulation of the counterregulatory response to hypoglycemia; UCNIII injected into the VMN acts on corticotrophin releasing factor 2 receptors (CRF2R) to dampen this counterregulatory response. Here we show using quantitative real-time PCR that there are rostro-caudal gradients of GK, UCNIII and CRF2R mRNA expression in serial sections of the MAN. There is a direct relationship between CRF2R and GK and inverse relationship between CRF2R and UCNIII mRNA levels. Next, we used fura-2 calcium imaging to assess glucose-induced changes in intracellular calcium oscillations in dissociated MAN neurons to asses their glucosensing capabilities. When glucose levels were lowered from 2.5mM to 0.5 and then back to 2.5mM glucose, 6 % (32 out of 522) of MAN neurons were GE, 8% (39 out of 522 neurons) were GI and the rest did not respond to glucose. These results demonstrate for the first time that the MAN contains glucosensing neurons which may either co-express UCNIII and/or synapse with UCNIII neurons which project to the VMN. Such findings provide a mechanism whereby MAN UCNIII neurons can respond to and modulate the counterregulatory response to hypoglycemia.

11-LB

Mild Hypoglycemia Provokes Increases in Neuronal Activity Specifically in the Hypothalamus and Prior to the Counterregulatory Hormonal Response.

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The hypothalamus plays a central role in the regulation of metabolism by sensing fuel status and relaying this information to effector areas involved in maintaining energy homeostasis. Neuroimaging studies have shown an inhibition of hypothalamic activity in response to glucose ingestion in humans and animals. To determine the effect of acute hypoglycemia on brain activity we used magnetic resonance imaging (MRI) cerebral blood flow (CBF) mapping during hyperinsulinemic eu- and hypoglycemia in healthy human subjects. We hypothesized that acute hypoglycemia would stimulate hypothalamic blood flow, a marker of hypothalamic neuronal activation. Sixteen healthy volunteers underwent euglycemic (plasma glucose ~100mg/dl) and hypoglycemic clamp sessions (plasma glucose ~50 mg/dl) on two separate days in random order. Arterial spin labeling was performed during the study sessions and cerebral blood flow (CBF) was compared between the two conditions. In the hypoglycemic clamp studies CBF was measured as glucose levels were declining and in some cases before the glucose nadir was reached. Group differences showed that CBF to the hypothalamus significantly increased during hypoglycemia compared to euglycemia ($p<0.05$). Region of interest analysis showed that the hypothalamus was the only brain region with signficantly greater CBF during hypoglycemic conditions. Interestingly, hypothalamic CBF was significantly increased ($p<0.05$)

even in subjects in whom perfusion measurements were performed before glucose levels decreased below 70mg/dl and prior to significant elevations in counterregulatory hormones. Our data suggest that the hypothalamus is exquisitely sensitive to small decrements in systemic glucose levels and that changes in hypothalamic blood flow, and presumably neuronal activity, commonly precede the rise in counterregulatory hormones seen during hypoglycemia.

COMPLICATIONS—MACROVASCULAR - ATHEROSCLEROTIC CVD AND HUMAN DIABETES

12-LB

A Retrospective Analysis on Risk of Acute Myocardial Infarction (AMI) in T2 Diabetic Patients Following Initiation of Basal Insulin Therapies

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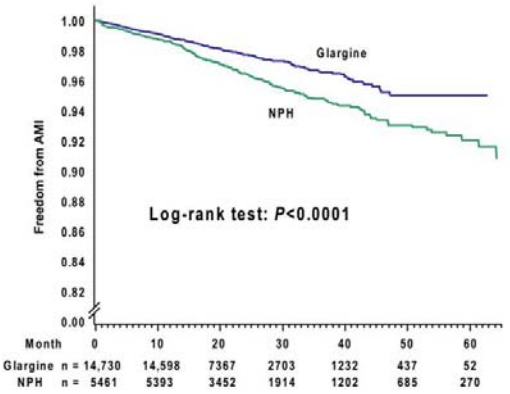
At 2007 American Heart Association meeting, we presented initial findings from a retrospective study comparing the event rate of AMI between diabetic patients who were initiated on basal insulin therapy with either NPH (n=5461) or glargine (GLAR, n=14730). We have since further examined the risk of AMI in the study cohort (mean age 55 years, 46% female) identified from the national database of >30 US managed care health plans with propensity score methods and additional sensitivity analyses to evaluate consistency of the findings between different statistical approaches. Unadjusted AMI event rate was higher with a mean follow-up of 24.4 months after initiation of NPH vs GLAR (17.6/1,000 person-years vs 11.5/1,000 person-years; OR=1.54, p<0.0001, 95% CI:1.30-1.82; also see figure for Kaplan-Meier curves). AMI incidence trended higher in NPH vs GLAR initiators across all age groups but statistically higher in 50-59 years (18.5 vs 10.1/1,000 person-years, OR=1.85, p<0.0001) and 60-69 years (22.0 vs 14.2/1,000 person-years, OR=1.56, p=0.0057). Primary Cox model (hazard ratio [HR]=1.39, 95% CI:1.14-1.69) and propensity matched (1:1) analysis ([OR=1.55, CI:1.23-1.96], AMI rates: 2.53% vs 1.64%) showed consistent excess risk for AMI with NPH compared to GLAR. Sensitivity analyses yielded HR's of AMI from 1.30 (p=0.004) to 1.56 (p=0.005) with Cox models and OR's of 1.47 (p=0.003) to 2.27 (p=0.02) with propensity-matched models for NPH vs GLAR. The analyses consistently showed that patients on oral anti-diabetic agents initiating NPH rather than GLAR experienced a higher risk of AMI. These findings need to be validated in prospectively designed investigations.



13-LB

Cardiovascular Outcomes of the Diabetes Subgroup of the ACCOMPLISH trial

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The Avoiding Cardiovascular events through COMbination therapy in Patients LIving with Systolic Hypertension (ACCOMPLISH) is the first cardiovascular outcome trial designed to compare initial use of two different fixed dose antihypertensive regimens, benazepril plus hydrochlorothiazide versus benazepril plus amlodipine, on cardiovascular endpoints in hypertensive patients at high cardiovascular risk secondary to previous major events or presence of diabetes mellitus (DM). Of the 11,464 patients, 60.4% have DM. At baseline, compared with non-DM patients; DM patients were less likely to have previous myocardial infarctions (15% vs. 37%) or strokes (8% vs. 21%). Those with diabetes were more likely to be female (43% vs. 34%), black (15% vs. 8%), overweight (BMI: 32 vs. 29). At baseline, DM patients were more like to have metabolic syndrome manifested by higher fasting glucose (145 vs. 101 mg/dL), higher triglycerides (178 vs. 150 mg/dL), and slightly lower HDL cholesterol (48 vs. 51 mg/dL) compared to the non-DM cohort. Although estimated GFR (80 and 76 mL/min/ 1.73 m2) was similar in the DM and non-DM groups, presence of both albuminuria (8.7% vs. 3.5%) and microalbuminuria (29% vs. 20%) were more prevalent in DM. After 6 months treatment, BP control rates (<140/90 mm Hg) using blinded data (both therapeutic groups combined) for DM demonstrated 42.8% of DM patients were at <130/80 mm Hg.

The main trial was event not time driven for outcomes and was stopped early by the DSMB due to fewer events in the group randomized to the ACE inhibitor/CCB combination. This was also seen in those with diabetes. Glycemic control was not a determinant of CV outcome.

14-LB

Diabetic Patients Do Not Have More Coronary Plaque but Do Have More Vulnerable Plaque: Results from a Prospective Multinational IVUS Registry

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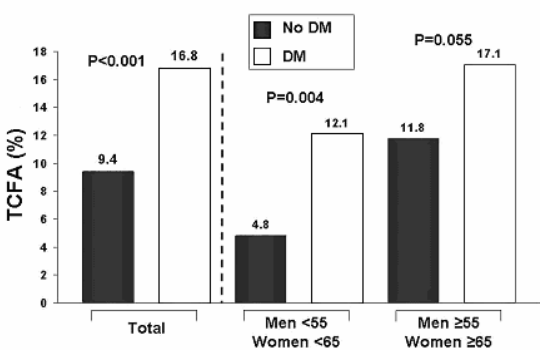
DM patients are at increased risk of cardiac death and nonfatal MI. Thin cap fibroatheroma (TCFA) is an inflammatory atherosclerotic lesion containing luminal confluent necrotic core, with a thin fibrous cap. TCFA is believed to cause plaque rupture, leading to sudden cardiac death and MI. Tissue characterization of atherosclerotic plaque in living subjects and, thus, identification of TCFA is now feasible using intravascular ultrasound (IVUS) assisted by Virtual Histology (VH).

We compared the prevalence of TCFA between DM and non-DM patients enrolled in the Global VH-IVUS Registry-the largest prospective, multinational IVUS registry. From 2004-2006, 3,000 subjects with suspected cardiovascular disease were enrolled at 37 worldwide centers. All patients underwent diagnostic coronary angiography and VH-IVUS. IVUS-defined TCFA was classified as >50% plaque burden and confluent necrotic core extending >14 pixels along the circumference of the lumen on 3 consecutive frames with or without confluent luminal dense calcium. We report interim findings on 990 patients, while results for the entire 3,000 patient cohort will be available for presentation.

There were 792 patients (DM=194) eligible for this analysis. Grayscale measures of normalized plaque volume were similar between DM and non-DM patients (median [IQR] 312.9 [236.4-394.7] vs 294.5 [231.1-371.6] mm^3, P=0.07). DM patients had a significantly greater proportion of TCFA plaque (Figure). When stratified by the age criteria commonly used to establish premature CHD, older DM patients had the greatest prevalence of TCFA. Unexpectedly, younger DM patients had nearly 3-fold more TCFA compared with their non-DM counterparts.

DM patients, particularly the young, have a higher frequency of vulnerable plaque (TCFA) compared with their non-DM counterparts. This may partially account for the greater risk of death and nonfatal MI in

both the young and old.



15-LB

High-dose Atorvastatin Provides Sustained Benefit in Reducing Risk of Cardiovascular Disease Among Patients with Diabetes or Metabolic Syndrome

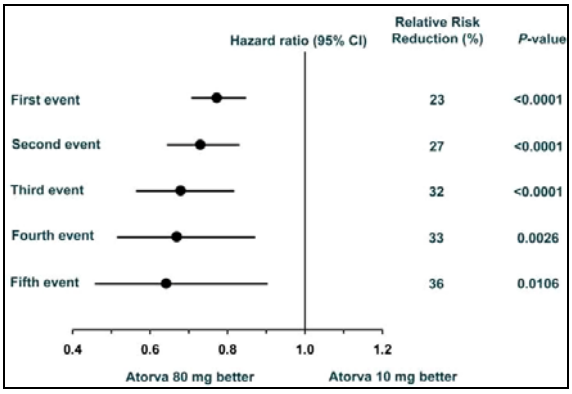
PRAKASH DEEDWANIA, JOHN LAROSA, JAMES SHEPHERD, ON BEHALF OF THE TNT INVESTIGATORS, *Fresno, CA, New York, NY, Glasgow, United Kingdom*

For methodological reasons, analyses of randomized clinical trials are usually restricted to analyses of time to occurrence of first endpoint event. However, since a significant number of patients in long-term trials experience multiple events this approach precludes much potentially useful clinical and health economic information. Based on analyses of time to first event, results of the TNT trial showed that intensive lipid lowering with atorvastatin (ATV) 80 mg significantly reduced the risk of CVD compared with ATV 10 mg both among stable CHD patients with diabetes and among those with metabolic syndrome (MetS). In this analysis, we evaluated the effect of treatment with ATV 80 mg vs 10 mg among patients with diabetes or MetS in that period after the occurrence of a first CV event.

In TNT, 5854 stable CHD patients with either diabetes or MetS were randomized to double-blind therapy with ATV 10 or 80 mg and followed for 4.9 years. Post hoc time-to-event analysis was used to estimate the treatment hazard ratio separately for time to first, second, third, fourth, and fifth occurrences of any CV event (defined as: any coronary event [CHD death, nonfatal MI, resuscitated cardiac arrest, revascularization procedure, procedure-related MI or documented angina]; a cerebrovascular event [fatal or nonfatal stroke, TIA]; PAD; hospitalization with primary diagnosis of CHF.

During TNT, 2002 patients with diabetes or MetS experienced a first CV event. The number with second, third, fourth, and fifth CV events were 1004, 478, 237 and 141, respectively. Among patients with diabetes or MetS receiving ATV 80 mg the relative risk of a first CV event was significantly reduced (23%; $p<0.0001$) compared with those receiving ATV 10 mg. Similar findings were made for the occurrence of second, third, fourth and fifth events (Figure).

Treatment with ATV 80 mg continued to significantly reduce the risk of any CV event over time compared with ATV 10 mg among patients with either diabetes or MetS who had survived previous events.



COMPLICATIONS—NEPHROPATHY

16-LB

Genome-wide Association Scan for Susceptibility Genes for End-Stage Renal Disease (ESRD) in Type 1 Diabetes Mellitus (T1DM): Results from the Genetics of Kidneys in Diabetes (GoKinD) Collection

MARCUS G. PEZZOLESI, G.D. POZNIK, JOSYF MYCHALECKYJ, DANIEL P. K. NG, GRZEGORZ PLACHA, LUIS H. CANANI, KRZYSZTOF WANIC, PISUT KATAVETIN, MASAHIKO KURE, JAN SKUPIEN, JONATHON S. DUNN, ADAM SMILES, WILLIAM H. WALKER, JOHN J. ROGUS, STEVEN S. RICH, JAMES H. WARRAM, ANDRZEJ S. KROLEWSKI, *Boston, MA, Charlottsville, VA*

Diabetic nephropathy (DN) contributes to declining renal function and is the primary cause of ESRD in patients with T1DM. Linkage studies have implicated several loci that may harbor genes that confer increased risk of DN or ESRD, but no gene that affects susceptibility to either complication has been identified unequivocally. To better grasp the genetic factors contributing to ESRD in T1DM, a genome-wide association scan using single nucleotide polymorphisms (SNPs) was implemented on the GoKinD DNA collection as part of the Genetic Association Information Network (GAIN) Database. We first analyzed data from the Joslin component of GoKinD, comprised of 455 T1DM normoalbuminuric Caucasian controls and 351 T1DM ESRD Caucasian cases (203 from the GoKinD collection enriched with 148 additional ESRD patients recruited as part of the Joslin Kidney Study). We performed association analysis using ~400,000 genotyped and imputed SNPs in this panel and sought replication of nominally significant SNPs ($P \leq 0.0005$) in a second non-Joslin component of the GoKinD collection (comprised of 370 T1DM normoalbuminuric Caucasian controls and 315 T1DM ESRD Caucasian cases, both recruited from 27 clinical centers throughout the United States). We confirmed associations at 4 genetic loci in this replication panel. Significant associations were identified in the beta chimerin (*CHN2*) gene using the Cochran-Mantel-Haenszel statistic (combined $P=4.5\times10^{-5}$) and the cysteinyl-tRNA synthetase (*CARS*) gene ($P=2.8\times10^{-6}$). Both genes are highly expressed in human kidney. Strong association were also confirmed at intergenic regions located on chromosomes 9q22 (rs13300603, $P=8.9\times10^{-6}$) and 13q33 (rs9521445, $P=1.9\times10^{-6}$). Although these findings require further replication, they are bolstered by earlier linkage results and implicate *CHN2* and *CARS* as novel susceptibility genes for ESRD in T1DM. If confirmed, they provide insight into novel pathways involved in its pathogenesis.

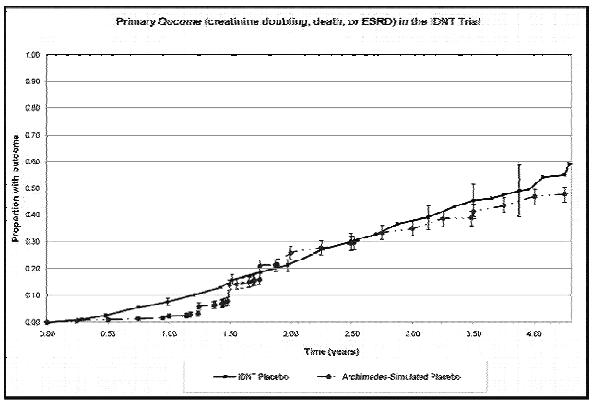
17-LB

Validation of a Mathematical Model of Renal Disease

LENA SHERBAKOV, ANDREI CHTCHEPROV, DAVID KENDRICK, LEONARD SCHLESSINGER, *San Francisco, CA*

Archimedes is a trial-validated model simulating human physiology, diseases (such as diabetes and its complications), and healthcare systems. Archimedes' mathematical representation of renal disease

incorporates the progression of glomerular filtration rate, urinary albumin, and serum creatinine, which are dependent on demographic characteristics as well as diabetes status and social habits. The interactions among these variables are continuously recalculated as a person ages, starts or stops medications, or undergoes diagnostic tests and interventional procedures. The accuracy of Archimedes is tested by simulating real clinical trials. Two randomized controlled trials, Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) and Irbesartan in Diabetic Nephropathy Trial (IDNT) were used to validate the progression of renal disease. The RENAAL trial was used to inform the model and thus its control arm provided a dependent validation. The IDNT's control and treatment (losartan) arms provided independent validations of Archimedes for the time-series primary outcomes, as well as the end of trial secondary cardiovascular outcomes. Log rank analysis estimated the likelihood that simulated results were statistically different (α=0.05) from those of the comparison trials (a p-value above 0.05 indicates no statistically significant difference). In the simulated control arms of both trials, all primary outcomes were not statistically different than those observed in the respective trials (RENAAL: composite outcome p=0.09, ESRD p=0.051, creatinine doubling p=0.24, death or ESRD p=0.051; IDNT: composite outcome p=0.24 [see graphic], ESRD p=0.31, creatinine doubling p=0.14, and death p=0.29). In the losartan arm of the IDNT trial, all primary outcomes predicted by Archimedes were again not found to be statistically different from the actual study results (composite outcome p=0.073, ESRD p=0.43, creatinine doubling p=0.051, and death p=0.21). Archimedes provides accurate, trial-validated estimates of the progression of nephropathy and its complications, as well as effects of therapy in diabetics.



COMPLICATIONS—OCULAR

18-LB

The ADORA2a Gene Is Associated with Incidence and Prevalence of PDR in Type 1 Diabetes

BASHIRA A. CHARLES, SUSAN M. SEREIKA, YVETTE P. CONLEY, ROBERT FERRELL, RACHEL MILLER, JANICE S. DORMAN, TREVOR J. ORCHARD, *Pittsburgh, PA*

Genetic susceptibility is thought to play a role in development of Proliferative Diabetic Retinopathy (PDR). Adenosine is an intermediary substance which attenuates tissue injury, inflammation and hypoxia in vascular cells and the tissues they nourish. Our aim was to determine if the adenosine A2a receptor (ADORA2a) gene or its alleles are associated with PDR.

We included participants (n=496) from the Pittsburgh Epidemiology of Diabetes Complications (EDC) prospective study of childhood onset type 1 diabetes (baseline mean age 28 yrs and mean diabetes duration 19 yrs) for whom banked DNA was available. Stereoscopic images of the retinal fundus were obtained at baseline (1986-1988) and biennially for 18 yrs. PDR was defined as grade ≥ 60 in one eye or < 60 but with panretinal photocoagulation scars consistent with laser therapy, according to the Airlie House system.

Two tagging single nucleotide polymorphisms (tSNPs) of the ADORA2a gene were selected using HAPMAP. The tSNPs were genotyped using TaqMan allelic discrimination assays.

	Allele Frequencies		
	Rs2236624	Rs2236624	Rs4822489
	CC CT TT	CC CT/TT	GG GT TT
Total	308 154 24	308 159	195 212 84
%PDR	26 37 20.8	26 34.8	26.2 32.1 29.8
Total Undetermined	10 (<3%)		5(<1%)

Univariate analysis showed the CT variant was associated with increased prevalence of PDR (OR=1.68, 95%CI 1.11-2.54) compared with the CC genotype. Controlling for traditional risk factors: diabetes dur, HDL, LDL, HTN, sex, glucose, trig., BMI, and smoking, the CT's variant significance was sustained. Prospectively, the incidence of PDR declined in those with the CC and TT genotypes compared to the CT genotype.

Baseline and Prospective Risk Factors Associated with PDR			
Genotype Grouping CC, CT, TT for Logistic Regression			
	Odds Ratio	SE	95% CI
Duration (DUR)	1.20	0.04	1.12-1.29**
Hypertension (HTN)	3.77	0.34	1.92-7.38**
Low Density Lipoprotein (LDL)	1.01	<0.01	1.00-1.02*
CT	2.18	0.27	1.24-3.81**
Genotype Grouping CC vs. CT/TT for Logistic Regression			
DUR	1.2	<0.04	1.12-0.87**
HTN	3.78	0.34	1.93-7.41**
LDL	1.01	<0.01	1.002-1.02*
Ever Smoker	1.19	0.09	1.002-1.40*
CT/TT	0.51	0.27	0.30-0.87*
Genotype Grouping CC, CT, TT for Cox Proportional Hazards Regression			
	Hazard Ratio	SE	95% CI
Glyclosylated Hemoglobin (GHB)	1.24	0.04	1.12-1.36**
Body Mass Index (BMI)	1.07	0.03	1.02-1.12*
TT	0.10	1.01	0.01-0.69*
CT	1.68	0.18	1.17-2.40**
*<0.05 **<0.01			

The CT variant is associated with increased prevalence of PDR at baseline; while the CC and TT genotypes are prospectively associated with decreased incidence of PDR. The region of DNA tagged by the rs2236624 SNP infers susceptibility to/protection from development of PDR in the EDC population.

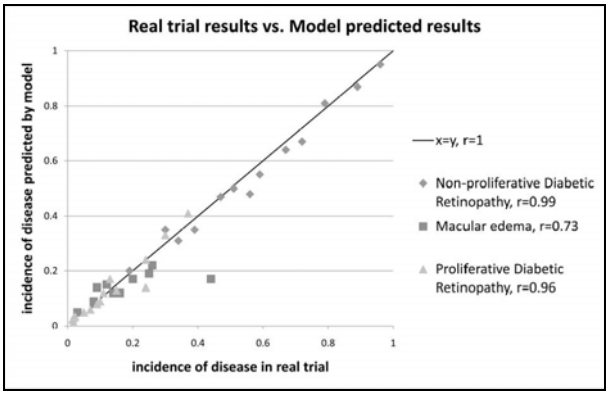
19-LB

Validation of a Model of Eye Disease in Diabetes

STUART SAMUEL, DAVID KENDRICK, *San Francisco, CA*

A model of eye disease in diabetes is developed for Archimedes, which is a trial-validated simulation model of human physiology, diseases and healthcare systems and the basis for the online risk assessment tool Diabetes PHD. We use algebraic and calculus-based equations that incorporate the risk factors of duration of diabetes, glycemia, blood pressure and diabetes type (1 or 2) to represent the physiological development of non-proliferative diabetic retinopathy (NPDR), macular

edema (ME), and proliferative diabetic retinopathy (PDR). For patients with PDR, we assess the probability of progression to legal blindness. We constructed the model from several major retinopathy studies and then used the model to predict the results of other research publications. Studies used to validate this model include the Wisconsin Epidemiological Study of Diabetic Retinopathy, the Epidemiology and Prevention of Diabetes (EURODIAB), the New Jersey 725, the Barbados Eye Studies, the United Kingdom Prospective Diabetes Study, the Taiwan Diabetes Study, the Diabetes Incidence Study in Sweden, the Helsingborg Study, and the Diabetes Control and Complications Trial. The figure shown compares the cumulative incidence of NPDR, ME and PDR for each of 39 independent validations with the incidence predicted by the model. The correlation coefficient for actual trial results compared with the model's predicted results is 0.99 for NPDR, 0.73 for ME, and 0.96 for PDR. One validation of ME failed and the removal this single outlier improves the correlation coefficient for ME to 0.92. Predictions of the three outcomes versus duration of diabetes at baseline agree with data to within one or two standard errors [graphs not shown]. We also independently validate distributions of NPDR levels and two- and three-step progressions [results not shown]. We conclude that the model accurately predicts the retinopathy outcomes of trials spanning a variety of diabetes populations and healthcare systems, and that it could be useful for predicting the results of a variety of changes in healthcare processes and treatments.



DIABETES EDUCATION

20-LB

Use of a Decision Aid for Patients with Type 2 Diabetes (T2D) Intensifying Treatment. A Randomized Clustered Trial

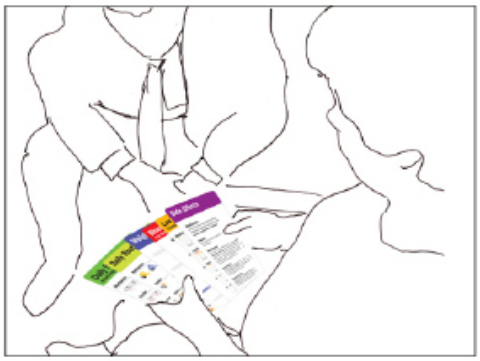
REBECCA J. MULLAN, VICTOR M. MONTORI, ROBERT J. STROEBEL, BARBARA P. YAWN, STEVEN A. SMITH, VICTOR P. YAPUNCICH, RICHARD SCHINDLER, SANDRA C. BRYANT, TERESA J. CHRISTIANSON, *Rochester, MN, Austin, MN*

Patients with T2D delay insulin use and do not participate in making choices about diabetes medications. We developed a decision aid for patients with poorly controlled T2D consisting of 6 issue cards that describe the impact five common medications have on A1c level, the incidence of hypoglycemia, weight changes, daily routine, blood sugar monitoring and side effects.

We conducted a clustered randomized trial at 11 primary care and family medicine clinics in Southeastern Minnesota. Eligible patients had T2D for ≥ 1 year, HbA1c 7-9.5% in the last six months, were not on insulin and were using < 4 anti-hyperglycemic medications. We randomized 21 clinicians and 48 patients to using the decision aid and 19 clinicians and 37 patients to usual care. Immediately post visit, patients and clinicians completed a questionnaire measuring outcomes related to the decision making process. At 6 months, we measured A1c and drug adherence using pharmacy records.

The decision aid promoted patient involvement in the decision making process: 96% (vs 84%) of patients who used the decision aid agreed with "my provider identified blood sugar control as a choice that I could participate in" (OR 3.71, 95% CI 0.72, 19.1); 92% (vs. 74%) of patients who used the decision aid agreed with "my provider asked about my expectations and fears regarding how my blood sugar is managed" (OR 7.97, 95% CI 1.5, 42.1) ; and 68% (vs. 58%) of participants would strongly recommend the decision aid to others facing a similar decision (OR 1.62, 95% CI 0.65, 4.02); 90% of clinicians want to have access to the decision aid for future diabetes visits. Video analysis showed that, compared to usual care, clinicians and patients using the diabetes cards more frequently discussed issues of importance to patients (such as the effect of medication on weight and the impact of medication on daily routine) and always discussed insulin.

A patient decision aid was acceptable in practice and enhanced patient-centered diabetes treatment intensification. The impact of this intervention on A1c and medication adherence awaits completion of 6-month follow-up with results due in April 2008. **ADA-Funded Research**



DIABETIC DYSLIPIDEMIA

21-LB

A Common Variant in the ABCA12 Gene Accounts for Linkage of Total Cholesterol to Chromosome Region 2q34 and is Associated with Obesity

WEI-DONG LI, GUANGMING YUAN, R. ARLEN PRICE, *Philadelphia, PA*

We previously found significant linkage for total cholesterol in human chromosome region 2q34 in 320 nuclear families (D2S2944, LOD=4.36, p<0.00001). We further tested 65 families with the highest family specific lod score for quantitative associations using 125 SNPs (single nucleotide polymorphisms) over a 6 Mb region. We found significant associations between the gene ABCA12 (ATP-binding Cassette, Superfamily A, Member 12) SNPs and total cholesterol (rs1980846, p=0.0001). A significant association (rs4673937, p=0.00009) was also found for fasting glucose.

We further tested ABCA12 polymorphisms using cases and controls, including 592 cases (BMI>35kg/m^2) and 548 controls (BMI<25kg/m^2). We genotyped 14 SNPs in the ABCA12 gene, including 4 non-synonymous coding region polymorphisms. Associations were found among ABCA12 gene SNPs and total cholesterol and body weight related phenotypes (BMI, %fat, waist circumferences and waist/hip ratio). The SNP rs4673937 yielded the most significant result for BMI ($P=7.6 \times 10^{-7}$, chi-square analysis) and BMI adjusted total cholesterol (P=0.00007). One-way ANOVA showed that the SNP rs4673937 associated with BMI (p=0.003), %fat (P=0.001) and waist (P=0.003). Flanking ABCA12 SNPs also showed strong to moderate associations for lipid and body weight related phenotypes.

The ABCA12 gene belongs to the ATP-binding cassette (ABC) transporters super family. The ABC genes are involved in lipid transport. ABC genes mutations have been identified in Harlequin ichthyosis and Tangier Disease. In our study, we have found no truncation mutations in our samples. Four non-synonymous coding region mutations were tested in our study and only marginal associations were found for total cholesterol and waist circumferences with rs726070. ABCA12 gene polymorphisms are among the strongest found for body weight and lipid profiles by the positional-candidate strategy. Additional replications are needed to substantiate the associations and functional studies are needed to identify causal variation. Associations with SNPs like these for ABCA12 may aid in identifying causal variation that contributes to common diseases.

EPIDEMIOLOGY

22-LB

Are the Disparities in the Prevalence of Diabetes a Result of Race/Ethnicity or Socioeconomic Status? Results from the Boston Area Community Health (BACH) Survey

CAROL L. LINK, JOHN B. MCKINLAY, *Watertown, MA*

The American Diabetes Association reports that the prevalence of diabetes is 2 to 4 times higher in minority populations compared to Whites. In the United States, minority populations are often disadvantaged socioeconomically compared to Whites and the question arises: Is the disparity in the prevalence of diabetes due to race/ethnicity or socioeconomic status and its associated disadvantages?

Using data from a community based epidemiologic survey of 5503 residents of Boston, Massachusetts (2301 men, 3202 women; 1767 Black, 1877 Hispanic, 1859 White), we find that the odds of having diabetes are 2.04 (95% confidence interval (CI) 1.42, 2.94) for Blacks and 2.35 (95% CI 1.60, 3.44) compared to Whites after adjusting for gender and age. However, if one adds socioeconomic status (a combination of education and income), trouble paying for basics, health insurance status, body mass index (BMI), physical activity, smoking history, and family history of diabetes (parent, sibling, or child has diabetes) to the model the odds are reduced to 1.19 (95% CI 0.81, 1.76) for Blacks and 1.41 (95% CI 0.90, 2.20) for Hispanics compared to Whites. Using a generalized R squared statistic we find that we can only explain 11.8% of the variation for the prevalence of diabetes. If we enter modifiable risk factors before non-modifiable factors, the explained variation is due to: BMI (24.3%), socioeconomic status / trouble paying for basics (13.2%), physical activity (6.9%), health insurance status (1.7%), smoking history (0.4%) (46.5% modifiable risk factors), age (32.3%), family history of diabetes (19.4%), gender (1.2%), and race/ethnicity (0.6%) (53.5% non-modifiable risk factors).

These results suggest that socioeconomic status (potentially modifiable) trumps race/ethnicity (non-modifiable) as a contributor to variation in the prevalence of diabetes. These findings have profound implications for social policy, public health interventions, and clinical practice.

23-LB

Diabetes and the Risk of Cancer Death: A Case-Cohort Study in aiwanese Men

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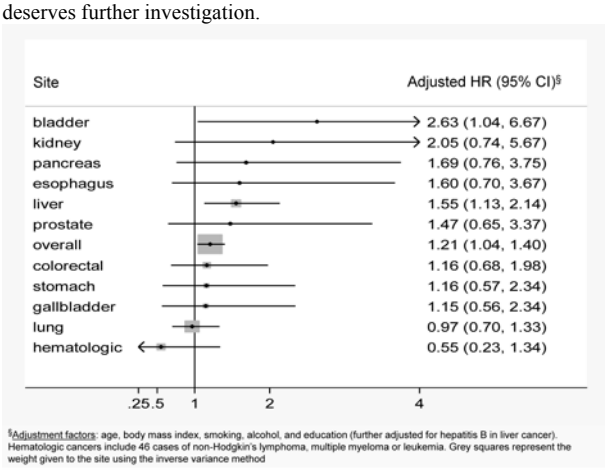
Whereas recent evidence suggests that diabetes mellitus may be a risk factor for the development of cancer, few prospective studies have addressed the impact of diabetes on cancer in Asian populations. We conducted a case-cohort study using longitudinal data from MJ Health Study, a private health screening program based in Taiwan, to determine the risk of cancer death in adults with vs. without diabetes. At baseline in 1996 to 2003, 54,751 men aged 40-80 years completed a detailed health screening, and were followed through 2005 for mortality (mean follow-up, 5.8±2.5 years) by linking with the National Death Registry. We excluded men with cancer at baseline and cancer deaths occurred within the first 2 years of follow-up. Therefore, a total of 951 cancer deaths, including 238 liver, 222 lung, and 80 colorectal cancers, were included for analysis. For comparison, a sub-cohort of 4,000 men (mean age 54±10) was randomly selected from the original population. The prevalence of diabetes (defined by self-reported diabetes, use of anti-diabetes medication, or fasting plasma glucose ≥126 mg/dl) was 44% and 24% in the case and in the sub-cohort, respectively. In the multivariate analysis using Cox proportional hazards model with variance correction for the case-cohort design, diabetes was associated with significantly elevated risk for overall cancer mortality [Hazard Ratio 1.21, 95% CI, 1.04 - 1.40], after adjustments for age, body-mass index, smoking, alcohol intake, and education. In particular, adults with diabetes were 2.6 times more likely to die from bladder cancer [HR, 2.63, 95%CI, 1.04 - 6.67], and 55% more likely to die from liver cancer [HR, 1.55, 95%CI, 1.13 - 2.14, with further adjustment for the hepatitis B status] compared to their non-diabetic counterparts (see Figure). Increased but non-significant risks of cancer death were also observed for other sites, but not for lung or hematologic malignancies. Our study suggests that diabetes is a risk factor for cancer mortality in this Asian population. Whether improvements in diabetes prevention and care for people with cancer could reduce mortality deserves further investigation.



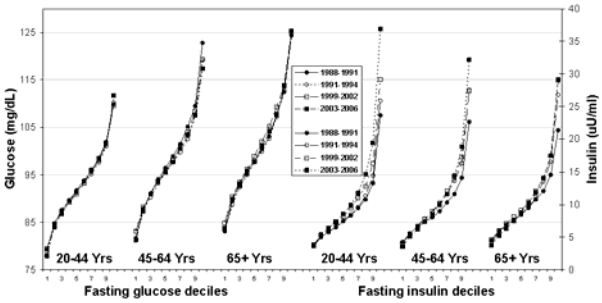
ADA-Funded Research

24-LB

Did the Distributions of Fasting Glucose and Insulin in the US Adult Population Without Diabetes Change in the Past 18 Years?

YILING J. CHENG, EDWARD W. GREGG, GIUSEPPINA IMPERATORE, HENRY S. KAHN, LINDA S. GEISS, *Atlanta, GA*

An increase in incidence and prevalence of diabetes in the U.S. population has been well documented, but whether this has been accompanied by recent shifts in the distribution of fasting glucose and insulin concentrations of the U.S. population without diabetes is unknown. We analyzed data on 15,322 participants from 4 periods of NHANES (year 1988-1991 n=4250; 1991-1994 n=4450; 1999-2002 n=3400; and 2003-2006 n=3222) to examine the changes of fasting plasma glucose (FPG) and fasting serum insulin (FI) of their period-specific distributions by age groups. Analyses excluded persons with diagnosed diabetes or with FPG >=140 mg/dl. Changes in means by period-specific deciles were used to describe changes of distribution. Analyses were stratified by 3 age groups (age 20-44, 45-64, and 65+ years) and we used survey weights to account for the complex sampling design. All results were controlled by age, sex, and race/ethnicity. From the survey period 1988-1991 to 2003-2006, the mean FPG did not change in adults age 20-44 (-0.2 mg/dL, p=0.153), was slightly lower in adults age 45-64 (-1.4 mg/dL, p<0.001), and slightly higher in adults age 65+ (+0.4 mg/dL, p=0.016). Among the overall population age >=20, there was only a small mean decrease (-0.5 mg/dL, p=0.047) in the FPG level across the period 1988-1991 to 2003-2006. For insulin during the same time period, however, mean FI increased by 3.0, 1.8, 1.3 uU/ml for adults 20-44, 45-64, and 65+, respectively (all p<0.001). Increases in FI were notable at the high end of the distribution; mean increases of FI for the 10th decile were 13.3, 9.5, and 7.8 uU/mL for adults age 20-44, 45-64, and 65+, respectively, between 1988-2006 (all p<0.001). Meanwhile, at the 1st decile there were small decreases of FI levels among adults age 20-44 (-0.3 uU/mL, p=0.057), adults age 45-64 (-0.7 uU/mL, p<0.001), and adults age 65+ (-0.8 uU/mL, p<0.001). Our analyses indicate that during the past 18 years there has been little change in the distribution of FPG across the population without diabetes but FI levels have increased substantially, particularly among the younger adults.



25-LB

Fueling the Diabetes Epidemic? Artificially Sweetened Beverage Consumption and Diabetes Incidence in the San Antonio Heart Study

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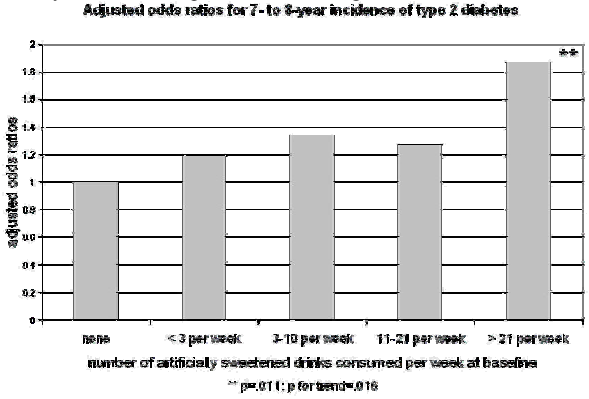
Propelled by rising obesity levels, the epidemic of type 2 diabetes has spread worldwide. Against this backdrop, artificial sweeteners (AS) have been widely promoted as healthy alternatives to sugar. Recently, however, AS consumption has been associated with weight gain in animal research, and artificially-sweetened beverages (ASB), including diet sodas, have been linked to long-term weight gain, and incidence of overweight, obesity, and even metabolic syndrome. We have examined the association between AS use and diabetes incidence (DM_{inc}) in the San Antonio Heart Study.

From 1979 to 1988, oral glucose tolerance tests, medical histories, and dietary questionnaires were administered to 5158 residents of San Antonio, TX, of whom 3682 (71%) returned to follow-up examination 7 to 8 years later. Of 3085 follow-up participants without baseline diabetes (per 1999 World Health Organization criteria), 282 (9.1%) had developed diabetes by follow-up.

Figure 1 shows ORs for DM_{inc} by baseline ASB consumption quartiles, adjusted for gender, ethnicity, socioeconomic status, education, family history of diabetes, and the following baseline variables: age, BMI, systolic blood pressure, serum triglycerides, serum HDL cholesterol, fasting and two-hour post-glucose-load plasma glucose, and exercise frequency.

Adjusted ORs for DM_{inc} rose from 1.19 (0.72-1.96) in ASB quartile 1, through 1.34 (0.83-2.18) and 1.27 (0.80-2.02) for quartiles 2 and 3, to 1.87 (1.15-3.03, p=.01) in quartile 4 (p=.016 for trend). Diabetes incidence thus almost doubled among those consuming 22 or more artificially sweetened beverages per week, vs. none.

While these data do not prove that artificially sweetened beverage consumption increases diabetes risk, they are congruent with recently published data in suggesting the possibility that frequent consumption of artificial sweeteners may increase - rather than reduce - the risk of chronic, obesity-related health problems, including diabetes itself.



EXERCISE—HUMAN

26-LB

Combining Short-Term Metformin Treatment and Acute Exercise Does not Improve Insulin Sensitivity

CARRIE G. SHAROFF, TODD A. HAGOBIAN, STEVEN K. MALIN, STUART R. CHIPKIN, HAIYAN YU, MICHAEL F. HIRSHMAN, LAURIE J. GOODYEAR, BARRY BRAUN, *Amherst, MA, Boston, MA*

The results from the Diabetes Prevention Program highlight the effectiveness of metformin and regular physical activity in the prevention of Type 2 diabetes. The mechanism by which these treatments prevent diabetes is by improving insulin sensitivity. Metformin and exercise independently increase insulin sensitivity; however, the combined effects have not been studied. To assess the combined effects, we studied 2 groups of insulin resistant subjects matched for age (33±10 vs. 32±10 yrs), weight (87±16 vs. 91±23 kg), body fat (41±7 vs. 36.4± 6%), fitness (27±6 vs. 29±6 ml/kg/min) and degree of insulin resistance (composite insulin sensitivity index 2.8±1 vs. 2.2±1). The 1st group (n=9:6W,3M) was studied before treatment (B), after 2-3 weeks of 2000 mg/day metformin (MET), and after metformin plus 40min of exercise at 65%VO2peak (MET+Ex). The 2nd group (n=7:5W,3M) was studied at baseline and after an acute bout of exercise at 65%VO2peak (Ex). Biopsies of the vastus lateralis were taken at B, after MET, immediately after MET+Ex (group 1) or immediately after Ex only (group 2), and used to measure AMPKα2 activity and muscle glycogen. Insulin sensitivity was assessed 3 hrs post-exercise with a euglycemic hyperinsulinemic (40 mU/m2/min) clamp enriched with [6,62H]glucose. Data were compared using ANOVA with repeated measures. Exercise alone increased insulin sensitivity by 54% ($p<0.01$); however, there was no increase in insulin sensitivity with MET+Ex. Muscle glycogen was reduced by ~50% with both Ex and MET+Ex suggesting that differences in muscle glycogen concentrations are not responsible for the differences in post-exercise insulin sensitivity. Skeletal muscle AMPKα2 activity was increased by 3-fold ($p<0.01$) with Ex alone but did not increase with MET+Ex. Circulating plasma free fatty acids were not different across conditions and therefore are not likely to be responsible for the differences in post-exercise insulin sensitivity. These surprising findings suggest that adding short-term metformin treatment to an acute bout of exercise does not enhance insulin sensitivity and may actually attenuate the well documented effects of exercise. These results highlight the importance of future studies designed to examine the effects of long-term metformin treatment combined with exercise training on whole-body insulin sensitivity. Supported by ADA 7-04-JF-10 and ADA-7-04-MN-16. **ADA-Funded Research**

EXERCISE—REGULATION OF MUSCLE METABOLISM

27-LB

Evidence for a Consistent Association between the Effects of Prior Exercise on AS160 Phosphorylation and Insulin-stimulated Glucose Transport in Rat Skeletal Muscle

KATSUHIKO FUNAI, GEORGE G. SCHWEITZER, GREGORY D. CARTEE, *Ann Arbor, MI*

Exercise leads to an increase in insulin-stimulated glucose transport (GT) in skeletal muscle that persists for a day or longer in fasted rats and is reversed with post-exercise (PEX) chow feeding. This persistent increase in PEX insulin-stimulated GT can occur in the absence of increases in many upstream insulin signaling events, including insulin receptor tyrosine kinase activity, insulin receptor substrate-associated phosphatidylinositol 3-kinase, or Akt serine phosphorylation. In contrast, phosphorylation (detected using the phospho Akt substrate, PAS, antibody) of Akt substrate of 160 kD (AS160), the most distal insulin signaling step that has been linked to GLUT4 translocation, remains elevated 3h PEX in the absence of insulin. The aims of this study were to determine if: 1) the increase in insulin-stimulated GT is associated with a persistent increase in PAS-

AS160 at 27h PEX; and 2) if reversal (by chow feeding) of insulin-stimulated GT would be accompanied by loss of the increased PAS-AS160. Wistar rats were assigned to sedentary (SED) or PEX (2h swim) groups. After exercise, rats were either chow fed ad libitum for 3h (SED-Chow3 or PEX-Chow3), fasted for 3h (SED-Fast3 or PEX-Fast3), or fasted for 27h (SED-Fast27 or PEX-Fast27). One epitrochlearis from each rat was incubated without insulin, and the contralateral muscle was incubated with 50μU/ml insulin. 3-O-[^{3}H]Methyl-D-glucose transport (3MGT), PAS-AS160 and Akt threonine phosphorylation (pThrAkt) were determined. Data were analyzed by 1-way ANOVA. 3MGT, PAS-AS160, and pThrAkt were greater in insulin-stimulated muscles from PEX-Fast3 vs. SED-Fast3 and PEX-Fast27 vs. SED-Fast27. In addition, PAS-AS160 from non-insulin-stimulated muscles was greater in PEX-Fast3 vs. SED-Fast3 and PEX-Fast27 vs. SED-Fast27. However, 3MGT, PAS-AS160, and pThrAkt were not increased in PEX-Chow3 vs. SED-Chow3, regardless of insulin concentration. These results: 1) confirm the previous finding that increased PAS-AS160 and pThrAkt are associated with increased insulin-stimulated GT at 3h PEX in fasted rats; 2) demonstrate that these associations persist for up to 27h PEX with continued fasting; and 3) indicate that each of these exercise effects can be reversed to SED levels with 3h of chow feeding. Further studies will be necessary to determine if increased phosphorylation of AS160 and/or Akt is necessary for increased insulin-stimulated GT after exercise.

28-LB

Inhibition of Contraction-stimulated AMPK Partially Inhibits the Contraction-stimulated Increases in Glucose Transport and PAS-150kD without Altering PAS-160kD in Rat Skeletal Muscle

KATSUHIKO FUNAI, JAMES G. MACKRELL, GREGORY D. CARTEE, *Ann Arbor, MI*

Two members of the TBC1 domain family of proteins, Akt substrate of 160kD (AS160) and TBC1D1, become phosphorylated in response to contraction by skeletal muscle. AMP-activated protein kinase (AMPK) and Akt, which are activated by contraction, can phosphorylate both AS160 and TBC1D1. We previously found that wortmannin (inhibitor of phosphatidylinositol 3-kinase, PI3K, which is upstream of Akt) can eliminate the contraction-stimulated (CS) increase in AS160 phosphorylation (detected using phospho-Akt substrate, PAS, antibody) of rat epitrochlearis muscle without altering the CS increase in glucose transport (GT), indicating that CS PAS-AS160, but not GT, is Akt dependent in rat epitrochlearis. To evaluate the roles of AMPK and Akt on CS GT and phosphorylation of AS160 and TBC1D1, rat epitrochlearis were incubated ± inhibitors of AMPK (Compound C) or PI3K/Akt (wortmannin) prior to and during contraction (2ms twitch, 2Hz for 20min) or resting conditions. Neither inhibitor altered tension development. Muscles were used for immunoblotting or 3-O-[^{3}H]methyl-D-glucose transport measurement. CS kinases were assessed with phospho-specific antibodies. In samples immunoblotted using antibodies against each TBC1 domain protein, we found that AS160 migrated at ~160kD and TBC1D1 migrated slightly lower at ~150kD. We also found that immunoprecipitation (IP) of samples with PAS antibody, followed by immunoblotting with the PAS antibody revealed 2 CS PAS-bands at ~160 and ~150kD, and their respective locations corresponded to bands identified by IP with PAS followed by immunoblotting with anti-AS160 and anti-TBC1D1. Contraction increased pAMPK, phosphorylated acetyl CoA carboxylase (pACC; an AMPK substrate), pGSK3 (an Akt substrate), PAS-160kD, PAS-150kD, and GT. Wortmannin eliminated CS pGSK3 ($P<0.05$) and PAS-160kD ($P<0.01$), but did not significantly alter pAMPK, pACC, PAS-150kD or GT. Compound C completely inhibited the CS increase in pACC ($P<0.001$), partially blocked the CS PAS-150kD ($P<0.05$) and GT ($P<0.001$), but did not significantly alter pGSK3 or PAS-160kD. These data suggest that, in CS rat epitrochlearis, 1) PI3K/Akt activation is essential for the increase in PAS-160kD (apparently AS160), but not for the increase in PAS-150kD (apparently TBC1D1) or GT, and 2) AMPK activation is necessary for the full increase in PAS-150kD and GT, but not for the increase in PAS-160kD.

29-LB

The B_2 Receptor of Bradykinin Is Not Essential for the Increase in Insulin-Stimulated Glucose Uptake Following Acute Exercise

GEORGE G. SCHWEITZER, CARLOS M. CASTORENA, TAKU HAMADA, EDWARD B. ARIAS, GREGORY D. CARTEE, *Ann Arbor, MI*

Previous studies have shown that bradykinin can modulate skeletal muscle glucose uptake by acting through the B_2 receptor of bradykinin (B2R) and that exercise can increase skeletal muscle bradykinin release, raising the possibility that bradykinin may play a role in exercise effects on glucose uptake. The primary aim of this study, therefore, was to determine if the B2R is essential for the post-exercise increase in insulin-stimulated glucose uptake. Male wildtype (WT) or B2R knockout (B2RKO) mice were either sedentary (SED) or performed a 60min treadmill exercise protocol (EX) that has been shown to increase insulin-stimulated glucose uptake in the soleus, but not in the extensor digitorum longus (EDL) of WT mice. Blood was sampled from SED and EX mice for measurement of glucose concentration. Paired soleus and EDL muscles were excised from WT and B2RKO mice (SED or EX) and incubated with [^{3}H]-2-deoxyglucose with or without submaximally effective insulin. Insulin-stimulated glucose uptake was calculated by subtracting basal glucose uptake values from values for paired muscles incubated with submaximal insulin. Data were analyzed by two-way ANOVA. There was not a significant genotype effect on blood glucose concentration ($P=0.739$), but there was a trend ($P=0.051$) for glycemia to be lower for EX vs. SED mice. There was also a trend ($P=0.090$) for an exercise-induced increase in insulin-independent glucose uptake in the soleus, but not the EDL. There was not a significant genotype and exercise interaction for insulin-independent glucose uptake in the soleus ($P=0.939$) or EDL ($P=0.848$). EX vs. SED had increased insulin-stimulated glucose uptake in the soleus ($P<0.05$), but not in the EDL. There was no significant interaction between genotype and exercise effects on insulin-stimulated glucose uptake in the soleus ($P=0.940$) or EDL ($P=0.542$). There was no significant effect of exercise or genotype on insulin-stimulated Akt threonine phosphorylation (pThrAkt) in either muscle, and there was no significant interaction between genotype and exercise for pThrAkt in the insulin-stimulated soleus ($P=0.760$) or EDL ($P=0.588$). These results indicate neither the B_2 receptor of bradykinin nor an increase in insulin-stimulated pThrAkt is essential for the post-exercise increase in insulin-stimulated glucose uptake in mouse soleus muscle.

FOOT CARE—LOWER EXTREMITIES

30-LB

The Interaction between Pressure and Skin Blood Flow in Normal and Diabetic Populations at 3 Environmental Temperatures

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As a protective response, skin blood vessels dilate with applied pressure, but, due to diabetic endothelial dysfunction, this response may be absent or diminished. The present study investigated this response in the foot at 3 environmental temperatures. Subjects were older (O) (n=15, average age 66.9 ± 18.4 yrs), had diabetes (D) (n=15, average age 62 ± 5.9 yrs, HB A1c 6.77 ± 1.19 %, mean duration 13.2 ± 9.1 yrs) or were younger (Y) (n=15, average age 25 ± 2.9 yrs). An infared laser dopler flow meter was used to measure skin blood flow on the bottom of the foot during and after applications of pressure at 7.5, 15, 30, 45, and 60 kPa. After 30 seconds of pressure, the pressure-induced vasodilation (PIV) was significantly lower in the group with diabetes ($p<0.05$). (Figure 1a) The blood flow for all three groups was significantly lower in the 16°C environment ($p<0.05$). Circulation at rest was significantly less in the D group ($p<0.05$) in all three global temperatures. After pressure was released, Y and O showed a marked increase in blood flow for every pressure application except 7.5 kPa at all three global conditions. D had significantly lower PIV blood flows after all application of pressure in the colder (16°C) environmental temperature ($p<0.05$). (Figure 1b) Thus, the protective mechanism of PIV is absent

or diminished in diabetic populations especially in colder environments were skin blood flow is already reduced.

Figure 1a. The pressure-induced vasodilation following 30 seconds of local pressure in the 24°C gl temperature for all groups of subjects.

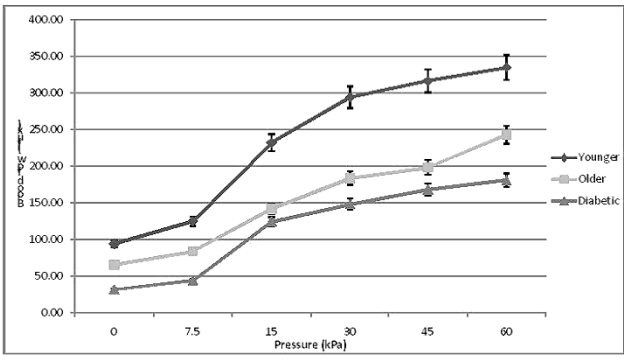
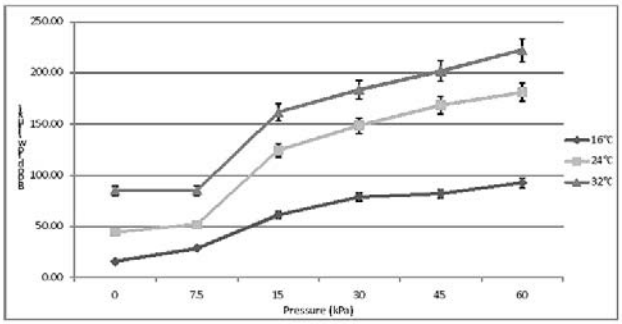


Figure 1b. The pressure-induced vasodilation following 30 seconds of local pressure in all global temperatures for the subjects with diabetes.



GENE EXPRESSION—CHIPS AND MICROARRAYS

31-LB

Global Placental Gene Expression in Gestational Diabetes Mellitus

DANIEL ENQUOBAHRIE, MICHELLE A. WILLIAMS, CHUNFANG QIU, MARGARET MELLER, TANYA K. SORENSEN, *Boston, MA, Seattle, WA*

Gestational diabetes mellitus (GDM), a disorder of glucose metabolism that complicates 4-7% of pregnancies in the US, is associated with short and long-term morbidity in both the mother and the offspring. In addition to an exaggerated glucose intolerance and insulin resistance, GDM is characterized by inflammation, altered plasma leptin and adiponectin concentrations as well as alterations in placental transcriptome. Global gene expression profiling tools can potentially enhance understanding of the molecular basis of GDM pathophysiology. However, only one small study has previously investigated global placental gene expression in GDM.

We conducted a microarray and a confirmatory quantitative real time polymerase chain reaction (QRT-PCR) study to identify global placental gene expression patterns among 19 GDM cases and 21 controls. RNA was extracted from placental samples. Oligonucleotide probes representing 22,000 genes (Operon's Human Genome Array Ready Oligo Set™) were used to measure gene expression. Differential gene expression was evaluated using Students T-test, fold change assessment and Significance Analysis of Microarrays. In path analysis, functions and functional relationships of differentially expressed genes were assessed using two independent tools: Database for Annotation, Visualization and Integrated Discovery (DAVID) and Ingenuity Pathway Analysis (IPA).

Sixty-six genes, participating in cell functions involving cell activation, immune response, organ development and regulation of cell death, were differentially expressed in GDM placentas. These genes include those with strong *a priori* evidence for involvement in GDM pathogenesis (such as LEP, MIF, CD63, UTS2, and FLT1), those involved in putative pathways (such as CEBPA, ADFP and STEAP4), and novel genes (such as AQP3). Results from our confirmatory QRT-PCR study of 9 selected genes (ADFP, AQP3, CEBPA, FLT1, INHA, ITGAX, MIF, STEAP4 and TUSC3) were generally comparable with results from microarray experiments. These findings potentially advance understanding of GDM pathogenesis that may lead to early diagnosis, treatment, and improved outcome.

32-LB

Lymphocytes from Patients with Type 1 diabetes Display a Distinct Profile of Histone Lysine Methylation

FENG MIAO, DAVID D. SMITH, LINGXIAO ZHANG, ANDREW MIN, WEI FENG, RAMA NATARAJAN, *Duarte, CA*

Type I diabetes (T1D), is an autoimmune disorder characterized by T-lymphocyte mediated destruction of the pancreatic islet beta cells. Several identified T1D susceptibility genes have implicated in cell-mediated autoimmunity from T cell activation. However, the complexity of interactions between genes and the environment is a major challenge for T1D studies, as well as most human diseases.

Histone modifications in chromatin have been linked to gene transcription and epigenetics. Along with DNA methylation, histone methylation contributes to epigenetic heritable changes in gene function. We hypothesized that, apart from genetic changes, epigenetics and the persistent changes in chromatin histone methylation of key genes play vital roles in the etiology of T1D, metabolic memory and complications. We used the chromatin immunoprecipitation coupled to DNA microarray analysis (ChIP-chip) approach to compare genome-wide histone H3 lysine 9 dimethylation (H3K9me2) patterns in blood lymphocytes and monocytes from T1D patients versus normal subjects. H3K9me2 was studied since this chromatin mark is generally associated with inactive or repressed genes. Use microarray analyses tools, we observed that a subset of genes in the T1D cohort shows significant increase in H3K9me2 in lymphocytes (at both coding and promoter regions), but not monocytes. This was T1D specific since no statistical difference was noted when subjects were grouped as males versus females, or young versus old. To validate the microarray results, ten probes displaying increased H3K9me2 were selected for follow-up conventional ChIP assays and quantitative real-time PCRs. Nine of these showed at least 2-fold significant increase in H3K9me2. Next, we applied the bioinformatics tool, Ingenuity Pathway Analysis, and identified a high scoring biological network that included 35 of these altered methylated genes. Notably, these genes were part of key autoimmune and inflammation related pathways such as TGF-β, NFkb, p38Mark, TLR, IL6.and PPAR. Additional bioinformatics queries demonstrated close links between these 35 genes and T1D through their connections with known T1D related genes such as IL1B, IFNG IL4R, TAB2, IL1A, IL3, IL10, IL2 and CD28. This biological relations network with our candidate methylated genes suggests that ***the concerted and synergistic alteration of histone methylation within the identified network in lymphocytes may eventually result in T1D and its complications. In addition, these novel approaches***, for the first time, provide clear evidence of the association between T1D and altered histone methylation of key genes.

33-LB

Regulation of Micrornas Expression by Pro-Inflammatory Cytokines In Islets.: A Microrna Microarray Study

VALIA BRAVO-EGANA, SAMUEL ROSERO, DAMARIS MOLANO, ANTONELLO PILEGGI, LUCA INVERARDI, CAMILLO RICORDI, RICARDO L. PASTORI, *Miami, FL*

MicroRNAs (miRNAs) are non-coding gene products that regulate gene expression through specific binding to target mRNAs. Advances in microarray technology made possible the use of chip arrays to study the expression of miRNAs in various cells and tissues. In the insulitis lesion of type 1 diabetes the infiltrating immune cells produce proinflammatory cytokines such as TNF-α, IL-1β and IFN-γ, which combined together have the ability to induce beta-cell death. In this study we investigated the effect

of the cytokine cocktail TNF-α, IL-1β and IFN-γ on the expression of miRNA in rat islets using miRNA array technology.

Islets were cultured for 6hs or 18hs with or without the cytokine cocktail: TNF-α (2,000 U/mL), IL-1β (50 U/mL), IFN-γ (100 U/mL). Total RNA was isolated with a method that preserves <200 bp RNA molecules (Ambion). The expression of miRNAs was examined using the Exiqon LNA based miRNA array platform containing the Sanger mirBase release 9.2 list. To identify significantly expressed genes across all replicate arrays one-class SAM (Significant Analysis of Microarray) analysis was used with FDR<1% (False Discovery Rate).

A combination of SAM analysis, fold change > 2 and a cutoff, based on the miRNA abundance was utilized. The effect of cytokines on miRNA expression was detected after 6hs but not after 18hs of treatment. We identified 67 miRNAs that are differentially expressed in cytokine treated islets. From these only 5 were downregulated; 14 miRNAs were upregulated 10 to 25 fold. Among them were miR-375, an islet specific miRNA, described previously and miR-7 which we described recently as the most abundant pancreatic endocrine miRNA. The change in expression levels was confirmed by quantitative real-time PCR. Inhibition of NF-κB followed by cytokine treatment led to the inhibition of most cytokine-induced miRNAs.Our results indicate that miRNA expression in islets is modulated early on by diabetogenic cytokines. Cytokine-mediated change of most induced miRNAs is dependent on the activation of NF-κB, a key pathway controlling endoplasmic reticulum stress, NO and cytokine production. These studies may lead to the identification of therapeutic targets to modulate miRNA expression and influence selected pathways associated with ß-cell death and diabetes development.

GENETICS—TYPE 1 DIABETES

34-LB

Association of HLA Class I Alleles with Type 1 Diabetes: Results from the Type 1 Diabetes Genetics Consortium

JANELLE A. NOBLE, ANA MARIA VALDES, HENRY A. ERLICH, JOYCE A. CARLSON, MIKE VARNEY, PAT CONCANNON, JOSYF C. MYCHALECKYJ, JOHN A. TODD, PERSIA BONELLA, ANNA LISA FEAR, EVA LAVANT, ANTHONY LOUEY, PRISCILLA MOONSAMY, TYPE 1 DIABETES GENETICS CONSORTIUM, *Oakland, CA, Zola Predosa (BO), Italy, Pleasanton, CA, Malmo, Sweden, Melbourne, Australia, Charlottesville, VA, Cambridge, United Kingdom*

The Type 1 Diabetes Genetics Consortium (T1DGC) is an international effort to generate a large collection of type 1 diabetes (T1D) families, cases, and controls with sufficient power to detect all of the loci that contribute to the genetic basis of T1D susceptibility. The genes encoding the HLA class II proteins, particularly DR and DQ, are known to make the greatest contribution; however, alleles of the genes encoding the classical HLA class I loci A, B, and C can confer T1D risk or protection as well. We utilized data from the April, 2006 data freeze of the T1DGC, which included 607 Caucasian, multiplex families, to look for HLA class I associations and compared those results to our previous results from 283 Caucasian, multiplex T1D families from the Human Biological Data Interchange (HBDI) collection. No HBDI families are included in the April 2006 data freeze, so the data represent an independent replication cohort. All data were adjusted to account for linkage disequilibrium with HLA DR-DQ haplotypes. T1D predisposing alleles seen in both data sets include A*2402, B*1801, and B*3906. A*3002, which is frequently found on a conserved, high-risk B18-DR3 haplotype, was observed more often than expected in the T1DGC data, but the result was not significant, suggesting that the allele itself may not be conferring the high T1D risk for this haplotype. T1D protective alleles seen in both data sets include A*3201, B*4403, C*0802, and C*1601. B*5701 was the most significantly predisposing allele in the T1DGC (p = 4 x 10-8). B*5701 was also seen less frequently than expected in the HBDI data, although the result did not reach statistical significance. The T1DGC effort is ongoing; the data freeze from October, 2007 will be available for analysis at the end of April, 2008 and will be used as an additional replication cohort for these studies. The availability of the large data set will allow stratification of the data to examine susceptibility effects in both haplotypic and genotypic context. This in-depth analysis will not only allow refinement of prediction of T1D risk but also likely reveal insights into the mechanism of HLA-based T1D susceptibility.

35-LB

Increased Type 1 Diabetes Risk for HLA B18-DR3 Haplotypes Carrying the Minor Allele of the G(-376)A Polymorphism in the TNF Gene

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The gene encoding TNFα sits within the HLA region of chromosome 6. Alleles of the TNF gene have been extensively studied for association with a number of autoimmune diseases, including type 1 diabetes (T1D), with conflicting results. Because of the extensive linkage disequilibrium (LD) in the HLA region, and the strong contribution of the HLA DR- and DQ-encoding loci to T1D susceptibility, detection of true associations of TNF alleles with disease is very challenging. Our previous studies of single nucleotide polymorphisms (SNPs) in the TNF promoter region showed that all apparent association for the SNPs at positions -238 and -308 could be accounted for by LD with HLA. An additional TNF promoter SNP, G(-376)A, appears to add additional risk to the high-risk B18-DR3 haplotype. Genotype data were generated for 1636 samples, representing 341 Caucasian, multiplex T1D families from the Human Biological Data Interchange, which were previously genotyped for all HLA loci as well as the TNF -308 and -238 SNPs. The TNF -376A allele was present at low frequency (3.9% in the parents) and was invariably found on chromosomes also carrying the TNF -238A allele, and 49 of 53 (92.5%) chromosomes carrying the -376A allele were B18-DR3, a haplotype known to be high-risk for T1D. Our previous data showed that the presence of the TNF -238A allele on B18-DR3 haplotypes did not significantly affect the transmission of the haplotype (80.0% and 81.1% transmission for the -238 G and A alleles, respectively). Addition of the -376 A allele to the B18-DR3-TNF-238A haplotype increases the transmission proportion to 84.8%, suggesting that the presence of the TNF -376A allele may confer additional risk to this already high-risk haplotype. Whether the polymorphism itself plays a causative role, or whether it is in LD with another, causative polymorphism, remains to be determined. Either way, the TNF G(-376)A polymorphism may be useful as an additional risk marker for T1D.

GENETICS—TYPE 2 DIABETES

36-LB

Genes Involved in Insulin Secretion or Sensitivity in Mothers are Associated with Offspring Size at Birth

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Results of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study demonstrate that in mothers with glucose values below those diagnostic of diabetes, there is a continuous, graded relationship between measures of maternal glucose and size at birth or fetal adiposity. Our hypothesis is that genetic factors also contribute to these phenotypes, impacting both maternal glucose and birth size or adiposity. To test this, we examined genetic variation in loci previously implicated in insulin secretion or sensitivity to determine associations with maternal glycemia and insulin secretion at ~28 weeks gestation and/or offspring size at birth. Using DNA collected from 2133 Thai mothers and their offspring who participated in HAPO, we genotyped 1536 single nucleotide polymorphisms (SNPs) using the Illumina GoldenGate platform in 79 candidate genes. The SNPs tagged common haplotypes spanning the

coding region of each gene as well as 20 kb up- and 15 kb downstream, and were informative for all three major geographic human populations (Europeans, Africans, and Asians). We investigated association of maternal genotype with maternal traits [fasting glucose (FG) and C-peptide (FCP) and 1-hr glucose (1hG) from the oral glucose tolerance test (OGTT)] and fetal genotype with birth outcomes [birth weight (BW), birth length (BL), head circumference (HC), and sum of skinfolds (SSF)]. Associations were assessed through linear regressions with the single trait/outcome under an additive genetic model adjusting for confounders (maternal age, BMI, blood pressure, and height at OGTT, gestational age at delivery, neonatal gender, and parity). We found significant associations ($p<0.01$) between maternal FG or 1hG and maternal SNPs in known diabetes/hyperglycaemia genes: *ABCC8* (best SNP is rs2073583; p=0.004 1hG), *CAPN10* (rs11683693; p=0.005 FCP), *GCK* (rs917793; p=0.002 FG), *HNF4A* (rs2071200; p=0.004 1hG), *PPARG* (rs4498025; p=0.009 FG), and *TCF7L2* (rs290484; p=0.009 FG). In a subset of these genes, fetal SNPs were associated with fetal BL (*PPARG* rs2972164, p=0.004), HC (*CAPN10* rs4676422, p=0.007), or SSF (*ABCC8* rs1055574, p=0.007) suggesting that genes involved in insulin secretion or sensitivity may additionally impact size at birth or fetal adiposity. **ADA-Funded Research**

37-LB

Genetic and Non-Genetic Prediction of Future Type 2 Diabetes

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Type 2 diabetes is considered to develop from an interaction between environmental and genetic factors. We examined whether genetic and/or non-genetic factors could predict progression to diabetes in two prospective cohorts. We genotyped 11 single nucleotide polymorphisms (SNPs) which have recently been identified in several genome-wide association studies and examined clinical and metabolic factors in 16,061 Swedish and 2,770 Finnish subjects, 2,201 of whom developed diabetes during 400,000 follow-up years. Effect of genetic variants on change in insulin secretion and action over time was studied in 2444 non-diabetic participants followed for a mean of 8 years in the Botnia study . A family history of diabetes (OR (95% CI), 1.64 (1.42-1.89), $P=9.06\times10^{-12}$), body mass index (1.21 (1.19-1.22), $P=2.00\times10^{-155}$), smoking (1.52 (1.38-1.68), $P=1.15\times10^{-16}$), liver enzymes (ALT, 2.06 (1.85-2.30), $P=1.91\times10^{-38}$; GT, 1.67 (1.54-1.82) $P=2.95\times10^{-34}$) and measures of insulin secretion (disposition index (DI), 2.23 (1.85-2.67), $P=1.27\times10^{-17}$) and action (insulin sensitivity index, 1.68 (1.43-1.97), $P=3.02\times10^{-10}$) strongly predicted diabetes. Common variants in 10 genes, e.g. *TCF7L2* (OR 1.32, $P=3.32\times10^{-13}$), *KCNJ11* (1.16, $P=3.70\times10^{-5}$), *PPARG* (1.22, $P=1.65\times10^{-4}$), *CDKAL1* (1.13, $P=1.31\times10^{-3}$), *IGF2BP2* (1.12, $P=2.34\times10^{-3}$), *FTO* (1.11, $P=2.35\times10^{-3}$), *WFS1* (1.19, $P=7.00\times10^{-4}$), *HHEX* (1.10, $P=1.07\times10^{-2}$), *SLC30A8* (1.11, $P=7.35\times10^{-3}$) and *JAZF1* (1.11, $P=3.53\times10^{-3}$) predicted independently of non-genetic factors future type 2 diabetes. There was a stepwise increase in diabetes risk with increasing number of risk alleles and increasing quartiles of body mass index yielding an odds ratio of 8.12 (5.68-11.61, $P=1.49\times10^{-30}$) for carriers of high risk group and highest body mass index quartile and of 6.78 (3.54-13.03, $P=8.41\times10^{-9}$) for a low disposition index. A time-dependent increase in BMI and concomitant decrease in insulin sensitivity was a consistent finding with no difference between carriers of high (20% of the participants with more than 13 risk alleles) and low genetic risk (20% with less than 8 risk alleles). However, individuals with a high genetic risk had lower insulin secretion at baseline (insulinogenic index: P=0.008; DI, $P=3.22\times10^{-5}$) and were not able to increase their insulin secretion to compensate for the increase in insulin resistance as efficiently as individuals with low genetic risk (insulinogenic index: $P_{trend}<0.01$; DI: $P_{trend}<0.02$). In conclusion, a combination of genetic and non-genetic factors was associated with a 8-fold increased risk of future type 2 diabetes. Most genetic variants result in impaired capacity of beta-cells to increase insulin secretion in response to increased needs imposed by obesity and insulin resistance.

38-LB

Joint Analysis of Early Insulin Secretion and Type 2 Diabetes in a 100K Genome-wide Association Study

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Reduced early insulin release is a strong risk factor for type 2 diabetes, and both insulin secretion and diabetes are strongly heritable. We therefore conducted a joint analysis of type 2 diabetes and acute insulin response (AIR), measured at 3-5 minutes in a 25g intravenous glucose tolerance test, within a 100K genome-wide study.

The scan included 243 American Indians with AIR measurements who had normal glucose tolerance; 71 of whom subsequently developed type 2 diabetes. Analysis included 59,486 markers with minor allele frequency>0.10 from the Affymetrix 100K array. Logistic regression models were used to assess the association of genotype with diabetes and with AIR (adjusted for age, sex, percent body fat and insulin sensitivity), and to construct a combined test of association for both traits. To identify polymorphisms that could contribute to both traits pleiotropically, path analysis was conducted using Q transformations of the odds ratios to approximate correlation coefficients.

Low AIR strongly predicted subsequent diabetes (odds ratio=0.50 per SD, $p<0.0001$). In general, the distribution of p-values for both AIR and diabetes was similar to that expected by chance, including several with p-values<0.001. SNPs with the strongest associations with AIR included rs6776600 on chromosome 3 ($p=6\times10^{-6}$) and rs6461153 on chromosome 7 ($p=1\times10^{-4}$). Among SNPs with evidence for pleiotropy by path analysis, rs10496149 on chromosome 2 ($p=2\times10^{-5}$ for joint association) had the strongest combined association. Other potentially pleiotropic SNPs included rs9302144 near *CEP152* on chromosome 15, rs2274070 on chromosome 14, rs218428 near *GRIK3* on chromosome 1 and rs5757091 near *KNCJ4* on chromosome 22 (all $p\sim3\times10^{-4}$ for joint association). These SNPs were not among those most strongly associated with diabetes in our larger diabetes genome-wide scan.

This genome-wide association analysis has identified several loci that may influence both insulin secretion and risk of diabetes. These analyses are limited by the small number of subjects and confirmation is required in additional subjects.

39-LB

Meta-Analysis of Four Genome-Wide Association Scans of Type 2 Diabetes in Diverse Ethnic Groups: Results from the Type 2 Diabetes 100K Consortium

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Objective: In 2007, four genome-wide association studies (GWAS) of type 2 diabetes (T2D) were performed in different ethnic populations - Caucasians, Mexican-Americans, and Pima Indians- using the same Affymetrix 100K genotyping platform. In the present study, we performed a full meta-analysis of these data as part of the Type 2 Diabetes 100K Consortium with the hope of identifying polymorphisms which potentially increase risk of type 2 diabetes in multiple ethnic groups.

Research Design and Methods: A total of 89,549 SNPs that passed quality-control (QC) criteria in at least two studies were included in the meta-analysis. The number of cases in each study ranged from 91 to 287. *P*-values for each study were combined using the Stouffer-Liptak method (a weighted sum of the inverse normal transformation of the *P*-value with weights determined by sample size) and Fisher's method, both of which required consistency in the direction of effect size estimates across studies.

Results: In general, the distribution of *P* -values for both methods was similar to that expected by chance; however, a significant excess of SNPs with low *P*-values was observed (e.g 124 and 132 with *P*-value<0.001 for Stouffer-Liptak and Fisher methods, respectively compared with ~90 expected). None of the combined results achieved genome-wide

significance ($p < 10^{-7}$). Based on the more conservative Stouffer-Liptak meta-analytic approach, we observed our strongest association signals for rs4809590 on chromosome 20 and rs2863389 on chromosome 3, both with combined *P*-values~10^{-6}. Also among the top results is rs10502147, a SNP in intron 4 of *SNF1LK2* (SNF1-like kinase 2) with a combined *P*-value of 1.14×10^{-4} with Fisher's method and observed replication in the WTCCC GWAS (*P*-value = 0.003). In the recently published DIAGRAM meta-analysis of high-density GWAS, its combined *P*-value is 0.12. *SNF1LK2* is highly expressed in insulin-stimulated adipocytes in insulin-resistant rats and is likely involved in insulin signal transduction. The significance of the remaining set of SNPs is unclear.

Conclusions: We have performed a full meta-analysis of four GWAS for T2D in ethnically diverse populations. Although none of our results achieved genome-wide significance, several loci merit further exploration. Replication attained in larger samples will be important for further prioritization of our findings.

40-LB

TCF7L2 in the Type 2 Diabetes Mouse Model TALLYHO/Jng: Association with a GLUT4 Translocation Defect and Reduced Levels of IRS1

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The TALLYHO/Jng (TH) mouse strain is a polygenic model for type II diabetes characterized by obesity, impaired glucose tolerance, insulin resistance, and hyperinsulinemia. TH mice show reduced uptake of glucose into muscle and adipose tissue. The goal of this study was to elucidate the molecular mechanisms responsible for the reduced glucose uptake in TH mice and to test the involvement of TCF7L2, a candidate gene for a quantitative trait locus, tanidd1, controlling plasma glucose levels in the TH model.

Immunhistochemistry and immunprecipitation were used to examine TCF7L2 and IRS1 in adipose tissue of male TH and B6 control mice and in B6 males congenic for a 2Mb segment of the TH *tanidd1* region including *Tcf7l2*. GLUT4 translocation as well as protein levels and phosphorylation status of insulin signaling pathway components were also analyzed.

TCF7L2 protein, but not mRNA, was significantly reduced in TH adipose tissue. IRS1 protein was also reduced by 50%. Immunoprecipitation of TCF7L2 unexpectedly co-precipitated IRS1. Double immunocytochemical localization showed TCF7L2 protein co-localized with IRS1 in proteasomal structures in TH. In contrast, both molecules showed nuclear co-localization in control B6 adipocytes. The phenotype of reduced IRS1 protein was recapitulated in congenic B6 mice carrying the TH alleles across the *tanidd1* region. Sequestration/degradation of IRS1 in TH was correlated with reduced IRS1 tyrosine phosphorylation, increased IRS1 serine phosphorylation, reduced PI3 kinase activity, and associated with impaired GLUT4 translocation in TH adipocytes.

Our findings suggest that the association of TCF7L2 and IRS1 may play a role in IRS1 degradation in insulin resistant states. The TH mouse is an attractive model to investigate this relationship and recapitulates many of the defects observed in human patients with type 2 diabetes.

ADA-Funded Research

41-LB

Transcriptional Network Analysis to Identify Genes for Risk of Kidney Damage By Long-Term Diabetes Mellitus

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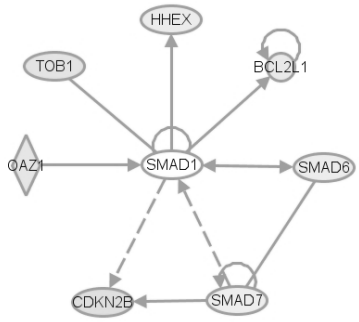
The pathways that link prolonged diabetes mellitus (DM) to kidney damage are still incompletely understood; in particular, we have little knowledge of the genetic factors that may lead to differential risk of diabetic nephropathy (DN) in the presence of DM. We have acquired genome-wide RNA expression profiles in lymphocytes from 1,240 participants in Mexican American families of the San Antonio Family Heart Study (SAFHS). In another presentation at this meeting (204-OR) we provide data on a subset of genes that show significant evidence of genotype x duration of DM (GxDOD) interaction influencing levels of expression. Notable among the top 23 genes so identified is SMAD1 (MADH1), which has been identified by other investigators as a possible early biomarker of DN. Here we report results of a bivariate polygenic analysis of SMAD1 with other heritably-expressed transcripts in SAFHS. Of 12,249 validated autosomal gene transcripts examined, 2007 were nominally (P<0.05) genetically correlated with SMAD1, suggesting that they may share common genetic regulation. 65 of these genetically correlated transcripts also show nominally significant GxDOD. Protein kinase C, eta subunit (PRKCH or PKCH), which has also been implicated in cerebral infarction, was highly ranked for both GxDOD (P=1.6 x 10-11) and genetic correlation with SMAD1 (P=0.00075). We examined the relationship of these SMAD1-correlated and DOD-interacting transcripts within functional pathways using the proprietary bioinformatic software Ingenuity Pathway Analysis (IPA; Ingenuity Systems, Inc.). Of 122 first-degree regulatory pathway connections with SMAD1 in IPA, 7 are included in our set of genetically correlated genes (see figure). 45 genetically correlated genes show second-degree connections. Thus, a substantial number of genes with known functional relationship to SMAD1 also show correlated heritable variation in expression in the SAFHS. Functional assignments by IPA of the genetically correlated transcripts include 6 genes associated with renal damage (ALOX5, FGR, HCK, MIF, STAT4, TLR2) and a much larger number (92 genes) associated with inflammatory disease. Thus, through a combination of transcriptomic, bioinformatic, and genetic epidemiological data, we have begun to identify networks of genes likely to be involved in the development of DN. In particular, the evidence of GxDOD interaction suggests that many of these genes harbor genetic variants that confer differential heritable risk of DN in the presence of prolonged DM.



HEALTH CARE DELIVERY—ECONOMICS

42-LB

Assessing Differences in Glycemic Control, Utilization and Costs between Insulin Detemir (Levemir®) and Insulin Glargine (Lantus®) Users

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The introduction of new basal insulins has raised questions about the relative effects of determir and glargine in a real world setting. In this study, we assessed differences in glycemic control, overall and diabetes-related costs of type 2 diabetes patients treated with insulin detemir and insulin glargine using **r**etrospective data of patients enrolled in a large US health plan with medical and pharmacy benefits. Patients were identified if their first prescription claim (index) for insulin detemir or insulin glargine occurred between 05/01/2006 and 12/31/2006. Eligible patients were required to be age 18 or above, had 6 months of continuous enrollment pre- and post-index date, had HbA1c readings during the pre- and post-index periods and had no evidence of any insulin use during pre-index.

Thus, only insulin naïve patients switching to or adding insulin detemir or insulin glargine in the post-period are included. Primary outcomes include daily average consumption (DACON) of insulin detemir or insulin glargine, post-index HbA1c, and overall and diabetes-related cost. Differences in outcomes between insulin detemir and insulin glargine users were adjusted for baseline characteristics through generalized linear modeling (GLM). Propensity score matching was used to reduce selection bias between the two groups. The study included 48 insulin detemir patients and 258 insulin glargine patients. Adjusted DACON for basal only insulin detemir and insulin glargine cohorts were 29.3 and 29.6 units/day respectively (p=0.93). The corresponding HbA1c values were 8.2 and 7.9 (p =0.15) for insulins detemir and glargine respectively. Adjusted diabetes-related follow up total and medical costs for the insulin detemir cohort were lower than the insulin glargine cohort ($2,261 vs. $3,408; p<0.03) and ($707 vs. $1,510, p < 0.03) respectively. Moreover, adjusted overall medical cost for insulin detemir users also was marginally lower ($2,319 vs. $3,704; p=0.07). No difference in overall or diabetes-related pharmacy cost was observed ($1,277 vs. $1,149; p=0.23).

Conclusion: No significant difference in DACON or adjusted pharmacy cost between insulin detemir and insulin glargine users were observed during the study period despite equivalent but not target levels of glucose control (HbA1c). However, insulin detemir patients experienced a significantly lower diabetes-related and overall adjusted medical cost during the follow up period.

43-LB

Ethnicity/Race and the Extent of Physician Ordered Hemoglobin A1C During U.S. Office-Based Visits by Patients with Diabetes Mellitus

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Diabetes mellitus (DM) has emerged as a global epidemic. The number of persons with diabetes in the United States (U.S.) is projected to reach 48 million by 2050. In 2002, U.S. direct and indirect expenditures for DM were estimated to be $132 billion.

In the U.S., ethnic/racial minorities now account for the largest increase in the prevalence of DM; specifically Hispanics (H), followed by non-Hispanic Blacks (B). It is estimated that H and B are more than twice as likely to develop DM, and suffer the clinical consequences of insufficient monitoring, relative to Whites (W). Measurement and monitoring of glycemic control is widely considered a cornerstone of management for persons with DM. Likewise, availability of Hemoglobin A1C (A1C) results at the point-of-care has been associated with intensification of therapy, and improvement in glycemic control, lending support to the utility of A1C testing in the monitoring and optimization of patient outcomes.

The present study was designed to discern: (i) the characteristics and population-adjusted rate of U.S. office-based physician-patient encounters (visits) for DM among ambulatory patients aged >20 years, in total, and by ethnicity/race (W; B; H); (ii) the extent of physician ordered A1C, in total, and by ethnicity/race; and (iii) factors predictive of physician ordered A1C. Data were derived from the 2005 U.S. National Ambulatory Medical Care Survey (NAMCS).

Our findings indicate physician ordered A1C occurred in 27% of all office-based visits; 31.3% for W; 15.9% B; and 11.5% H. The rate of office-based visits per 100 U.S. population for DM were 17.5, in total, and 17.9 for W; 15.9 for B; and 16.8 for H. A factor predictive of physician ordered A1C was an office-based visit with the patient's primary physician (OR=9.6; 95% CI = 5.4-17.1). However, having adjusted for patient's age, gender, insurance coverage, and whether the office-based visit occurred with the primary physician, both B and H were significantly less likely (2.5 fold for B; 3.3 fold for H) to have an A1C ordered, relative to W (OR=0.4; 95% CI = 0.2-0.8 for B; OR=0.3; 95% CI = 0.2-07 for H).

Our findings reveal a disparity in physician ordered A1C by ethnicity/race. Previous research documents, that when measured, B and H have higher A1C readings relative to W; and this fact may portend a greater propensity toward morbidity and mortality.

44-LB

Practice-linked Online Personal Health Records for Type 2 Diabetes: A Randomized Controlled Trial

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Web-based Personal Health Records (PHRs) have been advocated as a means to improve diabetes care. However, few web-based systems are linked directly to the electronic medical record used by physicians.

We randomized eleven primary care practices in Eastern Massachutts that were linked by a common electronic medical record within an academic health center network. Intervention practices (n = 4) received access to a diabetes-specific PHR that imported clinical and medications data, provided patient-tailored decision support, and enabled the patient to author a "Diabetes Care Plan" for electronic submission to their physician prior to upcoming appointments. Active control practices (n = 7) received a PHR to update and submit family history and health maintenance information. All patients attending these practices were encouraged to sign up for on-line access.

We enrolled 244 patients with type 2 diabetes (37% of the eligible population with registered on-line access, 4% of the overall diabetes population). Study participants were younger (56.1 vs. 60.3 years, p<0.001) and lived in higher income neighborhoods (median income $53,784 vs. $49,713, p<0.001) but had similar baseline glycemic control compared to non-participants. More intervention patients had their diabetes treatment regimens adjusted (53% vs. 15%, p<0.001) compared to active controls. Among participants with Hba1c > 7.0% at baseline, intervention patients were more likely to reach HbA1c goal at study end compared to control patients (45% vs. 25%, 79 patients with available data, p = 0.07). However, overall there were no significant differences in risk factor control between study arms after one year.

Pre-visit use of on-line PHR linked to the electronic medical record increased rates of diabetes-related medication adjustment. Low rates of on-line patient account registration and good baseline control among participants limited the intervention's impact on overall risk factor control. (ClinicalTrials.gov number, NCT00251875)

45-LB

Relation of Obesity and Severe Obesity to Quality Of Life in 6th Graders at Risk for Type 2 Diabetes: Preliminary Results from the HEALTHY Study

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Childhood obesity is a significant public health issue and is associated with poorer health-related quality of life (QOL). Prior studies have assessed this effect in samples of severely obese children or mixed samples of obese and severely obese children. Here we evaluate QOL in healthy weight, overweight, obese, and severely obese children.

Baseline measurements were taken among 6th grade participants in the school-based HEALTHY primary prevention trial to assess the association between QOL and body mass index percentile (BMI%). HEALTHY is being conducted in 42 schools at 7 field centers. BMI% was categorized as normal weight (<85), overweight (85 to <95), obese (95 to <99), and severely obese (99+). We assessed QOL by the Health Utilities Index Mark 2 and 3 (HUI2 and HUI3) and a feeling thermometer (FT). Statistical inference was based on linear mixed model analysis.

Preliminary data were available from 5969 students. The mean age was 11.3 years (SD 0.6); 50% of the sample was healthy weight, 20% overweight, 23% obese, and 7% severely obese; 53% were Hispanic, 20% Black, 19% White. Mean QOL scores were 0.842 (0.161) for the HUI2, 0.790 (0.239) for the HUI3, and 0.806 (0.161) for the FT. BMI% was negatively associated with all 3 QOL measures (p<0.001). After controlling for age, parental educational attainment, race/ethnicity, family history of diabetes, fasting glucose and insulin, sex, and Tanner stage, obesity (BMI% 95 to <99) was associated with significantly lower QOL scores that ranged between -0.016 (p=0.01) for the HUI2 to -0.042 (p<0.0001) for the FT. The lower QOL scores associated with severe obesity (BMI% 99+) ranged between -0.036 (p=0.0007) to -0.077

(p<0.0001). Lower QOL scores associated with overweight (BMI% 85 to <95) were significant for the FT only (-0.021, p=0.0008).

In this large, ethnically diverse cohort of 6th grade middle school students, the association between reductions in QOL and BMI% begin at lower levels of BMI% than has been reported in the past. The impact may begin with overweight, and becomes more meaningful with obesity and severely obesity. This finding supports efforts to prevent obesity and its consequences in this population through public health approaches and strategies.

INSULIN ACTION— GLUCOSE TRANSPORT

46-LB

A Novel PH Domain Containing Protein Phldb1 Regulates Insulin-Induced Glucose Transport and GLUT4 Translocation in Adipocytes

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Insulin stimulates GLUT4 glucose transporter translocation from intracellular membranes to the plasma membrane, enhancing glucose transport in adipocytes and muscle cells. In the search for new molecules which regulate insulin's actions, we identified a novel PH domain containing protein, Pleckstrin Homology-like domain, family B, member 1 (Phldb1), We report here the potential role of Phldb1 on glucose transport and GLUT4 translocation stimulated by insulin. Phldb1 contains PH and FHA domains in addition to coiled coil regions, and its expression is up-regulated during adipocyte differentiation. Depletion of Phldb1 by about 75% using siRNA inhibited deoxyglucose uptake in 3T3-L1 adipocytes in response to insulin by approximately 50%, while knockdown of Phldb2, a Phldb1 isoform which contains a PH domain reported to bind phosphatidylinositol(3,4,5)P3 (PIP3), had little effect. Furthermore, RNAi-based silencing of Phldb1 in cultured adipocytes attenuated insulin-stimulated myc-GLUT4-EGFP translocation by about 39%. TIRF microscopy indicates high concentrations of Phldb1-GFP in membrane ruffles and knockdown of Phldb1 by siRNA inhibited insulin-induced movement of endogenous GLUT4 vesicles into the TIRF zone by 42%. Expressed Phldb1-EGFP displayed a cytoplasmic disposition in the basal state, but translocated to the plasma membrane in response to insulin, while 10 nM wortmannin, a PI3 kinase inhibitor, blocked this insulin-stimulated Phldb1-EGFP translocation. These results suggest that Phldb1 is an insulin-responsive protein in respect to its cellular localization, potentially through the binding of its PH domain to PIP3 in the plasma membrane. The data indicate that Phldb1 is required for optimal insulin stimulation of glucose transport and GLUT4 translocation in 3T3-L1 adipocytes, suggesting it may be a component of the insulin signaling mechanism that regulates GLUT4-containing vesicle movements or fusion with the plasma membrane.

ADA-Funded Research

47-LB

A Novel Protein Interacting with IRAP Regulates Insulin-Stimulated Glucose Uptake.

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In striated muscle and adipose tissue, insulin stimulates the translocation and fusion with the plasma membrane of intracellular vesicles containing the glucose transporter Glut4. These vesicles also contain a transmembrane protein named IRAP for Insulin responsive aminopeptidase. Using the yeast two hybrid technique we have screened a skeletal muscle cDNA library and identified a novel protein interacting with the cytoplasmic N-terminus of IRAP. This protein which we have named IRAP -Regulin, interacts with IRAP *in vitro* in pull down assays. When IRAP -Regulin is overexpressed in cultured 3T3L1 adipocyte cells it inhibits both IRAP and Glut4 redistribution to the plasma membrane in response to insulin. Expression of IRAP regulin does not affect the intracellular trafficking of the Glut1 glucose transporter or trafficking of other intracellular markers such as the Mannose 6-Phosphate receptors. The interaction between IRAP Regulin and IRAP does not depend on the dileucine motifs of IRAP. IRAP- Regulin has two potential sites for phosphorylation by AKT. Interestingly, overexpression of a double mutant of IRAP Regulin in which these phosphorylation sites have been mutated to alanines, does not inhibit IRAP or Glut4 trafficking in 3T3L1 adipocytes. Taken together these findings suggest that IRAP-Regulin may be a new protein involved in the regulation of glucose transport in adipocytes.

48-LB

Differential Regulation of Akt Isoforms by Insulin and its Implications in Glucose Transport

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Insulin stimulates the uptake of glucose into muscle and fat cells by inducing the redistribution of the glucose transporter, GLUT4, from intracellular sites to the plasma membrane (PM). Insulin activated kinase Akt is a critical signal transducer regulating GLUT4 translocation. Although adipocytes expressAkt1 and Akt2 isoforms, the results of genetic studies suggest that insulin signaling to GLUT4 is primarily through Akt2. How this specificity is achieved remains unknown. The differential functions of Akt isoforms might be due to differences in expression patterns, activity or regulation by insulin. We observed that despite differential expression levels of Akt1 and Akt2, insulin can effectively activate both isoforms in 3T3-L1 adipocytes. siRNA-mediated knockdown of Akt2, but not Akt1, impaired insulin induced GLUT4 translocation in adipocytes (32±5% reduction, n=3). Moreover, knockdown of Akt1 and Akt2, did not further impair insulin-induced GLUT4 translocation to the PM compared to Akt2 knockdown alone (35±7% reduction, n=5). The defects in GLUT4 translocation in transiently Akt2 knocked-down adipocytes were effectively rescued by overexpression of Akt2 but not Akt1, indicating that differences in the activity or regulation of these kinases might determine their distinct contribution to GLUT4 translocation. We characterized the dynamic subcellular distribution of Akt1 and Akt2 upon insulin stimulation. We used total internal reflection fluorescence microscopy (TIRFM) in live adipocytes to measure the effect of insulin on Akt translocation to the PM. Similar fractions of total Akt1 and Akt2 were detected in the vicinity of the PM in serum starved adipocytes, however insulin induced a significantly higher accumulation of Akt2 at the plasma membrane environment compared to Akt1 (57±7% increase in TIRF for Akt2 versus 23±3% for Akt1, n=12). These differences were observed in the context of full length kinases and not the isolated pleckstrin homology domains, suggesting that interactions between several Akt domains are required to confer specificity to Akt1 and Akt2 subcellular location. Furthermore Akt mutants that failed to accumulate at the PM environment did not regulate GLUT4 translocation. There is evidence that Akt regulates the late stages of GLUT4 exocytosis; we propose that differential regulation and subcellular distribution of Akt isoforms in response to insulin might determine the specific requirement of Akt2 in insulin-induced GLUT4 translocation in adipocytes.

49-LB

Identification of New Akt binding Molecule Regulating GLUT4 Trafficking

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We took advantage of the yeast two hybrid system to screen for Akt/PKB (protein kinase B) binding partners in a human skeletal muscle cDNA library. Using delta PH (PH domin was deleted)-Akt2 as bait, we identified a protein belonging to the Tctex-1 family that we tentatively termed, Akt binding molecule M (AktBM). In 3T3L1 adipocytes, AktBM was co-immunoprecipitated with endogenous Akt. However, insulin stimulation had no effect on the interaction of AktBM with Akt. Acute insulin stimulation (up to 30 min) in 3T3L1 adipocytes over expressing AktBM had no effect on the phosphorylation of the insulin receptor beta subunit, IRS-1, Erk, or Akt. Interestingly, over expression of AktBM

significantly increased the basal state phosphorylation of the Akt substrate AS160 and enhanced the GLUT4 translocation to the plasma membrane in the basal state. In contrast, AktBM over expression reduced insulin stimulated GLUT4 translocation. Thus, AktBM appears to be a novel Akt binding partner that specifically affects signaling pathways leading to GLUT4 translocation in adipocytes.

INSULIN ACTION—METABOLISM

50-LB

Chronic Inhibition of the mTOR/S6K1 Pathway Causes Insulin Resistance by Uncoupling PI 3-kinase Activity from Akt Phosphorylation in Insulin Target Tissues.

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The mammalian target of rapamycin (mTOR)/S6K1 pathway has emerged as a critical signaling component in the development of obesity-linked insulin resistance. This effect is triggered by both nutrients and an insulin-mediated negative feedback loop toward PI 3-kinase activation. We and others have previously demonstrated in various cell culture models that short term treatment with rapamycin, a specific inhibitor of mTOR, improves insulin action on glucose transport. Therefore, mTOR inhibition could be considered as a potential therapeutic strategy for alleviating insulin resistance. We have further tested whether chronic inhibition of the mTOR pathway with rapamycin can modulate insulin action and metabolism in animal and cellular models. As expected, chronic treatment (15 days) of Sprague-Dawley rats with rapamycin (2mg/kg/day) was found to improve insulin signaling through inhibition of the negative feedback loop, as revealed by an increased activation of IRS-associated PI 3-kinase in skeletal muscle, liver and adipose tissues. Unexpectedly, however, chronic rapamycin treatment also caused marked inhibition of Akt phosphorylation, uncoupling its activity from the PI 3-kinase signaling cascade. This resulted in significant hyperinsulinemia, hyperglycemia and impaired glucose tolerance despite enhanced PI 3-kinase activation in insulin target tissues. Chronic rapamycin treatment of 3T3-L1 adipocytes and Fao hepatic cells also increased insulin signaling to PI 3-kinase while impairing Akt phosphorylation and kinase activity. siRNA-mediated silencing of either mTOR or its downstream effector S6K1 also uncoupled insulin signaling to PI 3-kinase from Akt, resulting in inhibition of insulin-mediated glucose transport in 3T3-L1 adipocytes. In conclusion, chronic inhibition of the mTOR/S6K1 pathway with rapamycin or following RNA interference improve PI 3-kinase activation by insulin, yet causes insulin resistance by uncoupling the PI 3-kinase/Akt signaling cascade. These findings emphasize the essential role of the mTOR/S6K1 pathway in the negative regulation of PI 3-kinase activity but our data also highlight the limitation of using mTOR and S6K1 inhibitors for combating insulin resistance and type 2 diabetes.

51-LB

Enhanced Insulin Sensitivity and Beta-Cell Function in Patients with a Rare Cancer Syndrome due to *PTEN* Mutations: Further Evidence for the Yin-Yang Hypothesis

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Emerging data from genome-wide scans for association in Type 2 diabetes (T2DM) suggest that genes involved in cell cycle control play key roles in susceptibility to disease. Moreover, recent studies have demonstrated that common genetic variants predisposing to cancer protect against T2DM; variants in *HNF1B* have been shown to predispose to prostate cancer and also confer protection against T2DM. Cowden syndrome (CS) is a rare cancer predisposition syndrome (prevalence 1 in 200 000) caused by mutations in the phosphatase and tensin homolog tumour suppressor gene (*PTEN*). PTEN, expressed in pancreatic beta-cell, liver, muscle and adipose tissue, is a lipid phosphatase which reverses a step in PI_3-kinase signalling (a key pathway in insulin action)**.** In mice, *PTEN* haploinsufficiency promotes insulin sensitivity, and beta-cell specific knockouts have increased beta-cell mass and hypoglycaemia. We hypothesised that *PTEN* haploinsufficient patients with CS could have enhanced insulin sensitivity and beta-cell function. To test our hypothesis we recruited patients with CS (n=7) and age, BMI and sex-matched controls (n=7). Subjects underwent an OGTT (75g) and blood samples were analysed for glucose and insulin. Insulin sensitivity and beta-cell function were assessed with the homeostasis model of insulin resistance (HOMA2 IR) and disposition index (DI) respectively. Results are shown as geometric mean [SD range]; CS patients vs controls. Patients with CS and control subjects were well-matched for gender, age (P=0.88) and BMI (32.6kg/m^2 [25.8, 41.3] vs 32.9kg/m^2 [26.6, 40.6], P=0.79). Patients with CS have greater insulin sensitivity (HOMA2 IR 0.51 [0.19, 1.38] vs 1.35 [0.67, 2.69], P=0.028) and a tendency for improved beta-cell function (DI 136.2 [65.9, 281.2] vs 75.5 [29.6, 192.5], P=0.29) compared to matched controls. Our data support the hypothesis that humans with *PTEN* haploinsufficiency have improved insulin sensitivity and suggest that the beneficial affects of loss of PTEN are most marked in tissues of insulin action rather than in improved beta-cell function *per se*. Understanding the role of PTEN in insulin secretion/action is not only of biological importance but will also clarify the potential for adverse oncogenic events related to manipulation of these pathways to treat diabetes.

52-LB

Homozygous Deletion of TRPM2 Ca^{2+}-Channels Increases Insulin Sensitivity and Cardiac Glucose Metabolism In Vivo in Mice

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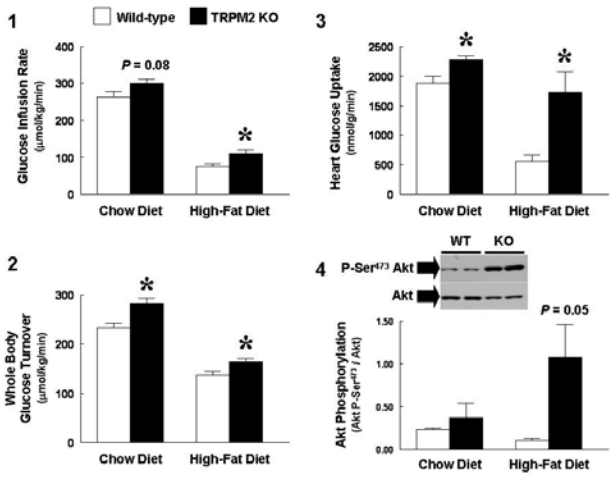
Increasing evidence indicates the role of inflammation and oxidative stress in the development of insulin resistance. The transient receptor potential (TRP) protein superfamily is a group of Ca^{2+}-permeable cation channels, and TRPM2 was shown to regulate cell death induced by oxidative stress and TNF-α. We examined the role of TRPM2 in glucose homeostasis by measuring insulin sensitivity during hyperinsulinemic-euglycemic clamps in awake TRPM2 KO mice fed standard chow (n=5~8) or high-fat (55%) diet (HFD; n=12) for 8 wks. On chow diet, glucose infusion rate (GINF) during clamps tended to increase in TRPM2 KO mice (Fig.1), while insulin-stimulated whole body glucose turnover was significantly increased in these mice compared to wild-type (WT) littermates (Fig.2; $*P<0.05$). This was mostly due to a 20% increase in cardiac glucose metabolism, whereas glucose uptake in muscle and fat were not affected (Fig.3). Following HFD, WT mice developed insulin resistance as indicated by significant reductions in GINF and whole body glucose turnover. In contrast, HFD-fed TRPM2 KO mice became less insulin resistant and showed significantly elevated GINF and whole body glucose turnover compared to HFD-fed WT mice. Diet-induced insulin resistance caused a 70% reduction in heart glucose uptake in WT mice (Fig.3). Remarkably, TRPM2 KO mice were completely protected from diet-induced cardiac insulin resistance, which was associated with increased Akt serine phosphorylation in the heart of HFD-fed TRPM2 KO mice (Fig.4). Overall, these results demonstrate that homozygous deletion of TRPM2 increases insulin sensitivity and prevents diet-induced insulin resistance in heart. Our findings indicate a novel role of TRPM2 in cardiac glucose metabolism and identify TRPM2 as a potential therapeutic target in the treatment of type 2 diabetes and cardiovascular complications.



ADA-Funded Research

53-LB

Mitochondria Dysfunction in Diabetic Cardiomyopathy: Alterations of Oxidative Phosphorylation Complexes, Mitochondria Biogenesis, and Acute Insulin Signaling to Mitochondria Matrix

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Cardiomyopathy is one of the most challenging diabetic complication. Mitochondria (mito) play key roles in the regulation of myocardial energy production, oxidative stress, and function. However, the exact nature of mito dysfunction in diabetic cardiomyopathy is not clear and its mechanism of regulation poorly understood. The aims of this study were to characterize mito oxidative phosphorylation (OxPh) complex activities and oxidative stress in diabetic myocardium, and to define how insulin receptor signaling modulates mitochondria in vivo. Diabetes was induced by streptozotocin injection in mice. OxPh complex I+III, II, IV, and V activities per mito unit were respectively reduced by 31%, 27%, 24% and 50% in the diabetic myocardium. Mito abundance was significantly reduced in the diabetic myocardium by 23% (assessed by the ratio of mtDNA to nuclearDNA). Taking reduction of mito content into consideration, total complex I+III, II, IV, and V activities per myocardial unit were respectively reduced by 40%, 35%, 31%, and 64% in the diabetic myocardium. Oxidative stress was increased in the diabetic myocardium, possibly in part due to OxPh dsyfunction. Treating diabetic mice with Phlorizin could not normalize OxPh complex activities or mito abundance, thus hyperglycemia did not cause mito OxPh dysfunction. However, chronic insulin therapy restored OxPh complex activities and mito abundance in diabetic myo-cardium, which suggests insulin may regulate OxPh and mito biogenesis. Acute insulin injection (20 minutes) increased OxPh V activities by 20%, without changing mito abundance. Neither the abundance of complex V subunit nor phosphorylation of subunit was altered by diabetes or insulin. To explore whether insulin receptor signaling can reach mitochondria, insulin was injected in vivo and myo-cardium was isolated. The results showed that upon insulin stimulation phosphorylated Akt/PKB entered mito inner membrane and translocated into mito matrix. In contrast, insulin did not stimulate ERK translocation into mito. The translocated Akt returned to the cytosolic compartment 20 minutes after injection of insulin. Blocking phosphorylation of Akt did not inhibit insulin-stimulated Akt translocation to mito, suggesting that Akt translocation was independent of Akt phosphorylation. Insulin-stimulated Akt translocation to mito is reduced in an insulin-resistant Type 2 DM mice model. These findings indicate that mito dysfunction could be a fundamental mechanism underlying the development of diabetic cardiomyopathy. Insulin-stimulated Akt translocation to mito matrix may play a key role in the regulation of mito function and biogenesis.

54-LB

Novel Insights into the Role of the Covalent Phosphate of Glycogen in its Metabolism and Structure

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Glycogen is a branched polymer of glucose, which acts as a repository of energy. Skeletal muscle and liver comprise the largest glycogen reserves and play critical roles in maintaining whole body glucose homeostasis. The metabolism of glycogen in these tissues is linked hormonally to nutritional status involving regulation of enzymes that participate in both the synthesis and degradation of the polymer. Glycogen contains small amounts of covalently linked phosphate with unknown function and origin. We hypothesize that this phosphate is important for the metabolism and/or structure of glycogen. Lafora disease (LD) is a fatal form of progressive myoclonus epilepsy characterized by the formation of Lafora bodies (LB), which contain poorly branched, insoluble glycogen-like polymers (polyglucosan). LB are most common in organs with the highest rates of glucose metabolism, including the brain, muscle and liver, all of which express the Epm2a gene product, laforin, which is mutated in ~50% of LD cases. Laforin contains a dual specificity phosphatase and a carbohydrate binding domain that associates with glycogen. We report here that laforin removes phosphate from glycogen *in vitro*, in a time-dependent reaction with Km for glycogen of 4.5 mg/ml. Mutation of the carbohydrate binding domain eliminated the ability of laforin to dephosphorylate glycogen. Epm2a-/- mice accumulate LB in several tissues, including liver and muscle. Liver and muscle glycogen from these mice showed an age dependent increase in the associated covalent phosphate, up to 3-fold ($p < 0.005$) and 6-fold ($p < 0.001$), respectively compared to WT. Analysis by electron microscopy of muscle glycogen purified from 9 month old Epm2a-/- mice revealed a strikingly distinct morphology compared to WT controls, with granules of more even electron density and a greater variability in size. The particles tended to aggregate into large clumps. This structural abnor-mality was largely reversed by treatment with laforin to remove phosphate. Glycogen isolated from these Epm2a -/- mice has a poorly branched structure and a greater affinity for binding glycogen synthase. Our results suggest that the covalent phosphate normally present in glycogen has an important role in its metabolism and/or the maintenance of a physiologically functional polysaccharide structure.

INTEGRATED PHYSIOLOGY—ADIPOCYTE BIOLOGY

55-LB

Knockdown of TRB3 Expression by Antisense Oligonucleotides Enhances Peripheral Insulin Sensitivity in a Rat Model of Type 2 Diabetes

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TRB3 belongs to a family of kinase-like proteins with potent signaling regulatory functions. *In vitro* studies have shown that TRB3 can inhibit AKT activation in hepatocytes and PPARγ activation in adipocytes, thus impairing insulin action in both tissues but its physiologic function *in vivo* is not known. We hypothesized, that ASO induced knockdown of TRB3 would improve insulin sensitivity in a rat model of type 2 diabetes. STZ treated rats were fed a high fat (HF) diet and treated with TRB3-ASO (75 mg/week ip) for 4 weeks. ASO treatment specifically targets gene expression in adipose tissue and liver, resulting in an 80% and 70% reduction of TRB3 expression, respectively. Despite the effective knockdown, no differences in AKT activity were observed in liver or white

adipose tissue (WAT). Fasting plasma glucose and insulin concentrations as well as endogenous glucose production were not significantly altered with TRB3 ASO. However, under hyperinsulinemic-euglycemic conditions, TRB3 ASO treatment increased insulin stimulated whole body glucose uptake by 50%. 2-deoxyglucose uptake was 40% and 30% higher in soleus and tibialis anterior muscles. Though there was no difference of 2-DG uptake in WAT on a per mass basis, there was an overall 70% increase in epididymal WAT mass, which may also have contributed to the increased whole body glucose uptake in ASO treated animals. TRB3 inhibition may have augmented adipogenesis through increased PPARγ action. A 45% increase in PPARγ expression was apparent, resulting in increased circulating adiponectin levels. Finally, a 40% increase in total cholesterol was observed which could largely be attributed to an increase in HDL concentration. This likely results from a 70% increase in SREBP2 expression in liver. In conclusion, these data support the hypothesis that knockdown of TRB3 increases peripheral insulin sensitivity in a T2DM rat model via a PPARγ mediated mechanism and increased adipogenesis.

ADA-Funded Research

56-LB

Leptin for the Treatment of Nonalcoholic Steatohepatitis in the Setting of Relatively Low Leptin Levels

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Recombinant leptin therapy improves insulin sensitivity and dyslipidemia and reverses nonalcoholic steatohepatitis (NASH) in leptin deficient lipodystrophy in humans. Based on these prior observations, we sought to determine if recombinant leptin therapy would be effective in reversing the histopathological changes as well as fat deposition in patients with biopsy proven NASH (NASH activity score 3 or higher with a minimum score of 1 on steatosis, inflammation and hepatocellular injury and/or fibrosis) and relative leptin deficiency (circulating leptin levels<25th percentile of BMI matched controls from NHANES III population). To date, 9 non-diabetic men (age: 32 to 53 years, weight: 77.9 to 106.2 kgs, BMI 26.2 to 31.8 kg/m2, circulating leptin levels 2.7 to 8.1 ng/dL, NASH Activity Score 3 to 11, ALT:26 to 257 IU/L, 7/9 on lipid lowering therapy with a statin or a fibrate for dyslipidemia, 1/9 on metformin for IGT, 2/9 on antidepressive medications) with intact hepatic synthetic function were enrolled in a pilot efficacy trial. No dosing changes are allowed in the cited drugs during the one year study period. The first 4 of these subjects have completed 6 months of recombinant leptin therapy (METRELEPTIN, Amylin Corp, San Diego, CA) given subcutaneously daily at a dose of 0.1 mg/kg/day. As shown in Table 1, body weight and liver fat as measured by MRI and MR spectroscopy (MRS) decreased by 6 months in 3 of the 4 subjects. All subjects underwent a liver biopsy at baseline and are expected to undergo another liver biopsy at 12 months of the study. The first subject who completed his 12 months of therapy had a reduction of his NASH score from 6 to 3.

Table 1: Preliminary findings from the first 4 subjects

Subject	Weight Baseline (kg)	Weight 6-mo.(kg)	Liver Fat Baseline (%)	Liver Fat 6-months (%)
NASH-1	82.3	76.0	9±3 by MRI 15 by MRS	7±3 by MRI 9 by MRS
NASH-2	83.8	78.7	16±4 by MRI 16 by MRS	8±2 by MRI 4 by MRS
NASH-3	106.2	106.8	32±3 by MRI 29 by MRS	31±3 by MRI 32 by MRS
NASH-4	89.0	86.9	22±3 by MRI 25 by MRS	17±3 by MRI 10 by MRS

Overall, recombinant leptin therapy was well-tolerated: 2/9 subjects experienced transient injection site reactions (starting around 3 weeks and subsiding within 4 weeks). Our preliminary results suggest that a subset of patients with NASH with lower leptin levels at baseline may benefit from leptin therapy for weight reduction as well as improvement in hepatic fat content. These results also suggest that the therapeutic window for leptin may be wider than just absolute leptin deficiency.

57-LB

Rapid activation or recruitment of macrophages in mesenteric fat and skeletal muscle of C57Bl/6 mice after short-term exposure to a high-fat diet

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Chronic low-grade inflammation associated with consumption of a high-fat diet (HFD) is hypothesized to be an important contributor to obesity-induced insulin resistance. Because hepatic insulin resistance occurs within a few days after exposure to a HFD, we asked whether rapid changes in inflammation might contribute to this development. The gene expression of proinflammatory cytokines (TNF-α, IL-1β, IL-α, and MCP-1) as well as the macrophage marker, CD68, was directly compared by Q-PCR in insulin- and leptin-target tissues including liver, skeletal muscle, hypothalamus, mesenteric fat, epididymal fat and inguinal fat of mice fed a low-fat diet (LFD) or HFD for 2 days. Weight gain and energy intake were significantly elevated in the HFD mice. In mesenteric fat, expression of CD68 and MCP-1 were significantly elevated after 2 days on the HFD, and there was a non-significant increase in the expression of proinflammatory cytokines as well. CD68 was also significantly increased in the skeletal muscle of HFD mice relative to that of LFD mice. In contrast, there were no changes in the epididymal fat of HFD mice. CD68 was slightly decreased ($P < 0.1$) with no change in proinflammatory cytokines in the liver of HFD mice relative to LFD mice. These data suggest that the rapid recruitment or activation of macrophages in mesenteric fat after short-term exposure to HFD constitutes an early change that may contribute to the early occurrence of hepatic insulin resistance, perhaps by releasing proinflammatory cytokines into the hepatic portal circulation.

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

58-LB

A Novel Fatty Acid Synthase Inhibitor Suppresses De Novo Lipogenesis but induces Hepatic Steatosis, Dermatitis and does not Enhance Insulin Sensitivity in Obese Zucker Rats

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Inhibition of de novo lipogenesis (DNL) by blockade of fatty acid synthase (FAS) might improve insulin sensitivity and dyslipidemia associated with the metabolic syndrome by decreasing tissue lipid accumulation eg in liver. This concept was tested by oral treatment of insulin resistant obese Zucker rats for 10 days with a FAS inhibitor (FASi, 60 mg/kg/day), with an in vitro inhibitory potency of 0.35 µM against rat enzyme. Insulin sensitivity was assessed using the euglycemic hyperinsulinemic clamp method in two groups: FASi treated and vehicle treated (Con) rats. At clamp steady state, hepatic DNL (rate of glucose incorporation into lipid), hepatic glucose output (HGO) and glucose disappearance (Rd) were assessed using ^{14}C-glucose. Hepatic triglycerides (TG) and malonyl-CoA were analysed.

As expected, FASi reduced hepatic DNL (Con: 6.1 ± 0.9, FASi treated: 2.6 ± 0.3 µmol/100g/min) and increased hepatic malonyl-CoA (by ~250 %). In addition, FASi prevented the rapid body weight gain seen in Con. There was no difference in glucose infusion rate (GIR) between the FASi treated and Con during the clamp (Con: 64.9 ± 9.4, FASi treated: 48.0 ± 9.1 µmol/kg/min). HGO (Con: 91.6 ± 6.7, FASi treated: 77.8 ± 7.5 µmol/kg/min) and Rd (Con: 26.7 ± 4.3, FASi treated: 29.9 ± 2.2 µmol/kg/min) were also similar. Hepatic TG levels were increased in response to FASi treatment (Con: 2.5 ± 0.6 g/100g, FASi treated: 6.9 ± 1.4 g/100g). After 9 days treatment, cutaneous lesions were observed in FASi treated rats, which microscopically showed hyperkeratosis and inflammation.

In conclusion, we showed that a novel FAS inhibitor which decreased DNL and increased malonyl-CoA levels, actually worsened hepatic

steatosis (a probable consequence of malonyl-CoA induced inhibition of carnitine palmitoyltransferase-I) and failed to improve whole body insulin sensitivity. These results question the usefulness of peripheral inhibition of FAS as a therapeutic principle for treatment of the metabolic syndrome.

INTEGRATED PHYSIOLOGY— OTHER HORMONES

59-LB

Antisense Reduction of Retinol-Binding Protein 4 Expression in Liver and Adipose Tissues Causes Robust Improvements in Insulin Sensitivity in Diabetic and Obese Mice

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Previous studies indicated that insulin sensitivity is inversely correlated with plasma retinol-binding protein 4 (RBP4) in both mice and humans. To further investigate the role of RBP4 in glucose metabolism and insulin sensitivity, antisense approach was applied to two different mouse models of diabesity. Male, 7 week old *ob/ob* mice and high-fat diet-induced obese male C57BL/6J mice (DIO mice) were treated with a RBP4-specific antisense oligonucleotide (ASO) or a control ASO at 25 mg/kg BW, or with saline, twice a week for 5 and 6 weeks, respectively. RBP4 ASO reduced RBP4 gene expression by 97% in white fat and 90% in liver in *ob/ob* mice, and 71% and 67%, respectively, in DIO mice, whereas the control ASO did not change its expression in either tissue. RBP4 ASO, but not control ASO, treatment also resulted in > 80% decrease in plasma RBP4 levels as early as 2-3 weeks after treatment initiation. In parallel, RBP4 ASO caused a significant reduction in plasma glucose (319.0 ± 28.7 *vs* 500.4 ± 50.9 mg/dl in saline group in *ob/ob* mice and 198.7 ± 3.7 *vs* 236.7 ± 9.0 mg/dl in saline group in DIO mice; $P < 0.01$ for both) and insulin levels (13.80 ± 1.48 *vs* 27.10 ± 4.12 ng/ml in saline group in *ob/ob* mice and 2.30 ± 0.18 *vs* 4.91 ± 0.76 ng/ml in saline group in DIO mice; $P < 0.01$ for both) compared to either saline group or control ASO group in both models. Additionally, RBP4 ASO improved glucose tolerance during GTT and improved insulin sensitivity during ITT in both models. RBP4 ASO lowered plasma FFA levels by 17% (1.23 ± 0.04 *vs* 1.47 ± 0.08 mEq/L in saline group; $P < 0.05$) in *ob/ob* mice and plasma resistin levels by 44% (2.86 ± 0.61 *vs* 5.06 ± 0.34 ng/ml in saline group; $P < 0.01$) in DIO mice, which are consistent with the observed improvements in insulin sensitivity in these mice. Furthermore, RBP4 ASO treatment improved ketosis in *ob/ob* mice and caused a 58% reduction in fasting plasma β-hydroxybutyrate levels (214.8 ± 13.2 *vs* 510.9 ± 119.6 μMol/L in saline group; $P < 0.01$). ASO treatment did not change BW or adiposity in either of the two models, indicating that lowered plasma glucose levels and improved insulin sensitivity were not secondary to changes in these parameters. These data demonstrate that RBP4 plays an important role in glucose metabolism and could be a potential therapeutic target for type 2 diabetes.

60-LB

No Effect of GLP-1 on Human Brain Glucose Delivery During Hypoglycaemia

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The brain almost exclusively depends on circulating glucose. Hypoglycaemia therefore is deleterious to brain function. The Michaelis-Menten formalism describes the magnitude of blood-brain glucose transfer across the blood-brain barrier (BBB) at glucose concentrations in arterial plasma ranging from hypo- to hyperglycaemia. Glucagon-like-peptide-1 (GLP-1) inhibits blood-brain glucose exchange at normoglycaemia and predictably also at hyperglycaemia. We believe that this action may explain GLP-1's neuroprotective effect at normal or higher glucose levels. However, the Michaelis-Menten formalism predicts that hypoglycaemia minimizes the effect on glucose transport of GLP-1's action on the maximum glucose transport capacity.

To test this prediction of the effect of GLP-1 in hypoglycaemia, we determined glucose transport and consumption rates in 7 healthy men in a randomized, double-blinded placebo-controlled cross-over experimental design. The acute effect (independent of insulin) of GLP-1 on glucose transfer in the brain was measured by positron emission tomography (PET) during a pituitary-pancreatic stepwise hypoglycaemic clamp with 18-fluoro-deoxy-glucose (FDG) as tracer of glucose.

The plasma glucose (PG) during PET averaged 3.0 mM with an insulin infusion rate of 0.8 mU/kg*min. We initially maintained growth hormone (GH) and glucagon close to baseline but both increased significantly in the hypoglycaemic phase, as did epinephrine levels. PG, insulin, glucagon, GH, epinephrine levels and glucose infusion rate were similar with GLP-1 and placebo. Total and intact GLP-1 levels stayed in the pharmacological range.

In total cerebral grey matter, the cerebral glucose uptake remained unchanged during hypoglycaemia with GLP-1, 0.37 ± (SEM) 0.03 (GLP-1) vs. 0.40 ± 0.02 micromol/cm³/min (placebo), (P=0.25). The cerebral metabolic rate for glucose remained unchanged at 0.29 ± 0.01 vs. 0.30 ± 0.01 micromol/cm³/min, (P= 0.28) with GLP-1, as was the intracerebral glucose concentration at 0.63 ± 0.05 vs. 0.76 ± 0.16 mmol/L, (P=0.39).

The results complement our previous study of GLP-1 in normoglycaemia. The confirmation that the effect of GLP-1 on glucose transport rates is too small to measure at low glucose concentrations contributes to the understanding of GLP-1's generally beneficial action also in hypoglycaemia.

INTEGRATED PHYSIOLOGY— INSULIN SECRETION IN VIVO

61-LB

Pioglitazone Enhances Beta Cell Function in Normal Glucose Tolerant, Insulin Resistant, Subjects.

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Thiazolidinediones (TZD) improve glucose homeostasis and beta cell function in Type 2 diabetes and impaired glucose tolerance (IGT). TZD effects in insulin resistant (IR) subjects who have both normal glucose tolerance (NGT) and normal fasting glucose (NFG, <100mg/dl) are unknown. We therefore tested whether IR subjects may benefit from use of an insulin sensitizer prior to deterioration of glucose homeostasis (IGT or IFG). We evaluated the effect of pioglitazone (Pio) on IR and the insulin (Ins) secretory response to glucose (G) in two groups of volunteers; one with NFG/NGT+IR (N-IR), (n=11) and another with IGT (n=12). Participants were studied using a graded glucose infusion (GGI) to study Ins secretion and the frequently sampled intravenous glucose tolerance test to evaluate Ins sensitivity (Si). Subjects were placed on Pio for 10 weeks and the studies repeated. N-IR subjects were 8F/3M, mean (±SEM) age 36±3.4 yrs and BMI 36.6±0.8 Kg/m^2; IGT were 9F/3M, age 45.3±4 yrs and BMI 34.9±1.64 Kg/m^2. As expected after Pio, Si increased from 1.17±0.2 to 3.3±0.8 in N-IR (p=0.025) and from 2.5±0.4 to 3.95±0.6 (uU/ml)$^{-1}$.min^{-1} in IGT (p=0.001). Fasting Plasma Glucose (FPG) after Pio was unchanged in N-IR (p=0.6) and decreased in IGT by -10.5±2.5 mg/dl (p=0.002); Insulin (FPI) was reduced by 46% in both N-IR and IGT (p<0.006). In response to G infusion in GGI, plasma G concentrations were unchanged after Pio; mean AUC in N-IR was 55.5±4.7 before and 50.4±2.8 mg/dl.min after; in IGT, 73.9±7.4 before and 68.9±4 mg/dl.min after (p=NS). The Ins response during GGI was lower after Pio; mean AUC was 63.4±12 vs. 29.9±6.6 uU/ml.min in N-IR (p<0.001) and 32.3±6.3 vs. 17.7±3.5 uU/ml.min, in IGT. However, C-peptide AUC was unchanged, 1.57±0.13 vs. 1.42±0.17 pmol/ml.min and 0.96±0.11 vs. 0.97±0.08 pmol/ml.min, indicating a strong effect of Pio to enhance insulin clearance. Disposition Index (using Si*AUC C-peptide to reflect Ins secretion rather than clearance), was significantly increased after Pio, 1.74±0.31 vs. 4.19±1 in N-IR (p=0.04) and 2.25±0.44 vs. 3.68±0.63 units in IGT (p=0.001). In summary, in NFG/NGT subjects with IR, increasing Ins sensitivity with Pio enhances the Ins secretory response to G, even though an effect on plasma G is not observed. This is in contrast to IGT

subjects, where FPG also improved along with beta cell function. We conclude that in appropriate, high-risk IR, insulin sensitization may be a useful approach to enhance B-cell function to prevent diabetes before glucose homeostasis is impaired.

62-LB

Targeted Disruption of Exchange Protein Directly Activated by Cyclic-AMP 1 Alters Glucose Homeostasis and Modulates Pancreatic β-cell Survival

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Previously, the cAMP analog 8-pCPT-2'-O-Me-cAMP (also known as 007), which activates exchange protein directly activated by cAMP (Epac) without affecting PKA, has been shown to act on insulin-secreting cells using *in vitro* model. However, the underlying mechanism(s) by which Epac mediates such function is not fully understood since both isoforms of Epac (Epac1 and Epac2) can be activated by 007. Here, we generated homozygous Epac1 knockout (Epac1-/-) embryonic stem cells (ESC) and mice in order to investigate whether Epac1 also contributes to insulin secretion. The insulin-producing cells differentiated from the Epac1-/- ESC exhibited impaired glucose-responsive insulin secretion *in vitro* (102.8 ± 1.33 mM in Epac1+/+) vs 61.7 ± 5.9 mM in Epac1-/-) ; $P<0.05$). However, the Epac1-/- mice maintained similar random blood glucose levels (7.40 ± 0.21 mM vs 7.27 ± 0.21 mM) to those of the sex- and age-matched wildtype mice (Epac1+/+). To further examine the glucose homeostasis in these mutant mice, we performed intraperitoneal glucose tolerance test (IPGTT) and demonstrated that Epac1-/- mice exhibited impaired glucose tolerance compared to Epac1+/+ mice (AUC: 1461 ± 83.61 mM·min vs 1716 ± 64.81 mM·min; $P=0.028$). Preliminary data from measurements of serum insulin levels following glucose challenge revealed a tendency for a reduction of glucose-stimulated insulin secretion in Epac1-/- mice, suggesting that the Epac1-/- β-cells might have insulin secretion defect. Intriguingly, we also performed insulin tolerance tests and showed that Epac1-/- mice are less sensitive to insulin (AUC: 565.1 ± 33.0mM·min in Epac1+/+ vs 715.5 ± 43.4mM·min in Epac1-/-; $P=0.020$). To gain insights into the role of Epac1-/- in β-cell survival, we administrated multiple-low-dose Streptozocoin (MLDS) for 5 consecutive days and examined the development of hyperglycemia during 1-4 weeks. The Epac1-/- mice developed hyperglycemia earlier and were more severely hyperglycemic than those of Epac1+/+ mice (AUC: 402.9 ± 11.8mM·day in Epac1+/+ vs 477.2 ± 22.8mM·day in Epac1-/-; $P=0.006$), implicating that Epac1 protects β-cell against MLDS-toxicity. Taken together, the present study provides evidences suggesting that Epac1 may have a dual role of regulating glucose homeostasis as well as pancreatic β-cell survival.

INTEGRATED PHYSIOLOGY—LIVER

63-LB

Targeted Disruption of CEACAM1 in Mice Reveals a Novel Mechanism Linking Hepatic Steatosis to Impaired Insulin Action and Clearance in the Liver

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The liver is a major site of insulin resistance and perturbed lipid metabolism in obesity-linked type 2 diabetes. Although a close relationship exists between hepatic steatosis and insulin resistance, the molecular mechanisms implicated remain poorly understood. The carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM1, CC1), known to regulate glucose homeostasis by facilitating hepatic insulin clearance, has also been shown to modulate lipid metabolism as revealed by its ability to lower hepatic fatty acid synthase activity. We have further explored the role of CC1 in hepatic insulin action and lipid metabolism in mice lacking CC1 ($Cc1^{-/-}$). Microarray analysis of livers from these mice revealed a 4-5 times overexpression of sterol regulatory element binding protein 1c (SREBP-1c) -regulated genes within the lipogenesis pathway. Their livers displayed signs of hepatic steatosis as shown by oil red staining, hepatic cholesterol and triglyceride content, and plasma ALT levels. This was more evident when $Cc1^{-/-}$ mice were challenged with a high fat diet as compared to their wild type littermates. A major defect in the ability of insulin to suppress hepatic glucose production, as evidenced by hyperinsulinemic-euglycemic clamp studies, further suggested that these animals are insulin resistant. This insulin resistance was more pronounced after high-fat feeding of $Cc1^{-/-}$ mice, as were their elevated fasting blood glucose and plasma insulin levels compared to the wild type controls. We also determined *in vivo* insulin clearance rates using a new fast-sampling method and found that insulin clearance is reduced in $Cc1^{-/-}$. These results indicate that CC1 plays a key role in the regulation of lipid synthesis in the liver as well as in the control of glucose metabolism through modulation of hepatic glucose production and insulin clearance. Thus, defective CC1 expression or activity could potentially contribute to the development of the metabolic syndrome in obesity-related type 2 diabetes.

INTEGRATED PHYSIOLOGY—MUSCLE

64-LB

A new transgenic mouse model to study GLUT4(*myc*) regulation in skeletal muscle

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Type 2 diabetes is associated with insulin resistance in peripheral tissues. Skeletal muscle is the major site for glucose uptake and insulin-stimulated muscle glucose uptake occurs via regulation of glucose transporter isoform 4 (GLUT4). However, this tissue has proven to be refractory to detailed molecular analysis of GLUT4 traffic. By comparison, rat L6 myoblasts stably expressing GLUT4 with a *myc*-epitope in the first exofacial loop (GLUT4*myc*) have greatly enhanced our knowledge of GLUT4 regulation, although they lack many characteristics of the mature tissue. We hypothesized that transgenic expression of GLUT4*myc* in skeletal muscles of mice would provide a useful model to investigate GLUT4 regulation in health and disease. Here we describe the generation of such mouse model and provide proof of concept for its usefulness to detect changes in cell surface GLUT4 and to identify proteins that interact with cytosolic regions of the transporter. A homozygous mouse colony was generated expressing the GLUT4*myc* transgene driven by the muscle creatine kinase (MCK) promoter. GLUT4*myc* protein levels were 4-fold higher in MCK-GLUT4*myc* transgenic mice compared to littermate controls ($p < 0.05$). The *myc* epitope co-localized with C-terminus GLUT4 and insulin-responsive aminopeptidase (IRAP) in permeabilized muscle fibers bundles prepared from transgenic mice. Stimulation with insulin (12 nM for 30 min) induced a 2.1-fold increase ($p < 0.05$) in surface GLUT4*myc* detected by immuno-fluorescence of the exofacial *myc* epitope in non-permeabilized muscle fiber bundles. Glucose uptake and surface GLUT4*myc* levels were 3.5- and 3-fold higher ($p < 0.05$) in giant membrane vesicles blebbed from hind-limb muscles of insulin-stimulated transgenic mouse muscles compared to unstimulated counterparts. The *myc* epitope also allowed us to immunoprecipitate GLUT4 without interfering with associated proteins. The intracellular tether TUG co-immunoprecipitated with GLUT4*myc* from muscles of transgenic mice or L6-GLUT4*myc* cells, and insulin-stimulation decreased TUG association with GLUT4*myc* in both systems. The results indicate that GLUT4*myc* is a faithful reporter gene in MCK-GLUT4*myc* transgenic mice that can be used to investigate the regulation of GLUT4 in skeletal muscle. The model should be an asset to study dynamic regulation of GLUT4 in muscle as it pertains to insulin action, exercise, insulin resistance and Type 2 diabetes.

65-LB

Deficiency of Electron Transport Chain in Human Skeletal Muscle Mitochondria in Type 2 Diabetes Mellitus

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The well recognized defect in the oxidative metabolism in skeletal muscle in Type 2 diabetes mellitus (T2DM) can be a result of a deficiency in mitochondria mass, a deficiency in mitochondrial function or both. A sedentary life style can contribute to diminished skeletal muscle oxidative capacity. The purpose of this study was to directly compare the effects of exercise plus weight loss to improve insulin sensitivity and influence mitochondrial mass and function in sedentary lean (N=9), obese (N=8) and T2DM (N=10) subjects. The NADH oxidase activity of mitochondrial electron transport chain (ETC) and mitochondria content (cardiolipin, citrate synthase, mtDNA) were analyzed in skeletal muscle biopsies of vastus lateralis. Insulin sensitivity (GIR), determined using the hyperinsulinemic, euglycemic clamp, improved similarly in both obese and T2DM. Prior to intervention, mitochondria content in sedentary healthy lean, obese and T2DM was practically the same. Exercise increased mitochondria content similarly in all groups. However, the specific activity of mitochondrial ETC (per mg cardiolipin) prior to intervention was significantly lower in T2DM and obesity, and remained two-fold lower after exercise intervention.

Post-Intervention	Lean (N=9)	Obese (N=8)	T2DM (N=10)
GIR[¶]	13.8±1.1	8.7±1.1*	6.3±0.9*
Cardiolipin [#]	101.3 ± 7.2	98.5 ± 9.5	87.5±8.9
Citrate synthase[§]	3.5 ± 0.3	5.5 ± 0.8*	5.1 ± 0.9
NADH oxidase[§]	0.62±0.11	0.30±0.05*	0.28±0.06*
NADH oxidase/CL[£]	5.9 ± 0.9	2.9 ± 0.3*	3.0 ± 0.4*

*P<0.03;Lean vs. Obese or T2DM; [¶]mg/kgFFM/min; [#]µg/mU CK; [§]U/mU CK; [£]U/mgCL

The reduced activity of ETC in skeletal muscle mitochondria in obesity and T2DM can not be explained by insufficient physical activity or reduced mitochondrial content. These data demonstrate for the first time a distinct impairment of mitochondrial ETC activity in T2DM and obesity.

66-LB

DNA Methylation in the Promoter Region of PGC-1α in Muscle is Induced by Low Birth Weight and High Fat Diet and Influence the Risk of Developing Type 2 Diabetes

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Low birth weight (LBW) subjects exhibit increased risk of type 2 diabetes (T2D). Epigenetic modifications including DNA methylation is speculated to play a role in the susceptibility of LBW subjects to T2D, but proof of principle is lacking. Insulin resistance in patients with overt T2D has been associated with decreased expression of genes involved in oxidative phosphorylation (OXPHOS) and their transcriptional regulator peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) in skeletal muscle. Overfeeding may unmask early diabetic traits in predisposed subjects. The aim of the study was to determine the expression of PGC-1α and 4 OXPHOS genes relevant to T2D, and to study DNA methylation in the promoter region of PGC-1α in the skeletal muscle. The study was conducted in 22 normal birth weight (NBW) and 17 LBW men, aged 23-27 years, with no family history of diabetes, who received a 3-day control isocaloric diet and a 5-day high fat (60E%), high energy (+50%) diet, in a randomized sequence. Muscle biopsies were obtained on both occasions before and after a euglycemic hyperinsulinemic clamp. Total RNA and DNA was extracted. Expres-sion of ATP5O, COX7A1, NDUFB6, UQCRB and PGC-1α was assessed by quantitative real-time PCR. DNA methylation was assessed by bisulfite sequencing and data processed with ESME software. Five days of high fat diet induced a higher degree of peripheral insulin resistance (P=0.03) in LBW subjects as well as a lower expression of ATP5O (P=0.03) and UQCRB (P=0.05) at basal state, and of PGC-1α (P=0.04) during insulin stimulation, relative to baseline, compared with NBW subjects. LBW subjects had a significantly higher methylation (integrated over 3 CpG sites) of PGC-1α during control diet compared to NBW subjects (14±5 vs. 9±4%, P=0.01). Interestingly, NBW but not LBW subjects increased PGC-1α methylation significantly during overfeeding eliminating the difference between LBW and NBW subjects. Nevertheless, the excess methylation was reversed in NBW subjects when shifted from initial overfeeding (14±4%) to subsequent control diet (9±4%) (P=0.07), whereas the LBW subjects remained hypermethylated regardless of diet. In conclusion, the data indicate a role for DNA methylation of the PGC-1α promoter induced by LBW and diet in the development of insulin resistance and T2D.

ISLET BIOLOGY—BETA CELL GROWTH AND DIFFERENTIATION

67-LB

The Role of Gene 33 in Beta Cell Proliferation

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The limited availability of pancreatic islets from cadaver donors has hindered the broad application of islet transplantation as a cure for type 1 diabetes. Because of this, it is important to develop methods to stimulate islet proliferation. We have discovered that trefoil factor 3 (TFF3) can stimulate rat islet proliferation *in vitro* and have accumulated evidence that this effect is mediated by epidermal growth factor (EGF) receptor signaling. Interestingly, EGF, a potent mitogen, has little ability to stimulate islet proliferation alone, perhaps due to the upregulation of Gene 33 (a negative regulator of EGF signaling). We therefore sought to determine if Gene 33 is induced by EGF and/or TFF3 overexpression in rat islets and if Gene 33 serves as a "molecular brake" for beta cell proliferation. Gene 33 mRNA was induced in rat islets cultured overnight in media containing 0.5 ng/ml EGF compared to islets cultured in media alone. Similarly, Gene 33 mRNA was increased in islets transduced with an adenovirus expressing TFF3 (AdCMV-TFF3) compared to a control adenovirus expressing beta-galactosidase. To determine if Gene 33 has the ability to negatively regulate beta cell proliferation, INS-1-derived 832/13 cells were transfected with a siRNA duplex directed against Gene 33 (siGene 33) or a control siRNA with no known sequence homology (siControl). [^{3}H]-thymidine incorporation was increased in siGene 33 compared to siControl-transfected cells (157 ± 9 vs. 100 ± 9 %). Transduction of isolated rat pancreatic islets with an adenovirus containing siGene 33 stimulated [^{3}H]-thymidine incorporation by 280 ± 54 % and potentiated the normal ability of AdCMV-TFF3 to stimulate islet replication by 503 ± 56 %. In summary, both EGF and TFF3 signaling induce Gene 33 expression, thereby activating a negative feed back control loop for islet proliferation. Therefore, methods that combine activation of TFF3 and/or EGF signaling and the inhibition of Gene 33 induction or activity are predicted to be optimally efficacious for stimulation of islet beta cell replication.

ISLET BIOLOGY—HORMONE SECRETION AND EXOCYTOSIS

68-LB

Critical Role of Rim2 in Insulin Granule Exocytosis

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Various proteins are involved in the exocytotic process of insulin granules, including recruitment, docking, and fusion to the plasma

membrane. Although, like neurons, SNARE proteins (syntaxin, SNAP-25, and VAMP) and their regulators (α-SNAP, NSF, synaptotagmin, Munc18, etc) are thought to be core proteins in fusion events of insulin granules, many other proteins functioning in the exocytotic process of insulin granules have been identified recently. Among these, Rim2, an isoform of Rim1, was originally identified as a molecule interacting with cAMP-GEFII (Epac2) in our laboratory. We previously showed that overexpression of deletion mutant Rim2, which lacks the zinc-finger and C_2 domains but retains Epac2 binding region, inhibited cAMP-induced, Ca^{2+}-dependent exocytosis in PC12 cells and insulin-secreting clonal β-cells. Although Rim2 is expressed mainly in endocrine cells, the role of Rim2 in exocytosis remains unclear. To investigate the physiological function of Rim2 directly, we generated Rim2 knockout (KO) mice. Rim2 KO mice exhibit glucose intolerance with impaired insulin secretion, and glucose-induced insulin secretion is decreased in Rim2 KO islets. To clarify the function of Rim2 in more detail, we generated Rim2-deficient pancreatic β-cell lines by crossbreeding Rim2 KO mice and transgenic mice developing insulinoma. Rim2-deficient pancreatic β-cells did not respond to glucose stimulation, but glucose responsiveness was restored when Rim2 was exogenously introduced by adenovirus-based gene transfer. In addition, Rim2 is localized just below the plasma membrane and is partially co-localized with insulin granules. By using total internal reflection fluorescence microscopy, we show that the number of insulin granules near the plasma membrane is significantly decreased in Rim2-deficient pancreatic β-cells, compared to that in normal β-cells. When Rim2 is exogenously introduced, the number is restored to almost normal level. These results demonstrate that Rim2 is critically involved in the process of insulin granule exocytosis.

69-LB

Tiam1, A Guanine Nucleotide Exchange Factor For Rac1, Regulates Glucose-Stimulated Insulin Secretion In Pancreatic B-Cells

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Using various biochemical, pharmacological and molecular biological approaches, we previously reported key regulatory roles for Rac1 in glucose-stimulated insulin secretion [GSIS]. Tiam1, a specific guanine nucleotide exchange factor for Rac1, has been shown to play key regulatory roles in cellular activation. To this end, we determined regulatory roles for Tiam1 in signaling events leading to GSIS in INS 832/13 cells. Western blot analysis indicated that Tiam1 is predominantly cytosolic in distribution. NSC23766, a specific inhibitor of Tiam1, markedly attenuated glucose-[~ 80%], but not KCl-induced insulin secretion in INS 832/13 cells. *In vitro* G-protein activation assays confirmed the specificity of NSC23766 to inhibit Rac1, but not Cdc42 or Rho. A significant reduction in glucose-mediated activation [i.e., GTP-bound configuration] and membrane association of Rac1 was also demonstrable in NSC23766-treated cells. Effects of NSC23766 on GSIS were specific since it elicited no effects on the expression of Rac1, total protein content, insulin content or metabolic viability in these cells. Specific depletion [~ 80%] of Tiam1 by RNAi approach markedly inhibited not only glucose-induced activation of Rac1, but also its trafficking to the membrane compartment. Paradoxically, GSIS was significantly potentiated in Tiam1-depleted INS 832/13 cells. Together, our data indicate that: [i] Tiam1 represents one of the GEFs for Rac1 in insulin-secreting cells; [ii] Tiam1-mediated Rac1 activation is necessary, but not sufficient for GSIS in these cells; and [iii] additional regulatory factors/mechanisms might underlie GSIS in Tiam1-depleted cells. Potential significance of these findings in relation to novel regulatory roles for GEFs in GSIS will be discussed.

ISLET BIOLOGY— SIGNAL TRANSDUCTION

70-LB

Protein Farnesylation Is Necessary for Glucose-Induced Activation of ERK1/2 and Insulin Secretion in Pancreatic B-Cells

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Post-translational prenylation of small G-proteins is felt to increase their hydrophobicity culminating in their translocation to their relevant membranous sites for interaction with their respective effectors. In the current study, we studied regulatory roles for protein farnesylation in glucose-induced ERK1/2 activation and insulin secretion in normal rat islets and INS832/13 cells. Two structure-specific inhibitors of farnesyltransferase [e.g., FTI-277 and FTI-2628] significantly attenuated glucose-induced ERK1/2 activation and insulin release in these cells. siRNA-mediated selective depletion of endogenous H-Ras, a farnesylated protein, elicited minimal effects on glucose-induced ERK1/2 activation and insulin secretion in INS 832/13 cells. Interestingly, GW-5074, a specific inhibitor of Raf-1, which is an effector protein for H-Ras, markedly reduced glucose-induced ERK1/2 activation and insulin secretion in INS 832/13 cells. Overexpression Raf-1BXB, a constitutively-active mutant of Raf-1, potentiated glucose-stimulated ERK1/2 activation and insulin secretion. Finally, mastoparan, a global activator of G-proteins and insulin secretion, also stimulated ERK1/2 activity. Together, our findings suggest that glucose-mediated ERK1/2 activation and insulin secretion in pancreatic β-cells involves activation of a farnesylated protein, which is distinct from H-Ras. It also requires the intermediacy of a Raf-1 activation step. Identity of the putative farnesylated protein, which promotes glucose-mediated ERK1/2 activation and insulin secretion, remains to be determined.

NUTRITION–CLINICAL

71-LB

Can a Single High-Fat Meal Impair Endothelial and Autonomic Function?

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A major marker for diabetes is chronic inflammation due to visceral obesity. It is known that a chronic high-fat diet is directly responsible for endothelial dysfunction and exacerbation of diabetes. However, the effect of an acute single high-fat meal on endothelial and autonomic function is not well understood and needs further investigation. Therefore, the purpose of this study was to determine the effect of a single high-fat (HF) versus low-fat (LF) meal on endothelial and autonomic function. Nineteen healthy subjects participated in a randomized controlled breakfast cross-over feeding study (mean age 26.6 ± 4.4 years, mean BMI 24.5 ± 1.62 kg/m^2). Endothelium dependent vasodilation was assessed in the forearm using Whitney strain gauge Plethysmography (BF). Skin flows (SF) were measured by Laser Doppler flowmetry. Blood pressure (BP), heart rate (HR), cardiac work, and arterial resistance were used to assess autonomic function. Subjects fasted the night before the experiment and were randomly fed either a HF (50.1g total fat) or a LF (5.1g total fat) meal with a one week interval before crossover to the alternate breakfast-meal. BF and cardiovascular measurements were taken at multiple baselines and at 2-and 4-hours following the test meal. After the LF meal , resting BF at 4-hour postprandial was significantly increased ($p=0.029$) compared to the baseline. Two-and 4-hour postprandial peak and excess BF were significantly increased from baseline ($p=0.0019$ and $p<0.0001$, respectively). HR and cardiac work (HR x BP) were significantly increased ($p<0.05$ and $p=0.03$, respectively) after the LF meal ingestion. Mean BP during hyperemia at 2-hr postprandial increased ($p=0.0016$) whereas forearm vessel resistance decreased ($p=0.0015$), compared to baseline. No endothelial or autonomic function change was found in either BF or cardiovascular measures subsequent to HF meal ingestion. Our findings show that a single HF meal ingestion can impair endothelial and

autonomic function for at least up to 4 hours. The extrapolation to a pattern of high fat meal ingestions, as present in the typical American diet is likely to have profound implications for diabetes and CVD. **ADA-Funded Research**

OBESITY—ANIMAL MODELS

72-LB

Antiobesity and Antidiabetic effects of the Melanocortin Receptor Modulating Agent AP1030 in Selective Breed DIO Rats

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AP1030, the lead compound within a novel group of phenyl pyrrol aminoguanidines with melanocortin type 1 and 4 receptor modulating effects is an orally available small molecule intended for once daily treatment. After single dose administration to normal Sprague-Dawley (SD) rats AP1030 dose dependently reduced food intake without concomitant effects on locomotor activity.

The aim of the present study was to examine the effects of 33 days administration of AP1030 (25 mg/kg po) on feeding and metabolic parameters in male selectively breed SD, diet-induced obesity (DIO) rats (18 weeks of age) on a high fat diet (Fat 4.41 kcal/g - ~32 % energy from fat). Rats treated with vehicle (20% PEG 200) were used as controls and Sibutramine (5 mg/kg po) were used as positive control. N=10 in all groups (all values given as mean ± SE).

Food intake at the end of the treatment period as % of baseline levels: Veh: 96.8±7.6%; AP1030: 85.0±17.1%, $p<0.01$ vs Veh; Sibutramine: 84.3±16.4% $p<0.01$ vs Veh.

Furthermore, AP1030 reduced weight gain with ~10% BW from baseline and with ~15% when compared to vehicle treated animals (Veh: 106±2%; AP1030: 90±6%, $p<0.01$) (Sibutramine: 93±2%).

AP1030 treatment was associated with a ~50% reduction in retroperitoneal and mesenteric fat depots in comparison to vehicle treated and by ~25% reduction in comparison to sibutramide treated rats.

When compared to Vehicle treatment, AP1030 significantly reduced fasting levels of glucose, insulin and cholesterol (Glucose: Veh: 7.1 ±0.6mM; AP1030: 6.4±0.9mM, $p<0.01$), (Insulin: Veh: 545 ± 138pM; AP1030: 376± 84pM, $p<0.01$), (Cholesterol: Veh: 2.7±0.8mM; AP1030: 1.3±0.3mM, $p<0.01$).

Finally, an oral glucose tolerance test was conducted at study day 28. Both AP1030 and sibutramine had beneficial effects on glucose metabolism: Peak glucose (t=15 min): Veh: 12.0±2.0 mM; AP1030: 9.8±1.2 mM, $p<0.01$ vs Veh; Sibutramine: 11.1±1.1 mM, NS vs Veh; Peak Insulin (t=15 min): Veh: 1063± 437 pM; AP1030: 429±212 pM, $p<0.01$ vs Veh; Sibutramine: 492±207 pM, $p<0.01$ vs Veh; Insulin sensitivity index (120 min): Veh: 2.4±1.1; AP1030: 4.6±1.2, $p<0.01$ vs Veh; Sibutramine: 4.4±1.7, $p<0.01$ vs Veh.

In conclusion, these results show that once daily treatment with AP1030 in DIO rats exerts marked antiobesity and antidiabetic effects and may prove to be feasible therapeutic route for the treatment of obesity and type 2 diabetes and related metabolic disorders.

73-LB

Receptors for Tumor Necrosis Factor Play A Protective Role Against Obesity

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Tumor necrosis factor-α (TNF) is a potent inflammatory cytokine with many metabolic effects mediated by receptors TNFR1 (R1) and TNFR2 (R2). Reducing tissue levels of TNF by drugs or ablation by genetic engineering in mice improves the outcome of inflammatory diseases and reduces obesity. However, loss of both TNF receptors (RKO) does not lead to reduced body weight as compared to control mice and as seen for TNF deficient animals. The purpose of this study is to identify the molecular mechanisms by which TNF receptors modulate body weight in the absence of the TNF ligand. TNF-/-, RKO and wild-type (WT) mice (on C57BL/6) were fed rodent chow or a high fat and sucrose (HFHS) diet for up to 14 weeks. At 14 wks of HFHS, weight gain was significantly greater for RKO mice (41±1.3 g) than TNF-/- (29±0.6 g) and wild-types (32±0.7 g; $p<0.05$, n=8-12). Also, percent adiposity was increased 2-fold in RKO as compared to TNF-/- and WT mice based on quantitative NMR (qNMR) (RKO, 40%; TNF, 20%; WT, 27%; $p<0.05$). Increases in adiposity were distributed among all fat depots. The RKO obesity was not explained by hyperphagia as food intake was comparable among strains, and reinforced by quantitative PCR levels for NPY, a mediator of food intake which was not elevated for RKO mice (RKO and WT, 0.6 + 0.2 rel. units versus TNF-/-, 2.2±0.9; $p<0.05$). Peripheral metabolism was assessed by calorimetry (Dr. Michael Schwartz, University of Washington) performed 3 weeks into the diet, when we expected an initiation of metabolic changes. Twenty-four hour VO2 consumption was not different among strains. However, light cycle oxygen consumption and dark cycle cumulative activity were significantly higher for TNF-/- mice as compared to WT and RKO. While TNF-/- mice were slightly hypermetabolic, RKO mice showed metabolism comparable to WT mice. Thus, increased adiposity of RKO may be mediated at the level of the adipocyte. To test this concept, adipogenesis was examined by isolation and differentiation of preadipocytes from TNF-/-, RKO and WT mice. Triglyceride accumulation (expressed as relative fluorescent units) was significantly decreased for TNF-/- adipocytes as compared to adipocytes taken from WT and RKO mice (63 for TNF vs 123 for WT and RKO, $p<0.05$). Taken together, these data indicate that loss of receptors (RKO) leads to an obese phenotype, opposite of that due to loss of ligand (TNF). This may occur due to increased accumulation of triglyceride in adipocytes which will be discussed.

74-LB

TSC-mTOR Signals Regulate Energy Homeostasis by Hypothalamic POMC Neurons

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mTOR (mammalian Target Of Rapamycin) is known to be a key regulator of energy homeostasis. Its activity is negatively regulated by TSC (Tuberous Sclerosis Complex). Arcuate nucleus in hypothalamus has been shown to have relatively high mTOR activity among other regions in the brain, and inhibiting mTOR in arcuate nucleus stimulates appetite. We found that in TSC2 heterozygous (TSC2+/-) mice, in contrast to the wildtype littermates (TSC2+/+), mTOR activity was low in the arcuate nucleus, whereas the nearby ventromedial hypothalamus exhibited high mTOR activity. Furthermore, among the different types of neurons in arcuate nucleus, more than 90% of proopiomelanocortin (POMC) expressing neurons exhibited high mTOR activity, while less than 60% of neuropeptide Y (NPY) expressing neurons exhibited detectable mTOR activity. We also generated conditional knockout (CKO) mice lacking *TSC1* in proopiomelanocortin (POMC) neurons ($Tsc1^{flox/flox}$) to study the long-term manipulation of hypothalamic TSC-mTOR signals on energy homeostasis. While $Tsc1^{flox/+}$ CKO mice showed lower body weight than wildtype siblings, $Tsc1^{flox/flox}$ CKO mice exhibited a biphasic change in body weight: a severe weight loss between 4-6 weeks old followed by a progressive adult-onset obesity started from 6 weeks old. Our findings suggest that TSC-mTOR in hypothalamic POMC neurons plays a pivotal role in regulating energy homeostasis. This study will help us understand how TSC and mTOR regulate body weight and thus provide leads for treating diabetes, obesity and eating disorders. **ADA-Funded Research**

75-LB

XOMA 052, an Anti-IL-1β Antibody, Preserves Beta-Cell Function and Reduces Hyperglycemia in the Diet-Induced Obesity Mouse Model of Type 2 Diabetes

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The cytokine interleukin-1β (IL-1β) is central to the pathology of many inflammatory diseases, and neutralization of the IL-1 pathway shows

efficacy in both animal models and human disease. Recent evidence points to the role of elevated IL-1β in glucose-induced beta-cell apoptosis and dysfunction. Clinical data also have shown that IL-1Ra treatment improves glycemic control and beta-cell function, thus validating this pathway for targeted therapy. XOMA 052 is a potent antibody that binds human IL-1β (hIL-1β) with very high affinity (K_D = 0.3 pM) and may modulate the inflammatory component of early Type 2 diabetes. XOMA 052 binds mouse IL-1β (mIL-1β) with a 3 nM affinity and can neutralize the bioactivity of mIL-1β in vitro and in vivo. It has been previously reported that IL-1Ra treatment prevented diabetes by improving glucose tolerance and insulin secretion in the diet-induced obesity (DIO) model of Type 2 diabetes (T2D). Here, we report efficacy results in studies using 10-fold less XOMA 052 per dose and less frequent dosing to prevent diabetes in DIO mice, as compared with IL-1Ra. In a 14-week study, C57BL/6 male mice were fed either normal diet (ND) or high fat/high sucrose Surwit diet (HFD). Subsets of mice were treated with twice weekly injections of XOMA 052 (i.p., 1 mg/kg) or daily injections of IL-1Ra (i.p., 10 mg/kg). After 14 weeks, mice on HFD had impaired glucose tolerance, elevated serum glucose and cholesterol, and impaired insulin secretion. XOMA 052 treatment preserved insulin secretion during intraperitoneal glucose tolerance test (ipGTT). In addition, fasting glucose and cholesterol levels were reduced compared to isotype control treated mice. The beneficial results with XOMA 052 were statistically significant and comparable to the results observed with IL-1Ra treatment. Thus, blocking IL-1β alone was sufficient for preserving beta cell function during 14 weeks of HFD. XOMA 052 is currently in Phase I clinical trials in the United States and Switzerland in Type 2 diabetes patients.

OBESITY—PATHOGENESIS

76-LB

Intrahepatic Fat, not Visceral Fat, is Associated with Liver, Muscle and Adipose Tissue Insulin Resistance in Obese Subjects

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Both increased visceral fat (VF) and increased intrahepatic fat (IHF) are associated with insulin resistance and diabetes. However, it is not clear whether VAT or IHF are independent risk factors for insulin resistance, because an increase in one fat depot is usually correlated with an increase in the other. Therefore, we evaluated insulin action in liver (glucose production), skeletal muscle (glucose uptake), and adipose tissue (lipolysis) in 3 groups of obese subjects (BMI range: 29-45 kg/m^2) who were matched on either VF volume or IHF content: 1) normal IHF (3.7±1.8%) and high IHF (25.3±11%) (n=10 for each group), matched on VF volume (1290±754 and 1335±564 cm^3); 2) low VF (744±276 cm^3) and high VF (1474±840 cm^3), with normal IHF content (3.7±2% and 3.4±2%) (n=7 in each group); and 3) low VF (1093±372 cm^3) and high VF (2112±1070 cm^3), with high IHF content (21±15% and 22±12%) (n=7 in each group). In all studies, subjects were matched on sex, body mass index, and percent body fat. A euglycemic-hyperinsulinemic clamp procedure in conjunction with stable isotope tracer infusion, were used to assess hepatic, muscle, and adipose tissue insulin sensitivity. Magnetic resonance spectroscopy and magnetic resonance imaging were used to detect IHF content and VF volume, respectively. Hepatic insulin sensitivity, assessed as a function of glucose production rate and plasma insulin concentration, was 41±11% higher in subjects with low IHF than those with high IHF, matched on VF volume ($p<0.01$). Adipose tissue insulin sensitivity, assessed as the decrease in palmitate release into plasma during insulin infusion, was greater in subjects with low IHF than those with high IHF, matched on VF volume (72±2% vs 62±3% reduction from basal, $p<0.01$). Muscle insulin sensitivity, assessed as the increase in glucose uptake during insulin infusion, was greater in subjects with low IHF than those with high IHF, matched on VF volume (273±28% vs 175±6% increase from basal, $p<0.05$). However, differences in VF volume did not affect adipose tissue, liver, or skeletal muscle insulin sensitivity when subjects were matched on IHF content. These data demonstrate that IHF content, not VF volume, is associated with liver, adipose tissue, and skeletal muscle insulin resistance in obese non-diabetic subjects. The relationship between VF volume and insulin resistance observed in previous studies might be due to the correlation between VF and IHF.

PEDIATRICS—TYPE 1 DIABETES

77-LB

Hyperglycemia Associated with Brain Volume Changes Over Time in Youth with Type 1 Diabetes Mellitus (T1DM)

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Debate continues about the long-term effects of blood glucose extremes on the developing brain. In a previous retrospective analysis in youth with T1DM (7-16 years old), we observed differences in regional brain volumes associated with a history of severe hypoglycemia or hyperglycemia exposure. We followed this cohort prospectively for 2 years and repeated structural brain imaging. This study is the first to quantify brain development in youth with T1DM. T1-weighted magnetic resonance images (MRI) in youth with T1DM (n=74) and healthy sibling controls (HC, n=24) were acquired at baseline and after 2 years. During the interval between sessions, all severe hypoglycemic episodes and hemoglobin A1c (HbA1c) values were recorded. Images representing gray and white matter volumes were aligned and placed into atlas space, and difference images (Time 2 minus Time 1) were created for each subject. Whole brain voxel-based morphometry (SPM5) with multiple comparison corrections was used to determine effects of hyperglycemia (average HbA1c), exposure to severe hypoglycemia (any vs. none) and group (T1DM vs. HC) on change in regional volume, covarying age, age of onset, and gender. Among T1DM subjects, those with higher average HbA1c were more likely to have decreases in gray matter volume in the right inferior temporal gyrus ($p<.001$) and thalamus ($p=.04$) and decreases in white matter volume in the splenium of the corpus callosum ($p<.001$). When compared with HC on a whole brain analysis, the 24 T1DM subjects with the highest average HbA1c (mean=10.1%, SD=1.4) had decreased white matter volume on the left in the splenium ($p=.04$; T1DM =-1.9 mm^3, SD=18.7; HC = 16.9 mm^3, SD=12.4). There were no significant differences in gray or white matter volume change between the HC and total T1DM groups or between T1DM subjects reporting any (n=15) vs. no severe hypoglycemia (n=59). The small number of subjects experiencing severe hypoglycemia during follow-up may have limited our ability to detect any hypoglycemia-associated effects on brain volumes. In summary, greater exposure to hyperglycemia was associated with measurable differences in gray and white matter development in youth with T1DM over a 2-year time period. These data suggest a potential risk of hyperglycemia for optimal brain development.

PEDIATRICS—TYPE 2 DIABETES

78-LB

Prevalence of Prediabetes in US Youth, 2005-2006

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The health consequences of the rising prevalence of overweight and obesity among U.S. youth are a national concern. We present the first nationally representative estimates of the total prevalence of prediabetes (predm), including impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), for US adolescents.

We used data from the 2005-2006 National Health and Nutrition Examination Survey. The present analysis included 788 participants, 12-19 y of age, who completed an oral glucose tolerance test after fasting at least 8 h. We defined predm as having IFG (fasting glucose of 100-125 mg/dl) or IGT (2-hour glucose of 140-199 mg/dl). We used logistic regression to test whether the prevalence of predm, IFG, and IGT differed by sex, age

(12-15 y, 16-19 y), race or ethnicity (non-Hispanic white, non-Hispanic black, Mexican American), or BMI (normal weight, at risk for overweight, overweight). We defined overweight as having body mass index (BMI) ≥ the 95th age-sex-specific percentile, and at risk for overweight as having BMI between the 85th and 95th percentiles. We computed predictive margins to adjust our prevalence estimates for the covariates listed above.

The prevalence among US adolescents of predm, IFG, and IGT was 17%, 13%, and 4%, respectively. The combined prevalence of diagnosed and undiagnosed diabetes was <1%. Boys had notably higher prevalence of predm (23% vs 10%, p<.01) and IFG (20% vs 6%, p<.01) than girls, but IGT was not significantly different between the sexes (3% vs 5%, p=.4). The prevalence of predm was higher in youths 12-15 y than in those 16-19 y (21% vs 13%, p=.05). Non-Hispanic whites were more likely than non-Hispanic blacks to have predm (18% vs 11%, p<.01), IFG (15% vs 9%, p=.02), and IGT (5% vs 2%, p=.05). Estimates for Hispanic youth were between those for the other 2 groups (16% predm, 12% IFG, 4% IGT). Overweight youth were more likely than normal weight youth to have predm (33% vs 11%, p<.01), IFG (26% vs 9%, p=.05), and IGT (9% vs 2%, p=.03). Estimates for youth at risk for overweight were between those for overweight and normal weight youth (21% predm, 15% IFG, 7% IGT).

In 2005-2006, one in six US adolescents had prediabetes. Among overweight youth, the prevalence was nearly one in three. Studies should examine the long-term consequences of the prediabetes in youth on adult health

PSYCHOSOCIAL—BEHAVIORAL MEDICINE

79-LB

Impact of a Theory-Based Intervention to Support Medication Adherence for Patients with Type 2 Diabetes: an Open Parallel Group Randomized Trial

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Up to half of oral glucose lowering medication (OGLM) for type 2 diabetes may not be taken as prescribed. Improving medication adherence could substantially enhance patient outcomes, but evidence for effective interventions is limited. A promising approach aims to strengthen motivation to take medication by targeting beliefs based on the Theory of Planned Behavior, and facilitate adherence by asking patients to define where and when they will take their medication.

We evaluated whether this intervention is more effective than standard care in improving objectively measured medication adherence in a 14-week parallel group randomized trial based in 13 family practices. Patients ≥18 years with type 2 diabetes, HbA_{1c} ≥7.5%, and prescribed at least one OGLM were randomly assigned in a 3:2 ratio to receive either the trial intervention, delivered by trained and quality-assured practice nurses, or standard general practice care. The primary outcome was the mean percentage of days on which the correct number of medication doses was taken, measured objectively over 14 weeks with an electronic medication-monitoring device (TrackCap, Aardex, Switzerland). Secondary outcomes included glycemic control (HbA_{1c}) and the diabetes treatment satisfaction questionnaire (DTSQ). Analysis was intention-to-treat.

273 patients were screened, 211 met inclusion criteria. Mean (sd) age was 63.2 (10.7) years, weight 96.2 (20.9) kg, and HbA_{1c} 8.33 (1.24)%. 65% (138) were male and 87.2% (177) were prescribed metformin. Mean (sd) percentage of adherent days was 77.4 (2.5)% in the intervention group and 69.0 (3.4)% in standard care (mean difference between groups 8.4, 95% confidence interval 0.3 to 16.7, p=0.044). There was no impact on HbA_{1c} (mean difference between groups 0.06, -0.19 to +0.32, p=0.64) or DTSQ, (-0.73, -2.22 to +0.77, p=0.34).

Delivery of a theory-based intervention was feasible in primary care, acceptable to patients and clinicians and was associated with an increase in objectively measured medication adherence. Follow-up may have been too short to detect an impact on glycemic control. A pragmatic trial evaluating longer-term impact on clinical outcomes is warranted.

TRANSPLANTATION

80-LB

A Peptide-MHC II Chimera Favors Survival of Pancreatic Islets Grafted in Type1 Diabetes Mice

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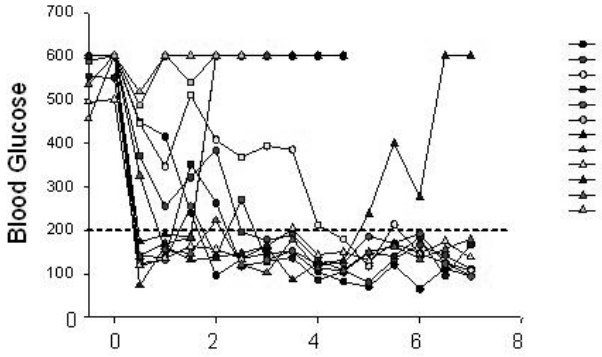
There has been rapid progress in islet transplantation over the last years. The Edmonton protocol initially demonstrated a 90% success rate in producing insulin independence after transplant. However, insulin production has been found to decrease over time, even with administration of immunosuppressive drugs. Followup studies show a reduction to less than 10% of recipients being insulin independent. We hypothesized that the pre-existing autoimmune attack against the islet cells persists after transplant, leading to reduced insulin production and eventual return to diabetes. We report here the use of a soluble peptide-MHC class II chimera (DEF) aimed at producing an antigen-specific therapy for down-regulation of anti-islet T cell responses after trasplantion.

Pancreatic islets from transgenic mice expressing the hemagglutinin antigen in the beta cells under the rat insulin promoter (RIP-HA) were grafted under the kidney capsule of diabetic, double-transgenic (dTG) mice expressing hemagglutinin in the beta cells and T cells specific for hemagglutinin (RIPHA/TCRHA). The recipient dTg mice were treated or not with the DEF, and progression of diabetes, graft survival, and T cell responses to the grafted islets were analyzed.

The DEF protected syngeneic islet transplants against the islet-reactive CD4 T cells and prolonged the survival of the transplanted islets. Protection of the transplants occurred by polarization of the antigen-specific memory CD4 T cells toward a TH2 anti-inflammatory response.

The use of DEF after islet transplant is an effective and specific therapeutic approach to down-regulate anti-islet T cells responses, leading to long-term survival of grafted pancreatic islets.



81-LB

The Impact of Malglycemia on Mortality and Infection for Patients Undergoing Allogenic Hematopoietic Stem Cell Transplants

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Non-relapse mortality (NRM) rates for patients undergoing allogenic hematopoietic cell transplant (HCT) depend on several factors, including underlying disease, but average 15-20% during the first 200 days. Due to high-dose immunosuppression and insulin, malglycemia [which we define as hyperglycemia, hypoglycemia and glycemic variability, measured as the standard deviation of plasma glucose (PG) values] is common in this population. We hypothesized that malglycemia is associated with increased mortality in HCT patients.

We retrospectively reviewed 1175 adults receiving allogeneic HCT

between 2000 and 2005 at the Fred Hutchinson Cancer Research Center in Seattle, WA. PG values through day 100 were considered, and patients had a median of 0.55 values per patient-day (range of 0.09-3.62) with a total of 66,062 measurements. Sixty-four percent and 34% of patients had at least one PG above 200 mg/dL and 300 mg/dL respectively. Overall, there were 215 cases of NRM by day 200 post-HCT and 601 deaths from any cause. A multivariable Cox regression model was fit to examine the association of malglycemia with day-200 NRM, where various parameters for malglycemia were modeled as time-dependent covariates. Other non-glycemic factors known to be associated with mortality were also included. Our model demonstrates a non-monotonic association between PG and day-200 NRM, with all three components of malglycemia being associated with increased NRM relative to normoglycemia as shown in the table (associations with overall mortality were qualitatively the same).

Category	Hazard Ratio	95% CI*	p-value
Plasma Glucose (mg/dl)			
101-150	1	---	---
0-70	2.40	0.87-6.66	0.09
71-100	0.67	0.41-1.11	0.12
151-200	1.39	0.96-2.00	0.08
201-300	1.93	1.31-2.83	0.0009
>300	2.78	1.58-4.92	0.0004
Standard Deviation PG			
0-16	1	---	---
16-26	2.28	1.03-5.09	0.04
26-46	4.83	2.22-10.50	0.0001
>46	14.57	6.83-31.06	<0.0001
Minimum PG			
>89	1	---	---
0-89	2.18	1.59-3.03	<0.0001

*CI, confidence interval

Fungal infection, CMV disease, and gram-negative bacteremias are the leading infectious complications in HCT. Combining these infections into a composite infection endpoint, there were 1073 episodes observed among 506 patients (43%) who had at least one occurrence. As with NRM, all three components of malglycemia were associated with an increased infection rate but glycemic variability was found to be an even stronger predictor of infection than hyperglycemia or hypoglycemia.

We conclude that all components of malglycemia, particularly glycemic variability, impact mortality and infection in patients receiving allogenic HCT. Further study is now indicated to assess the feasibility and impact of reducing malglycemia in this population of HCT recipients. If confirmed in this and other populations, new inpatient treatment targets for glucose may be justified.

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