Late Breaking Abstracts

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01-LB

In Obese Subjects with Type 2 Diabetes, Are Short Acting Insulin Analogues That Short?

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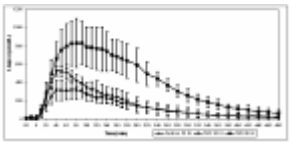
Pharmacokinetics of short acting insulins (SAIs) were established following small (4-14^{IU}) subcutaneous (sc) injections in normal weight healthy or type 1 diabetes subjects and in a rare few moderately overweight subjects with type 2 diabetes (T2D). However, most patients using insulin are obese and receive much larger dosages. Insulin absorption depends mainly on blood flow in adipose tissue (ATBF). However, in obese subjects, ATBF is 40-70% lower than in healthy subjects and, after sc injection of saline, decreases for a period which correlates with the volume of injection.

This study aims to show that pharmacokinetics of SAIs are delayed in obese subjects with T2D.

Plasma SAI (lispro) was measured by RIA (Linco Inc) in 6 healthy subjects (age: 23±2 yrs, BMI 22±1 kg/m2) following 10^{IU} lispro sc injection and in 8 subjects with T2D (age: 60±7 yrs, BMI: 37±6 kg/m^2, duration of T2D 18±9 yrs, insulin therapy 5±4 yrs, A1c 8.1±1.1%) following 10^{IU} and 30^{IU} (random order).

In controls, results were as expected: Tmax (time to maximal concentration) = 50±9 min, Cmax (maximal concentration) = 552±55 pmol/L, AUC = 79794±21420 pmol.min/L, TRmax (time to max infusion rate) = 65.0±10.0 min. In T2D subjects, **a)** following 10^{IU}, results were: similar Tmax (52.5±16.7 min, p=0.75), lower Cmax (-42%, 353±96 pmol/L, p<0.001), smaller AUC (63735±12557 pmol.min/L, p=0.10), longer TRmax (123.8±10.6 min, p<0.0001); **b)** following 30^{IU}, results were: longer Tmax (86.3±23.3 min, 36 min later than after 10^{IU}, p=0.004), higher Cmax (879.5±281 pmol/L), AUC 2.8 times larger than after 10^{IU} sc (197155±36552 pmol.min/L, p<0.001), but only 2.5 times larger than in controls (p<0.001), and longer TRmax (161.9±20.9 min, p=0.002).

In healthy normal weight subjects, our results reproduced data accepted and used in daily practice for insulin prescriptions. However, in a population of obese subjects with T2D, we show for the first time that plasma levels of SAIs are blunted, at low dosage, and severely delayed at average dosage. Our data therefore question the timing of injection and the effectiveness of SAIs in obese subjects with T2D, so commonly treated by GPs and endocrinologists.



02-LB

Results of a Randomized, Single-Dose, 2/3-Way Crossover Comparison Study of Intranasal Insulin Spray (Nasulin$^{TM)}$ Injectable Fast-Acting Insulin (Humalog^{R}) in Normal Nonsmoking and Smoking Subjects

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Results of a Randomized, Single-Dose, 2/3-Way Crossover Comparison Study of Intranasal Insulin Spray (Nasulin™) and Injectable Fast-Acting Insulin (Humalog®) in Normal Nonsmoking and Smoking Subjects.

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The pharmacokinetic characteristics of the investigational drug, Nasulin, were compared to those of Humalog in normal nonsmoking and smoking subjects to determine the effects of smoking on the absorption of insulin delivered intranasally. Eighteen (18) normal nonsmoking subjects were randomized to receive 2 treatments (Nasulin and Humalog) in 2 different periods, and 18 chronic smoking subjects were randomized to receive 3 treatments (Nasulin immediately after smoking 2 cigarettes, Nasulin after cessation of smoking for 10 hours, and Humalog) in 3 different periods. All subjects were normal, healthy men. A series of samples for insulin levels were obtained predose and over 4 hours postdose (16 time points). For each of the dosing periods, subjects received an alternate treatment, and blood samples were obtained at the same time points for PK analysis.

PHARMACOKINETIC CHARACTERISTICS OF INSULIN

Treatment	C_{max} (µIU/mL)	t_{max} (h)	Mean AUC_{0-1} (µIU·h/mL)	Mean AUC_{0-2} (µIU·h/mL)	Avg. Relative $Bioavail_{0-1}$ of Nasulin (%)	Avg. Relative $Bioavail_{0-2}$ of Nasulin (%)
Humalog NS (n=18)	28.27	0.76	16.61	31.91	—	—
Nasulin NS (n=18)	29.10	0.27	12.11	13.58	18.76	9.65
Humalog S (n=18)	29.33	0.76	16.79	31.22	—	—
Nasulin S (n=18)	28.18	0.35	12.37	15.01	20.44	14.8
Nasulin S+10 (n=18)	26.72	0.37	10.20	10.97	17.31	10.2

NS=nonsmoker, S=smoker immediately after smoking 2 cigarettes, S+10=smoker 10 h after smoking.

Nasulin insulin levels peaked sooner than Humalog in both nonsmokers (0.27 vs 0.76 h) and smokers (0.35 and 0.37 vs 0.76 h). C_{max} levels were comparable for Nasulin and Humalog. A plot of 95% CI for mean AUC values of Nasulin showed no difference between smokers and nonsmokers in either the first hour or the first 2 hours. Relative bioavailability (0-1 h) of Nasulin in nonsmokers was 18.76% and in smokers after smoking and after cessation of smoking for 10 hours was 20.44% and 17.31%, respectively. There was no significant difference in insulin AUC values among subjects from the 3 Nasulin groups (P=0.3526, 0.2622, and 0.2480 for 0-1 h, 0-2 h, and 0-4 h, respectively).

03-LB

Pharmacokinetics and Pharmacodynamic Effects of Oral Glp-1 And Pyy3-36: A Proof Of Concept Study in Healthy Subjects

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Context: Oral formulations of GLP-1 and PYY3-36, two satiety peptides, were studied. An oral dosage form of these peptides would be preferable in many of the possible therapeutic indications.

Objectives: Our objective was to establish the pharmacological profile of increasing oral doses of GLP-1 and PYY3-36 in healthy volunteers. In addition, the pharmacological effects of GLP-1 and PYY3-36 were investigated. GLP-1 and PYY3-36 were delivered by the oral-enteric route by means of the eligen® technology.

Setting: Single center escalating dose study with oral applications.

Subjects and Methods: In the first part, GLP-1 was given orally to 6 male subjects; the treatment consisted of one of the following oral doses of either GLP-1 (0.5, 1.0, 2.0 and 4.0 mg) or placebo. In the second part, PYY3-36 was given orally to another 6 healthy male subjects; the treatment consisted of one of the following oral doses of either PYY3-36 (0.25, 0.5, 1.0, 2.0 and 4.0 mg) or placebo.

Results: The oral administration of both peptides induced a rapid and dose-dependent increase in plasma drug concentrations (linear dose-response relationship for GLP-1: R=0.96; P=0.011 and a curve linear dose response for PYY3-36). In addition, oral application of GLP-1 induced a potent effect (p<0.05 vs placebo) on insulin release, whereas oral PYY3-36 induced a trend for inhibition of ghrelin secretion, which was significant (p<0.05 vs placebo) for the highest dose.

Conclusions: This study showed for the first time that satiety peptides such as GLP-1 and PYY3-36, can be delivered orally in humans; GLP-1 was active with regard to stimulation of insulin release and PYY suppressed ghrelin levels after oral administration.

Cmax after oral application of GLP-1 or PYY3-36					
Doses	PLACEBO	0.5 mg Peptide	1 mg Peptide	2 mg Peptide	4 mg Peptide
Cmax, plasma GLP-1 (pmol/L)	0.2 ± 0.1	63.5 ± 18.9	108.8 ± 42.3	83.7 ± 14.9	300.6 ± 99.4
Cmax, plasma PYY3-36 (pg/ml)	113 ± 2	257 ± 59	450 ± 169	418 ± 49	630 ± 150

04-LB

Initial Combination Therapy with Sitagliptin and Metformin Provides Effective and Durable Glycemic Control Over 1 Year in Patients with Type 2 Diabetes: A Pivotal Phase III Clinical Trial

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Background and Aim: Initial monotherapy is often unsuccessful at getting patients to glycemic goals, and due to the progressive nature of type 2 diabetes, patients require intensified treatment in order to maintain or achieve optimal glycemic control. Long-term efficacy and safety of initial combination therapy with sitagliptin and metformin was assessed in patients with type 2 diabetes and inadequate glycemic control (A1C 7.5% to 11%) on diet and exercise.

Methods and Materials: After completing an initial 24-week placebo-controlled phase, patients continued in a 30-week, double-blind, active-controlled phase. At Week 24, patients remained on their previous active treatments: sitagliptin 100 mg/metformin 2000 mg (S100/M2000), sitagliptin 100 mg/metformin 1000 mg (S100/M1000), metformin 2000 mg (M2000), metformin 1000 mg (M1000) (all as divided doses administered b.i.d.); and sitagliptin 100 mg q.d. (S100). Patients on placebo were switched to M2000. This report presents 54-week results for patients who received active treatment throughout the study.

Results: Of the 1091 patients who were randomized, 748 patients continued into the active-controlled phase: S100/M2000 n=157, S100/M1000 n=148, M2000 n=137, M1000 n=122, S100 n=106. In the all-patients-treated analysis, mean A1C changes from baseline were -1.8% (S100/M2000), -1.4% (S100/M1000), -1.3% (M2000), -1.0% (M1000), and -0.8% (S100). With a mean baseline A1C of 8.7% (8.5% to 8.8% across groups), the proportions of patients with an A1C <7% at Week 54 were 67% (S100/M2000), 48% (S100/M1000), 44% (M2000), 25% (M1000), and 23% (S100). Glycemic response was generally durable over time across treatments. For the patients completing treatment through Week 54, mean A1C changes from baseline were -1.9% (S100/M2000), -1.7% (S100/M1000), -1.6% (M2000), -1.2% (M1000), and -1.4% (S100). The incidence of hypoglycemia (1.1%-2.7%) was low across treatment groups. The incidences of gastrointestinal adverse experiences were similar for the combination groups compared with the respective metformin monotherapy groups.

Conclusions: In this study, the initial combination therapy of sitagliptin and metformin provided durable and substantial glycemic improvements and was generally well-tolerated over 54 weeks in patients with type 2 diabetes.

05-LB

The AGE/ALE Inhibitor LR-90 Prevents Development of Insulin Resistance and Nephropathy in Zucker Diabetic Fatty Rats

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Resistance to insulin action is a common abnormality present in major human diseases such as diabetes mellitus and obesity. Abdominal obesity contributes to insulin resistance, a metabolic abnormality linked to the development of type 2 diabetes and cardiovascular disease. Advanced glycation end products (AGEs) as well as advanced lipoxidation end products (ALEs) are pro-oxidant and pro-inflammatory compounds that have been linked to impaired insulin sensitivity. Recently, an increase in intracellular methylglyoxal (MGO), a key source of AGE/ALE, was reported to impair insulin- signaling, leading to the development of insulin resistance. We have previously demonstrated the renoprotective effects of LR-90 in experimental Type 1 diabetes, and its anti-inflammatory effects on activated monocytes. In this study, we investigated the effects of LR-90 in the prevention of insulin resistance and nephropathy in ZDF (*fa/fa*) rats. After 30 weeks, ZDF rats were hyperglycemic, hyperlipidemic, and had increased C-reactive protein (CRP) levels than age-matched lean (*fa*/+) animals. ZDF rats also had increased plasma insulin levels and reduced pancreatic insulin content, which was associated with significant disruption of islet cell architecture and fibrosclerosis. In addition, ZDF rats exhibited nephropathy as demonstrated by elevated urinary albumin excretion and increased glomerulosclerosis, concomitant with increased gene and protein expression of transforming growth factor (TGF)-β1 and connective tissue growth factor (CTGF) and accumulation of AGE and its receptor (RAGE) in the kidney tissues. Treatment of ZDF rats with LR-90 significantly decreased hyperlipidemia and CRP levels in the plasma. Moreover, LR-90 reduced the levels of plasma insulin and significantly ameliorated pancreatic insulin contents and islet cell morphology. LR-90 markedly reduced albuminuria and glomerulosclerosis, prevented renal AGE and RAGE deposition, and attenuated TGF-β1 and CTGF mRNA and protein expression. These new data provide further evidence that LR-90, in addition to its renoprotective effects, may also protect against β-cell dysfunction associated with Type 2 diabetes.

06-LB

PC-DAC:Exendin-4 (CJC-1134-PC) Demonstrates Safety and Efficacy as an Adjunct Therapy to Metformin: A Randomized, Double-Blind, Placebo-Controlled, One Month Phase I/II Study in 70 Patients with Type 2 Diabetes Mellitus

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This multiple-dose study evaluated safety, tolerability, PK and efficacy parameters of CJC-1134-PC in patients with stable Type 2 diabetes on metformin. Seventy patients were enrolled and randomized in US and Canada to 1mg (n=18), 2mg (n=17), 3mg (n=17) or placebo (n=18) groups. Sixty-nine patients received 5 weekly doses injected with a 30G needle. All three treatment groups experienced reductions in mean fasting plasma glucose (FPG) that were statistically significant versus baseline ($p<0.005$) and placebo ($p<0.03$) over the 5-week treatment period. The average reductions from baseline values for the 1mg, 2mg, 3mg and placebo groups were 9%, 11%, 7% and 1%, respectively. HbA1c improved in all three treatment groups with median HbA1c decreasing 0.5%, 0.8% and 0.6% in the 1mg, 2mg, and 3mg groups at the end of the 5-week period (Day 35), 0.7%, 0.6%, and 0.7% on Day 49, and 0.7%. 0.8% and 0.9% at the end of the study period (Day 63) versus baseline. The placebo group declined 0.35% on Day 35, 0.3% on Day 49, and 0.2% on Day 63. The reduction for the pooled three treatment groups was significant versus placebo both on Day 49 and Day 63 (p=0.03). There was no significant change in weight in any cohorts (baseline 81-85kg) at the end of the treatment period. The drug was generally well tolerated. The most common side effects during treatment included headache occurring in 3/18 (17%) placebo patients and 15/52 (29%) treated patients, and nausea which was reported in 3/18 (17%)placebo patients and 11/52 (21%) treated patients. There were no cases of drug-related vomiting in either the 1mg or 2mg cohorts during the treatment period; vomiting occurred in 5 patients in the 3mg cohort, none of which led to patient drop-out. There were no drug related injection site reactions in the 2mg and 3mg treatment groups; skin reactions were reported in 4 placebo patients and 1 patient in the 1mg cohort. Generally low-titered antibodies were detected in 11/52 treated patients. There were no drug-related serious adverse events during the study. In summary, once-weekly CJC-1134-PC was very well tolerated and effectively lowered blood glucose, especially at the 2mg dose.

07-LB

Lapse of Glycemic Control Despite Higher Daily Insulin Dose in a Switch From Insulin Glargine to Detemir in Type 1 Diabetes Mellitus

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Recently, Iowa Care (Iowa Medicaid) switched insulin Glargine to Detemir in subjects with Diabetes Mellitus (DM) without the knowledge or approval of healthcare providers. Therefore, retrospective review to assess glycemic control (HbA1c), body weight (BW), daily insulin dose (Units), total (T) as well as Glargine (GI) or Detemir (DI) and rapid acting insulin, Novolog (NI) in 15 subjects with Type 1 DM, duration 17 ± 3 years with age 38 ± 5 years who were switched from GI to DI (Group 1) for whom HbA1c records were available pre-switch and at the end of 6 months. Records of 15 subjects matched for age (42 ± 5 years) and continuing GI (Group 2) during the same period were examined for comparison. Initially, subjects were switched from GI to DI in the same daily dose in AM. The daily doses of DI and NI in Group 1 were adjusted by telephone at weekly intervals based on self blood glucose monitoring until stabilization was achieved. They were also followed in the outpatient clinic at interval of 3 months similar to subjects continuing GI. All subjects in Group 1 were changed to DI twice a day because of significant rise in hypoglycemia with the whole daily dose used once a day. Glycemic control remained stable on continuing GI once daily while it worsened on switching to DI despite a higher daily dose being injected twice a day and greater total insulin dose. (Table) Therefore, switching to Detemir insulin from Glargine insulin is likely to result in lapse of glycemic control as well as higher costs and undue hardship to both patients and providers because of the resulting higher insulin dose as well as an increased number of injections, and need for frequent recurrent evaluations during the initial period of switch.

Table

Group	Basal Insulin (BI)	BI DOSE (U/D)	NI DOSE (U/D)	TI DOSE (U/D)	HbA1C (%)	BW (Kg)
1	Glargine QD ↓ Detemir BID	37 ± 6 49 ± 9*	33 ± 5 36 ± 6	70 ± 10 86 ± 12*	7.8 ± 0.2 8.5 ± 0.8*	80 ± 5 83 ± 5
2	Glargine QD ↓ Glargine QD	30 ± 6 31 ± 6	26 ± 5 27 ± 6	56 ± 10 57 ± 12	7.6 ± 0.3 7.7 ± 0.2	86 ± 6 85 ± 7

* p <0.05 Vs. Glargine

08-LB

Contrasting Actions of Sitagliptin and Exenatide on Food Intake, Body Weight, Glucose Stimulated Insulin Secretion and Gastric Emptying in Rodents

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Exenatide (EXN) is an incretin mimetic that shares similar glucoregulatory properties to human GLP-1 including enhancement of insulin secretion, regulation of gastric emptying (GE), decreased food intake (FI) and glucagon secretion. Sitagliptin (SGP) is a DPP-IV inhibitor and acts by increasing active endogenous GLP-1 in the circulation. The present studies compared the pharmacological effects of acute treatment with EXN vs SGP. To assess duration of action of SGP, C57BL/6J male mice received an oral dose of 10 or 50 mg/kg. After 3h, plasma DPP-IV activity decreased by 75% and 90% from baseline, respectively, and returned to basal levels after 24h. OGTT's were performed in *db/db* mice with EXN (20 µg/kg IP) and SGP (50 mg/kg PO) administered 15min before a glucose challenge (2g/kg). In the EXN group, plasma insulin (assessed as $AUC_{0\text{-}120\ min}$) increased by 86% (P< 0.05) and plasma glucose ($AUC_{0\text{-}120\ min}$) decreased by 47% (P<0.01 vs control). There was no significant difference in insulin and glucose AUC between SGP-treated mice and controls. SGP (60 mg/kg PO) did not affect mean cumulative FI (CFI) measured hourly over a 24h period, while EXN (5µg/kg IP) significantly decreased CFI at each hour over the 24h period. Mean CFI at 12h and 24h was reduced in the EXN group by 45% and 33%, respectively (P<0.01 vs control). BW gain after 24h was significantly reduced by 2.4% in the EXN group (P<0.01) but not in SGP-treated rats. GE was measured by appearance of acetaminophen (ACET) in plasma 30min after ACET gavage at t=0. Oral administration of SGP (60mg/kg) at t= -45min decreased GE by 39±4% vs 90±2% inhibition with EXN (10nmol/kg) administered by SC injection at t=-5 min (P<0.0001). In these GE studies, DPP-IV activity measured at t=30min was reduced only in SGP-treated rats (91% from control P<0.001). These studies reveal significantly greater in vivo efficacy with EXN vs SGP for modulating several physiological parameters that contribute to improved glucoregulation.

09-LB

Effects of PHX1149, a Selective DPP4 Inhibitor, in Subjects with Type 2 Diabetes Mellitus

PHENOMIX PROTOCOL 201 STUDY GROUP, HANS-PETER GULER, *San Diego, CA*

DPP4 inhibitors prolong the half-life of incretins which leads to enhance insulin secretion and suppression of hepatic glucose production. PHX1149 is a highly water soluble, potent and selective, small molecule DPP4 inhibitor that stays in the extracellular space. The compound is not metabolized, is excreted renally and has a half-life of 10 to 13 hr. This was a randomized, double-blind, placebo-controlled, multi-center 28 day study in subjects with Type 2 diabetes mellitus. The primary efficacy endpoint was change in the area under the curve (AUC) of postprandial glucose values from Day 1 to Day 28 as measured after a test meal administered in the morning after fasting overnight. A total of 174 subjects were enrolled into 3 active dose groups and one placebo group (1:1:1:1) at multiple clinical sites in the US, Mexico, and Australia. Subjects had a mean age of 52 years, a mean BMI of 33 kg/m^2, and a mean baseline HbA_{1c} of 8.7%. The majority (59%) were female. Almost all of the subjects (93%) were on a stable background therapy of metformin only, while 7% were on a combination of metformin and a glitazone.

	Δ AUC pp glucose [mmol/L*hr]	Δ HbA1c [%]	Δ AUC pp GLP-1 [pmol/L*hr]	Ex vivo DPP4 [%] inhibition during test meal
Placebo	0.11±0.50	-0.45±0.09	3.90±2.83	-
100 mg	-2.08±0.51, p=0.002	-0.46±0.09	11.63±2.86, p=0.053	83
200 mg	-1.73±0.49, p=0.008	-0.42±0.08	16.42±2.72, p= 0.001	89
400 mg	-1.88±0.48, p=0.004	-0.73±0.08	15.75±2.71, p= 0.0002	96

Δ refers to the comparison between Day 28 and Day 1. ANCOVA, all results are shown as Δ LS Mean ± SE, p-value vs. placebo. pp = postprandial

DPP4 inhibition at 24 hr after the dose remained high at ~ 80, 70, and 50% for the 100, 200, and 400 mg dose groups, respectively. Overall, there was no discernable pattern of drug related adverse events and drug treated patients were similar to placebo. 63.8% of all subjects reported at least one adverse event and all but 2 events were rated mild or moderate. Two serious adverse events were reported, both in the placebo group. The most frequently observed adverse events were headache (10.3%), followed by hyperglycemia (8.6%), diarrhea (6.9%), UTI (6.3%), and nausea (5.7%). Edema was reported in the placebo and drug treated subjects; there was no dose relationship in the drug treated subjects.

PHX1149 was tolerated very well, no apparent safety issues emerged. Postprandial blood glucose control in this short term study improved significantly. GLP-1, the biological mediator was increased in all drug treated subjects. HbA1c was reduced in the high dose group (p=0.019). Longer term studies will further explore safety and efficacy of this DPP4 inhibitor in subjects withType 2 diabetes mellitus.

10-LB

Inhibition of DPP-4 Leads to Improved Glycemic Control and Restoration of Islet Cell Mass and Function in a Rodent Model of Type 2 Diabetes

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Inhibition of dipeptidyl peptidase-4 (DPP-4) activity has been shown to improve glycemic control by prolonging and potentiating the actions of incretin hormones such as GLP-1 and GIP. In this study, we compared the effects of chronic treatment of sitagliptin, a potent and selective DPP-4 inhibitor, and the sulfonylurea agent glipizide on islet function and glycemic control in the HFD/STZ ICR mice, a model with insulin

resistance induced by high fat diet (HFD) feeding and impaired insulin secretion resulting from a low dose streptozotocin (STZ) treatment. Significant reduction of blood glucose, HbA1c, and circulating glucagon and improvement in oral glucose tolerance were observed in mice during the entire course of the 10-week treatment with sitagliptin. Conversely, glipizide only improved glycemic control during the early weeks and to a lesser degree compared to sitagliptin, and had no effect on circulating glucagon levels or glucose tolerance measured at 9-10 week of the treatment. In perfused pancreas, sitagliptin, but not glipizide, caused significant improvement on glucose-dependent insulin secretion (insulin AUC of sitagliptin vs. diabetic control: 9.5 +/- 2.8 vs. 3.8 +/- 0.5 ng/ml; $p < 0.03$, n=7) and the insulin secretion capacity assessed by arginine stimulation (insulin AUC of sitagliptin vs. diabetic control: 105 +/- 34 vs. 32 +/- 3 ng/ml; $p < 0.05$, n=7). Importantly, sitagliptin restored normal β-cell mass and α/β cell ratio; the effects were detectable at week 4 of the treatment and sustained through week 10. In contrast, glipizide had no effect on α or β cell mass during the study. These data indicate that DPP-4 inhibition by sitagliptin provided better durability of glucose lowering compared to glipizide in this mouse model. The ability of sitagliptin to improve islet cell mass and function may offer the potential for disease modification in the treatment of diabetes and warrants further studies in the clinic.

50-LB

Effects of Timed Cycloset™ (a Quick Release Formulation of Bromocriptine Mesylate) Administration on Safety, Cardiovascular Event Rate, and Glycemic Control in Subjects with Type 2 Diabetes Receiving Diet, Oral Hypoglycemic, and/or Insulin Treatment Regimens

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Prior clinical studies revealed that treatment with Cycloset™ improves glucose intolerance, insulin resistance, glycemic control and dyslipidemia in obese subjects with insulin resistance or type 2 diabetes. However, the impact, if any, of Cycloset™ therapy upon cardiovascular adverse event rate in subjects with type 2 diabetes has not been prospectively studied in a large population. The current trial therefore investigated the influence of Cycloset on all-cause serious adverse events and cardiovascular event rate among subjects with type 2 diabetes.

This trial was a 52 week, double blind, 2:1 randomized, multicenter study in patients with type 2 diabetes receiving a diabetes therapeutic regimen consisting of diet or no more than two hypoglycemic agents or insulin with or without one additional oral agent that were randomized to treatment with Cycloset™ (titrated from 1.6 mg/day to a maximal tolerated dose up to 4.8 mg daily; n= 2,054), or placebo (n= 1,016). Inclusion criteria included subjects aged 30-80 with type 2 diabetes, an HbA1C of ≤ 10.0 and BMI ≤ 43. The primary and secondary endpoints were time to first all-cause serious adverse event (SAE) and cardiovascular SAE (composite of myocardial infarction, stroke, coronary revascularization, hospitalization for angina and hospitalization for congestive heart failure), respectively, which were adjudicated by an independent review committee. A pre-specified analysis of the between-treatment differences in HbA1c following 24 weeks of therapy among a subpopulation of subjects receiving metformin and sulfonylurea and HbA1c of ≥ 7.5 at baseline was also performed.

There were 176 Cycloset™ and 98 placebo subjects that experienced a SAE, yielding a rate ratio of 0.88 and a hazard ratio of all cause SAE of 1.023 (96% one sided confidence limit of 1.27). There were 31 (1.5%) cardiovascular SAEs in the Cycloset™ group and 31 (3.0%) events in the placebo group resulting in a 43% reduction in cardiovascular outcomes in Cycloset™ treated subjects versus placebo (HR = 0.57, 95% CI: 0.34 - 0.93; P = 0.025). The incidence rate ratio for each of the components of the cardiovascular composite was less than 1.0. Among the metformin and sulfonylurea treated subpopulation of subjects, Cycloset™ (n= 121) treatment resulted in an HbA1c reduction of -0.674 from baseline versus an increase for placebo (n = 71) of 0.015 to give a placebo-adjusted change from baseline of -0.69 (P <0.0002). Of these Cycloset™ treated subjects, 39% (vs. 11% placebo) reached the American Diabetes Association goal of HbA1c ≤ 7.0 (P<0.0007) and 53% (vs. 21% placebo) experienced a minimum reduction in HbA1c from baseline of 0.7 (p<0.0001).

Cycloset™ significantly reduced the risk for the a priori adjudicated cardiovascular adverse event endpoint and was comparable to placebo for all other serious adverse events for the entire study population. Among individuals inadequately controlled on metformin and sulfonylurea, 24 weeks of Cycloset™ therapy significantly improved glycemic control relative to placebo.

Trial registration: *clinical trials*.gov NCT00377676

11-LB

Increased Circulating Total and High Molecular Weight Adiponectin (HMW) Concentrations and Improved Insulin Sensitivity After Treatment with Antisense Oligonucleotides Targeting Protein Tyrosine Phosphatase-1B (PTP-1B) in Obese Insulin-Resistant Rhesus Monkeys

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PTP-1B is an attractive therapeutic target for type 2 diabetes (T2DM), as PTP-1B-deficient mice display improved sensitivity to insulin and leptin, and resist diet-induced obesity. However, the precise role of PTP-1B in human obesity and insulin resistance is undefined, due to lack of specific small molecule inhibitors. ISIS 113715 is a specific antisense inhibitor of PTP-1B being developed for the treatment of T2DM. Its binding site is completely conserved in mice, rats, monkeys and humans. In a Phase 2A trial, ISIS 113715 treatment reduced fasting serum glucose levels and HbA1c in treatment-naïve patients with T2DM.

Adiponectin is an adipocyte-derived hormone with insulin-sensitizing actions. Plasma adiponectin concentrations are reduced in obesity and insulin resistance. Adiponectin circulates as low-, medium- and high-molecular weight multimers (LMW, MMW, HMW), and HMW has been proposed to be the most active form with respect to hepatic insulin sensitivity. The aim of the present study was to investigate whether increases of total and HMW adiponectin may be involved in the improvement in insulin sensitivity in ISIS 113715-treated monkeys. ISIS 113715 was administered to five obese, insulin-resistant non-diabetic rhesus monkeys (20 mg/kg SC x 6 doses over 4 wk). ISIS 113715 treatment reduced fasting plasma insulin concentrations by ~50% (baseline: 67±7 μU/ml vs. treated: 31±9 μU/ml (mean ± SEM), p=0.0001). Plasma glucose levels were unchanged. During IVGTT, a marked reduction in insulin area under the curve was observed (p=0.0002) and insulin sensitivity (slope of glucose disappearance (5-20 min)/insulin AUC) was significantly increased. ISIS 113175 treatment increased plasma adiponectin levels by 136±17%, from 3.7±1.0 μg/ml to 8.0±1.6 μg/ml at 4 wk (p=0.0023). The proportional increase of HMW was larger than for total adiponectin (+360±146%), from 2.0±1.0 μg/ml at baseline to 5.2±1.5 μg/ml (p=0.0070). The proportion of adiponectin in the HMW form (S_A) increased from 42±11% to 60±7% at 4 wk (p=0.038). MMW and LMW adiponectin increased by 70±16%, from 1.7±0.2 μg/ml to 2.8±0.1 μg/ml (p=0.0008). These results suggest that one of the mechanisms by which ISIS 113715 improves insulin sensitivity is by increasing the production of adiponectin; and in particular, the HMW form.

Complications

12-LB

Thalamic Activation and Hypoglycemia-Associated Autonomic Failure in Diabetes

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Hypoglycemia, the limiting factor in the glycemic management of diabetes, is the result of the interplay of insulin excess and compromised glycemic defenses. The key feature of the latter is an attenuated sympathoadrenal response to falling glucose levels, typically the result of recent antecedent hypoglycemia, that causes both defective glucose counterregulation and hypoglycemia

unawareness. This has been termed hypoglycemia-associated autonomic failure (HAAF) in diabetes. Normally, hypoglycemia increases brain synaptic activity in a discrete system of interconnected brain regions including the medial prefrontal cortex (MPFC), the thalamus and the periaqueductal grey (PAG) in humans (*Proc Natl Acad Sci USA* 2004; 101:6217). We sought to determine which components of this system showed alterations in this response after recent hypoglycemia, reasoning that those regions would be involved in the pathogenesis of HAAF. Accordingly, we used [^{15}0]water and positron emission tomography to measure regional cerebral blood flow, and thus regional brain synaptic activity, during clamped euglycemia (90 mg/dL) and hypoglycemia (55 mg/dL) before (Day 1) and after (Day 2) ~24 hours of interval hypoglycemia (~55 mg/dL) in 9 healthy adults. Interval hypoglycemia produced a model of HAAF - reduced adrenomedullary epinephrine and largely sympathetic neural symptom responses to hypoglycemia on Day 2 - the phenomenon we sought to explain. It resulted in greater increments in, and absolute levels of, synaptic activity during hypoglycemia on Day 2 (both P=0.020) only in the medial thalamic nuclei, a region that included the paraventricular nucleus of the thalamus (PVNT). In rats, the PVNT inhibits the hypothalamic-pituitary-adrenal response during recurrent stress as part of a circuit involving the hypothalamus, MPFC, accumbens nucleus, and PAG (*Endocrinology* 2006; 147:4917). Our data suggest that, in humans, the mechanism of HAAF involves similar increased activity in the medial thalamic nuclei (including the PVNT), induced by antecedent hypoglycemia, that inhibits the hypothalamic glucose sensor initiated sympathoadrenal response to hypoglycemia.

13-LB

Glucose Fluctuations and Activation of Oxidative Stress in Type 1 Diabetes Patients

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Background and aims: Hyperglycemia is a major risk factor for microvascular complications and accelerated atherosclerosis in diabetes, through hyperglycemia-induced oxidative stress. Also short-term glucose fluctuations showed a strong correlation with oxidative stress in 21 type 2 diabetes patients (Monnier, JAMA 2006) and may be valuable to predict diabetic complications. We evaluated the relation between glucose variability and oxidative stress in type 1 diabetes patients.

Material and Methods: Continuous glucose monitors (CGMS gold) were inserted subcutaneously in 25 patients for 48 h. During the measurement, patients collected 2 subsequent 24-h urine samples, while 24 age and sex-matched healthy controls collected 1 24-h urine sample for determination of 15(S)-8-*iso*-$PGF_2\alpha$ using HPLC MS/MS (Mass Spectroscopy). Mean Of the Daily Differences (MODD), Mean Amplitude of Glycemic Excursions (MAGE) and Continuous Overlapping Net Glycemic Action (CONGA) over 1 h were calculated as markers for inter-, intra-day and rapid glucose variability, and correlation with 15(S)-8-*iso*-$PGF_2\alpha$ excretion was calculated.

Results: Median (IQR) urinary excretion of 15(S)-8-*iso*-PGF2α was higher in patients than in healthy controls: 159 (137 - 216) pg/mg of creatinine versus 118 (101 - 146) pg/mg of creatinine (P = 0.001). Median (IQR) MODD was 3.7 (3.2 - 5.0) mmol/L, MAGE 7.6 (6.4 - 9.0) mmol/L and CONGA-1 2.3 (2.1 - 2.8) mmol/L. Univariate regression did not reveal an association for inter-day (r^2 = 0.01), intra-day (0.08) and rapid glucose variability (0.07) with 15(S)-8-*iso*-PGF2α excretion, neither did analysis when corrected for HbA1c, age, gender and smoking. Correlations between 15(S)-8-*iso*-PGF2α excretion and MODD, MAGE and CONGA-1 were -0.148, -0.399 and -0.207, all non-significant.

Conclusion: We report no relationship between inter-, intra-day and rapid glucose variability and urinary 15(S)-8-*iso*-PGF2α, determined with highly specific HPLC MS/MS and confirm that type 1 diabetes patients have higher levels of urinary 15(S)-8-*iso*-PGF2α than healthy controls, suggesting the existence of an oxidative stress favouring factor other than glucose variability. We did not see a relation between high glucose variability and elevated levels of oxidative stress in type 1 diabetes patients.

14-LB

VEGF Protein Expression in the Retina of Diabetic Rats is Rapidly Upregulated Despite Decreased VEGF mRNA Expression

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Diabetic retinopathy is the leading cause of blindness among working-age adults, appearing in the majority of individuals who have had diabetes for twenty or more years. Among the causes implicated in the development of diabetic retinopathy both abnormal neovascularization and increased vascular permeability have been linked to enhanced expression of the angiogenic factor VEGF. The present study examined the hypothesis that diabetes leads to signaling pathway changes resulting in enhanced translation of VEGF mRNA. Retinas from streptozotocin-induced diabetic rats were isolated at 1, 2, 4, 6, and 12 weeks after treatment and were used to explore translational control mechanisms leading to increased VEGF protein expression. VEGF mRNA expression decreased through 12 weeks of diabetes. In contrast, VEGF protein expression was upregulated over the controls, as assayed by Western analysis, as early as 2 weeks following the induction of diabetes. Complementing the change in VEGF protein expression was increased expression of 4E-BP1, the eukaryotic initiation factor (eIF)4E binding protein 1, a negative regulator of m7-GTP cap-dependent mRNA translation. Relative phosphorylation on the inhibitory T37/46 site of 4E-BP1 was unchanged. 4E-BP1 mRNA remained constant, as did the message levels of it's transcription factor, FoxO1 and the FoxO targets, Gadd45 and angiopoietin2. eIF4E immunoprecipitation in 4-week animals revealed significantly more 4E-BP1 bound to eIF4E in the diabetic as compared to the control retina. The increased association of 4E-BP1 with eIF4E would be expected to repress global rates of protein synthesis due to decreased cap-dependent translation, while permitting cap-independent translation to proceed unimpaired. Studies are in progress to determine the mechanism of 4E-BP1 protein upregulation. Overall, the data indicate that diabetes-induced signaling, resulting in elevated 4E-BP1 expression and increased binding of 4E-BP1 to eIF4E, may act to repress global mRNA translation while selectively enhancing the translation of a subset of mRNAs including the mRNA encoding VEGF.

Diabetes Education

15-LB

Improving Quality of Care for Diabetic Patients

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Objective: To redesign the systems of diabetes care in the Yale New Haven Hospital Adult Primary Care Center (PCC) in order to improve control of patients' hemoglobin A1c, lipids, blood pressure, and to ensure appropriate preventive management of complications associated with diabetes. Diabetes was selected as the target condition because prior evidence has suggested large gaps between evidence based care processes and actual process for vulnerable patients with diabetes.

Methods: Our team included representation from podiatry, pharmacy, nursing, nutrition, and medical staff. This comprehensive initiative involved 3 distinct and complementary domains: 1) *medical informatics*, which used an electronic medical record to drive evidence-based practice using decision support tools and clinical prompts, and to capture real-time clinical data for provider feedback and monitoring; 2) enhancing *clinical services* by providing access to a diabetes educator/nurse practitioner and establishing a multi-disciplinary diabetes clinic which allowed diabetic patients to see multiple practitioners in a single visit; and 3) *provider feedback/education* including academic detailing, a process improvement (PI) curriculum, and a physician champion.

Results: All measures improved substantially. Hemoglobin A1c (HBA1c) testing rates improved from 94% in 2002 to 99% in 2005. Glucose control improved substantially: patients with HbA1c levels ≤ 8.0 increased from 57% in 2002 to 72% in 2005 ($p<0.05$). Control of blood pressure (≤ 140/90) improved, increasing from 48% in 2002 to 76% of patients in 2005 ($p<0.001$). Urine protein testing showed marked improvement, rising from 44% in 2002 to 72% in 2005 ($p<0.001$). Adequate lipid control (LDL < 100 g/dl) improved from 41%

in 2002 to about 68% in 2005 (p<0.001).
Discussion: Overall diabetic care received by PCC patients improved markedly and now exceeds national benchmarks. This improvement occurred using a combination of interventions (specific goals, administrative support, clinical support, and use of data) that have been demonstrated to be a critical part of successful (PI) initiatives. Outpatient quality of care efforts can be feasible, sustainable, and implemented with minimal incremental cost. The electronic medical record can be leveraged to improve clinical care and to simplify data collection. Key components of our success included a physician champion, an interdisciplinary team, iterative tailoring of the electronic medical record system, and the ability to adapt clinical services to provide additional care for our most vulnerable patients.

16-LB

Effectiveness of Diabetes (DM) Self Management Education (DSME) at an Urban Public Library

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In the US, < 40% of persons with DM receive DSME. DSME is associated with lower rates of DM complications which disproportionately affect African Americans (AA). Interventions that target improved DM-related outcomes are particularly important in the District of Columbia where about 60% of residents are AA and over 45,000 persons have DM. We examined whether a concise, modular DSME program can be used as an effective education tool to impact outcomes and feasibility of using public library resources to offer the program.
Methods: A free, community-based DM Learning Center was placed in a central public library (partnership between a health system, local hospital, public library system and DC Dept. of Health). Target audience was AA residents with DM. DSME program (two 2-1/2 hour classes), support group; and diabetes resource center were offered. Management targets, focusing on CVD risk reduction (A1C, BP & LDL cholesterol) and empowerment to communicate with primary-care physician were taught to enable attainment of clinical outcomes. McNemar's and paired t-test were used for comparison analysis.
Results: N= 354 subjects. Knowledge of ADA recommended targets for A1C (7%), blood pressure (BP<130/80mmHg) and cholesterol (LDL < 100mg/dl) increased from baseline to follow-up at 6 mos post-intervention respectively as follows: A1C 37.13% to 67.65% correct (p=<0.001); BP 5.88% to 18.75% (p<0.001); and LDL 16.91% to 40.44% (p=<0.001). Clinical outcomes were also impacted positively. Self-reported Emergency Room visits for hyper- and hypoglycemia significantly decreased from 8.05% to 2.68% (p=.0043); a significant means change in A1C (-0.55%) from 7.99% at baseline to 7.44% follow-up (p=0.001) was observed. Logistic regression model showed that participants who started on or remained taking insulin were 2.27 times more likely to decrease or maintain A1C level to below 7% compared to those who did not take insulin or stopped taking insulin during the study period. Conclusions: Offering DSME at an urban public library was both feasible and had significant clinical impact.

17-LB

Adolescent Females' Mental Models Of Diabetes and Reproductive Health

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Preconception counseling aims to increase women's chances of having a healthy pregnancy and a healthy baby, by teaching them how to achieve and maintain tight glucose control, and to postpone pregnancy until it is safe and wanted. The American Diabetes Association recommends preconception counseling for all women with diabetes with child-bearing potential, thus including adolescent females. Effective preconception counseling targets recipients' informational needs. This study aimed to examine adolescent females' "mental models" of diabetes and reproductive health. We interviewed 29 teenaged girls recruited from the diabetes clinic at a large university-based medical center in Detroit, MI. Participants were aged 13-18 (mean=15.1, SD=1.5); 73% were African-American; half had type 1 and half had type 2 diabetes. Participants were asked to share their knowledge on a variety of topics, identified as relevant by our panel of diabetes experts. Our results suggest a substantial lack of appreciation of the risks of pregnancy to women with diabetes, and a near complete lack of awareness of preconception counseling. Not a single participant had heard the term before. Most did not know where to get information about diabetes and planning a pregnancy, with just 20% saying that their doctor or nurse would be a good source of such information. However, about half of participants believed that doctors or nurses would tell their parents if they asked about pregnancy. We had been concerned that young women might be overly fearful about their pregnancy risks, but this did not seem to be the case. All participants believed that women with diabetes could have healthy babies, and many were unaware of the risks that they faced. Only half of participants made any reference to complications during pregnancy; only 20% appeared to know that elevated blood sugars can affect a fetus. When asked to speculate about when such effects might happen, fewer than a third of participants guessed that anything could happen as early as the beginning of pregnancy. These results suggest a need for preconception counseling for adolescents with diabetes.

Epidemiology

18-LB

Dietary Intake Trends Among U.S. Adults with Diabetes: 1988-2004

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The quantity and quality of the diet is considered a crucial aspect of the control and management of diabetes, but few data exist on trends in dietary intake of U.S. adults with diabetes. We examined secular trends in dietary intake among a nationally representative sample of adults aged 20-74 years using the National Health and Nutrition Examination Survey (NHANES) III (Phase I/II: 1988-1990/1991-1994), 1999-2000, 2001-2002, and 2003-2004. Multiple linear regression was used to compute predictive marginals of total calories/day by time period and self-reported diabetes, controlled for age, sex, and race/ethnicity. Among people with diabetes, we also stratified analyses by body mass index (BMI; <25 kg/m^2 normal weight; 25-29.9 kg/m^2 overweight; $\geq$30 kg/m^2 obese). Linear tests for trend were done using Joinpoint regression analysis.
From 1988 - 2004, total caloric consumption increased among people with diabetes (1979-2192 kcals; p=0.02), and non-significantly among people without diabetes (2181-2319; p=0.07). People with diagnosed diabetes consumed fewer total calories than people without diagnosed diabetes, possibly indicating attempts to make nutrition related lifestyle modifications.
Among people with diabetes, increases in total calorie consumption were observed in overweight (1950-2118; p=0.02) and obese categories (1986-2274; p=0.2), but not in the normal weight category (2012-1925; p=0.9). Increases among women with diabetes were significant (1677-1927; p=0.02) as were increases among women without diabetes (1770-1908; p=0.002). The increase for men with diabetes approached significance (2296-2481; p=0.07). There were no significant differences among people with diabetes across age categories (20-44, 45-64, 64-74). Among people without diabetes, there was an increase in total calorie consumption among those aged 45-64 (2126-2282; p=0.03).
Our data indicate that total calorie consumption has increased over time among people with diabetes, especially among overweight and obese people with diabetes. These results have major public health implications for targeting calorie reductions for those at highest risk.

19-LB

Quantification Of Serum Protein Biomarkers Can Identify Early Converters To Type 2 Diabetes

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Botnia is a large, prospective study that has been used to successfully discover genes which predispose subjects to T2DM. We identified a panel of serum biomarkers which could predict the development of T2DM within 5 years in this cohort**.** An ultrasensitive protein detection method was used to quantify 42 proteins (~1.3 µL/ assay) in 444

samples. The case/control study tested baseline serum from 148 subjects who developed T2DM during follow-up and matched 1:2 for BMI, family history, age, gender, and glucose tolerance status with non-converters. Baseline values were combined with protein results to test marker search algorithms and predictive statistical models. In this analysis subjects who converted to T2DM within 5 years were compared to non-converters with at least 8 years of follow-up. Seven of the 48 biomarkers provided most of the statistical distinction between the groups. As expected based on initial matching, BMI, family history, age and glucose were not significant. The best models generated from repeated 10-fold cross-validations involved only protein markers (c statistic = 0.73-0.78). Although individual proteins had p-values ≤ 0.0001, no single protein could distinguish the groups as well as models constructed from multiple proteins. Existing risk indices based upon age, gender, family history, SBP, HDL and FGT were less useful for segmenting the groups (c statistic = 0.58-0.63). The seven protein biomarkers in this panel are involved in glucose and lipid metabolism, inflammation, and coagulation and complement cascades. In this study a panel of biomarkers provides a tool for identifying individuals at risk of developing T2DM with a sensitivity and specificity of approximately 80%.

20-LB

Development And Testing Of A Novel Platform For Measuring Multiple Biomarkers In Serum Samples Demonstrated In 5 Yr Prospective Study Of Progression To Type 2 Diabetes (Inter99)

JANICE KOLBERG, ROBERT GERWIEN, EDDIE MOLER, MICHAEL P. MCKENNA, MICKEY URDEA, OLUF PEDERSEN, TORBEN HANSEN, TORBEN JORGENSEN, KNUT BORCH-JOHNSEN, *Emeryville, CA, Copenhagen, Denmark*

From both public health and clinical practice perspectives, identification of a subset of serum biomarkers that could precisely and accurately predict future development of type 2 diabetes (T2DM) would represent a major medical advance. The aim of the present study was to develop and test an ultra-sensitive protein detection method to enable this discovery. Molecular Counting Technology (MCT), which on average uses 1.3 µl serum/ assay, was applied to case-cohort samples in the population-based Inter99 study of 6,784 middle-aged Danish whites. All 200 individuals who progressed to T2DM (converters) during the 5-yr. study period were compared with 472 randomly selected non-converters. 18 serum protein biomarkers were measured for each subject. Univariate and multivariate statistical analyses were conducted employing quantification of protein biomarkers and clinical annotations including age, BMI, waist, systolic blood pressure, and diastolic blood pressure. Additional laboratory results included fasting glucose, insulin, total, HDL and LDL and triglycerides.

MCT assays had dynamic ranges of 10^{2} to 10^{3}, intra-plate CVs of 5%, and sensitivities ranging from sub-picogram to ng/mL. Univariate analysis of converters vs. non-converters revealed that 6 serum biomarkers had p- values less than or equal to 0.0003, whereas 4 clinical variables had p-values between 0.001 and 0.0001.

In this study, serum biomarkers were measured reliably. Proteins significantly associated with conversion to diabetes are known to be involved in carbohydrate and lipid metabolism, inflammation, coagulation and complement cascades. The results suggest that serum protein biomarker panels provide information over and above known risk markers and will be useful tools for prediction of conversion to diabetes type 2 in middle-aged subjects in the general population.

21-LB

Hemoglobin A1c Values Have Remained Stable Since 2004: Evidence from Analysis of a Large Reference Laboratory Data Base

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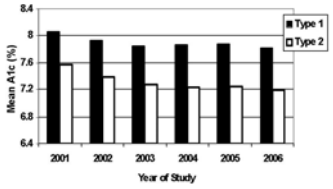
From 1/01 to 12/06 Quest Diagnostics, a large national reference laboratory, performed 44.4 million (M) A1c analyses using the Roche Diagnostics COBAS INTEGRA system with standardization to the NGSP. Subjects were identified as having type 1 or 2 diabetes (DM) based on ICD9 codes and were considered to have a specific type of DM only if all samples for that subject had an appropriate ICD9 code. This yielded 0.13M subjects being classified as type 1 DM and 2.13M as type 2 and a total of 15.3M A1c analyses. For inclusion in the longitudinal analysis the subject had to have at least two A1c measurements.

Mean A1c values decreased in both DM types. The decline was significantly greater from 2001 to 2003 than from 2004 to 2006 (Figure). Longitudinal analysis showed an average absolute decrease in A1c of 0.13% in the year following entrance into the database but a gradual increase thereafter. No gender differences were observed in patients with type 1 DM but the average absolute A1c values were 0.33% higher in men with type 2 DM aged 20-39 ($p<0.0001$) compared with women. A1c values decreased after age 60 in both groups.

Our analysis indicates that mean A1c values for patients with DM declined from 2001 to 2006 but the rate has slowed significantly since 2003. Longitudinal analysis also indicates that A1c values have remained steady. Since 45% of the A1c values remain above the ADA target of 7%, new strategies are necessary for these patients to meet this goal. Strategies targeting men age 20-39 with type 2 DM may be particularly beneficial.



22-LB

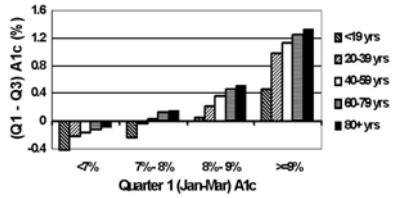
Factors Affecting the Seasonal Pattern in Hemoglobin A1c Values: Analyses of Data from a Large Reference Laboratory Database

XIAOHUA HUANG, SAM REICHBERG, HARVEY KAUFMAN, MARSHA GREEN, EILEEN KOSKI, FRANCINE KAUFMAN, RICHARD W. FURLANETTO, *Lyndhurst, NJ, Bronx, NY, Los Angeles, CA, Chantilly, VA*

We have used data generated at Quest Diagnostics, a large national reference laboratory, to examine factors affecting the seasonal pattern in hemoglobin A1c (A1c) values. From 1/01 to 12/06 approximately 44.4 million (M) A1c measurements were performed at Quest Diagnostics using the Roche COBAS INTEGRA system with standardization to the National Glycohemoglobin Standardization Program. From this group subjects having type 1 or 2 diabetes (DM) were identified based on ICD9 codes. Subjects were considered to have a specific type of DM only if all samples for that subject had an appropriate ICD9 code. This restriction yielded 21.9M A1c analyses on 4.8M individual subjects.

Temporal analysis of the mean A1c levels in this cohort indicated a seasonal variation in A1c values of about 0.3% (i.e. 0.3g/100g Hgb), with the highest levels being observed in Jan-March (Q1) and the lowest in July-Sept (Q3). Of the 4.8 M subjects, 1.36M had paired Q1-Q3 A1c analyses; in these subjects the magnitude of the seasonal variation correlated strongly with both the subject's age and the Q1 A1c value (Figure). Similar seasonal patterns were observed in those with type 1 and 2 DM but the variation was smaller in the type 1 subjects at all A1c levels. Men and women showed similar patterns but the variation was slightly smaller in the women, primarily the result of lower winter peak values (7.43% vs 7.49%).

In summary, A1c values show significant seasonal fluctuations, the magnitude of which is dependent on age, diabetes type and winter A1c value. This seasonal effect should be considered when interpreting serial changes in A1c values for individual patients and for interpreting studies aimed at assessing the quality of DM care or effectiveness of therapies. The effect of seasonal variations can be minimized by obtaining serial A1c measurements in late spring and autumn.



Exercise

23-LB

Skeletal Muscle Deoxygenation and Microvascular Blood Flow Kinetics during Exercise in Type 2 Diabetes

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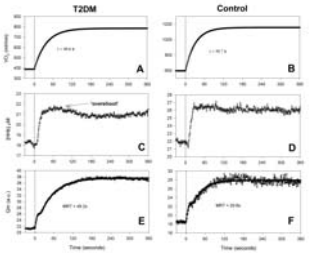
People with type 2 diabetes (T2DM) have impaired maximal and submaximal exercise tolerance even in the absence of cardiovascular complications. One key feature of the submaximal exercise abnormalities of T2DM is abnormally slowed oxygen uptake (VO_2) kinetics at the onset of constant load exercise that may lead to an increased oxygen deficit and premature fatigue. The mechanisms of this delayed adaptation during exercise are unclear but likely relate to impairments in skeletal muscle blood flow or metabolic abnormalities. We hypothesized that people with T2DM would have altered skeletal muscle oxygen extraction responses and concordantly slowed microvascular blood flow (Qm) kinetics compared with healthy subjects following the onset of moderate constant work rate exercise. Skeletal muscle deoxygenated hemoglobin/myoglobin [HHb] concentration changes (determined using near infrared spectroscopy) and pulmonary VO_2 kinetics were assessed in 11 T2DM and 11 healthy subjects. Subjects performed two bouts of cycling exercise at 85% of individually calculated lactate threshold. Qm responses were estimated from the VO_2 kinetics and measured [HHb] responses via rearrangement of the Fick principle. Following the onset of exercise, the time constant of VO_2 kinetics was slowed in T2DM compared with controls (panels A & B, 43.8±9.6s vs. 34.2±8.2s, P<0.05), and the [HHb] temporal profile of T2DM subjects was altered demonstrating an 'overshoot' of oxygen extraction in T2DM muscle (panel C). The mean response time of estimated Qm increase was significantly prolonged in T2DM compared with healthy subjects (panels E & F, 47.7±14.3s vs. 35.8±10.7s, P<0.05). We conclude that at the onset of moderate exercise, T2DM skeletal muscle demonstrates a transient imbalance of muscle O_2 delivery relative to O_2 uptake suggesting the adaptation of muscle microvascular blood flow during exercise is slowed compared with healthy subjects. Impaired muscle oxygen delivery during exercise secondary to microvascular abnormalities and/or altered vascular control are likely major contributors to the abnormal exercise responses and exercise intolerance observed in T2DM.



24-LB

Contraction-Stimulated Phosphorylation of AS160 is Temporally Coupled with Phosphorylation of CaMKII, but not AMPK or Akt

KATSUHIKO FUNAI, GREGORY D. CARTEE, *Ann Arbor, MI*

Akt substrate of 160kD (AS160), the most distal insulin signaling protein known to be important for insulin-stimulated glucose transport (GT), also becomes phosphorylated in response to contractile activity by skeletal muscle. AMP-activated protein kinase (AMPK) and Akt are contraction-stimulated kinases that can phosphorylate AS160. Ca2+/calmodulin-dependent kinase (CaMK)-II has been implicated as a trigger for contraction-stimulated GT, but its role in regulating phosphorylation of AS160 is unknown. The primary purpose of this study was to identify the temporal relationship among GT ([3H]-3-O-methylglucose), phosphorylation of AS160 (pAS160) and phosphorylation of contraction-stimulated kinases (pAMPK, pAkt, pCaMKII and their respective substrates: pACC, pGSK3, pSRF). Rat epitrochlearis muscles were studied without (resting controls) or with twitch electrical stimulation (2ms twitch, 2Hz for 5, 10, 20, 40 or 60min). In vitro twitch contraction significantly increased pAMPK (3.3-fold), pCaMKII (2.1-fold), pAS160 (2.4-fold) and GT (2.9-fold) at 20min of contraction. At 60min of contraction, pAS160 and pCaMKII returned to baseline although pAMPK and GT remained elevated. Pearson correlation analysis revealed that pCaMKII and pAS160 were significantly correlated (r=0.724, p<0.0001). Contraction effects on pACC and pSRF were similar to their respective kinases. pAkt was not significantly affected, but pGSK3 was increased at 10min. The temporal dissociation of pAS160 from pAMPK and pAkt suggests there may be an alternative AS160 kinase, and the close, temporal relationship between pCaMKII and pAS160 provides suggestive evidence that CaMKII may influence pAS160. A sustained increase in pAS160 was not essential for the maintenance of contraction-stimulated GT, although it is possible that transiently elevated pAS160 was involved in triggering the initial increase in GT. These results, which are consistent with previous data indicating that the contraction-stimulation of pAS160 and GT can be uncoupled, further elucidate the complex relationship between pAS160 and contraction-stimulated GT.

Insulin Action

25-LB

RalA Facilitates Glut4 Trafficking to the Plasma Membrane via the Exocyst and the Molecular Motor Myo1c

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Insulin stimulates glucose transport in muscle and adipose tissue by producing translocation of the glucose transporter Glut4. The exocyst, an evolutionarily conserved vesicle tethering complex, is crucial for targeting Glut4 to the plasma membrane. Here we report that insulin regulates the final steps in the assembly of the exocyst via the G protein RalA, which is present in Glut4 vesicles in adipocytes. Insulin stimulates the activity of the G protein in a PI 3-kinase-dependent manner. Upon activation, RalA interacts with the exocyst components Exo84 and Sec5, producing the unification of the complex. Disruption of RalA function by expression of dominant negative mutants or siRNA-mediated knockdown attenuates insulin-stimulated glucose transport, as does depletion of Sec5 or Exo84. RalA also interacts with Myo1c, a molecular motor previously implicated in Glut4 trafficking. This interaction is not influenced by the activity state of RalA, but is modulated by calmodulin, which functions as the light chain for Myo1c in adipocytes, and is also required for insulin-stimulated glucose uptake. Thus, RalA serves two functions in insulin-stimulated Glut4 targeting: as a cargo receptor for the molecular motor Myo1c, and as a signal for the unification of the exocyst complex to target Glut4 vesicles to the plasma membrane.

26-LB

A Common Trafficking Route for GLUT4 in Cardiomyocytes Following Insulin and Energy-Status Signalling

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Analysis of GLUT4 localisation and trafficking in heart muscle cells has been achieved by using a transgenic mouse line which expresses GLUT4, with both a C-terminal GFP tag and an exofacial HA tag (HA-GLUT4-GFP), under the control of a muscle specific promoter. Cardiomyocytes were isolated by Langendorf perfusion and treated with insulin or oxidative metabolism stress, or stimulated to contract, and the localisation of HA-GLUT4-GFP analysed by immunofluorescence. The exofacial HA tag enabled fluorescent labelling of only GLUT4 that was exposed at the external surface. The results show that HA-GLUT4-GFP is totally excluded from the sarcolemma under basal conditions, and is trafficked to the sarcolemma when treated with 30 nM insulin, stimulated to contract, and upon induction of metabolic stress when incubated with 5 µM oligomycin, 5 µg/mL berberine chloride, or in hypoxic buffer. The exofacial tag was only apparent at the sarcolemma and not at the t-tubule membrane. To monitor the internalization process, GLUT4 was pulse-labelled with anti-HA antibody after treating the cells under different conditions (10 nM insulin, contraction, hypoxia), followed by washing and incubation for a further 40 min. Anti-HA antibody-labelled GLUT4 was internalized, by a clathrin-mediated route, from the sarcolemma to predominantly a peri-nuclear compartment that was

indistinguishable among the different stimuli. This implies a common pathway for internalisation independent of the initial stimulus. Moreover, since the internalized reservoir compartment is common, the data suggest that different stimuli which invoke GLUT4 translocation to the sarcolemma of cardiomyocytes may recruit it from the same intracellular pool of GLUT4.

27-LB

Cdk5 Negatively Regulates Glut4 Translocation Through TC10 Phosphorylation

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Differentiated 3T3L1 adipocytes were found to express cyclin-dependent kinase-5 (CDK5) and its regulatory subunit p35 localized to lipid raft domains. The Rho family GTP binding protein TC10 alpha was also lipid raft localized and underwent a transient insulin-stimulated CDK5-dependent phosphorylation on threonine 189 that was olomoucine sensitive. Expression of a phosphorylation mimetic mutant (T189D-TC10) was also lipid raft localized whereas a phosphorylation deficient mutant T189A-TC10 was not. Moreover, over expression of wild type (WT-TC10) and the T189D-TC10 mutant resulted in depolymerization of cortical F-actin whereas the T189A-TC10 mutant was without effect. Consistent with these results, WT-TC10 and T189D-TC10, but not T189A-TC10, inhibited insulin-stimulated GLUT4 translocation. Taken together, these data demonstrate that CDK5 phosphorylates T189 of TC10 alpha and negatively regulates insulin-stimulated GLUT4 translocation through cortical F-actin rearrangement.

28-LB

Transcriptional Repression of Glucokinase by Orphan Nuclear Receptor SHP

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The orphan nuclear receptor SHP plays important roles in lipid metabolism. Our recent studies observed markedly improved liver insulin sensitivity and islet insulin secretion in *SHP*-deficient mice. This phenotype was accompanied by the increased mRNA levels for both the hepatic glucokinase (LGK) and the pancreatic glucokinase (BGK) in the *SHP*-null mice. We hypothesize that SHP directly represses glucokinase gene expression in the liver and islet, and that upregulation of the glucokinase gene by the loss of SHP repression explains the improved insulin secretion and sensitivity in *SHP*-null mice. We generated mice with hepatocyte and beta-cell specific overexpression of SHP (designated Alb-SHP and Rip-SHP, respectively). The mRNA levels of LGK or BGK were markedly down-regulated in Alb-SHP or Rip-SHP mice, demonstrating the transcriptional repression of LGK and BGK by SHP. To determine the molecular basis for SHP regulation of glucose homeostasis through the glucokinase pathway, both the LGK and BGK luciferase reporters were constructed. Transient transfection showed that SHP inhibited LGK and BGK transactivation by the transcription factor HNF4a in a dose-dependent manner. ChIP assay demonstrated that SHP and HNF4a were co-immuno precipitated on LGK or BGK promoter. Our studies identified both LGK and BGK as direct SHP target genes. Future studies are required to elucidate the changes of glucokinase signaling pathway in the Alb-SHP livers and Rip-SHP islets and the association of SHP with GK activities that affect liver and islet glucose homeostasis.

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29-LB

Hepatic Foxo1 Critically Mediates Insulin Regulated Glucose Homeostasis

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Forkhead transcription factor Foxo1 plays an important role in multiple cellular functions including metabolism. To elucidate the molecular mechanism in the insulin regulated Foxo1 functions, we generated liver-specific single Foxo1 (Foxo1-LKO) and Irs1, Irs2, and Foxo1 triple knockout mice (LTKO). In contrast to previous reports, we observed that either Irs1 or Irs2 could mediate insulin signaling to phosphorylate Foxo1 at Thr24 and Ser256 residues. Hepatic deficiency of Irs1 and Irs2 (LDKO) caused early onset diabetes in mice by 4-6 weeks of age. The LDKO mice also manifested severe glucose and insulin intolerance. In addition, gluconeogenic genes like PGC-1α and G6pc in the LDKO liver were not responsive to feeding. Ablation of Foxo1 in the hepatocytes of LDKO mice normalized both fed and fasting blood glucose and strikingly improved glucose tolerance, although Foxo1-LKO mice did not display significant alteration in blood glucose levels under fasting or fed conditions. Fasting serum insulin levels in the LTKO mice were comparable to those in the control floxed mice and the fed serum insulin levels were intermediate between control and LDKO mice. Expression of PGC-1α, Pck1, and G6pc was completely normalized in the LTKO liver. Interestingly, glucokinase expression was significantly increased in the liver of LTKO mice compared to that in the LDKO mice under both fasting and refeeding conditions and its expression was also responsive to feeding. The improvement in glucose metabolism and tolerance in the LTKO mice was not attributable to any change in hepatic insulin signaling because this remained defective. In contrast to restored glucose tolerance, insulin tolerance was not significantly improved in the LTKO mice. In conclusion, our data suggest that both Irs1 and Irs2 can regulate Foxo1, and hepatic Foxo1 is critically involved in the regulation of glucose homeostasis.

Integrated Physiology

30-LB

Rapid and Weight-Independent Improvement of Glucose Tolerance by a Peptide that Induces Apoptosis in Endothelium of White Adipose Tissue

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When the cell-surface molecule prohibitin found on endothelial cells in white adipose tissue is bound by a specifically engineered peptide, apoptosis of the critical supporting vasculature of the white adipose tissue occurs. We and others have found that one consequence is dramatic weight loss resulting from reduced food intake and no associated signs of illness. A key question is whether compromising adipose tissue function in this way has a beneficial or a detrimental effect on glucose homeostasis. In mice maintained on a high-fat diet (HFD), insulin levels were lower in the propapoptic peptide (PP)-treated mice as compared to mice treated with a vehicle (CP). However, because PP-treated mice lost significant weight, it was unclear whether the effect was secondary to weight loss. To address this more directly, PP-treated HFD mice, CP-treated HFD mice, HFD mice pair-fed (PF) to the reduced caloric intake of PP-treated mice, and a group of mice maintained on a low-fat diet (LFD), were assessed for glucose tolerance (1.5 mg/g; Day 3) and insulin tolerance (1 mU/g; Day 10). By Day 3, PP mice had gained significantly less body weight than CP mice, and there was no significant difference between PP and PF mice. Cumulative energy intake of PP mice was lower than that of CP mice by Day 2. HFD CP mice were significantly glucose intolerant relative to LFD controls and to PP mice, and glucose tolerance of PF mice was comparable to that of CP mice and worse ($P<0.1$) than that of PP mice. LFD mice were significantly more sensitive to the ability of insulin to reduce plasma glucose than CP mice. PP and PF mice had improved insulin sensitivity ($P<0.1$) relative to CP mice. These data indicate that selectively suppressing white-fat vasculature produces rapid and weight-independent improvement of glucose tolerance.

31-LB

Orphan Nuclear Receptor COUP-TFII is a Novel Suppressor of Adipogenesis

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Adipogenesis is a precise process controlled coordinately by many transcription factors. We have sought to identify novel transcriptional pathways regulating adipogenesis using a variety of experimental and computational methods. One factor that emerged from this analysis is chicken ovalbumin upstream promoter-transcription factor II (COUP-

TFII), an orphan member of the nuclear receptor superfamily known to exert effects in the development of several non-adipose tissues. We show that COUP-TFII is present in mouse adipose tissues and that COUP-TFII levels decline during differentiation. Over-expression of COUP-TFII in 3T3-L1 pre-adipocytes deprives these cells of the ability to differentiate into mature adipocytes. Conversely, shRNA-mediated reduction of COUP-TFII in 3T3-L1 pre-adipocytes potently promotes adipogenesis, as shown by increased lipid accumulation and elevated expression of fat-cell marker proteins. In addition, uncommitted NIH-3T3 fibroblasts can be induced to differentiate into fat cells if COUP-TFII levels are reduced. COUP-TFII represses the expression of a number of pro-adipogenic factors such as EBF, KLF and C/EBP family members in adipocytes, and in vitro reporter assays and chromatin immunoprecipitation analysis suggests that COUP-TFII is associated with and represses the C/EBPα promoter. Hedgehog and GATA signaling have been shown to participate in an anti-adipogenic pathway; we show that COUP-TFII is required for these factors to exert their full effects on differentiation. Moreover, COUP-TFII and GATA2 are physically associated with each other and repress target gene expression in an additive manner. Consistent with our in vitro results, COUP-TFII mRNA levels are decreased in white adipocytes of ob/ob mice, a well-established model of obesity. Taken together, our data demonstrate that COUP-TFII represents a novel, endogenous suppressor of adipogenesis with physical and functional links to key components of the adipogenic transcriptional cascade.

32-LB

Ccdc80, a Novel Adipokine Involved in Metabolic Regulation

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Metabolic crosstalk between adipocytes and peripheral tissues has been proposed to be instrumental for the development of obesity-linked insulin resistance. Establishment of the adipose tissue not only as a fat storage site, but also as an endocrine organ prompted us to identify new adipocyte-secreted proteins potentially involved in metabolic regulation. Using transcriptional profiling, we searched for novel genes 1) expressed in white adipose tissue, 2) encoding putative secreted proteins and 3) regulated under various metabolic paradigms. We identified coiled-coil domain containing 80 (Ccdc80) as a novel mouse adipocyte-specific gene. Proteomic and immunoblot analysis of conditioned medium from transiently-transfected HEK293T or adenovirus-transduced HepG2 cells confirmed that Ccdc80 is a secreted protein. Ccdc80 gene expression was decreased during fasting in C57BL/6 mice. In addition, Ccdc80 mRNA levels were significantly reduced in *ob/ob* mice and restored upon treatment with the PPARγ agonist rosiglitazone. Temporal analysis of Ccdc80 gene expression in 3T3-L1 cells revealed a biphasic pattern with low mRNA levels during proliferation and clonal expansion, and increased expression in growth-arrested fibroblasts and fully differentiated adipocytes. Retrovirus-mediated silencing of Ccdc80 by RNAi compromised the ability of 3T3-L1 cells to differentiate into mature adipocytes and accumulate lipids. Impaired adipogenesis in Ccdc80-knockdown cells was accompanied by reduced expression of C/EBPα, PPARγ and aP2, and increased expression of Pref-1, a preadipocyte marker. In summary, Ccdc80 was identified as a novel adipokine whose expression suggests a role in metabolic regulation. We further found that Ccdc80 is a novel regulator of adipocyte differentiation.

33-LB

Ncb5or is Critical for White Adipose Tissue Maintenance and is Involved in Delta 6 Fatty Acid Desaturation

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The novel reductase Ncb5or (b5+b5R, Cyb5r4) is critical for β-cell survival. Ncb5or-null mice are viable and initially have normal blood glucose levels. However, at around 4 weeks of age these mice begin to lose β-cells from the pancreatic islets. At 6 weeks of age, these mice are hyperglycemic owing to a decrease in serum insulin. Insulin tolerance in these animals is normal. Ncb5or-null animals lose weight as they age and demonstrate classic signs of frank diabetes. Transplant of healthy islets into the kidney of Ncb5or-null animals effectively corrects the diabetes. Interestingly, these animals continue to lose weight, predominantly in the form of white adipose tissue. Gonadal fat pad was slightly reduced in Ncb5or-null animals at 3-6 weeks, with the difference becoming more pronounced after 6wks. The decreased mass appears to result from a loss of lipid in adipose cells. Compositional analysis of fatty acid species in liver, including the chains making up each lipid class, indicated impaired desaturation of 16:1(n-7) and 18:1(n-9) fatty acids, suggesting a potential role for Ncb5or in delta-6-desaturase catalyzed fatty acid desaturation.

34-LB

Acute Administration of a Sulfonylurea Compound Restores the Insulinotropic Effect of Glucose-Dependent Insulinotropic Polypeptide in Patients with Type 2 Diabetes

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Mutations in the kir 6.2 gene, encoding the pore-forming unit of the ATP-sensitive potassium channels (KATP-channels) of the beta-cell, are part of the genetic polymorphism seen in type 2 diabetes. Defects in the function of the kir 6.2 subunit in mice are known to inhibit the insulinotropic effect of glucose-dependent insulinotropic polypeptide (GIP).

Our aim with this study was to evaluate a possible role of the KATP-channels in the lost insulinotropic effect of GIP. We hypothesized that closing the KATP-channels through acute administration of sulfonylurea (SU) would re-establish the insulinotropic effect of GIP. Twelve patients (8 men) with type 2 diabetes (Age: 54 [45-70] years; BMI: 30 [26-35] kg/m^2; HbA_{1c}: 7.7 [7-8.6] %; values mean [range]) on mono-therapy with metformin (1 diet) were studied over the course of 4 days. Patients were examined using hyperglycaemic *clamps* (15 mM, 2 hours) with concomitant infusion of GIP (4 pmol/kg body weight/min), GIP + administration of 10 mg SU (glipizide) as tablets 1 hour before the clamp, saline + administration of 10 mg SU as tablets 1 hour before the clamp or saline alone. During the examinations blood was sampled at intervals of 10 to 15 minutes to measure plasma concentrations of glucose, intact and total GIP, glucagon, insulin, C-peptide, FFA and SU.

Insulin secretion, calculated as the *incremental area under the curve* (iAUC) from time 0 to 120 minutes, was significantly higher during GIP+SU (38 ± 9 nmol/l x 120 min) compared to GIP alone (15 ± 3 nmol/l x 120 min; p=0.0005), to SU alone (12 ± 5 nmol/l x 120 min; p=0.001) and to infusion with saline alone (6 ± 2 nmol/l x 120 min; p=0.0005). The iAUC during GIP+SU was also significantly higher than the sum of SU alone and GIP alone (38 ± 9 vs. 21 ± 5 nmol/l x 120 min; p= 0.02). There was no significant difference between examinations with GIP alone compared to SU alone (15 ± 3 vs. 12 ± 5 nmol/l x 120 min; p=NS).

Our results imply that in patients with type 2 diabetes, treated with metformin as mono-therapy, acute administration of a SU compound seems at least partially to restore the insulinotropic effect of GIP. This effect may be caused by SU closing the K-ATP channels in the beta cell, hereby uncoupling any influence from K-ATP channel malfunction on the effect of GIP. We suggest that K-ATP channel malfunction contributes to the lost insulinotropic effect of GIP observed in patients with type 2 diabetes.

35-LB

Beta-Cell Dysfunction Causes Impaired Glucose Tolerance in Friedreich Ataxia

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Friedreich ataxia (FA) is a rare neurodegenerative disease caused by a GAA trinucleotide repeat expansion in the frataxin gene. FA patients have an increased prevalence of diabetes, but the pathogenesis of this monogenic diabetes is poorly understood. Our aim was to study insulin secretion and sensitivity in these patients.

We studied 13 non-diabetic FA patients (6F/7M) and 14 controls (10F/4M) matched for age (patients 32±2 years vs controls 31±2 years) and BMI (22.8±1.2 vs 23.3±1.2 kg/m^2). Glucose tolerance was assessed by oral glucose tolerance testing. Frequently-sampled

intravenous glucose tolerance tests were used to measure insulin secretion (acute insulin response to glucose, AIRg) and determine insulin sensitivity (insulin sensitivity index, SI) using the minimal model. The disposition index, a measure of beta cell adaptation to the prevailing insulin sensitivity, was calculated as AIRg x SI. Body composition was measured by injection of ^3H_2O and resting energy expenditure by indirect calorimetry. The size of the GAA expansion in the frataxin gene was determined by PCR.
Fasting glucose levels were similar in the FA and control groups (90±3 vs 89±2 mg/dl). The 2-hour glucose level during the oral glucose tolerance test was 143±9 in patients vs 118±4 mg/dl in controls (p=0.01). The prevalence of impaired glucose tolerance and diabetes was 31% and 15%, respectively, in FA patients, compared to 0% in the controls. FA patients were insulin resistant compared to controls (SI 21±3 vs 36±3 x 10^{-5} min^{-1}/(μU/ml), p=0.001). Body composition (fat-free mass 46±3 vs 49±3 kg) and basal energy expenditure (28.6±1.0 vs 28.3±0.9 kcal/kg lean mass/24 h) were similar in patients and controls. In the patients, SI correlated inversely with the number of GAA repeats (r=-0.58, p<0.05). Insulin secretion was not different between the two groups (AIRg 70±10 vs 62±10 μU/ml). Hence, the disposition index tended to be lower in FA patients (1488±231 vs 2071±328 x 10^{-5} min^{-1}). While the FA patients we studied were young and lean, they had a high prevalence of impaired glucose tolerance. Compared to controls, the patients were insulin resistant, the severity of which was correlated with the number of trinucleotide repeats. Their insulin secretion in response to glucose was not different from the controls. Thus, the expected compensatory increase in insulin secretion in response to insulin resistance did not occur. Our findings suggest that patients with FA have markedly impaired pancreatic β-cell function/adaptation.

36-LB

Acute Inhibition of Liver PKC-Delta Prevents Hepatic Insulin Resistance Caused by Short-Term Lipid Infusion

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The mechanism of free fatty acid (FFA)-induced hepatic insulin resistance remains unclear. Our previous studies have found an association between PKC-δ membrane translocation and hepatic insulin resistance caused by short-term (7h) lipid infusion; however, it is unknown whether PKC-δ is a causal mediator in the process. In the present study, we utilized a specific antisense oligonucleotide against PKC-δ to determine whether inhibiting liver PKC-δ protein synthesis abolishes FFA-induced hepatic insulin resistance. Wistar rats (250-300g; n=5-6/group) were injected i.p. with either antisense oligonucleotide against PKC-δ (PKC-δ ASO) or control antisense oligonucleotide (CON ASO) at a dose of 20 mg/kg, 3 times per week, for 2 weeks. In preliminary studies, we found that this dose of PKC-δ ASO decreased liver PKC-δ protein expression by ~ 50%. After the last injection, the rats were overnight fasted and subjected to a 7h intravenous infusion of either saline or Intralipid plus 20U/ml heparin (IH; to elevate plasma FFA by ~2-fold), during the last 2 hours of which a hyperinsulinemic-euglycemic clamp (insulin infusion: 5 mU/kg/min) with tracer infusion was performed to test hepatic and peripheral insulin sensitivity. Whole body insulin sensitivity, as indicated by glucose infusion rate during the clamp, was decreased with IH infusion compared to SAL infusion in CON ASO treated rats (P<0.01). Treatment with PKC-δ ASO, however, partially prevented the IH-induced decrease in whole body insulin sensitivity (P<0.05 vs CON ASO + IH). Furthermore, treatment with PKC-δ ASO completely prevented the effect of IH infusion to decrease insulin-stimulated suppression of hepatic glucose production (CON ASO + SAL: 62±4%; CON ASO + IH: 22±7%; PKC-δ ASO + IH; 47±10%; PKC-δ ASO + SAL: 53±2%) without affecting IH-induced decrease in peripheral glucose utilization. Administration of CON ASO did not affect peripheral or hepatic insulin sensitivity. These results provide evidence for a potential causal role of PKC-δ in hepatic insulin resistance caused by short-term (7h) lipid infusion and point to PKC-δ as a potential therapeutic target for treatment of hepatic insulin resistance.

37-LB

Txnip is a Critical Regulator of Hepatic Glucose Production

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Thioredoxin-interacting protein (Txnip) has emerged as a possible link between cellular redox state and glucose metabolism. Txnip binds thioredoxin and inhibits its disulfide reductase activity *in vitro*, while a naturally occurring strain of Txnip-deficient mice has hyperlipidemia, hypoglycemia, and ketosis exacerbated by fasting. Recent data in humans demonstrates that Txnip expression is potently upregulated by high glucose and that Txnip expression, in turn, regulates glucose transport. We generated Txnip-null mice to investigate Txnip's role in glucose homeostasis. Txnip-null mice had fasting hypoglycemia, hypoinsulinemia, and blunted glucose production following a glucagon challenge, consistent with a central liver glucose-handling defect. Glucose release from isolated Txnip-null hepatocytes was 2-fold lower than wildtype hepatocytes, while β-hydroxybutyrate release was increased 2-fold, supporting an intrinsic defect in hepatocyte glucose metabolism. While hepatocyte-specific gene deletion of Txnip did not alter glucose clearance compared to littermate controls, Txnip expression in the liver was required for maintaining normal fasting glycemia and glucose production. In addition, hepatic over-expression of a Txnip transgene in wildtype mice resulted in elevated serum glucose levels and decreased ketone levels. Liver homogenates from Txnip-null mice had no significant differences in effective thioredoxin reducing activity or in glutathione oxidation state. However, over-expression of wildtype Txnip in Txnip-null hepatocytes rescued cellular glucose production while over-expression of a C247S mutant Txnip, which does not bind thioredoxin, had no effect. These data demonstrate that Txnip is required for normal glucose homeostasis in the liver. While changes in thioredoxin activity are not observed in Txnip-null mice, Txnip's effects on glucose homeostasis are abolished by a single cysteine mutation that inhibits binding to thioredoxin.

38-LB

Resveratrol Effects on Insulin Action are Mediated Through a Hypothalamic Sirt1 Dependent Pathway

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Recent reports have suggested that resveratrol (RSV) a potent activator of the deacetylase Sirt1 can modulate lifespan in part by ameliorating several markers associated with the metabolic syndrome and insulin resistance. Studies done in our lab and others have revealed that the arcuate nucleus within the medio-basal hypothalamus (MBH) is a key regulatory center for determining nutrient availability and regulating glucose homeostasis. We hypothesize that RSV induced activation of hypothalamic Sirt1 may play a key role in glucose regulation. We utilized both pharmacological and molecular tools to address this issue. First we investigated the effect of hypothalamic RSV on regulating glucose metabolism. RSV (200 uM) or vehicle (5% DMSO) was infused into the MBH of 10-week-old Sprague-Dawley male rats during a 6-hour pancreatic-basal insulin clamp. Intra-hypothalamic (IH) infusion of RSV significantly suppressed hepatic glucose production by 50% (from 11.1± 0.8 at baseline to 5.95±0.85 mg/kg.min) with no appreciable change in glucose uptake. As a result, the animals infused with RSV required a 4 fold increase in the glucose infusion rate (GIR) compared to controls (4.46±0.35 vs 1.3±0.6 mg/kg.min) to maintain euglycemia. Secondly to determine that the effect of central RSV on glucose homeostasis is indeed mediated through a Sirt1 dependent pathway we co-infused a cell permeable Sirt1 inhibitor (SI) with RSV into the MBH during a basal insulin clamp. We found that the SI significantly negated the effect of RSV on hepatic glucose production (SI:11.35±0.87 vs RSV:5.95±0.85) which resulted in a reduced GIR as well (SI:1.79±0.35 vs RSV:4.59±0.31). Similarly when Sirt1 expression in the MBH was silenced using a short-hairpin RNA targeted specifically against Sirt1, RSV no longer had a significant effect on reducing hepatic glucose production. Taken together these studies suggest that IH infusion of RSV suppresses hepatic glucose production and that this action is mediated mostly through a Sirt1 dependent pathway.

39-LB

Central Effects of Oleoyl-Estrone on Feeding and Body Weight in Rats

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Oleoyl-estrone [O-E] is a synthetic form of oleoyl-estrone, an endogenous acyl-estrone released from adipose tissue in proportion to body fat mass. Previous studies have shown that peripheral administration of O-E to rodents reduces food intake [FI] and body weight [BW]. This study tested the hypothesis that O-E acts centrally by examining the effects of intracerebroventricular [ICV] administration of O-E on FI and BW in normal lean rats.

Young adult male (mean BW = 485 g) and female (mean BW = 295g) Sprague-Dawley [S-D] rats were implanted with stainless steel guide cannula targeted to the lateral or 3rd ventricles, respectively, then microinjected with ascending doses of O-E dissolved in 0.5% DMSO and diluted with aCSF. Microinjections (4.0 and 2.0 uL, respectively) of O-E were given in ascending order: vehicle, 100, 250, and 500ng. FI and BW were measured 5, 24 and 48 hrs post-microinjection. Tests were separated by a minimum of 3 days.

In males, microinjection of O-E significantly and dose-dependently reduced BW at 24 hrs post-injection ($p < 0.05$), with a maximum reduction vs. pre-test BW at the 500 ng dose of -10.6 g. In females, microinjection of O-E reduced BW at 5 hrs post-injection at the 250 and 500 ng doses ($p < 0.01$), and at 24 hrs post-injection at the 250 ng dose ($p < 0.01$). A maximum BW reduction of -5.1 g at 24 hrs at the 250 ng dose was observed. Also in females, O-E reduced first 24-hr post-injection FI at the 250 and 500 ng doses ($p < 0.05$), with a maximum reduction vs. pre-test FI baseline of -4.6 g at the 250 ng dose. FI remained reduced ($p < 0.01$) in the females during the second 24-hr post-injection interval.

In summary, ICV administration of O-E dose-dependently reduced BW in lean female and male S-D rats. O-E also reduced 24-hr food intake in females on the first and second days following microinjection. Significant reductions of BW in females prior to reductions of FI indicate that O-E enhanced energy expenditure as well as inhibited feeding. Our data indicate that in addition to its demonstrated peripheral anti-lipogenic effects, O-E acts centrally to inhibit feeding and reduce BW in rats. These results support the rationale for ongoing clinical studies of O-E in common and morbid obesity.

Islet Biology

40-LB

Constitutive Expression of *PAX4* Enhances Differentiation of Human Embryonic Stem Cells to Insulin-Producing Cells

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Human embryonic stem cells (hESC) differentiated *in vitro* to produce insulin-secreting cells represent a potential source of material for transplantation-based therapy for diabetes. We investigated whether constitutive expression of PAX4, a key transcription factor during murine pancreatic development, could promote differentiation of hESC towards a functional β-cell phenotype.

H7 hESC stably-transfected with Pax4 (H7.Px4) and untransfected H7 controls (H7) were differentiated over a 3-week period following embryoid body (EB) formation. Expression of key genes important during hESC maintenance (*OCT4*), pancreatic development (*PDX1*, *ISL1*, *NEUROD1, KRT19*) and mature β-cell function (*SLC2A1*, *GCK, ABCC8*, *KCNJ11, CACNA1A-E, CACNB1-4,* and *INS*) were assessed using standard RT-PCR and quantitative PCR (qPCR) at days 0, 7, 14 and 21 following initiation of differentiation. Physiological function was also assessed at each time point using Ca^{2+} microfluorimetry of EBs challenged with depolarising concentrations of KCl (40 mM) at days 7, 14 and 21 of differentiation. After 21 days differentiation, EBs were enzymatically dissociated and subjected to FACS using Newport Green to isolate a Zn^{+} positive population of cells. The Zn^{+} positive cells were subsequently assayed for C-peptide secretion using ELISA. qPCR revealed substantially higher levels of expression of *PDX1* and *INS* mRNA in H7.Px4 EBs than H7 control EBs (n=3) at the mid- to late- stages of differentiation and this was coupled with upregulated expression of voltage-dependent Ca^{2+} channel (VDCC) genes *CACNA1A* and *CACNA1C* over the same time period. The increase in VDCC gene expression correlated with an enhanced responsiveness of EBs to 40 mM KCl-induced Ca^{2+} signalling in the H7.Px4 EBs relative to H7 control EBs. Many other β-cell-related genes were also expressed in the H7.Px4 EBs.

Following FACS, the Zn^{+} positive cells were found to be positive for *INS* expression by RT-PCR and qPCR , they also contained C-peptide protein (77±7 pg/10^4 cells; n=3) and secreted C-peptide in response to stimulation with tolbutamide (100 μM; 23 ± 5 vs 68±4 pg/10^4 cells/15 min; n=3; Student's t-test $p<0.001$).

These studies describe for the first time the enhancement of β-cell differentiation in hESC by constitutive expression of PAX4, and also a novel method to separate differentiating insulin-secreting cells from undifferentiated precursors.

41-LB

A New Mathematical Model for Islet Architecture Explains Beta-Cell Function and Pathology Better Than Previous Ones

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It has been found recently that animal models may have a distinct islet architecture compared to humans. However, it is still not clear how these findings translate into functional differences. In this study, for the first time, we investigate the quantitative dependence of the islet oscillatory insulin release on islet architecture. We have developed a new mathematical model for pancreatic islet oscillation based on the hexagonal closest packing (HCP) of β cells in an islet. This is closer to the real islet cell organization than the simple cubic packing (SCP) that has been used in the past. In addition, we found that the HCP clusters are functionally more desirable and more robust than the SCP clusters. Using our HCP model, we examined the fraction of β cells that is able to burst, the synchronization index of the bursting β cells, the bursting period, plateau fraction, and the amplitude of intracellular calcium oscillation. The architectural parameters include the number of β cells in the islet n_β (range from 8 to 343), number of β-β couplings of each β cell n_c (0-12), and the intercellular coupling strength g_c (25-1000 pS). To investigate the properties of real islets under both physiological and pathological conditions, we have also simulated β-cell clusters with varying amounts (0-90%) of non-β cells, or damaged β cells. We find that the islet function depends quantitatively on its architecture. Reduced β-cell cluster size, intercellular coupling number, or impaired coupling strength could all compromise normal islet function. We also find that normal HCP β-cell clusters are functionally robust against significant perturbations to its architecture. Most strikingly, they can function with up to 70% of their β-cell mass lost, which is consistent with laboratory and clinical findings. These results are important in understanding the regulation mechanism of insulin secretion, as well as in translational studies of animal models, and in the surgical selections of islets for transplantation.

42-LB

3D Structure-Function Studies of the Insulin Biosynthetic Pathway by Whole Cell Electron Tomography of Islet Beta Cells

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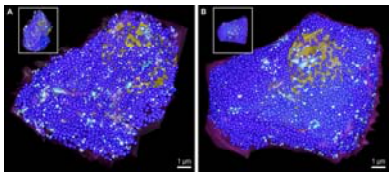
We are focused on understanding the basic mechanisms that underpin normal beta cell/islet function, so that we can elucidate the steps that lead to beta cell/islet dysfunction and ultimately, diabetes. To this end, we combine fast-freezing with electron microscope tomography (ET) to conduct comparative structure-function studies of pancreatic islets isolated from mice and humans. To move toward a more 'holistic' approach to understanding the mammalian cell as a unitary example of an ordered complex system, we have undertaken a multi-scale/multi-resolution approach to reconstructing mammalian (beta) cells in toto in 3D at the EM level. Two 3D reconstructions of glucose-stimulated mouse islet beta cells that demonstrate 15-20nm resolution are

providing unique insights into structure-function relationships among key organelles of the insulin biosynthetic pathway. Most notably, these data provide direct evidence for an apparent inverse relationship between the number of mitochondria, the extent of mitochondrial branching and mitochondrial proximity to the Golgi ribbon versus insulin granule biosynthesis. Further, we provide proof-of-concept data for imaging and reconstructing entire beta cells in 3D at 4-5nm resolution. By providing complete sets of 3D spatio-temporal coordinates for islet cells at a range of resolutions that will uniquely inform advanced in silico studies of 3D cell and molecular organization, we are working to develop the world's first navigable 'Visible Cell'. This work requires the parallel development and implementation of new mathematical tools for extracting useful structural/biological information from the data in a rapid, reliable and quantifiable manner. Such an interactive high-resolution map of the 3D landscape of insulin-secreting cells imaged and reconstructed in their entirety by ET will serve as a unique international resource for protein and organelle annotation, database integration and 3D visualization, and as a framework for 4D animations of cells at pseudo-molecular resolution.



Nutrition

43-LB

Root Extract of Salacia Oblonga Plant Lowers Postprandial Glycemia in Patients with Type 2 Diabetes After a Carbohydrate-Rich Meal

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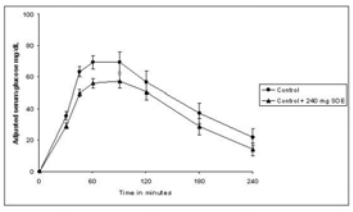
Because postprandial hyperglycemia is a risk indicator for micro- and macrovascular complications in patients with type 2 diabetes, decreasing the blood glucose rise following a meal is an important facet of diabetes management. An extract of *Salacia oblonga* (SOE) has been previously shown to significantly lower postprandial glycemia in subjects with type 2 diabetes. This earlier research tested the herbal extract with a liquid test meal; whereas, the results presented here tested a commercial pasta meal with meat sauce. The purpose of the present study was to evaluate the effect of alpha-glucosidase inhibitor SOE on postprandial glycemia following a solid carbohydrate-rich test meal. We studied 49 patients with type 2 diabetes within a multicenter, randomized, controlled, double-blinded, crossover study design. While in a fasted state, subjects consumed one of the following two meals: 1) control = commercial frozen spaghetti with meat sauce; 2) control plus 240 mg SOE. Postprandial serum glucose samples were analyzed for 240 min. The net changes in serum glucose concentrations are shown in Figure 1. SOE significantly lowered the postprandial positive area under the glucose curve by 19% compared with the control meal alone (p=0.0002). Additionally, adjusted peak glucose response was reduced by 19% when the herbal extract was consumed with the control meal (p=0.0001). Because SOE lowers acute glycemia in persons with type 2 diabetes after meals, this herbal extract may be useful for greater flexibility in meal-planning and increased postprandial glucose control.

FIGURE 1
The points are net changes from baseline for serum glucose concentrations (mean ± SEM, n = 45-49) for the control meal and control + 240 mg SOE at each time point.



Obesity

44-LB

Lack of Erythropoietin Receptor in Non-hematopoietic Tissues Disrupts Glucose Metabolism and Results in Insulin Resistance

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Erythropoietin (Epo), a cytokine known for its role in erythropoiesis, functions via specific binding to its cell surface receptor (EpoR) on erythroid progenitor cells to promote cell viability, proliferation and differentiation. However, Epo receptor expression and Epo function extends beyond hematopoietic tissues. Through the investigation of EpoR null mice rescued with EpoR expression restricted to hematopoietic tissue, we observed the development of obesity and glucose intolerance. Rescued mice increased body weight significantly faster than control mice from the first week after birth, and continued beyond 18 months. These mice were glucose intolerant as early as 8 weeks old, but insulinemia and insulin resistance were not recognized until 6 months when their obesity became more apparent. Body composition of rescued mice at 8 months showed that fat mass was 2 to 3 times that of control mice with no difference in lean mass. There is no diffrence in food intake in these mice. Indirect calorimetry assay on these mice demonstrated no difference in young mice but a significantly decreased metabolic rate and activity in old, obese mice (18 months), suggesting that reduced activity is not the primary cause of weight gain. Conversely, Epo treatment of ob/ob mice decreased blood glucose level and weight gain. Although EpoR is not expressed on mature skeletal muscle, we observe EpoR mRNA and protein expression in adipose tissue of wild type mice, raising the possibility that Epo/EpoR signaling is involved in adipocyte metabolic activity. The decrease in fat mass in ob/ob and control mice on a high fat diet further suggests that Epo may also play a role in modulating adipocyte differentiation or proliferation.

45-LB

Weight Loss with Bupropion and Naltrexone Improves Markers of Insulin-Resistance

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Contrave ™ is a combination of Naltrexone (N) with Bupropion (B) in phase III development for weight loss. This combination approach is designed to both promote hypothalamic POMC activity (appetite and energy expenditure) while negating the auto-inhibition of this pathway by endogenous beta-endorphin. While sustained weight loss has been reported with the combination, the effect on markers of insulin resistance, not accounted for by weight loss, was unexpected. A double-blind, placebo-controlled, multi-center trial which randomized 285 healthy, non-diabetic, obese subjects to either: B 200 mg bid, placebo (P), N 48 mg/d (N_1) or B with naltrexone 32mg qd (BN_2), of which 182 completed, for 24 weeks of treatment. A subset of 60 subjects from two participating centers had Dexa and Multislice CT to measure body fat, lean tissue and visceral fat. Groups were matched at baseline. Markers of insulin resistance improved more with BN_2 than expected from the weight loss alone; a robust effect on decreasing visceral fat was also evident (see table treatment adjusted mean ± SE, *p<0.05, **p<0.01, ***p<0.001 vs. BN_2).

	Placebo, N=17	Naltrexone 48 mg, N=11	Bupropion 400 mg, N=7	Naltrexone & Bupropion 32/400 mg, N=22
Weight (% change)	-1.1 ± 0.6 ***	-1.74 ± 0.9 ***	-3.14 ± 0.7 ***	-7.1 ± 0.7
Waist (cm)	-1.0 ± 5.4 **	-3.8 ± 12.7	-2.9 ± 6.0	-5.4 ± 7.6
Fasting Glucose (mg/dL)	1.9 ± 1.3*	3.4 ± 1.7*	3.5 ± 1.5*	-2.0 ± 1.5
Insulin (mcU/mL)	0.9 ± 0.9**	1.7 ± 1.3**	-0.5 ± 1.1	-3.0 ± 1.1
Triglyceride (mg/dL)	-15.0 ± 7.7 *	-17.6 ± 10.4	-18.4 ± 9.0 *	-43.6 ± 8.8
Visceral fat (% change)	-4.6 ± 2.4 **	-0.1 ± 3.2 ***	-2.3 ± 3.3*	-13.7 ± 2.1

We concluded that BN_2 improves markers of insulin resistance beyond that expected by weight lost. This effect was not only superior to that seen with placebo, but exceeded that seen with either of the individual therapeutic components. This apparent pharmacological synergy is a novel observation and invites additional research to better understand the Contrave ™ mechanisms of action and resulting potential for metabolic/glycemic control in obesity.

Pregnancy

46-LB

Elevated Fasting Free Fatty Acids are Associated with a Positive Glucose Challenge Test and Adverse Pregnancy Outcomes in the Absence of Gestational Diabetes Mellitus

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Prior data from the Camden Study suggested that elevated levels of fasting free fatty acids (FFAs) were a biomarker for the development of gestational diabetes mellitus (GDM). Approximately 9-19% of pregnant women have a positive glucose challenge test (GCT) when screening for GDM but a normal diagnostic oral glucose tolerance test (OGTT). It is not known whether increased FFAs prognosticate a positive GCT or adverse pregnancy outcomes.

We subsequently examined associations between fasting plasma FFAs during the 3rd trimester (30.96 ± 0.16 wks) with a positive GCT without GDM and adverse pregnancy outcomes in a cohort of 505 healthy young pregnant women (African-American 35%, Hispanic 53%, and Caucasian 12%) aged 22.6 ± 0.2 (yr), pregravid BMI (kg/m2) 25.99 ± 0.28, from Camden, NJ. Women with GDM were not included in this analysis.

FFA concentration was significantly increased in women with positive GCT and normal OGTT (n=41) compared to those with a normal GCT (n=464) (419.7 ± 23.6 vs 372.4 ± 7.1 μM, mean ± SE, p=0.05). Using multiple logistic regression, with control for confounders, women in the highest tertile of FFA (>432 μM) showed a 2-fold increased risk of developing a positive GCT (adjusted odds ratio (AOR) 2.07, 95% confidence interval (CI) 1.06-3.82, p<0.01), a nearly 2-fold increased risk of infant admission to neonatal intensive care unit (AOR 1.91, 95% CI 1.06-3.45, p<0.03) and a 2.8-fold increased risk of spontaneous preterm delivery (AOR 2.80, 95% CI 1.16-6.76, p<0.03). High FFAs did not show a direct effect on the development of large-for-gestational age infants (LGA), but women with positive GCT had a 3-fold increased risk of LGA (AOR 3.38, 95% CI 1.24, 9.23, p<0.03) compared to those with a normal GCT. Our data thus suggest that a positive GCT with a normal OGTT may be a state of abnormal glucose metabolism that intermediates between normal glucose homeostasis and GDM and influences risk of fetal overgrowth. Consequently, elevated fasting plasma FFAs may play an important role in mild glucose intolerance and poor pregnancy outcomes.

47-LB

High Fat Feeding in Utero and Maternal Insulin Resistance Program Growth and Metabolic Profile in Offspring

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To determine the effects of in utero high fat diet (HFD) and maternal insulin resistance (IR) on metabolic programming of offspring.

Female CD1 mice aged 10-12 wks, either wild type (WT) or GLUT4 heterozygous knockout (G4) (genetic model of IR), were fed a HFD (36% fat) or control chow (10% fat) for 2 wks prior to mating, through pregancy and lactation. Pregnant mice were either sacrificed on embryonic day 18.5 (n=31) or allowed to deliver spontaneously (n=108). WT male offspring (n=51) were weaned onto low-fat diet (5% fat). At 6, 12 and 20 wks of age, blood was collected from WT offspring for determination of serum metabolites and hormones.

Pregnancy outcomes: G4 females exhibit a 45% decreased in insulin action (determined by euglycemic hyperinsulinemic clamp studies)(p<0.05). During pregnancy, G4 mothers, exhibit increased fed glucose and decreased triglyceride (TG) and lactate levels compared to WT (p<0.05). HFD was associated with decreased maternal BW gain, increased insulin and NEFA and decreased TG levels. HFD and maternal IR resulted in decreased fetal BW, crown rump length, and placental weight (p=0.001). Glucose and insulin levels were significantly higher in embryos of G4 compared to WT mothers (p<0.05). Offspring growth and metabolism; IU diet had a significant impact on prepubertal growth. HFD was associated with a decrease in BW gained from 3 to 6 weeks of age (p<0.05). At 6 wks HFD was associated with increased glucose, glucose insulin ratio (G/I), PAI-1 levels and decreased adiponectin levels (p=0.03). After puberty, maternal IR was associated with an increase in circulating glucose, G/I and resistin levels, and a decrease in BW gain in offspring (p<0.05). At sacrifice (24-26 wks) HFD was associated with higher fed and fasted glucose, enlarged heart and kidney/body weight ratios, decreased TG and lactate levels (p<0.05), and altered hepatic expression of PPAR alpha, stearoyl-coenzyme A desaturase 1, and glucose 6 phosphatase. Maternal IR was associated with a higher G/I ratios, suggesting a lower insulin response to glucose. Pups born to WT mothers had a 50% decrease in skeletal GLUT4 expression and impaired glucose tolerance, suggesting that GLUT4 expression can be programmed in utero.

HFD and maternal IR independently program offspring growth and metabolic profile at different stages of development. Altered metabolic profile may be mediated in part by alteration in expression of GLUT4 and genes that regulate substrate utilization.

Signal Transduction

48-LB

Redox-Dependent Inactivation of Tuberin and Downregulation of Ogg1 by High Glucose in Renal Proximal Tubular Epithelial Cells

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Oxidative stress is involved in the pathogenesis of diabetic nephropathy. Superoxide and hydrogen peroxide generating NAD(P)H oxidases have been shown to be a major source of these radicals in the diabetic kidney. Reactive oxygen species (ROS) are also an important endogenous source of DNA damage, i.e. formation of 8-Oxo-deoxyguanine (8-oxodG) by ROS primarily as a result of GC to TA transversions. 8-oxodG levels are increased in the diabetic kidney in man and in rodents. 8-oxodG in DNA is repaired primarily by an enzyme called 8-oxoG-DNA glycosylase (OGG1). In this study, we investigated the effect of high glucose concentration (HG) on tuberin phosphorylation and OGG1 expression in murine proximal tubular epithelial cells (MCT), as well as in primary culture of rat proximal tubular epithelial cells isolated from Long Evens rats (RPTECs). We also tested the redox sensitivity of this pathway. We show that HG caused a rapid time-dependent increase in NADPH-dependent superoxide and peroxide generation that is associated with an increase in Akt and tuberin phosphorylation that remain sustained up to 1 h in both cell types. Exposure of the cells to HG also induced downregulation of OGG1 protein expression that paralleled its effect on Akt and tuberin. Inhibition of phosphoinositide 3-kinase/Akt pathway significantly reduced HG-induced tuberin phosphorylation and restored OGG1 expression. Hydrogen peroxide stimulates Akt and tuberin phsophorylation and decreases OGG1 protein expression. In a rat model of streptozotocin-induced type 1 diabetes, we demonstrate that hyperglycemia is associated with an increase in NADPH oxidase activity, 8-oxodG accumulation, increase in Akt and tuberin phosphorylation and downregulation of OGG1 in kidney cortex. These results provide the first evidence that high glucose leads to phosphorylation/inactivation of tuberin and downregulation of DNA repair enzyme OGG1 via the redox-dependent activation of Akt in renal tubular epithelial cells. This signaling cascade may play a role in oxidative stress-mediated DNA damage induced by hyperglycemia during diabetic nephropathy.

49-LB

Long Term Insulin Independence after Supplemental Islet Infusion Under Exenatide Treatment in Subjects with Islet Graft Dysfunction

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To re-establish insulin independence (IND) and prevent severe hypoglycemia, supplemental islet infusions (SI) were performed at our Center in islet transplant recipients with graft dysfunction under Edmonton-like immunosuppression. Five subjects underwent SI in 2003 without exenatide (SI-C group) and four in 2006 under exenatide treatment (SI-EXN group), to improve islet engraftment and long-term graft function. The SI-EXN group had been on this drug for median time of 171 days (range 155-197) without achieving IND. Outcomes evaluated: IND achievement/duration and metabolic function. The SI-C and SI-EXN groups received a mean 8713±4714 and 5613±970 IEQ/kg, respectively (NS). In the SI-C only 2/5 patients achieved IND, one up to POD 273 and the other is still IND at 1423 days. In the SI-EXN group, all patients have achieved IND (for > than 405, 374, 394 and 407 days respectively). Probability of IND at 1 year was 20% in SI-C group compared to 100% in SI-EXN group (p=0.02) [Fig. 1].

At 12 mo A1c, C-peptide glucose ratio (CPGR) and 90-min Glucose after mixed meal tolerance test were 6.0±0.8 vs 6.1±0.45% (NS), 1.1±0.4 vs 2.0±0.25 ng/mg (p<0.01) [Fig 2] and 187±41 vs 144±19 mg/dl (NS) in SI-C and SI-EXN groups, respectively.

This study suggests that SI under EXN treatment are more successful achieving and maintaining IND. The significant increase in CPGR in the SI-EXN group suggests better preservation of functional islet mass. Exenatide is a beneficial adjuvant, which may assist with single donor protocols and long-term islet transplant outcomes.

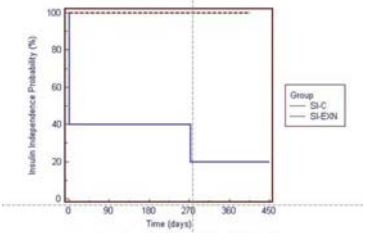

Figure 1: Kaplan Meier for probability of IND after SI

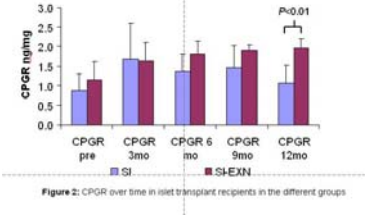

Figure 2: CPGR over time in islet transplant recipients in the different groups