2813-PO

WITHDRAWN

INSULIN ACTION—ADIPOCYTE BIOLOGY

2815-PO

WITHDRAWN

TRANSPLANTATION

2814-PO

The Human Pancreas as a Source of Extracellular Matrix Scaffold for a New Generation Bio-artificial Endocrine Pancreas

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Our study aims at producing acellular extracellular matrix scaffolds from the human pancreas (hpaECMs), as a first critical step towards the production of a new generation, fully human-derived bio-artificial endocrine pancreas (BAEP). In this BAEP, the hardware will be represented by hpaECMs, while the software will consist in the cellular compartment generated from patient's own cells. To achieve our goal, human pancreata were decellularized with Triton-based solution and thoroughly characterized. Primary endpoints were: complete cell and DNA clearance, preservation of ECM components, growth factors (GFs) and stiffness, ability to induce angiogenesis, and conservation of the framework of the innate vasculature. Secondary endpoint was hpaECMs' ability to sustain growth and function of human islet and human primary pancreatic endothelial cells (hPPEC). Results show that hpaECMs can be successfully and consistently produced from human pancreata, maintain their innate molecular and spatial framework and stiffness, as well as vital GFs. Importantly, hpaECMs are cytocompatible and supportive of representative pancreatic cell types. We therefore conclude that hpaECMs has the potential to become an ideal platform for investigations aiming at the manufacturing of a regenerative medicine-inspired BAEP.

Supported By: Liberitutti Onlus Foundation

2816-PO

Erythropoietin Stimulated Erythropoiesis, Metabolic Response and Reduced Body Mass in Mice

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Erythropoietin (Epo) is the primary cytokine regulating red blood cell production that acts to promote survival, proliferation and differentiation of erythroid progenitor cells. Increasing evidence in animal models suggest that in addition to increasing erythropoiesis, Epo treatment affects metabolic processes. Epo treatment in wild type (WT) C57Bl/6 mice increases hematocrit and decreases fasting blood glucose levels and fat mass, and improves glucose tolerance. While Epo and leptin share some homology as members of the hematopoietic cytokine super family, leptin is not required for the metabolic effect of Epo that is also observed in ob/ob mice. Epo receptor (EpoR) is highest on erythroid progenitor cells, and primary human erythroid progenitor cells in culture show an Epo dose dependent increase in glucose uptake that peaked at maximal expression of EpoR and Glut1. EpoR is also expressed in white adipose tissue (WAT) and fat specific knockout mice (ΔEpoRWAT) compared with WT mice exhibit increased body weight, blood glucose and serum insulin levels that are more apparent when on a high fat diet (HFD). ΔEpoRWAT mice treated with Epo show only a modest change in body mass, indicating that white adipose tissue contributes significantly to the Epo metabolic response. Fat mass increases with age and older WT mice exhibit greater metabolic Epo responses compared with young mice while the increase in hematocrit appear comparable. These data suggest that Epo metabolic response appears to increase with increasing fat mass/obesity and age, and includes contributions from increased glucose uptake with Epo stimulated erythropoiesis as well as direct Epo response in WAT.

2817-PO

WITHDRAWN

2818-PO

Short-Term Regulation of Adiponectin Multimerization and Secretion by the Aminothiol Cysteamine

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Adiponectin levels are reduced in non-alcoholic fatty liver disease (NAFLD). The objective of this study was to determine if the thiol-containing antioxidant cysteamine directly modulates fat cell (FC) formation and secretion of adiponectin.

Freshly isolated human FCs were treated with cysteamine for 36 hr and media adiponectin was quantified by ELISA. Total intracellular adiponectin and intracellular and media adiponectin multimers were assessed by Western blot. The cysteamine-mediated adiponectin secretion rate was calculated.

Treatment of FC with 90 μM cysteamine reduced intracellular adiponectin by 32% (*P = 0.05)* and increased adiponectin secretion by 28% (*P=0.001*). This effect was dose-dependent with a peak increase of 33% at 180 μM (*P=0.004*). 90 μM cysteamine increased the rate of adiponectin secretion by 24% over the first 10 min (*P=0.03*), but no differences were observed at later time intervals. 90 μM cysteamine increased media high molecular weight adiponectin (HMW) by 52% (*P=0.002*) and reduced both intracellular (47%, *P=0.003*) and media (62%, *P < 0.0001*) medium molecular weight adiponectin (MMW). Media low molecular weight adiponectin (LMW) tended to increase in response to 180 μM cysteamine (*P=0.11*). In both the cell and the media 90 μM cysteamine significantly increased the % HMW (cell: *P=0.01*, media: *P=0.007*) and decreased the % MMW (cell: *P=0.0006*, media: *P=0.007*). There was a trend towards an increase in the media % LMW at 90 μM (*P=0.12*). Cysteamine acts directly on FC to increase total and HMW adiponectin formation and secretion and may have therapeutic potential in conditions such as NAFLD and T2DM, in which augmentation of adiponectin levels may improve underlying pathology.

2819-PO

Insulin-Sensitivity Phenotypes of Obese and Nonobese Subjects and Incident Prediabetes in the Pathobiology of Prediabetes in a Biracial Cohort (POP-ABC) Study

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Background: In the Framingham Offspring Study (mostly white subjects), insulin-sensitive obesity (ISO) was associated with a lower rate of incident diabetes, but data are lacking for prediabetes and diverse populations.

Subjects and Methods: The POP-ABC study followed normoglycemic African American (AA) and European American (EA) offspring of T2DM parents for 5.5 y, with serial assessments (OGTT, anthropometry, body composition, beta-cell function, and insulin sensitivity by euglycemic clamp (Si-clamp). Using the 25th percentile for Si-clamp (umol/kg fat free mass/min.pmol/L^{-1}) and the 75th percentile for HOMA-IR at enrollment, we defined 4 groups: 1) insulin resistant obese (IRO) (BMI $\geq$ 30 kg/m^2, Si-clamp <0.1, HOMA-IR $\geq$2.5); 2) insulin sensitive obesity (ISO) (BMI $\geq$ 30 kg/m^2, Si-clamp $\geq$0.1, HOMA-IR <2.5; 3) insulin resistant nonobese (IRN) (BMI < 30 kg/m^2, Si-clamp <0.1, HOMA-IR $\geq$2.5); and 4) insulin sensitive nonobese (ISN) (BMI < 30 kg/m^2, Si-clamp $\geq$0.1, HOMA-IR <2.5). The primary outcome was incident prediabetes (impaired fasting glucose/impaired glucose tolerance).

Results: We analyzed data from 321 (177 AA, 144 EA) participants. At enrollment, the mean ($\pm$ SD) age was 44.2$\pm$10.6 y, BMI was 30.2$\pm$ 7.23 kg/m^2, fasting plasma glucose was 91.8$\pm$6.77 mg/dl, 2-hrPG was 124$\pm$25.8 mg/dl, and A1c was 5.56$\pm$0.44%. During 5.5yrs of followup, 109 of the 321 subjects developed incident prediabetes (N=100) or T2DM (N=9). The cumulative incidence of prediabetes/T2DM was 48.7% in IRO, 30.9% in ISO, 47.1% in IRN and 26.0% in ISN (*ANOVA* P=0.0051). The relative prediabetes incidence was 40% lower in ISO (vs. IRO) and 80% higher in IRN (vs. ISN).

Conclusion: Insulin-sensitive obesity protects against incident prediabetes, whereas insulin-resistant non-obesity predicts accelerated progression to prediabetes, among AA and EA offspring of T2DM parents. The mechanisms underlying the ISO and the IRN phenotypes remain to be elucidated.

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2820-PO

Native Human LDL Uptake Decreases Adipocyte Differentiation

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Subjects with high plasma apoB, or numbers of apoB-lipoproteins, have dysfunctional white adipose tissue (WAT). Moreover, LDL, the most common form of apoB-lipoproteins, decrease adipocytes function measured as reduced hydrolysis and storage of triglyceride-rich lipoproteins; however, the mechanisms for these findings remain unexplored. Our hypothesis was that high concentrations of LDL provoke WAT dysfunction by reducing adipocytes' differentiation.

To address this, 3T3-L1 murine preadipocytes were differentiated in the presence or absence of high but physiological concentrations of human native LDL (1.4 g apoB/L) for 7 days. Preadipocytes and adipocytes were treated with human native LDL coupled to a fluorescent probe (DiI-LDL) for 3hrs to follow its internalization by the cells. Adipogenesis was measured by AdipoRed™ (lipid droplet-specific fluorescent probe) and cellular proliferation was assessed by Hoechst nuclear staining. Preliminary data suggest that, in control preadipocytes, adipogenesis, cellular-uptake of DiI-LDL and LDL receptor protein expression increased in a differentiation dependent manner over the 7 days. On the other hand, LDL-differentiated adipocytes had higher proliferation rate (RFU: 172,8% of control; RFU: Relative Fluorescence Units) and lower adipogenesis (RFU:51.5% of control) and DiI-LDL uptake (RFU:53,2% of control). Moreover, LDL-differentiated adipocytes showed lower LDL receptor protein expression than the controls as measured by immunoblots.

Taken together these data suggest that high concentrations of LDL interfere with the differentiation of preadipocytes as measured by reduced accumulation of lipid droplets together with reduced LDL receptor expression and LDL uptake. This may explain why subjects with high plasma apoB have dysfunctional WAT, hypertriglyceridemia and increased risk for T2D.

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2821-PO

Role of Circulating Free Fatty Acids on Insulin Resistance in Chronic Endogenous Hypercortisolism (Cushing's Disease)

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The aim of the present study was to investigate the role of free fatty acids (FFA) in impaired insulin resistance (IR) and glucose tolerance that occur very often in patients with Cushing's syndrome. Acipimox (AC) or placebo was administrated overnight to five groups of female patients: lean (n=8), obese normotolerant (NGT; n=10), obese who had impaired glucose tolerance (IGT; n=8), diabetic obese (DM; n=9), and Cushing's disease (CD; n=7). After treatment all patients underwent indirect calorimetry during an oral glucose challenge and

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ADA-Funded Research

euglycemic hyperinsulinemic clamp on different days. CD group had higher basal and post-glucose FFA leveis than lean and NGT groups. After AC, FFA leveis were reduced in all groups with no difference between them. FFA leveis reduction was accompanied by basal glucose leveis decrease in all but IGT group, and insulin leveis were decreased in all but the lean group. Basal glucose oxidation was similar and increased after AC in all groups. On calorimetry, non-oxidative glucose disposal was significantly lower in CD group compared to ali other groups and it increased post AC in lean and obese diabetic patients, but not in CD group. On clamp study, CD group had lower glucose infusion rate (GIR) value versus lean and NGT groups. After AC all groups showed increased insulin sensitivity (IS), but CD patients GIR was lower than that observed in the other groups. We concluded that FFA have a role on CD glucose homeostasis impairment, and that acipimox partially reversed impairment in IS and glucose tolerance.

INSULIN ACTION—CELLULAR AND MOLECULAR METABOLISM

2822-PO

Effect of Subcutaneous Insulin Detemir on Glucose Flux, Lipolysis, and Electroencephalography during Euglycemia and Hyperglycemia in People with Type 1 Diabetes

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Insulin detemir leads to less weight gain when compared with human insulin but the mechanisms are not understood.

To investigate the effects of detemir on glucose flux, lipid metabolism and brain function twelve people with type 1 diabetes received in random order 0.5Units/kgBW subcutaneous insulin detemir or NPH insulin. Glucose concentration was clamped at 5mmol/L for 210 minutes then over 90 minutes increased to 10mmol/L. Glucose production rate (Ra) and uptake (Rd) and glycerol Ra were measured with a constant iv infusion of [6,6 2H2] glucose and [2H5] glycerol. Electroencephalography (EEG) direct current (DC) and alternating current (AC) potentials was measured throughout.

Blood glucose concentration in euglycemia and hyperglycemia was not different between treatments. While detemir induced comparable effects on glucose Ra, glucose Rd and glycerol Ra during euglycemia, compared with NPH insulin, it triggered a distinct negative shift in DC-potentials, with significant treatment effect in the frontal cerebrocortical channels ($p<0.001$). AC spectral power showed significant differences in insulin action in the theta and alpha frequencies during the euglycaemic clamp ($p=0.03$).

Detemir exerts different effects on brain function when compared with NPH insulin. This may be an important mechanism behind the limitation of weight gain observed with insulin detemir.

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2823-PO

Short-Term Curcumin Gavage Improves Insulin Signaling in Dexamethasone-treated C57bl/6 Mice

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Understanding mechanisms underlying the beneficial effects of the polyphenol curcumin in metabolic homeostasis is essential for broadening its application in the prevention and treatment of metabolic disorders. Here we assessed the effect of short-term curcumin gavage in mice, with and without dexamethasone (DXM) injection. DXM (100 mg per kg per day for five days) did not increase fasting plasma glucose level, but increased mouse plasma and liver lipid contents, associated with impaired insulin tolerance ($P < 0.05$, $n=4$) and elevated basal plasma insulin levels ($p<0.01$ and $n=6$). Simultaneous curcumin gavage did not improve the plasma lipid profiles of DXM-injected mice, but reduced the expression profiles of both gluconeogenic and lipogenic genes in the liver ($P < 0.05$, $n=6$). Interestingly, simultaneous curcumin gavage improved insulin signaling, assessed by both intraperitoneal insulin tolerance test and the AKT and GSK3 phosphorylation in the mouse liver tissues. The sensitizing effect of curcumin on insulin signaling was also demonstrated in vitro via stimulating the IRS-1/pAKT signaling cascade and repressing glucose production in mouse primary hepatocytes. Finally, we found that curcumin increases the expression of fibroblast growth factor 21 (FGF-21), which is known to sensitize insulin signaling. Our observations suggest that a rapid beneficial effect of curcumin administration is the sensitization of insulin signaling, involving the stimulation of hepatic FGF-21 production. These events are ahead of its long-term effect in decreasing lipogenesis.

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2824-PO

Superoxide Dismutase Activity in Patients with Type 2 Diabetes Is Correlated with the Pancreatic Islet Function

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Purpose: In clinical study, the precise relationship between alterations in SOD activity and the progression of T2DM remains incompletely understood. Here, we measured SOD activity in different stage of T2DM and investigated the possible mechanism.

Methods: We recruited 205 patients and divided them into four groups according to the stage of diabetes as follows: 54 non-diabetic (Non-DM), 54 newly-diagnosed T2DM (New-DM), 47 early-stage T2DM with the duration of diabetes less than 5 years (Early-DM), and 50 late-stage T2DM with the duration of diabetes more than 5 years (Late-DM). The SOD activities, metabolic markers and fasting C-peptide (CP) levels as the pancreatic islet function indicator were measured in the four groups.

Results: SOD activity in New-DM was higher than that in Non-DM (138.33±4.20 IU/L vs. 128.33 ± 2.70, $P = 0.040$), while this increased SOD activity gradually declined along with progression of T2DM, with the Late-DM group showing the lowest SOD activity among these four groups ($P < 0.013$). In the New-DM group, age was significantly negatively associated with SOD ($r= -0.307$, $P=0.045$), while BMI and CP were positively associated with SOD ($r=0.413$, $P=0.008$, and $r=0.322$, $P=0.035$, respectively). However, in the Non-DM group these associations were not found. In the three multiple linear regression analysis models, among all the variables (age, BMI, fasting glucose and lipid profiles, and CP), CP played the most important role in the activity of SOD ($R2=0.062$, $P=0.004$; $R2=0.074$, $P=0.002$; $R2=0.078$, $P=0.001$, respectively).

Conclusions: We demonstrate that SOD activity is able to predict the progression of diabetes and the failure of β-cell function. The measurement of SOD activity is helpful for the clinician to evaluate the progression of diabetes and the failure of β-cell function.

2825-PO

WITHDRAWN

2826-PO

WITHDRAWN

2827-PO

AICAR Decreases Cell Apoptosis through Regulation YY1 in Renal Proximal Tubular Cells and Diabetic Kidney Mouse

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Apoptosis of tubular epithelial cells is a major feature of diabetic kidney disease, and generation of free radicals and oxidant stress in tubular cells. Several approaches were demonstrated to investigate the mechanism by which AICAR regulates oxidative DNA damage and cell apoptosis in renal mouse proximal tubular cells exposed to high glucose (HG). Cells pretreated with AICAR before exposure to HG showed significant increase in protein expression of p-AMPK and AMPK activity at 48hrs. AICAR pre-treated cells showed significant decrease in oxidative DNA damage (8-oxodG) compared to cells exposed to HG. Moreover, cells pretreated with AICAR before exposed to HG showed significant decrease in PARP activity and cytochrome C expression compared to cells treated with HG. EMSA analysis was performed to test the role of YY1 that has putative binding site in PARP promoter site in nuclear extracts isolated from cells grown in NG or HG for 48h. Treatment of cells with HG significantly increased binding of YY1 to PARP promoter compared to cells grown in NG. The specificity of binding of the DNA/protein complex to YY1 was demonstrated by adding an YY1 antibody to the reaction mixture resulted in marked reduction of the specific DNA/protein complex and confirmed that YY1 is a major transcription factor involved in regulation of PARP and cell apoptosis. Moreover, kidney of dbdb mice showed higher levels (800 pg of 8-oxodG/ml) of oxidative damage and significant (40%) increase in number of apoptotic cells (measured by TUNEL assay) compared to kidney from wild type animals. Db/db mice treated with AICAR (2 mg/kg body weight for 4 weeks) showed significant decrease (50%) in total oxidative DNA damage and total number of apoptotic cells (78%) compared to non-treated mice. These data showed that AICAR activates AMPK to decrease oxidative DNA damage and cell apoptosis through regulation of YY1 and PARP activity to prevent renal cell damage and improve kidney function in diabetes.

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2828-PO

Metformin Attenuates Vascular Smooth Muscle Cell Migration and Proliferation via Insulin-Independent Pathways

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We have shown that vascular smooth muscle cells [VSMCs] exposed to high glucose and palmitate, metformin had little effect on insulin signaling pathways, whereas inflammatory signals were attenuated. To examine whether these changes are accompanied by reductions in migration [M] and proliferation [P] rates, we incubated human coronary VSMCs in similar growth medium [C-22062 and C-39267, Promo Cell] with either high glucose [25mM=25G] and/or palmitate [200 μM=PALM] ± metformin [1mM=MET] for 24 hrs, followed by 20min of insulin [100nM]; BSA and 5mM glucose were used as control. [M] was analyzed in re-suspended cells and assessed by: (i) polycarbonate membrane barrier with chemo-attractants and extended cell protrusions quantified by optical density [OD_{595}nm]; and (ii) inverted microscopic images and calculated % area closure {[ϖ^2 (BEFORE)-ϖ^2 (AFTER)/ϖ^2 (BEFORE)]*100}, [2D-Assay]. Cell Absorbance [A^{550}-A^{690}] was used to evaluate [P]. Results in each experimental condition were compared using [ANOVA] with repeated measures and Bonferroni post-hoc testing (n=5). As determined by [OD_{595}nm], [M] was 0.10±0.02 in [control] and increased to 0.18±0.02 [25G], 0.22±0.02 [PALM] and 0.20±0.03 [25G+PALM], but only to 0.15±0.02 [25G+MET], 0.12±0.01 [PALM+MET] and 0.17±0.03 [25G+PALM+MET], (all p<0.01). Using the % area chamber closure calculation, [M] was 60% in [control] and increased to 78% [25G], 95% [PALM] and 82% [25G+PALM] (p<0.05), but only to 65% [25G+MET], 40% [PALM+MET] and 50% [25G+PALM+MET] (all p<0.001). A rise in [P] from 0.24±0.02 [control] to 0.30±0.03 [25G] in the absence, but not the presence of metformin [0.25±0.03, 25G+MET], (p<0.05) was seen.

Conclusion: Our results indicate that metformin attenuates the increase in coronary VSMC migration and proliferation induced by high glucose and palmitate. These data likely reflect anti-inflammatory effects of metformin and are independent of insulin signaling pathways.

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2829-PO

Mitochondrial Content Is Reduced in Prediabetic Patients

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Individuals with prediabetes (or intermediate hyperglycemia) account largely for the population at higher risk of developing type 2 diabetes (T2D) and related health problems (e.g., heart disease and stroke). Mitochondria are the primary metabolic platform, and the changes in mitochondrial content and function have been identified in patients with T2D. However, whether mitochondria alteration occurs to prediabetic individuals has not been well addressed. In this study, we investigated mitochondrial content by measuring mitochondrial DNA copy number in leukocytes from 11 prediabetic and 8 healthy subjects (serving as the control group). The fasting plasma glucose levels were 111±3 mg/dL (prediabetic) and 84±1 mg/dL (control), p< 0.0001; fasting plasma insulin levels were 26±5 mcU/ml (prediabetic) and 8±1 mcU/ml (control), p<0.05; and HbA1c levels were (5.8±0.1)% and (5.4±0.1)% for the prediabetic and control groups, respectively, p=0.07. The prediabetic group had a HOMA-IR value of 7.3±1.6, which was 4.3-fold higher than that in the control group (1.7±0.2) and indicative an impaired insulin action. The mitochondrial DNA copy number decreased in prediabetic group (3.1±0.1 in logarithm scale) in comparison with the control group (3.8±0.2), while the difference was statistically insignificant (p=0.052). In contrast, evaluation based on HOMA-IR revealed a significantly lower mitochondrial DNA copy number in insulin resistant subjects than in insulin sensitive individuals (p=0.034). Such correlations persisted after adjustment for age and gender. These results suggest that mitochondrial content is reduced in prediabetic patients, and it may arise from impaired insulin action. Further study is underway to elucidate the underlying mechanism.

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INSULIN ACTION—GLUCOSE TRANSPORT AND INSULIN RESISTANCE IN VITRO

2830-PO

Decrease Irs1 Protein Increases Cell Matrix Protein Accumulation through Regulation of Yy1 in Renal Proximal Tubular Cells and Diabetic Mice

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Tubular cells are primary targets of hyperglycemia, and chronic exposure to elevated blood glucose levels contribute to the tubulointerstitial changes seen in overt diabetic nephropathy. Alterations in renal structure may occur that are not specific to nephropathy but reflect a consequence of long-standing diabetes/hyperglycemia. The target gene(s) involve in transcription regulation of cell matrix proteins is not characterized. Mouse proximal tubular cells were grown in normal glucose (NG, 5mM) or treated with high glucose (HG, 25mM) for 24h or pretreated with AICAR or compound C or transfected with siRNA of IRS1, DN-AMPK, DN-Akt before expose to HG. HG significantly reduced IRS-1 and resulted in increased matrix protein expression as well as promoter activity of fibronectin. Downregulation of IRS1 by siRNA in cells treated with HG resulted in significant increase in protein expression and promoter activity of fibronectin. On the other hand, pre-treatment the cells with AICAR before expose to HG resulted in increase IRS1 and decrease YY1 protein expression as well as decrease the promoter activity and protein expression of fibronectin. Downregulation of AMPK by DN-AMPK or treat the cells with compound C resulted in significant decrease in YY1 protein expression and increase in protein expression and promoter activity of fibronectin. In addition, downregulation of Akt by DN-Akt significantly decreased the promoter activity and protein expression of fibronectin. These data showed that IRS1 regulates fibronectin expression through YY1 while pretreatment the cells with AICAR reversed these changes suggesting that

For author disclosure information, see page A810.

YY1 is a major transcription factor in fibronectin regulation. These data shed the light on one of major transcription factor that involve in regulation of fibrosis in diabetes which may consider as a potential therapeutic target for treatment of renal complications in diabetes.

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2831-PO

Mitochondrial Dysfunction Induces Insulin Resistance in Differentiated 3T3-L1 Adipocytes via NF-κB Activation Pathways

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Growing body of evidence suggests that activation of NF-κB signaling pathways is among the inflammatory mechanism responsible for various metabolic disorders. Nevertheless, the regulatory roles of NF-κB inhibition in modulating mitochondrial function of the adipose tissues is not well-established. Noting that mitochondrial dysfunction alter oxidative metabolism in adipose tissues, the present study was to investigate the direct effects of NF-κB inhibitor upon mitochondrial dysfunction-induced insulin resistance in 3T3-L1 adipocytes. NF-κB inhibitor ameliorated mitochondrial dysfunction induced by commonly used mitochondrial inhibitor, oligomycin by altering mitochondrial fusion and fission in adipocytes. The level of oxidative DNA damage, protein carbonylation, and lipid peroxidation were improved. Further, the reduced lipolysis was observed in treated cells with morphology and quantification of intracellular lipid droplets was reduced. The insulin stimulated glucose uptake activity was restored with the enhancement of insulin signaling activity via increased phosphorylation of IRS1, Akt/PKB and AS160 in the adipocytes with mitochondrial dysfunction co-treated with inhibitor. The accumulation of pro-inflammatory mediators TNF-α and IL-1β was markedly depleted. These findings may therefore provide a novel insight into the roles of inflammatory inhibitor as a potential avenue for developing effective therapeutic intervention of mitochondrial dysfunction in the pathogenesis of insulin resistance and type 2 diabetes.

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INSULIN ACTION—SIGNAL TRANSDUCTION, INSULIN, AND OTHER HORMONES

2832-PO

Insulin Signaling in Brain Is Increased in Insulin-resistant States—A Link to Alzheimer's?

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Alzheimer's disease (AD) and insulin-resistant (IR) states of obesity and type 2 diabetes mellitus (T2DM) commonly co-exist, and it is postulated that IR in brain predisposes to AD development. Here, we examined brains harvested from: (a) three mouse models of IR, including, high-fat-fed mice, ob/ob mice, and mice with impaired glucose transport in muscle owing to heterozygous muscle-specific knockout of protein kinase C(PKC)-λ (Het-MλKO); and (b) IR monkeys with diet-dependent long-standing T2DM. Surprisingly, in brains of all IR mouse models, resting/basal activities of the two major insulin-regulated protein kinases, Akt and atypical PKC (aPKC), were maximally increased by the existing hyperinsulinemia, as acute insulin treatment, which activated Akt and aPKC in brains of normal mice, did not provoke further increases in brains of IR mice. Moreover, excessive Akt activity in brain was accompanied by increased phosphorylation of Akt substrates, glycogen synthase kinase-3β, mammalian target of rapamycin, and forkhead homeobox class O proteins, FoxO1, FoxO3a and FoxO4. However, tau phosphorylations, thought to be improved by insulin, were not diminished in IR mice. Increases in Akt phosphorylation were also seen in individual neurons of both anterior cortical and hippocampal brain regions of IR mice, wherein adverse alterations might impact memory function. Importantly, with full correction of hyperinsulinemia in Het-MλKO mice (by inhibition of a key factor needed in the pathogenesis of hepatic and systemic IR in each model, viz., hepatic aPKC), brain insulin signaling to Akt, aPKC and FoxO reverted to normal. Increased activation of Akt and aPKC was also seen in brains of T2DM monkeys. Given the apparent importance of FoxO proteins for maintaining neuronal stem cell function in older mice (as judged from FoxO KO studies), the persistent activation of Akt and the subsequent inactivation of FoxO family members in hyperinsulinemic states may abet development of AD or other CNS disorders.

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2833-PO

Insulin Modulates the Enhanced Rewarding Effects of Nicotine in Diabetic vs. Control Rats

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Rationale: The underlying mechanisms that promote tobacco use in persons with diabetes are not clear. Work in our laboratory has demonstrated that diabetic rats display enhanced rewarding effects of nicotine in conditioned place preference (CPP) and intravenous self-administration (IVSA) procedures. This study examined whether the latter effects are insulin-mediated.

Methods: Male rats first received administration of streptozotocin (STZ), a drug that destroys insulin-producing cells in the pancreas and produces hyperglycemia. After STZ administration, the rats were either surgically implanted with an insulin pellet or they received a sham surgery. Two-weeks later, the rats were implanted with IV catheters and were tested for nicotine IVSA and others were conditioned with repeated nicotine injections in the presence of distinct environmental stimuli in our CPP apparatus.

Results: Insulin replacement normalized the rewarding effects of nicotine in diabetic rats to control levels in the IVSA procedure. However, insulin did not alter nicotine reward in the CPP paradigm.

Conclusion: Our results suggest that insulin modulates the direct reinforcing effects of nicotine, as measured by the IVSA studies. However, insulin may be less critical for modulating the conditioned reinforcing effects of nicotine as assessed in CPP procedures. Future studies are needed to further explore the mechanisms by which insulin modulates the rewarding effects of nicotine in diabetic rats.

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2834-PO

Plasma Angptl8 Levels Correlated with Insulin Secretion and LDL Cholesterol but Not with Triglycerides in Japanese Subjects with Type 2 Diabetes

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ANGPTL8 (Betatrophin) is a secreted peptide that plays a role in lipid metabolism through inhibition of lipoprotein-lipase (LPL). Although its pancreatic beta cell proliferative capacity is controversial, its pathophysiological role in diabetes was suggested by the recent reports of its higher levels in type 1 and type 2 diabetes patients than in non diabetic subjects. In this study, we aim to investigate the ANGPTL8 levels in relation with insulin secretion capacity, lipid profiles, and renal function. The subjects included 99 inpatients with type 2 diabetes. Insulin secretion capacity was evaluated by urinary 24-hour C-peptide excretion (U-CPR) and the increment of C-peptide 6 min after 1mg glucagon injection(ΔCPR). Plasma ANGPTL8 levels of blood samples taken in P800 tubes after overnight fasting were measured using enzyme-linked immunosorbent assay kits. We examined the correlation of ANGPTL8 levels with age, body weight (BW), BMI, duration of diabetes, total cholesterol (TC), LDL cholesterol (LDL), triglycerides (TG), serum creatinine (Scr), 24-hour creatinine clearance (Ccr), U-CPR and ΔCPR. ANGPTL8 levels were positively correlated with age ($r=0.44$, $P<0.001$) and duration of diabetes ($r=0.35$, $P<0.001$), but negatively correlated with BW ($r=0.24$, $P=0.01$), Scr ($r=0.31$, $P=0.001$), Ccr ($r=0.44$, $P<0.001$), ΔCPR ($r=0.26$, $P=0.007$) and U-CPR ($r=0.21$, $P=0.03$). No significant correlation was found between ANGPTL8 levels, BMI and TG. Age and ΔCPR were independent predictors of ANGPTL8 levels in multivariate modeling. In subjects without statin (n=69), ANGPTL8 levels were negatively correlated with TC ($r=0.33$, $P=0.005$) and LDL ($r=0.26$, $P=0.025$) but not with TG ($r=0.13$ $P=0.19$). In conclusion, our results provide new evidence on the association of ANGPTL8 with insulin secretion and cholesterol, but not with TG which is inconsistent with the results of animal models suggesting that the role of ANGPTL8 in human is different from rodents.

2835-PO

WITHDRAWN

2836-PO

Synergic Effect of AKT1 and SGK1 in the Modulation of eNOS Activation in HCAEC

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Cardiovascular diseases (CVD) are the first cause of mortality in patients with diabetes. The first step in development of CVD is the impairment in endothelial homeostasis, which is regulated by nitric oxide (NO) production. Studies conducted in primary cultures of endothelial cells identified several pathways, such as the activation of PI3K-AKT1 signalling, that after insulin stimulation enhance endothelial nitric oxide synthase (eNOS) activation. Indeed, Akt1 directly phosphorylates human eNOS at Ser^{1177} resulting in an increase of eNOS activity, conversely the role of Serum and glucocorticoid-inducible kinase (SGK)1 in modulation of eNOS activity is still sparse. In the present study we investigated the role of SGK1 in modulating eNOS activation in human coronary artery endothelial cells (HCAEC). A retrovirus system was used to infect HCAEC with SGK1wt, SGK1Δ60 (lacking of the N-60 amino acids), and SGK1Δ60KD (kinase dead constructs). eNOS phosphorylation was measured in cells infected with different SGK1 constructs in diverse experimental conditions: 1. insulin stimulation alone (10^{-7}M for 1h); 2. SGK1 inhibitor (GSK650394, 103nM, added 30 min before insulin); 3. AKT1 inhibitor (iAKT, 10μM, added 18h before insulin) in presence or absence of insulin. AKT1 inhibitor completely inhibited phosphorylation of AKT1 in Ser^{473} in all constructs, but inhibited only partially eNOS phosphorylation in Ser^{1177}. Comparably, SGK1 inhibitor, inhibited only partially eNOS activity but this inhibition was significantly less ($p<0.05$) in SGK-1Δ60 cells than other constructs, probably due to an increased activity of SGK1 in this cells. Taking together, these results, suggest that SGK1 and AKT1 may have a synergistic and compensatory effect in inducing phosphorylation and activation of eNOS in Ser^{1177}, by modulating NO production, in coronary cells. Further studies in different cellular systems and in vivo are needed to better understand this process related with diabetic cardiovascular disease.

2837-PO

An Attempt to Designate the Cut-off Point for Circulating Sex Hormone Binding Globulin (SHBG) for Hepatic Insulin Resistance in Young Women Diagnosed with Polycystic Ovary Syndrome

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Insulin resistance and its compensating hiperinsulinemia seems to play an important role in the pathogenesis of PCOS. One of factors that participate in the increased bioavailabity of androgens and estrogens in PCOS is decreased synthesis of SHBG. This protein is synthesized in the liver and increased circulating insulin level is the factor that inhibits its synthesis. It has also been shown that circulating SHBG level is a potential marker of liver insulin resistance. So far there is the lack of data assessing the cut-off point for SHBG level for liver insulin resistance in PCOS women. Therefore, aim of the study was determination of the cut-off point for SHBG level in young women diagnosed with PCOS.

Four-hundred-seventy-six women diagnosed with PCOS were enrolled in the study. Fasting serum glucose, insulin and SHBG were measured and oral glucose tolerance test (OGTT) was performed. Insulin resistance was assessed on the basis of homeostasis model assessment index (HOMA-IR).

There was a negative correlations between HOMA-IR values and circulating SHBG levels ($R= -0.50$; $p<0.001$). On the basis ROC analysis cut-off point of circulating SHBG levels for diagnosis of liver insulin resistance in young women diagnosed with PCOS was 47.7 nmol/L (sensitivity 40.1% and specificity 80.9%, accuracy of the classification 46.0%).

In conclusions, serum SHBG levels below 48 nmol/L characterize young PCOS women with liver insulin resistance. However, low sensitivity of this parameter limits its practical application.

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2838-PO

WITHDRAWN

2839-PO

Insulin Resistance and Nutritional Complications in Rheumatoid Arthritis

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Although insulin resistance is well-known in rheumatoid arthritis (RA), its relations with the characteristics and the nutritional complications of the disease are not clear, and they may involve changes in the energy expenditure.

Among patients with well-characterized RA (duration, activity: DAS28VS), we measured the insulin sensitivity by HOMA-IR, and the energy expenditure (EE): Resting EE (REE) by indirect calorimetry and physical activity-EE by actimetry (Sensewear Armband). The metabolic syndrome (MS) was defined according to the IDF criteria and Rheumatoid Cachexia (RC) from DXA body composition analysis if the Fat Free Mass (FFM) index was 25th percentile.

Fifty-seven patients were included (73% women, age 57±10 years). The duration of the disease and its activity were independently related to the HOMA-IR: mean 3.8±3.0 years, $r=0.61$; $p<0.001$ and DAS28VS 3.0 ±1.4, $r=0.56$; $p<0.001$. Both these associations kept significant after adjusting for age and BMI.

The MS and RC were present in 24% and 19% of the patients respectively, and were not significantly associated with each other. The MS was related to the duration of the disease ($p=0.007$): 5% MS before 4 years, 25% between 4 and 9 years, 50% after, and independently to the BMI ($p=0.012$). The patients with RC had a +10% higher REE reported to their FFM ($p=0.007$). Two modifiable factors were independently associated with nutritional complica-

For author disclosure information, see page A810.

tions (MS and/or RC): a low level of physical activity (METs) (exp(B)=0.03; p=0.009) and a treatment by corticosteroids (exp(B)=4.08; p=0.046).

Insulin resistance associates with the duration and inflammatory activity of RA. Contrasting effects on energy expenditure (high REE with inflammation and low physical activity energy expenditure with duration of disease), probably contribute to the different nutritional complications: metabolic syndrome, or rheumatoid cachexia. More physical activity and less corticosteroids may help to prevent these complications.

2840-PO

Pgc-1a Ameliorates Insulin Resistance Induced by High Fat through Regulating STARS in Muscle Cells

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Objectives: To explore the effect of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) on insulin resistance induced by palmitic acid and its potential molecular mechanism in in skeletal muscle cells.

Methods: Rat L6 muscle cells were then randomly divided into two groups: control group and palmitic acid (PA) treatment group. The control group was randomly divided into normal control (NC) group, normal empty-vector (pcDNA) group and PGC-1α overexpression (PGC-1α) group; Palmitic acid (PA) group was randomly divided into palmitic acid (PA) group, PA cells infected with empty-vector (PpcDNA) group and PGC-1αoverexpression of PA cells (PPGC-1α) group. Expression of PGC-1α, striated muscle activator of Rho signaling (STARS) and insulin signaling pathway related genes were measured.

Results: 1. Compared with NC group, the expression of PGC-1αmRNA and protein was significantly decreased and STARS was increased (P<0.05). Insulin signaling pathway related genes such as IRS-1,AKT, GLUT4 mRNA and protein expression were reduced (P<0.05). (2) Compared with NC group and pcDNA group, expression of PGC-1α, IRS - 1, AKT, and GLUT4 were increased (P < 0.05), while the expression of STARS was increased in PGC-1α group (P < 0.05). (3) Compared with PA group and PpcDNA group, expression of PGC-1α, IRS-1, AKT and GLUT4 were increased in PPGC-1α group (P < 0.05), while the expression of STARS mRNA reduced (P < 0.05), (P < 0.05).

Conclusions: Expression of PGC-1α and insulin signaling pathway related genes were decreased, and STARS was increased in L6 muscle cells cultured by palmitic acid. Expression of STARS was lower and insulin signaling related genes were higher by up-regulating the expression of PGC-1α. It suggested PGC-1α may improve insulin sensitivity in skeletal muscle cells through regulating the expression of STARS.

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2841-PO

Association between Serum Thyroid Hormones and Insulin Resistance in Healthy Euthyroid Subjects

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The association between insulin resistance and thyroid function in euthyroid subjects has not yet been clarified. We aimed to investigate the association between thyroid function within the normal reference range and insulin resistance in participants of the Tehran Thyroid Study (TTS).

This cross-sectional study was conducted within the framework of TTS. Of 5786 subjects, aged ≥20 years, 2758 euthyroid subjects without a history of thyroid disorders, diabetes, chronic kidney diseases, cardiovascular disease or consumption of steroid and lipid lowering agents, were studied. Serum concentrations of lipids and lipoproteins, fasting blood glucose, insulin, free T4 and TSH were measured. The homeostasis model assessment index for insulin resistance (HOMA-IR) was used to evaluate IR.

After adjustment for age, smoking and physical activity, HOMA-IR increased positively in the first FT4 tertile compared to the third, in both men [B_1=0.12, P<0.05] and women [B_1=0.09, P<0.05]. However, after adjustment for age, smoking, physical activity and WC, HOMA-IR was positively associated with the first tertile of FT4, only in men. The prevalence of insulin resistance decreased from 27.2 to 19.1 with increasing tertiles of FT4 only in men (p=0.01). Multiple logistic regression analysis showed that the first tertile of FT4 was accompanied with higher odds of insulin resistance, compared to third [OR=1.82, (1.25-2.65) p=0.03] only in men. Finding also showed that serum TSH levels did not influence the odds of being insulin resistant either in men or in women.

Low FT4 was independently associated with insulin resistance in healthy euthyroid Iranian men.

2842-PO

TrkA Receptor in Streptozotocin-induced Diabetic Rat Brain

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Diabetes has an impact on central nervous system with impairment in learning, memory and problem solving ability. Neuronal loss and cerebral cortex degeneration has been reported in diabetic patients. Diabetes has been associated with an increased risk of developing Alzheimer disease (AD). Nerve growth factor (NGF) plays a crucial role in differentiation, survival, maintenance of sensory and sympathetic neurons. Two cell surface receptors for NGF have been identified: TrkA and p75. Here we show that tyrosine phosphorylation sites in TrkA sequence are similar to insulin receptor. Binding of NGF to TrkA induces auto phosphorylation, and interaction of IRS-1. Interestingly, we found that TrkA interaction with IRS-1 is impaired in streptozotocin (STZ) treated rat brain. The interaction of IRS-1 with TrkA requires the kinase activity of TrkA. Moreover, the activation of Akt and MAPK is dependent upon TrkA kinase domain. These results suggest that NGF-TrkA receptor may be involved in insulin signaling.

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INTEGRATED PHYSIOLOGY—INSULIN SECRETION IN VIVO

2843-PO

WITHDRAWN

2844-PO

Anti-aging Gene Klotho Protects against Type 2 Diabetes via Preserving the Function of β Cells

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Type 2 diabetes mellitus (T2DM) impairs β cells leading to β cell failure. Thus, it is important to preserve β cell function in pancreatic islets in T2DM. Klotho is a recently discovered anti-aging gene. Klotho protein expression levels were decreased in pancreatic islets in db/db mice, a mouse model of T2DM. Interestingly, β cell-specific expression of mouse Klotho (mKL) attenuated the development of diabetes in db/db mice. β cell-specific expression of mKL decreased hyperglycemia and enhanced glucose tolerance. The beneficial effects of mKL were associated with significant increases in the number of β cells, insulin storage levels in pancreatic islets, and glucose-stimulated insulin secretion from pancreatic islets which lead to the increased blood insulin levels in diabetic mice. β cell-specific expression of mKL decreased the intracellular superoxide levels, oxidative damage, apoptosis, and DNAJC3 (ER stress marker) in pancreatic islets. Furthermore, β