1831-P

Islet Cell Autotransplantation (IAT): A Successful Endocrine Procedure after Total Pancreatectomy in Children with Chronic Pancreatitis

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Introduction: Total pancreatectomy (TP) markedly improves quality of life in children with chronic pancreatitis (CP), but results in brittle, insulinopenic diabetes. TP with IAT (TPIAT) may preserve insulin secretion. Minimal data are available regarding TPIAT in pediatric patients (Pts).

Methods: Between 2009-2016, TPIAT was performed in 13 Pts (7 boys) with median age 10.3 yrs (range 7-17 yrs). Six (46%) had a PRSS1 (protease, serine, 1) mutation, 2 (15.4%) had a CFTR (Cystic fibrosis transmembrane conductance regulator) mutation, 2 (15.4%) had combined CFTR/SPINK1 (serine protease inhibitor, Kazal-type, 1) mutations. All were euglycemic prior to TPIAT (normal fasting glucose and HbA1c; normal response to mixed meal tolerance test in a subset). Islet cells, isolated after TP, were infused in the portal vein. Pts were kept on an insulin infusion for (average) 6.8 days, then switched to MDI, with tight glycemic control.

Results: Six months after TPIAT, 5 Pts (38%) did not require insulin (HbA1c 5.5-6.1%), 3 Pts (23%) were on basal insulin only (0.03-0.35 U/Kg/day), with HbA1c of 5.5-6.7%, and 5 Pts (39%) required basal/bolus insulin therapy (0.5-1 U/Kg/day, median 0.6) with a HbA1c of 6.8-10.2%, median 7.6. Insulin requirements did not correlate with BMI-SDS (r=0.18) or number of islets/kg infused (r=-0.21). Pts with PRSS1 mutation had borderline lower (p=0.05, t-test) insulin requirements (0.1 U/kg/day) than Pts with CFTR mutation (0.46 U/kg/day). Ten Pts (77%) discontinued pain medication with complete pain resolution within 3 months. Complications including pyloric stenosis, intra-abdominal adhesions, and gastroparesis occurred in 3 Pts; Spontaneous/exercise-induced hypoglycemia in 2 Pts.

Conclusions: TPIAT is an effective treatment for CP in children and adolescents. Within 6 months, it provided pain resolution in 77% and allowed good glycemic control with no insulin or low dose basal insulin in 61% of patients in this series.

1832-P

The Role and Mechanism of Exosomes in Regulation of β-Cell Function

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Objective: In this study, we used the exosomes derived from the pancreatic islet β cells and transplant the exosomes into the diabetic mice model to observe their effects on β cell function in vivo and explore the underline mechanisms.

Methods: Beta-cell lines were cultured in exosome-free medium for 48 hours. Ultracentrifugation method was used to collect the exosomes derived from the cells. Transmission Electron Microscopy (TEM), Tunable Resistive Pulse Sensing (TRPS) technique and Western blot were used to identify the exosomes. Streptozotozin was used to establish diabetic mouse model.

Results: With ultracentrifugation method, the exosomes we collected from beta-cell culture medium. TRPS exhibited the mean or median diameters of the exosome were 77nm or 101nm with a cup or round-shaped morphology. The Western blot showed that CD9 was highly expressed in the exosomes. Transplantation of the exosomes into diabetic mice resulted in a longer median survival time compared with control mice ($P < 0.01$). In mice with abnormal glucose tolerance, transplantation of the exosomes could not only improve the glucose tolerance, but increase the insulin contents in pancreas as well. Moreover, expression of CD31, a marker of endothelial cells, increased significantly in pancreas after the exosome transplantation.

Conclusion: Transplantation of the exosomes derived from beta-cells improved glucose metabolism in mice treated with STZ. Such protective effect was associated with increased CD31 expression in pancreas.

Supported By: Shanghai Natural Science Foundation (16ZR1425800)

INSULIN ACTION—ADIPOCYTE BIOLOGY

Moderated Poster Discussion: Signaling in Adipose Tissue
(Posters: 1833-P to 1838-P), see page 16.

1833-P

Characterizing the Wilms Tumor 1 (Wt1) Adipose Subpopulation

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Although white adipose tissue was once thought to serve only as an energy reservoir, it is now known to play an active role in energy homeostasis and insulin sensitivity. Excess visceral fat has been strongly associated with an increased risk of metabolic and cardiovascular diseases. Wilms Tumor 1 (Wt1) has been shown to be expressed in mesothelial cells and a major regulator of developing kidney and heart. Notably, a subset of visceral adipocytes has been shown to be derived from Wt1 positive mesothelial cells, and adipocyte-specific ablation of Wt1 has been shown to lead to altered thermogenic and inflammatory pathways in adipose tissue. To further characterize the adipocyte population derived from the Wt1 lineage, we labelled these adipocytes by crossing the tamoxifen-induced Wt1-CreERT2 mice to the cell membrane-targeted $ROSA26^{mTmG}$ fluorescent Cre reporter mice. Recombination was induced during embryogenesis by a single administration of tamoxifen (8mg/40g body weight) at e14.5. At 6 months, adipose tissues from recombined mice were studied using confocal microscopy and fluorescence-activated cell sorting (FACS) analysis. Confocal microscopy revealed the presence of adipocytes from the Wt1 lineage in visceral depots, including the pericardical fat (22.9 +/- 8.8%), perigonadal fat (30.7 +/- 4.2%), and perirenal fat (18.0 +/- 2.9%). On the other hand, no adipocytes from the Wt1 lineage were observed in either subcutaneous depots examined, including the inguinal subcutaneous and scapular white fat. Moreover, a lineage-dependent effect on adipocyte size was not observed. FACS analysis showed that pre-adipocytes from the Wt1 lineage made up 10.0 +/- 3.7% and 6.6 +/- 4.1% of total adipocytes in perigonadal and perirenal fat, respectively, while pre-adipocytes from the Wt1 lineage were undetectable in the subcutaneous inguinal and scapular white fat. Future work will demonstrate the effects of high-fat diet induced obesity on Wt1 derived adipocytes and their roles in regulating adipocyte function and inflammation.

1834-P

Inverse Association between Fasting Insulin Levels and Postprandial Changes of Plasma Asprosin Concentration in Patients with Type 2 Diabetes

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Background and Aims: Asprosin, a fasting-induced protein secreted by adipose tissue, has recently been discovered as a glucogenic hormone that promotes hepatic glucose release in mice and humans. While circulating Asprosin levels are reported to physiologically decrease after feeding, the pathophysiological role of Asprosin in patients with glucose intolerance remains poorly understood. The aim of this study was to evaluate the relationship between Asprosin postprandial concentration kinetics and other biomarkers.

Materials and Methods: 11 healthy subjects and 23 type 2 diabetes mellitus (T2DM) patients underwent a 2-h meal tolerance test in the morning after an overnight fast; the meal consisted of 460 kcal of total caloric load with 56.5 g of carbohydrates, 18 g of protein and 18 g of fat. Blood samples were collected immediately before and 2 hrs after meals. HbA1c, fasting plasma glucose (FPG), total cholesterol and other biomarkers were measured in all subjects. Plasma Asprosin levels was determined by enzyme-linked immunosorbent assay according to the manufacturer's protocol (Eiaab, Catalogue No. E15190h). Correlations were evaluated by Spearman's rank test. P values <0.05 were considered statistically significant.

Results: Fasting Asprosin levels in healthy subjects were significantly less than those in T2DM patients ($p < 0.05$). In T2DM patients, postprandial reduction of Asprosin levels showed a significant negative association with fasting insulin levels ($r = -0.41$ $p < 0.05$). No significant correlation was found between Asprosin levels and other biomarkers including FPG.

Conclusion: To our knowledge, this is the first report of a negative correlation of fasting insulin levels with postprandial reduction in Asprosin levels and higher fasting Asprosin levels in T2DM patients than healthy controls. Our results suggest that insulin resistance could be associated with the regulation of circulating Asprosin levels.

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1835-P

DBC1, Deleted in Breast Cancer 1, Is a Novel STAT5A-Interacting Protein in Fat Cells

ASHLEY A. ABLE, ALLISON J. RICHARD, JACQUELINE M. STEPHENS, *Baton Rouge, LA*

STAT5A (signal transducer and activator of transcription 5A) is a transcription factor that has been shown in play a role in adipocyte development using both in vitro and in vivo approaches. In addition, STAT5 mediates the actions of growth hormone and prolactin. Despite well-documented evidence of the role of STAT5 in adipocyte differentiation, its specific roles in mature fat cells are largely unknown. To further understand the function of STAT5 proteins in adipocytes, we employed a non-biased co-immunoprecipitation and mass-spectrometry based approach to identify novel STAT5A-interacting proteins. One of the proteins we identified was DBC1, deleted in breast cancer 1. DBC1 is a protein that is typically localized in the nucleus and has been primarily studied in tumor cells. However, very little is known about the function of DBC1 in adipocytes. Using mouse fat cells, we confirmed that there is a physical interaction between endogenous STAT5A and DBC1 proteins under physiological conditions. Moreover, we have shown that this association occurs in the nucleus but is not dependent upon STAT5 tyrosine phosphorylation. Knockdown of DBC-1 in 3T3-L1 adipocytes was achieved to determine if a decrease of DBC1 can affect the ability of STAT5 to regulate gene expression. We have shown that the expression of five STAT5A target genes, including Socs3, Cish, Bcl-6, Socs2, and Igf-1, were not affected by a loss of DBC1 protein levels. These studies suggest that STAT5A transcriptional activity may not be affected by DBC1. On-going studies are being performed to examine the expression of additional STAT5A target genes and to determine the function of the DBC1/STAT5A interaction.

In summary, DBC1 is a novel STAT5A-associating protein in adipocyte nuclei.

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1836-P

ES1 Plays an Essential Role in Adipocyte Differentiation in Mice via Regulating Mitochondrial Function

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ES1 (C21orf33, also named KNP-I or GST335) is a ubiquitously expressed and abundant mitochondrial protein in metabolic active tissues in mouse and humans. However, it remains unclear what is the functional role of ES1 in mitochondrial structure and function. To study the functional role of ES1 in mamallian animals, we generated an ES1 knockout (ES1-KO) mouse line (C57/B6). Under the basal condition, the ES1-KO mice showed a slower increase of body weight in mice approached adulthood. MRI body composition analysis revealed that fat mass was not increased as much in the ES1-KO relative to the wild type (WT) mice littermates. Micro-CT scanning revealed that both sucutaneous and viscous fat in the ES1-KO mice were not as much as those of controls. Glucose tolerance assay revealed that the ES1 showed improved glucose toleration. Primary cultures of adipose derived stromal-vascular (SV) cells (preadipocytes) from both the ES1-KO and WT control mice showed that the differentiation of the ES1-KO preadipocytes were markedly repressed compared with the WT cells based on morphological features and Oil-red O staining. Additionally, markers of adipocytes such as aP2, CEBP/α and PPARγ were correspondingly lower in the ES1-KO than the WT controlled preadipocytes. We further investigated the molecular mechanisms underpinning the impaired adipocyte differentiation in ES1-KO mice. ES1 interacted with the α and β subunits of the ATP synthase F1 sector, promoting ATP synthesis and repressing ATP hydrolysis. As a result, the lack of ES1 in cells led to impaired mitochondrial respiration with decreased oxygen consumption. The results indicates that ES1 is a novel mitochondrial protein that plays a key role in regulating mitochondrial respiration and hence the differentiation of adipocytes. The impaired adipogenesis may slow the fat accumulation and distribution in adult mice and improved basal glucose tolerance. Therefore, ES1 may serve as a potential therapeutic target for obesity and type 2 diabetes.

1837-P

CB1-Receptor Antagonism in Fat-Fed Dogs Promotes Subcutaneous and Visceral Adipose Tissue Beiging Mainly via Activation of the Sympathetic Nervous System and Not Direct Effect of the Drug

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We have recently demonstrated, that after 16 weeks of high fat diet (HFD), dogs treated with Rimonabant (RIM) had an upregulation of UCP1, PGC1α, Prdm16, β3-adrenergic receptors and other genes involved in adipose tissue beiging in the subcutaneous (SC) and visceral (VIS) depots when compared to control treated dogs. The mechanism(s) by which the CB1-receptor antagonist RIM promotes adipose tissue beiging is unknown. We hypothesized that the beiging effect might be induced via the sympathetic nervous system (SNS) and not via a direct effect of RIM on adipocytes per se. To test whether the RIM effect was direct, primary adipocytes were isolated from VIS depot from dogs fed the HFD. The effects of RIM and the β_3-adrenergic receptor agonist CL-316253 were studied in vitro following application to adipocytes using two different time points, 4 and 24 hours (h). To test the ability of the cells to 'beige' ex vivo, we first used CL-316253 and found that it increased the expression of UCP1 by 2.4 fold after the 4 h exposure and 2.7 fold after the 24 h exposure. Additionally, we measured gene expression changes for PGC1α which was increased by 12 fold after 4 h and 5 fold after 24 h. Prdm16 expression increased by 4.7 fold after 4 and 2.7 after 24 h. In contrast to CL-316253, RIM did not directly affect the expression of the beiging genes. Whereas the CL compound can induce upregulation of markers of beiging in vitro, our data suggest RIM either alone or in combination with CL does not upregulate further, indicating that the ability of RIM to induce beiging requires direct sympathetic innervation of the adipocytes which may have its origin in the CNS. Increasing of the browning process in the SC and VIS depots is an important mechanism by which the CB1 antagonist regulates energy homeostasis.

1838-P

Blockade of Peripheral Cannabinoid 1 Receptor Attenuates Insulin Resistance through Inhibition of NLRP3 Inflammasome Activation in Diabetic Obese Mice

JI HYE HAN, JUN GI RHO, HAN HO SHIN, JUHWAN YOON, SUNGEUN HEO, SUNG HWA YOON, WOOK KIM, *Suwon, Republic of Korea*

Obesity and diabetes are associated with chronic mild inflammation and the NLRP3 inflammasome, a component of the innate immune system, instigates obesity-induced inflammation and insulin resistance through inducing the maturation of the inflammatory cytokines. Then, a recent article reported that Cannabinoid 1 receptors (CB1Rs) have been implicated in activation of the NLRP3 inflammasome in macrophages infiltrating into pancreatic islets, leading to β-cell loss in T2DM. Here, we show that blockade of CB1R inhibits infiltration of macrophages and the activation of the NLRP3 inflammasome in white adipose tissue (WAT) in db/db and diet-induced obesity (DIO) mice. Similar to a central CB1R antagonist (rimonabant), peripheral CB1R antagonists (AJ-5012 and AJ-5018) reverse or prevent these inflammatory responses and restore normoglycemia and insulin sensitivity. These findings implicate CB1R and NLRP3 inflammasome activation in insulin resistance and suggest macrophage-expressed CB1R as a therapeutic target in obesity and diabetes.

1839-P

C-C Chemokine Ligand 5 Gene Deletion Facilitates Beige Fat Biogenesis in Mice under Cold Exposure

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Emerging studies suggest that certain white adipose tissue (WAT) depots are able to convert to a "brown-like" state and is also an important site of thermogenesis especially under cold stress. However, the potential mechanism under the browning of WAT is largely unknown. The aim of our study was to investigate the role of C-C chemokine ligand 5 (CCL5) in the regulation of adaptive thermogenesis under long-term cold exposures. Here, using a CCL5 knockout mouse model, we found that both CCL5$^{-/-}$mice and wild type (Wt) mice suffered a drop in body weight after a 1-week exposure to 4 °C. CCL5$^{-/-}$mice showed a significant increase in the amount of beige adipocytes in inguinal WAT (iWAT) and epididymal WAT (eWAT) compared to those in Wt mice. Long-term exposure to cold increased the expression of UCP-1 and PGC-1α mRNA in both Wt and CCL5$^{-/-}$mice in BAT, iWAT and eWAT. However, the expression of UCP-1 protein level was greater in iWAT and eWAT of CCL5$^{-/-}$mice than in the Wt mice. Enhanced lipolytic activities in iWAT

For author disclosure information, see page A751.

Moderated Poster Discussion   ADA-Supported Research

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and eWAT of CCL5-/- mice were also indicated by increased protein level of adipocyte triglyceride lipase, a key lipase in adipocytes, when compared with Wt mice. To directly assess the role of adipocyte CCL5 in the browning of WAT, 3T3-F442A cells were engineered to silence CCL5 proteins using inducible silenced CCL5 upon doxycycline (Dox) addition during adipocyte differentiation period D0-D8. The mRNA levels of browning associated transcription factors such as PPAR-γ and CEBP-α and thermogenetic genes such as Prdm16 and PGC-1α significantly increased during D6 to D8 while Dox was added in 3T3-F442A adipocytes to induce CCL5 gene suppression. Taken together, the present finding suggests that CCL5 mediated signaling negatively regulates beige adipocyte biogenesis under cold exposure.

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1840-P

Bone Marrow Fat Relationship with Insulin Resistance (IR) and Adiponectin Secretion in Lean and Obese Women

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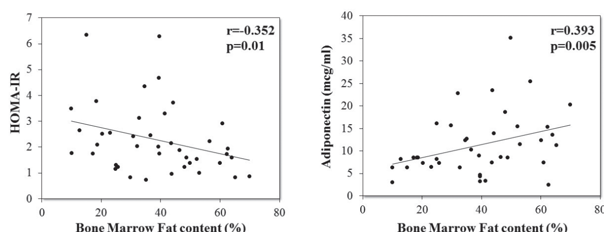
Intramyocellular ectopic fat correlates with IR. Fifty obese and nonobese premenopausal women underwent proton magnetic resonance spectroscopy to quantify vertebral bone marrow fat (BMF) content and unsaturation index (UI). Abdominal visceral, subcutaneous and epicardial fat were measured with magnetic resonance imaging. We measured fasting serum glucose, insulin, lipids, adiponectin and calculated HOMA-IR.

BMF content negatively correlated with insulin and HOMA-IR (r=-0.342; r=-0.352, respectively, p=0.01) and positively with adiponectin and HDL cholesterol (r=0.393, p=0.005; r=0.270, p=0.043, respectively). From a multivariate regression model including lnHOMA-IR as dependent variable and visceral, subcutaneous, epicardial and BMF as independent variables lnHOMA-IR was significantly associated with BMF content (β=-0.008±0.004, p=0.04) and subcutaneous fat (β=0.003±0.001, p=0.04). BMF, among the other adipose depots, was a significant predictor of circulating adiponectin (β=0.147±0.060, p=0.021). BMF content and UI were similar in obese and nonobese women while visceral fat negatively correlated with BMF UI (r=-0.316, p=0.026).

In conclusion, in premenopausal women BMF correlates with insulin sensitivity, possibly through adiponectin release. The amount of visceral fat may affect BMF composition. BMF may be a valuable predictor of whole-body IR.

Figure.



1841-P

Inhibition of Either HDAC5 or -6 Can Significantly Modulate the Expression of Specific Growth Hormone-Regulated STAT5 Target Genes in Adipocytes

PAULA MOTA DE SA, ALLISON J. RICHARD, JACQUELINE M. STEPHENS, *Baton Rouge, LA*

Unpublished studies from the lab have revealed that the transcription factor STAT5 physically associates with pyruvate dehydrogenase complex (PDC) in both mouse and human adipocytes following growth hormone stimulation. This interaction occurs in the nucleus. This is surprising since PDC is normally found in the mitochondrial matrix where it converts pyruvate into acetyl-CoA. The discovery of PDC in the adipocyte nucleus and its interaction with STAT5 suggests that PDC could be providing acetyl-CoA for acetylation of proteins in the STAT5 transcriptional complex. Since histone deacetylases (HDACs) are responsible for removing acetyl groups from proteins, we used general and specific HDAC inhibitors to examine changes in the expression of STAT5 target genes as well as HDAC translocation to the nucleus upon GH stimulation. Treatment of adipocytes with two non-specific HDAC inhibitors, TSA and sodium butyrate, increased expression of the STAT5 target gene SOCS3 while decreasing the expression of CISH, another STAT5 target gene. The same results were obtained when HDAC5 and 6 were specifically inhibited. However, the use of HDAC1 or 3 inhibitors or SIRT inhibitors did not affect the expression of SOCS3 or CISH in adipocytes. Moreover, GH treatment promoted the translocation of HDAC5 from the cytosol to the nucleus in murine adipocytes. Our results demonstrating that inhibition of HDAC5 or 6 modulates the expression of STAT5 target genes suggests that protein acetylation within the STAT5 transcriptional complex plays a significant role in the ability of STAT5 to both increase and decrease transcriptional regulation of specific genes. These novel observations are being further investigated to identify the specific proteins that are acetylated in the STAT5 transcriptional complex.

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1842-P

Lipocalin-2 Suppresses Autophagy and Promotes Beiging of White Adipose Tissue

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We have previously characterized lipocalin-2 (Lcn2) as a positive regulator of brown adipose tissue (BAT) thermogenesis. Recent investigations have shown that inhibition of autophagy controls BAT thermogenesis and beiging of white adipose tissue. Herein, we determined the role of Lcn2 in autophagy and WAT beiging by utilizing both Lcn2 gain- and loss-of-function mouse models. WT and Lcn2-/- mice were fasted for 24 h to induce autophagy, resulting in increased LC3-II, beclin-1, and atg7 protein levels in Lcn2-/- inguinal WAT (iWAT) vs. WT. After 6 hours of refeeding, autophagy was suppressed as demonstrated by decreased LC3-II and increased p62 levels in WT iWAT, while less suppression of autophagy was observed in Lcn2-/- iWAT. Primary differentiated Lcn2-/- inguinal adipocytes had higher levels of LC3-II and lower levels of p62 under both nutrient-starved and sufficient conditions. Further, recruitment of LC3-II and p62 to the mitochondria was increased in Lcn2-/- inguinal adipocytes in the presence of sufficient nutrients and insulin, suggesting increased mitophagy in the absence of Lcn2. After stimulation with nutrients and insulin following 3 h starvation, Lcn2-/- inguinal adipocytes had decreased activation of Akt-mTORC1-ULK1 Ser757 pathway, suggesting an ineffective suppression of autophagy in the absence of Lcn2. Overexpression of adipose Lcn2 in ap2-driven Lcn2 transgenic (Lcn2 Tg) mice resulted in lower levels of LC3-II and higher levels of p62 along with significantly increased UCP1 and PGC1-α gene expression in iWAT. Lcn2 Tg mice developed decreased fat mass and inguinal adipocyte size on a regular chow diet and maintained a higher body temperature after 3 h cold exposure. Additionally, Lcn2 Tg mice had a trend towards a lower respiratory exchange ratio compared to WT mice, suggesting increased preference for fat oxidation. Together, this data indicates a crucial role of Lcn2 in promoting autophagy inhibition and activating a beige-adipocyte phenotype in iWAT.

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INSULIN ACTION—CELLULAR AND MOLECULAR METABOLISM

Moderated Poster Discussion: Cellular and Molecular Metabolism *(Posters: 1843-P to 1848-P), see page 19.*

1843-P

Genetic Ablation of Acetyl-CoA Carboxylase 2 Reduces Intramyocellular Lipid without Competing Glucose Utilization and Prevents Lipid-Induced Insulin Resistance

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Intramyocellular lipid (IMCL) accumulation in skeletal muscle greatly contributes to lipid-induced insulin resistance. Since acetyl-CoA carboxylase 2 (ACC2) negatively modulates mitochondrial fatty acid oxidation (FAO) in skeletal muscle, inhibition of ACC2 is expected to reduce IMCL by enhancing FAO and attenuate insulin resistance. Contrary to this concept, there is the substrate competition theory that enhanced FAO results in reduced glucose utilization due to excessive acetyl-CoA pool in mitochondria, providing a plausible explanation for lipid-induced insulin resistance. Here, we generated ACC2 knockout (KO) mice and examined energy substrate metabolism in whole-body and skeletal muscle, focusing on the balance of glucose and lipid utilization. KO mice showed an elevation in whole-body energy expen-

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Insulin Action/ Molecular Metabolism POSTERS

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diture and a higher capacity to cope with either glucose or lipid overload. Notably, muscle metabolite analysis revealed that reduced IMCL levels in KO mice [68% lower than wild type (WT) mice, $p < 0.05$] were accompanied with decreased acetyl-CoA content (38% lower than WT mice, $p < 0.001$). The levels of acetylcarnitine and TCA-cycle metabolites, the major products of acetyl-CoA metabolism in mitochondria, were significantly higher in muscle of KO mice than that of WT mice. Furthermore, similar differences in metabolites were also observed in mice fed on a high-fat diet (HFD). KO mice showed a marked improvement in HFD-induced insulin resistance as reflected by a 1.9-fold increase in the steady-state glucose infusion rate in hyperinsulinemic-euglycemic clamp, compared to WT mice ($p < 0.05$). These findings suggest that both FAO and glucose utilization work actively in ACC2-deleted skeletal muscle via elevation of acetyl-CoA metabolism, offering new mechanistic insight into improvement in insulin resistance by ACC2 inhibition.

1844-P

NAT1 Ablation Causes Insulin Resistance and Hepatic Steatosis Associated with Mitochondrial Dysfunction in Mice

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GWAS has identified a single nucleotide polymorphism in the human arylamine N-acetyltransferase 2 (NAT2) gene to be associated with insulin resistance. To understand the mechanism by which alterations in NAT2 activity might cause insulin resistance, we examined the murine ortholog NAT1 knockout (NAT1 KO) mice fed either a regular chow (RC) or high-fat (HFD) diet. NAT1 KO mice manifested whole-body insulin resistance as reflected by lower glucose infusion rates (RC: 25% P<0.01, HFD: 62% P<0.001 vs. WT) during a hyperinsulinemic-euglycemic clamp, which could be attributed to both reduced suppression of endogenous glucose production (RC: 62% P<0.01, HFD: 85% P<0.001 vs. WT) and reduced insulin-stimulated muscle glucose uptake (37% RC, 30% HFD, P<0.01 vs. WT). Insulin resistance was associated with marked increases in both liver and muscle triglyceride (Liver: 58% RC, 108% HFD, P<0.01 vs. WT; Muscle: 160% RC, 105% HFD, P<0.01 vs. WT) and diacylglycerol (DAG) (Liver: 58% RC, 71% HFD, P<0.01 vs. WT, Muscle: 89% RC, 45% HFD, P<0.01 vs. WT) content. Increased liver and muscle DAG content was associated with increased activity of both hepatic PKCε (74% RC, 65% HFD, P<0.01 vs. WT) and muscle PKCθ (90% RC, 55% HFD, P<0.01 vs. WT). NAT1 KO mice also displayed reduced whole-body energy expenditure (10%, P<0.05 vs. WT). Consistent with these results, oxygen consumption was reduced by 44%, 55%, and 34% in white adipose tissue, brown adipose tissue, and hepatocytes, respectively obtained from NAT1 KO mice.

In conclusion, NAT1 KO mice manifest liver and muscle insulin resistance, which in turn can be attributed to reduced mitochondrial activity. Reduced mitochondrial energy expenditure in turn predisposes NAT1 KO mice to increased ectopic lipid (DAG) accumulation in liver and muscle, nPKC activation and hepatic and skeletal muscle insulin resistance. These studies provide new insights into how variants in the NAT2 gene may predispose humans to insulin resistance and type 2 diabetes.

1845-P

Persistent Vitamin C Deficiency Promotes Insulin Resistance and Dyslipidemia in Mice

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Rationale: An inverse relationship between plasma vitamin C (VitC) levels and the incidence of type 2 diabetes (T2DM) has been suggested. VitC deficiency, an unrecognized condition, may be contributing to this increased incidence of T2DM. Unlike animals, humans are dependent upon dietary VitC due to mutations in L-gulono-γ-lactone oxidase (Gulo), the final enzyme for VitC biosynthesis. Therefore, there is a need to generate humanized animal models to determine whether low VitC levels predispose to T2DM. We addressed this by using $Gulo^{-/-}$ knockout mice which, like humans, require VitC in their diet.

Methods: VitC "sufficient" $Gulo^{-/-}$ mice were fed ad libitum with regular chow and water supplemented with VitC (0.330 g/L). VitC "deficient" mice were generated by reducing VitC supplementation (0.033 g/L) for 12 weeks. Plasma insulin levels were determined using a Mouse Insulin ELISA Kit. Blood glucose was monitored using an Accu-Chek glucometer. Real time QPCR was used to examine liver expression of genes associated with lipogenesis and lipid oxidation such as Acaca, Cpt1a, Fasn, Ppar-α and SREBP.

Results: Over a 12 week period, VitC "deficient" mice progressively gained weight, with significant increases being observed as early as 4 weeks (p<0.05). VitC "deficient" mice had significantly higher baseline circulating insulin levels and were also glucose intolerant (p<0.05). They also had significantly higher HOMA-IR (4.2 vs. 1.1, p<0.05). In addition to circulating lipid abnormalities, VitC "deficient" mice exhibited increased lipid deposition in livers and higher mRNA expression of genes associated with lipogenesis.

Conclusions: Induction of VitC deficiency in $Gulo^{-/-}$ mice was associated with a phenotype characterized by insulin resistance, weight gain, dyslipidemia and hepatic steatosis. VitC supplementation could potentially be used as a preventative measure to delay the onset of insulin resistance and metabolic syndrome.

1846-P

Stimulation of Cardiac Glucose Oxidation by Insulin Glargine Improves Cardiac Function in db/db Mice

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Diabetes increases the risk of developing cardiovascular disease. In diabetes, the heart has decreased glucose oxidation rates and increased fatty acid oxidation rates. These changes in energy metabolism decrease cardiac efficiency and contribute to decreased cardiac function. The ability of insulin to stimulate glucose oxidation and inhibit fatty acid oxidation may be beneficial. We treated 18 week old db/db mice, a well characterized model of diabetes and diastolic dysfunction, for 4 weeks with either vehicle, NPH insulin (150 U/kg BW), insulin degludec (Deg, 150 U/kg BW), or insulin glargine (Gla, 150 U/kg BW). At the end of the treatment period we assessed in vivo cardiac function via echocardiography (Echo). Long acting insulins improved whole body glucose tolerance comparably as measured by OGTT and fasting blood glucose levels. Echo did not show any negative effect on cardiac function of any long acting insulin. However, compared to vehicle, Gla improved db/db mouse cardiac output (vehicle, 23±2; Gla, 28±2 ml/min) and stroke volume (vehicle, 52±3; Gla, 66±4 ml). If the in vivo active Gla metabolite M1 (500 μU/ml) was added acutely to isolated working db/db mouse hearts, stimulation of glucose oxidation (from 189.8±38.9 to 528.0±81.2 nmol·g dry $wt^{-1} \cdot min^{-1}$) and inhibition of fatty acid oxidation (from 985.7±128.5 to 594.9±59.4 nmol·g dry $wt^{-1} \cdot min^{-1}$) was seen. This contrasted with the effect of insulin (500 μU/ml) which inhibited cardiac fatty acid oxidation but had no significant effect on glucose oxidation, and Deg (1000 μU/ml) which had no effect on cardiac glucose oxidation or fatty acid oxidation. Effects of Gla were associated with improved cardiac insulin signaling, as evidenced by increase in p-Akt and decrease in p-IRS (ser307). We speculate that stimulation of glucose oxidation may be a contributing factor to the improved cardiac function seen after chronic administration of Gla in vivo. Our data suggest that Gla may reduce cardiac dysfunction in diabetes.

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1847-P

FoxO Transcription Factors Regulate Diabetes-Induced Muscle Atrophy

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Uncontrolled type 1 diabetes is a catabolic state with loss of both fat and lean mass. Muscle atrophy, characterized by loss of muscle mass or strength, can lead to disability, impaired recovery from illness or increased mortality. FoxO transcription factors (of which three isoforms FoxO1, FoxO3 and FoxO4, are expressed in muscle) are suppressed by insulin signaling and are critical regulators of muscle atrophy in response to starvation or denervation, but their role in diabetic muscle disease is incompletely characterized. To determine the role of FoxOs in muscle loss from insulin-deficient diabetes, we created mice with muscle specific knockout of FoxO1, O3, and O4 (M-FoxO TKO), and induced diabetes with streptozotocin (STZ). STZ treatment induced diabetes equally in both control and M-FoxO TKO mice 14 days after STZ. Fed blood glucoses were >500 mg/dl and insulin levels decreased by >75% compared to non-STZ treated mice. While individual muscle weights and myofiber cross sectional area decreased by 22% at day 14 in STZ-diabetic mice, muscle loss was prevented in diabetic M-FoxO TKO mice and body weight loss was also mitigated. Protein markers of autophagy such as ULK1, p62, and LC3-II, were increased in muscle from STZ diabetic mice relative to controls, but remained unchanged in diabetic M-FoxO TKO. STZ-diabetes increased mRNA levels of autophagy markers, Ulk1, LC3A, Bnip3L, and Gabarapl, as well as proteasomal E3-ubiquitin ligases Atrogin-1 and MuRF-1, but these increases were again prevented in diabetic M-FoxO TKO. To determine if these changes were relevant in humans, we in obtained cDNA of muscle biopsies from 8 individuals with type 1 diabetes before and after 8

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hours of withdrawal from insulin. Expression of the FoxO target p27^{Kip1} as well as Bnip3L, Gabarapl, Atrogin-1 and MuRF-1 all increased by 1.4-3.3 fold after insulin withdrawal. These data indicate that insulin-deficient diabetes induces protein degradation pathways in muscle leading to muscle atrophy, which is mediated by FoxO transcription factors.

1848-P

WITHDRAWN

1849-P

Metabolomic Profiling of Dynamic and Basal Measures of Glucose Homeostasis Identifies Distinct Metabolic Signatures: The Insulin Resistance Atherosclerosis Family Study (IRASFS)

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Metabolomics provides biochemical fingerprints which, coupled with clinical phenotypes, provide disease insight. We evaluated signatures of dynamic and basal glucose homeostasis in a cross-sectional, pedigree-based analysis of 1,034 Hispanics, an ethnic minority disproportionately impacted by metabolic disease. Univariate analysis with metabolite predictors (n=801) was adjusting for age, gender, center and BMI. Insulin sensitivity (S_I), a dynamic measure (intravenous glucose tolerance test, FSIGT), was associated with 92 metabolites (P<6.2E-5, threshold for 801 tests). Metabolites explained 42% of the variance in S_I as compared to 28% by BMI. Signatures of amino acids (proportion of variance, R^2=9%), phospholipids (R^2=15%) and long chain fatty acids (R^2=11%) persisted after adjusting for dietary fat intake, suggesting the influence of fat storage. These signals were minimal for basal measures, i.e., fasting glucose and homeostasis model assessment of insulin resistance ($HOMA_{IR}$) (R^2<7%). Signatures for acute insulin response (dynamic measure of insulin secretion, FSIGT), identified three metabolites (glucose, R^2=15%). The complementary basal measure ($HOMA_{2B}$) had significant signatures from the urea cycle (R^2=8%), suggesting utilization of amino acids as energy under basal conditions or increased protein degradation in insulin resistance. Prevalent T2D was associated with 64 metabolites including glucose (P=3.5E-57) and 1,5-anhydroglucitol (P=8.0E-53; two week glucose). The latter had a stronger genetic component (heritability=0.53) making it a target for genetic studies. Half of T2D metabolites (n=31) were not associated with basal or dynamic measures of glucose homeostasis. Metabolomic analysis reveals distinct signatures which differentiate dynamic and basal measures of glucose homeostasis providing unique insight into the pathophysiology of metabolic disease.

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1850-P

RhoGDIα in the Regulation of Insulin-Stimulated Rac1 Activity and Glucose Metabolism in Skeletal Muscle

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Introduction: Skeletal muscle is important in the regulation of whole body glucose homeostasis. The small GTPase Rac1 controls insulin-stimulated glucose uptake into skeletal muscle, but it is unresolved how Rac1 activity is regulated. In non-muscle cells, the GDP-dissociation inhibitor, RhoGDIα keeps Rac1 in the inactive GDP-loaded state and interestingly, in adipocytes RhoGDIα has been shown to regulate insulin-stimulated GLUT4 translocation.

Methods: To investigate the role of RhoGDIα in skeletal muscle glucose metabolism, L6 rat skeletal muscle cells overexpressing myc-tagged GLUT4 (L6-GLUT4myc) were transfected with siRNA targeting RhoGDIα and insulin-stimulated Rac1 activity and GLUT4 translocation were measured. Furthermore, RhoGDIα was overexpressed specifically in skeletal muscle of mice after a single intravenous injection using adeno-associated virus-mediated delivery of DNA. Mice were fed a high fat diet (HFD, 60E%) or standard chow diet and body weight assessed every week. At week 6, body composition and glucose tolerance (i.p. 2 g glucose/kg body weight) were investigated.

Results: Reducing RhoGDIα protein content in L6-GLUT4myc cells increased Rac1 activity in both the basal and insulin-stimulated state compared to control cells (p<0.001). This was followed by a ~35% and ~15% increase in basal and insulin-stimulated GLUT4 translocation to the surface membrane, respectively, compared to control cells (p<0.001). Body composition was similar in mice overexpressing RhoGDIα and control mice. Interestingly, mice overexpressing RhoGDIα displayed markedly impaired glucose tolerance when fed both HFD and chow compared to control mice (p<0.05) despite a tendency to augmented insulin response (p<0.06).

Conclusion: RhoGDIα is a negative regulator of Rac1 activity and GLUT4 translocation in skeletal muscle cells and impairs whole body glucose tolerance when overexpressed in mouse skeletal muscle.

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1851-P

Effect of Insulin-Degrading Enzyme Inhibitors on Cellular Insulin Processing and Degradation

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It has long been suggested that inhibition of insulin-degrading enzyme (IDE) would be a means to treat diabetes, by increasing the availability of secreted insulin to peripheral tissues. This potential therapy has been hampered by the lack of a specific inhibitor that does not have effects on other proteases. However, specific inhibitors have recently become available. This strategy presumes that insulin clearance is dependent primarily on the activity of IDE, despite the fact that cellular degradation is rate limited by insulin binding and internalization. We examined the effect of two IDE inhibitors, 6bK and ML345, on cell association and degradation by HuH-7 cells (N=4 separate experiments). Cells were preincubated with vehicle, 6bK (1 uM) or ML345 (5 uM); concentrations that should inhibit greater than 90% and 85% of IDE activity, respectively. ^{125}I-insulin was added and after 2 hours medium assayed for degradation by trichloroacetic acid precipitation. Cells were washed with PBS and collected for determination of cell associated radioactivity. 6bK and ML345 inhibited insulin degradation by 51% and 37%, respectively (p<0.001 for both). Further, 6bK increased the cell associated ^{125}I by 40% (p<0.05) and ML345 increased it 24%, though it did not reach statistical significance. Our results indicate that while IDE inhibitors can decrease insulin degradation by hepatocytes, the inhibition is less than expected if IDE is solely responsible. This suggests there is a redundant pathway for insulin degradation. A likely candidate is cathepsin D, a lysosomal enzyme that has been shown to degrade insulin. Alternatively, the retention of insulin suggests inhibition of IDE alters receptor recycling, thereby limiting the amount of insulin that is internalized and degraded within the cell. Our results indicate insulin degradation is not solely dependent on IDE, and suggest use of IDE inhibitors to control insulin clearance may not be entirely effective.

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1852-P

Sumoylation, a Novel Regulator of HNF-1A Protein Function

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Hepatocyte nuclear factor-1 alpha (HNF-1A) is a transcription factor regulating several pancreas and liver specific genes. Mutations in HNF1A are associated with Maturity-Onset Diabetes of the Young (MODY3; HNF1A-MODY). Mechanisms for tissue-specific regulation of HNF-1A, including the role and function of post-translational modifications, have so far been poorly investigated. In this study, we show the first evidence that HNF-1A is a substrate of SUMOylation both in vitro and in cellullo and that its level of SUMOylation is increased in transiently transfected HEK293 when co-transfecting with the SUMO E3 ligase; protein inhibitor of activated STAT (PIASγ). Using a rat albumin promoter-linked luciferase reporter assay in transiently transfected MIN6 β-cells, HNF-1A transactivation was found reduced when co-transfecting with human SUMO-2/3, Ubc9 (E2) and PIASγ. Overexpression of PIASγ alone reduced the activity even more, suggesting that the repression of HNF-1A transcriptional activity by PIASγ is SUMOylation-independent. Moreover, we identified three lysines (K) as potential SUMOylation sites in HNF-1A; K205, K273 and K506. Interestingly, overexpression of the SUMOylation machinery in K506R transfected in HEK293 cells seemed to considerably reduce the protein level of HNF-1A. Whether SUMOylation of HNF-1A influences glucose-stimulated insulin secretion, will be investigated in the future.

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1853-P

Targeting PHD3 Using Antisense Oligonucleotides for the Treatment of Type 2 Diabetes

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Type 2 diabetes is a chronic metabolic disorder characterized by high blood glucose, insulin resistance, and/or relative lack of insulin. It can cause a number of complications including cardiovascular disease, kidney failure, and diabetic retinopathy. As of 2015, an estimated 415 million people were affected by type 2 diabetes.

Prolyl hydroxylases (PHDs) are enzymes that regulate the ubiquitination and proteasomal degradation of transcription factors known as hypoxia inducible factors (HIFs). Under normoxia, PHDs hydroxylate proline residues on Hif-1α and Hif-2α resulting in von Hippel-Lindau tumor suppressor-dependent proteasomal degradation. It has been shown that acute deletion of hepatic PHD3 improves gluconeogenesis and insulin sensitivity in mice on a 6-week high fat diet (Taniguchi et al. Nature Med 2013). Using a selective antisense oligonucleotide (ASO) against PHD3, we evaluated the effects of PHD3 inhibition in a high fat diet (HFD) induced type 2 diabetes mouse model.

C57B/6 mice were treated with a PHD3 ASO for 6 weeks after being placed on a 60% high fat diet (D12492, Research Diets) for 15 weeks. ASO treatment resulted in hepatic PHD3 mRNA reduction of 97% causing an 83% decrease in liver triglyceride levels and a decrease in total liver lipid accumulation based on Oil Red O staining. A glucose tolerance test demonstrated 24% improvement in glucose tolerance (AUC) and a 22% reduction in fasted plasma glucose levels compared to the control group. An insulin tolerance test showed 23% improvement in insulin tolerance (AUC) compared to controls. These data demonstrate that ASO-mediated reductions in PHD3 improve glucose tolerance and insulin sensitivity, in addition to other metabolic endpoints, in HFD-fed mice. Inhibition of PHD3 could be a potential therapeutic approach for people affected by type 2 diabetes.

1854-P

TP63 Is a New Candidate Gene Involved in Insulin Resistance

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Background: Insulin resistance is key player in the development diabetes complication. Several evidences suggest that metabolic abnormalities cause renal dysfunction and inflammation, play a major role in precipitating diabetic kidney disease.

Methods: RNA extracted from kidney of type 2 diabetic mice (db/db) at age of 10 months were sequenced to identify genes that involved in insulin resistant. In addition, siRNA of TP63 was transfected into mouse renal proximal tubular cells exposed to high glucose (HG) for 48 h. Other genetic manipulations to upregulation of tuberin and activation of AMPK were performed to determine the mechanism by which TP63 regulates IRS-1 under hyperglycemia conditions.

Results: RNA sequencing and qRT-PCR showed 3-fold increase in TP63 expression in 10 months of kidney of diabetic mice compared to wild type mice. TP63 protein expression measured by Western blot analysis and immunostaining showed significant increase in proximal tubular cells in kidney sections of diabetic mice compared to wild type mice. Cells treated with HG for 48 hrs showed significant increase in TP63 compared to cells grown in normal glucose (NC). Downregulation of TP63 by siRNA significantly increased IRS1 and activated AMPK (p-AMPK-Th^{172}) under NG and HG conditions. In addition, activation of AMPK by pretreated the cells with AICAR resulted in significant downregulation of of TP-63 and increased IRS1 expression. Moreover, upregulation of tuberin by infected the cells with Ad-c-DNA resulted in significant decrease in TP63 and upreguation of IRS-1 expression under NG and HG conditions.

Conclusion: Our data suggest that TP63 is a major regulator of IRS-1. Hyperglycemia enhances TP63 (RNA/Protein) expression to involve in degradation of IRS-1 and induce insulin resistant. These data showed a potential role of TP63 as a new candidate gene involves in insulin resistant in diabetes that may be used as a new therapeutic target to prevent insulin resistant in diabetes.

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1855-P

CA 19-9 and Glucose Tolerance in Cystic Fibrosis

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CA19-9 (carbohydrate antigen 19-9) is synthesized by normal human pancreatic and gallbladder ductal cells and by gastric, colon, and salivary epithelia. This short carbohydrate motif is attached to glyco-lipids or -proteins including membrane mucins. CA 19-9 is present in small amounts in serum. Its estimates are hampered by the influence of the Lewis histo-blood group system. It can be over expressed in several benign or malignant digestive tract disorders. Otherwise, it is used as an immuno-histochemical marker of pancreatic ductal cells, which have been shown to be able to differentiate in beta cells.

The aim of this work was to study the relationship between CA19-9 and glucose tolerance. A cohort of 187 cystic fibrosis patients followed in a reference center with a yearly assessment was split into 2 groups according to the CA19-9 blood level < (n= 111) or > (n=76) 36UI/L. Compared to the normal CA19-9 group, the high CA19-9 group was younger both at the age of assessment (27±10 vs. 34±14 years; p=0.009) and of diabetes occurrence (22±6 vs. 38±14 years), was more frequently delta F508 homozygous, had a lower BMI (19±2 vs. 22±4 kg/m2(all 3 p<0.0001) and VEMS (61±26 vs. 72±28; p=0.006), a higher alkaline phosphatasis level (146±113 vs. 92±82 UI/L; p=0.002), a higher need for pancreatic extracts (200 000 vs. 150 000 U/d; p<0.0001)), and a higher 2 hours-OGTT (154±67 vs. 116± 60 mg/dL; p=0.0002) and CGM-maximum glucose (250±76 vs. 200±75 mg/dL; p=0.02). CVF, A1c, CGM-SD tended to differ (p=0.07 to 0.08). FBG, CGM mean and minimum glucose were similar. Also, compared to patients with normal glucose tolerance CA19-9 levels were significantly higher in patients with glucose intolerance (p=0.006) or diabetes (p=0.0003).

Conclusion: CA19-9 is associated with markers of cystic fibrosis severity in terms of age, BMI, VEMS, liver disease, genetics, but also exo- and endocrine pancreatic dysfunction. High blood glucose could stimulate synthesis/proliferation of ductal cells or alterations of mucins related to the CFTR mutation could induce CA19-9 increase.

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INSULIN ACTION—GLUCOSE TRANSPORT AND INSULIN RESISTANCE IN VITRO

1856-P

Insulin Resistance Is Associated with Iron Overload through the Transferrin Receptor 1 in Human Skeletal Muscle Cells and Muscle Biopsies from T2DM Patients

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Altered iron homeostasis is associated with insulin resistance (IR) and diabetes. Iron perturbations are well studied in several tissues, such as adipose tissue and the liver in diabetic animal models. However, the relationship between iron metabolism and IR in skeletal muscle has not been well studied. In this study, we investigated the relationship and molecular mechanism between iron overload and IR in human skeletal muscle cells and

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muscle biopsies from patients with type 2 diabetes (T2DM). To clarify the iron homeostatic brake in IR muscle cells and muscle biopsies from patients with T2DM, we measured iron metabolism-related protein levels such as transferrin receptor 1 (TfR1), heavy chain ferritin (FTH), light chain ferritin (FTL) and divalent metal transporter 1 (DMT1). Immunoblotting analysis showed that treating human skeletal muscle cells with palmitate increased the levels of TfR1 and FTH, but not iron regulatory protein 1. In addition, the levels of TfR1, FTH, FTL and DMT1 increased in muscle biopsies from patients with T2DM compared to those in normal subjects. In addition, the intracellular labile iron pool increased in palmitate-induced IR skeletal muscle cells. Knockdown of TfR1 using siRNA in human skeletal muscle cells protected against palmitate-induced IR and preserved the intracellular iron pool. Treatment with an iron donor ($FeSo_4$ or $FeCl_3$) stimulated phospho-JNK and phospho-p38 and significantly induced mitochondrial dysfunction in human skeletal muscle cells. In particular, the chemical iron chelator deferoxamine recovered mitochondrial dysfunction and palmitate-induced IR in human skeletal muscle cells. The current study showed that iron overload in skeletal muscle cells induced mitochondrial dysfunction and IR through TfR1, whereas reducing the intracellular iron overload protected against IR. Therefore, attempts to block iron overload might be a strategy for preventing IR and diabetes.

1857-P

Mechanically Physiological Microenvironment Sensitizes Primary Cardiomyocytes to Glucotoxicity: New In Vitro Diabetic Heart Research Model

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Type 2 diabetes patients exhibit cardiac insulin resistance and dysfunction. However, understanding of the impact of glucotoxicity on these pathological features in the diabetic heart is still incomplete. One reason could be the current limitation of in vitro cardiomyocyte culture in modeling disease conditions. Emerging evidence suggests that mechanical properties of the microenvironment affect cardiomyocyte functions. Nevertheless, the effect of high glucose on cardiomyocytes cultured on substrates whose stiffness matches that of the heart (approximately 15 kPa) is untested. Thus, we investigated the molecular events caused by high glucose in rat neonatal cardiomyocytes seeded on either 15 kPa polyacrylamide gels or glass coverslips. As reported previously, immunostaining actinin and F-actin revealed organized sarcomeres on both 15kPa gels and glass at the normal glucose level (5 mM). However, 15 mM glucose, a glucose level frequently observed in diabetic patients, deregulated these cytoskeletal structures in cells on 15 kPa gels, but not in cells on glass. Deregulation of cytoskeletal structures by high glucose was independent of alterations in osmolality. Mitochondrial membrane potential at 5mM glucose in cells on 15kPa gels was significantly higher than that in cells on glass. Furthermore, high glucose significantly decreased mitochondrial membrane potential in cardiomyocytes on 15kPa gels in a dose- and time-dependent manner, but not in cells on glass. Attenuated mitochondrial membrane potential was accompanied by an increased level of reactive oxygen species (ROS), and these high-glucose-dependent events were restored by ROS scavengers. Our data provide evidence that a mechanically physiological microenvironment enhances susceptibility of primary cardiomyocytes to moderately elevated glucose levels, as frequently observed in diabetic patients, providing an innovative model for diabetic heart research.

1858-P

ATP2B1 Silencing Increase Insulin Sensitivity through Facilitating Akt Activation via $Ca2^+$/Calmodulin Signaling Pathway and Ca^{2+}-Associated eNOS Activation in Endothelial Cell

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Endothelial cell insulin resistance may be partially responsible for the higher risk of atherosclerosis and cardiovascular disease in populations with insulin resistance and type 2 diabetes mellitus (T2DM). A genome-wide association study revealed a significant association between ATP2B1 and T2DM in two community-based cohorts from the Korea Association Resource Project. While, we know little about the implication of ATP2B1 on T2DM. In the present study we investigated the role of ATP2B1 in endothelial cell insulin sensitivity. Silencing of ATP2B1 resulted in enhanced intracellular calcium concentration and increased insulin-induced Akt activation than negative siRNA transfected HUVEC cells. Elevated insulin sensitivity mediated by ATP2B1 silencing was Ca^{2+}/calmodulin-dependent, as verified by the administrations of calcium chelator BAPTA-AM or a specific calmodulin antagonist W7. Moreover, higher levels of phosphorylation of eNOS (ser1177) were observed in ATP2B1 silencing HUVEC cells. In addition to BAPTA-AM and W7, L-NAME, a eNOS antagonist, abolished the insulin-induced Akt phosphorylation on Ser473 in both si-Neg and si-ATP2B1 endothelial cells. These results indicated that enhanced insulin sensitivity in ATP2B1 silencing endothelial cells was alternatively dependent on increasing intracellular Ca^{2+} and subsequently activating Ca^{2+}/calmodulin/eNOS/Akt signaling pathway.

In summary, silencing of ATP2B1 increased insulin sensitivity in endothelial cells through Ca^{2+}/calmodulin signaling pathway directly and also Ca^{2+}/calmodulin/eNOS/Akt signaling pathway.

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INSULIN ACTION—SIGNAL TRANSDUCTION, INSULIN, AND OTHER HORMONES

Moderated Poster Discussion: Signal Transduction *(Posters: 1859-P to 1863-P), see page 21.*

1859-P

SPARC Regulates GLUT4 Expression and Translocation through AMPK Mediated Signaling: Augmenting Effect of Metformin and Rosiglitazone

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Secreted Protein Acidic and Rich in Cysteine (SPARC) is a multifunction secretory protein. SPARC is secreted from human adipose tissue and is predominantly derived from adipocytes. SPARC dysregulation has been associated with a wide range of obesity-related disorders, including T2DM. In this study, the role of SPARC overexpression to GLUT4 was investigated in L6 myocytes. Overexpression of SPARC increased GLUT4 expression and translocation to the membrane in L6 myocytes. The expression of SPARC significantly increased both protein and mRNA levels of GLUT4, as compared to the respective control. Additionally, SPARC siRNA diminished GLUT4 protein expression. The results showed that GLUT4 levels increased almost 2-fold as a result of SPARC overexpression. We tested the effect of rosiglitazone and metformin on SPARC expression in L6 myocytes, treatment of muscle cells with rosiglitazone and metformin markedly increased expression of both SPARC protein and SPARC mRNA, and markedly enhanced the effect of SPARC on regulating GLUT4 expression. To understand the involvement of the AMPK pathway in SPARC-mediated regulation of the glucose metabolism signaling cascade, L6 myocytes were transfected with mock, empty vector, pSPARC or with siRNA against SPARC, After 24 hrs, the cells were incubated with rosiglitazone, metformin or AICAR for 12 hrs, Our result showed that rosiglitazone and metformin enhanced SPARC-stimulated GLUT4 expression and tranlocation that was mediated by AMPKα phosphorylation in L6 myocytes. These studies confirmed the active involvement of SPARC in regulating GLUT4 metabolism depending on the phosphorylation of AMPK.

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1860-P

Identification of Basolateral but No Luminal Distribution of Insulin and IGF-1 Receptors in Gut Epithelia

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Due to the robust barrier properties of the gastrointestinal tract, appreciable amounts of insulin are expected to remain unabsorbed following its oral administration. Given this, it is critical to understand the expression in the intestinal epithelia of receptors, such as the insulin and IGF-1 receptors (InsR and IGF-1R, respectively), which could potentially interact with the insulin present in the intestinal lumen.

In the present study, we investigated the luminal (apical) and basolateral distribution of InsR and IGF-1R via the assessment of insulin- and IGF-1-mediated signalling in in vitro systems of intestinal epithelial or goblet-cell function (i.e., Caco-2, T84 and HT29-MTX-E12 cell monolayers). In addition, insulin-mediated signalling was also investigated in rat intestinal tissue mounted in Ussing chambers.

Apical stimulation of the cell monolayers with either human insulin (HI) or IGF-1 did not result in significant InsR or IGF1-R activation. In contrast, application of the ligands on the basolateral side induced the phosphorylation of their respective receptors in a concentration-dependent manner and with EC_{50} values in the low nanomolar range. Mirroring the activation of the

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receptors, basolateral (but not apical) administration of HI or IGF-1 resulted in a clear phosphorylation of Akt.

Similarly, basolateral exposure of an enzymatically-stabilised insulin analogue to rat colonic mucosa induced a significant increase in Akt phosphorylation compared to that of apically-treated tissue ($p<0.05$).

Visualisation of the InsR in murine intestinal samples by means of immunohistochemical (IHC) analyses further confirmed its localisation in the basolateral membrane.

Collectively, these results indicate a clear basolateral distribution of InsR and IGF-1R in the intestinal epithelia, which essentially renders these receptors inaccessible to any unabsorbed insulin which may be present in the intestinal lumen following oral dosing of insulin.

1861-P

Studies in Insulin Receptor/Insulin-Like Growth Factor-1 Receptor Double Knock-Out Cells Imply a Role For Insulin-Like Growth Factor-2 Receptors in Pluripotency and Differentiation of Stem Cells

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Pluripotent stem cells (PSCs) express insulin receptor (IR), insulin-like growth factor-1 receptor (IGF1R), and insulin-like growth factor-2 receptor (IGF2R). IGF1R is essential to the maintenance of an undifferentiated state and is necessary for stem cell renewal. Although insulin-like growth factor-2 (IGF2) is well established as a significant modulator of embryonic growth through interactions with IGF1R, the role of IR and IGF2R remain elusive in PSCs. IGF2R is believed to be a scavenger receptor for IGF2; details of downstream signals mitigated by this interaction have yet to be fully explored. Here we report the relevance of IR, IGF1R and IGF2R in the maintenance of stem cell pluripotency and growth. We generated inducible PSCs (iPSCs) expressing only IGF2R by using IGF1R and IR double knockout mouse embryonic fibroblasts (DKO MEFs) from embryonic day 14.5 harboring double floxed IR and IGF1R sites using an adenoviral-Cre vector. DKO iPSCs were then derived using a cocktail of Yamanaka reprogramming factors. Six iPSC clones from 2 MEFs (3 from Cre-treated MEFs, 3 from Control-treated MEFs) were selected and propagated in a 2-inhibitor feeder-free system. DKO iPSCs had 37% decreased cell viability, 30% decreased cellular proliferation and a 5% decrease in cell diameter. Gene expression and protein analysis confirmed knock-out of IGF1R and IR in MEFs and iPSCs. Basal signaling analysis demonstrated that DKO cells had down regulation of key proteins involved in pluripotency and growth including phospho-Stat3, phospho-Akt, phospho-mTor and phospho-Erk. Interestingly, IGF2R protein levels were elevated in DKO iPSCs; this implicates IGF2 signaling in stem cell homeostasis and warrants further investigation. Studies of IR and IGF1R DKO iPSCs are useful in exploring the role of IR, IGF1R and IGF2R in stem cell proliferation and maintenance of pluripotency.

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1862-P

Bailcalin Attenuates Glucose Intolerance by Regulating Pancreatic Hormones in High-Fat-Induced Obese Diabetic Mice

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Objective: Baicalin, a naturally occurring flavonoid found in in the genus Scutellaria, was originally known as an allosteric modulator of the benzodiazepine sites of the γ-aminobutyric acid A receptor ($GABA_AR$) producing anxiolytic effects in mice without sedative or myorelaxant effects. Baicalin has been recently shown to exert metabolic effects by attenuating hyperglycemia-induced mitochondrial damage in β-cells in diabetic rats, high-fat diet- (HFD-) induced body weight gain, and lipid deposits in the liver and systemic inflammation in mice. This study investigated the effects of baicalin on islet functions and diabetic status in HFD-induced obese diabetic mice.

Methods: C57BL/6 mice were grouped into normal control, HF control, HF sham, and 4 different baicalin dose-administered groups (25, 50, 100, and 150 mg/kg). HFD containing 60% fat and water were fed ad libitum for 24 weeks. Designated doses of baicalin or 0.9% saline were administered intraperitoneally 5 d/wk. Body weight (BW) and conventional glucose homeostasis parameters (FPG, FPI, AUC-glucose, AUC-insulin, and etc.) were monitored; in addition, ex vivo glucose-stimulated insulin secretion (GSIS) and glucagon secretion (GSGS) with isolated islets were performed.

Results: Baicalin attenuated HFD-induced hyperglycemia in dose-dependent manner without affecting BW. Insulin secretion in response to high glucose stimulation (16.7mM) was significantly higher in islets isolated from the groups administered 50, 100, 150 mg/kg baicalin compared to HF control; glucagon secretion was significantly suppressed by high glucose stimulation in islets from the same groups.

Conclusion: This study showed that baicalin had positive effects on glycemic control by regulating secretion of insulin and glucagon, possibly through $GABA_AR$ mediation.

1863-P

TCF7L2 Regulates Pancreatic β-Cell Proliferation and Function through PI3K/Akt Signal Pathway

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Type 2 diabetes (T2DM) is the result of interplay between genetic predisposition and environment. Our group identified, for the first time, that variants in TCF7L2 was associated with diabetes in Chinese Han population in Shanghai district. Researches show that, variants in TCF7L2 are associated with decreased insulin secretion and declined sensitivity of insulin. But the underlying mechanism is still unclear. Using ChIP-seq technique, we previously found the gene Pik3r1 encoding p85 protein of PI3K was one of the target genes transcriptionally regulated by TCF7L2. In vitro experiments on min6 cells indicated that over expression of TCF7L2 inhibited expression of the gene Pik3r1 and its encoding protein PI3K p85 and activated Akt/BAD signaling pathway. With the increased concentrations of glucose or insulin in the culture of min6 cells, the expression of TCF7L2 increased and the gene Pik3r1 decreased, followed by the activation of Akt signaling. In vivo studies on high glucose perfusion rat models showed that after high glucose perfusion for 48 hs and 72 hs, islets proliferation and mass increased significantly. At the same time, TCF7L2 expression enhanced, p85 declining, along with the increased phosphorylations of Akt and BAD.

In summary, we presented evidence here that TCF7L2 activated PI3K/Akt signaling pathway by transcriptionally inhibiting Pik3r1 expression, thus stimulating insulin secretion and enhancing β cell proliferation. It is proposed that there may exist a TCF7L2-PI3K/Akt signaling pathway in β cell which exerts an important role in islets proliferation and insulin secretion.

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1864-P

iGlarLixi Combination Is More Effective in Activation of Insulin Receptor Downstream Signaling and Blood Glucose Lowering in db/db Mouse than Treatment with Insulin Glargine or Lixisenatide Alone

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Pharmacodynamic effects of low dose of iGlarLixi, a combination of insulin glargine (iGlar) and GLP-1R agonist of lixisenatide (Lixi), vs. either drug alone on tissue insulin signaling and blood glucose were evaluated in diabetic, insulin resistant (db/db) mice. 12-13 weeks old db/db received either 1 µg/kg SC Lixi, 0.3 U/kg SC iGlar or same doses as iGLarLixi. Akt phosphorylation status (pAkt) at 0.5 h were measured in various tissues (skeletal muscle, heart, adipose and liver) and compared in treated vs. control mice. Blood glucose (BG) levels were also determined 0.5 h and 1 h after injection. In both muscle and heart there was a highly significant increase in pAkt in db/db vs. lean control mice indicative of the insulin resistant state of db/db's. After Lixi and iGlar alone pAkt levels were further significantly increased vs. db/db. In muscle, pAkt was highest with iGlarLixi and showed statistical significance vs. both iGlar ($p<0.0001$) and Lixi ($p=0.0313$) alone. Similar effects were obtained in heart where iGlarLixi increased pAkt significantly more than iGlar ($p=0.0103$) and showed at least a trend towards higher pAkt vs. Lixi ($p=0.0596$) alone. In adipose and liver tissues pAkt was not significantly changed. BG was significantly higher in db/db compared to lean litter mates. Neither 1 µg/kg SC Lixi nor 0.3 U/kg SC iGlar had a significant effect on BG while the same doses of iGlarLixi significantly reduced BG at both 0.5 h ($p=0.013$) and 1 h ($p=0.0032$). Thus, it could be shown that the iGlarLixi improves insulin signaling in skeletal and heart muscle of diabetic, insulin resistant db/db mice. This resulted in BG lowering by iGlarLixi even at doses that were too low for being effective when given as monotreatment of iGlar or Lixi alone. These data confirm the synergistic effects obtained with iGlarLixi on the level of insulin receptor downstream signaling and glucose control in a model of type 2 diabetes.

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1865-P

Inhibition of Ceramidesynthase-6 as a Novel Therapeutic Approach to Treat Obesity and Type 2 Diabetes

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High fat diet couples with increased production of ceramides that contribute to development of insulin resistance and type 2 diabetes as amelioration of ceramide biosynthesis improves the metabolic phenotype. However, as sphingolipids are involved in multiple fundamental cellular processes, concerns exist about possible risks or adverse effects for treatment of chronic diseases. Therefore inhibiting the specific ceramide species, which are pathogenic offers new strategies for pharmaceutical intervention. In this perspective recent research demonstrated that specifically CerS6 mediated C16 ceramide plays a key role in the development of obesity mediated insulin resistance. Here we evaluated the therapeutic validity of CerS6 using anti-sense oligonucleotide (ASO) to knock-down the protein in three different rodent models for diabetes and obesity. In db/db and ob/ob mice CerS6 expression is significantly elevated compared to control animals (1.5 and 3 fold) which correlated with a 3 and 4 fold increase of C16 ceramide in the plasma, respectively, whereas in DIO mice neither enzyme levels or ceramide levels are significantly elevated. CerS6 ASO treatment led to selective and significant ~80% knock-down of the CerS6 expression in the liver and correlated with normalization of plasma C16 ceramide levels compared to control ASO-treated animals. CerS6 knockdown protected in all animal models against body weight gain and was associated with significant reduction in whole body fat content and fed/fasted blood glucose levels. Moreover, insulin resistance was significantly ameliorated as evidenced by significant improvement of oral glucose tolerance tests. Thus CerS6 dependently generated C16 ceramide represents a distinct sphingolipid species, which contributes to the development of obesity and insulin resistance and therefore represents a unique and attractive novel target to treat obesity and type 2 diabetes.

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1866-P

Akt in Macrophages Regulate the Response to Feeding

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Inflammatory processes have been reported in the pathogenesis of obesity and type 2 diabetes, although the precise mechanisms remain poorly understood. We explored the role of the serine/threonine protein kinase Akt in macrophages, using conditional knockout models. Myeloid specific Akt1/Akt2 double knockout mice (MAktDKO mice), fed a normal chow diet, showed insulin resistance and higher glucose levels before they gained more body weight after 15 weeks of age and onward. Among the various responses to feeding, the liver of MAktDKO mice showed impaired suppression of gluconeogenic gene expression. Lipopolysaccharide (LPS), stored in vast amounts in the intestinal flora, and which increases in the blood after feeding, is one of the factors that may play a role in feeding responses in the liver regulated by macrophages via Akt. We therefore screened for Akt dependently expressed genes in response to LPS in primary macrophages. Among genes Akt dependently induced by LPS, IL-10 was induced in 72 hours when stimulated by LPS alone, but this induction was markedly shortened to 3 hours when co-stimulated by insulin. Deficient IL-10 induction in macrophages of MAktDKO mice was rescued when mTOR signaling was strengthened by deletion of TSC2, suggesting Akt-mTOR signaling induces IL-10 in response to insulin/LPS stimulation. Adenovirus mediated gene transfer of IL-10 decreased plasma glucose levels and gluconeogenic gene expression, further supporting a model in which macrophages respond to insulin and LPS after feeding, regulating glucose homeostasis in the liver by expressing IL-10 in an Akt-mTOR dependent process.

1867-P

Arrestin Domain-Containing 3 (ARRDC3) Is a Novel Downstream Target of the Insulin-Signaling Pathway in Skeletal Muscle

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Insulin signaling is critical for skeletal muscle growth and metabolism. To gain more insight into insulin action on gene expression in vivo at physiological level of insulin, quadriceps muscle was isolated from mice during a euglycemic insulin clamp (at 4 or 12 mU. kg-1. min-1) and submitted to RNA-sequencing analysis. One of the most strongly regulated transcripts was the adapter protein ARRDC3 (arrestin domain-containing 3), which was suppressed by both doses of insulin by 75-80%. ARRDC3 belongs to the subfamily of α-arrestins and has been implicated in the regulation of obesity and energy metabolism, although its role in skeletal muscle has not been addressed. ARRDC3 down-regulation was confirmed in differentiated C2C12 myotubes in vitro and found to be time dependent and insulin-specific, with no effect of IGF1. Consistent with negative regulation by insulin, ARRDC3 gene expression was increased in muscle of insulin resistant mice fed a high fat diet and up-regulated by 2- to 6-fold by pharmacological inhibition of PI3K, MAPK or AKT. At the protein level, expression of human ARRDC3 in HEK293 cells was markedly reduced when co-expressed with the human insulin receptor (IR). When co-expressed with IR, ARRDC3 expressing cells also showed a marked reduction of insulin stimulated IR tyrosine-phosphorylation, and a reduction of basal and insulin-induced ERK1 phosphorylation.

In conclusion, ARRDC3 is a novel target downstream of the insulin-signaling pathway, which is down-regulated by insulin and up-regulated by insulin resistance in skeletal muscle. ARRDC3 interacts with IR reducing its stimulation by insulin and reducing downstream signaling particularly to the ERK/MAP kinase pathway.

1868-P

WITHDRAWN

1869-P

Excitation-Contraction Coupling in the Absence of Insulin Receptors in the Mouse Heart

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Cardiovascular disease is the main complication in the diabetic population even after correcting for the presence of comorbidities like hypertension and obesity. An improper insulin signaling is thought to be one of the hallmarks of diabetes mellitus so it is essential to understand the relationship between a deregulated insulin signaling and the development of abnormal Ca^{2+} handling that is found in animal models of diabetes. Here, I propose to use a mouse model that avoids the systemic complications of diabetes that can per se alter excitation-contraction coupling (ECC). We measured Ca^{2+} transients in isolated cardiomyocytes from 8 weeks cardiomyocyte-selective insulin receptor knockout (CIRKO) mice (Cre-$IR^{lox/lox}$) and compared them with their wild type (WT) littermates ($IR^{lox/lox}$). Cardiomyocytes loaded with the Ca^{2+} dye Fluo-4 were field stimulated at 3.3 Hz at 37°C and intracellular Ca^{2+} was monitored using a custom-made epifluorescence system. Ca^{2+} transient

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amplitude and maximum rate of rise were found to be reduced in CIRKO compared with WT (F/F0 0.79±0.05 vs. 2.2±0.2, n= 20/group, p<0.05 and F/F0 per sec 28±2 vs. 77±7, n=20/group, p<0.05 respectively). We also found a reduction in $Ca_v1.2$ expression (main trigger for Ca^{2+} transient) and current in CIRKO mice with no difference in the steady-state activation properties of the channel (gene expression 44±5%, n=10/group, p<0.05; protein expression 62±1%, n=3/group, p<0.05; 35% decreased in the macroscopic current at +10 mV (-21±1 A/F, n=6 in WT vs. -14±2 A/F, n=8 in CIRKO, p<0.05).

In conclusion, our finding suggest that impaired cardiac insulin signaling per se is involved in the Ca^{2+} mishandling observed in the systemic models of diabetes.

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1870-P

Sustained Activation of Beta-Adrenergic Receptors Enhances de Novo Lipogenesis in Adipose Tissue

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The de novo lipogenesis (DNL) pathway in adipocytes appears to influence whole body metabolic homeostasis, perhaps by producing bioactive lipids that modulate systemic insulin sensitivity. Thus, the identification of regulators of DNL in adipose tissue (AT) is of high interest, in particular the mechanisms by which obesity downregulates DNL. Here we investigated whether the sympathetic nerve system (SNS), via activation of β-adrenergic receptors, modulates the DNL in AT. Stimulation of SNS outflow to AT depots in cold-exposed mice (6°C) enhanced the expression of several genes in the DNL pathway, including ChREBPβ, ACLY, FASN and ELOVL3 in AT. Similarly, treatment of mice housed at 22°C with the β_3-adrenergic receptor (β_3AR) agonist CL316243 increased the expression of DNL genes in AT. Conversely, when mice were housed at thermoneutral conditions, in which SNS outflow and the βAR signaling pathway in AT is negligible, a strong downregulation of the DNL genes in AT depots were observed. In the absence of endogenous SNS drive, DNL in AT could be partially overcome by treatment with CL316243. Based on our recent findings that mTORC1 is required for β_3AR-induced thermogenesis in AT, we investigated whether mTORC1 was also necessary for β_3AR-regulation of DNL genes in fat. While the mTORC1 inhibitor rapamycin completely blocked induction of the key thermogenic mediator UCP1 by CL316243 or by cold-exposure treatment, it failed to inhibit DNL gene expression in AT under these same conditions. In contrast, adipocyte deletion of the mTORC1 subunit Raptor prevented both thermogenic and DNL gene expressions in concert with a strong inhibition of β_3AR expression. These data suggest multiple outputs from the mTORC1 complex in response to βAR signaling. Overall, these results indicate that SNS outflow and sustained βAR activation stimulate the DNL in AT. Our findings suggest that dysregulation of βAR signaling might contribute to suppression of DNL genes and AT dysfunctions associated with obesity.

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INTEGRATED PHYSIOLOGY—CENTRAL NERVOUS SYSTEM REGULATION OF METABOLISM

1871-P

Increased Insulin Resistance Is Associated with Decreased Cognitive Function in Elderly Koreans: A Prospective Community-Based Cohort Study

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Objective: It is well-known that diabetes is associated with increased risk of cognitive dysfunction. However, it is controversial that whether peripheral insulin resistance effects on cognitive function in longitudinal studies. We aimed to evaluate whether change of insulin resistance was associated with cognitive decline in elderly Korean.

Method: The study was conducted in the Ansung Cohort Study, the ongoing prospective community-based cohort study in Korea. A total of 5,018 participants aged over 40 years at baseline were followed up for a mean of 9 years. Of them, the final analysis was performed in 450 participants aged over 65 years who were evaluated with Korean mini-mental status examination (K-MMSE) at both baseline and follow-up. Pearson's correlation analyses and multivariate linear regression analyses for change of K-MMSE score were carried out.

Results: Mean age at baseline was 69.2 ± 2.9 years, and 234 participants (48.0%) were men. Mean duration of education was 7.3 ± 3.4 years. During a mean follow-up of 6 years, K-MMSE score was significantly decreased with 1.0 (interquartile range of 4.0), homeostasis model assessment of insulin resistance (HOMA-IR) was not significantly changed during follow-up.

Results: Participants with more decreased K-MMSE score (Δ K-MMSE) had shorter duration of education (p = 0.006), older age (p = 0.015), higher baseline K-MMSE score (p < 0.001), and increased insulin resistance (Δ HOMA-IR, p = 0.021). The correlation between Δ K-MMSE and Δ HOMA-IR remained significant after adjustments for age, gender, baseline K-MMSE score, duration of education, baseline Korean geriatric depression scale, smoking status, history of diabetes and hypertension, body mass index, and apolipoprotein E4 genotype status (B = -0.168, p = 0.028).

Conclusions: During 6 years of follow-up in elderly Korean population, increased HOMA-IR was significantly associated with decreased cognitive function.

1872-P

O-Linked β-N-Acetylglucosamine Signaling in Pro-Opiomelanocortin Neurons Mediates Brain-to-Fat Communication to Coordinate Whole-Body Metabolism

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Proopiomelanocortin (POMC) neurons in the arcuate nucleus of hypothalamus suppress feeding and promote energy expenditure. The function of POMC neurons is regulated by a variety of metabolic hormones. Known as a metabolic sensor, O-linked β-D-N-acetylglucosamine modification (O-GlcNAcylation) of intracellular proteins is catalyzed by O-GlcNAc transferase (OGT). Here we report that OGT is required for POMC neuron action on adipose tissue and whole-body metabolism. POMC neuron-specific OGT deletion reduces neuronal excitability, increases food intake, lean mass and fat mass. These correlate with impaired lipid catabolism in white fat depots and reduced thermogenesis. The respiratory quotient is increased in OGT knockout mice, suggesting an increased preference toward carbohydrate utilization. Under the high fat diet condition, POMC neuron-specific OGT knockout mice exhibit pronounced body weight gain and adiposity, but improved glucose tolerance and insulin sensitivity. Collectively, these results reveal that O-GlcNAc signaling in POMC neurons is a key node of brain-to-fat communication to coordinate whole-body metabolism.

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1873-P

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