dose of Streptozotocin (150mg/kg), at a dose of 1500 IEq per recipient. The mice were monitored for a period of 60 days after which the kidneys were explanted and analyzed for insulin and glucagon staining.

Mean blood glucose was 552±24mg/dL pre-transplant (mean±sem). All mice treated with islet xenografts (I&II) under the kidney capsule in diabetic nude mice maintained euglycemia until nephrectomy (Day 60). At two and four weeks post-transplant, recipient mice were responsive to an oral glucose challenge. Both transplant groups remained non-hyperglycemic at 30 (167±7 mg/dL, I; 185±8 mg/dL, II) and 60 days (172±11 mg/dL, I; 176±8 mg/dL, II) post-transplant. All recipients reverted to hyperglycemia within 48 hours of explant (nephrectomy). Histological examination of the islets within the explanted kidney demonstrated positive staining for insulin and glucagon.

Young pig islets can survive and function when transplanted in diabetic mice after prolonged *in vitro* culture. This data is critical to enhance our understanding of islet biology and design pivotal large animal xenotransplantation trials using isolated cultured porcine islets.

Supported By: Islet Sciences, Inc.

2573-PO

WITHDRAWN

INSULIN ACTION—ADIPOCYTE BIOLOGY

2574-PO

WITHDRAWN

2575-PO

Are Both the Omental and Mesenteric Adipose Tissues Considered as Representatives of Visceral Fat?

DAISUKE SATO, KANAKO ODA, MASATAKA KUSUNOKI, ATSUYOSHI NISHINA, ZHONGGANG FENG, TAKAO NAKAMURA, *Yamagata, Japan, Nagoya, Japan, Tokyo, Japan, Yonezawa, Japan*

We previously reported that fatty acid (FA) compositions in rat adipose triglyceride (TG) depends on anatomical site, and relates to insulin resistance. It is known that the greater omentum regulates immune system, and that the immune reaction is involved with insulin resistance. However, differences in role of greater omental (OME) and mesenteric (MES) adipose tissues, recognized as representatives of visceral fat, on insulin sensitivity have not been clarified yet. In this study, we compared FA composition in the rat adipose TG in OME with that in MES. Male rats were randomized into normal (n=6), HFF (n=5), and HFF+PIO (n=5) groups. The normal group was fed with a standard laboratory chow whereas the HFF and HFF+PIO groups received a high-fat diet from 6 weeks of age. At 11 weeks of age, pioglitazone (30 mg/kg/day) was administered to the HFF+PIO group to improve insulin resistance. At 15 weeks of age, rats were killed, and 24 FA contents in TG fraction in OME and MES were analyzed by gas chromatography. Insulin sensitivity was assessed with hyperinsulinemic euglycemic clamp in additional rats in the three groups. Glucose infusion rate in the HFF group was significantly lower ($p<0.05$) than those in the normal and HFF+PIO groups. Stearic (18:0), oleic (18:1n-9), and eicosanoic (20:1n-9) acids contents in OME in the HFF group were significantly lower than those in MES ($p<0.05$, respectively), and palmitic acid (16:0) content in OME tended to be lower ($p=0.05$). In contrast, docosatetraenoic acid (22:4n-6) content in OME was significantly higher ($p<0.05$). However, no significant difference was detected between FAs in the OME and MES in the normal and HFF+PIO groups. The difference of FA composition between OME and MES may take place only under high insulin resistance, and disappear at normal state and under improved insulin resistance condition. In addition, the results may imply that OME should be considered not same as MES, especially in case of insulin-resistance related studies.

2576-PO

WITHDRAWN

2577-PO

Chronic Inflammation in Dysmetabolic NHP: Bridging the Gap between Rodents and Humans

KARNI SCHLESSINGER, FRANKLIN LIU, SHU-CHENG CHEN, NYKIA WALKER, DIANE LEVITAN, EFFIE TOZZO, DAVID E. KELLEY, SANDRA C. SOUZA, *Kenilworth, NJ*

Chronic inflammation is a hallmark of diabetes in rodents and in humans. Adipose tissue from obese human patients, as well as from obese and type 2 diabetic rodents shows increased macrophage infiltration and a shift in macrophage phenotype from anti-inflammatory to pro-inflammatory. This has been associated with decreased mRNA expression of anti-inflammatory genes and increased mRNA expression of pro inflammatory genes and levels of pro-inflammatory cytokines and chemokines. In addition, activation of intracellular kinases such as c-Jun N-terminal kinase (JNK) and IkB kinase (IKK), which inhibit insulin receptor signaling, have been implicated in the progression and severity of diabetes. To understand the significance of inflammation to insulin resistance in higher species, we have characterized chronic inflammation in dysmetabolic/insulin resistant Rhesus. Increased infiltration of macrophages was confirmed by the increased presence of crown-like structures in sub cutaneous adipose tissue of dysmetabolic NHPs in comparison to lean animals. RNA profiling of adipose tissues from dysmetabolic NHPs showed a robust increase in gene expression of several inflammatory genes such as PAI-1, C4A, osteopontin, CCL3, IL1RN, MCP1, resistin and CLEC7A, as well as decrease in adiponectin and insulin receptor gene expression. Phospho-protein analysis of adipose tissue revealed an upward trend in JNK phosphorylation in dysmetabolic NHPs. Finally, a significant increase in plasma inflammatory markers such as IL-6, MCP-1 and CRP was detected in dysmetabolic NHP compared to lean. In summary, we confirmed the presence of multiple inflammatory markers in sub cutaneous adipose tissue and plasma of dysmetabolic NHPs, which allows future anti-inflammatory strategies to be tested preclinically in a NHP model.

2578-PO

WITHDRAWN

2579-PO

WITHDRAWN

INSULIN ACTION—CELLULAR AND MOLECULAR METABOLISM

2580-PO

Decreased Oxygen Consumption Rate in Liver Mitochondria from Insulin-degrading Enzyme (IDE) Knockout Mice

FREDERICK G. HAMEL, GERRI L. SIFORD, ROBERT G. BENNETT, *Omaha, NE*

Insulin-degrading enzyme (IDE) has a mitochondrial form whose function is not known. This mitochondrial IDE has been associated with SIRT4, which has ADP-ribosyltransferase activity. Glutamate dehydrogenase (GDH), which is important for the use of glutamate as a substrate in the TCA cycle, can undergo ADP-ribosylation by SIRT4, decreasing activity. We therefore hypothesized that mitochondria (mitos) from IDE KO mice would have decreased activity when glutamate was used as substrate. Mitos were isolated from wild type (WT) and IDE KO mice by differential centrifugation. Using MitoProfile® Total OXPHOS Rodent Antibody Cocktail, no difference was seen between WT and KO for any Complex I to V. Oxygen consumption rates (OCR; n=5 for each phenotype) were measured using a Seahorse XF24 Flux Analyzer using 10 µg of mitochondrial protein per well. Measurements were made sequentially of State 2 (mitos + glutamate and malate), State 3 (ADP added), State 4o (oligomycin added) and State 3 uncoupled (FCCP added), and expressed as pmoles O_2/min/10 µg protein. OCR was expressed as mean ± SEM and compared with a one-tailed t-test. In State 2, OCR was slightly lower for KO than WT (56.4 ± 5.1 vs. 64.9 ± 6.9; $p=0.1731$). In State 3, the difference was more dramatic (KO 118.2 ± 17.2 vs. WT 152.7 ± 12.8; $p=0.0728$) State 4o showed a similar trend (KO 52.5 ± 9.8 vs. WT 71.7 ± 8.2; $p=0.0848$). State 3 uncoupled showed the most significant difference (KO 163.5 ± 21.4 vs. WT 215.1 ± 22.1; $p=0.0661$). For States 3, 4o, and 3 uncoupled, OCR in KO mitos were 77, 73 and 76 percent of WT. Thus, the absence of IDE in the mitos leads to a decrease in OCR with glutamate as a substrate. We speculate that IDE interacts with SIRT4, decreasing its

action and thereby decreasing GDH ribosylation and increasing its activity. Loss of IDE thus increases GDH ribosylation, decreasing the conversion of glutamate to α-ketoglutarate and its use in the TCA cycle. IDE may have a role in controlling cellular metabolism beyond the degradation of insulin.

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2581-PO

Influence of 5 Days High-Fat Overfeeding on Genome-Wide Gene Expression and DNA Methylation Levels in Adipose Tissue in Healthy Young Men

LINN GILLBERG, ALEXANDER PERFILYEV, CHARLOTTE BRØNS, TINA RÖNN, ALLAN VAAG, CHARLOTTE LING, *Copenhagen, Denmark, Malmö, Sweden*

We have previously shown that 5 days high-fat overfeeding (HFO) induced DNA methylation changes in 45% of all genes in skeletal muscle from healthy young men. Subjects born with low birth weight (LBW) who have an increased risk of type 2 diabetes (T2D) seem however to be epigenetically less flexible in muscle tissue in response to HFO. We hypothesized that increased risk of T2D in LBW subjects may be explained by differential epigenetic and transcriptional responses in subcutaneous adipose tissue (SAT) when exposed to HFO. Therefore, we investigated genome-wide gene expression and DNA methylation in SAT from 16 LBW and 24 matched normal birth weight (NBW) men during control and 5 days HFO diets. Gene expression was measured with Affymetrix Human Gene 1.0 ST arrays and DNA methylation with Illumina Infinium 450K BeadChips. *In vivo* hepatic insulin resistance ($p<0.01$), increased serum insulin ($p<0.01$) and decreased FFA ($p<0.001$) levels indicated glucose intolerance and lipid storage with overfeeding. The genome-wide gene expression in SAT from LBW men was not significantly different from NBW men and all 40 men were therefore analyzed together. After the HFO, 3,890 corresponding to 12% of all transcripts on the array were differentially expressed in SAT ($q<0.05$, Benjamini-Hochberg correction). Oxidative phosphorylation ($q=10^{-26}$) and citrate cycle ($q=10^{-10}$) were the top-two significant pathways of transcripts upregulated by HFO. In total, 5% of the 64,920 analyzed methylation sites annotated to the differentially expressed genes changed DNA methylation with HFO ($p<0.05$), but none was significant after correction for multiple testing. In conclusion, the transcriptional profile and response to HFO was not different in SAT from LBW compared to NBW men. Our data indicate extensive adipocyte gene expression changes after only 5 days HFO.

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2582-PO

WITHDRAWN

2583-PO

Effects of Linagliptin on Glucose-induced Neuronal Damage in Caenorhabditis elegans

KANYANAT WONGCHAI, ANDREAS SCHLOTTERER, JIHONG LIN, MICHAEL MORCOS, THOMAS KLEIN, HANS PETER HAMMES, *Mannheim, Germany, Heidelberg, Germany, Biberach, Germany*

Caenorhabditis elegans (C.elegans) lacks a vascular system and is therefore an ideal model system to investigate the mechanisms of glucose-mediated damage to neurons. Experimental hyperglycaemia causes neuronal dysfunction in *C.elegans* and reduces lifespan by overproduction of reactive oxygen species (ROS) and advanced glycation end products (AGEs). On the biochemical level, a key regulator for longevity and stress response is DAF-16, homolog of mammalian FOXO transcription factor proteins, which up-regulates MnSOD, an enzyme detoxifying ROS. In the current study, we used wild-type and the gene *daf-16* to study the potential molecular mechanisms underlying the effects of linagliptin on *C.elegans* with relation to the diabetic retina.

A total of 150 - 300 *C.elegans* were used per group. Linagliptin was administered to *C.elegans* by feeding 13 µmol/L linagliptin daily for 12 days, resulting in an intracellular linagliptin concentration of around 8 nmol/L. Under HG conditions in wild-type *C.elegans* linagliptin normalised the reduced lifespan and improved relative head motility (a parameter for neuronal activity) by 10% ($p<0.01$), and 36% ($p<0.05$), respectively. These positive effects were blunted in *daf-16* knock-out worms. Importantly, under HG conditions linagliptin significantly decreased ROS (by 19% ($p<0.05$) and 12% ($p<0.05$)) and Methylglyoxal(MG)-derived AGEs formation (by 15% ($p<0.05$) and 8% ($p<0.05$)) in both wild-type and *daf-16* knock-out worms, respectively.Linagliptin exhibits anti-MG-derived AGEs and antioxidative properties in a *daf-16* independent manner. In contrast, the neuroprotective and lifespan-enhancing effects may be *daf-16* dependent, suggesting that the protective effects of linagliptin may involve different molecular pathways. Our studies reveal a positive role of linagliptin in a neuronal system, which may concomitantly with its protective effect in a vasculature system of the diabetic retina, as a result of a combined effect on neurovascular unit.

2584-PO

WITHDRAWN

2585-PO

Major Regional Differences in Brain Glucose Metabolism Determined by Imaging Mass Spectrometry in Normal and Diabetic Mice

HEATHER A. FERRIS, ANDRE KLEINRIDDERS, MICHELLE REYZER, JEFFREY SPRAGGINS, RICHARD CAPRIOLI, C. RONALD KAHN, *Boston, MA, Nashville, TN*

Glucose is the major energy substrate of the brain. In the setting of diabetes there can be excessive or reduced glucose availability, as well as impairment of insulin signaling in the brain. These may lead to both short and long term effects on brain function, ranging from confusion and seizures to increased risks of depression and Alzheimer's disease. In the present study we measured metabolites across mouse brain sections at a resolution of 100 microns using imaging mass spectrometry (IMS), and coupled this with immunohistochemistry (IHC), qPCR, western blotting and enzyme assays of dissected brain regions to determine the relative contributions of the glycolytic and pentose phosphate pathways to regional glucose metabolism and how it is altered in diabetes. In normal murine brain the thalamus and corpus callosum showed high levels of the pentose phosphate pathway enzyme glucose-6-phosphate dehydrogenase (G6PD) compared to other brain regions by qPCR, western blotting, enzyme assay and IHC. This agreed with very low levels in these regions of hexose bisphosphate (a glycolytic intermediate) as measured by IMS. Conversely, the amygdala and hippocampus which had low levels of G6PD and higher levels of the glycolytic enzyme hexokinase by IHC and qPCR showed very high levels of hexose bisphosphate by IMS. Interestingly, in streptozocin diabetic mice, IMS imaging of brain revealed an increase in hexose bisphosphate levels, particularly in the hippocampus, compared to control, without a change in level in other regions with high expression of hexokinase, such as the amygdala. These data indicate the importance of direct measurement of metabolic intermediates to determine the differential pathways of glucose utilization in brain and suggest that under diabetic conditions these pathways are altered favoring increased glycolysis in the hippocampus.

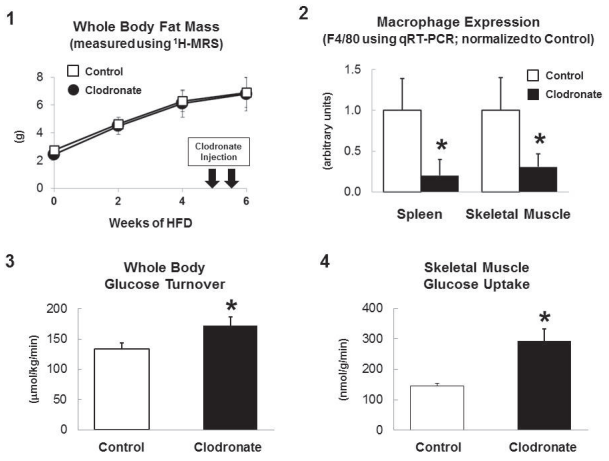
2586-PO

Clodronate-mediated Depletion of Macrophages Prevents Diet-induced Insulin Resistance in Mice

SEZIN DAGDEVIREN, PAYAL R. PATEL, KIMBERLY A. NEGRIN, XIAODI HU, NICHOLAS TSITSILIANOS, ANDREW V. TSITSILIANOS, DUY A. TRAN, KUNIKAZU INASHIMA, JONATHAN M. DURGIN, JASON K. KIM, *Worcester, MA*

We have previously shown that local inflammation and macrophages play an important role in obesity-mediated insulin resistance in skeletal muscle. Here, we examined the effects of macrophage depletion using clodronate-containing liposomes on insulin sensitivity in obese mice. Male C57BL/6 mice were fed a high-fat diet (HFD) for 6 wks, and clodronate (n=6) or PBS-containing liposomes (control; n=7) were intravenously administered twice during the last 1 wk of HFD. Clodronate treatment did not affect obesity in mice (Fig. 1). qRT-PCR showed that F4/80 expression was reduced by 70% in spleen and skeletal muscle following clodronate treatment (Fig. 2; *P<0.05). A 2-hr hyperinsulinemic-euglycemic clamp was performed to measure insulin sensitivity in awake mice. Whole body glucose turnover was significantly increased in clodronate-treated mice as compared to controls (Fig. 3). This was mostly due to a 2-fold increase in skeletal muscle glucose uptake following clodronate treatment (Fig. 4). In contrast, hepatic glucose production and glucose uptake in white and brown adipose tissues were not significantly affected by clodronate. Thus, our findings support an important role of macrophages in obesity-mediated insulin resistance in skeletal muscle and therefore suggest a therapeutic role of suppressing local inflammation to treat insulin resistance.

For author disclosure information, see page A743.



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INSULIN ACTION—GLUCOSE TRANSPORT AND INSULIN RESISTANCE IN VITRO

2587-PO

Mapping Sites of Insulin Regulation in Glut4 Trafficking: Insulin Regulates Fusion, Sequestration into and Release from GSVs

PAUL D. BREWER, ESTIFANOS N. HABTEMICHAEL, IRINA ROMENSKAIA, ADELLE COSTER, CYNTHIA MASTICK, *Reno, NV, New Haven, CT, Sydney, Australia*

Insulin increases glucose uptake into muscle and fat by redistributing glucose transporter 4 (Glut4) from intracellular storage compartments to the cell surface, a process known as Glut4 translocation. Under both basal and insulin conditions, intracellular Glut4 is constantly exchanging with cell surface Glut4. Therefore, the cell surface level of Glut4 is determined by the relative ratios of exocytosis (insertion into the plasma membrane) and endocytosis (internalization from the plasma membrane). Insulin promotes Glut4 translocation by increasing the rate constant of Glut4 exocytosis (kex) and redistributing Glut4 from slow to fast recycling pathways.

Previously we developed flow cytometric assays to characterize the trafficking kinetics of Glut4 and other endosomal proteins in adipocytes. By mathematically modeling these data, we constructed a trafficking model which identified six trafficking steps that are unique to Glut4 compared to the transferrin receptor, and identified steps which are regulated by insulin. Interestingly, perturbation of a trafficking step produces a unique kinetic phenotype which can then be used as a diagnostic to assign the protein(s) targeted to their given step.

In the current study, we independently knocked down Sortilin, CDP138, and Hugl1 using shRNA expressing lentiviruses. We also expressed Glut4 constructs containing mutations in endosomal trafficking motifs. The effects of these perturbations were then characterized by carefully measuring the trafficking kinetics of Glut4. To test hypotheses about where these proteins/motifs could function, mathematical modeling was used to map their site of action to specific steps in the Glut4 trafficking itinerary.

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2588-PO

Midkine Is a Potential Link between Obesity and Insulin Resistance

YI LIN, NENGGUANG FAN, HAIYAN SUN, YONGDE PENG, *Shanghai, China*

Obesity is associated with increased production of inflammatory mediators in adipose tissue, which contributes to chronic inflammation and insulin resistance. Midkine (MK) is a heparin-binding growth factor with potent proinflammatory activities. We aimed to test whether MK is associated with obesity and has a role in insulin resistance. It was found that MK was expressed in adipocytes and regulated by inflammatory modulators (TNF-α and rosiglitazone). In addition, a significant increase in MK levels was observed in adipose tissue of obese ob/ob mice. In vitro studies further revealed that MK impaired insulin signaling in 3T3-L1 adipocytes, as indicated by reduced phosphorylation of Akt in response to insulin. Thus, MK may provide a novel link between obesity and insulin resistance.

Supported By: NSFC (81370904)

INSULIN ACTION—SIGNAL TRANSDUCTION, INSULIN, AND OTHER HORMONES

2589-PO

Transcription Factor Sp1 Is Crucial for the Mouse Disulfide-bond A Oxidoreductase-like Protein (DsbA-L) Gene Expression

QICHEN FANG, WENJING YANG, WENXIU HU, HUATING LI, KUN DONG, QIANQIAN SONG, FENG LIU, WEIPING JIA, *Shanghai, China, San Antonio, TX*

Disulfide-bond A oxidoreductase-like protein (DsbA-L) has been demonstrated as a key regulator of adiponectin biosynthesis and multimerization. The fat-specific DsbA-L transgenic mice displayed resistance to diet-induced obesity, insulin resistance, and hepatic steatosis. The level of DsbA-L is negatively correlated with obesity in mice and humans. Although DsbA-L possesses beneficial effect on insulin sensitivity and energy metabolism, the transcriptional regulation of DsbA-L is largely unknown. To gain insights into the transcriptional mechanisms that regulate DsbA-L gene expression, we generated reporter gene constructs containing the promoter sequence of the DsbA-L gene. Deletion analysis showed that the region between -186 to -34 was essential for basal level expression of the DsbA-L gene. In addition, there was a putative Sp1 transcription factor binding site in the first intron of DsbA-L gene. Removing the putative Sp1 regulatory site by mutagenesis resulted in a significant increase in DsbA-L promoter activity. Electrophoretic mobility shift assay and chromatin immunoprecipitation analysis indicated that Sp1 bound to this region in vitro and in vivo. Overexpression of Sp1 significantly down-regulated DsbA-L promoter activity whereas suppression of Sp1 by siRNA significantly enhanced the DsbA-L transcriptional activity. Moreover, the binding activity of Sp1 was gradually decreased during 3T3-L1 cell differentiation. These results demonstrated that Sp1 regulated the transcription of mouse DsbA-L gene through interaction with the cis-acting inhibitory element within intron region and this inhibition might be reduced during adipocyte differentiation.

2590-PO

Characteristics of a Nonmetabolizable Research Tool Analog of Insulin Glargine in Rats

NORBERT TENNAGELS, MARCUS KORN, RONALD SCHMIDT, THOMAS WENDRICH, ULRICH WERNER, *Frankfurt, Germany*

Insulin glargine (GLA) is a basal insulin with safe and effective 24-h glycemic control. GLA is rapidly metabolized in humans and animals to its main metabolite M1 (comprising 76-92% of metabolite total) and has an in vitro metabolic and mitogenic profile comparable to human insulin (HI). GLA exerts insulin receptor (IR) signaling similar to that of HI in various tissues of rats even at suprapharmacological doses and does not stimulate IGF-1 receptor (IGF1R) phosphorylation in vivo. The aim of this study was to determine how glargine itself affects signaling pathways by characterizing a research tool-based, non-metabolizable insulin glargine analog (NMI) in vitro and in vivo.

In vitro NMI was stable towards proteolytic degradation by carboxypeptidases. NMI had an IR activity and occupancy profile similar to GLA, as well as a slightly higher affinity for the IGF1R than HI. For in vivo characterization, male Wistar rats were injected s.c. with 1, 12.5 & 200 IU/kg of HI, GLA or NMI, and effects on blood glucose and phosphorylation status of IR, IGF1R and AKT in tissue samples derived from muscle and heart were investigated after 1h. For all analogs and doses, blood glucose lowering was comparable. LC-MS/MS analysis of the in vivo metabolite plasma profile indicated rapid metabolism of GLA to M1 at all doses; no metabolism of NMI was detected. Both GLA and NMI treatment resulted in phosphorylation levels of IR and AKT comparable with that of HI through all dose levels. Importantly, even at a suprapharmacologic dose of 200 U/kg, neither HI nor GLA nor NMI resulted in significant IGF1 phosphorylation in muscle and heart tissue.

Our results indicate that injection of suprapharmacological doses of HI, GLA, or NMI does not lead to activation of IGF1R in vivo. This supports the view that mitogenic effects of insulin and insulin analogs seem to be based solely on the growth promoting activity of the IR itself. Consequently, IGF1R activation by insulin analogs may be less relevant than previously discussed.

Supported By: Sanofi

2591-PO

WITHDRAWN

2592-PO

The Effect of Ambient Light Intensity on Postprandial Glucose and Insulin Levels

RUTH I. VERSTEEG, DIRK JAN STENVERS, ERIC FLIERS, M.J. SERLIE, ANDRIES KALSBEEK, SUSANNE E. LA FLEUR, PETER H. BISSCHOP, *Amsterdam, Netherlands*

The increasing prevalence of type 2 diabetes correlates to the increased exposure of artificial light. Specialised retinal ganglion cells transmit light information to the hypothalamus via the retino-hypothalamic tract and thus modulate hormonal secretion and autonomic activity. Light has time dependent autonomic effects in humans and administration of a light pulse to rats directly alters metabolic gene expression in liver. However, the direct effects of light intensity on human carbohydrate and lipid metabolism have never been investigated. The aim of this study was to investigate the effect of ambient light intensity on postprandial glucose and lipid levels. In a randomised cross-over design, 8 healthy lean men (age: 22.4 ± 1.7yrs; BMI: 22.1 ± 1.3 kg/m2) with a normal sleep/wake pattern were admitted to the clinical research unit in the evening. After a standardised mixed meal (800 kcal) they were allowed to sleep in darkness (1 lux) for 8 hours. In the morning they were exposed to either bright light (4000 lux) or dim light (10 lux) for 5 hours. Sixty minutes after lights on, subjects consumed a liquid mixed meal (600 kcal, 54 energy% carbohydrates, 29 energy% fat, 17 energy% protein). Blood samples were taken at fixed time points for 5 hrs. The incremental area under the curve (AUC) of postprandial glucose levels was not different between dim (12,439 ± 123) and bright (12,439 ± 133) light (P = 0.24). Furthermore, the AUC of plasma insulin (521,908 ± 32,067 vs. 534,799 ± 26,779; P = 0.73), C-peptide, free fatty acids and triglycerides were not different between the two light conditions. In conclusion, in our study light intensity did not affect postprandial levels of glucose and insulin in healthy lean men.

Supported By: Dutch Technology Foundation (12189)

2593-PO

Effects of AICAR and Metformin on TNF-α-induced Serine Phosphorylation of IRS-1: Mediation by Activation of AMP-activated Protein Kinase in 3T3-L1 Adipocytes

KUMI SATOH, AKIRA TAKAGURI, *Otaru, Japan*

Adenosine monophosphate-activated protein kinase (AMPK) plays a key role in the regulation of energy homeostasis and monitors cellular energy charge, acting as a "metabolic master switch" for regulating adenosine triphosphate concentrations in the face of stressors that decrease cellular energy levels. Although AMPK activation is known to contribute toward the acceleration of insulin signaling, the mechanism by which AMPK regulates insulin signaling remains unclear.

Serine phosphorylation of insulin receptor substrate (IRS)-1 negatively regulates insulin signaling. Here we investigated the role of 5-aminoimidazole-4-carboxamide-1-d-ribofuranoside (AICAR) and metformin, an AMPK activator, in serine phosphorylation of IRS-1 at 636/639, which is induced by tumor necrosis factor (TNF)-α in 3T3L1 adipocytes.

AICAR significantly inhibited the TNF-α-induced serine phosphorylation of IRS-1 at 636/639 by suppression of extracellular signal-regulated kinase (ERK) phosphorylation. In contrast, although metformin inhibited the TNF-α-induced serine phosphorylation of IRS-1, there was no effect on ERK phosphorylation. Moreover, ICAR stimulation enhanced the interaction between ERK and MAP kinase phosphatase-4 (DUSP9/MKP-4) without affecting DUSP9/MPK4 mRNA synthesis.

Intraperitoneal AICAR administration (0.25 g/kg) or metformin (0.40 g/kg) to db/db mice improved blood glucose levels. AICAR but not metformin inhibited ERK phosphorylation in adipose tissues of the mice.

In conclusion, AICAR and metformin improved blood glucose levels through suppression of TNF-α-induced serine phosphorylation of IRS-1 at 636/639 by different mechanisms.

2594-PO

Effect of Extracellular Visfatin on Glucose Production in HepG2 Cells through the PKA/CREB Pathway

HAE JIN KIM, SUNG-E CHOI, YONG JUN CHOI, EUN SUK HA, YUP KANG, JA YOUNG JEON, SO YOUNG OCK, SEUNG JIN HAN, DAE JUNG KIM, KWAN WOO LEE, *Suwon, Republic of Korea*

Adipokines reportedly affect hepatic gluconeogenesis, and the adipokine visfatin plays a role in insulin resistance and type 2 diabetes. However, whether visfatin contributes to hepatic gluconeogenesis remains unclear. Visfatin, also known as nicotinamide phosphoribosyltransferase (NAMPT), modulates sirtuin1 (SIRT1) through the regulation of nicotinamide adenine dinucleotide (NAD). Therefore, we investigated the effect of extracellular visfatin on glucose production in HepG2 cells, and evaluated whether extracellular visfatin affects hepatic gluconeogenesis via a NAD+-SIRT1-dependent pathway. Treatment with visfatin significantly increased glucose production and the mRNA expression and protein levels of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) in HepG2 cells in a time- and concentration-dependent manner. Knockdown of SIRT1 had no significant effect on the induction of gluconeogenesis by visfatin. Subsequently, we evaluated if extracellular visfatin stimulates the production of gluconeogenic enzymes through the classical protein kinase A (PKA)/cyclic AMP-responsive element (CRE)-binding protein (CREB)-dependent process. The phosphorylation of CREB and PKA increased significantly in HepG2 cells treated with visfatin. Additionally, Knockdown of CREB and PKA inhibited visfatin-induced gluconeogenesis in HepG2 cells. In summary, extracellular visfatin modulates glucose production in HepG2 cells through the PKA/CREB pathway, rather than via SIRT1 signaling.

2595-PO

T Cell-derived OSM in Adipose Tissue Promotes Metabolic Dysfunction in Adipocytes via OSMRβ and STAT5

CARRIE M. ELKS, PENG ZHAO, RYAN W. GRANT, URSULA A. WHITE, DAVID SANCHEZ-INFANTES, JACQUELINE M. STEPHENS, *Baton Rouge, LA, Barcelona, Spain*

The gp130 cytokines are known to mediate many biological processes, and several members of this cytokine family have been targeted as anti-obesity therapeutics. Oncostatin M (OSM) is a gp130 cytokine that is distinct from other members of this family, in that it signals through a transmembrane receptor specific to the OSM protein (OSMRβ). We have recently shown that OSM expression is highly elevated in the adipose tissue of obese/T2D mice and humans. In follow-up studies, we have shown that T cells are the primary source of elevated OSM expression in murine adipose tissue in conditions of obesity/T2D. These new results support our previous studies indicating that OSM is not produced from adipocytes in adipose tissue of obese humans. Our new data also indicates that OSMRβ is highly expressed in adipose tissue compared to many other tissues. We have also previously demonstrated that OSM induces inflammatory markers in adipocytes *in vitro*. We therefore hypothesized that T cell-derived OSM promotes metabolic dysfunction in adipocytes. To test this hypothesis we used siRNA to knockdown OSMRβ in murine adipocytes. OSM treatment normally results in a substantial induction of MCP1 and IL-6 expression, but these effects were absent in adipocytes lacking OSMRβ. OSM is normally a potent activator of STAT3 and STAT5, but interestingly, in adipocytes lacking OSMRβ, the ability of OSM to induce STAT5, but not STAT3, tyrosine phosphorylation was significantly blunted. Collectively, these data demonstrate that the effects of OSM are mediated by OSMRβ; and suggest that OSM produced from non-adipocyte cells in adipose tissue, such as T cells, may directly affect adipocytes in a metabolically unfavorable manner. We have utilized adiponectin-Cre mice and floxed OSMRβ mice to create a mouse that lacks OSMRβ in adipocytes. We are currently phenotyping this mouse and predict these mice may be protected from the metabolically detrimental effects of high fat feeding.

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2596-PO

WITHDRAWN

2597-PO

Thyroid-related Hormones Are Associated with Glucose Control, Pancreatic Function, and Insulin Sensitivity in Type 2 Diabetes Patients

XUEFEI RUI, NI ZHONG, JIE HAO, JUNLEI SU, LE BU, HONG LI, HUI SHENG, SHEN QU, *Shanghai, China*

To investigate the association between serum thyroid-related hormones and glucose control, pancreatic function and insulin sensitivity in Han Chinese patients with type 2 diabetes mellitus (T2DM).104 T2DM patients were recruited (Male: 54, Female: 50, Average age: 67.24 ± 11.11 years). The fasting plasma glucose (FPG), insulin (FINS), C peptide (FC-P), HbA1C and thyroid-related hormones (total triiodothyronine (TT3), total thyroxine (TT4), free triiodothyronine (FT3), free thyroxine (FT4), and thyroid stimulating hormone (TSH)) were measured. Homeostasis model assessments (HOMA) were calculated using oral glucose tolerance tests (OGTT) to assess beta cell function (HOMA-%B) insulin sensitivity (HOMA-%S), and insulin resistance (HOMA-IR). After controlling for age and BMI, TT4 was positively correlated with HOMA-IR (P<0.05). FT3 was negatively correlated with HbA1C (P<0.05).

For author disclosure information, see page A743.

In males, after controlling for age and BMI, TT3 was negatively correlated with HOMA-%S (P<0.05) and positively correlated with HOMA-IR (P<0.05). TT4 was positively correlated with HOMA-IR (P<0.05). FT3 was negatively correlated with HbA1C (P<0.05), and positively correlated with HOMA-%B and HOMA-IR (P<0.05). TSH was positively correlated with HOMA-%S (P<0.05). In females, after correcting for age and BMI, TT4 was positively correlated with course of diabetes (P<0.05). FT4 was positively correlated with FPG (P<0.05). Multivariable linear regression analysis showed that HOMA-IR was the independent variable influencing TT3, HbA1C was the independent variable influencing FT3, and HOMA-%S and HOMA-IR were the independent variables influencing TSH in males (P<0.05).These results suggest that thyroid-related hormones might promote insulin secretion, increase insulin sensitivity, and improve insulin resistance. Thyroid hormone may be a target to regulate insulin secretion.

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WITHDRAWN

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Fetuin-A, Insulin Resistance, and Obstructive Sleep Apnea

ALICE LIU, SHIMING XU, JAMES CARDELL, CLETE KUSHIDA, GERALD M. REAVEN, *Stanford, CA*

Elevated plasma fetuin-A levels have been associated with insulin resistance in obese individuals, as well as patients with clinical syndromes such as type 2 diabetes (T2DM). Obstructive sleep apnea (OSA) is associated with excess adiposity, increased prevalence of insulin resistance, and risk for T2DM. The present analysis was initiated to evaluate the possibility that differences in fetuin-A may contribute to the insulin resistance seen in overweight/obese patients with OSA.

Overweight obese, non-diabetic volunteers (n=96) were recruited from the Stanford Sleep Medicine Center and surrounding communities. Nocturnal polysomnography was performed to diagnose OSA. Fasting plasma fetuin-A levels were determined, and steady-state plasma glucose (SSPG) concentrations derived during the insulin suppression test were used to quantify insulin-mediated glucose uptake; higher SSPG concentrations indicated greater insulin resistance.

Mean age was 50 years old, average body mass index (BMI) was 30.6 kg/m^2 (range 26.1 - 37.9 kg/m^2), and 36.5% were women. SSPG concentrations varied 6-fold (range 51-309 mg/dL). Correlations between fetuin-A and BMI, SSPG, or apnea-hypopnea index (AHI) were not statistically significant. Fetuin-A did not differ between the upper (insulin-resistant, IR) as compared with the lower (insulin-sensitive, IS) SSPG tertile, or between patients with severe (n=40) as compared with mild (n=27) OSA. Comparison of IR patients with severe or moderate OSA (n=24) with IS patients with mild OSA (n=12) yielded no appreciable differences in fetuin-A levels.

The lack of an association between fetuin-A concentrations and degree of obesity, magnitude of insulin resistance, or severity of OSA, suggests the absence of a major role of fetuin-A in the pathophysiology of insulin resistance in OSA.

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In Vitro Functional Study of Missense Mutation of R1174W in the Human Insulin Receptor Gene

ZHIMIN HUANG, XIAOYING HE, LIJUAN XU, HONGYU GUAN, YANBING LI, *Guangzhou, China*

Previous study showed that missense mutation of R1174W in human insulin receptor gene was associated with type A insulin resistance syndrome due to defect in glycogen synthesis in the muscle. Functional studies in the transduced human skeletal muscle cells expressing the mutant insulin receptor gene (R1174W), wild type human insulin receptor gene (HIRWT) and null plasmid (GFP) were carried out, comparing the post insulin receptor glycogen synthesis pathway (PDK1/Akt/GSK-3/GYS) in RNA transcription and protein expression level.

Splice overlapping extension polymerase chain reaction was employed to construct the mutant R1174W gene. Expression clone carrying R1174W, HIRWT and GFP was constructed separately using Gateway protocol. The 293ft cells were transfected with the target genes, followed by transduction into the human skeletal muscle cells. Quantitative real-time PCR was employed to compare RNA transcription, while western blot was performed to compare the expression of phosphorylated and total PDK1, Akt, GSK-alpha, GSK-beta and GYS proteins. Glycogen synthesis content was also measured.

Glycogen content in the HIRWT transduced cells with insulin stimulation was significantly higher than R1174W transduced cells (P=0.042).The expression of phosphorylated proteins of PDK1(Ser241), Akt(Thr308) and GSK-3 α/β(Ser21/9) in the HIRWT transduced cells with insulin stimulation were significantly higher than those without, and also significantly higher than those of R1174W transduced cells (P<0.05). The RNA transcription of PDK1, Akt1, GSK-3α/β and GYS1 were comparable in the 3 transduced cells.

The missense mutation of R1174W in the human insulin receptor gene is associated with the defect of phosphorylation of PDK1/Akt/GSK-3 proteins, impairing insulin-stimulated glycogen synthesis in the human skeletal muscle cells and leading to severe insulin resistance.

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INTEGRATED PHYSIOLOGY—INSULIN SECRETION IN VIVO

2601-PO

Syndecan Knockdown in the Insulin-Producing Cells of Drosophila melanogaster Leads to Increased Carbohydrate Levels and Decreased Tolerance to a Glucose Meal

JONATHAN L. WARREN, MARIA DE LUCA, *Birmingham, AL*

The fruit fly *Drosophila melanogaster* has emerged in recent years as a powerful model for studying human metabolic disease and pathways. *D melanogaster* produces seven insulin-like peptides (DILP1-7), with well-studied homology to mammalian insulin. DILP2 is the most highly expressed of these in the insulin producing cells (IPCs) of the fly brain. Strong evidence exists of a conserved role of DILP2 in the control of glucose metabolism. Previous findings from our lab showed decreased expression of the gene coding for DILP2 in the brain of flies homozygous for a hypomorphic mutation in the *Drosophila* syndecan (*dSdc*) gene. Syndecan is a transmembrane proteoglycan that binds a variety of circulating ligands, such as growth factors and cytokines, as well as members of the extracellular matrix proteins. The objective of this study was to further characterize the role of *dSdc* in the IPCs. Using a GAL4/UAS approach, we crossed *dilp2*-GAL4 male driver flies with UAS-RNAi-*dSdc* or w^{1118} female flies to generate flies with reduced expression of *dSdc* in the IPCs and controls, respectively. We measured food intake by capillary feeding assay (CAFÉ) and metabolic rate by indirect calorimetry. Whole-body levels of glucose and trehalose (main circulating carbohydrate in flies) were measured by colorimetric assay. An oral glucose tolerance test was performed by subjecting flies to an overnight fast, feeding for 2 hrs in a 10% glucose medium, and measuring glucose 0 hrs, 2 hrs, and 4 hrs following the meal. T-tests and analysis of covariance models were used to analyze the data. While no difference in food intake or metabolic rate was observed between mutant and control flies, we found significantly higher glucose (p<0.001) and trehalose (p<0.01) levels in the mutants. The latter also had increased levels of glucose post meal compared to controls (p<0.001). Our findings suggest that *dSdc* may play a cell-autonomous role in the proper functioning of the IPCs.

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Short-term Ad Libitum Feeding with a Chow Diet Induces Highly Dynamic Changes in Pancreatic Beta-Cell Response in Rhesus Macaques

MICHAEL STAUP, FRANCINE GREGOIRE, *Kannapolis, NC*

Aging rhesus macaques develop insulin resistance in a manner that mirrors the progression of metabolic disorder and the onset of type 2 diabetes in humans. This makes them an excellent translational animal model. Disease progression in rhesus monkeys was described as occurring in a stepwise fashion, with a slow, spontaneous transition taking place over 5 to 10 years. However, more rapid changes in metabolic parameters are known to occur in insulin resistant humans and therefore the present study sought to investigate physiological responsiveness to dietary changes among rhesus monkeys after only two months of *ad libitum* feeding with a regular chow diet. Thirteen monkeys on a controlled regimen were assessed via Intravenous glucose tolerance test (IVGTT) before and after only two months of *ad libitum* feeding. Glycemic index, insulin, triglycerides and total cholesterol levels were assessed *throughout* the study. Daily food consumption and body weight were monitored throughout the study. Body fat was profiled using dual x-ray absorptiometry imaging. After only two months of *ad libitum*