Results: Mean age was 54.3 (8.1) years and BMI 26.2 (5.8) kg/m2. 23 patients (26.4%) had overt diabetes diagnosed by FG. 79.1% of patients who underwent OGTT (all of them with normal FG) were diagnosed of diabetes, and 9.8% of IGT. 30.4% of patients who had diabetes diagnosed by FG, didn´t meet criteria for diabetes after transplantation. The insulin requirements in patients who continued to have diabetes after transplantation decreased from 0.48 IU/kg/day to 0.17 IU/kg/day ($p < 0.05$). 6 patients (9.4%) of the remaining 64 patients without overt diabetes before transplantation had diabetes diagnosed by FG after transplantation, 4 (6.3%) refused to undergo OGTT and 7 (10.9%) died. Among the patients who underwent OGTT after transplantation, 26.4% met criteria for diabetes and 30.3% for IGF. Altogether, 49% of patients with diabetes before transplantation didn´t meet criteria for diabetes 12 months after transplant (confirmed by FG and OGTT). The choice of immunosuppressive therapy was not associated with persistence of diabetes after transplant.

Conclusions: Prevalence of diabetes in patients with end-stage liver disease is high. After liver transplantation impaired glucose metabolism improve. Further studies are needed to explain the mechanisms involved.

2832-PO

Effects of Transplantation of Mesenchymal Stem Cells in High-Fat Diet-Induced Type 2 Diabetes Mellitus

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Mesenchymal stem cells (MSC) are immunomodulatory and hypoimmunogenic. These characteristics make MSC transplantation an attractive strategy to treat DM. The aim of this study is to investigate the effects of systemic MSC transplantation on glycemia regulation and pancreatic morphometric analysis of high fat diet-induced diabetes in *Swiss* mice. High fat diet-induced diabetic mice received one single or four multiple intraperitoneal injections of rat bone marrow-derived MSCs ($5\text{-}8 \times 10^6$) (MSCD group). Control high fat diet-induced diabetic mice received only PBS injections (D group) and control non-diabetic (C group) mice did not receive injections. Fasting and nonfasting glycemia were determined weekly and glucose (GTT) and insulin (ITT) tolerance tests were performed at one, two, three and four months after MSC transplantation. Four months after MSC injection, animals were killed and pancreas was collected for histological analyses. Four months after 1 single injection of MSC, 67% of MSCD animals were considered responders (fasting glycemia <180mg/dl) and 37% were considered non-responders. Four months after 4 multiple MSC injections, 70% of MSCD animals were considered responders and 30% were considered non-responders. Pre- and post- transplant fasting glycemia of responders were significantly different. After 4 injections, fasting glycemia and glycemia response to ITT of responder MSCD animals were significantly lower compared to diabetic animals. After 4 injections, the total islet area of the responder MSCD animals was significantly bigger than the islet area of both diabetic and control animals. Beta cell volume was not different among the groups. However, alpha cell volume was significantly smaller in responder animals than in control animals. In conclusion, the results demonstrate that MSC transplantation via intraperitoneal injection restored glucose homeostasis and induced pancreatic morphometric changes in high fat diet-induced diabetes in mice.

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INSULIN ACTION—ADIPOCYTE BIOLOGY

2833-PO

Effects of Insulin Glargine on Proliferation, Differentiation and Adipose Function of Human Primary Preadipocytes from Different Fat Depots

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Insulin glargine is a long-acting human insulin analogue which is widely used as basal insulin to treat diabetes, but its role on proliferation, differentiation of human primary preadipocytes and adipose function still remain unclear. In this study,we cultured primary preadipocytes from human subcutaneous (*sc.*) and omental (*om.*) adipose tissue; compare their morphological and differentiation differences. Then two kinds of preadipocytes were induced to differentiation with different dose of insulin glargine (20nM, 200nM, 500nM,1000nM and 1500nM). We observed the effects of insulin dose on adipogenic genes expression, adipokines secretion and lipolysis.Our results showed that both cells can be successfully cultured from adipose tissue and amplificated *in vitro*.

Human *sc.* preadipocytes is more slender, and proliferates more quickly, while *om.* preadipocytes were polygonal, and easier to aging. MTT results showed that insulin glargine could inhibit *om.* preadipocytes proliferation after 72h incubation, and this effect is dose-dependent, but it had no effect on the proliferation of *sc.*preadipocytes, whether in low dose or high dose. We also found that insulin at 500nM is a suitable concentration to induce differentiation. RT-PCR analysis showed that adipogenic genes such as *PPAR*γ, *C/EBP*α had the highest expression and preadipocytes gene *Pref-1* had the lowest expression at this concentration, ELISA results showed that this concentration had the strongest adipokines (leptin, adiponectin, RBP4 and TNF-α) secretion function. Too higher insulin concentration (1000nM and 1500nM) will induce lipolysis, and lower insulin concentration (200nM) will lead to incomplete differentiation. In conclusion, insulin glargine could inhibit *om.* preadipocytes proliferation, but it had no effect on the proliferation of *sc.*preadipocytes, insulin concentration at 500 nm is a suitable concentration to induce differentiation.

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INSULIN ACTION—CELLULAR AND MOLECULAR METABOLISM

2834-PO

Metabolic Effects of Insulin in Human Skeletal Muscle Myotubes With Reference to Phosphatidic Acid: An Activator of mTOR

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Insulin increases expression or activates numbers of lipogenic enzymes involved into de novo lipogenesis (acetyl-CoA carboxylase, fatty acid synthase, GPAT). Phosphatidic acid, the intermediate metabolite of de novo lipogenesis is a signaling lipid that was verified as an activator of mechanistic target of rapamycin (mTOR). Currently, mTOR is emerging as a central metabolic regulator involved in nutritional control, insulin resistance, cell growth, cell proliferation and aging. The acute effects of insulin on the content of molecular species of phosphatidic acid, its precursors- long-chain acyl-CoAs and lysophosphatidic acids, and also a terminal product of de novo lipogenesis- cardiolipin were analyzed in human myotubes obtained from vastus lateralis of athlete volunteers (n=2). Myotubes were serum-starved for 4 hs and then insulin stimulated (2.0-60 nM) for 40 min in the presence of 5 mM glucose. NAD+ was measured as a marker of myotubes content.

In the basal state, the myotubes contain 16±2 pmol of long chain acyl-CoAs, 1.5±0.5 pmol of lysophosphatidic acids, 70±10 pmol of phosphatidic acids, and 300±50 ng of cardiolipin per 1 nmol of NAD^+. The major molecular species for all the metabolites are palmitoleate, palmitate, oleate and stearate containing species. Insulin induces relative increase in palmitoyl-CoA at concentration as low as 2 nM that indicates the activation of malonyl-CoA/fatty acid synthase axis. At higher concentrations (5-40 nM) insulin induces ~30% increase in the content of both lysophosphatidic acids and phosphatidic acids. There is no significant increase in cardiolipin.

In summary, the activation of malonyl-CoA/fatty acid synthase axis is an early event in the effects of insulin in human skeletal muscle myotubes. The accumulation of phosphatidic acids follows this event. We hypothesize that glucose entrance into myotubes can play role in insulin-induced increase of the phosphatidic acid in myotubes.

2835-PO

WITHDRAWN

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2836-PO

Ecdysterone Increases Glycogen Content by Enhancing Insulin Signaling in C2C12 Muscle Cells

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Ecdysterone (ECDY), structurally similar to insect molting hormones, produces a range of effects in mammals, including increasing growth, physical performance, altering lipid and carbohydrate metabolism. We sought to study effects of ECDY on glycogen content and insulin signaling in cultured muscle cells (C2C12 cells. At day 6 of differentiation, myotubes were treated with various concentrations of ECDY overnight (14 h); glycogen content and insulin signaling protein abundance were measured using a spectrophotometric enzymatic microassay and western blotting. Results show that ECDY significantly increased both basal and insulin stimulated glycogen content in muscle cells at a dose response manner (P<0.05 - P<0.001). ECDY modestly increased IRS-1 and reduced PI 3K content at high dose (50 µg/ml), but significantly increased IR β abundance at the doses of 10, 25 and 50 µg/ml (P<0.01, P<0.001 and P<0.05, respectively) as well as IRS-2 at dose of 25 µg/ml (P<0.05) when compared with control cells. ECDY also significantly increased tyrosine phosphorylation of IRS-1 and Akt-2, but not Akt-1. Moreover, PI-3 Kinase activity at baseline and insulin stimulated condition was significantly higher in ECDY treated than in control cells at a concentration-depending manner. Collectively, this study suggests that ECDY improves glucose metabolism by enhancing insulin signaling in cultured muscle cells.

2837-PO

Effect of Lifestyle Intervention on Metabolic Characteristics in Pre-Diabetes Patients

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In order to discover the predictors for lifestyle intervention effects of diabetes and understand the development process of diabetes, we established a method of using liquid chromatography-triple quadrupole linear ion trap (LC-Qtrap) mass spectrometer to screen metabolomics biomarkers.

Subjects accepting the intensive lifestyle intervention in "Beijing Prediabetes Reversion Project"(BPRP) were selected. The 50 subjects progressed to diabetes after 1 year were enrolled, and so were 50 subjects reversed to normal glucose group who matching the diabetes group in gender, age and body mass index(BMI) as control. Clinical characteristics and laboratory tests data at baseline and 1 year follow-up were both documented. Measurements of biomarkers in fasting serum and urine were conducted in the Agilent1200 High Performance Liquid Chromatography System. Basing on these data, the method of using LC-Qtrap mass spectrometer to screen metabolic biomarkers was established.

Through this method we found the following results. (1)Compared with blood lipid and inflammatory factors, the baseline fasting blood glucose and 2 hour postprandial glucose levels had a significant correlation with long-term risk of diabetes. (2)The decline in the ability of glucose metabolism at baseline (decreased citric acid, lactic acid, oxaloacetate and α-ketoglutaric acid levels) and decreased glucogenic amino acids might predict the effect of lifestyle intervention. (3)The levels of intermediates of lipid and bile acid metabolism, and amines were different between the two subject groups in baseline and after 1 year intervention. With the glucose metabolic disorder aggravating, the changed metabolites were also differed. This suggested in different stage of glucose tolerance disorder, the disturbed metabolic pathways were different. And also the changes of amines level suggested intestinal flora might play a role in the development of diabetes.

2838-PO

Genetically Determined Insulin Resistance Alters Cellular Metabolism in iPS Cells

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Insulin resistance is associated with altered insulin action and oxidative metabolism, yet it is unknown whether cellular metabolism defects are primary features of insulin resistance and thus present in an undifferentiated state. To address this question, we created and characterized induced pluripotent stem (iPS) cells from patients with Donohue syndrome (leprechaunism). Each patient had severe insulin resistance due to an insulin receptor mutation. These iPS cells were compared to iPS cells generated from fibroblasts of 3 healthy age-matched controls. iPS cells were generated by expression of *c-MYC*, *SOX2*, *KLF4* and *OCT4* reprogramming factors. Western blot analysis of patient-derived iPS cells demonstrated a 40% reduction of insulin-stimulated phosphorylation of the insulin receptor (p<0.05), consistent with genotype. This was paralleled by reductions in downstream pathways including AKT, ERK and GSK phosphorylation. Similarly, expression of key genes involved in insulin signaling and glucose metabolism, as assessed by qRT-PCR, was also decreased in patient-derived iPS cells, including *INSR* (down 40-80%; p<0.05), *IGF1R* (down 35%; p<0.05) and *GLUT1/4, HK1* and *PKM2* (all down ~40%; p<0.05). Interestingly, genes regulating mitochondrial metabolism were relatively unchanged. Notably, catalase and *GPX3* mRNA were decreased by >60% and 25%, respectively, in both insulin-resistant lines (p<0.05). Direct assessment of mitochondrial ROS production using CM-H_2DCFDA staining revealed increased ROS levels in the patient lines. Patient iPS cells also exhibited increased energetic stress, as demonstrated by increased phosphorylation of AMPK and its downstream target ACC (~6 fold increase; p<0.05). Together, these data indicate that mutations in the insulin receptor gene in undifferentiated iPS cells increase oxidative and energetic stress.

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2839-PO

IGF1 and Insulin Signaling Chain are Upregulated in a Subgroup of Colorectal Cancer

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Obesity and Diabetes Mellitus Type 2 are associated with an increased incidence of different malignancies, in particular mamma carcinoma, colorectal carcinoma, pancreas carcinoma and renal carcinoma. It is hypothesized that hyperinsulinaemia as a consequence of insulin resistence might be part of a molecular link explaining these associations. It is speculated, that individual variation of the IGF1 and Insulin signaling chain in the tumor cell might define individual susceptibility to hyperinsulinaemia. We tested this hypothesis in 54 colorectal carcinoma Patients. mRNA levels of IGF1- Receptor (IGF1R), Insulin Receptor A and B (IRA, IRB), IRS1, IRS2, PI3Kinase (PI3K), AKT2, mTOR and Girdin were determined in cancer tissue and normal intestinal tissue of each patient.

Out of the 54 Patients more than 20fold increased respectively more than 100fold increased RNA levels were found in the following Patient numbers: IGF1R 16/ 6 Patients; IRA 10/ 3; IRB 11/ 3; IRS1 22/ 7; IRS2 17/ 5; mTOR 7/ 2; Girdin 9/ 2; AKT2 10/ 5; PI3K 2/0. In 10 Patients all elements of the signaling chain were concomitantly upregulated. In two of these Patients upregulation of all mRNA levels were more than 100fold. In three of the Patients with upregulated IGF1R mRNA enough material was available for western blotting. Receptor upregulation could be confirmed in two of the three samples.

We conclude, that in a subgroup of colorectal carcinoma Patients the molecular bases of an increased susceptibility for hyperinsulinaemia is observed, in very few individuals an extreme upregulation of the signaling chain was found. The clinical relevance of these observations is presently studied in a post-operative follow-up of the 54 Patients.

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INSULIN ACTION—GLUCOSE TRANSPORT AND INSULIN RESISTANCE IN VITRO

2840-PO

WITHDRAWN

INSULIN ACTION—SIGNAL TRANSDUCTION, INSULIN, AND OTHER HORMONES

2841-PO

Insulin Glargine (GLA) Metabolites Do Not Stimulate Cell Growth in Human Cancer Initiating Cells (CICs)

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[AspB10]insulin (X10) is a long-acting insulin analog that displays greater affinity for IGF1R than either the long-acting insulin analog GLA or human insulin (HI) in vitro. X10 is tumorigenic in animal models whereas GLA is not. GLA is rapidly metabolized to the metabolites, M1 and M2. These metabolites exhibit metabolic and mitogenic profiles similar to that of HI in vitro. CICs are cancer cells with self-renewing, stem cell-like properties with the ability to proliferate and differentiate into specific cells found in tumor types and thus they are able to maintain tumor bulk. Many cancers are thought to be initiated by CICs, and there is evidence that they cause cancer recurrence, metastatic progression and resistance to therapies. Avoiding growth factors, like insulin, estrogen or testosterone, is a current strategy to keep cancer incidence or recurrence under control. Our aim was to compare the mitogenic activity of HI, GLA, X10, M1 and M2 on human CICs in vitro.

CICs derived from 10 human cell lines including glioma, lung, breast, colon, and prostatic cancers were isolated and grown in defined growth medium (no serum) with either HI, GLA, X10, M1 or M2. Growth was estimated using the MTS colorimetric assay.

HI, GLA and X10 stimulated CIC growth to a similar extent, whereas M1 and M2 had growth promoting activity similar to medium without insulin. Similar results were obtained when measuring diametric sphere growth. Additionally, AKT phosphorylation levels were increased 2-fold after stimulation with HI, GLA or X10, whereas stimulation with M1 or M2 did not produce a significant increase.

This is the first study that has explored the biology of CICs treated with insulin analogs. The data show that GLA displays a mitogenic profile comparable to that of human insulin for all CIC lines tested, whereas M1, the main

GLA metabolite in vivo, has even less growth promoting activity. Our results do not support GLA increasing CICs growth and thus the involvement of GLA in cancer promotion or relapse.

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2842-PO

Insulin Glargine and Human Insulin Promote Proliferation of Human Hepatocellular Carcinoma HepG2 Cells via MEK/ERK1/2

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In the present study, we investigated the effect of insulin glargine and human insulin on proliferation of human hepatocellular carcinoma HepG2 cells and the role of PI3K/Akt and MEK/ERK1/2 in the process. HepG2 cells were incubated with insulin glargine and human insulin at different concentrations and for different time courses. Specific inhibitors for PI3K, LY294002, and for MEK1/2, PD98059 and U0126, were used either alone or in combination with insulin or glargine, respectively, to test the involvement of PI3K/Akt and MEK/ERK1/2 pathways. Cell proliferation was evaluated using cell counting kit-8 reagents. Protein phosphorylation of Akt and ERK1/2 were analyzed by Western blot. Insulin glargine and human insulin similarly enhanced HepG2 cell proliferation at the concentrations of 10 and 100IU/L after treatment for 72 h. No significant difference was observed between insulin glargine and human insulin in their effects on HepG2 cell proliferation. HepG2 cell proliferation was significantly inhibited by LY294002 and U0126, in association with reduction in phosphorylation of Akt and ERK1/2, respectively. Insulin- and glargine-induced HepG2 cell proliferation and phosphorylation of ERK1/2 were blocked by PD98059 and U0126. However, in the presence of LY294002, both drugs still promoted cell proliferation as compared with cells treated with LY294002 alone. These data suggested that insulin glargine and human insulin similarly promoted HepG2 cell proliferation via activation of MEK/ERK1/2, not PI3K/Akt.

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2843-PO

Comparison of the Rabbit, Diabetic Miniature Swine and Non-Human Primate to Evaluate the Clinical Biopotency of Insulin Products

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The potency of human insulin has classically been evaluated following the U.S. Pharmacopeia (USP) guideline for comparison to an international standard (IS) of activity (IU). However, since insulin analogues are intentionally different and cannot be standardized against IS, there is a need for a bioassay to assess clinical specific activity (U). To this extent, we compared the biopotency (U) of different insulin products in rabbits, in type 1 diabetic miniature swine and in normal non-human primates, and used human and pork insulin as reference standards. New Zealand White rabbits were fasted and injected subcutaneously (s.c.) at a dose level of 0.5 U/kg. Yucatan miniature swine (Sus scrofa) were made diabetic by intravenous administration of alloxan and insulin products were injected s.c. at dose level of 0.1 U/kg in overnight fasted animals (no feed or insulin for 18 h). Normal insulin suppression tests (nIST) were conducted in fasted male cynomolgus primates (Macaca fascicularis) receiving a bolus intravenous (i.v.) infusion of glucose (ivGTT; 0.25 g/kg) and treated with somatostatin. Insulin products were given at dose level of 0.05 U/kg. Glucose levels were recorded using handheld glucometer devices. The blood glucose kinetic (BGPK) and the blood glucose area under the curve (BGAUC) were used to assess biopotency in the rabbit and in the diabetic miniature swine. The slope of the blood glucose clearance (k_G) was used to assess biopotency during the nIST. Our data indicate that the biopotency of insulin products can be assessed using BGAUC and k_G, but that only the type 1 diabetic miniature swine can discriminate between differences in biopotency for all aspects of BGPK. For example, the BGPK for short-acting human and pork insulin were similar in the rabbit assay but their respective BGAUCs were different (ratio of 1.2). Accordingly pork insulin was more potent during nIST in the non-human primate (slope of -0.012 vs. -0.010; pork and human, respectively).

2844-PO

WITHDRAWN

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