2460-PUB

A Functionalized Photo Tunable Hydrogel Promoting Islet Transplant Engraftment and Function

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Islet transplantation is emerging as a promising cell replacement therapy for type 1 diabetes. To date, however, several hurdles remain for the widespread adoption of this approach. First, revascularization of islet transplants remains inefficient leading to substantial tissue loss during the first few days after transplantation. Second, immune recognition of allogeneic histocompatibility antigens and recurrence of autoimmunity requires systemic immunosuppressive regimens that have been shown to negatively impact islet engraftment, survival and function. To overcome these limitations, the identification of biocompatible materials that can be used to encapsulate islet grafts offers new opportunities. Yet, encapsulation materials tested so far have met limited success for the long-term survival, function and protection of islet grafts in vivo.

In this study, we have designed and tested a new generation of biocompatible materials whose properties can be readily tuned to integrate user-defined biophysical and biochemical cues that support islets function, promote interaction with the host vasculature and incorporate immune modulatory moieties capable of mitigating host immune responses. The material is a new poly (ethylene glycol)-based hydrogel whose chemistry render it phototunable, allowing for the dynamic material functionalization of the hydrogel with pro-angiogenic cues and immunoregulatory moieties upon mild exposure to cytocompatible light. Our results demonstrate that this new programmable biomaterial supports islet cell survival and function by providing a tissue-like niche whose three-dimensional architecture and biochemical composition is designed to incorporate extracellular matrix moieties that we previously identified in the human pancreas, support angiogenesis, and promote leukocyte exclusion from the grafts. We anticipate that this new approach will have a significant translational application to human islet transplantation.

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INSULIN ACTION—ADIPOCYTE BIOLOGY

2461-PUB

Omics Analysis of Adiponectin in African Americans to Elucidate the Biology Underlying Metabolic Disease

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There is extensive epidemiological literature examining adiponectin in T2D and obesity. Despite higher prevalence, few studies have examined African Americans (AAs) where differences are accentuated, i.e., lower protein levels compounded by higher insulin resistance and cardiometabolic disease risk. Beyond statistical implication, we assessed the biological contribution of adiponectin to cardiometabolic phenotypes using omics technologies. Plasma adiponectin, transcriptional profiling of subcutaneous adipose and muscle, glucose homeostasis (fasting and FSIGT), and genotypes (Omni5+) were assessed in 240 fasting nondiabetic AAs. Plasma adiponectin was negatively correlated with insulin resistance ($HOMA_{IR}$; r=-0.36, P=1.7E-8) and adiposity (BMI; r=-0.25, P=9.3E-5), with similar relationships for the ADIPOQ transcript (r=-0.25, P=4.6E-5 and r=-0.37, 1.2E-9, respectively) in adipose (age, gender, admixture adjusted). The correlation between protein and transcript was weak (r=0.28, P=1.5E-5) suggesting additional modulators. Despite no ADIPOQ muscle expression, correlation of the adipose transcript with downstream targets in muscle, e.g., ADIPOR1 and AKT1, indicates tissue-tissue crosstalk. A positive correlation ($r \geq 0.4$) of 247 transcripts (e.g., CS, DLST, ECH1) was enriched for mitochondrial function and oxidative phosphorylation (P=1.2E-22). A negative correlation ($r \leq -0.4$) of 44 transcripts (e.g., CD68) was enriched for immune response (P=2.32E-28). Association analysis of the ADIPOQ locus with transcript level identified rs17846866 (P=1.1E-5), in ADIPOQ which was not associated with plasma adiponectin (P=0.9). These results suggest higher order regulation of protein pathways, e.g., protein multimerization. Analysis is currently underway for high molecular weight adiponectin. These results elucidate the metabolic role of adiponectin in human physiology and reveal novel insights into the regulation of metabolic disease.

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INSULIN ACTION—CELLULAR AND MOLECULAR METABOLISM

2462-PUB

WITHDRAWN

2463-PUB

WITHDRAWN

2464-PUB

WITHDRAWN

2465-PUB

WITHDRAWN

2466-PUB

Insulin Activated AMPK through Inhibiting Rho Kinase in Insulin-Resistant Skeletal Muscle Cells

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Lipotoxicity has been associated with type 2 diabetes mellitus. Our recent study indicated that SREBP-1c, a transcription factor that controls cellular lipogenesis, participated in fatty acid-induced insulin resistance through a direct effect of suppressing the transcription of insulin receptor substrate-1 in skeletal muscle cells, addressing its potential importance in metabolic disease. The molecular mechanism of insulin reducing SREBP-1c protein level is unclear.L6 myotubes were added with a final concentration of 0.5mM palmitic acid (PA) for 0 h to 48 h and then treated with 100nM insulin for 12 h. L6 myotubes were added with 0-100nM insulin after 0.5mM PA intervention. DN-AMPKα2 lentivirus, siRNA-AMPKα2 and AMPK inhibitor were used for determining the role of AMPK in vivo and in vitro. In order to investigate the specific mechanisms responsible for the insulin-induced AMPK activation, siRNA-ROCK1 and siRNA- LKB1 were transfected into PA-induced L6 myotubes. The protein levels were measured by western blot.We found that insulin increased AMPK phosphorylation and reduced SREBP-1c protein

expression in PA-treated L6 cells. Importantly, the inhibition of SREBP-1c activity by insulin was notably attenuated with the use of DN-AMPKα2 lentivirus, siRNA-AMPKα2 and AMPK inhibitor in vivo and in vitro, suggesting that the activation of AMPK is sufficient and necessary for insulin suppression of SREBP-1c activity in lipid energy surplus. Furthermore, to provide the molecular mechanism by which insulin activated AMPK, the potential role of Rho kinase/LKB1 signaling was studied. Our results showed that PA stimulated Rho kinase and inhibited LKB1 expression to inhibit AMPK. Accordingly, insulin intervention reversed the activity of Rho kinase induced by PA. In summary, our studies for the first time demonstrated that insulin activated AMPK through inhibiting Rho kinase in insulin resistant skeletal muscle cells for ameliorating insulin resistance.

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2467-PUB

Antidiabetic Effect of Trans-S-1-propenyl-L-cysteine Sulfoxide, from Allium Cepa

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Studies have shown that Allium cepa, onions, possess appreciable antidiabetic effects. Of the compounds in A. cepa, Alk (en) yl cysteine sulfoxides (ACSO) are believed to play a major role in onion's pharmacological properties. This study was therefore aimed at determining the antidiabetic effects of trans-S-1-propenyl-l-cysteine sulfoxide (PeCSO), the major ACSO in onions, in streptozotocin (STZ) induced diabetic mice. To isolate PeCSO, onion bulbs were macerated and subjected to a series of chromatographic techniques to obtain PeCSO with a yield of 1.65 mg/g fresh weight and purity of 98%. Type 1 diabetes in mice was induced by a single high dose STZ i.p injection while type 2 diabetes was induced by a combination of high fat diet (HFD) and low dose STZ. Oral administration of purified PeCSO in type 1 diabetic mice for 4 weeks reduced blood glucose to an average of 189 mg/dL. In contrast, diabetic control mice that received saline had their blood glucose exacerbate to 553 mg/dL, suggesting that PeCSO was either suppressing the effects of, or ameliorating the damage caused by STZ. Oral administration of the onion extract in type 2 diabetic mice tended to decrease the elevated blood glucose levels. Preliminary results from in vivo experiments indicate that PeCSO stimulates insulin secretion in isolated islets from mice. In vitro antioxidant effect of PeCSO was evaluated by monitoring its 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and H_2O_2 scavenging abilities. Results showed that at high concentration of 50mg/ml, PeCSO is an excellent scavenger of DPPH radical in DMSO, with a DPPH radical formation inhibition ability of 91%, as compared to that of N-Acetyl cysteine (NAC), a potent antioxidant, which was 51%. On the contrary, PeCSO did not show any significant H2O2 scavenging ability. From bioavailability assay, the highest concentration of PeCSO in blood was observed 1 hour post administration. Although more studies are needed, our results suggest that PeCSO possess appreciable antidiabetic potential.

INSULIN ACTION—SIGNAL TRANSDUCTION, INSULIN, AND OTHER HORMONES

2468-PUB

Effect of the Branched (Hydroxy) Fatty Acid Esters 5- and 9-PAHSA on Metabolic Control In Vitro and In Vivo

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Branched (hydroxy) fatty acid esters (FAHFAs) have been proposed as a novel class of endogenous mammalian lipids and suggested to improve glucose control in mice by stimulation of insulin and GLP-1 secretion, activation of Glut4 translocation and anti-inflammatory properties. Especially the isomers 9-PAHSA and 5-PAHSA seem to be promising for treatment of hyperglycemia. Here we describe the synthesis of 5-/9- PAHSA and their specific R- and S- enantiomers and their effects on metabolic parameters in vitro and in vivo. An enantioselective synthesis of 5-/9- PAHSA using cross metathesis was developped to access enantiopure compounds in good yields (55% over 5 steps). Neither racemic 5- or 9- PAHSA or their isolated enantiomers were able to i) improve insulin stimulated glucose uptake in L6 myocytes or human primary adipocytes, ii) to stimulate GLP-1 release in GLUTag cells or iii) to induce glucose stimulated insulin secretion (GSIS) in primary rat islets, respectively. In DIO mice, acute oral treatment with 5- and 9-PAHSA (45mg/kg) or a mix of both isomers (22.5mg/kg each) did not significantly affect glucose tolerance when compared to vehicle treated mice. Furthermore, no significant stimulatory effects on GSIS and GLP-1 secretion were observed. We speculated that the endogenous PAHSA-concentrations in mice are affected by the dietary FAHFA intake and that FAHFA concentrations in high-fat diets are determined by the dietary fat source. We therefore repeated the in vivo experiments using 6 different rodent diets (3 standard diets and 3 HFD). However, treatment with 5- or 9-PAHSA did not show beneficial effects on glucose metabolism irrespective of the diet. In summary, PAHSAs failed to improve metabolic end effects in cellular in vitro models. In DIO animal models, 5'-PAHSA and 9'-PAHSA alone or in combination were not able to improve the metabolic status. Therefore PAHSAs might not be considered as therapeutic glucose lowering principle.

2469-PUB

Insulin Resistance in Patients with Multinodular Goiter and Auto-immune Thyroiditis

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Introduction: Insulin resistance and the metabolic syndrome are important risk factors for cardiovascular disease, even in individuals without diabetes.

Aims: To evaluate the presence of insulin resistance and cardiovascular risk factors in patients with multinodular goiter (MNG) and auto-immune thyroiditis (AIT) with normal thyroid function.

Methods: We analyzed thyroid function, BMI, waist circumference, lipid profile, C-reactive protein (CRP), antithyroid antibodies, homocysteine, folic acid, vitamin B12 levels and insulin resistance indexes such as HOMA-IR, QUICKI, ΔAUC glucose, HISI (hepatic insulin sensitivity index), ΔAUC insulin and IGI (insulinogenic index) in OGTT in 181 patients with MNG and 232 patients with AIT. The OGTT was performed in the morning with measurements for glycose and insulin at 0, 30, 60, 90 and 120 minutes after 75 g of glycose *per* os. For the statistical analysis we used the Mann-Whitney test and Spearman correlations. A two-tailed $p<0.05$ was considered statistically significant.

Results: There was no significant differences in age, BMI, waist circumference, TSH, FT3, FT4, CRP, thyroglobulin, glycose, total cholesterol, HDL, triglycerides, ApoA1, ApoB, Lp(a), folic acid and vitamin B12 levels. The patients with MNG had significantly higher levels of LDL (125.84 ± 109.11 vs. 116.57 ± 109.42 mg/dl, p=0.01), homocysteine (8.85 ± 98.99 vs. 6.72 ± 39.98 µmol/L, p=0.02), insulin (67.62 ± 1677.15 vs. 51.13 ± 574.73 µU/ml, p=0.01) and C-peptide (9.44 ± 9.36 vs. 8.18 ± 5.86 ng/ml, p=0.02). The values of HOMA (0.24 ± 0.03 vs. 0.17 ± 0.01, p=0.03), HISI (334.89 ± 703.77 vs. 92.89 ± 97.10, p=0.02) and ΔAUC glucose (63.32 ± 2046.28 mg/dl x min, p=0.02) were also significantly higher in the MNG group.

Conclusion: The patients with MNG had significantly higher level of insulin resistance, homocysteine and LDL cholesterol when compared with the patients with AIT. It's possible that there are common etiopathogenic factors between insulin resistance and MNG.

2470-PUB

1,25-(OH)2D3 Increases NO and Inhibits ET-1 Synthesis in Human Umbilical Vein Endothelial Cells through Akt/eNOs and Erk/ET-1 Pathways

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Vitamin D functions are not limited to skeletal health benefits and may extend to preservation of insulin secretion and insulin sensitivity. This study investigated the effects of 1,25-Dihydroxyvitamin D3($1,25\text{-}(OH)_2D_3$) on the synthesis of NO and ET-1, the changes of Akt/eNOs and Erk1/ET-1 signal pathway in HUVECs pretreated with or without palmitic acid. Our results showed a significant increase in NO production in VD(1nM) +PA(0.6 mM) group compared with the PA group at 30s and 1 min. ET-1 mRNA expression sharply decreased in VD+PA group compared to the PA group at 0.5 h, 1 h, 2 h, 4 h, 8 h, 16 h. Western blot tests showed the protein relative ratio of pAkt/Akt and peNOs/eNOs were up-regulated and pErk/Erk was down-regulated dose dependently in the VD+PA group compared to the PA group. We conclude that $1,25\text{-}(OH)_2D_3$ increase NO synthesis through activate Akt/eNOs pathway and decrease the ET-1 production through inactivate Erk/ET-1 pathway. Our results indicate $1,25\text{-}(OH)_2D_3$ is likely a good candidate for insulin sensitizer and may have potential vascular protection effect.

For author disclosure information, see page A696.

Figure 1.

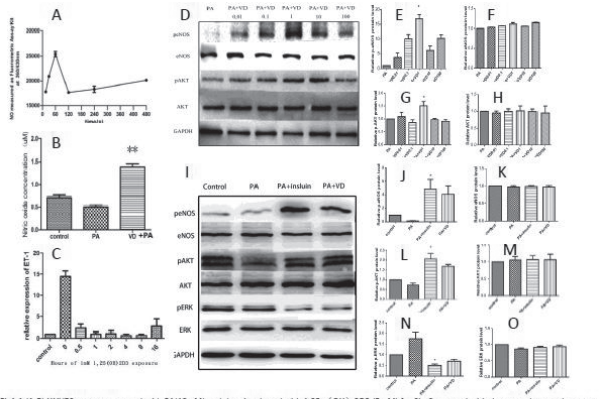

Fig1.A (A-B) HUVECs were pretreated with PA(10 μM) and then incubated with 1,25-（OH）2D3 (5 μM) for 6h. Compared with the control group,there was a significant decrease of the NO release in PA group （0.5065±0.0301μM vs 0.7102 ±0.0438μM，p <0.01) and a significant increase in VD+PA group at 30s and 1min. C.ET-1 mRNA expression significantly decreased in VD+PA group at 0.5h,1h,2h,4h,8h,16h （p all< 0.05）. （D-O）Western blotting results showed :the relative ratio of pAkt/Akt and peNOs / eNOs were up-regulated dose dependently in the VD+PA group compared to the PA group （ p all< 0.05） . the expression of peNOS and pAkt proteins were up-regulated after vitamin D（1nM） and insulin (100nM) treatment (1.6928±0.0972 vs 0.7379 ±0.0996, 2.0563±0.2785 vs 0.7379 ±0.0996 , p <0.05), and the expression of p-Erk was down -regulated after vitamin D（1nM） and insulin (100nM) treatment (0.5064 ±0.1241 vs 1.7563 ±0.5326, 0.7044 ± 0.1634 vs 1.7563 ±0.5326 , p <0.05).*P <0.05, **P <0.01. All data from 3 independent expriments.

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INTEGRATED PHYSIOLOGY—INSULIN SECRETION IN VIVO

2471-PUB

GPR40 Knockout Rats Have Diminished Lipid-mediated Potentiation of Insulin Secretion

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Chronic activation of GPR40, a fatty acid sensing G-protein-coupled receptor, improves glycemic control in type 2 diabetic humans.

We used zinc finger nuclease technology to generate GPR40 null rats in the Sprague-Dawley genetic background with a 2 bp deletion resulting in a truncated GPR40 protein. GPR40 Knockout (GPR40 KO) rats and their wild type (WT) littermates were fed either high fat (HFD, Research Diets 12492, 60% kcals fat) or regular chow (LabDiets 5001, 13% kcals fat) for ten weeks.

On regular chow, GPR40 KO rats showed fasting hyperglycemia when compared to WT rats (118 ± 4.5 vs. 94 ± 3.0 mg/dl, $p < 0.05$); there were no other differences observed between GPR40 KO and WT rats.

HFD-GPR40 KO rats showed fasting hyperglycemia (121 ± 5.1 vs. 93 ± 2.5 mg/dl, $p < 0.05$) with decreased fasting insulin (471 ± 124.3 vs. 994 ± 105.2 pg/ml, $p < 0.05$). HFD-WT rats were hyperinsulinemic compared to WT rats on regular chow (1201 ± 166 pg/ml vs. 517 ± 80.4 pg/ml, $p < 0.05$). Insulin levels in HF-GPR40 KO rats were similar to GPR40 KO rats on regular chow. After a glucose challenge, the glucose area under the curve (AUC) of HFD-GPR40 KO rats was higher than, but not statistically different from, HFD-WT rats (24411 ± 660 vs. 22224 ± 1237 mg/dl/120 min). However, the insulin AUC was markedly decreased in the HFD-GPR40 KO rats (264,737 ± 25,702 vs. 479,989 ± 40,157 pg/ml/120 min, $p < 0.05$).

We examined pancreatic islets from the WT and KO rats. WT and KO islets responded similarly to increased concentrations of glucose; however, consistent with the in vivo data, when the islets were challenged with glucose and palmitate, the islets from the KO rats showed a diminished insulin secretory response.

Taken together, these data demonstrate that GPR40 is required for the maintenance of fasting glycemia and the compensatory response of the β-cell to lipid-induced hyperinsulinemia.

2472-PUB

WITHDRAWN

2473-PUB

Application of Translational Pharmacokinetic and Pharmacodynamic Modeling in the Development of GPR40 Partial Agonists

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Partial GPR40 agonists have been clinically validated as a mechanism for glucose lowering in patients with type 2 diabetes. A prior GPR40 partial agonist TAK-875 by Takeda was discontinued in Phase 3 due to liver toxicity with unknown mechanism. Discovery efforts for identifying novel GPR40 partial agonists have since focused on compounds with low anticipated clinical dose as a mitigation strategy. Given the critical nature of human dose projection for this target, a translational pharmacokinetic and pharmacodynamic model was developed to enhance predictions of clinical dose-response for novel chemical matter.

A semi-physiologic glucose and insulin model was developed based on a human model published by Jauslin et al. [J Clin Pharmacol 2007;47:1244–55]. This compartmental model was allometrically scaled and applied to glucose and insulin data observed in man and in Goto-Kakizaki rats. Published and internal data from human trials and rodent experiments under fasted or oral glucose tolerance test conditions in single dose and chronic administration were included in the analysis. A model-based in vitro-in vivo correlation relating potency from a cell-based inositol phosphatase 1 accumulation assay to in vivo response was established to enable predictions.

The translational model adequately described data for 5 compounds evaluated in preclinical experiments and was predictive of the published human dose-response relationship of TAK875. Simulations from the quantitative model were shown to be consistent with the observed dose-response glucose data both in the fasted state and following meals in the completed MK-8666 proof of concept study. This model was further used in making quantitative predictions of the dose-response relationship for novel GPR40 agonists in discovery phase, and has been used by the development team as a basis for prioritization and decision making around proposed clinical development programs.

2474-PUB

WITHDRAWN

INTEGRATED PHYSIOLOGY—LIVER

2475-PUB

Triple Therapy Utilizing Vitamin E, Milk Thistle, and Carnitine Improves ALT and the Metabolic Abnormalities Associated with NAFLD

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Evidence of scientific data in peer reviewed journals indicates that antioxidant supplementation may improve abnormal liver chemistries, glucose control, and hyperlipidemia, in patients with NAFLD. The primary objective was to determine the effects of Vitamin E 200IU, Silymarin 750mg, L carnitine 1gm (VSC) on normalization of abnormalities in liver function testing in patients with NAFLD and to determine possible improvements in blood glucose, hemoglobin A1c, cholesterol, LDL, triglycerides and CRP. Patients with NAFLD were treated with either VSC (n=15) or placebo (n=17) for a period of 24 weeks. The following laboratory values were assessed AST, ALT, triglycerides, HDL, LDL, insulin levels, glucose and CRP. Eleven out of 15 (70%) patients in the VSC group had normalization of ALT whereas 9 of 17 patients (54%) had normalization of ALT in the placebo group (p=0.1). Patients treated with VSC had a 3% reduction in serum glucose levels after 24 weeks of treatment, whereas patients on placebo had a 6% increase in serum glucose levels. A 17% increase in serum insulin levels was noted in the placebo group, while a 18% reduction in insulin was seen in the VSC group. Serum triglycerides were reduced by 12% at week 24 in the VSC treated subjects whereas a 18% increase in serum triglycerides were seen in the placebo group. A trend in the reduction of serum cholesterol, HDL, LDL and CRP was seen in the VSC treated patients in comparison to the placebo treated group. In this 24 week study patients treated with VSC had normalization of ALT and significant reductions in serum glucose, insulin and triglycerides in comparison to the placebo group. Also noted in this study were reductions in AST, cholesterol, HDL and LDL and CRP levels. The ability of this compound