INSULIN EFFECTS ON PANCREATIC BETA CELLS-NOT ALWAYS WHAT YOU MAY THINK: INSULIN ACTIVATES APOPTOTIC PATHWAYS AND CONTRIBUTES TO BETA CELL DETERIORATION

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Insulin resistance leads to increased levels of insulin necessary to control plasma glucose levels and results in persistent hyperinsulinemia followed by hyperglycemia Some studies indicate that signs of mitochondrial dysfunction in beta cells may precede evidence of beta cell damage(1;2). Hence, hyperinsulinemia may contribute to the development of beta cell deterioration in T2DM. Indeed, we could show that insulin increases death of pancreatic beta cells and exacerbates the effects of H_2O_2on their viability(3). Here, we investigated the possibility that insulin may adversely influence pancreatic β cells through activation of apoptotic pathways. Effects of insulin on cell viability were conducted on 3 pancreatic β cell lines, isolated mouse and human islets, as well as on 3 non-pancreatic beta cell lines. Cell viability was estimated by measurements of LDH and by Cell-Titer Blue viability assay. Caspase activity was also determined. Treatment of beta cells or islets with insulin for 24 hr caused a decrease in viability and increase in caspase 3/7 activity. Decreased viability induced by H_2O_2could also be increased by insulin. These effects were specific to insulin and selective for pancreatic β cells. Viability of non-pancreatic beta cells was not decreased by insulin. The caspase inhibitor z-VAD-fmk abrogated both the increase in caspase activity and the decrease in viability of Min6 cells induced by insulin. The findings indicate that prolonged elevated levels of insulin, alone or in combination with other factors, may adversely affect pancreatic beta cells by activating apoptotic mechanisms that lead to beta cell deterioration and death.

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HEPATIC MICRORNAS 34A AND 221 ARE ELEVATED IN OBESITY AND REGULATE ADIPOR1 AND ADIPOR2 PROTEIN EXPRESSION

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Aim: Obesity is associated with several pathological conditions including insulin resistance and type 2 diabetes. As miRNAs have been identified as a new class of gene expression regulatory molecules, we hypothesized that obesity-induced deregulation of hepatic miRNAs may promote insulin resistance.

Results: To evaluate the effect of obesity on hepatic miRNAs, we compared the miRNAs expression pattern in livers of ob/ob mice, a genetic model of obesity, and C57/BL6 lean mice, using the TaqMan Low-Density Arrays (TLDAs) technology. This analysis revealed significant up-regulation of 18 miRNAs and down-regulation of 4 miRNAs in livers of ob/ob compared with control mice. Evaluating 9 of the 22 deregulated hepatic miRNAs by qPCR in a large group of ob/ob mice as well as in diet-induced obese mice has confirmed that 6 miRNAs were significantly upregulated in both models, including miR-34a and miR-221. Using bioinformatics analysis, we identified adiponectin receptor-1 (AdipoR1) gene product as a potential miR-221 target, and AdipoR2 as a potential miR-34a target. These receptors have been shown to mediate the insulin-sensitizing, anti-inflammatory effects of adiponectin.To examine whether miR-34a and miR-221 negatively regulate AdipoR1/2 expression, their precursors were transfected into HepG2 cells and AdipoR1/2 protein levels were assessed by western blotting. miR-34a or miR-221, respectively, decreased AdipoR2 and AdipoR1 protein levels, without affecting their mRNA level. Moreover,miR-34a or miR-221 overexpression respectively decreased reporter activity of AdipoR2- or AdipoR1- 3' UTR-containing luciferase constructs in HepG2 cells, confirming AdipoRs as miR-34a and miR-221 targets.

Conclusions: Our findings uncover deregulation of hepatic miRNAs in obesity and support a role for miR-34a and miR-221 in controlling AdipoRs expression.

AHNAK ROLE IN OBESITY AND INSULIN RESISTANCE

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AHNAK is a giant phospho-protein that participates in hyperlipidemia - mediated cellular signaling in cardiac muscle. We have previously shown in vitro that in rat adipocytes AHNAK negatively regulates the GLUT4-Promoter activity (GLUT4-P); impairs Glut4 translocation and cellular glucose-uptake and inversely correlates with Glut4 protein level in adipose tissue obtained from obese patients. However AHNAK in vivo role in obesity and DM2 is yet unclear.

Compared to lean non diabetic patients, AHNAK serum levels were 1.85 and 2.4 fold ($p \leq 0.05$) increased in obese and obese/DM2 patients, respectively. AHNAK serum levels significantly correlated with BMI ($R=0.385$, $p=0.05$),and negatively correlated with HDL levels ($R=-0.465$, $P=0.022$).

Compared to control rats AHNAK protein levels were increased by 230±0.3% (Mean ±SEM; $p \leq 0.05$) and 608±22% ($p \leq 0.05$) and Glut4 protein decreased by 80±6.5% and 78±0.01% in adipose and skeletal muscle obtained from aged/obese rats, respectively.

This inverse relation between AHNAK and Glut4 cellular protein levels was also observed in adipose tissue obtained from ob/ob mice where three fold increase in AHNAK protein level was associated with 65% reduction in Glut4 protein levels ($p<0.05$).

In vivo, 6 weeks AHNAK KO mice fed normal chow have significant alterations in body composition compared to control mice. AHNAK-KO had 50% less fat mass and significant higher lean body (muscle) mass 86 vs 82% ($p<0.05$).

Most importantly, AHNAK-knockout mice displayed protection against high fat diet (HFD) induced obesity. After 4 weeks of HFD AHNAK-KO mice weight was 27 gr while WT mice on normal chow or HFD weight weighed 28 and 34 gr, respectively ($p<0.05$ compared to WT-HFD).

In conclusion, we introduce AHNAK as a new player in the “diabesity” syndrome and therefore as a potential molecular target for obesity and type 2

AUTOLOGOUS PERIPHERAL BLOOD-DERIVED STEM CELL PRODUCTS FOR THE TREATMENT OF DIABETES COMPLICATIONS

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Background: Recent data shows that dendritic cells (DCs) have a role in pro- and anti-angiogenic processes. We describe here a process in which human Hematopoietic Stem/Progenitor Cells (HSPC) are specifically stimulated by activated DCs, making it possible to use unmobilized peripheral blood as a source for therapeutic cells, thus eliminating both surgical bone marrow harvesting and G-CSF mobilization.

Goal: To show that DCs can direct the generation from blood of an Enriched Endothelial Progenitor Cell (EnEPC) population, which includes Endothelial Progenitor Cells (EPC) and HSPCs, addressed to treat critical limb ischemia.

Methods: Blood-derived immature DCs were used to generate EnEPC from HSPCs by an overnight culture. A specific EnEPC formulation [BGC101] was tested in-vitro as well as in a nude mouse hind limb ischemia model) in order to evaluate its safety and therapeutic potential, following intramuscular transplantation.

Results: After *in-vitro* testing showing that BGC101 contains 84±10x10^6 cells with a viability of 97±2% is composed of a mixture of 56±3% EPC and 16±2% HSPC *in-vivo* results on day 21 after BGC101 treatment show improved limb function and doubled the blood flow to the legs from an average of 23±5% after injury to an average of 51±3% ($p<0.005$).

Conclusions: Results shown here indicate that blood-derived DCs can direct *in-vitro* cellular interactions resulting in a potentially therapeutic EnEPC population after a short-term culture of HSPC. The cellular product was found efficient and safe in the hind-limb ischemia model. This process makes it possible to use unmobilized blood as the raw material for generating stem/progenitor cell products.

diabetes therapy.

GENERATION OF HUMAN ADIPOSE TISSUE FOAM CELLS CORRELATES WITH CLINICAL PARAMETERS ASSOCIATED WITH THE METABOLIC SYNDROME

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Inflammation is one mechanism proposed to link obesity with diabetes. Macrophages accumulation in adipose tissues is proportional to obesity and suggested to contribute to insulin resistance and diabetes. We discovered in histological sections of human fat biopsies a sub-group of macrophages loaded with lipids which resemble atherosclerotic foam cells. Here we investigated whether adipose tissue foam cells (ATFC) correlate with clinical parameters associated with the metabolic syndrome. ATFC accumulated in crown-like structures surrounding the adipocytes in omental fat biopsies of obese patients. Omental ATFC significantly increased with BMI (spearman correlation, p: 0.0181, n=25).Among obese non-diabetic persons, increased number of ATFC were found in omental depots with intra-abdominal (central) fat distribution compared with subcutaneous adiposity (3.4 ±0.4 fold, n=11 and 12 for central and subcutaneous adiposity, respectively). Importantly in another group of patients, elevated number of ATFC were detected in diabetic compared with non-diabetic patients (2.5 ±0.3 fold, n=11 and 15, respectively). Using flow cytometry to quantify ATFC, we gated on CD45+, CD14+, CD64+ cells and analyzed their lipid content by Bodipy staining for neutral lipids. ATFC were more abundant in obese males and significantly increased in omental compared with subcutaneous adipose tissues (2.6 ±0.3 fold in mean lipid content, p: 0.012, n=18 and 14, respectively).

In conclusion, we discovered in human adipose tissues formation of foam cells in crown-like structures surrounding the adipocytes. Foam cells were abundant in omental adipose tissues and correlated with males, BMI, intra-abdominal adiposity and diabetes, which are clinical parameters associated with the metabolic syndrome. We propose that the foam cells participate in clearance of lipids from dead adipocytes accumulated in response to nutritional overload in obesity and its linked morbidities.

GLUCOSE TOLERANCE FACTOR (GTF) - AN ANTI DIABETIC MATERIAL EXTRACTED FROM YEAST, DECREASES GLUCOSE DAMAGE TO THE EYE LENS

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High glucose causes damages to the eye lens by inducing deleterious changes in its optical quality, its enzymes activity and its crystallins. The purpose of our study was to investigate the mechanisms involved in the damage caused by high glucose on the eye lens, and to examine the effect of Glucose Tolerance Factor (GTF), an anti diabetic agent extracted from yeast as anti-cataractogenic factor.

In vivo studies done in our laboratory showed that treating diabetic rats with repeated doses of GTF decreased significantly the development of diabetic cataract. In the current research we studied the direct influence of high glucose medium on the eye lens, and the effect of the addition of GTF.

Bovine lenses were placed in specially designed culture containers for 14 days incubation. The lenses were divided into four groups: (1) Control group. (2) Lenses incubated in medium containing 450-mg % glucose. (3) Control lenses incubated with GTF. (4) Lenses incubated in high glucose medium and GTF. An automated scanning laser system monitored lens optical quality throughout the culture period, and lens epithelial samples were taken daily for enzymatic analysis.

No change in the optical quality of control lenses was observed during 14 days of the culture period. Lenses incubated in high glucose medium showed reduction in their optical quality. GTF decreased the damage caused by high glucose. The activities of Na/K ATPase, and Aldose reductase were affected by high levels of glucose. GTF partially corrected the enzymatic changes. We also found changes induced by high glucose in lens' specific proteins. These changes were prevented by the addition of GTF.

In summary, High levels of glucose induced deleterious changes to lens optics, to lens enzymes and to its cristallins. GTF prevented the optical damage and the alterations in lens' enzymes activity induced by high levels of glucose.

NOVEL FAST AND REVERSIBLE LEPTIN ANTAGONIST-INDUCED MICE MODEL OF METABOLIC SYNDROME AND T2DM.

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Obesity and its major consequence, type II diabetes mellitus (T2DM), is epidemic throughout Western society. T2DM accounts for 95% of the diabetes worldwide. One limitation to the development of new diabetes treatments has been a lack of effective animal models to use in research. There are no rodent models that recapitulate the pancreatic β-cell lesions of humans with T2DM. Moreover, animal models of obesity either require overfeeding which is expensive and takes weeks to months to establish, or specific genetic mutations that cause lifelong metabolic dysfunction. Thus the ability to rapidly induce obesity in healthy rat and mouse strains would be a major advance that could enhance research in diabetes and obesity and the development of novel therapies. We have recently developed superactive mouse leptin antagonist (D23L/L39A/D40A/F41A). In a mono-pegylated form this compound (PEG-SMLA) has strong orexigenic properties, and when given to mice every 24-48 hrs for 2-3 weeks leads to weight gain of nearly 50% with primarily fat accumulation. PEG-SMLA -induced weight gain is accompanied by elevated fasting glucose and glucose intolerance with hyperinsulinemia, higher plasma cholesterol and TGs, and a dramatic rise of corticosterone. These changes were fully reversible with cessation of PEG-SMLA injections, and disappeared within 10-14 days. One month exposure to PEG-SMLA altered the expression of key regulatory genes such as UCP 2 , UCP3, PPAR-gamma, CASP3, GLUT4 and others in adipose tissue, muscle and brain. These findings indicate that leptin antagonism induces systemic dysmetabolism in a rapid, practical manner, and provides a valuable tool for research in obesity and diabetes.