population level, we used genetically-encoded fluorescent indicators selectively expressed in alpha cells. Imaging intact mouse islets with these indicators in 3D responding to treatments in real time yields hundreds of individual alpha cell recordings per experiment. Calcium imaging showed reproducible heterogeneous responses to a panel of known physiological potentiators of glucagon secretion such as arginine vasopressin, epinephrine, and amino acids. Separate dose response experiments revealed that the proportion of alpha cells responding to each signal plateaus at different proportions of alpha cells. The calcium data correlate both with direct glucagon secretion levels as well as cAMP measurement. Our findings highlight previously unappreciated levels of functional heterogeneity among alpha cells and demonstrate that alpha cells are not a single uniform unit. Our observations suggest that dose-dependent increases in glucagon secretion in response to different physiological cues may be the result of mobilizing progressively larger proportions of the total alpha cell mass. We hypothesize that this functional heterogeneity is a built-in mechanism through which different physiological cues elicit graded glucagon responses from the alpha cells.

Diabetes Mellitus and Glucose Metabolism

BENCH TO BEDSIDE: NOVEL MECHANISMS IN DIABETES AND METABOLISM

Hyperglycemia-Induced Metabolic Reprogramming Mediates a Proatherogenic Phenotype in Healthy Human Monocytes

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Introduction: Poor glycemic control is considered an important contributor to cardiovascular disease in patients with diabetes. Episodic hyperglycemia as a surrogate for glycemic variability promotes monocyte adhesion and increases the prevalence of proinflammatory monocytes within atherosclerotic plaques of patients with diabetes. We previously found that acute hyperglycemia-induced a pro-inflammatory phenotype and promoted the development of foamy monocytes by increasing total cholesterol deposition, cholesterol ester, and free cholesterol content by enhancing oxidized LDL uptake. However, the mechanism by which acute hyperglycemia induces monocyte cholesterol deposition and inflammation remains unknown. **Methods:** Monocytes isolated from healthy individuals (age range 20–40; n=5) were cultured in low (5mM) or high (16.7mM) glucose conditions with or without a glycolysis inhibitor (2-deoxyglucose, 2DG, 5 mM) or an endoplasmic reticulum stress inhibitor (4-phenylbutyric acid, PBA; 20mM) for 6 hrs. After treatment, cytokine release, oxidized LDL uptake, and metabolic assays using Seahorse Technology were performed. **Results:** Healthy human monocytes exposed under high glucose conditions showed a pro-atherosclerotic phenotype with higher levels of the pro-inflammatory cytokines, TNFα(median of differences 6.34 pg/ml, p=0.002) and IL1β(12.04 pg/ml, p=0.003), and increased oxidized LDL uptake (5062ug Dil-Ox LDL/mg, p=0.001). Furthermore, hyperglycemia resulted in higher levels of glycolysis (basal glycolysis 12.94 pmol/min, p=0.01; basal proton efflux rate 15.5 pmol/min, p=0.03) and mitochondrial respiration (percentage of respiratory capacity 16pmol/min p=0.04), suggesting a significant alteration in the metabolic programming of these monocytes. Treatment with 2-DG or PBA attenuated the pro-atherosclerotic phenotype induced by hyperglycemia, promoting a reduction of cytokine release, a reduction of oxidized LDL uptake, and near normalization of the glycolic rate and mitochondrial respiration, stabilizing cellular bioenergetics. **Conclusions:** Altogether, our results suggest that monocyte ER stress in response to acute hyperglycemia promotes a hypermetabolic state characterized by a proinflammatory and proatherogenic monocyte phenotype. Therefore, acute hyperglycemia is a potential mechanism promoting atherosclerosis in patients with type 2 diabetes.

Diabetes Mellitus and Glucose Metabolism

BENCH TO BEDSIDE: NOVEL MECHANISMS IN DIABETES AND METABOLISM

LGR4 and Its Extracellular Domain as Novel Regulators of ß-Cell Survival and Proliferation

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Our lab has shown that RANK (Receptor activator of the NF-κB) by interacting with its ligand, RANKL, inhibits ß-cell proliferation and survival; which can be reversed by Osteoprotegerin (OPG). Recently, the G protein-coupled receptor LGR4 (leucine-rich repeat-containing G protein-coupled receptor 4), which binds R-spondin (RSPO), was identified as a novel receptor for RANKL in osteoclast precursor cells. Thus, RANKL can bind two distinct receptors, RANK and LGR4 in osteoclasts, leading to opposite effects on osteoclastogenesis. LGR4 is expressed in rodent and human ß-cells, but the role of this receptor in ß-cells remains unknown. We postulated that LGR4 through its interaction with RANKL is involved in regulating ß-cell survival and proliferation. Our data indicate expression of specific LGR4 family members, Lgr4, Rank, Rankl, is modulated by stressors, such as cytokines, ER stress, diabetes and aging, in INS1 cells, rodent and human islets. Knocking down Lgr4 in INS1 cells or rodent islets has no significant effect on ß-cell proliferation but is detrimental for ß-cell survival in basal and cytokine-stimulated conditions. We also propose that the soluble extracellular domain of LGR4 (LGR4-ECD), which binds to its ligands (RSPO/RANKL), holds therapeutic potential like OPG, by inhibiting the interaction between RANKL/RANK. At 200ng/ml LGR4-ECD significantly enhances young adult (8-12-week-old) and aged (1.y.o.) rodent ß-cell proliferation, as well as human ß-cell proliferation, in islets from not only control subjects (45±17 y.o.), but also with Type 2 diabetes (48±7 y.o.). Additionally, LGR4-ECD significantly promotes

mouse and human ß-cell survival against cytokine-induced cell death. Future studies will determine the physiological role of LGR4 and the therapeutic potential of LGR4-ECD on the beta cell *in vivo* in basal conditions and in the setting of diabetes.

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BENCH TO BEDSIDE: NOVEL MECHANISMS IN DIABETES AND METABOLISM

Medicsen Smartpatch: A New Approach to Diabetes Management

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Introduction: Diabetes is a metabolic disorder characterized by a dysregulation of the glucose levels. With insulin being the main drug to be administrated for glucose levels modulation, it needs to be injected subcutaneously with daily injections, which can lead to poor patient compliance, apart from several side-effects. Although other administration methodologies have been investigated (oral or inhaled insulin), they show enough drawbacks to not to be consider as feasible alternatives for diabetes therapy. That´s why Medicsen has developed a Smartpatch that integrates a wide range of technologies, with the purpose of ensuring the correct insulin delivery from the skin´s surface to the bloodstream using a non-invasive and painless drug delivery method through a phenomenon induced by sonophoresis. **Materials and Methods:** Several *in vitro* and *in vivo* tests have been performed to prove the efficacy and safety of the technology, allowing us to collect experimental evidence through different methodologies that demonstrate the therapeutic potential of the device. Among these methodologies, permeability studies using Franz diffusion Cell and swine models (that prove efficacy of the technology) as well as safety studies, for both the insulin and the skin are highlighted. **Results:** In voltage experiments, the mean time for the disappearance of the membrane potential between the compartments separated by skin was: 334.7 (SD+/-103.6) seconds. Regarding the slope of the voltage line, as an approximation to the transfer speed, an arithmetic mean of (μ)= 0.0164 Mvolts/sec (+/- SD(σ): 0.006) was obtained. No significant differences were found between the circular dichroism spectra of samples (minimum peak at 219nm (sd+/-8.31) and that of the standard, which suggests that the molecular structure of insulin maintains stability. In the same way, HPLC studies shows no variability between the standard and all groups tested. Regardin skin safety, SEM images shows no significant damage to the skin, and ELISA test for TNF α and IL-2, as well as other biochemical tests, show no differences between control and samples. On In vivo experiments with our technology, glucose changes are comparable to those evoked through direct drug injection using conventional syringes. Lastly, the technology proved to be effective in the delivery of insulin through the skin in a non-invasive way, as observed in a Franz Diffusion Cell system and in the in vivo model of blood glucose reduction. **Conclusions:** Results observed during in vitro and in vivo studies indicate that the technology developed by Medicsen is effective and safe for the patient and the insulin. Following steps, including human trials, will be critical to fully demonstrate its potential in the treatment of diabetes.

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BENCH TO BEDSIDE: NOVEL MECHANISMS IN DIABETES AND METABOLISM

Pre-Conception Weight Loss Improves Reproductive, Metabolic and Kidney Health in Obese Mice and Their Offspring

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Background and Aims: An alarming 40% of women of reproductive age have obesity and during pregnancy obesity adversely impacts metabolic health in mothers and offspring. Maternal complications include diabetes, preeclampsia and chronic kidney disease (CKD). Our previous work showed that offspring have increased risks of obesity, diabetes, and CKD. While pre-pregnancy weight optimisation is advocated, evidence of benefits for mother and offspring are lacking. We aimed to determine if weight loss prior to pregnancy, either with diet modification or liraglutide, improves maternal and offspring metabolic outcomes, and reduces kidney complications in obese mothers and the offspring.
Methods: C57BL/6 female mice were fed a high-fat-diet (HFD) for 8 weeks and compared to lean chow-fed controls. HFD-fed dams were administered liraglutide (0.3mg/kg, s.c., for 4weeks) or switched to chow, to induce pre-conception weight loss. Pregnancy rates were observed after mating. Maternal anthropometry and glucose tolerance were measured before and after intervention, and at late gestation. Pregnant dams were either culled at gestational day 18–20 with blood and kidney harvested, or allowed to deliver their offspring. Offspring anthropometry, and glucose tolerance were assessed at postnatal week 12 after either HFD or chow feeding. Immunohistochemistry (IHC), western blotting and RT-PCR were used to measure kidney metabolic (FAS, SREBP) and inflammatory markers (CD-68,TGF-b).
Results: HFD-fed dams had reduced glucose tolerance compared to chow-fed dams ($p<0.0001$), and higher expression of renal metabolic and inflammatory markers in late gestation (eg FAS <0.05, TGFb <0.05). Intervention with liraglutide or diet lowered body weight, improving glucose tolerance (both $p<0.001$), and fecundity. Markers of kidney damage, namely albuminuria and fibronectin (by RT-PCR and IHC) were reduced (both $p<0.05$). Liraglutide treated mice exhibited greater gestational weight gain than mice switched to chow ($P<0.001$). Markers of inflammation and oxidative stress were significantly lower in obese mice with preconception weight loss via diet compared to liraglutide