processes and that at high glucose islets convert a significant portion of the sugar into glycerol and FFA, which are exported outside the β-cell. The identification of fuel surfeit detoxification mechanisms by a targeted metabolomic approach should reveal additional pathways of β-cell fuel detoxification. Targeting fuel detoxification processes may provide a novel approach to preserve β-cell mass and function.

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Role of Adipose Triglyceride Lipase and Lipolysis in the Regulation of Insulin Secretion: Study in β-Cell-Specific ATGL-Deficient Mice

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Previous studies have suggested that lipolysis-derived lipid signalling molecules play a role in the regulation of glucose-stimulated insulin secretion. To directly assess the role of lipolysis of β-cell endogenous lipid stores in glucose signalling for insulin secretion, β-cell-specific adipose triglyceride lipase (ATGL)-deficient mice (KO) were generated.

β-cell-specific tamoxifen-inducible ATGL deletion in MIP-Cre-ER/ATGL-LoxP mice (KO) was compared to tamoxifen-injected-MIP-Cre-ER and -ATGL-LoxP mice (controls).

Two weeks after tamoxifen treatment in 8-week-old mice, ATGL protein level was dramatically decreased in KO islets from both sexes. KO male mice showed a decrease in body weight gain compared to the controls, which became significant 6 weeks after tamoxifen injection. Fasting plasma levels of free fatty acids and triglycerides were unchanged in KO mice of both sexes. Male KO mice showed a 60% decrease in insulinemia without altered glycemia in fed and fasted states. They also showed a 70% reduction in plasma insulin levels in response to a glucose challenge and a trend toward glucose intolerance. However, despite similar ATGL deletion in female KO mice, none of these changes in glucose homeostasis and insulin secretory response were observed.

These results demonstrate that lipolysis via ATGL is involved in glucose-stimulated insulin secretion, at least in male mice. Female mice are often protected from diabetes in various animal models due to estrogens and a number of compensatory processes. We are currently studying the compensatory mechanisms that occur in KO female mice as they may reveal signalling and compensatory pathways involved in insulin secretion.

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Functional Genomic HTS to Study Primary Human Beta-Cell Proliferation

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Human beta cells rarely proliferate in the adult, which limits their use in beta-cell replacement strategies. Functional genomic high throughput screening (HTS) is a powerful tool to elucidate novel components of signalling pathways and to identify genes underlying human disease.

Performing HTS in primary human islets poses numerous obstacles due to limited donor islet availability, low initial cell mass, rare proliferation, donor-to-donor variation and cell clustering. Recent work identified cyclin-dependent kinase 6 (CDK6) as a regulator of human beta-cell proliferation. In this study, we optimized an HTS to monitor the subcellular localization of CDK6 (from cytoplasm to nucleus) in dissociated human islets as a proxy for entry of beta cells into the cell cycle. We demonstrate that dispersed human islet cells cultured in poly-D lysine-coated 384-well plates maintained glucose responsiveness, showed a high infection rate (~90%) using a GFP+-lentiviral shRNA delivery system and were susceptible to shRNA-mediated gene knockdown as quantified by immunoblotting. We performed a proof-of-principle screen comparing the effect of knockdown of key cell cycle regulators and components of the LKB1/AMPK pathway on CDK6 localization. We used an algorithm to quantify subcellular localization of CDK6 in GFP^+/$insulin^+$ dispersed islets and showed that over-expression of CDK6 could drive nuclear localization of CDK6 and increase Ki67 expression. Taken together, we have optimized conditions for genomic HTS in human islets and engineered a novel strategy to study beta-cell proliferation with a view to develop novel therapies for the treatments of diabetes.

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Lkb1 Depletion Enhances Insulin Secretion and K^+_{ATP} Channel Closure Despite Impairing Glucose Metabolism

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The Lkb1 tumour suppressor is a serine/threonine protein kinase that exerts its biological effects through activation of the AMPK family of ser/thr protein kinases. Extensive genetic and biochemical evidence supports a role for Lkb1 in cell cycle arrest, establishment of cell polarity and in the control of cellular energy metabolism. We previously reported that knockout of Lkb1 in the β cell compartment of pancreatic islets of adult mice improves glucose tolerance and protects from hyperglycemia induced by a high-fat diet. Lkb1-/- β cells are hypertrophic, proliferate more and secrete more insulin in response to glucose. Here we report that loss of Lkb1 stimulates insulin secretion by increasing K^+_{ATP} channel closure and calcium influx, in effect "short circuiting" the classical pathway of insulin secretion, which is comprised of glucose metabolism, mitochondrial respiration, ATP generation, K^+_{ATP} channel closure and calcium entry. Indeed, this model has been challenged in recent years by evidence that an increase in ATP/ADP and consequent closure of K^+_{ATP} channels does not correlate with insulin output following glucose stimulation, suggesting that additional coupling factors exist. Among those proposed are NADPH, malonyl-coA, mitochondrial GTP and phosphoenolpyruvate (PEP) as well as several amino acids. Our data suggest that Lkb1 regulates coupling of glucose metabolism to the insulin secretion machinery, and that β cells lacking Lkb1 can be used as a system to uncover novel coupling factors in the insulin secretion pathway.

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Role of Protein Kinase D 1 in Pancreatic Beta Cells

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In pancreatic beta cells, the second phase of glucose-stimulated insulin secretion (GSIS) is markedly amplified by fatty acids. We