Down Regulation of Glucokinase and Mitochondrial ATP Synthase in Islets from Type 1 Organ Donors

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Purpose: Glucose metabolism and the resulting ATP production are essential for stimulus coupled insulin secretion and maintenance of normal blood glucose levels. Using human pancreatic organ samples obtained from the Network for Pancreatic Organ donors with Diabetes (nPOD) initiative, we sought to identify differences in essential components of glucose stimulated insulin secretion (GSIS) in individuals with Type 1 diabetes (T1D) and individuals at risk for T1D, as determined by circulating autoantibody positivity (AAb+/non-T1D), compared to T1D free controls.

Methods: Slides with tissues from the following groups were requested from nPOD: T1D free controls (n=4), AAb+/non-T1D individuals (n=6), and T1D subjects with insulin positive (INS+) beta cells remaining within the islets (n=4). Interestingly, approximately one-third of the organs from patients with long-standing T1D in the nPOD collection have a significant number of INS+ islets. Samples were age and sex-matched. Slides were stained for insulin (INS), as well as for glucokinase (GCK) and the β subunit of the F1Fo ATP Synthase (ATPase), two essential proteins for GSIS by β cells. A Zeiss Axioskop Microscope was used to capture images that were subsequently analyzed using ImageJ/Fiji (NIH). Microscope and camera settings were identical for each islet on every slide. Normalizing protein expression levels by exocrine staining minimized the contributions of potential differences in staining from sample to sample due to their specific collection and preparation. We compared the mean pixel intensity of GCK and ATPase in INS+ endocrine cells to their expression in the exocrine tissue, and determining differences, if any, between the groups tested.

Summary of Results: The expression of ATPase was decreased in the INS+ cells of T1D patients, compared to those in AAb+/non-T1D or T1D free samples. GCK was significantly reduced in the INS+ cells of both T1D+ and AAb+/non-T1D samples compared toT1D free sections. GCK levels were identical when comparing INS+ cells from T1D+ samples to AAb+/non-T1D samples. Further, the staining intensities of both GCK and ATPase in INS+ cells correlated to c-peptide levels in the circulation of the organ donors [GCK: $R^2=0.7$ & $p=0.02$, and ATPase: $R^2=0.58$ & $p=0.048$]. The staining intensity of insulin within the beta cells did not differ between the three groups and also did not correlate with circulating C-peptide in the organ donors.

Conclusions: Insulin positive beta cells are present in a number of the T1D cases in the nPOD collection despite reduced beta cell mass. In addition, deficiencies in GCK and the mitochondrial ATPase in T1D+ subjects may be responsible for decreased beta cell function. This suggests that in patients with long-standing diabetes, beta cells are present but function is impaired. Understanding this process could have a major impact on how T1D is treated. The reduction in GCK in the AAb+/non-T1D cases, may demonstrate the presence of the ongoing T1D processes at the beta cell level.