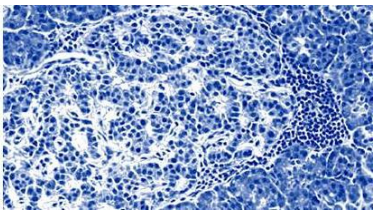
nPOD
Network for Pancreatic Organ Donors with Diabetes



Proinsulin-Insulin pancreatic islets *in-situ* expression mirrors metabolic defects observed in type 2 diabetic and glucose intolerant living donors

AUTHORS

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PURPOSE

Islets beta cell dysfunction in type 2 diabetes (T2D) can be a consequence of alteration of proinsulin processing. It has been reported that circulating levels of proinsulin (PI), and proinsulin-to-insulin ratio are increased in patients with metabolic alterations and in particular in T2D. It has been hypothesized that an elevated PI/INS ratio is caused by increased secretory demand on β cells due to insulin resistance and hyperglycemia, which promotes the release of immature granules with a higher relative content of PI and its conversion intermediates. The exact mechanism behind this increase in T2D, as well as in T1D, is unknown.

For this reason, the aim of this study was to analyze proinsulin and insulin expression in pancreatic islets of tissues biopsy of patients undergoing partial pancreatectomy (PP) with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and T2D, in order to explore the alterations that occur in islets during metabolic stress.

METHODS

In order to explore the alterations that occur in PI and INS staining pattern in pancreatic islets, Oral Glucose Tolerance Test (OGTT) was performed in n=17 patients on the waiting list for PP, classified into n=5 NGT, n=9 IGT and n=3 T2D. β-cell glucose sensitivity (calculated as the ratio of insulin secretion and glucose increments), basal insulin secretion, insulin secretion rate (ISR) and glucose levels 2-hours following OGTT were analyzed. Frozen sections of pancreatic tissue biopsy were stained for INS and PI through double immunofluorescence staining. Image analysis was performed on each individual islet to measure colocalization coefficient (M_1), islet area (μm^2), PI and INS positivity through Volocity software. *In-situ* staining measurements were correlated with patients' clinical parameters. Statistical analysis was performed using one-way ANOVA multiple comparison test and Pearson correlation.

SUMMARY OF RESULTS

PI-INS colocalization gradually increased from NGT to IGT and T2D pancreatic islets ($p<0.01$), indicating an altered PI processing due to the localization of PI and INS in the same compartment. The area (μm^2) of PI positivity and PI/INS ratio were significantly increased in T2D compared to IGT and NGT pancreatic islets ($p<0.01$), suggesting the release of immature granules with a higher relative content of PI, as previously demonstrated for in-vivo circulating levels. Moreover, we observed that the increase of PI-INS colocalization was positively correlated to ISR and glucose levels 2-hours following OGTT ($r=0.6$ $p<0.01$). The increase of PI/INS ratio was associated with higher basal INS and glucose levels 2-hours following OGTT ($r=0.6$ $p<0.01$). Finally, we also observed that the reduction of β-cell glucose sensitivity is linked to increase of *in-situ* PI-INS ratio ($r=-0.6$ $p<0.03$).

CONCLUSIONS

In conclusion, we demonstrated that: (*i*) *in-situ* PI-INS staining patterns are altered in T2D and IGT patients; (*ii*) pancreatic islets PI/INS ratio as well as PI area and PI-INS colocalization coefficient might mirror *in-vivo* the increased insulin secretion rate and β cell function reduction in the same patients. Our findings, suggest that poor β-cell glucose sensitivity is linked to increased *in-situ* PI/INS ratio, highlighting the importance of correct PI processing and folding in the maintenance of glucose homeostasis.