Title:

Single-cell-resolution imaging of alpha/beta-cell metabolic response to glucose stimulation in living human-derived langerhans islets

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Background and aims

A cascade of highly regulated biochemical processes connects glucose stimulation to hormone secretion in specialized cells within Langerhans islets of the mammalian pancreas, the α and β cells. Given the importance of this process for systemic glucose homeostasis, non-invasive and fast strategies capable to monitor quantitatively α- and β-cells metabolic responses in living islets are highly desirable. Despite the efforts, however, no report thus far was able to probe the specific signature of α- and β-cells response to glucose stimulation in living human islets (HIs).

Materials and methods

To tackle this issue we used here a combination of label-free Fluorescence Lifetime Imaging Microscopy (FLIM) in living HIs with post-fixation immunofluorescence. More in detail, by the phasor approach to FLIM we discriminated the free and protein-bound forms of NAD(P)H molecules in optical sections of living HIs and, by means of their ratio, we defined the tissue metabolic shift upon pulsed glucose stimulation. Then, by using post-fixation immunofluorescence, we identified α and β cells, finally matching single-cell identities with their metabolic response. A cohort of 4 healthy donor patients was included in this study.

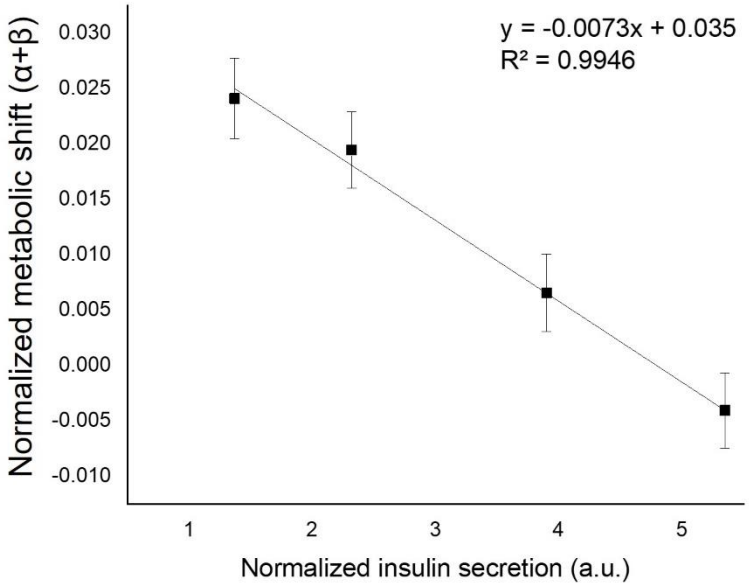
Results

A total of 15 islets were measured from the above-mentioned cohort, with 312 α cells and 654 β cells identified. We observed a neat metabolic shift towards oxidative phosphorylation in the great majority of β cells, in keeping with previous results from rat/mouse β cells. By contrast, we observed a wide spectrum of metabolic shifts in α cells, from glycolysis- to oxidative-phosphorylation-oriented ones, apparently contradicting previous reports which assessed an univocal glycolytic response to glucose in rat/mouse α cells. Interestingly, at the patient level, the heterogeneous α-cell responses reveal an inverse proportionality with respect to the amount of insulin secreted by the 4 donors (independently probed by an ELISA assay): the higher the insulin secreted, the more glycolysis-oriented the metabolic shift measured

in α cells. Noteworthy, such inverse proportionality transforms into a very marked linear anti-correlation (Pearson's correlation coefficient: -0,997) if α- and β-cell metabolic shifts are summed together and plotted against the amount of insulin secreted by the 4 donors (see Graph).

Conclusions

The emerging picture indicates the synergistic action of α and β cells as key signature of HI metabolic response to glucose and, in turn, as a basic constituent for the regulation of systemic glycaemia. While demonstrating the effectiveness of an optical-microscopy-based protocol to measure the specific responses of α and β cells in a living human Langerhans islet, present results pave the way to similar investigations to be conducted in diabetes and/or using drugs to restore cell functionality.



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