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Palmitate-induced impairment of glucose-stimulated insulin secretion by pancreatic beta cells is disconnected from concomitant mitochondrial respiratory defects

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Background and aims: Pancreatic beta cell mitochondria couple the oxidative breakdown of glucose to the synthesis of ATP and are essential for glucose-stimulated insulin secretion (GSIS). Indeed, mitochondrial respiratory activity might prove a good indicator of functional beta cell integrity, and insight in islet respiration may thus benefit transplantation protocols. With this study we aimed to clarify the connection between mitochondrial function and GSIS in pancreatic beta cells subjected to glucolipotoxic conditions.

Materials and methods: Islets isolated from C57BL/6 mice and INS-1E insulinoma cells were exposed for 24 or 48 hr to BSA-conjugated palmitate (free concentration 20-40 nM) in the presence of 11 mM glucose. Glucose sensitivity of oxidative phosphorylation was derived from absolute oxygen uptake rates measured in a Seahorse XF24, and insulin was quantified by ELISA. Respiratory activity and insulin secretion were normalised to islet DNA or INS-1E cell number determined from PicoGreen and DAPI fluorescence, respectively. Significance of palmitate effects was evaluated with Student's t-tests.

Results: Islets exposed to palmitate for 48 hr consume oxygen at a rate of 3.9 ± 0.5 pmol oxygen x min^{-1} x ng DNA^{-1} when incubated at 5.5 mM glucose. This respiratory rate is corrected for non-mitochondrial oxygen uptake and is indistinguishable from that exhibited by control islets exposed to BSA alone. Basal mitochondrial respiration increases to 8.2 ± 1.5 and 6.4 ± 0.8 pmol oxygen x min^{-1} x ng DNA^{-1} in control and palmitate-exposed islets, respectively, when glucose is raised to 28 mM. Normalised to basal mitochondrial oxygen uptake, palmitate lowers the islets' respiratory response to glucose from 2.5- to 1.7-fold ($P < 0.05$). This respiratory defect is echoed by GSIS impairment: 48-hr palmitate exposure lowers the islet insulin secretory response to glucose from 10.5-fold ± 1.8 to 3.4-fold ± 0.6 ($P < 0.001$). GSIS impairment results from an inhibitory palmitate effect ($P < 0.01$) on absolute insulin secretion at 28 mM glucose and a stimulatory effect ($P < 0.001$) on absolute basal insulin release at 5.5 mM glucose. GSIS impairment is also apparent after 24-hr palmitate exposure when the islet insulin secretory response to glucose has been lowered from 6.5-fold ± 0.5 to 3.5-fold ± 0.6 ($P < 0.01$). Importantly, after this relatively short exposure, palmitate has not yet significantly affected islet mitochondrial respiration. A disconnect between palmitate-induced respiratory defects and GSIS impairment is also seen in INS-1E cells. After 24-hr exposure, palmitate lowers the insulin secretory response (secretion at 28 mM glucose normalised to insulin release by nutrient-starved cells) from 4.4-fold ± 1.1 to 1.2-fold ± 0.1 ($P < 0.05$). This GSIS annulment results from palmitate stimulation of basal insulin release ($P < 0.05$) and a small non-significant inhibition ($P = 0.45$) of insulin secretion at 28 mM glucose. Concomitantly, palmitate lowers the normalised mitochondrial respiratory response to glucose of nutrient-starved INS-1E cells from 1.7 ± 0.1 to 1.2 ± 0.1 ($P < 0.05$). This respiratory defect is exclusively caused by an inhibitory effect ($P < 0.01$) of palmitate on absolute respiration at 28 mM glucose.

Conclusion: Palmitate-induced GSIS impairment in pancreatic beta cells is disconnected from mitochondrial respiratory defects.