# 1 SUMMARY

Increased ATP/ADP ratio resulting from enhanced glycolysis and oxidative phosphorylation represents a plausible mechanism controlling the glucose-stimulated insulin secretion (GSIS) in pancreatic β-cells. Using high resolution respirometry and parallel mitochondrial membrane potential ($\Delta\Psi_m$) monitoring we have quantified bioenergetics in rat insulinoma INS-1E cells representing a suitable model for studies of insulin secretion *in vitro*. Upon glucose addition to glucose-depleted cells, we have demonstrated a simultaneous increase in respiration and $\Delta\Psi_m$ during GSIS and shown that the endogenous state 3/state 4 respiratory ratio hyperbolically increases with glucose, approaching the maximum oxidative phosphorylation rate at maximum GSIS. Attempting to assess the basis of the "toxic" effect of fatty acids on insulin secretion, GSIS has been studied after linoleic acid addition. The linoleic acid addition diminished the observed respiration increase, $\Delta\Psi_m$ jump, and magnitude of insulin release, and reduced state 3/state 4 dependencies on glucose, which was caused by mitochondrial uncoupling.

Energetic status of mitochondria, the rate of oxidative phosphorylation, is expressed not only by bioenergetic parameters measurable by classical biophysical methods (*e.g.* above mentioned respiration and mitochondrial membrane potential), but it is also reflected by morphology of the mitochondrial network. Therefore, also pathology of diabetic β-cells might be reflected by the altered morphology of mitochondrial network. Its characterization is however hampered by the complexity and density of the three-dimensional (3D) mitochondrial tubular networks in these cell types. Conventional confocal microscopy does not provide sufficient axial resolution to reveal the required details; electron tomography reconstruction of these dense networks is still difficult and time consuming. We tried to visualize mitochondrial network of intact β-cell within Langerhans islet of Goto-Kakizaki (type-2 diabetes model) and Wistar rats using 4Pi microscopy, a laser scanning microscopy technique which provides an approximately 7-fold improved z-axial resolution (approximately 100 nm) over conventional confocal microscopy. We have found that mitochondrial network from diabetic Goto-Kakizaki rats exhibits a more disintegrated pattern compared to that from control Wistar rats.

One of the pancreatic β-cell features is enhanced expression of uncoupling protein 2 (*UCP2*). Likewise chemical uncouplers, UCP1 and most likely also UCP2, although in much lower amounts, both uncouple respiration from ATP synthesis by their protonophoric activity. However, the mechanism by which these proteins exert their protonophoretic activity is still a matter of debate. Two models regarding the role of fatty acids in UCP-mediated uncoupling have been proposed. Data presented in this thesis for UCP1 are consistent with the fatty acid cycling hypothesis. Moreover, on the basis of EPR studies we provide evidence of existence of a fatty acid binding site on UCP2.