Type II diabetes mellitus is characterized by defective pancreatic β-cell insulin secretion and loss of glucose sensitivity. Pancreatic β-cells as a glucose sensor respond to glucose addition by enhanced oxidative stress in the cytosol, whereas superoxide release is diminished in the mitochondrial matrix. The nature of the enzymatic source of cytosolic oxidative stress in diabetes or as a response on the exposure of cells to high glucose is not precisely understood. Using a few superoxide- and H$_2$O$_2$-reactive fluorescent probes, we have definitively proven that although cytosolic reactive oxygen species (ROS) increase, mitochondrial matrix superoxide production decreases upon exposure of pancreatic β-cells to high glucose. The most probable cytosolic ROS source were identified to be the NADPH oxidase isoform-4 and Q$_o$ site of respiratory chain Complex III, since the glucose-stimulated ROS production was inhibited by DPI and stigmatellin. Such enhanced oxidative stress is also accompanied by fragmented mitochondrial network, visualized by 3D 4Pi confocal microscopy at 100 nm resolution. Imaging of model pancreatic β-cells - insulinoma INS1E cells cultivated at 11 mM glucose (Fig.1a) or 5mM glucose, i.e., with insufficient autocrine insulin (Fig.1b) and β-cells in Langerhans islets isolated from diabetic Goto-Kakizaki rats (Fig.1d) showed high fragmentation when compared to highly interconnected tubular mitochondrial network in control Wistar rats (Fig.1c) and insulinoma INS1E cells (Fig.1a).

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