The sensitivity of pancreatic β-cells to mitochondrial injuries triggered by lipotoxicity and oxidative stress

Ning Li, Francesca Frigerio and Pierre Maechler

Department of Cell Physiology and Metabolism, Faculty of Medicine, University of Geneva, rue Michel-Servet 1, CH-1211 Geneva 4, Switzerland

Abstract
Pancreatic β-cells are essential for the maintenance of glucose homeostasis, and dysfunction of these insulin-secreting cells results in the development of diabetes. In the course of events leading from obesity to Type 2 diabetes, several mechanisms are currently envisaged. Among them, lipids and oxidative stress are considered as toxic candidates for the β-cell. The cellular link between fatty acids and ROS (reactive oxygen species) is essentially the mitochondrion, a key organelle for the control of insulin secretion. Mitochondria are the main source of ROS and are also the primary target of oxidative attacks. The present review presents the current knowledge of lipotoxicity related to oxidative stress in the context of mitochondrial function in the β-cell.

Introduction
Pancreatic β-cells are poised to sense glucose and other nutrient secretagogues to regulate insulin exocytosis, thereby maintaining glucose homeostasis. Translation from nutrient stimuli to intracellular signals, ultimately activating the secretory machinery, requires efficient metabolic coupling in which mitochondria play a crucial role [1]. Type 2 diabetes is characterized by insufficient insulin release by the β-cells that should compensate for insulin resistance of peripheral tissues [2]. In this context, several mechanisms have been proposed to explain β-cell dysfunction; among them are lipotoxicity and oxidative stress [3]. In the present review, we discuss sources of oxidative stress, putative links with lipotoxicity and deleterious actions of ROS on β-cells.

Basis of oxidative stress
Oxidative stress refers to a persistent imbalance between excessive production of ROS (reactive oxygen species) and/or RNS (reactive nitrogen species) and limited antioxidant defences. ROS are either charged species, such as superoxide (O_2^•−), precursor of ROS and hydroxyl radical (OH•); or uncharged species, e.g. H_2O_2. Superoxide can be converted into less reactive H_2O_2 by SOD (superoxide dismutase) isoenzymes. H_2O_2 can in turn be degraded to O_2 plus H_2O by catalase, GPx (glutathione peroxidase) and peroxiredoxin, enzymes which constitute antioxidant defences [4]. ROS formation is an inherent and essential phenomenon of life, and small fluctuations in the steady-state concentrations of oxidants may contribute to physiological control of cell functions [4–7]. However, uncontrolled increase in oxidants, or reduction of their detoxification, leads to free radical-mediated chain reactions ultimately targeting cellular proteins, lipids, polysaccharides and DNA [8–10]. This might initiate a pathogenic cascade of events [6,11].

Oxidative stress and diabetes
Increased oxidative stress and free radical-induced damage have been proposed to be implicated in diabetic state [12]. In Type 1 diabetes, ROS participate in β-cell dysfunction initiated by autoimmune reactions and inflammatory cytokines [13]. In Type 2 diabetes, excessive ROS impair insulin synthesis [6,11,14,15] and activate β-cell apoptotic pathways [14,16].

Hyperglycaemia induces generation of superoxide at the mitochondrial level in endothelial cells and triggers a vicious cycle of oxidative reactions implicated in the development of diabetic complications [17]. In the rat ZDF (Zucker diabetic fatty) model of Type 2 diabetes, direct measurements of superoxide in isolated pancreatic islets revealed ROS generation coupled with mitochondrial metabolism and perturbed mitochondrial function [18]. Alloxan-induced Type 1 diabetic animal models provide an example of the involvement of free radicals in experimental diabetogenesis. After its selective uptake by β-cells through the glucose transporter GLUT2, alloxan generates ROS in a cyclic redox reaction along with its reduction product dialuric acid. This leads to selective necrosis of β-cells (reviewed in [19]). Supplementation of free radical scavengers, such as SOD and vitamin E, efficiently removes oxidants and protects against the diabetogenic action of alloxan [20,21].
Sources of ROS in diabetes
Several conditions leading to ROS generation in diabetes have been proposed, among which are glucolipotoxicity and mitochondrial pathways. Hyperglycaemia, defining diabetes, can be directly associated with increased ROS generation through a variety of mechanisms [3]. During chronic hyperglycaemia, \( \beta \)-cells are exposed to high glucose concentrations for an extended period of time. In this context, glucose saturates the normal route of glycolysis and a fraction of the sugar is shifted towards alternative pathways, from which ROS are generated through distinct metabolism within and outside mitochondria [22].

In addition to hyperglycaemia, the Type 2 diabetic state is also characterized by increased fasting and postprandial plasma levels of triacylglycerols and non-esterified fatty acids, molecules known to favour ROS generation [23]. Specifically, excessive levels of palmitate are associated with abnormal islet functions that lead to increased lipid esterification, in turn producing ceramides that induce oxidative stress [24,25]. Noteworthy, antioxidant therapies have been reported to protect \( \beta \)-cells from glucolipotoxicity. For instance, metformin and troglitazone, both of which exhibit antioxidant properties, can prevent hyperglycaemia in the ZDF rat [26,27]. Troglitazone also prevents increased levels of lipid hydroperoxide in another rat model of Type 2 diabetes [28].

Mutations in the mitochondrial genome may be associated with maternally transmitted diabetes, i.e. MIDD (mitochondrial inherited diabetes and deafness). We have recently reported that one of the most frequent mutations linked to this syndrome results in elevated ROS levels in cells carrying such mutant mtDNA (mitochondrial DNA) [29]. This raises the possibility that ROS is implicated in the development of mitochondrial diabetes [30].

The lipid connection to ROS
Type 2 diabetes and \( \beta \)-cell dysfunction are associated with adiposity and high levels of circulating non-esterified fatty acids. The deleterious effects of non-esterified fatty acids on \( \beta \)-cell function and viability have been ascribed to several factors, such as progressive \( \beta \)-cell failure, while compensating for peripheral insulin resistance [31], fatty acid-induced apoptosis [25], accumulation of malonyl-CoA leading to cytoplasmic accumulation of long-chain fatty acyl-CoAs [32], increased fatty acid oxidation and esterification [33], decreased insulin gene promoter activity [34] and decreased binding of the transcription factor PDX-1 (pancreatic duodenal homeobox-1) to the insulin gene [35]. As discussed above, accumulating evidence shows that ROS are implicated in \( \beta \)-cell dysfunction [36] and that cells exposed to fatty acids increase ROS production [23]. Exposure of pancreatic islets to olate and palmitate induces apoptosis, an effect that can be counteracted by treatment with nicotinamide, pointing to oxidative stress as a key player in fatty acid-induced apoptosis [37].

A link between non-esterified fatty acids and ROS production in \( \beta \)-cells was already described in the late 1990s. It was observed that chronic treatment of rat islets with palmi-tate increases ROS production [38]. However, slight uncoupling of the electron transport chain by the fatty acids, acting as protonophores, partially counteracted oxidative stress [38]. The mechanisms by which non-esterified fatty acids promote ROS generation in mitochondria remain unclear. Oleate-induced inhibition of the respiratory chain has been regarded as a mechanism for ROS elevation in insulin-secreting cells [39]. Non-esterified fatty acids, and their derivatives ceramides, modulate the efficiency of the respiratory chain, mainly by inhibiting complexes I and III [40]. Specifically, ceramides inhibit complex III and thereby promote ROS generation, mimicking the pharmacological effects of antimycin A [41,42].

ROS generation induced by non-esterified fatty acids could also be explained by activation of NADPH oxidase. Palmitate activates NADPH oxidase in a PKC (protein kinase C)-dependent manner in cultured aortic smooth-muscle and endothelial cells [23]. In insulin-secreting cells, palmitate was shown to induce ROS production along with an increase in the p47(phox) component of the NADPH oxidase [43].

Association between lipid exposure, ROS generation and \( \beta \)-cell dysfunction remains a matter of debate. It was shown in insulinoma cells that olate increases ROS and inhibits glucose-stimulated insulin secretion, although normalization of ROS production failed to restore secretory response [44]. Moreover, no relation was found between impairment of glucose-stimulated insulin secretion after a 72 h exposure of rat islets to fatty acids and ceramide synthesis or ROS production [45].

Mitochondria as a source of ROS
Mitochondrial respiratory chain is the major site of ROS production within the cell. Electrons from sugar, fatty acid, and amino acid catabolism accumulate on the electron carriers NADH and FADH\(_2\), and are subsequently transferred through the electron transport chain to oxygen, promoting ATP synthesis. ROS formation is coupled with this electron transportation as a by-product of normal mitochondrial respiration through the one-electron reduction of molecular oxygen [46,47]. The main submitochondrial localization of ROS formation is the inner mitochondrial membrane, i.e. NADH dehydrogenase at complex I and the interface between ubiquinone and complex III [17]. Increased mitochondrial free radical production has been regarded to be a result of diminished electron transport occurring when ATP demand declines or under certain stress conditions impairing specific respiratory chain complexes [48–50]. This is consistent with the observation that inhibition of the mitochondrial electron transport chain by mitochondrial complex blockers, antimycin A and rotenone, leads to increased ROS production in INS-1 \( \beta \)-cells [51].

Mitochondria as a target for ROS
Mitochondria not only produce ROS but are also the primary target of ROS attacks. mtDNA carries 37 genes (16569 bp)
encoding 22 tRNAs, 2 rRNAs and 13 polypeptides of electron transport chain complexes [52]. In general, mtDNA is more vulnerable to oxidative stress, and consecutive damages are more extensive than those in nuclear DNA due to the lack of protective histones and low repair mechanisms [53,54]. Being in close proximity to the site of free radical generation, mitochondrial inner membrane components are at a high risk of oxidative injuries, eventually resulting in depolarized mitochondrial membrane and impaired ATP production. Such a sensitivity has been shown for mitochondrial membrane proteins such as the ANT (adenine nucleotide translocator) and ATP synthase [55,56]. In the mitochondrial matrix, aconitase was also reported to be modified in an oxidative environment [57]. Furthermore, mitochondrial membrane lipids are highly susceptible to oxidants, in particular the long-chain polyunsaturated fatty acids. ROS may directly lead to lipid peroxidation, and the production of highly reactive aldehyde species exerts further detrimental effects [58]. The mitochondrial membrane-specific phospholipid diphasphatidylglycerol is particularly vulnerable to oxidative damage, altering the activities of the ANT and cytochrome c oxidase [59].

**ROS and the β-cell**

ROS may have different actions depending on whether cellular concentrations are either below or above a specific threshold, i.e. signalling or toxic effects respectively. Accumulating observations suggest that low concentrations of ROS could participate in signalling events triggering glucose-stimulated insulin secretion [51]. Conversely, robust oxidative stress caused either by direct exposure to oxidants or by secondary exposure to glucolipotoxicity has been shown to impair β-cell function [3,22,36]. A short transient exposure to oxidative stress is sufficient to impair glucose-stimulated insulin secretion in pancreatic islets [36]. Specifically, ROS attacks in insulin-secreting cells result in mitochondrial inactivation, thereby interrupting transduction of signals normally coupling glucose metabolism with insulin secretion [36].

The degree of oxidative damage also depends on the protective capability of ROS scavengers. Mitochondria have a large set of defence strategies against oxidative injuries. Superoxide is enzymatically converted into H$_2$O$_2$ by the mitochondrial-specific manganese SOD [60]. Other antioxidants such as mitochondrial GPs, peroxiredoxin, vitamin E and coenzyme Q$_{10}$ and various repair mechanisms contribute to maintaining redox homeostasis in mitochondria [61,62]. However, one characteristic of β-cells is a relatively weak expression of the free radical-quenching enzymes SOD, catalase and GPs [63]. Overexpression of such enzymes in insulin-secreting cells inactivates ROS attacks [64]. Besides ROS inactivation, UCP2 (uncoupling protein-2) was shown to reduce cytokine-induced ROS production, an effect independent of mitochondrial uncoupling [65].

**Conclusion**

Much evidence, derived from experimental models, point to ROS as candidates for β-cell impairments. However, it should be emphasized that the link between oxidative stress and Type 2 diabetes has not been demonstrated in patients. Therefore oxidative stress remains a putative mechanism participating in the progression of β-cell dysfunction associated with Type 2 diabetes.

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**References**


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