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Review Article

Redox Homeostasis in Pancreatic β Cells

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We reviewed mechanisms that determine reactive oxygen species (redox) homeostasis, redox information signaling and metabolic/regulatory function of autocrine insulin signaling in pancreatic β cells, and consequences of oxidative stress and dysregulation of redox/information signaling for their dysfunction. We emphasize the role of mitochondrion in β cell molecular physiology and pathology, including the antioxidant role of mitochondrial uncoupling protein UCP2. Since in pancreatic β cells pyruvate cannot be easily diverted towards lactate dehydrogenase for lactate formation, the respiration and oxidative phosphorylation intensity are governed by the availability of glucose, leading to a certain ATP/ADP ratio, whereas in other cell types, cell demand dictates respiration/metabolism rates. Moreover, we examine the possibility that type 2 diabetes mellitus might be considered as an inevitable result of progressive self-accelerating oxidative stress and concomitantly dysregulated information signaling in peripheral tissues as well as in pancreatic β cells. It is because the redox signaling is inherent to the insulin receptor signaling mechanism and its impairment leads to the oxidative and nitrosative stress. Also emerging concepts, admiting participation of redox signaling even in glucose sensing and insulin release in pancreatic β cells, fit in this view. For example, NADPH has been firmly established to be a modulator of glucose-stimulated insulin release.

1. Why to Deal with Redox Homeostasis in Pancreatic β Cells

Due to its complex health and economic sequels as well as steadily increasing prevalence, type 2 diabetes mellitus (T2DM) represents one of the serious burdens of the 21th century. Its pathogenesis is complex and different factors may prevail in individual cases. The typical feature of progressed T2DM is insulin resistance as well as β cell dysfunction [1, 2]. Excellent recent reviews cover gathered knowledge of all aspects of pancreatic β cell biology, development, molecular physiology, and medical aspects [1–19]. A great progress has been achieved in understanding molecular mechanism of physiological phenomena [1–5], etiology of T2DM and medical aspects or treatment [6–9], microscopic anatomy of human islets of Langerhans [10], and their *in vivo* imaging [11], as well as in understanding β cell biology, specifically longevity and development and differentiation of β cells [13–19]. This paper attempts to focus on aspects that determine β cell dysfunction and possess a common denominator in oxidative stress origin and/or dysregulated information signaling, while emphasizing dysregulated redox signaling. The impact is striking, since redox signaling is the inherent part of β cell physiology and contributes to, for example, insulin secretion. We left out of scope β cell development, cell cycle, longevity and differentiation, attempts to produce β cells from stem cells, and extensive description of pathophysiology. We had a chance only to touch a topic of information signaling and its dysregulation as it substantiates a subject for another extensive review. Due to the same reason, we leave also the themes of nitrosative stress and lipotoxicity which are, however, closely related to the oxidative stress. In turn, we focus more closely on some recent emerging aspects, such as possible signal modulating role of mitochondrial uncoupling protein UCP2 [20], reviewing and emphasizing the role of mitochondrion in β cell molecular physiology as well as pathology. Likewise, we focus on action of insulin itself on the β cell, that is, autocrine insulin secretion and its link to the redox homeostasis. We strictly distinguish mitochondrial and cytosolic sources of

reactive oxygen species (ROS) and their antioxidant defense, and when possible, we distinguish also mitochondrial versus cytosolic redox regulations.

Why to deal with redox homeostasis in pancreatic β cells at all? Are not the above described emerging aspects of β cell biology superior to our focus? The answer lies in the necessity to establish, whether T2DM is the inevitable result of progressive self-accelerating oxidative stress [21, 22] and concomitant progressively dysregulated information signaling, including redox signaling that both lead to diabetic complications. This view becomes even more skeptical, when one realizes that redox signaling manifested by transient ROS burst at least locally is an inherent part of numerous molecular mechanisms, some of which will be reviewed here. Thus redox signaling is inherent to mechanism of insulin receptor signaling and emerging concepts admit its role even during glucose sensing and insulin release in pancreatic β cells [4]. For example, the role of NADPH has been already firmly established in modulation of insulin release. One may consider NADPH as antioxidant since it is an important metabolite usually shifting redox homeostasis towards the reduced state. However, when used by NADPH oxidases to produce ROS, it becames an evil of the *pro*-oxidant side. It is not surprising that H_2O_2 is another such key molecule and in further description we shall recognize many more metabolites and proteins with the Janus angel/devil double face.

2. Mitochondrial Generation and Scavenging of Reactive Oxygen Species (ROS) in Pancreatic β Cells

2.1. Mitochondrial ROS Sources. Likewise in other cell types, mitochondrial respiratory chain is the main source of superoxide ($O_2^{\bullet -}$, and its conjugated acid-hydroperoxyl radical, $HO_2^{\bullet}$, pKa 4.9) in mitochondrion of pancreatic β cells [21]. Specifically, Complex I, an H^+-pumping NADH: quinone oxidoreductase, produces maximum superoxide only when both electron transport and H^+ pumping are retarded [22, 23]. H^+ pumping may be attenuated by highly established electrochemical gradient of protons at IMM (termed proton motive force, Δp, when expressed in mV units) or inhibited by oxidative stress-related mutations of ND5 subunit (or other mitochondrion-coded subunits) [22]. Intermediate $O_2^{\bullet -}$ formation results from fully reduced flavin as reported for isolated Complex I [24–26]. Binding of rotenone and similar inhibitors in proximity to the Q-site (a ubiquinone binding site) highly retards electron transport throughout the peripheral arm of Complex I. This was originally ascribed to the formation of longer-lived semiquinone species having a higher probability of reacting with oxygen which thus would form $O_2^{\bullet -}$ [27]. Detailed mechanism of $O_2^{\bullet -}$ formation within Complex I and its relation to H^+-pumping have yet to be established. It is well recognized, however, that nearly all Complex I-produced $O_2^{\bullet -}$ is released to the matrix compartment [27]. Complex III, a ubiquinol-cytochrome c reductase, contributes to $O_2^{\bullet -}$ generation by autooxidation of the ubisemi-quinone anion radical ($UQ^{\bullet -}$) within so-called Q cycle [21, 27, 28], while it releases $O_2^{\bullet -}$ about equally to both sides of the inner mitochondrial membrane (IMM) [28, 29].

A fast electron flux via the whole respiratory chain at a high substrate pressure (NADH/NAD^+ ratio) produces more $O_2^{\bullet -}$ than under conditions, when slower flux occurs at the same relative retardation (same oxidation/reduction states). Hence, in intact respiratory chain, mostly effectors that retard cytochrome c turnover between Complex III and IV (cytochrome c oxidase), slow down Q cycle or Q migration between Complex I and III, accelerate superoxide production [30].

2.2. Mild Uncoupling Attenuates Mitochondrial ROS Generation. The oxidative phosphorylation (OXPHOS) terminating at ATP synthase (Complex V) is driven by the proton motive force, Δp, formed by the respiratory chain H^+ pumping at Complex I, III, and IV. The IMM part of ATP synthase, so-called F_OATPase, thus consumes the adequate portion of Δp in non-dormant mitochondrion in a state, historically termed state-3. *In vivo* cell mitochondrial respiration is governed by the metabolic state and/or availability of substrates, and one can recognize various states-3 differing by distinct respiration rates, depending on the substrate load. A state-4, never existing in cells, is then given by zero ATP synthesis, hence by zero H^+ backflux via the F_OATPase. Respiration and H^+ pumping at state-4 is given only by the other protonophores, either of protein character or by the native H^+ permeability of IMM. As in mitochondrion Δp is predominantly in a form of IMM electrical potential, it is valid that $\Delta\Psi_m$ is maximum at state-4 at the maximum substrate load. The H^+ backflux excluding F_OATPase is termed an H^+ leak. Also other proteins may contribute to the H^+ leak, such as the ADP/ATP carrier. A short-cut of proton circuit within IMM is also known as uncoupling and can be physiologically provided also by mitochondrial uncoupling proteins (UCPs) [20, 31–34]. When UCP-mediated protonophore activity plus IMM H^+ leak does not overwhelm the F_OATPase protonophoric activity, ATP synthesis, hence OXPHOS, still takes place. Such a mild uncoupling (mild in contrast to a complete uncoupling by agents termed uncouplers) is, however, beneficial in terms of lowering mitochondrial $O_2^{\bullet -}$ formation. The $O_2^{\bullet -}$ formation in both Complex I [22] and Complex III [27–29, 35] is diminished by mild uncoupling. Due to a relative predominance of mitochondrial ROS source within the cell, one can predict that even accumulated oxidative stress might be attenuated by mild uncoupling. Note, however, that oxidative stress originating from irreversible changes due to mutated subunits encoded by mitochondrial DNA (mtDNA) cannot be improved by mild uncoupling [22]. An example is given by certain mutations of ND5 subunit of Complex I (ensuring H^+ pumping in intact wt form) that inhibit H^+ pumping and lead to increased $O_2^{\bullet -}$ formation. Such a block is not withdrawn by uncoupling [22]. In conclusion, the retardation of H^+ pumping which accelerates Complex I $O_2^{\bullet -}$ formation rater initiates further turn of a vicious spiral of self-accelerated oxidative stress.

2.3. Uncoupling Protein UCP2 Attenuates Mitochondrial ROS Generation. Among five UCP isoforms, UCP2 was identified in pancreatic β cells and it has been deduced that UCP2 exerts an important antioxidant role in β cells while preventing excessive superoxide formation within the respiratory chain [20]. There are still, however, controversies, on how UCP2-mediated uncoupling is initiated, since mutually incompatible models for the uncoupling mechanism of UCP2 (or other UCPs) have been developed [36–39]. In fact, the functional roles of UCP2 that were originally suggested—including the attenuation of ROS production [40–42], regulation of GSIS [42–44] (see Section 3.1), and regulation of Ca^{2+} levels in mitochondria [45–47]—are in dispute. We have previously documented the fatty acid (FA) cycling model [39] using reconstituted UCPs into liposomes [37, 38, 48–53] and black lipid membranes [54–57] and demonstrated that transport of polyunsaturated FAs (PUFAs), including hydroperoxy FAs [52] is faster [51, 56, 57]. According to the FA cycling model, FA anions are the true substrates transported by UCP2 and other UCPs [53, 57]. After protonation on the *trans*-side of the IMM, protonated FAs are internalized into the lipid bilayer core and subsequently flip to the *cis*-side of IMM and thus carry a proton across the membrane [38]. Opposing models have postulated a pathway that requires only protons, for which FAs are enhancers of basal H^+ transport [36, 40]. Lipid peroxidation products, for example, 4-hydroxy-2-nonenal, may also act as enhancers of proton transport by chemical modification of UCPs [58]; however, recently, we have provided an evidence that they do so only when FAs are present (Pohl E, Jabůrek M, et al., unpublished).

In pancreatic β cells it has been observed that the UCP2-mediated mild uncoupling decreases the yield of ATP from glucose [43, 59]. Further studies suggested superoxide activation of UCP2-mediated uncoupling on the basis of observation of elevated $\Delta\Psi_m$ in islets treated with a superoxide dismutase (SOD) mimetic manganese [III] tetrakis (4-benzoic acid) porphyrin (MnTBAP) or overexpressing MnSOD, absent in islets from UCP2 KO mice [60]. Upon presumed inhibition of UCP2-mediated uncoupling by Genipin, $\Delta\Psi_m$ increased in wt islets but not in UCP2 KO islets [61]. UCP2 overexpression in INS-1 cells attenuated IL1β-induced ROS formation [62]. With UCP2 silencing, a mild uncoupling in mitochondria isolated from INS-1E cells was linked to UCP2, while accounting for up to 30% of H^+ leak [63]. UCP2-mediated uncoupling was detectable also in intact INS-1E cells as compared to those silenced for UCP2 [64]. In turn, Galetti et al. could not demonstrate any effect of UCP2 overexpression on mitochondrial coupling in INS-1 cells, neither after oleate addition [65]. Chronic absence of UCP2 in UCP2 KO mice of three highly congenic strain backgrounds caused oxidative stress reflected by decreased GSH/GSSG ratio in blood or examined tissues while their islets had elevated levels of antioxidant enzymes and increased nitrotyrosine content [66]. Pancreatic β cells from UCP2 KO mice had chronically higher ROS when compared to wt mice, as estimated by dihydro-dichlorofluorescein diacetate fluorescent probe (CM-H2DCFDA, further abbreviated DCF) [67]. Mice with selective knock-out of UCP2 in pancreatic β cells (UCP2BKO mice) exhibited somewhat increased glucose-induced $\Delta\Psi_m$ [20]. UCP2BKO mice had also elevated intracellular ROS levels as determined by DCF [20]. These results comply with the antioxidant function of UCP2-mediated mild uncoupling. UCP2 may also modulate redox signaling, if could be effectively switched on and off.

2.4. Mitochondrial Superoxide Dismutases and Glutathione Peroxidases. $O_2^{\bullet-}$ in the matrix is converted to H_2O_2 by matrix MnSOD [68], while $O_2^{\bullet-}$ released to the intramembrane space (IMS) is partly dismuted by IMS CuZnSOD [69, 70]. Any residual $O_2^{\bullet-}$ which diffuses into the cytosol is similarly converted by the cytosolic CuZnSOD. If any mitochondrial $O_2^{\bullet-}$ can reach the extracellular space, it is then detoxified by extracellular CuZnSOD (SOD3). Once H_2O_2 is produced, it can easily penetrate through membranes, thanks to its uncharged property and poor reactivity. H_2O_2 can be degraded by peroxisomal catalase (rare in mitochondria with exception of the heart) and by three isoforms of selenium-dependent glutathione peroxidases (GPX) which, with a cofactor glutathione, convert H_2O_2 to water and also convert free FAOOHs to their corresponding hydroxy acids (FAOH). The fourth isoform, GPX4, specifically acts on hydroperoxy groups of peroxidized phospholipid side chains and on cholesterol hydroperoxides [71, 72]. Resulted GSSG is reduced to GSH by glutathione reductase with NADPH as a cofactor. Mitochondria have their own GSH pools independent of the cytosolic GSH pool. Both key mitochondrial ROS detoxifying enzymes MnSOD and GPX were demonstrated to be essential not only for balancing redox homeostasis but also for insulin secretion [73, 74].

3. Mitochondrial Redox Homeostasis and Glucose-Stimulated Insulin Secretion (GSIS)

3.1. Tuned Oxidative Phosphorylation (OXPHOS) as Determinant of Glucose-Stimulated Insulin Secretion (GSIS) but Also Mitochondrial ROS Generation. The intimately specific feature of pancreatic β cells lies in glucose sensing through the oxidative phosphorylation [75–79]. Respiration and OXPHOS rates, leading to a certain ATP/ADP ratio, are governed by the availability of glucose, whereas in most other cell types, cell demand dictates respiration/metabolism rates and the ATP/ADP ratio. It is because of a specific enzyme/regulation pattern of β cells. At first, unlike in numerous other cell types, pyruvate cannot be diverted towards lactate dehydrogenase for lactate formation in β cells. Consequently, glucose cannot be metabolized by anaerobic glycolysis, which provides so-called Warburg phenotype in cancer cells and under physiological cell responses to hypoxia and other adaptations [80, 81]. Thus nearly 100% of glucose is metabolized by OXPHOS in β cells (likewise in hepatocytes and numerous differentiated OXPHOS cells). The pattern of pyruvate dehydrogenase kinase genes is surely responsible for this. Thus, β cell PDK1 and PDK3 are "constitutively blocked" [82], and PDK2 is "inefficient" so that it does not phosphorylate PDH E1α subunit of pyruvate

dehydrogenase (PDH), hence does not inhibit its activity. At low basal glucose, PDH is 90% active, whereas at maximum glucose PDH is inhibited only by 22% [82]. Also hexokinase IV (glucokinase) in β cells is not inhibited by glucose-6-phosphate like in for example, skeletal muscle cells [83]. The lack of such a feedback inhibition of glycolysis directly connects glycolysis to pyruvate. Finally, the human glucose transporter GLUT1 or rodent GLUT2 are not dependent on insulin [84, 85], so glucose in β cell cytosol is proportional to bloodstream glucose [86]. This is perfect setting for a sensor.

Consequently, glucose metabolism in β cells is finely adjusted to the blood glucose levels [87]. At starvation with ~3 mM glucose levels, β cell respiration is relatively low, as well as the intensity of ATP synthesis, corresponding to the established state-$3_{[Glc = 3\,mM]}$ [88–90]. The $\Delta\Psi_m$ is still lower than would be at state-4 with 3 mM glucose. Increasing glucose intake into β cells may increase up to OXPHOS-saturating ~12 to 15 mM glucose, when maximum OXPHOS takes places with the established state-3_{max}, maximum respiration and maximum $\Delta\Psi_m$ [90]. The resulting increased ATP/ADP ratio in the cell cytosol initiates closure of plasma membrane ATP-sensitive K^+ channels [1, 91, 92], leading to plasma membrane depolarization and opening of voltage-sensitive Ca^{2+} channels [1, 93]. Increased cytosolic Ca^{2+} initiates insulin granule exocytosis [3, 94–96]. The above description represents a simplified schema of glucose-stimulated insulin secretion (GSIS). It has been hypothesized that β cells maintain a relatively high [ATP]/[ADP] value even in low glucose and that glucose metabolism leads to dramatically decreased free ADP with only modestly increased ATP [97]. If a high [ATP]/[ADP] ratio exists even at low glucose levels, as a result, the total adenine nucleotide concentration is unchanged during a glucose-induced elevation.

GSIS was also reported to be modulated or accelerated by other metabolic pathways related to mitochondria, such as phosphocreatine shuttle, additional Ca^{2+} signaling due to glutamate metabolism [98, 99], citrate export [100], phosphoenolpyruvate [101], and pyruvate cycling [102, 103]. A common denominator in these modulations is NADPH, the role of which on insulin secretion has yet to be established. Overall, GSIS possesses also a component due to the autocrine function of insulin (see Sections 3.3 and 5.2).

3.2. ROS Generation Dependence on Glucose. For cells not completely depleted of glucose we hypothesize (Figure 1) that the release of superoxide to the mitochondrial matrix upon the GSIS onset is diminished with regard to release rates at lower glucose concentrations. GSIS should simultaneously result in a decrease of the mitochondrial oxidative stress. The incremental increase of electron flow through the respiratory chain is not high at ~3 mM glucose, and its raise due to a further glucose intake is relatively lower when compared with the effect of H^+ backflow via the F_O part of ATP synthase that elevates respiration (classic respiratory control for isolated mitochondria). Thus the effect of elevated OXPHOS intensity prevails and ROS production is attenuated. This should be valid also for decrease of mitochondrial ROS formation with decreasing ADP, hence increasing ATP [97] and has been experimentally observed [104]. In turn, at extensive glucose depletion, the effect of substrate load (a directly proportional increase in superoxide formation, e.g., on Complex I, with increasing NADH or respiration) should overcome the suppressing role of H^+ returning via F_OATPase at higher intensity of OXPHOS. Hence, experimentally, results of increasing mitochondrial ROS upon GSIS might be observed using DCF [105] or other means [106, 107] as well as increasing reducing equivalents [108].

Since H_2O_2 of mitochondrial origin may readily access cytosol, one may report on mitochondrial ROS contribution, when measuring cytosolic ROS sensitive to mitochondrial inhibitors [105]. As explained above, a various extent of glucose depletion may provide distinct outcome in ROS assays, which are further dependent on the employed probe. Thus using dihydroethidium fluorescent monitoring in primary rat β cells, Martens et al. have found that unlike in non β cells, oxidative stress diminishes with increasing glucose upon GSIS [109]. ROS decrease monitored by DCF in isolated Langerhans islets upon GSIS has also been indicated [110]. Other laboratories have reported increases in ROS upon GSIS [105–107]. Note, that insulin secretion in INS1 cells was also induced by exogenous H_2O_2 and diethyl maleate [111], or by mono-oleoyl-glycerol [112] which both elevate intracellular H_2O_2.

3.3. Autocrine Insulin and Mitochondrial ROS Generation. Autocrine insulin has acute (4 hour) effects on GSIS in healthy humans [113]. Studies of Poderoso group have pointed out an emerging role of mitochondrial NO synthase (mtNOS) activated upon insulin signaling via the Akt-2/protein-kinase-B-mediated phosphorylation in skeletal muscle [114]. Released nitric oxide, a freely permeable radical, $NO^\bullet$, having a half-life of 1 to 10 s, causes a mild oxidative and nitrosative stress but also transiently diminishes respiration. In skeletal muscle and liver $NO^\bullet$ can facilitate conversion of glucose to glycogen. Experimentally, it has been proven by a sustained insulin dosage that the insulin-Akt-2-mtNOS pathway mediates $NO^\bullet$ burst in skeletal muscle [114]. Also, nitric oxide donors increase glucose uptake in primary human skeletal muscle cells [115]. Signaling via phosphatidylinositol-3-kinase (PI3K) (and hence downstream Akt-2 signaling) was responsible for insulin receptor activation by nonpeptidyl mimetic L-783,281 which inhibited GSIS as well as basal insulin secretion in human islets of Langerhans [116]. Also a direct observation in isolated mitochondria that insulin signaling regulates mitochondrial function in β cells has been reported [117].

Since pancreatic β cells contain a functional insulin receptor [117–120], an acute autocrine insulin signaling may lead to the similar acute effects as in skeletal muscle and liver, besides chronic positive effects on stimulation of β cell proliferation [118], hence being beneficial for regulation of adult β cell mass. Transgenic mice lacking insulin receptor in pancreatic β cells (βIRKO mice) exhibited increased apoptosis, decreased proliferation, and reduced β cell mass [119]. The insulin receptor has also been found essential

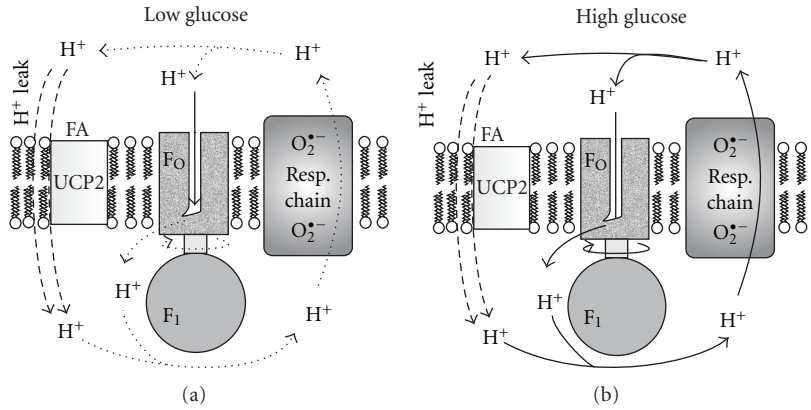

Figure 1: Mitochondrial superoxide formation should decrease upon a sudden glucose increase. Schemas depict the basic proton circuit within the inner mitochondrial membrane at low and high glucose, while higher rates, and therefore higher respiration rates, are depicted by thicker arrows. Thus the right schema depicts situation at a sudden glucose intake when state-3 respiration is maximum, as well as ATP synthesis, and hence also H^+ backflux through the F_O part of ATP synthase. Under these conditions superoxide formation within the respiratory chain complexes I and III is low (depicted by smaller fonts) and should be lower than at low glucose (left schema) at slower coupled respiration rate, where much higher superoxide should be formed (depicted by bigger fonts).

for islet compensatory growth response to insulin resistance [120]. There are two arms of autocrine insulin signaling via insulin receptor, the Raf-1 kinase arm and the Akt kinase arm. Insulin stimulates primary β cell proliferation via Raf-1 kinase and suppresses apoptosis. The Akt arm increases β cell mass and improves glucose tolerance. A signalosome complex of glucokinase, pro-apoptotic protein, Bcl-2-associated death promoter, BAD_S, and protein kinase A has been reduced in βIRKO mice, thus linking a lack of autocrine insulin with development of type 2 diabetes [117].

If mtNOS is indeed activated upon insulin signaling in β cells, the predicted outcome may substantiate different roles than in skeletal muscle cells and hepatocytes, just due to impossibility to switch to a partial aerobic glycolysis and provide a spectrum of anaplerotic pathways. The released $NO^\bullet$ may transiently inhibit Complex I and cytochrome c oxidase. $NO^\bullet$ may also reacting with superoxide, thus forming peroxynitrite which can further act against otherwise diminishing mitochondrial superoxide production.

4. Key Players Contributing to Cytosolic ROS Homeostasis in Pancreatic β Cells

4.1. Cytosolic ROS Sources in Pancreatic β Cells. Among the cytosolic ROS sources in pancreatic β cells, a family of NADPH oxidases (NOX) is the most important as the major plasma membrane or cytosolic superoxide sources. They catalyze the one-electron reduction of oxygen to generate $O_2^{\bullet-}$, while utilizing NADPH as electron donor. Isoforms NOX1, NOX2, and NOX4 may play a significant role in β cells [121, 122], hypothetically related to GSIS regulation and cell integrity [123]. Decreased NOX2 expression may contribute to regulatory mechanisms diminishing ROS upon high levels of metabolism [123]. NOX enzymes are composed by six hetero-subunits, which must associate, usually in a stimulus-dependent manner [124], with exception of constitutively assembled NOX4. Malic enzyme conversion of malate to pyruvate [125] or mitochondrial shuttles [100–103] may provide NADPH for NOX enzymes, since there is a relatively low pentose phosphate pathway activity in β cells. The catalytic core is formed by the two integral membrane protein subunits gp91phox and one p22phox plus by flavocytochrome b558. Additional subunits, p67phox, p47phox, p40phox, and the small GTPase Rac, are located in the cytosol during the resting state and upon activation assemble with the core [124]. Enzyme activation is initiated by p47phox phosphorylation through various protein kinases, such as protein kinase C (PKC) [124, 126, 127]. The upregulation of gp91phox and p22phox was demonstrated in β cells from rodent models of type 2 diabetes [128]. Another ROS source may be provided by cytochrome 450 enzymes such as CYP2E1 which determines mechanism of ketone-stimulated insulin release in pancreatic β cells [129]. Peroxisomes in β cells contribute also to endoplasmic reticulum (ER) stress [130].

4.2. Cytosolic Redox Buffers and Antioxidant Enzymes in Pancreatic β Cells. Redox buffers and antioxidant enzymes detoxify the produced ROS and frequently exert specific roles in β cell ROS homeostasis. Thus an increased antioxidant output from the pentose-phosphate pathway was suggested to decrease ROS upon GSIS [131]. Acute reduction in ROS by glucose was correlated with the increased pentose-phosphate pathway activity [132]. Catalase, glutathione peroxidase (GPX), and superoxide dismutase (SOD1 or CuZnSOD) represent the three of most important intracellular antioxidant enzymes, a primary defense system. However, the expression and activity of antioxidant enzymes is low in rodent β cells compared to other organs [132]. This property increases their susceptibility to an oxidative insult. When compared to liver content, pancreatic islets contain only 1% catalase, 2% GPX and 29% SOD1 activites [73, 133–135]. β cells posses also low repair machinery for oxidatively damaged

DNA [136]. In turn, β cells are rich in peroxidase-based antioxidant defenses, such as glutaredoxin and thioredoxin [137]. Human β cells seem to be less prone to oxidative stress than are rodent β cells, possibly because they have greater catalase and SOD activity [138]. Yet, GPX activity is poorly detectable in human islets [139]. Besides vitamin E (α-tocopherol), ascorbate, and uric acid, among small antioxidant molecules, glutathione provides an important mechanism protecting the β cells against oxidative damage [140, 141]. Glutathione, present in mM concentrations, is kept in the reduced state (GSH) by glutathione reductase. GSH transfers its reducing equivalents to ascorbate, GPX, and glutaredoxins.

The main protein antioxidant defense is composed of disulfide reductases namely, thioredoxin (TRX), glutaredoxin (GRX), peroxiredoxins (PRX) and glutamate-cysteine ligase. Thioredoxin represents a disulfide reductase for protein sulfhydryl groups, maintaining proteins in the reduced state [142]. Thioredoxin reductase uses electrons from NADPH and regenerates oxidized TRX. Similarly, glutaredoxin reductase-2 [143] reduces H_2O_2 or hydroperoxy-FA lipid chains to water or hydroxyFA lipid chains, respectively, on expense of conversion of GSH to oxidized glutathione GSSG, which is regenerated by glutathione reductase. Peroxiredoxins are a family of thiol peroxide reductases which uses TRX or other thiol-containing proteins to clear H_2O_2 or lipid peroxides [144]. Peroxiredoxin reaction product is sulfenic acid. At the TRX shortage, peroxiredoxin is inactivated to PRX-SO_2 [145], which can be reversed by sulfiredoxins, at expense of ATP, yielding PRX-SOH.

Interestingly, being localized at *peri*-plasma membrane cytosol, glutaredoxin GRX1 has been also implicated in modulation of Ca^{2+}-dependent insulin exocytosis, which was suppressed by GRX1 silencing [143]. The stimulatory action of NADPH on the exocytotic machinery was found to correlate with ~30% inhibition in whole-cell Ca^{2+} currents. Upon GRX1 silencing, NADPH did not amplify insulin release, but still inhibited Ca^{2+} currents [143].

4.3. Redox Information Signaling. The deviation in redox state towards *pro*-oxidation or reduction is always given by balance between production of ROS and antioxidant defense. Since in pancreatic β cells mainly NADPH-dependent systems operate, such as the thioredoxin or glutaredoxin system, the elevated ROS may activate stress-sensitive second messengers such as p38MAPK, JNK [146], and PKC [147]. Also, the transcription factors MAF-A and PDX1 participating in β cell proliferation and insulin biosynthesis were shown to be sensitive to oxidative stress [148, 149]. Moreover, the evidence of redox signaling exists in GSIS of pancreatic β cells. First, the exocytosis of insulin, namely, soluble NSF attachment protein receptor (SNARE) complex, is significantly reduced upon H_2O_2 treatment [150]. NADPH as an important component of antioxidant defense system was also proposed as a parallel mediator GSIS or modulator of canonical GSIS mechanism, since an increase in the NADPH pool is usually accompanied by the increase in insulin granule exocytosis [137]. In the β cell cytosol, NADPH is formed by reduction of its oxidized counterpart NADP via pyruvate cycling pathways mediated by cytosolic malic enzyme (ME1) and cytosolic isocitrate dehydrogenase (IDH1) as well as via glucose-6-phosphate dehydrogenase, the rate limiting enzyme of the pentose phosphate pathway shuttle. In mitochondria, NADPH is regenerated via NADP dependent reduction mediated by ME3 and mitochondrial IDH2 as well as via nicotinamide nucleotide transhydrogenase [151]. All above reactions change only reduced/oxidized form of NADPH, without changes in total NADPH and NADP pool. This can be performed through NAD kinase whose single cytosolic isoform was found to regulate insulin secretion in β cells [152]. NAD kinase was found to be activated by glucose stimulated increase in Ca^{2+}. Because NAD kinase is cytosolic, the produced NADP(H) can be used by other NADP/NADP(H) dependent enzymes. Also NADPH oxidases (implicated in GSIS) belong to NADPH consuming enzymes working in *pro*-oxidant mode. In conclusion, the redox couple NAD(P)/NAD(P)H plays an important role for GSIS. A *pro*-oxidative state can also induce ER stress, which can further impair β cell function by activation of PERK to decrease insulin synthesis [153].

5. Oxidative Stress in Pancreatic β Cells and Its Role in Type 2 Diabetes

5.1. Type 2 Diabetes Mellitus. The progressed T2DM is manifested by both insulin resistance in peripheral tissues as well as β cell dysfunction [1, 2, 6–9, 154–157]. Insulin resistance in skeletal muscle and fat tissue, increased liver gluconeogenesis, abnormal secretion of incretins and impaired central regulation of food intake and energy expenditure are indicated for T2DM [1, 2, 6–9, 153–159]. Overt hyperglycemia results mainly from an interaction between insulin resistance in the peripheral tissues and failing insulin secretory capacity. In both cases, the metabolic abnormalities typical for diabetes are linked to insufficient β cell mass, which is unconditional in type 1 DM (T1DM) or may be only relative in T2DM. The impaired glucose tolerance and later diabetes are manifested in the progressed stage, when already pancreatic β cells cannot defeat increased metabolic demands and their function fails. It is proposed that impaired GSIS might be a primary cause or, alternatively, it may result from the globally deregulated metabolism.

A clear disproportion between fuel intake and energy expenditure in T2DM etiology suggests participation of a metabolic disorder. This may be reflected also by impairment of redox homeostasis, impairment of insulin signaling, and redox signaling and dysfunctions at mitochondrial level in both, primarily (or earlier) in peripheral insulin-sensitive tissue, but also may play a significant role in failing pancreatic β cells [153–159]. Description of emerging role of redox signaling and ROS in β cell biogenesis and maintenance of β cell mass and in its loss in diabetes is out scope of this paper. However, we emphasize that progressive oxidative stress does not represent only chronic exposure to ROS *per se*, leading to oxidation of proteins, lipids, and DNA, notably mitochondrial DNA that results in further turn of self-accelerating metabolic deterioration. Progressive oxidative

stress also impairs redox signaling [4, 131, 149, 160–163], insulin signaling [1, 2, 74, 164], autocrine insulin signaling [117–120], and housekeeping mechanisms of cells, namely autophagy and mitochondria-specific autophagy, mitophagy [5, 165–167], besides initiating an inappropriate apoptosis [168–170]. Another component of oxidative stress comes from intake of excessive fatty acids and lipid peroxidation products, generally termed as lipotoxicity [88, 171–173]. Yet another component results from elevated blood glucose as is known as glucotoxicity affecting β cells as well [174]. It can be mentioned that distinct early events of impairment may converge upon T2DM progress towards the same consequences (Figure 2).

5.2. Mitochondrial Oxidative Stress. The impaired mitochondrial function belongs to key dysfunctions leading to insulin resistance and diabetes progression [1, 2, 4, 5, 123, 131, 132, 154–159, 175–191]. Typically, the decrease of extent between minimum and maximum OXPHOS leads to impaired GSIS. Mitochondrial dysfunction and excessive oxidative stress of mitochondrial origin may lead to lipid accumulation and peroxidation, shifts of the cellular redox balance towards oxidative stress, to the endoplasmic reticulum stress, and to a secondary inflammatory response [159]. All these events take place not only in peripheral tissues but also in β cells [156–159, 175–177]. Whereas energy metabolism of peripheral tissues in relation to T2DM etiology has been intensively investigated, β cell bioenergetics, metabolism, and pathogenesis related to T2DM are not fully understood.

The first target of oxidative stress in mitochondrion is mtDNA [158, 159, 177–184] and its maintenance proteins and proteins of mtDNA transcription and replication machinery [185, 186]. It is exemplified for Goto Kakizaki rats, a T2DM model, characterized by an onset of Langerhans islet pathology, indicated by islet hypertrophy with decreasing the number of insulin-secreting β cells [187, 188]. Indeed, degradation of mtDNA in the remaining β cells has been found [189, 190] as well as fragmented mitochondrial reticulum morphology [191]. Especially, mtDNA variants in the coding and control regions can have combined effects influencing T2DM development [178]. MtDNA encodes seven subunits of respiratory chain, Complex I ND1 to ND6 and ND4L, cyt b (subunit of Complex III), three subunits of Complex IV, that is, cytochrome c oxidase, subunits 1, 2, 3, and ATP synthase subunits 6 and 8, plus 22 tRNAs and two ribosomal RNAs. Certain mtDNA mutations in these mt genes should lead to oxidative stress and initiate β cell dysfunction [184], such as in the heart [192]. Thus an ATP8 subunit mutation has been associated with increased mitochondrial superoxide generation, impaired GSIS, and increased β cell mass adaptation [179]. Excessive ROS due to mtDNA mutation can induce apoptosis [180]. Similar to Goto Kakizaki rats, age-related decline in mtDNA copy number has been indicated in human Langerhans islets [183]. Nevertheless, a general link between clinically found mtDNA mutations and β cell dysfunction is not yet established (e.g., [182]). The reason lies in robust mtDNA genetics, when heteroplasmy has to exceed enormous threshold until deleterious outcomes arise. However, when the important feature of mtDNA genetics is impaired, such as impaired mtDNA maintenance protein transcription factor B1, mitochondrial, TFB1M, there is a risk of T2DM development [185]. Hence mitochondrial disorder of rather metabolic origin prevails in T2DM development [158, 177].

5.3. Cytosolic Oxidative Stress. Likewise in mitochondrion, when ROS sources exceed the antioxidant defense, oxidative stress prevails. Depending on the shift from the physiologically tolerant ROS homeostasis even mild oxidative stress may activate ROS-sensitive information signaling. Persistent oxidative stress is deleterious due to accumulation of oxidized proteins, lipids, and DNA. In pancreatic β cells an antioxidant defense can be considered as low. However, it may be set so to provide redox regulations involved in GSIS and β cell housekeeping processes.

Under diabetic conditions, oxidative stress markers have been frequently detected in β cells, such as 8-hydroxy-2′-deoxyguanosine (8-OHdG) reporting on DNA oxidation [136, 193, 194], 4-hydroxy-2,3-nonenal, that is, one of lipid peroxidation end-products [193, 195] or nitrotyrosine [194]. Studies using diabetic models showed improved insulin sensitivity by antioxidants [161]; however, antioxidant benefits to diabetic patient treatments may not be extrapolated to normal subjects for preventive purposes, since overly diminishing intracellular ROS by excessively high antioxidant enzyme activities deregulate GSIS.

As described in Section 4.2, β cell function may be easily impaired under yet mild oxidative stress. Such stress imposes also activation of ROS-sensitive second messengers, such as p38 mitogen-activated protein kinase, p38MAPK [146], or c-Jun N-terminal kinase, JNK/SAPK [148]. Activation of JNK pathway during oxidative stress results in decreased insulin gene expression by affecting the DNA binding activity of the epigenetic regulation of transcriptional factor pancreatic duodenal homeobox (PDX1). Thus, in turn, the beneficial effect of antioxidants on diabetic patient could be explained besides the protection of oxidative destructions of macromolecules also by maintenance of PDX1. PDX1 plays pivotal role in proliferation, survival, and function of β cells and activation of insulin gene expression [148, 196]. The epigenetic regulation of PDX1 involves histone acetylation of H3 and H4, which helps to remodel the chromatin in the PDX1 promotor to form more accessible structure for transcription and to maintain high level of functional PDX1 [196]. This promotes β cell differentiation and insulin synthesis for compensating insulin resistance. Also direct H_2O_2 effect on PDX1 was found to be induced through specific phosphorylation on Ser61 and/or Ser66, resulting in an increasing degradation rate and decreasing half-life of the protein [197]. PDX1 protein is also regulated through FOXA2 activator. SOD1 promotor was shown to contain four binding sites for FOXA2 [198, 199].

The cytokine-induced β cell dysfunction and apoptosis is also based on ROS-induced intracellular signaling pathways [200, 201]. Cytokine-generated ROS induce expression of inducible nitric oxide synthase (iNOS) which results in $NO^{\bullet}$ release and translocation of nuclear factor-κB (NFκB). In turn, NFκB induces NADPH oxidase as a

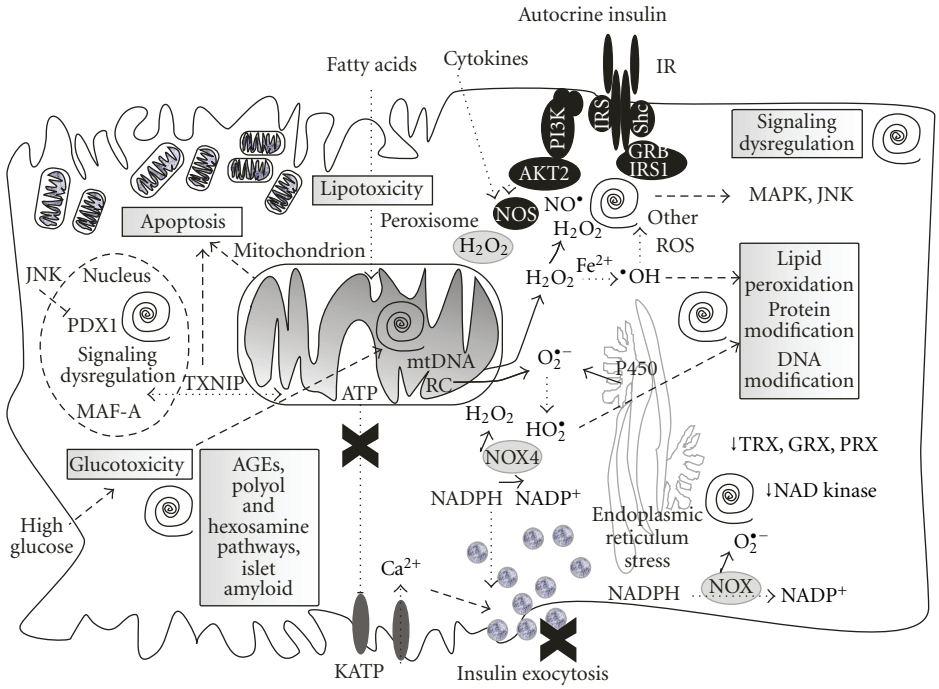

Figure 2: Vicious spirals of repeating self-accelerating oxidative stress and dysregulated redox and information signaling as possible causes of type 2 diabetes. Schema of cell events that occur at type 2 diabetes development, as related to oxidative stress and impaired redox homeostasis and signaling, dysfunctional insulin signaling in peripheral tissues, and autocrine insulin signaling in failing pancreatic β cells. Considered "vicious spirals" (depicted by black spirals) of progressive oxidative stress leading to oxidation of proteins, lipids, and DNA, notably mitochondrial DNA, all resulting in further turn of self-accelerating metabolic deterioration and specifically impairment of the glucose sensing. Progressive oxidative stress also impairs redox signaling and autocrine insulin signaling which further deteriorates fitness of β cells and their housekeeping mechanisms, specifically autophagy and mitochondria-specific autophagy, mitophagy, besides initiating an inappropriate apoptosis. Another component of oxidative stress comes from the intake of excessive fatty acids and lipid peroxidation products, generally termed as lipotoxicity. Yet another component results from elevated blood glucose as is known as glucotoxicity further accelerating cell oxidative stress, impairing cell maintenance, dysregulating information signaling and leading to advanced glycation end products (AGEs), (yet further accelerating oxidative stress and other cell stresses), activating polyol pathway and thus again contributing to *pro*-oxidation redox homeostasis, activating hexosamine pathway and dysregulating crucial survival pathways including insulin receptor (autocrine) signaling, and finally enhancing glycosylation and forming antiparallel crossed β-pleated sheet structure called amylin-derived islet amyloid, promoting β cell cytotoxicity.

major cytosolic ROS source. Recently, thiredoxin-interacting protein (TXNIP) was found to shuttle between nucleus and mitochondrion to which migrates upon oxidative stress and promotes apoptosis via matrix ASK1-induced release of cytochrome c [202]. This complies with results of TXNIP-KO mice studies [203, 204], in which streptozocin treatment is 50-fold less prone to apoptosis [203]. TXNIP also mediates ER stress induced β cell death and has numerous implications in diabetes development [205, 206].

5.4. Consequences of Chronic High Glucose. One would need to conclude that some important physiological aspects and numerous cell regulations in pancreatic β cells are dependent on glucose [174]. Surprisingly concentrations outside of physiological stimulatory range of 3 to ~10 mM glucose ("GSIS range") are deleterious especially when exposed to β cells at prolonged time. Thus, even low glucose can stimulate oxidative stress via AMPK activation [207]. Glucose at high end of GSIS range is one of the most important stimuli for β cell mass maintenance by stimulating proliferation, neogenesis and hypertrophy [174], most specifically via autocrine insulin signaling [117–120]. We may refer to glucotoxicity at much higher glucose levels, leading to effects that overwhelm the beneficial glucose "maintenance effects." Thus, for example, hyperglycemia deteriorates β cells after islets transplantation [208]. However, a hallmark of glucotoxicity is that hyperglycemia causes a profound oxidative stress that is possible to attenuate by overexpression of proteins of antioxidant defense [148, 193, 209–211]. Activation of JNK and impairment of PDX1 function (cf. above) belong to one of mechanisms involved. Also ER stress comes from glucotoxicity [206]. The classic pathway of glucotoxicity comes from the spontaneous reactions of glucose and other sugars with amine residues of proteins, lipids, and nucleic acids forming so-called advanced glycation end products (AGE) [212]. Polyol pathway is activated when excess glucose is converted to sorbitol in the presence of aldose reductase, consuming NADPH and thus contributing to *pro*-oxidation state [213]. By increased flux of glucose via so-called hexosamine pathway, resulting in induction of

O-glycosylation of signaling molecules, a crucial survival pathway is dysregulated leading to oxidative stress, as insulin receptor and insulin receptor substrates/PI3 kinase pathway [214]. Also elevated diacylglycerol under hyperglycemia activates protein kinase C which subsequently activates NADPH oxidase and ROS production [127]. Finally, persistent hyperglycemia/ROS exposure enhances glycosylation, thus unfolding of some proteins, lipids and nucleic acids, notably alters a 37 amino acid islet amyloid polypeptide (IAPP), termed amylin. Resulting antiparallel crossed β-pleated sheet structure called amylin-derived islet amyloid (ADIA) is sensitive to free radical polymerization and thus promotes β cell cytotoxicity [215–217].

6. Future Perspectives

In the future research it will be probably established whether T2DM is an inevitable disease and whether one may develop strategy to highly retard or completely exclude the pathological outcomes of progressive self-accelerating oxidative stress and nitrosative stress and concomitant dysregulated information signaling. The emerging role of redox signaling in GSIS and processes of molecular physiology of pancreatic β cells need to be elucidated as well. Unfortunately, neither targeted antioxidants might be able to defeat T2DM, since they simultaneously disrupt the inherent physiological redox signaling. Perhaps more focused strategies on yet unknown mechanisms will help to defeat T2DM world epidemy.

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