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REVIEW

Redox Status as a Key Driver of Healthy Pancreatic β-Cells

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Summary

Redox status plays a multifaceted role in the intricate physiology and pathology of pancreatic β-cells, the pivotal regulators of glucose homeostasis through insulin secretion. They are highly responsive to changes in metabolic cues where reactive oxygen species are part of it, all arising from nutritional intake. These molecules not only serve as crucial signaling intermediates for insulin secretion but also participate in the nuanced heterogeneity observed within the β-cell population. A central aspect of β-cell redox biology revolves around the localized production of hydrogen peroxide and the activity of NADPH oxidases which are tightly regulated and serve diverse physiological functions. Pancreatic β-cells possess a remarkable array of antioxidant defense mechanisms although considered relatively modest compared to other cell types, are efficient in preserving redox balance within the cellular milieu. This intrinsic antioxidant machinery operates in concert with redox-sensitive signaling pathways, forming an elaborate redox relay system essential for β-cell function and adaptation to changing metabolic demands. Perturbations in redox homeostasis can lead to oxidative stress exacerbating insulin secretion defect being a hallmark of type 2 diabetes. Understanding the interplay between redox signaling, oxidative stress, and β-cell dysfunction is paramount for developing effective therapeutic strategies aimed at preserving β-cell health and function in individuals with type 2 diabetes. Thus, unraveling the intricate complexities of β-cell redox biology presents exciting avenues for advancing our understanding and treatment of metabolic disorders.

Key words

Redox homeostasis • Pancreatic β-cells • NOX • Heterogeneity

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Introduction to redox milieu in β-cells with emphasis on pro-oxidative sources

There is no doubt that redox homeostasis is a critical feature of cellular signaling in a variety of cells. A transient increase in cellular reactive oxygen species (ROS) has been identified as an essential second messenger, while permanently increased production leads to oxidative stress, which has been linked to many diseases, including diabetes [1,2]. Many have shown that pancreatic β-cells, as the major glucose sensor in the body, are sensitive to perturbations in redox homeostasis and that long-term disruption of redox homeostasis leads to deterioration of β-cells and thus insulin secretion and glucose balance in the body, the prominent feature of diabetes (more in [3-7]).

It has been suggested that glucose-induced metabolism in β-cells can produce ROS *via* many pathways [8]. However, due to significant limitations in the methods used to detect ROS *in situ* (in terms of their species and cellular localization), the exact site of ROS production and oxygen species remained undetermined until recently [9,10]. By using redox probes targeting organelles that are also more ROS species-specific, we observed the increased production of ROS, particularly hydrogen peroxide (H_2O_2), under condition

of glucose stimulation in the cytoplasm by NADPH oxidase, isoform 4, NOX 4 [11] (Fig. 1). Interestingly, the production of ROS (especially superoxide) in the mitochondrial matrix was decreased upon glucose induction due to the decreasing $NADH/NAD^+$ ratio in the mitochondrial matrix [12]. This does not necessarily refute the role of mitochondria as a source of ROS during glucose induction, as the complex III of the respiratory chain is able to produce superoxide outside the mitochondria and is therefore undetectable with probes in the mitochondrial matrix (Fig. 1). Increased glucose-stimulated metabolism can also lead to increased autooxidation of glyceraldehyde, producing H_2O_2 and ketoaldehydes in diabetes [13]. Glycolysis produces dihydroxyacetone, which undergoes the reduction to glycerol-3-phosphate and acylation, producing diacylglycerol, which activates protein kinase C (PKC) [14-17]. PKC can phosphorylate NOX isoforms leading to the induction of their activity and thus increase in cytosolic ROS production. Metabolism of sorbitol, hexosamine, or methylglyoxal derived from glucose catabolism can also stimulate ROS production in diabetic conditions [8,18]. However, mitochondrial ROS production in β-cells has been documented mainly under conditions of the increased flux of fatty acids and amino acids as substrates, the conditions associated with diabetic pathology [11,19-24] (Fig. 1). Superoxide produced along the electron transport chain (complex I/III) is rapidly converted by superoxide dismutase, SOD2 isoform, to H_2O_2, being able to diffuse out of mitochondria [19,24-29] This production has been found to be controlled by the uncoupling activity of UCP2 in β-cells [30-32].

Be that as it may, H_2O_2 has been identified as a critical molecule for signal transduction in pancreatic β-cells. Its low concentration (nanomolar) is involved in signal transduction by cysteine oxidation of participating proteins, known as redox relay [33,34] (Fig. 1), whereas increased production in the micromolar concentration range causes oxidative stress because β-cells are unable to adequately increase antioxidant protection especially in long-term horizon [35] (Fig. 2). H_2O_2 is a small electroneutral molecule that can cross lipid membranes either alone or through peroxiporins, members of the aquaporin family (more in [36]). Its toxicity is tracked by the production of hydroxyl radicals (OH^-), which are generated when it encounters free iron or copper, rather than interacting with protective high-affinity thiols [37]. Hydroxyl radicals have a high oxidation potential, and their small radius and uncharged state provide great mobility so that chemical reactions can proceed at high rates [37]. Therefore, H_2O_2 is also a crucial mediator of toxicity under conditions of chronic oxidative stress.

The specific properties of H_2O_2 in terms of its reactivity allow it to specifically target reactive thiols of cysteine residues within protein structures. However, only some thiols are predetermined to undergo the oxidation reaction. This is given by the vicinity of the surrounding amino acids (local electrostatic environment) and pH. Thus, cysteines in proteins must display low pKa, to enhance the thiolate fraction, as a prerequisite for fast and efficient oxidation by peroxides, although its nucleophilicity (to attack the H_2O_2 electrophile) and its capacity to stabilize both the transition state with the reactant, H_2O_2, and the leaving group (which occurs after the rupture of the peroxidic bond) also must be preserved [38]. The reactive thiols then react with H_2O_2 to form sulfenic acid (reversible oxidation). The oxidized thiols are regenerated by reaction with other thiols groups on i) diverse proteins giving rise to the so-called redox relay system by which the redox signal is transferred or ii) proteins of the antioxidant defense system serving as the ROS scavengers [33,39].

The specific redox situation prevails in the endoplasmic reticulum (ER) of β-cells since ROS are involved in oxidation during insulin folding in β-cells (Fig. 1). Each proinsulin molecule folded at ER generates 3 molecules of H_2O_2 [40]. This is generated during protein disulfide isomerase oxidoreductase (PDI) reoxidation by ER oxidoreductin 1 (ERO1), which are involved in insulin folding. High amounts of H_2O_2 with limited efflux from ER and a low ratio of reduced to oxidized glutathione (GSH/GSSG) create a strong oxidative milieu in the ER of β-cells [41]. In the case of chronic nutrient excess, e.g., obesity, when β-cells struggle to maintain glucose homeostasis in the body by overproduction of insulin (hyperinsulinemia), ER stress develops [42]. This is associated with an imbalance in redox status in ER and leads to β-cell exhaustion over time.

The inappropriate composition of nutrition and especially its chronic overload leads not only to an increase in blood glucose levels but also to an increase in fatty acid levels circulating in the bloodstream, being sensed by β-cells. Under conditions of increased circulation of fatty acids in the blood, long-chain and medium-chain fatty acids can produce H_2O_2 during their β-oxidation in peroxisomes and mitochondria, where little catalase is present [41] (Fig. 1). The β-oxidation in

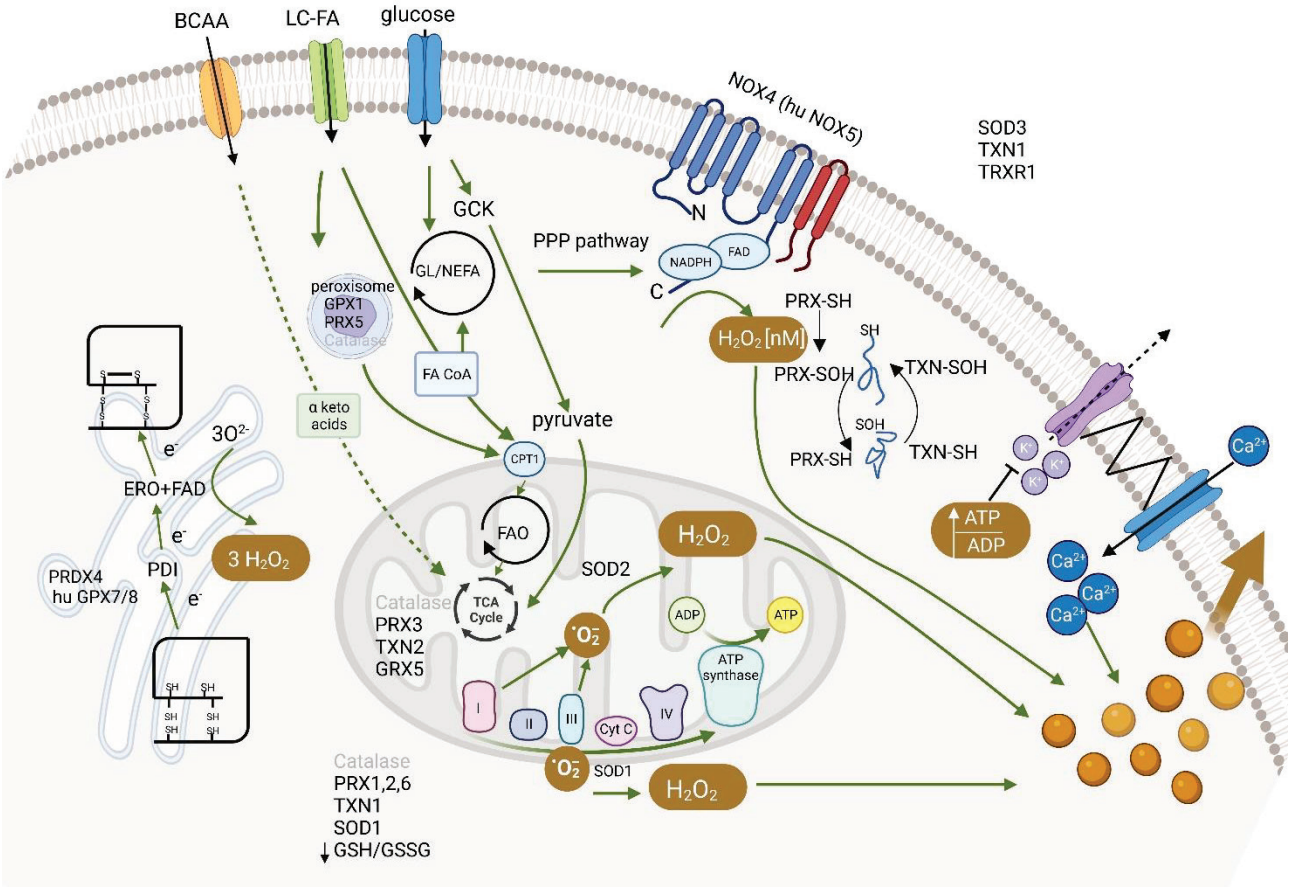

Fig. 1. Physiological amplification of insulin secretion by redox signaling in pancreatic β-cells. Glucose, the major trigger of insulin secretion, initiates glycolysis while being phosphorylated by glucokinase (GCK). As it proceeds, the metabolites also enter the pentose phosphate cycle (PPP), which generates NADPH in the cytosol, the substrate for NADPH oxidase 4 or human isoform 5 (NOX4, hu NOX5). Pyruvate, the product of glycolysis, enters the mitochondria to generate ATP and possibly superoxide ($^{\bullet}O_2$-) on Complex III directed out of the mitochondria. Glycerol-3-phosphate derived from glycolysis is involved in lipogenesis in β-cells. Stimulation by glucose leads to increased activity of NOX4/hu NOX5, generating H_2O_2, which at nM concentration is involved in redox relay signaling to target proteins involving peroxiredoxins (PRX) and thioredoxins (TXN). Redox signaling, H_2O_2 directly, and together with increased ATP lead to inhibition of the K_{ATP} channel, causing plasma membrane depolarization, calcium influx, and insulin granule release. Stimulation by glucose also increases endoplasmic reticulum (ER) activity, where folding of proinsulin requires a pro-oxidant environment, i.e., 3 molecules of H_2O_2 per 1 proinsulin. The presence of long-chain fatty acids (LC-FA) allows their accumulation in lipid droplets or, at low glucose, fatty acid oxidation (FAO) in mitochondria or peroxisomes, potentially generating ROS. Branched chain amino acids (BCAA) exhibit insulinotropic effects through α-keto acid metabolism, TCA cycling in mitochondria with the potential for ROS production. Pancreatic β-cells show low expression of catalase and glutathione peroxidases but express various forms of thioredoxins (TXN), peroxiredoxin (PRX), and superoxide dismutases (SOD) compartmentalized intracellularly. Created with Biorender.com.

peroxisomes produces H_2O_2 and shortens the length of the fatty acid chain before it is passed to mitochondria for complete oxidation, another site of ROS production [42]. Thus, mitochondria are functionally linked to peroxisomes in fatty acid processing. Chronically elevated concentrations of free fatty acids termed lipotoxicity together with hyperglycemia (often associated with diabetes) cause the impairment of insulin secretion and β-cell death. Another insulinotropic potentiator of β-cell is selected amino acids. The state of elevated dietary branched-chain amino acids (BCAAs) such as leucine, isoleucine, and valine, plays a critical role in stimulating insulin secretion by serving as both metabolic fuel and allosteric activator of glutamate dehydrogenase [43,44] (Fig. 1). Its acute action stimulates insulin secretion *via* direct inhibition of K_{ATP} channel currents in β-cells [45], where ROS may be part of the signaling action originating from mitochondria or NADPH oxidases [23,46].

Thus, it is clear that redox status reflects the nutritional conditions to which β-cells are subjected. The major signaling molecule is H_2O_2. Its involvement in redox signaling is required for efficient insulin secretion and function while its long-term increase leads to development of oxidative stress accompanying the development of diabetes.

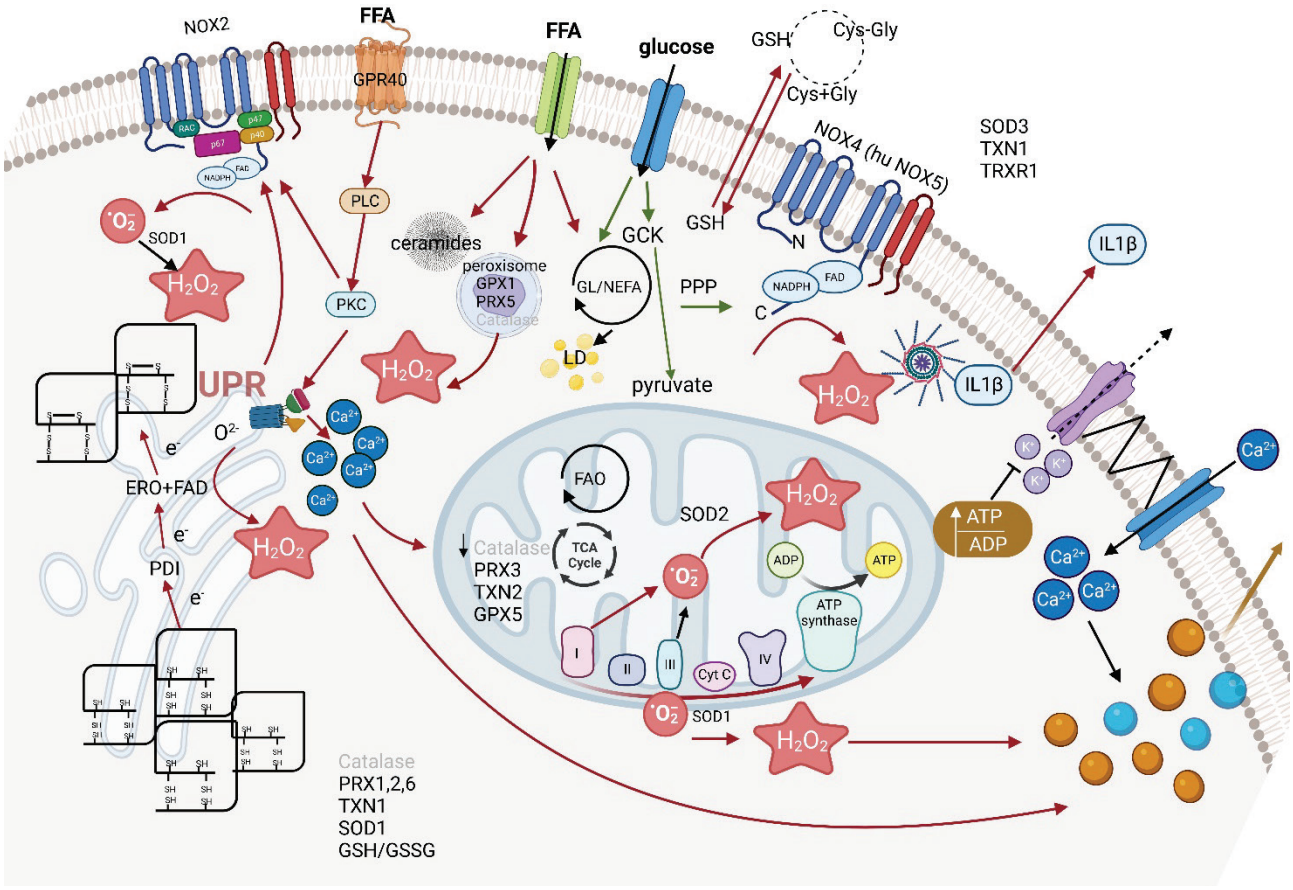

Fig. 2. Detrimental prooxidative intracellular environment upon chronic nutrient overload in pancreatic β-cells. Chronic hyperglycemia activates NADPH oxidase 4, which leads to increased assembly of inflammasome and maturation of IL1β, initiating local inflammation. Increased requirement for insulin secretion and enhanced pro-oxidative milieu in cytoplasm establish unfolded protein response (UPR), which is accompanied by oxidative stress in endoplasmic reticulum (ER). Increased amount of proinsulin is secreted (blue circles). Increased amount of free fatty acids (FFA) in plasma induces ceramide accumulation with the potential to induce apoptosis. FFA also signal through the GPR40 receptor to induce the activity of phospholipase C (PLC), protein kinase C (PKC) to regulate NADPH oxidase 2 (NOX2) to produce ROS and contribute to calcium release from ER for insulin granule release. FFA also enter peroxisomes for FAO, generating enhanced ROS because of the low expression of catalase. Prooxidative intracellular environment induces the degradation of glutathione outside of the cells to produce components for its synthesis inside the cells. Created with Biorender.com.

NOXs are good and evil in the redox homeostasis of pancreatic β-cells

There is an increasing number of reports showing that NOXs are significantly involved in β-cells physiology and pathology. They are essential regulators of physiological insulin secretion and promote superoxide/H_2O_2 for efficient signaling of insulin secretion. At the same time, they can have deleterious effects when chronically overactivated. There is ample evidence for the specific expression of isoforms of NOX1, NOX2, NOX4, and p22phox and their cytosolic regulators in rodent and human β-cells [43,44]. NOX5 is expressed only in human β-cells and no homolog was found in rats and mice [45]. Regarding its role and activity in β-cells, it was revealed that knocking down p47phox, the subunit of the originally phagocytic NOX2 isoform, significantly reduced glucose-induced H_2O_2 production and insulin secretion (GSIS) [46]. However, direct ablation of NOX2 in mice showed no impairment of GSIS. Until recently, many studies using nonspecific inhibitors also failed to shed light on the function of NOXs in β-cells [44,47]. Recently, we found that the NOX4 isoform, which is constitutively expressed and lacks many regulatory subunits present in other isoforms, is metabolically stimulated to activity, resulting in H_2O_2 production that supports GSIS [11] (Fig. 1). This was previously demonstrated with the nonspecific NOX4 inhibitor GLX351322 [48]. Its activity depends on the amount of NADPH normally derived from glucose metabolism in the vicinity of the enzyme. Unfortunately, the exact intracellular location in β-cells has not yet been defined due to a lack of reliable antibodies. However, NOXs are transmembrane proteins that transport electrons across biological membranes and reduce oxygen to superoxide or directly produce H_2O_2 (more in

[49]). Inhibition of NOX4 either by silencing of cultured β-cells or its ablation, specifically in mouse pancreatic β-cells, significantly increased the probability of K_{ATP} channel opening, thereby reducing the first phase of GSIS in particular [11]. Interestingly, β-cell specific *Nox4* knockout animals show suppressed insulin secretion upon glucose stimulation and constantly secrete low levels of insulin, prolonging the time needed to establish normoglycemia. This affects the feeding habits of these knock-out mice, as they consume less food to maintain the physiological glucose concentration [50]. NOX4 is thus an important redox molecule in the physiology of pancreatic β-cells. Recently, human NOX5 was also found to be important for proper GSIS [45].

However, NOXs may also contribute to β-cell dysfunction in chronic hyperglycemia in animals and humans with type 2 diabetes (T2D) (Fig. 2). Chronic overeating activates NOXs to produce superoxide/H_2O_2, leading to long-term oxidative stress and consequent β-cell damage and apoptosis [4,51]. Proinflammatory, hyperglycemic, and lipotoxic conditions have been shown to activate NOXs [11,52-55]. Gene expression profiling of islets from patients with T2D also showed increased expression of *NOX2/4/5* compared with nondiabetic individuals (Geoprofiles GDS3382). Proinflammatory cytokines upregulate the expression of 12-lipoxygenase, which can activate NOX1 in β-cells, leading to their failure [56,57]. Lipotoxicity, particularly from saturated free fatty acids, induces NOX2 activity, leading to impaired insulin secretion, calcium homeostasis, and viability [54]. It has been shown that GPR40, the free fatty acid receptor, can also activate NOX2 in β-cells [58]. Thus excess palmitate activates NOX2 triggering transient receptor potential melastatin 2 (TRPM2) channels. This can lead to an increase in mitochondrial zinc ions, resulting in a loss of membrane potential and mitochondrial fission, affecting energy metabolism and thus β-cell viability [53]. Upregulation of NOX5 has been shown to worsen insulin secretion under hyperglycemic/diabetogenic (palmitate-induced) conditions by increasing the depletion of cAMP, a critical enhancer component of insulin secretion [45]. We have observed that chronic overnutrition induced either by high-fat diet *in vivo* or by hyperglycemia *in vitro*, causes chronic activation of NOX4 and establishes an intracellular pro-oxidant status [50]. This leads to the assembly and activation of the inflammasome and further maturation of the proinflammatory interleukin 1β (IL1β). The release of IL1β from β-cells can then activate macrophages toward proinflammation, causing local inflammation (Fig. 2). Thus, chronic overproduction of ROS by NOXs in β-cells leads to their pathology and eventual loss of viability. Recent reports have shown that islets from *Nox2* knockout mice improved islet transplantation outcome. *Nox2*$^{-/-}$ islets showed decreased superoxide production, higher GSIS, and enhanced antioxidant defense by increased expression of Nrf2, and Sod1, improved Ho1 expression causing early revascularization. This leads to restoration of normoglycemia in diabetic transplanted mice [59].

New inhibitors of specific NOX isoforms have been developed and are being investigated in the treatment of diabetes (more in [60]). Many diabetic complications such as diabetic nephropathy, retinopathy, neuropathy, and cardiopathy, collectively termed diabetic vasculopathy, have been associated with chronic NOX activity. NOXs, especially NOX2 and 4, have been associated with endothelial dysfunction and are the target of the studies. However, the role of NOX overactivation and prooxidant signaling in pancreatic β-cells under conditions of chronic overeating require the development of antioxidants or inhibitors that target β-cells only. Their short-term treatment could be useful in restoring β-cell signaling and viability during the development of diabetes.

Pancreatic β-cells antioxidant defense system: Are β-cells so vulnerable towards oxidative stress?

Cellular antioxidant defense comprises enzymatic machinery and glutathione (GSH), which can buffer excessive reactive (oxygen, nitrogen, and sulfur) species produced by cellular metabolism. Cellular compartments differ in their pH, redox potential, and concentrations of reactive metabolites allowing redox signaling events or direct usage of these reactive compounds for metabolic reactions (e.g. protein folding in ER) in a specifically compartmentalized manner [61,62]. Consequently, the antioxidant enzymes are also strictly compartmentalized, with specific isoforms of the enzymes in these compartments. Also, the content of glutathione redox couple i.e., GSH/GSSG (reduced/oxidized glutathione) and its redox potential, differs throughout the compartments [63,64]. The difference is given not only by the diverse NADPH concentration and availability, ROS production, and expression of antioxidant enzymes but also because GSH is only synthesized in cytosol and can be only transported through the membranes in its reduced form [65]. Notably, the compartment-specific redox state of GSH affects not only the direct redox GSH partners but

also the specialized function given by the respective organelle [66]. GSH is synthesized by a two-step enzymatic reaction involving γ-glutamylcysteine synthetase (γ-GCS) and GSH synthetases (GSHS) in sequence. γ-GCS catalyzes the formation of the dipeptide γ-glutamylcysteine (γ-Glu-Cys), and GSHS catalyzes the binding of glycine to γ-Glu-Cys to form GSH [67]. The rate-limiting step of the synthesis is the presence of cysteine, which is mainly derived from the trans-sulfuration pathway of methionine and/or the reduction of cystine, which is transported from the extracellular space [67]. Except for these traditional pathways, a study done by Fu *et. al.* revealed that 25 % of carbon derived from glucose in human islets is directed to GSH synthesis *via* pyruvate carboxylase pathway and probably glutamate [68]. The degradation of GSH occurs in extracellular space, where it is converted to cysteinylglycine (CysGly) and then to Cys at tissue sites rich in the ectoenzymes γ-glutamyltranspeptidase (γ-GT) and dipeptidases (mainly kidney and lung) by the sequential action of these two enzymes [69] (Fig. 2). The amino acids can be taken by the cells again and used for GSH *de novo* biosynthesis. Interestingly pancreatic β-cells express significantly higher amounts of GSH degradation enzymes than those of the synthesis pathway, and as a result, rely on glutathione transferred from the liver [70].

From the historical perspective, β-cells were considered to contain low amounts of the main antioxidant enzymes [71,72] (Fig. 1). Still, this feature has always been compared with the liver and kidney, specialized organs for detoxification, which naturally contain high amounts of antioxidant enzymes [71]. An explanation for the low expression profile is nowadays interpreted by the fact that redox signaling, which is tightly associated with glucose sensing and its metabolism in pancreatic β-cells, is a required coupling factor for insulin secretion [11].

Superoxide dismutase (SOD) is considered one of the most important antioxidant enzymes. The enzyme accelerates the otherwise spontaneous dismutation of superoxide anion O^{2-} to less reactive H_2O_2 up to 10^4 [73]. SOD exists in three isoforms, whose activity is optimized according to the compartment-specific conditions. *Cu/Zn-SOD1* is expressed in the cytosol, the mitochondrial intermembrane space, the peroxisomes, and the nucleus. *Mn-SOD2* is expressed in the mitochondrial matrix, and Cu/Zn-SOD3 is targeted to extracellular space, but its expression in β-cells has only been detected on the mRNA level [74]. In addition to SODs other H_2O_2 degrading enzymes are expressed in β-cells. Interestingly, catalase is expressed at a very low level and is even considered one of the disallowed genes in β-cells [75]. Glutathione peroxidase 1 (*GPX1*) is expressed in cytosol and peroxisomes, *GPX7/8* and *PRDX4* in ER, and *PRDX3* exclusively in mitochondria [40]. However, the expression profile also depends on species, as *GPX1* and catalase were shown to have protective effects in human pancreatic islets but not in rodents [76]. In recent years the opinion of oxidative damage vulnerable β-cells is shifting as β-cells were shown to express thioredoxin (*TXN*) and thioredoxin reductase (*TXNRD*) isoforms, glutathione reductases (*GSR*), glutaredoxins (*GRX*), and peroxiredoxins (*PRDX*) [34,77-82].

Interestingly it has also been shown that cells are able to secrete oxidoreductases to extracellular milieu [83]. This phenomenon has been first observed for immune cells, but secretion of cytoplasmic isoforms of TXN1 and TXNRD1 by murine and porcine β-cells has been revealed under hypoxic and inflammatory conditions [84]. Extracellular TXN1 seems to cause autocrine or paracrine regulation of β-cells, overall having beneficial effect by improving cell viability and blood glucose control, preventing apoptosis, and preserving insulin secretion [84,85].

The β-cells therefore possess delicate redox balance in which a rich antioxidant system is involved. This enables effective redox signaling. Nevertheless, β-cells do not have such a robust antioxidant system and are therefore unable to withstand prolonged oxidative stress, leading to a deterioration in their function and ultimately contributing to the onset of diabetes.

Redox status in other endocrine cells of pancreatic islets

β-cells are the most studied and represented cell type of the pancreatic islets. However, there are other important endocrine cell types that are required for proper islet function. These are glucagon-producing α-cells, somatostatin-producing δ-cells, ghrelin-producing ε-cells, and pancreatic polypeptide-producing PP cells. There is little knowledge about these endocrine cells in terms of their redox status and signal transduction. However, their synchronized paracrine function with the β-cells is critical for proper islet activity. Cell lines of individual type of endocrine cells are scarce (this does not apply to α-cells, for which a cell line already exists), and individual cell types are difficult to isolate from islets, which probably explains the lack of knowledge [86]. Moreover, redox status setting of cell lines might differ from primary endocrine cells as the cell lines are adapted to cultivation conditions which significantly vary in terms of oxygen pressure, glucose levels etc.

As α-cells are the second best studied cell type after β-cells, pertinent information exists about their antioxidant capacity. Human α-cells are capable producers of antioxidant enzymes such as catalase and GPX. Interestingly, human α-cells express more of these enzymes than human β-cells. β-cells showed increased oxidative damage to DNA and decreased viability when exposed to H_2O_2 and NO donors, whereas α-cells showed no change in viability [87]. Expression of antioxidant enzymes is clearly associated with enhanced protection of α-cells from oxidative stress. In addition, the pancreas of T2D patients exhibited increased numbers of apoptotic β-cells but not α-cells [88]. However, both endocrine cell types showed increased volume density of ER [89]. Moreover, the better survival of α-cells in T1D was explained by differences in the expression of SOD2 compared to β-cells, in addition to the specificity of the immune response [90]. On the other hand, mouse cell cultures of α- and β-cells showed increased expression of mitochondrial *SOD* and catalase in β-cells under hyperglycemic conditions. In α-cells, these enzymes are decreased [91]. The question here is whether the difference in expression of these enzymes is due to species or to the different effects and timing of the various oxidants. Inhibition of phosphoinositide 3-kinases (PI3K) signaling in α-cells does reduce mitochondrial *SOD* expression under hyperglycemic conditions, suggesting that this pathway is involved in mitochondrial *SOD* expression [91].

Thus, we lack the knowledge about the paracrine redox signaling and involvement of redox status within individual endocrine cell types in synchronized action of insulin and other endocrine hormones secretion by pancreatic islet.

Heterogeneity of β-cells and the role of redox status

Pancreatic β-cells exhibit considerable heterogeneity, contributing to the complexity of islets [92]. Distinct subpopulations differ at the molecular, morphological, and functional levels [93,94]. Most importantly, individual β-cells have diverse sensitivity to glucose, which subsequently affects their ability to synthesize and secrete insulin [95,96]. Thus, the heterogeneity of β-cells and β-cell intercommunication within subpopulations is critical for the regulation of insulin secretion [94,97]. Changes in β-cell heterogeneity as well as altered redox homeostasis have been associated with T2D, however, their interconnection is far from being understood [98,99].

The origins of β-cell heterogeneity research go far back in history. Already in the 1940s, it was found that β-cell size differs between large and small pancreatic islets [100]. Later, regional differences in the nuclear size and the position of the nucleoli within the nuclei of various β-cells were identified [101]. Also, morphological differences in response to glucose stimulation were described with regards to β-cell localization. Hyperglycemia decreased the volume density of β-cell secretory granules and increased the size of the endoplasmic reticulum and Golgi apparatus earlier in β-cells within the core of the islets compared to the peripheral β-cells [102]. The distinct insulin secretory patterns broadly divided β-cells into two types – responsive and unresponsive [103]. The differences in insulin secretory response were also associated with altered gene expression of important enzymes, such as glucokinase [104]. Recently, it has been described that insulin secretion responses were orchestrated by two populations of cells: hub cells with pacemaker properties dictating the insulin secretion dynamics and follower cells controlled by the hubs through calcium waves [105]. The development of molecular methods has enabled the identification of many markers specific to distinct β-cell populations, including E-cadherin (CDH1) [106]; polysialylated-neural cell adhesion molecule (PSA-NCAM) [107]; Flattop (FLTP) [108]; CD9 molecule (CD9) [109,110]; ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase1 (ST8SIA1) [110]; Dickkopf WNT signaling pathway inhibitor 3 (DKK3) [111]; solute carrier family 18 member (A2SLC18A2/VMAT2) [112].

For instance, β-cells can be divided into two sub-populations with different glucose responsiveness according to the level of surface PSA-NCAM, a prominent marker of functional β-cells [107]. Low PSA-NCAM-labeled β-cell population (β^{low}-cells) exhibited altered gene expression profile (e.g. downregulation of *Neurod1, Pdx1, Nkx6.1, Pax6*, i.e. β-cell identity genes; and upregulation of *Ldha, Hk1m*, i.e. β-cell forbidden genes) which suggested that these cells were composed of immature and/or non-functional cells in contrast to high PSA-NCAM-labeled population (β^{high}-cells). Moreover, these two populations also differed in the expression of antioxidant enzymes. For example, *Gpx1* was upregulated, while *Sod2* and *Trx2* were significantly downregulated in β^{low}-cells. Besides antioxidant enzymes, the expression of nitric oxide synthase 1 (*Nos1*) was also decreased [107]. These results indicate extensive dysregulation of the redox state in the poorly glucose-responsive β-cells. Importantly, the distribution of β^{high} and β^{low}-cells was completely inversed in ZDF rats (a genetic model of T2D) as the

β^{low}-cells became the predominant population in these animals [107]. In ST8SIA1+ β-cells, which are also less responsive to glucose compared to ST8SIA1-β-cells, *Gpx3* was significantly enriched. Representation of these cells was found to be abnormally high in T2D islets [110].

Although subpopulations of β-cells have been shown to differ in redox homeostasis in response to altered metabolism, redox regulations independent of β-cell heterogeneity have also been reported. Inhibition of NOX4 protected human islets from glucolipotoxicity regardless of their size, activity, and reactivity to glucose. These results suggested that NOX4-induced β-cell death occurs in all types of islets and may involve a mechanism that acts independently of the insulin-releasing activity of the islet [113].

Since redox homeostasis is a key factor determining β-cell fate, identification of differences in redox regulations in distinct populations of β-cells is required to unravel the role of β-cell heterogeneity in the physiology and pathology of pancreatic islets. Our laboratory focuses on this topic. This knowledge could potentially lead to novel therapeutic approaches to restoring β-cell function and mass.

Interestingly, α-cells were reported also in several distinct subpopulations based on proglucagon-derived peptides [114]. It was suggested to be reflected by the α-cell maturity state. However, no link to the redox state was reported.

Conclusions

There is no doubt that redox status is a critical determinant of pancreatic β-cell function. Their fragile redox homeostasis is a key part of nutrition-induced insulin signaling. However, long-term chronic nutritional overload leading to metabolic dysregulation impairs insulin secretion, induce inflammation, and consequently glucose homeostasis in the body. This leads to the development of T2D. It is important to decipher the mechanisms of balanced homeostasis and its disruption leading to oxidative stress and subsequent dysregulation of signaling in pancreatic β-cells. It is known that to maintain glycemic control and reduce oxidative stress and inflammation, restrained caloric intake and physical activity show the strongest beneficial effects. Chronic physical activity reduces ROS production, increases antioxidant potential, and improves insulin sensitivity. However, we must also define how to act in the prediabetic phase, when the first glucose imbalance occurs. To do this, we need to determine the right targets and timing for ROS production in pancreatic β-cells. NOXs in pancreatic β-cells in prediabetic conditions could be one of them for pharmacotherapy.

Conflict of Interest

There is no conflict of interest.

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