REVIEW

Reactive oxygen and nitrogen species generation, antioxidant defenses, and β-cell function: a critical role for amino acids

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Abstract

Growing evidence indicates that the regulation of intracellular reactive oxygen species (ROS) and reactive nitrogen species (RNS) levels is essential for maintaining normal β-cell glucose responsiveness. While long-term exposure to high glucose induces oxidative stress in β cells, conflicting results have been published regarding the impact of ROS on acute glucose exposure and their role in glucose stimulated insulin secretion (GSIS). Although β cells are considered to be particularly vulnerable to oxidative damage, as they express relatively low levels of some peroxide-metabolizing enzymes such as catalase and glutathione (GSH) peroxidase, other less known GSH-based antioxidant systems are expressed in β cells at higher levels. Herein, we discuss the key mechanisms of ROS/RNS production and their physiological function in pancreatic β cells. We also hypothesize that specific interactions between RNS and ROS may be the cause of the vulnerability of pancreatic β cells to oxidative damage. In addition, using a hypothetical metabolic model based on the data available in the literature, we emphasize the importance of amino acid availability for GSH synthesis and for the maintenance of β-cell function and viability during periods of metabolic disturbance before the clinical onset of diabetes.


Introduction

Reactive oxygen species (ROS) such as superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and the hydroxyl radical (OH$^-$) plus the related peroxynitrite (ONOO$^-$) molecule are generally thought to cause cell dysfunction and ultimately death due to alteration of i) metabolic pathway activity (Newsholme et al. 2007a, 2009b) and/or ii) the structure of cellular membranes, DNA, or proteins (Boveris et al. 1972, Turrens 1997, Johnson et al. 1999, Chandra et al. 2000, Limon-Pacheco & Gonsebatt 2009). However, the half-life and reactivity of these various species are very different, which is an indication of the different biological functions of these molecules (Droge 2002).

Formation of the O$_2^-$ can be considered to be the initial step for the subsequent formation of other ROS. It is generated by the single electron reduction of molecular oxygen (O$_2$). Superoxide, compared with other free radicals, is a poorly reactive species and can exist in solution for a considerable time (and thus diffuse) before reacting with other free radicals or with specific clusters of iron–sulfur in target proteins. Being a charged species, superoxide cannot freely cross biological membranes but may do so via anion channels. However, the fate of superoxide in cells and tissues is mostly determined by the activity of various site-specific enzymes (extracellular, cytoplasmic, and mitochondrial), the superoxide dismutase (SOD) family, that convert superoxide into molecular oxygen and hydrogen peroxide (Fig. 1).

H$_2$O$_2$ is an even less reactive species that is uncharged and can diffuse across membranes through aquaporins. Despite its low reactivity, some proteins contain specific cysteine residues that are prone to oxidation by hydrogen peroxide, which are critical to hydrogen peroxide-based signaling systems. H$_2$O$_2$ can be converted to the OH$^-$, a highly reactive species (see Fig. 1). Superoxide can also react with nitric oxide (NO), resulting in the formation of peroxynitrite (ONOO$^-$), see Fig. 1. Cell-associated oxidative damage may not be simply a question of the overall concentration of ROS, but rather the type of ROS formed with respect to relative reactivity.

Hydrogen peroxide is the substrate for the majority of the antioxidant systems in the cell. These antioxidant systems (many of which are enzymes) are required to minimize the
reaction controlling the conversion of $H_2O_2$ to $HO^\cdot$, a highly reactive molecule. Superoxide may also chemically combine with the reactive nitrogen species (RNS), NO in a diffusion-controlled reaction, resulting in the formation of peroxynitrite ($ONOO\cdot$). When cellular antioxidant defenses are overcome, ROS specificity is lost leading to reaction with non-readily oxidizable amino acid residues, as well as with unsaturated lipids and DNA, therefore leading to oxidative stress.

Pancreatic $\beta$ cells are considered to be particularly vulnerable to oxidative damage (Lenzen 2008b), as it has been reported that they express relatively low levels of catalase and glutathione (GSH) peroxidase, which would contribute to lipotoxicity, glucotoxicity, or a combination termed glucolipotoxicity in $\beta$ cells chronically exposed to nutrients, favoring apoptosis (Robertson et al. 1992, Newsholme et al. 2007a,b, Gehrmann et al. 2010). Interestingly, impairment, or otherwise, of $\beta$-cell function may depend on the antioxidant system involved in the scavenging process, as specific manipulation in antioxidant defenses may result in different outcomes (Lenzen 2008b).

Herein, we critically revise the major mechanisms of ROS/RNS production and implications for the physiological function of pancreatic $\beta$ cells. In addition, using a hypothetical metabolic model, based on the data available in the literature, we emphasize the importance of amino acid availability for GSH synthesis and for the maintenance of $\beta$-cell function and viability during periods of metabolic disturbance before the clinical onset of diabetes.

**The impact of ROS in pancreatic $\beta$ cells**

$\beta$ Cells adequately deal with the physiological challenges of substrate availability imposed both acutely and chronically depending on the nutritional and metabolic states (Schmitt et al. 2011). The availability and the type of antioxidant defenses in these cells are dictated by demand. There is now a consensus that the chronically high circulating levels of glucose (glucotoxic concentrations) and/or lipid (glucolipotoxic or lipotoxic concentrations) associated with type 2 diabetes induce oxidative stress in different cell types (Newsholme et al. 2007a, Gehrmann et al. 2010). In type 1 diabetes, induced by autoimmune $\beta$-cell destruction, death is associated with cytokine-mediated oxidative stress (Morgan et al. 2007, Lenzen 2008a).

Oxidative stress is currently viewed as an imbalance between pro- and antioxidants in favor of the former, which implicates a loss of redox signaling. It can be triggered by excessive ROS production as well as by low antioxidant enzyme activities. A well-known source of electrons for reduction of molecular oxygen is the mitochondrion.
The increase in superoxide formation in the electron transport chain is associated with a high (inner) mitochondrial membrane potential. This causes a decrease in the electron flow through the respiratory chain, increasing the probability of superoxide formation by the retained electrons at various sites in the mitochondrial respiratory chain. However, an additional specialized enzyme-based system can also generate superoxide in a regulated fashion. This enzyme complex, the NADPH oxidase (NOX), is a member of a family of enzyme isoforms that are able to transfer electrons from NADPH to molecular oxygen to generate O$_2^−$. NOX activation is more widely associated with efficient killing of pathogens by phagocytes, such as macrophages, monocytes, dendritic cells, and neutrophils, that form ROS from NOX2 within the phagosomal membrane (Bylund et al. 2010). NOX-derived ROS have been shown to stimulate mitogenic signaling and proliferation (Murrell et al. 1990, Arnold et al. 2001). It is now evident that NOX isoforms (NOX1, NOX2, and NOX4) are also expressed in pancreatic β cells (Oliveira et al. 2003, Uchizono et al. 2006), where their function is related to regulation of insulin secretion and cell integrity (as discussed below).

Interestingly, exposure to high levels of metabolic fuels in vivo is not necessarily associated with NOX2-derived oxidative stress and decreased function of pancreatic β cells. Islets isolated from Wistar rats fed a high-fat diet for 13 weeks resulted in increased pancreatic islet functionality, associated with high levels of glucose metabolism and GSIS but also low levels of NOX2 expression and ROS production (Valle et al. 2011). On the other hand, when Sprague Dawley rats (which are prone to develop insulin resistance and obesity (Reaven & Reaven 1981)) were submitted to a high-fat diet for a longer period (24 weeks), diabetes ensued that was associated with increased islet ROS and reduced insulin expression (Yuan et al. 2010). Moreover, human islets isolated from type 2 diabetic patients demonstrated increased mRNA levels of the p22 subunit of NAD(P)H oxidase (Marchetti et al. 1998).

Therefore, a reduction in islet NOX2 expression and thus ROS production, in vivo, may constitute an adaptive response of the pancreatic β cell to handle high levels of metabolic fuels. However, if the stimulus is maintained for a sufficiently long period (taking into account the different susceptibility of animal models, for instance Wistar vs Sprague Dawley rats), this adaptive response may fail to operate and the system would then operate in a positive feedback loop mode, enhancing NOX2 expression and oxidative stress, leading to impaired insulin secretion and overt type 2 diabetes.

Chronic glucose exposure also induces the expression and activity of inducible NO synthase (iNOS) in pancreatic β cells (Meidute-Abaraviciene et al. 2009). Moreover, other toxic stimuli as pro-inflammatory cytokines, saturated non-esterified fatty acids, or direct addition of ROS induce stress resulting in upregulation of iNOS in the pancreatic β cells, thus causing generation of high levels of NO that are associated with reduced insulin secretion and apoptotic cell death (Michalska et al. 2010).

Numerous papers have described the negative effects of NO generation in the β cell including attenuation of glucose-stimulated insulin secretion and stimulation of apoptosis, originating from the observation that pro-inflammatory cytokines induced gene and protein expression of iNOS and subsequent NO generation, which was associated with caspase activation and cell death (Rizzo & Piston 2003). It is possible that NOX2-derived ROS in the latter conditions may also trigger concomitant nitrosative stress (Michalska et al. 2010), thus suggesting that in toxic conditions, a key mediator for dysfunction and apoptosis would be peroxynitrite.

However, another example of a deleterious pathway that mediates substrate or cytokine toxicity is the formation of hydroxyl radicals via the Fenton reaction. In this situation, an abnormal increase in mitochondrial electron leakage or in NOX2 activity could overcome the antioxidant defenses allowing the reaction of H$_2$O$_2$ with transition metals (i.e. Fe$^{2+}$) producing HO$^\cdot$. In addition, another toxic pathway was recently described, which was associated with cytokine-dependent β-cell death, which was derived from an interaction between RNS and ROS. This pathway for cytokine-induced β-cell apoptosis involves an interaction of mitochondrion-derived hydrogen peroxide with NO, in which the presence of trace metals leads to hydroxyl radical formation resulting in β-cell death (Gurgul-Convey et al. 2011).

Likewise, the cytokine death pathway involving either ONOO$^−$ or HO$^\cdot$ formation may increase ROS production via increased NOX2 activity, as the treatment of β cells with a cytokine mixture (IL1β, TNFα, and INFγ) increased the expression of p47, the key cytosolic regulator protein of NOX2 (Michalska et al. 2010). Additionally, iNOS and NOX2 share a same transcription activator factor, NF-κB, which in turn is increased in concentration by cytokine exposure in β cells, thus increasing the possibility of nitro-oxidative stress.

Interestingly, the stress pathways driven by either cytokine or chronically increased fuel levels appear to share commonality in the formation of nitro-oxidative species. An increase in intracellular oxidative stress triggers an increase in the proteins involved in the formation of NOX2 and iNOS (Michalska et al. 2010), which could provoke a deleterious effect, either by ONOO$^−$ or by HO$^\cdot$ formation (Fig. 1).

**Physiological function of RNS generated in the pancreatic β cell**

Numerous papers have described the negative effects of NO generation in the β cell, including attenuation of glucose-stimulated insulin secretion and stimulation of apoptosis, originating from the observation that pro-inflammatory cytokines induced gene and protein expression of iNOS and subsequent NO generation, which was associated with caspase activation and cell death (Tejedo et al. 1999).
However, it is now clear that enhanced glucose metabolism in the β cell, in response to an increase in glucose availability, apart from involving activation of glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation to produce ATP, also triggers additional signaling mechanisms involving the modulation of nitrogen species at intracellular levels (Rizzo & Piston 2003, Leloup et al. 2009, Gray & Heart 2010). Posttranslational modification by S-nitrosylation requires the modification of the sulphydryl side chain of specific cysteine residues within a protein by NO. This modification is dynamic, like that of phosphorylation, although it proceeds in the absence of a protein catalyst and is responsive to nutrient availability. A family of NOS enzymes, for example, nNOS, eNOS, and iNOS, can synthesize NO endogenously from l-arginine, oxygen, and NADPH. iNOS is increased in concentration in response to fatty acids, pro-inflammatory cytokines, and l-arginine (Michalska et al. 2010, Krause et al. 2011).

In human islets and MIN6 pancreatic β cells, S-nitrosylation of syntaxin 4 was reported to occur in response to an acute (5 min) glucose stimulation. S-nitrosylation mapped specifically to Cys141 and facilitated activation of syntaxin 4 as determined by association with its cognate v-SNARE VAMP2 (Wiseman et al. 2011). Stimulation of MIN6 β cells with inflammatory cytokines TNFα, IL1β, and IFNy for 2 h resulted in an induction of syntaxin 4, S-nitrosylation, and stimulation of insulin secretion in the absence of glucose (Wiseman et al. 2011). Thus, this site for S-nitrosylation, which is important for physiological processes, may also be involved in dysregulated NO-mediated processes that could contribute to the onset of secretory dysregulation in pancreatic β cells subjected to inflammatory stress and/or chronic stimulation.

AMP-activated protein kinase (AMPK) has been reported to be a target for NO action in the β cell (Meares et al. 2010). AMPK is critical to β-cell functional integrity as on activation AMPK can phosphorylate many downstream effectors so as to reduce ATP-consuming processes and promote ATP-producing processes (Meares et al. 2010). LKB1 can phosphorylate AMPK on threonine 172 and, together with increased AMP, activates AMPK (Hawley et al. 2005, Hardie 2007). LKB1 is thought to be the predominant kinase responsible for the activating phosphorylation of AMPK; however, calmodulin-dependent protein kinase kinase β (CaMKKβ) can act as an alternative activator of AMPK, independent of cellular energy status but responsive to changes in intracellular Ca2+ (Hawley et al. 2005).

AMPK was recently reported to play a critical role in the functional recovery of β cells from NO-induced damage. Indeed, pro-inflammatory cytokines activated AMPK in a NO-dependent fashion and AMPK functioned to attenuate death and promote the functional recovery of β cells from NO-mediated stress (Meares et al. 2010). Thus, it is possible to consider three interdependent mechanisms responsible for AMPK activation and promotion of β-cell functional integrity that includes insulin secretion (Sun et al. 2010), AMP, Ca2+, and NO.

Recent studies involving engineered liver cells demonstrated that expression of either human insulin or the β-cell-specific transcription factors PDX1, NeuroD1, and MafA in the Hepa1–6 cell line or primary liver cells via adenoviral gene transfer resulted in production and secretion of insulin. However, insulin secretion was not significantly increased in response to high glucose. Interestingly, l-arginine stimulated insulin secretion up to threefold, an effect dependent on the production of NO (Muniappan & Ozcan 2007).

Interactions of NO with K+ ATP channel activity are concentration dependent and complex. NO released from donors in the micromole range have been reported to produce membrane hyperpolarization and channel opening due to attenuation of glucose metabolism in β cells (Drews et al. 2010). NO diminished mitochondrial ATP production by depolarizing ΔΨ (Drews et al. 2010). NO may interact directly with K+ ATP channels or l-type Ca2+ channels (Drews et al. 2010) presumably via SH–groups in the channel protein. NO suppressed K+ ATP channel activity in β cells via a cGMP/PKG-dependent pathway but at higher concentrations increased the K+ ATP current by inhibition of ATP production (Drews et al. 2000). Genetic or pharmacological ablation of K+ ATP channels protected β cells against an oxidant insult (Drews et al. 2010). The NO donor sodium nitroprusside (SNP) attenuated glucose-induced but not glyceraldehyde-induced K+ ATP channel inhibition (Drews et al. 2010), suggesting that NO may target a glycolytic step upstream to glyceraldehyde-3-phosphate, hence reducing critical glycolytic NADH production. Indeed, it has been reported that SNP inhibits glyceraldehyde-3-phosphate dehydrogenase in insulin-secreting RINm5F cells (Drews et al. 2010).

Antioxidants in pancreatic β cells and the role of glucose

Although β cells express low levels of certain antioxidant defenses (i.e. SOD, catalase, and GSH peroxidase (Ivarsson et al. 2005, Lenzen 2008b)), it has been previously shown that they are particularly rich in other peroxidase-based antioxidant defenses, such as glutaredoxin and thioredoxin (Ivarsson et al. 2005). Interestingly, modulation of β-cell function may depend on the antioxidant system involved in the scavenging process, as specific manipulation in antioxidant defenses promoted differential results, e.g. modulation of either glutaredoxin or thioredoxin levels altered exocytosis in the presence of NADPH in a reciprocal fashion (Ivarsson et al. 2005) while mitochondrial SOD overexpression increased cytokine-induced apoptosis in β cells (Lortz et al. 2005).

While long-term exposure to high glucose induces oxidative stress in β cells, conflicting results have been published regarding the levels of ROS on acute glucose exposure and their role in GSIS. Despite evidence from mouse cells that ROS enhanced insulin secretion at low
glucose (Pi et al. 2007, Leloup et al. 2009), both previous and recent results in rat islets indicate that glucose acutely decreases ROS content in islets, an effect that may involve reduction in mitochondrial ROS production (Martens et al. 2005) and/or increased antioxidant output from the pentose–phosphate pathway (Rebelato et al. 2011). Indeed, the evidence from rat islets indicates that antioxidant production acutely surpasses ROS production on exposure to glucose load, (Martens et al. 2005) and that the control of ROS levels is important for insulin secretion (Zhang et al. 2010).

Another class of metabolic substrate, fatty acids, acutely enhanced ROS levels in islets, at least in part through activation of NOX (Morgan et al. 2007, Graciano et al. 2011, Santos et al. 2011). However, the mechanisms underlying this activation and their functional role seem to differ depending on the type of fatty acid analyzed. The palmitic acid activates NOX at high glucose level in a manner independent on fatty acid oxidation (Graciano et al. 2011), whereas oleic acid–induced NOX activation (Santos et al. 2011).

We wish to present a hypothetical model of the pro-oxidant and antioxidant behavior in β cells required for the maintenance of cell function and viability (Fig. 2). Events leading to ROS/RNS production are detailed in the lower part of Fig. 2 (increasing levels will result in clockwise direction change). Antioxidant (mainly GSH) production in response to oxidant challenge is indicated in the upper part of Fig. 2 (increasing levels will result in clockwise direction change). The arrows can change in direction independently. β Cells must maintain their function (insulin secretion) under normal physiological conditions, which is accomplished by the fact that the concentration/activity of antioxidants anticipates and adjusts to ROS/RNS production at a level that is able to protect the cells from oxidative damage but maintain redox signaling (Fig. 2).

If the pancreatic β cell is exposed to prolonged metabolic overload (i.e. hyperglycemia/hyperlipidemia), ROS and RNS production may cause oxidative damage and cell dysfunction. Under this condition, antioxidant production may increase to a higher level in order to maintain cell viability, shifting β-cell metabolism away from a key role in energy generation and stimulus–secretion coupling and toward a catabolic state, which may be related to cell defense (Fig. 2). As long as the pancreatic β cells receive all relevant macro- and micro-nutrients (i.e. amino acids, vitamins, and minerals) for the synthesis of antioxidants (especially GSH), they will be able to suppress the harmful effects of increased ROS/RNS generation.

**Proposed model for pancreatic β-cell dysfunction on the time course of diabetes**

During the progression of type 2 diabetes, deterioration of insulin secretion due to pancreatic β-cell dysfunction results in progressive hyperglycemia. This can result in glucose toxicity in various cell types and irreversible β-cell dysfunction driven, at least in part, by chronic oxidative stress caused by continuous hyperglycemia (Numazawa et al. 2008). ROS increases levels of protein oxidation, DNA oxidation, and lipid peroxidation. Consequently, oxidative stress originating from hyperglycemia would be a major cause of impaired islet function at the level of insulin synthesis and secretion.

In pancreatic β cells, GSH seems to be the most important tool to protect the cells against oxidative damage (Newsholme et al. 2009a, Krause et al. 2011). GSH is the major low-molecular weight thiol in mammalian cells. The cellular GSH redox buffer is present in cells at millimolar concentrations and maintains redox homeostasis critical to appropriate protein conformation (Schafer & Buettner 2001). Recent studies have demonstrated that an acute reduction in ROS concentration induced by glucose was correlated with increased pentose phosphate pathway activity (Lenzen 2008b) and increased insulin secretion. As increased pentose
phosphate pathway activity will generate NADPH, we would like to suggest that GSH levels could have been elevated under such conditions leading to an acute reduction in ROS concentration but increased insulin secretion. Synthesis of GSH involves the use of three key amino acids: glutamate, glycine, and cysteine. Changes in the availability of these amino acids may lead to decreased levels of GSH and ultimately cell death. The rate-limiting step in de novo synthesis of GSH is a reaction catalyzed by cystolic \( \gamma \)-glutamylcysteine synthetase (\( \gamma \)-GCS). On the other hand, \( L \)-cysteine availability is also an important factor to maintain GSH levels (Numazawa et al. 2008). In \( \beta \) cells, the transport of cysteine/cystine is mediated by a \( L \)-cystine/\( L \)-glutamate exchanger (system Xc\(^{-} \); Numazawa et al. 2008). Physiological flux via system Xc\(^{-} \) involves the entry of \( L \)-cystine and exit of \( L \)-glutamate. \( L \)-cystine imported by system Xc\(^{-} \) is reduced to form \( \alpha \)-cysteine and is used as a substrate for GSH synthesis. If the activity of the \( L \)-cystine/\( L \)-glutamate exchanger is restricted to reduce the levels of intracellular GSH, \( \beta \) cells become susceptible to oxidative stress (Numazawa et al. 2008). Importantly, excess extracellular glutamate reverses the activity of the glutamate/cystine antiporter system Xc\(^{-} \), thus depleting the cells of cysteine (Murphy et al. 1989). Thus, extracellular concentrations of glutamate can determine the availability of cysteine for the intracellular synthesis of GSH.

\( L \)-Glutamate can be released by pancreatic \( \alpha \) and \( \beta \) cells in pathophysiological conditions such as pro-inflammatory challenge (Kiely et al. 2007), a condition likely to occur in diabetes. Therefore, we propose that under low-grade inflammation and metabolic overload, both \( \alpha \) and \( \beta \) cells will release glutamate to the extracellular islet environment, causing accumulation of this amino acid and the inhibition of cysteine uptake leading to the failure of GSH production, resulting in oxidative stress and finally cell death (Fig. 3A). Under these conditions, \( L \)-arginine and \( L \)-glutamine availability are important for GSH synthesis as they participate in intracellular glutamate production (Kiely et al. 2007, Krause et al. 2011).

A physiological model is illustrated in Fig. 3B. Hyperglycemia and dyslipidemia are features of diabetes and pre-diabetes. They can induce oxidative stress in many tissues and, if associated with obesity, can lead to the production of pro-inflammatory cytokines that will culminate in a chronic pro-inflammatory state and further oxidative damage (Newsholme et al. 2009a). Under this condition, the liver will increase GSH production (Lord & Bralley 2008). Increased synthesis of cysteine is a key regulatory mechanism for GSH synthesis, and the oxidative stress state can induce the activation of cysteine formation from methionine and serine (Lord & Bralley 2008). Cystathionase catalyses the conversion of cystathionine into cysteine and also forms a by-product called \( \alpha \)-ketobutyrate (\( \alpha \)-KB) that can be converted into \( \alpha \)-hydroxybutyrate (\( \alpha \)-HB) in a reaction catalyzed by lactate dehydrogenase (LDH) and/or by hydroxybutyrate dehydrogenase (\( \alpha \)-HBDH). Indeed, when \( \alpha \)-HB levels are elevated, this condition can predict diabetes development (Gall et al. 2010). LDH and \( \alpha \)-HBDH catalyze the synthesis of \( \alpha \)-HB when the levels of NADH are high, a condition found in diabetic and obese patients as they have higher fatty acid oxidation rates that result in NADH production.

Glutamate, another key amino acid for GSH synthesis, can be synthesized from glutamine (Newsholme 2001). The likely source of glutamine is the skeletal muscle, which is known to increase the release of this amino acid in starvation and pathophysiological conditions such as insulin resistance (conditions that also result in branched chain amino acid release; Wang et al. 2011). The continuous uptake of \( L \)-glutamate by the liver and by immune cells makes a significant quantitative contribution to a reduction in plasma \( L \)-glutamine (Krause Mda & de Bittencourt 2008). Indeed, plasma glutamine levels are known to be significantly reduced in type 2 diabetes mellitus (T2DM) (Menge et al. 2010).

Glycine, the third key amino acid for GSH synthesis, is found decreased in the plasma of insulin-resistant \( ob/ob \) mice (Altmaier et al. 2008). The possible reasons for the decreased levels of glycine are increased glycine uptake by the liver and also that elevated glucagon levels can lead to increased oxidation of this amino acid (Gall et al. 2010). Serine, an important amino acid for glycine and cysteine synthesis, is known to be reduced in T2DM, confirming the importance of these amino acids for GSH synthesis under oxidative stress conditions (Gall et al. 2010).

Islet structure and thus its function display considerable diversity between vertebrate species. Moreover, islet structure is not static but undergoes changes in response to normal physiological and pathophysiological conditions, including diabetes. In general, human islets present with fewer \( \beta \) cells and more \( \alpha \) cells than rodent islets (for further information see Steiner et al. (2010)). Another important difference between human and rodent islets is the fact that human \( \beta \) cells do not express the key urea cycle enzyme ornithine transcarbamylase; thus, the ornithine produced from arginine activity may be converted to glutamate and GSH or used for polyamine synthesis, with profound implications for \( \beta \)-cell functional integrity (Ohtani et al. 2009).

The pro-inflammatory state associated with T2DM may lead to activation of immune cells, such as macrophages. These cells, in the presence of pro-inflammatory cytokines, can secrete other cytokines, such as IL1B (which causes dysfunctional effects in \( \beta \) cells), but they can also secrete the enzyme arginase (Krause et al. 2011). Increased levels of arginase and the subsequent degradation of extracellular \( \alpha \)-arginine to urea and ornithine may explain the low levels of this amino acid in T2DM (Pieper & Dondlinger 1997, Krause et al. 2011).

The repercussions of reduced availability of amino acids for pancreatic \( \beta \) cells are many: pro-inflammatory cytokines (from the plasma or from infiltrate macrophages) can stimulate glutamate release through specific glutamatergic transporters (and many other effects) that will culminate in the reversal or inhibition of cysteine transporters leading to lower intracellular cystine/cysteine levels. Secretion of arginase from
Figure 3 Hypothetical models for the failure of β-cell antioxidant production with respect to the time course of diabetes. (A) In β cells and pancreatic islets of Langerhans: failure in GSH synthesis can result in changes at redox state, loss of β-cell function, and cell death. GSH synthesis involves three key amino acids: glutamate, glycine, and cysteine. Changes in the availability of these amino acids may lead to decreased levels of GSH and cell death. L-glutamine and L-arginine are also important for intracellular glutamate synthesis. Extracellular glutamate plays an essential role on the β-cell function/survival. This amino acid is an inhibitor of cysteine transport. Increased levels of glutamate (from β and/or α cells) can be induced by pro-inflammatory cytokines and metabolic overload, leading to a blockade of cysteine uptake. When the level of cysteine/cysteine falls, glutathione levels decrease, leading to oxidative stress and cell death. (B) In vivo model: hyperglycemia and dyslipidemia are features of diabetes and pre-diabetes. They can induce oxidative stress in many tissues and, if associated with obesity (adipose tissue expansion), can lead to the production of pro-inflammatory cytokines that will culminate in a pro-inflammatory state (low-grade inflammation) and further oxidative damage. Under this condition, the liver is requested to increase glutathione production. Increased synthesis of cysteine is a key regulatory mechanism for GSH synthesis, and the oxidative stress state can induce the activation of cysteine formation from methionine and serine. Cystathionase (1) catalyses the conversion of cystathionine into cysteine and also forms a by-product called α-KB that can be converted into α-HB in a reaction catalyzed by LDH and/or α-HBDH. Indeed, α-HB levels are higher and also predict diabetes development (see text for reference) and prove that this pathway is very active in diabetes and pre-diabetes. Glutamate, another key amino acid for GSH synthesis, can be synthesized from glutamine. The likely source of glutamine is the skeletal muscle, which is known to increase the release of this amino acid especially in a state of insulin resistance (a condition that is also known to induce BCAA release). Continuous uptake of L-glutamine by the liver (and by immunological cells) can lead to a fall in the amino acid plasma concentration (as indicated by the arrow). Glycine, the third key amino acid for GSH synthesis, is found decreased in the plasma of diabetic subjects. The reasons for this are as follows: increased glycine uptake by the liver and elevated glucagon levels can lead to increased oxidation of this amino acid. Serine, an important amino acid for glycine and cysteine synthesis, is decreased in type 2 diabetes. The pro-inflammatory state may lead to activation of immunological cells, such as macrophages. These cells, in the presence of pro-inflammatory cytokines, can secrete inflammatory factors, such as IL1β (with several effects in β cells), but they can also secrete the enzyme arginase. Increased levels of arginase and the degradation of l-arginine to urea and ornithine may explain the lower levels of this amino acid in type 2 diabetic patients. Repercussion for the pancreatic β cells: pro-inflammatory cytokines (from the plasma or from infiltrate macrophages) can stimulate glutamate secretion (and many other effects) that will culminate in the inhibition of the cysteine leading to lower intracellular glutamate exchanger/cysteine levels. Secretion of arginase from infiltrated macrophages depletes l-arginine in the islet microenvironment leading to the lack of this amino acid. Together, lower arginine, glutamine, cysteine, and glycine lead to the decreased intracellular availability of these amino acids, essential for glutathione synthesis. A lower antioxidant defense culminates into oxidative stress, cell dysfunction, and β-cell death. *The key amino acids for GSH synthesis.
islet-infiltrated macrophages would deplete \( \gamma \)-arginine in the islet microenvironment leading to the lack of this amino acid. Together, lower arginine, glutamine, cysteine, and glycine lead to the decreased availability of amino acids for GSH synthesis. The resulting compromised antioxidant defenses culminate in oxidative stress and cellular dysfunction finally result in \( \beta \)-cell death.

**Conclusions and perspectives**

We conclude that pancreatic \( \beta \)-cell function and viability depends on the balance between ROS/RNS and GSH production in order to maintain normal cell function and insulin secretion but without causing oxidative damage. This balance must be maintained even in conditions of metabolic overload, when GSH synthesis plays a crucial role for the protection of the \( \beta \) cell. Under normal physiological conditions if the \( \beta \) cells receive all the required amino acids for GSH synthesis, this balance is maintained. However, during the development of T2DM when the availability of amino acids (such as cysteine, arginine, glutamine, and glycine) is reduced, GSH synthesis decreases, resulting in reduced \( \beta \)-cell protection and loss of integrity. We here propose that a key cause of \( \beta \)-cell failure, during the development of diabetes, is the reduced levels of critical amino acids required for GSH formation. Supplementation with these key amino acids may be a novel approach to support the maintenance and function of \( \beta \) cells in the face of nutrient overload, thus allowing adequate glycemic control to prevail.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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**Author contribution statement**

P N provided the initial ideas and stimulus for writing this review, which was conceived during a period of academic leave at the University Sao Paulo, Brazil. P N, E R, F A, and M K contributed ideas and input for writing and revision of the text of the review while M K additionally provided all Figures. A Carpinelli and R. Curi provided critical comment on the content of the review.

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