Free Radicals and Islet Function

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Abstract

Pancreatic β-cells secrete insulin in response to changes in extracellular glucose concentration. However, persistent hyperglycemia during diabetes creates a redox imbalance resulting from overproduction of reactive oxygen species (ROS). Although a cell's intracellular defense mechanism tries to cope with increased ROS levels, excess ROS impairs the integrity and physiological function of biomolecules and therefore affects viability and functionality of cells. This overproduction of ROS along with compromised antioxidant defenses leads to oxidative stress (OS). An organ's intrinsic defense mechanism protects against this excess generation of ROS and therefore its susceptibility to (OS) is determined by its defense mechanism and ability to repair DNA damage caused by ROS. Islets have poor defense mechanisms and also are not capable of efficiently

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I. Laher (ed.), *Systems Biology of Free Radicals and Antioxidants*,
DOI 10.1007/978-3-642-30018-9_154,

repairing oxidatively damaged DNA compared to other tissues. This explains the extraordinary sensitivity of islets to OS. In an attempt to strengthen their defense mechanisms, several strategies including oral administration of antioxidants and over-expression of genes responsible for antioxidant enzymes have proven to be successful. However, the reason for this low sensitivity of islets in comparison to other organs remains unresolved.

Keywords

Antioxidant defense • Islets • Oxidative stress • Reactive oxygen species

Abbreviations

8-OHdG	8-Hydroxy-2-deoxyguanosine
AOEs	Antioxidant enzymes
CAT	Catalase
ETC	Electron transport chain
GCL	Glutamate-cysteine ligase
GPx	Glutathione peroxidase
Grx	Glutaredoxin
GSH	Glutathione
GSIS	Glucose-stimulated insulin secretion
H_2O_2	Hydrogen peroxide
HNE	4-Hydroxy2-nonenal
JNK	c-jun NH2-terminal kinase
MCP-1	Monocyte chemoattractant protein-1
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
NK-κB	Nuclear factor-κB
NO	Nitric oxide
NOS	Nitric oxide synthase
$O_2^{\bullet -}$	Superoxide radical
$OH^{\bullet -}$	Hydroxyl radical
OS	Oxidative stress
p38MAPK	p38 mitogen-activated kinase
PDX1	Pancreatic and duodenal homeobox 1
Prx	Peroxiredoxin
PTP	Protein-tyrosine phosphatase
RNS	Reactive nitrogen species
$ROO^{\bullet}$	Peroxyl radical
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TCA	Tricarboxylic acid cycle
Trx	Thioredoxin
UCP	Uncoupling protein
ZDF rats	Zucker diabetic fatty rats
ZLF rats	Zucker lean fatty rats

Islets

The islets of Langerhans, named after the German physician Paul Langerhans who first discovered them in 1869, are irregularly shaped patches of endocrine tissue scattered within the pancreas of most vertebrates and constitute approximately 2–3 % of the mass of pancreas. Islets contain four major types of cells: α-, β-, δ-, and PP-cells (Fig. 147.1), secreting glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively, in the ratio of 68:20:10:2 % in adult islets (Rahier 1988). The cytoarchitecture of islets differs between species. Rodent islets are characterized by predominantly insulin-producing β-cells in the core of the cluster and scarce α-, δ-, and PP-cells in the periphery, whereas human islets display α- and β-cells in close relationship with each other throughout the cluster (Brissova et al. 2005; Ichii et al. 2005). The most common islet cell, the β-cell, produces insulin, the major hormone in the regulation of carbohydrate, fat, and protein metabolism. Insulin is crucial in several metabolic processes. In insulin responsive cells insulin promotes: (1) the uptake and metabolism of glucose; (2) prevents release of glucose by the liver; (3) causes muscle cells to take up glucose and amino acids, the basic components of protein; and (4) inhibits the breakdown and release of fats. Although the release of insulin from β-cells can be triggered by growth hormones or glucagon, with glucose being the most important stimulator of insulin release. When the blood glucose level increases – as it does after a meal – insulin is released and promotes glucose utilization, while glucagon released from the α-cell triggers glucose secretion when blood glucose levels are low. Thus, glucose metabolism represents a key process linking glucose sensing and the modulation of insulin

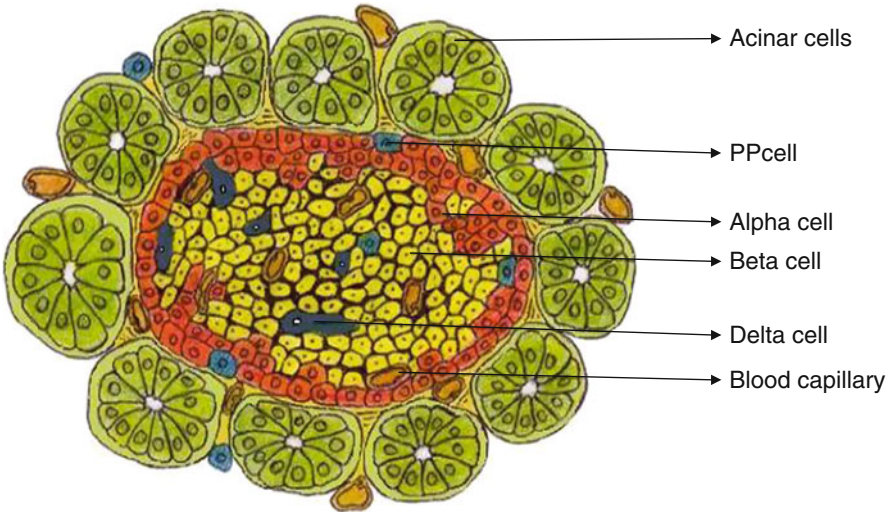

Fig. 147.1 Distribution of different cells in pancreatic islets where insulin secreting β-cells are shown in yellow, glucagon secreting αlpha cells are shown in red and somatostatin secreting δ-cells are shown in blue

release from the β-cell. The α-cells of the islets of Langerhans produce an opposing hormone, glucagon, which releases glucose from the liver and fatty acids from fat tissue. In turn, glucose and free fatty acids favor insulin release and inhibit glucagon release. The δ-cells produce somatostatin, a strong inhibitor of somatotropin, insulin, and glucagon; its role in metabolic regulation is not yet clear. Somatostatin secretion is stimulated by glucose and amino acids, whereas pancreatic polypeptide release is stimulated by hypoglycemia as well as by secretin. This interplay of regulators of hormone secretion from the pancreatic islets provides a means by which these hormones regulate overall body metabolism (Robertson and Harmon 2007).

Diabetes

Diabetes, a major health issue around the world, is a chronic disease which results from the impaired metabolism of glucose, leading to a high concentration of glucose in the circulation. Two main types of diabetes are type 1 and type 2 diabetes. Type 1 diabetes (T1D) is an autoimmune disorder where immune-mediated recognition of islet β-cells by auto-reactive T cells leads to the release of proinflammatory cytokines, resulting in destruction of pancreatic β-cells in the islets and resulting in complete loss of insulin secretion. Type 2 diabetes (T2D) results from insulin resistance and at later stages is characterized by defective insulin secretion by pancreatic islets (islet dysfunction) leading to a state of relative insulin deficiency. Since in either type of diabetes the underlying cause remains islet dysfunction, understanding the mechanisms by which islets secrete insulin and thereby meet the constantly changing demands of glucose metabolism during diabetes makes it critical to have a better understanding of islets functioning during glucose metabolism.

It is for these reasons that islets have been viewed as a possible in vitro system for studying syndromes that cannot be mimicked effectively using cell lines. Islets can be cultured as miniature organ systems wherein they retain their architecture, differentiated state and ability to secrete of insulin upon glucose stimulation, independent of nervous control. The ability to maintain islets in vitro has produced several studies on the pathophysiology of type 1 and 2 diabetes, islet transplantation, screening of hypoglycemic drugs, probing into causes of and mechanisms involved in diabetes, and prevention strategies (Bhonde et al. 2007).

Free Radicals and Diabetes

A free radical is any atom (e.g., oxygen, nitrogen) with at least one unpaired electron in the outermost shell and that is capable of independent existence. The presence of an unpaired electron in the outermost shell of an atom makes it highly reactive and therefore it reacts with biomolecules to stabilize itself and in the process causes cell death. Various types of free radicals are generated within the

body by enzymatic sources such as xanthine oxidase, nitric oxide synthase (NOS), and NAD(P)H oxidase (NOX), of which oxygen-derived free radicals (ROS) and reactive nitrogen species (RNS) are prevalent. ROS such as the superoxide anion ($O_2^{\bullet -}$), the hydroxyl radical ($OH^{\bullet -}$), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2) are produced as a normal product of cellular metabolism. For example, xanthine oxidase catalyzes the oxidation of hypoxanthine and xanthine to form $O_2^{\bullet -}$ (Lacy et al. 1998), while, NAD(P)H oxidase, a multi-subunit enzyme, catalyzes $O_2^{\bullet -}$ production by the 1-electron reduction of O_2 using NADPH or NADH. The nitric oxide (NO) radical is important for vasodilation in endothelial cells and is synthesized by the action of three isoforms of NOS (inducible NOS, neuronal NOS, and endothelial NOS) during the oxidative metabolism of amino acid L-arginine to L-citrulline in the presence of different cofactors. Nitric oxide at physiological concentrations has emerged as an important cell signaling molecule, in which it is pivotal to a number of physiologic functions, ranging from control of vascular tone, platelet aggregation, leukocyte adhesion, synaptic transmission to immune cell-mediated cytotoxicity, etc., as reviewed by Moncada et al. (1991). While the generation of nitric oxide ($NO^{\bullet}$) depends solely on the oxidation of L-arginine, NOS also produces superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) when the concentration of the substrate (L-arginine) is low. When excess $NO^{\cdot}$ and O_2 are produced simultaneously, they react with each other at a diffusion-controlled rate to form the highly reactive nitrogen species peroxynitrite ($ONOO^-$). Formation of these primary RNS can result in the cellular formation of secondary radicals such as peroxyl or alkoxyl radicals.

Under physiological conditions, glucose metabolism by the tricarboxylic acid (TCA) cycle generates electron donors which transfer electrons across the four complexes (complex I, II, III, and IV) of the electron transport chain (ETC) and finally to oxygen, which is reduced to water. This creates a voltage across the mitochondrial membrane and energy from this voltage gradient drives the synthesis of ATP by ATP synthase. This also leads to the generation of a basal level of $O_2^{\bullet -}$ within the mitochondria which is further dismutated by mitochondrial superoxide dismutase to H_2O_2. However, the rate of formation of $O_2^{\bullet -}$ in a cell fluctuates depending upon the amount of electrons flowing through the mitochondria at any given time. In diabetes, metabolism of excess glucose inside the cells via TCA cycle pushes more electron donors (NADH and $FADH_2$) into the ETC thereby overwhelming complex III of ETC where the transfer of electrons is blocked. Excess electrons, thus generated, causes coenzyme Q to donate electrons one at a time to molecular oxygen generating superoxide (Fig. 147.2) (Brownlee 2005). This superoxide in turn reacts with nitric oxide to form a very reactive peroxynitrite (Halliwell and Gutteridge 1999). Thus, generation of various ROS during diabetes stems from the excess generation of superoxide. Additionally, Houstis et al. (2006) demonstrated that ROS have a causal role in the establishment of insulin resistance by using two cellular models of insulin resistance, one induced by the proinflammatory cytokine tumor necrosis factor-α and the other by the anti-inflammatory glucocorticoid dexamethasone. In both these models increased levels of ROS mediate insulin resistance and reducing ROS activity ameliorated insulin resistance. Thus, ROS

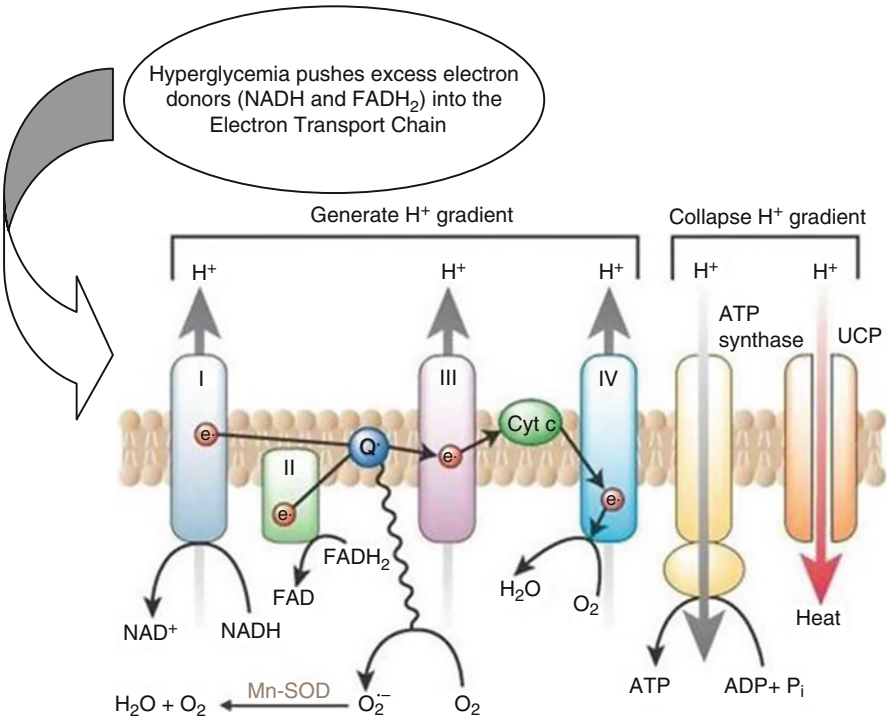

Fig. 147.2 Excess superoxide production. Excess electron donors generated during glucose metabolism by TCA cycle enter in electron transport chain and produce free radicals (Adapted from Brownlee 2005 and modified)

may have a causal role in establishment of insulin resistance, a cardinal feature of type 2 diabetes.

Glucose metabolism within islet β-cell is essential for coupling glucose sensing to insulin release and starts only after glucose uptake by GLUT2 receptors present on islets. The rise in blood glucose increases β-cell glucose metabolism, resulting in increased production of ATP from glycolysis, mitochondrial glucose oxidation, and active shuttling of reducing equivalents from the cytosol to the mitochondrial electron transport chain. The resultant increase in ATP/ADP ratio inhibits ATP-sensitive K^+ (K_{ATP}) channels, resulting in plasma membrane depolarization, activation of voltage-gated Ca^{2+} channels, and influx of extracellular Ca^{2+} which serves to activate insulin granule exocytosis. This in turn stimulates insulin secretion by β-cells in response to glucose and is also referred to as glucose-stimulated insulin signaling (GSIS). Uncoupling protein 2 (UCP2), a natural uncoupler of respiration and oxidative phosphorylation, is expressed in α- and β-cells (Diao et al. 2008; Gimeno et al. 1997) and is a negative regulator of insulin release. Increased activity of UCP2 reduces glucose-stimulated insulin secretion ultimately leading to β-cell dysfunction (Zhang et al. 2001). In support of this view, Chan et al. (2001) and

Hong et al. (2001) demonstrated that adenovirally mediated over-expression of UCP2 decreased GSIS in pancreatic islets and insulinoma cell lines, respectively. Superoxide produced as a result of excess glucose metabolism activates UCP2-mediated proton leak, thus lowering ATP levels and impairing GSIS. Mn(III) tetrakis(4-benzoic acid)porphyrin, a cell permeable SOD mimetic attenuates the endogenous production of superoxide radicals by catalyzing the conversion of superoxide to H_2O_2, blunts the UCP2-mediated proton leak in β-cells and improves GSIS (Krauss et al. 2003). UCP2 is therefore considered as a key link between oxidative potential, ATP synthesis, and insulin release from islet cells. Under physiological conditions, β-cells are constantly exposed to a fluctuating mix of glucose and fatty acids. Under gluco-lipotoxic conditions, UCP2 expression in β-cell cells are upregulated and cause increased uncoupling of oxidative phosphorylation and reduce cellular ATP (Fink et al. 2002). This results impairs K_{ATP} channel closure and decreases insulin secretion (Lee et al. 2004). Raised levels of FFA, such as with a high fat intake or during fasting, UCP2 expression is increased leading to reduced ATP production. The lower ATP/ADP ratio results in diminished insulin secretion (Fisler and Warden 2006). Thus, activation of UCP2 during excess glucose and fatty acids has two main purposes: to attenuate further superoxide production and act as a physiological signal to reduce insulin secretion (Brand and Esteves 2005).

Superoxide generated in excess reacts with H_2O_2 to form hydroxyl radicals via the Harber-Weiss reaction. Hydroxyl radicals, though extremely short-lived, are the most damaging radicals within the body. This state of excess free radicals generation during diabetes has deleterious effects deleterious effect on cells. In 2003, Bindokas et al. clearly demonstrated the production of superoxide in response to glucose by quantitating the rate of mitochondrial superoxide production in islets isolated from Zucker lean fatty (ZLF) and Zucker diabetic fatty (ZDF) rats. Stimulation with glucose led to a large increase in superoxide production in the islets of ZLF rats as compared to islets of ZDF rats, which exhibit an elevated basal rate of superoxide and was also associated with perturbed mitochondrial morphology contributing to abnormal insulin signaling. Several in vitro studies link glucose-induced β-cell dysfunction to oxidative stress, and also demonstrate the role of superoxide in glucose-induced β-cell dysfunction in vivo (Bindokas et al. 2003; Brownlee 2001; Maritim et al. 2003; Tang et al. 2007). Modak et al. (2011) show that excess superoxide generated as a result of persistent hyperglycemic conditions damages cellular biomolecules in islets and this is further increased in animals with uncontrolled diabetes; this damage was reduced and antioxidant defense status was restored in islets of insulin treated diabetic animals. These findings suggest that development and progression of diabetes is associated with defective mechanisms regulating ROS content within islet cells.

Though ROS generation due to excess metabolic substrates such as glucose and FFAs are implicated in development of T2D, additional factors contributing to excess free radical generation in T1D includes several inflammatory cytokines such as interleukin (IL)-1β, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α which are produced by auto-aggressive T lymphocytes and are therefore responsible for

inducing β-cell apoptosis. TNF-α reduces the level of glutathione, an important antioxidant in the cell (Reid and Li 2001). These inflammatory cytokines induce mitochondrial dysfunction (Todaro et al. 2003) which causes an increased leakage of superoxide from the electron transport chain resulting in oxygen radical accumulation in islet cells (Lakey et al. 2001). TNF-α also induces the expression of iNOS leading to the generation of ROS that in turn leads to translocation of nuclear factor-κB (NF-κB). NF-κB is a transcription factor that regulates NADPH oxidase, another major source of superoxide generation (Darville and Eizirik 1998). IL-1β, alone or in combination with interferon (IFN)-γ and TNF-α, induced the expression of iNOS in primary rodent β-cells and insulin-producing cell lines. On the other hand, blocking of both iNOS activity and iNOS gene expression by gene knockout prevented the deleterious effects of cytokines in β-cells (Eizirik and Pavlovic 1997; Liu et al. 2000). Additional evidence by Azevedo-Martins et al. (2003) demonstrates that improving mitochondrial antioxidant defense by over-expressing Mn-SOD prevented cytokine-mediated activation of NF- κB and iNOS in rat insulinoma cells.

There is much evidence to support a role for ROS generation in causing deleterious effects on the functionality of islet cells; in keeping with this, the relatively stable ROS H_2O_2 at lower concentrations is proposed to play a role as a second messenger for activating several signaling networks.

Free Radicals: Good or Bad?

ROS generated from any source has both beneficial and harmful effects for a cell. H_2O_2 is an important second messenger in GSIS (Pi et al. 2007; Sakai et al. 2003; Armann et al. 2007; Leloup et al. 2009; Morgan et al. 2009). A role for H_2O_2 in Ca^{2+} influx was demonstrated in studies where H_2O_2 and alloxan treatment caused a rapid elevation in intracellular Ca^{2+} and increased insulin release in rat islets at basal, non-stimulatory glucose concentrations. Isolated rat islets exposed to alloxan and xanthine oxidase/hypoxanthine ($\cdot O_2$ generating system) experience a temporary increase in insulin levels, thus providing additional support for the involvement of ROS in GSIS (Ebelt et al. 2000). Attenuation of glutamate-cysteine ligase (GCL) (which is known to reduce GSH synthesis and therefore raising intracellular ROS) enhanced insulin secretion in MIN6 cells (Kondo et al. 2000). Generation of ROS from glucose metabolism itself may be an important metabolic signal facilitating insulin secretion.

There is also evidence that H_2O_2 serves as a second messenger in insulin responsive cells for insulin-mediated signal transduction. The generation of cellular H_2O_2 enhances insulin signal transduction by inhibiting cellular protein-tyrosine phosphatases (PTPases) that negatively regulate insulin signal transduction pathway by catalyzing tyrosine dephosphorylation of the insulin receptor (IR) and its substrate proteins. The intracellular single-domain enzyme protein-tyrosine phosphatase 1B (PTP1B) is a physiological negative regulator of insulin action. Mice lacking PTP1B exhibit enhanced insulin sensitivity due to increased IR

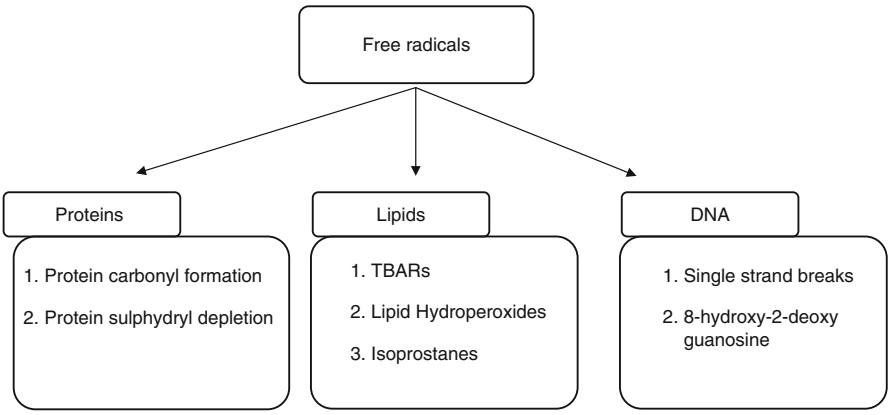

Fig. 147.3 The damaging effect of free radicals on proteins, lipids, and DNA which alters cell functionality

phosphorylation in liver and muscle (Elchebly et al. 1999; Zabolotny et al. 2002). Additionally, antisense oligonucleotides suppressing PTP1B expression in mouse and rat models of insulin resistance are able to enhance insulin sensitivity and normalize blood glucose levels (Zinker et al. 2002). PTPases have a common redox-sensitive catalytic cysteine residue, which is susceptible to oxidative damage by H_2O_2 and other oxidants, producing sulfenic acid intermediates which further react with thiols to form catalytically inactive PTP disulfides, thus enhancing insulin signaling (Mahadev et al. 2001; Meng et al. 2002, 2004).

Though H_2O_2 may well have a role in GSIS in β-cells and also in transduction of insulin signaling, continuous production of H_2O_2 due to persistent hyperglycemia results in chronic oxidative stress which impairs β-cell survival and function, a process known as glucose toxicity (Wu et al. 2004). Thus while the notion that ROS play an important role in GSIS in β-cells has been proven time and again, severe detrimental effects of high levels of ROS to cells cannot be overlooked. ROS impairs the integrity and physiological functions of cellular biomolecules such as lipids, proteins, and DNA leading to generation of chronic oxidative stress (Fig. 147.3). Excessive ROS induces lipid peroxidation that results in the formation of conjugated dienes, lipid hydroperoxides, and degradation products such as alkanes, aldehydes, and isoprostanes. ROS degrades polyunsaturated fatty acids, which is incorporated in all biological membranes, to peroxyl radicals ($ROO^{\bullet}$) which is further converted to malondialdehyde (MDA) through a series of chain reactions (Fedtke et al. 1990; Wang et al. 1996; Mao et al. 1999). MDA, a major aldehyde product of lipid peroxidation other than 4-hydroxy2-nonenal (HNE), is mutagenic in bacterial and mammalian cells and carcinogenic in rats (Valko et al. 2007). ROS damages proteins by attacking amino acids, particularly cysteine and methionine (Stadtman 2004). Direct oxidation of lysine, arginine, proline, and threonine residues yields carbonyl derivatives (Berlett and Stadtman 1997). Protein carbonyl derivatives are also formed when proteins react with aldehydes such as

HNE and MDA formed during lipid peroxidation (Esterbauer et al. 1991). Damage to proteins is of particular importance in vivo as it affects the function of several receptors, enzymes, transport proteins and also generates antigens that can provoke an immune response. The hydroxyl radical reacts with all the components of DNA, damaging both purine and pyrimidine bases and also the deoxyribose backbone (Halliwell and Gutteridge 1999) generating a wide range of oxidative DNA lesions. These mainly include C-8 hydroxylation of guanine to form 8-Oxo-7, 8-dehydro-2′-deoxyguanosine (8-OHdG), 2-OH-adenine, thymine glycol, 8-OH-adenine, cytosine glycol, etc. (Weitzman et al. 1994).

Thus, although physiological concentrations of endogenous ROS play a pivotal role in cell signaling, its excess production of ROS perpetuates its deleterious effects that can culminate in cellular apoptosis.

Antioxidant Defence Mechanisms in Diabetes

During normal metabolism, there exists a harmony between generation of ROS and its detoxification by antioxidant defense mechanisms. In a diseased state when this strong and persistent production of ROS overwhelms the antioxidant defense machinery of a cell to an extent that it cannot reset the redox homeostasis of a cell, it generates oxidative stress. Excess ROS thus generated within the body, if left unattended, can lead to cell death. The degree to which ROS can be detrimental to a cell is determined by its intrinsic antioxidative defense system which actively neutralizes these free radicals. Antioxidant defense comprises of agents that catalytically remove free radicals and other reactive species or low molecular agents that scavenge ROS and RNS. The primary defense includes free radical scavenging enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and several disulfide reductases, including thioredoxin (Trx) and glutaredoxin (Grx), peroxiredoxins (Prxs), and glutamate-cysteine ligase (GCL) (Fig. 147.4). Superoxide generated within mitochondria is readily dismutated to H_2O_2 by superoxide dismutase (SOD) as the first line of defense against free radicals. Hydrogen peroxide thus formed is unique in that it can either be converted to the highly damaging hydroxyl radical or be catalyzed and excreted harmlessly as water. Hydrogen peroxide is transformed to water and oxygen by catalase which decomposes H_2O_2 to water and O_2 or glutathione peroxidase (GPx) which removes H_2O_2 by coupling its reduction to H_2O with oxidation of reduced glutathione (GSH) (Halliwell and Gutteridge 1999). GSH is considered the major thiol disulfide redox buffer of the cell and measurement of the GSH/GSSG ratio is used to estimate the redox environment of the cell (Avanzo et al. 2002). In healthy cells and tissue, more than 90 % of the total glutathione pool is in the reduced form (GSH) and less than 10 % exists in the disulfide form (GSSG). To maintain the ratio of GSH/GSSG in the cell, glutathione reductase, a flavoprotein enzyme, though not directly involved in scavenging free radicals, is essential for regenerating GSH from GSSG at the expense of NADPH. Glutathione peroxidase/glutathione systems therefore are crucial during oxidative stress.

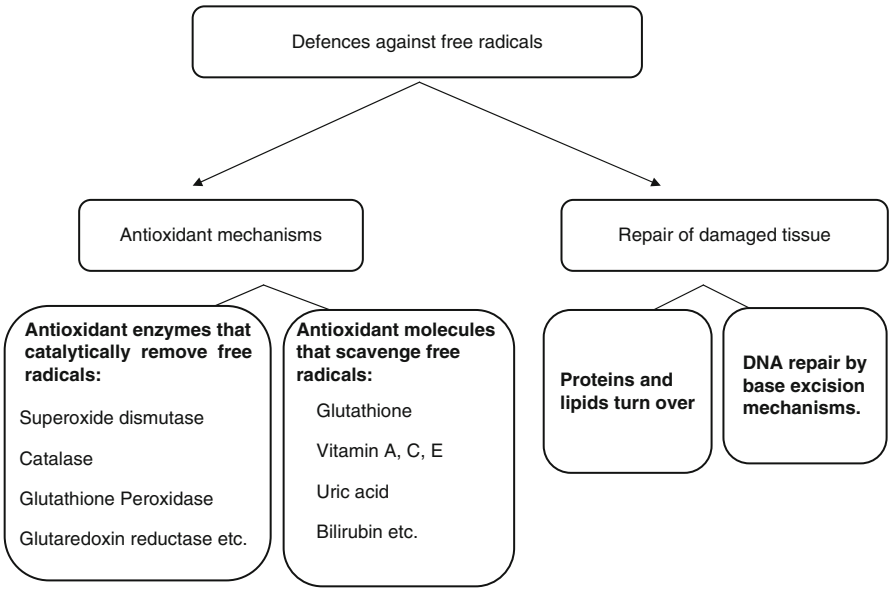

Fig. 147.4 Strategies to cope up against excess free radicals

The peroxiredoxin family of thiol-peroxidases also catalyze the reduction of H_2O_2 and alkyl hydroperoxides to water and alcohol, respectively, with the use of reducing equivalents provided by thiol-containing proteins; this results in the generation of sulfenic acid. Hyperoxidation of peroxiredoxins occurs when the H_2O_2 concentration exceeds the capacity of Prx regeneration by Trx rendering Prx inactive, and resulting in complete inactivation of its peroxidase activity (Yang et al. 2002; Rabilloud et al. 2002). This inactivation of Prxs is reversed by sulfiredoxins (Srxs) which catalyze ATP-dependent reduction of the hyper-oxidized peroxiredoxin (PrxSO2) into sulfenic peroxiredoxin (PrxSOH). Thioredoxin is a ubiquitous disulfide reductase responsible for maintaining proteins in their reduced state, caused by electron transfer from NADPH via thioredoxin reductase (TRX) (Holmgren 1985). TRX is induced under oxidative stress conditions and can protect proteins and DNA from oxidation by ROS by scavenging them.

A secondary defence system is in the form of small nonenzymatic antioxidant molecules such as GSH, uric acid, ascorbate, and α-tocopherol. GSH is the principal nonprotein thiol and has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. GSH also participates in the detoxification of xenobiotics as a substrate for the enzyme glutathione-S-transferase (Dickinson and Forman 2002). Glutathione is maintained in the reduced state by glutathione reductase and transfers its reducing equivalents to several metabolites and enzymes such as ascorbate in the glutathione-ascorbate cycle and GPx (Bindoli et al. 2008). Vitamin E,

a component of the total peroxyl radical-trapping antioxidant system, reacts directly with peroxyl and superoxide radicals and singlet oxygen and protects membranes from lipid peroxidation (Maritim et al. 2003). Uric acid is an antioxidant that scavenges peroxides, hydroxyl radicals, and hypochlorous acid (Becker 1993; Hellsten et al. 1997) and can also react chemically with singlet oxygen, superoxide, and hydroxyl radicals and therefore function directly as a free radical scavenger.

Antioxidant enzymes and molecules are involved in direct neutralization of free radicals. Among different biological molecules damaged by excessive free radicals, oxidatively damaged DNA is repaired while lipids and proteins are turned over. Therefore the ability of cells to with OS depends both on antioxidant defense mechanisms and on the ability to restore the oxidative damage.

Response of Islets to Oxidative Stress

Chronic exposure of β-cells to supraphysiologic concentrations of glucose causing oxidative stress induces defective insulin gene expression that is accompanied by marked decreases in insulin content and abnormal insulin secretion. In addition to oxidizing proteins, lipids, and DNA, ROS also activate various stress-sensitive signal transduction pathways such as c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (p38MAPK) (Purves et al. 2001), and protein kinase C (Koya and King 1998). The JNK (also referred to as SAPK) and p38MAPK are members of the complex superfamily of MAP serine/threonine protein kinases. Activation of the JNK pathway during oxidative stress affects the DNA-biding activity of PDX-1, resulting in decreased insulin gene expression. Adenoviral overexpression of dominant-negative c-Jun NH2-terminal kinase (DN-JNK) protected both PDX-1 binding to DNA and insulin gene mRNA from hydrogen peroxide-induced oxidative stress in β-cells as well as from the adverse effects of hyperglycemia (Kaneto et al. 2002).

PDX-1 is a member of the homeodomain-containing transcription factor family and plays an important role in pancreatic development (Jonsson et al. 1994; Holland et al. 2002), β-cell differentiation (Ber et al. 2003; Kojima et al. 2002; Noguchi et al. 2003; Taniguchi et al. 2003; Miyazaki et al. 2004), and in the maintenance of normal β-cell function by transactivating insulin gene and genes involved in glucose sensing and metabolism such as GLUT2, glucokinase, and islet amyloid polypeptide (Petersen et al. 1994; Bretherton-Watt et al. 1996; Carty et al. 1997; Waeber et al. 1996; Watada et al. 1996). Oxidative stress induces nucleo-cytoplasmic translocation of PDX-1 through activation of the JNK pathway. Since the PDX-1 gene is transactivated by PDX-1 itself, it has been proposed that the decrease of PDX-1 expression in nuclei results in further decrease of PDX-1 expressed in the cells (Kawamori et al. 2003). Thus, oxidative stress-induced nucleo-cytoplasmic translocation of PDX-1 plays a crucial role in suppression of insulin gene expression and its biosynthesis under diabetic conditions.

Low Antioxidant Defense of Islets

Most cells are well endowed with intrinsic mechanism to combat free radicals and through them try to escape the otherwise deleterious effects of free radicals. Islet cells have extraordinary low antioxidant defense system and therefore become more prone to get oxidatively stressed. Islets express exceptionally low activity of several key antioxidant defense enzymes compared with other tissues suggesting their vulnerability toward oxidative damage (Grankvist et al. 1981). This difference in the antioxidant defense status of different organs within an organism was reported by Lenzen et al. (1996). Gene expression profiling of several tissues like liver, brain, kidney, lung, skeletal muscle, heart muscle, adrenal gland, and pituitary gland revealed that pancreatic islets express substantially low amounts of SOD, CAT, and GPx in comparison with other tissues (Table 147.1). Additionally, markers of oxidative damage have also been observed to be significantly high in islets of diabetics than of control, and the levels of these markers correlate with the extent to which GSIS have been impaired (Tanaka et al. 2002). An increase in the expression of oxidative stress markers like 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 4-hydroxy-2, 3-nonenal (4-HNE) in islets under diabetic conditions also suggests the vulnerability of islets to oxidative stress (Ihara et al. 1999; Gorogawa et al. 2002; Guerra et al. 2005; Modak et al. 2009). It has further been proved (Modak et al. 2009) that islets exhibit very poor capacity to repair the oxidatively damaged DNA compared to liver cells. Cell-free extracts from islets and liver cells when examined for their ability to remove 8-OHdG from oxidatively damaged plasmid pBR322 in vitro as well as in vivo revealed that liver cells were much more efficient as compared to islet cells. Liver cells were able to remove 89 % of the DNA damage within 2 h as compared to only 23 % by islets.

Thus, excessive ROS assault due to hyperglycemia in the face of low antioxidant defense status of islets and its inefficiency to restore the oxidatively damaged DNA leads to islet dysfunction.

Table 147.1 Gene expression profiling of antioxidant enzymes in different tissues from albino mice in comparison to liver (Adapted from Lenzen et al. 1996)

Tissues	Cu/Zn-SOD (% of liver)	Mn-SOD (% of liver)	Catalase (% of liver)	GPx (% of liver)
Liver	100 ± 7	100 ± 17	100 ± 10	100 ± 5
Kidney	99 ± 7	125 ± 19	78 ± 8	91 ± 9
Brain	77 ± 8	67 ± 16	36 ± 10	39 ± 8
Lung	80 ± 12	66 ± 17	50 ± 10	58 ± 9
Skeletal muscle	59 ± 7	95 ± 14	41 ± 12	40 ± 10
Heart muscle	70 ± 10	142 ± 9	72 ± 11	39 ± 7
Pituitary gland	79 ± 19	47 ± 11	23 ± 2	66 ± 11
Adrenal gland	175 ± 16	239 ± 25	45 ± 7	77 ± 12
Pancreatic islets	38 ± 9	30 ± 5	Not detectable	15 ± 6

Antioxidant Therapy for Survival of Islets

Since the effects of ROS on islets are more deleterious to other cells, one of the strategies to overcome these damaging effects of ROS is by administration of several antioxidant supplements that can help strengthen the defense status of the cell. Antioxidants such as vitamin C, vitamin E along with N-acetyl-L-cysteine have been used for reducing the oxidative damage caused to islet cells from C57BL/KsJ-db/db mice (Kaneto et al. 1999). Several other antioxidants such as curcumin (Kanitkar et al. 2007), quercetin (Coskun et al. 2005), and probucol (Gorogawa et al. 2002) also protect islets from damage caused by free radicals. Puerarin, the main isoflavone glycoside found in a Chinese herb, protected islets against H_2O_2 mediated cell toxicity by inhibiting free radical generation (Xiong et al. 2006). Resveratrol (3,4′,5-trihydroxy stilbene), a phytoalexin, augmented cellular antioxidant defense capacity through induction of HO-1 via Nrf2-ARE signaling, thereby protecting PC12 cells from oxidative stress (Chen et al. 2005). Our laboratory demonstrated that Syndrex, a formulated antidiabetic drug containing powder of germinated fenugreek seeds, improved the functionality and viability of islet cells in vitro by protection against oxidative damage (Dixit et al. 2008).

Another strategy that could serve as a pharmacological approach for treating diabetes is by strengthening the antioxidant defense of islets through enhanced expression of radical scavenging enzymes in islets. Adenoviral-based over-expression of glutathione peroxidase in human islets not only increased GPx activity and protected islets against ribose-induced oxidative stress (Robertson et al. 2005; Tanaka et al. 2002) but also enhanced the resistance of rat β-cells to both ROS and RNS cytotoxicity (Moriscot et al. 2003). Over-expression of cellular enzymes such as mitochondrial catalase, Cu/Zn-SOD, and Mn-SOD also protects islet cells (RINmSF cells and INS-1 insulin secreting cells) from oxidative damage (Gurgul et al. 2004; Lortz et al. 2000; Hohmeier et al. 1998; Moriscot et al. 2000). Beta cell overexpression of thioredoxin in nonobese diabetic (NOD) transgenic mice reduced the severity of streptozotocin (STZ)-induced diabetes–where STZ toxicity in beta cells is mediated by its ROS generating ability (Hotta et al. 1998). Increasing GSH levels in islets by over-expressing the glutamyl cysteine ligase catalytic subunit, a primary regulator of de novo synthesis of glutathione (GSH) in mammalian cells, protected pancreatic islets against oxidative stress (Tran et al. 2004). Co-expression of several antioxidant enzymes such as glutathione peroxidase along with two isoforms of superoxide dismutase and catalase combined with glutathione peroxidase or catalase with SOD (Mysore et al. 2005; Tiedge et al. 1998; Lortz and Tiedge 2003) provided better protection to islets from oxidative injury compared with over-expression of individual antioxidant enzymes.

Enhancing the antioxidant defense status of islets either by oral administration of antioxidants or by increasing the expression of antioxidant enzymes, islets exhibit an improved ability to cope with oxidative stress and provides further support for the implication of oxidative stress in islet dysfunction in diabetes. Clinical trials using vitamin E show that this antioxidant can normalize beta-cell function and improve insulin resistance in type 2 diabetic patients (Gokkusu et al. 2001; Paolisso et al. 1993).

Another commonly used drug for treatment of T2D is metformin which acts by lowering glucose production through reduction in gluconeogenesis (Hundal et al. 2000). This drug has additionally been shown to exert antioxidant activity in streptozotocin-induced diabetic rats and to decrease erythrocyte susceptibility to oxidative stress in type 2 diabetic patients. Metformin also restores the normal secretory pattern of isolated rat pancreatic islets incubated with elevated glucose or free fatty acid (FFA) concentrations, which are known to generate OS. Thus, the beneficial effect of metformin seem to be due to both its antioxidant and anti-gluconeogenic properties.

Evolutionary Basis for the Low Defense Status of Islets

Although islets are not as well equipped with an antioxidant defense machinery as other cells, the reasons for this compromised status of islets are unclear. If ROS is detrimental because it impairs homeostasis, then it would be reasonable to expect islets to have sufficient capacity to combat ROS-induced harmful effects, and importantly without compromising its ability to produce insulin and thereby maintain homeostasis. It is possible that the low antioxidant capacity of islets may have a secondary role in beta cell function. This has been addressed by Rashidi et al. (2009) who hypothesized that ROS in β-cells have a fitness-enhancing role by exerting a negative effect on its ability to secrete insulin. During the course of evolution, the pancreas evolved (along with the brain and nervous system) to have an increased demand for energy to maintain its functionality, especially during stressful situation such as predation, competition, and infection which were more prevalent at that time. Although β-cells from pancreatic islets did provide an extra degree of freedom for the brain to evolve without having to organize an additional energy supply (Madsen 2007), this however was not sufficient to meet the energy demands of the brain. Cortisol and corticosteroid receptors which also coevolved receptors, which evolved nearly simultaneously, diverted surplus energy to the developing brain. Cortisol, a stress hormone, reduces the responsiveness of insulin-dependent tissues to insulin, resulting in insulin resistance (Qi and Rodrigues 2007). This results in hyperglycemia which in turn generates excessive ROS within islets. Excess ROS generated causes islet dysfunction and as a result hampers insulin production from islets. This then allows the diversion of glucose toward insulin-independent tissues such as the brain. However, if islets were to be sufficiently equipped with an antioxidant defense system, ROS would then not be able to regulate this diversion of glucose to tissues tissues with the highest demand at times of stress. However, to make frequent adjustments during stressful conditions may not be an efficient and cost-effective option. This requires a well-balanced evolutionary trade-off between brain and β-cells to allow the optimization of antioxidant defences and at the same time facilitate the metabolic demands during stressful situations.

Pregnancy in placental mammals is a period of physiological stress where maternal insulin resistance helps to divert more glucose to the increasing fetal

demands—this is accomplished by increasing cortisol concentrations. Low antioxidant defense mechanisms in islets thus allow ROS to perform its regulatory function by generating maternal insulin resistance. This corroborates the observation that females have lower antioxidant levels as compared to males, both in mice (Cornelius et al. 1993) and in humans (Tonooka et al. 2007). During evolution this strategy by which natural selection favored the maximization of fitness of the whole organism and ensuring offspring growth and viability may have caused beta-cells to retain a weak antioxidant profile. This hypothesis gains support from the observation that birds that have higher reproductive success (by increasing their brood size) also have lower levels of antioxidant enzyme expression and that senescence acceleration by increased reproductive effort is at least in part mediated by oxidative stress (Wiersma et al. 2004). Thus, for ROS to play its regulatory role, it may have become necessary that the antioxidant defense of islets be deliberately kept at a low level for the life span of an individual. The present-day compromised status of antioxidant defense mechanisms of islets could be attributed to this well-tuned coevolution of brain, the corticosteroid response and the adaptation of beta-cells in the endocrine pancreas.

Although increasing the defense mechanism by oral administration of antioxidants and over-expression of antioxidant levels in islets seems to be a better pharmacological strategy to alleviate oxidative stress, the benefits from increased resistance to ROS-induced damage derived from such an attempt would compromise physiological ROS signaling. Strategies to use antioxidant therapy should aim to balance protection of islets without altering basal ROS-mediated signaling in the pancraes (including insulin release) islets from ROS-induced oxidative damage.

Conclusion

Free radicals when present at lower concentrations play a major role as metabolic signaling molecules, for example, in GSIS in islets; however, when present in higher concentrations, they have a role in the pathogenesis of both type 1 and type 2 diabetes mellitus, based on both in vitro and in vivo studies. Endogenous antioxidant defense mechanisms neutralize excess free radicals to maintain redox homeostasis in a cell. However, a weak antioxidant defense and an inefficient repairing of oxidatively damaged DNA makes islets vulnerable to ROS assault during hyperglycemia. Since islet cells crucial maintaining glucose homeostasis and have exceptionally high vulnerability toward free radicals, strategies by which islet functionality can be maintained required vigorous investigations. Several attempts have been made to strengthen the antioxidant defense of islets through antioxidant therapy, while over-expressing antioxidant enzymes has proven beneficial in alleviating oxidative stress in animal models of diabetes. However, continuous use of antioxidant therapy may also interfere with ROS signaling required for GSIS. The compromised antioxidant defense status of islets may be a consequence of evolutionary conservation as it provides additional benefits to insulin-independent tissues during stressful conditions.

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