8

Mitochondria and Antioxidants: The Active Players in Islet Oxidative Stress

Tatyana V. Votyakova, Rita Bottino and Massimo Trucco
Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh
USA

1. Introduction

Diabetes mellitus (DM) of type 1 (T1D) and type 2 (T2D) are characterized by persistently high glucose (HG) blood levels known as hyperglycemia. The preponderance of evidence points to a significant role of oxidative stress in the development of complications in patients with DM. In particular, the pathogenic increase in reactive oxygen (ROS) and nitrogen species (RNS) as well as accumulation of oxidation and nitration products has been well documented in cases of diabetes. ROS and RNS affect all types of biological molecules: they cause oxidation of membrane lipids, modification of protein amino groups as well as deoxynucleotites. Insulin producing beta-cells, which are part of pancreatic islets of Langerhans, perform the energetically demanding function of sensing blood glucose and releasing insulin to sustain metabolic homeostasis. They are highly specialized endocrinal cells with a complex system of signal transduction and insulin producing capacity. In pathology of diabetes beta-cells are the ones which are most susceptible to oxidative stress.

2. Sources of oxidative stress in islets

In islets, reactive species can originate from several sources. NAD(P)H-oxidase located in the plasma membrane produces molecule of O^{2-}. Several isoforms of this enzyme were found in islets (Uchizono et al. 2006; Newsholme et al. 2007; Newsholme et al. 2009) and they are considered as substantial producers of ROS. The enzyme can be activated by exposure to fatty acids and in normal conditions it is believed to participate in glucose-stimulated insulin secretion (GSIS) (Graciano et al. 2011; Santos et al. 2011). Islets also possess both types of nitric oxide synthases (NOS): constitutive cNOS (Nakada et al. 2003) and inducible (iNOS) (Darville and Eizirik 1998; Kutlu et al. 2003). Nitric oxide produced by cNOS is part of normal beta-cell physiology, while activation of iNOS is associated with beta-cell destruction, in particular via cytokines produced by immune cells.

There is plentiful evidence that hyperglycemic conditions cause rise in ROS and RNS in beta-cells reviewed in: (Newsholme et al. 2007; Acharya and Ghaskadbi 2010). It was shown that high glucose triggers generation of ROS in rodent and human islets as well as in insulinoma cell lines (Tanaka et al. 1999; Tanaka et al. 2002; Bindokas et al. 2003; Robertson et al. 2003). A definitive role for mitochondria in glucose-induced ROS signal was proposed in a number of papers (Maechler and Wollheim 2001; Brownlee 2003; Fridlyand and

Philipson 2004; Newsholme et al. 2007; Newsholme et al. 2009). Thus, mitochondria are considered as an important ROS generator in islets.

3. Islet mitochondria as a source of reactive oxygen species

The main role of mitochondria in a cell is the production of ATP molecules for cellular energetic needs using for that energy of metabolite oxidation. ATP production by mitochondria is central for glucose sensing and insulin release in beta-cells, the fact that directs attention to mitochondria in diabetes studies. In the process of metabolite oxidation, mitochondria are dealing with the transfer of electrons along respiratory Complexes I through IV. Eventually four electrons are combined with four protons, H^+, and one molecule of oxygen, producing two molecules of water; the reaction takes place within Complex IV (for reference see (Nicholls and Ferguson 2002). However, as a side reaction, a low portion of unpaired electrons leaks from the respiratory complexes and interacts with molecular oxygen producing a molecule of superoxide anion radical, a form of ROS. Currently, it is believed that the sites of ROS production in the mitochondrial respiratory chain are Complex I and Complex III (Turrens 2003; Rigoulet, Yoboue, and Devin 2011).

For proper mitochondrial functioning with low levels of ROS production, the availability of end-point electron acceptor, molecular oxygen, is absolutely necessary. Temporal hypoxia, which causes a halt in electron flow followed by reoxygenation and resuming of electron flow, results in an increase of mitochondrial ROS (Selivanov et al. 2009). This phenomenon is related to a widely known phenomenon of ischemia-reperfusion injury and is a critical factor in the process of islet isolation from donor pancreata for transplantation purposes, as donor tissues inevitably become hypoxic when blood circulation stops. Presence of antioxidants in preservation and isolation media detoxifies mitochondria-derived ROS and substantially improves viability of islets and their potency to normalize blood glucose in recipient diabetic animals (Bottino et al. 2002; Bottino et al. 2004; Sklavos et al. 2010).

Manipulation of electron flow in the respiratory chain by specific inhibitors of mitochondrial respiratory Complexes can either increase or decrease ROS generation. In normal conditions, with low levels of glucose and low base level of ROS, inhibitors of Complexes I and III, rotenone and antimycin, respectively, increase ROS production in rat islets (Armann et al. 2007; Leloup et al. 2009). This is consistent with data obtained on isolated mitochondria *in vitro* (Votyakova and Reynolds 2001; Starkov, Polster, and Fiskum 2002; Starkov and Fiskum 2003; Rigoulet, Yoboue, and Devin 2011).

An important question is whether mitochondrial inhibitors are capable of down regulating ROS signals originating from the respiratory chain under conditions of hyperglycemia and hyperlipidemia. The answer depends on the type of chemical agent and the locus it binds in the respiratory chain. Oxidation of succinate in Complex II results in a high ROS generation because this prosess initiates forward electron flow to Complex III as well as reverse electron flow upstream to Complex I (Selivanov et al. 2011). Thus, inhibition of succinate oxidation would lead to a decrease in overall ROS. This effect was observed in a work by Sakai and collaborators (Sakai et al. 2003) where an inhibitor of Complex II thenoyltrifluoroacetone (TTFA) decreased glucose-stimulated ROS in human islets and MIN-6 cells. The inhibitor of Complex III antimycin A blocks Q-cycle in a way that increases free radical forms of respiratory chain components (Votyakova and Reynolds 2001; Starkov,

Polster, and Fiskum 2002; Starkov and Fiskum 2003). Leloup and co-authors observed a similar effect of antimycin A on rat islets (Leloup et al. 2009).

Complex I, or NADH-oxidoreductase, is the largest and the most sophisticated segment in the respiratory chain; its architecture, functioning and mechanisms of ROS generation has been extensively studied (Magnitsky et al. 2002; Vinogradov 2008). In mammals it consists of 47 subunits with a number of redox centers to transfer electrons. Among them, there are flavine mononucleotide at the entrance of the Complex, which binds NADH, several sulfur-iron clusters and several Coenzyme Q binding sites in the middle of the Complex. According to the current consensus, all inhibitors, which bind to subunits located in the middle of Complex I and block electron flow within the complex, such as rotenone, piericidin and others, increase ROS (Grivennikova and Vinogradov 2006). The only known inhibitor that decreases ROS generation in Complex I is diphenylene iodonium, which binds at the very entrance at the flavin mononucleotide (FMN) site (Liu, Fiskum, and Schubert 2002). This compound, though, is unspecific and also inhibits NAH(P)H oxidase of the plasma membrane. In other words, if an inhibitor blocks the very entry of Complex I at the site of FMN and prevents the access of electrons into Complex I at all, it would prevent ROS generation. If an inhibitor blocks electron flow somewhere in the middle of Complex I and allows redox centers upstream of the block to be over-reduced, it would result in an increase of ROS (Genova et al. 2003). In accordance with this notion, inhibitory effects of diphenylene iodonium on ROS induced by hyperglycemia in MIN-6 cells were reported by (Tsubouchi et al. 2005), though the researchers attributed this fact solely to the plasma membrane NAD(P)H oxidase inhibition. Regarding the other Complex I inhibitors, there is some controversy in the literature. The same authors reported no effect of rotenone, the effect could be expected if ROS was already increased, while earlier work by Sakai and collaborators reported that rotenone twofold decreased hyperglycemia-induced ROS in human islets and MIN-6 cells (Sakai et al. 2003).

There is a way to modulate mitochondrial ROS generation without interference into the activity of respiratory Complexes. In the cell this function belongs to a special group of proteins located in the inner membrane of mitochondria, called uncoupling proteins (USPs), which in a highly controlled manner modulate membrane potential, basically working as proton conductors (Ricquier and Bouillaud 2000). Islets express an UCP2 isoform of uncoupling protein (Gimeno et al. 1997), and in a number of publications it was shown that overexpression of UCP2 downregulated the levels of ROS (Pi et al. 2009; Affourtit, Jastroch, and Brand 2011).

The same effect can be achieved by using chemical compounds called uncouplers, which, while different in structure, have two properties in common: they can penetrate into mitochondrial membrane and are capable to easily accept or dissociate H^+ ion. Uncouplers decrease membrane potential by transporting protons into mitochondrial matrix and, in principle, their function is similar to that of UCPs, though they are more powerful modulators (see (Nicholls and Ferguson 2002) for detailed mechanism). Uncouplers were shown to decrease ROS production in glucose-stimulated human and rat islets as well as in MIN-6 cell line (Sakai et al. 2003; Leloup et al. 2009). This is consistent with the data observed on isolated mitochondria *in vitro* (Votyakova and Reynolds 2001; Selivanov et al. 2008).

Overall, the amount of data on the mechanism of mitochondrial ROS generation in beta-cell is limited and this issue is underexplored, mainly because islets are precious and less available for research compared with other more abundant tissues, like liver, muscle, heart and even brain. Mostly intriguing is the fact that, despite extensive evidence of increased level of free radicals in conditions of high glucose or high lipids, there is still no satisfactory molecular mechanism explaining exactly how high levels of cellular energy metabolites cause beta-cell mitochondria to produce more free radicals.

4. Antioxidant capacity of insulin producing beta-cells

Antiradical defense systems in the cell consist of a gamut of small antioxidant molecules and enzymes capable of interaction with reactive oxygen and nitrogen species. Endogenous antioxidant molecules are vitamins A, C and E, sulfur-containing compounds like amino acid cysteine and tripeptide glutathione (GSH – reduced form, GSSG – oxidized form). Coenzyme Q may also act as an antioxidant in particular conditions. These compounds possess different efficiency in scavenging harmful oxidants, glutathione being the most potent. The main players in enzymatic antiradical protection are superoxide dismutases (SOD), catalase and glutathione peroxidases (GPx). Within the cell two isoforms of SOD are found: Zn,Cu-SOD (SOD1) which is located in cytoplasm and Mn-SOD (SOD2) located in mitochondrial matrix. They are the first line of defense as they convert superoxide anion radical into O_2 and H_2O_2 by dismutation. The hydrogen peroxide next can be converted into H_2O and O_2 by catalase or, to H_2O, by GPx; the latter will use reduced glutathione molecule as a substrate. Glutathione is a recyclable molecule: its oxidized form is reduced back by glutathione reductase. Mitochondria have both enzymes (about of 10% of total cellular activity) which, working in concert, effectively detoxify peroxides and recycle GSH (for reference see (Halliwell 2001)). The proteins UCPs, which downregulate ROS generation in mitochondria, can also be formally added to antioxidant proteins.

Glucose sensor function and production of insulin on demand are the two dominating functions in beta-cell physiology and, apparently, this comes at a cost of downregulating some other functions. High susceptibility of islets to oxidative insults was well established long ago, and this feature was utilized to specifically target these cells, thus, creating animal models of diabetes. Compared to cells from other tissues, islet cells have profoundly lower activity of enzymes involved into antiradical defense. As early as in 1979 Grankvist and co-authors shown that alloxan effectively destroys rat islet cells through ROS-mediated mechanisms (Grankvist et al. 1979). Two years later they reported that beta-cell super-sensitivity to oxidative agent is due to a deficiency of anti-radical defense capacity (Grankvist, Marklund, and Taljedal 1981). The activity of the main anti-oxidant enzymes was found to be about 30% for both types of SODs and only in single percentage range for catalase and glutathione peroxidase (1.2% and 1.8%, respectively), as compared to liver. This data was confirmed by estimating the levels of mRNA of the respective genes by Lenzen and co-workers (Lenzen, Drinkgern, and Tiedge 1996). It should be noted, though, that a more appropriate comparison of islet enzymes' activities would be with that of tissues with similar functions and/or intensity of metabolism. As Table 1 shows, when compared with hypophysis or brain tissues, islets' activities of anti-oxidant enzymes were only 50% or lower.

Gene Expression	Cu/Zn SOD	Mn SOD	Catalase	Glutathione Peroxidase
Pancreatic islets % of Liver*	38	30	n.d. **	15
Pancreatic islets % Pituitary gland	48	64	n.d.**	23
Pancreatic islets % of Brain	49	45	n.d.**	38

Table 1. The levels of anti-oxidant gene expression in islets compared to brain and pituitary gland. *Data adopted from Lenzen & al. (1996). **In (Tiedge et al. 1997) it was determined to be around 5%.

Expression of anti-oxidant enzymes in islets may vary within the same species reflecting genome variations. Zraika and co-authors have found that mRNA levels of Mn-SOD in islets of a diabetes-prone DBA/2 mice were twofold higher than in islets of C57BL/6 mice (Zraika et al. 2006), while the level of catalase was the same. This means that at the same level of superoxide anion radical beta-cells of DBA/2 mice would produce H_2O_2 twice as fast compared to that of C57BL/6 mice, and consequently, having the same level of catalase, DBA/2 islets would deal with higher levels of peroxide.

Among species the differences in activities of antioxidant enzymes can also vary substantially. It was shown that human islets have more active catalase and SOD than rodent ones and, consequently, are more resistant to oxidative stress (Welsh et al. 1995).

The expression of antiradical enzymes can be changed in diabetes conditions. In islets of Goto–Kakizaki/Paris rats, a model of T2D, expression of the whole spectrum of antiradical defense genes is increased along with an increased level of reduced glutathione compared with normal healthy animals (Lacraz et al. 2010). In patients with T2D expression of Cu,Zn-SOD was found reduced (Sakuraba et al. 2002). Diabetes is a complex and dynamic disease, in which epigenetic and environmental factors can differently affect the expression of antioxidant enzymes at particular time points of its development. Thus, it is often difficult to compare data on diabetes-related oxidative stress in short-lived rodents to long-lived humans.

It is important to note that beta-cells possess a substantial activity of enzymes dealing with superoxide anion radicals, while the activity of the enzymes decomposing H_2O_2 is very low, especially that of catalase. This feature is in line with increasing evidence that suggests a signaling role for H_2O_2 molecule in the process of insulin secretion (Pi et al. 2009; Affourtit, Jastroch, and Brand 2011).

5. Application of chemical compounds with antioxidative properties as a strategy to offset oxidative stress

Low level of antioxidant defense in beta-cells suggested that supplementation of antioxidants could be beneficial. A number of chemical compounds with antioxidant properties were employed to prevent or counteract oxidative stress in islets. The studies were conducted on diabetic animals and on isolated islets. Both preventive and curing actions of antioxidants were studied; the antioxidative agents were administered either prior to induction of diabetes of oxidation stress, or in the course of developing processes.

Historically, the first to study were vitamins A, C and E which are naturally present in the body and possess the ability to scavenge free radicals. Unfortunately they offered very limited or no protection (Kaneto et al. 2001). Hence, a broad spectrum of chemical compounds was tested in search for agents capable to counteract oxidative burden experiencing by islets. They differ in chemical nature and by the mechanism through which they protect from oxidative stress. The most important aspect in search of these compounds is to pay attention to their potential side effects. We will focus on the most prominent ones.

5.1 Antioxidant properties of N-acetyl-L-cysteine (NAC)

Antioxidant properties of N-acetyl-L-cysteine (NAC), an acetylated derivative of amino acid L-cysteine) were employed in a range of medical conditions such as neurodegeneration (Pocernich et al. 2011), cardiovascular diseases (De Rosa et al. 2010), gastroenterological diseases (Ramudo and Manso 2010; Jegatheeswaran and Siriwardena 2011), transplantation (Czubkowski, Socha, and Pawlowska 2011) and diabetes (Kaneto et al. 2001). There are several mechanisms by which NAC can modulate oxidative stress: (i) it is a precursor in the synthesis of glutathione, an important component of the cellular antiradical defense system in the cell; (ii) NAC, as a thiol-containing compound, is able to directly reduce free radicals as well as S-S bonds in proteins, thus modulating redox signaling (Parasassi et al. 2010)

It was reported that NAC is protective or partially protective in animal models of diabetes. It reduces levels of oxidative stress markers and preserves beta-cell mass and function in STZ-treated hamsters (Takatori et al. 2004). Intravenous co-infusion of NAC with high glucose into Wistar rats offered only partial protection, as it quenched ROS, but did not restore beta-cell functions (Tang et al. 2007). However, the same group reported that NAC, co-infused with free fatty acids, not only decreased beta-cells ROS caused by prolonged exposure to fatty acids, but preserved their insulin and C-peptide responses to hyperglycemic clamps (Oprescu et al. 2007). These two studies show that effectiveness of particular antioxidant molecules depends on the type of oxidative stimuli and the metabolic pathways that are intervened. A study by Kaneto and co-workers also demonstrated that NAC can be protective in T2D-like metabolic deregulations. Using the db/db diabetic mouse model, the researchers found that NAC improved glucose-stimulated insulin response in these animals and, on a molecular level, NAC increased expression of pancreatic and duodenal homeobox factor-1 (PDX-1) in islets, a beta-cell-specific transcription factor (Kaneto et al. 2001).

NAC protects against oxidative stress stimuli *in vitro*. LDL oxidation level, which is relevant to pathology of T2D, significantly changes the expression of genes involved in the production and secretion of insulin and in cell survival mechanisms. This effect was offset by NAC (Favre et al. 2011). NAC counteracts the damaging effect of human amylin (hA), a small fibrillogenic protein, which accumulates in beta-cells in most subjects with T2D (Konarkowska et al. 2005). It is believed that NAC offers protection as a reagent capable of reducing protein SH groups, rather than a general ROS scavenger.

NAC is also protective against oxidative damage caused by direct short exposure of islets to H_2O_2 and free fatty acids, but is inefficient or partially efficient against long exposure islets to high glucose, or cytokines (Khaldi et al. 2006; Oprescu et al. 2007; Michalska et al. 2010). In some cases NAC, while decreasing ROS, did not restore beta-cell functions completely (Wang et al. 2004; Tang et al. 2007).

5.2 (R)-alpha-Lipoic acid (ALA)

(R)-alpha-Lipoic acid (ALA), (3R)-1,2-dithiolane-3-pentanoic acid, is a cyclic disulfide, being an oxidized form of its dithiol congener, (6R)-6,8-dimercaptooctanoic acid, or (R)-dihydrolipoic acid. Two sulfur atoms in ALA, which are connected to each other by a disulfide bond, can undergo facile and highly reversible redox processes (Arner, Nordberg, and Holmgren 1996). Thus, this compound can feature either antioxidant or pro-oxidant properties depending on particular redox context (Haramaki et al. 1997).

ALA is an essential co-factor in several mitochondrial oxidative complexes; the most important of these are pyruvate dehydrogenase (PDH) complex, 2-oxoglutarate dehydrogenase (OGDH) complex, and the complex for oxidation of branched chain amino acids (BCDH) (Nelson 2005).

Administration of alpha-lipoic acid to non-obese diabetic (NOD) mice decreased incidence of diabetes induced by cyclophosphamide from 60% to 30%. It also reduced severe intraislet infiltration and increases the percentage of islets with mild per-insular and periductular infiltrates (from 8.4 to 29.6 and 25.9%, respectively, $P < 0.01$) (Faust et al. 1994). The authors concluded that the anti-inflammatory action of lipoic acid may be due to its ability to scavenge oxygen radicals and to suppress nitric oxide production.

In the alloxan-induced diabetic mouse ALA lowered blood glucose, increased insulin release and prevented loss of beta cells and their dysfunction (Zhang et al. 2009). ALA prevented development of diabetes mellitus in obese Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a T2D animal model. It diminished glycosuria, reduced body weight and protected pancreatic beta-cells from destruction. ALA also reduced triglyceride accumulation in skeletal muscle and pancreatic islets (Song et al. 2005).

In vitro studies provided a deeper insight into the mechanism of ALA action on cellular and organellar levels. ALA counteracted oxidative stress induced by proinflamatory cytokines IL-1β, IL-6 and IFN-γ by preventing NF-κB activation (Zhang et al. 2009) and restoring insulin secretion (Schroeder et al. 2005). When a direct oxidative insult was applied in a form of H_2O_2 (Lee, Kwon, et al. 2009) or xantine-xantine oxidase (Burkart et al. 1993), pretreatment with ALA decreased cellular ROS and c-JNK activation, stabilized mitochondrial membrane potential and induced Akt phosphorylation, altogether offering protection to beta-cells from oxidative stress. In MIN6 cells and rat islets, this compound also offsets the deleterious actions of free fatty acids, which feature an *in vitro* model for conditions of T2D (Shen et al. 2008). In particular, it decreased levels of ROS, restored mitochondrial membrane potential, glucose-induced ATP and glucose stimulated insulin secretion.

It is worth noting that ALA, like many other redox active chemicals (Skulachev et al. 2009), has an optimum concentration for anti-radical actions, and, consequently, for protective activity. Optimum protective concentration for isolated mouse islets against oxidative stress induced by cytokine IL-6 was found to be 10^{-9} M; lower and higher concentrations were not effective (Schroeder et al. 2005). In the case of chemically-induced oxidative stress in INS-1 cells or isolated islets, ALA prevented apoptotic cell death in 150-300 μM concentrations, while higher concentrations caused apoptosis. Discrepancies in the effective concentration ranges reported in these studies may lie in different nature of oxidative stress stimuli and in

different protective mechanisms by which ALA acted in each case. Concentration of 2 mM, which is not physiologically relevant, impaired functions of isolated rat islets and MIN6 cells (Targonsky et al. 2006).

The nature of protective effects exhibited by ALA and NAC suggests that these compounds can act by multiple mechanisms, working as direct scavengers of free radicals, or by controlling of the thiol-disulfide level of reduction in signaling protein molecules (Pietta 2000; Pandey and Rizvi 2009; Parasassi et al. 2010). The antioxidant properties of ALA can be boosted by mitochondrial thioreductase (Trx), which restores its reduced form (Packer, Witt, and Tritschler 1995).

5.3 Coenzyme Q (CoQ)

Coenzyme Q (CoQ), a quinone, is an essential component of mitochondrial respiratory complexes I and III. In general, quinones can undergo two reversible one-electron redox processes, converting them into semiquinones and hydroquinones. High stability of semiquinone radicals renders the redox reversibility and is the basis for antioxidant properties of the quinone-related systems; both quinones and hydroquinones can serve as radical-protecting agents.

In cells, exogenous CoQ can exhibit either antioxidant or pro-oxidant properties, depending on conditions. As it was shown (Schroeder et al. 2005), a very low concentration of 10^{-12} M CoQ10 restored insulin production by mouse islets which were impaired by exposure to cytokine IL-1β. However, higher concentrations showed no effect or were even harmful. Overdoses of CoQ (50-200μM), although able to stimulate insulin release, were toxic to human islets and INS-1 cells. At these concentrations, CoQ, being a strong electrophile, covalently binds to E2 components of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes in mitochondria causing a substantial inhibition of the complexes and eventually triggering cell apoptosis (MacDonald et al. 2004). This circumstance put into a question the possibility to utilize CoQ10 as antioxidant to counteract diabetes-related oxidative burden as it would be difficult to find the right dose, while overdose would greatly outweigh potential benefits.

Some synthetic and plant-derived compounds may possess profound antioxidant properties, actively reacting with free radicals and ROS. These compounds belong to different chemical types; the most known of them are stable nitroxyl free radicals, metalloporphyrins, and plant polyphenols.

Superoxide anion radical is the first product resulting from the passing of unpaired electrons to molecular oxygen in the chain of chemical reactions causing oxidative stress. Thus, its detoxification would seem strategically advantageous. This notion prompted the introduction of a number of compounds which belong to different classes, but can all act as superoxide scavengers as they are able to effectively disproportionate superoxide to dioxygen and hydrogen peroxide. These compounds have a common name of SOD-mimetics.

5.4 TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl)

TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) is a nitroxyl stable radical. Being redox-active, TEMPOL facilitates the metabolism of many reactive oxygen and nitrogen

species (see review by (Wilcox 2010), in particular, TEMPOL catalyzes $O_2^{\cdot-}$ disproportionation. Its activity is not limited to this reaction, though, as it also catalytically converts H_2O_2 into water and dioxygen in a catalase-like reaction and inhibits generation of $OH^{\cdot}$ from H_2O_2 in the presence of redox-active transition metals in the Fenton reaction (Soule et al. 2007; Wilcox and Pearlman 2008).

Application of TEMPOL in diabetic animal models and *in vitro* on isolated islets was found to be protective against a variety of oxidative stress stimuli. Intravenous co-infusion of TEMPOL with high glucose or with free fatty acids into rats prevented islet dysfunction caused by hyperglycemia (Tang et al. 2007) or by hyperlipidemia (Oprescu et al. 2007). In Zuker rats, an animal model for T2D that features hyperglycemia, hyperinsulinemia as well as renal oxidative stress and high blood pressure, TEMPOL administered in drinking water reduced blood glucose, insulin secretion, renal oxidative stress and blood pressure which implies normalization of islet function (Banday et al. 2005).

5.5 Metalloporphyrins

Metalloporphyrins represent another well-known class of organic redox-active compounds. Manganese metalloporphyrins feature high chemical stability in different oxidation states and coordination of substrates at the central metal atom (Patel and Day 1999). Manganese ion can coordinate additional water or H_2O_2 in its axial sites, thus, performing its catalytic action. The stability of the porphyrin ring enables a variety of reversible catalytic metal-centered redox processes. Depending on substitutents, Mn-porphyrin compounds vary in their overall electric charge, redox potential and lipophic-hydrophylic properties. Eventually these physico-chemical properties translate into different catalytic activities and different abilities to penetrate into cells and cellular compartments. These compounds are capable of catalyzing not only the reaction of $O_2^{\cdot-}$ dismutation, but also the reduction or disproportionation of other reactive oxygen and nitrogen species, like H_2O_2, $HO^{\cdot}$, NO and $ONOO^-$ (Patel and Day 1999).

Manganese complexes of porphyrins were employed in studies of a number of biological models of oxidative stress (Batinic-Haberle et al. 2011). In an animal model of STZ-diabetic rats, Mn-porphyrin MnTM-2-PyP^{5+}, administered after the dose of STZ, counteracted the oxidative stress as judged by decreased levels of lipid peroxidation in blood plasma and erythrocytes (MDA products). Though it did not normalize blood glucose, it still decreased mortality of STZ-treated animals and increased their life span (Benov and Batinic-Haberle 2005). The data suggested that application of this antioxidant after a major STZ-induced oxidative stress did not restore islet function, but rather ameliorated following hyperglycemia-related complications. In the other experimental settings, the animals were preconditioned with Mn-porphyrin (MnTE-2PyP^{4+}, FBC-007), possessing a higher catalytic activity and a better ability to penetrate into cells. The animals were also given regular injections after STZ administration. Such treatment prevented development of diabetes in mice as monitored up to 120 days (Sklavos et al. 2010). It appears, therefore, that a preventive measure may play an important role in successful diabetes treatment.

In vitro, Mn-porphyrins showed a protective action also on the models of cultured isolated human and rodent islets as well as on insulin producing cell lines (INS-1 cells) subjected to oxidative insult. Bottino and co-workers demonstrated that the presence of MnTE-2PyP^{5+}

(AEOL10113) and MnTDE-2-ImP^{5+} (AEOL10150) as supplement to media during islet isolation resulted in up to three-fold increase of the viable mass of human islets, the fact of vital importance for transplantation medicine (Bottino et al. 2002). In a consequent publication a cascade of stressful events triggered by the procedure of islet isolation from the whole pancreas was studied in detail (Bottino et al. 2004). The islet isolation is a lengthy procedure that causes, apart of hypoxia-reoxygenation, mechanical and chemical stress to the cells. This results in activation of stress-related signals NF-κB and poly(ADP-ribose) polymerase (PARP) as well as increased levels of proinflamatory cytokines (Bottino et al. 2004). Mn-porphyrin effectively decreased NF-κB binding to DNA, PARP activation and release of cytokines and chemokines in islet cells, eventually resulting in higher survival and better insulin release(Bottino et al. 2004). Compound MnTE-2PyP^{5+} protected human islets from STZ-induced cell death and ensured better islet function after transplantation into immunodeficient diabetic mice. This holds true for transplantation of islets from the same mice strain (syngeneic), different strain (allogeneic) or in case of transplantation of human islets into mice (xenogeneic) (Sklavos et al. 2010).

Due to the complexity of metabolic and signal pathways, which vary in different cell types and physiological conditions, a possible protective effect of a Mn-porphyrin may depend on the cell type and the type of oxidative insult. Thus, it was reported that MnTMPyP preserved INS-1 cell viability and insulin secretion upon exposure to both NO and O_2^-, while human islets were protected by this compound only from NO, but not from superoxide anion radical (Moriscot et al. 2007).

5.6 Polyphenolic compounds

Polyphenolic compounds, naturally occurring in plants, were extensively studied in recent years as potential remedies against many diseases like cancer, cardio-vascular disorders and diabetes. They can be found in numerous dietary and medicinal plants and comprise an important part of the human diet, though they are generally viewed as nonnutrients. Particularly rich in polyphenols are red grapes, berries, tea leaves and some spices. These polyphenols comprise several types of compounds, i.e. phenolic acids, stilbenes, lignans and flavonoids, the latter are oxygen heterocycles (chromenes, for structures and more detailed classification see review by (Pandey and Rizvi 2009)). Flavonoids are formed in plants from aromatic amino acids phenylalanine and tyrosine, and malonate (Pietta 2000).

Polyphenols act as mild reductants in alkali and neutral pH by reducing common inorganic and organic oxidants and react with radicals both in reversible and irreversible ways, depending on their particular structure, thus, exerting direct antioxidant capacity (Pietta 2000; Pandey and Rizvi 2009). There are also other ways how polyphenols can chemically intervene into oxidative processes as shown in experiments *in vitro*: polyphenols can chelate transition metals like copper and iron, which catalyze propagation of radical chain reactions (Afanas'ev et al. 1995; Korkina and Afanas'ev 1997; Brown et al. 1998) and inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase (Arimboor et al. 2011) and protein kinase C (Ursini et al. 1994). However, interaction of flavonoids with isolated beef heart mitochondria in vitro caused an additional production of ROS due to inhibition of respiratory complex I (Hodnick et al. 1986; Hodnick et al. 1988; Hodnick, Duval, and Pardini 1994).

Several papers reported the protective effects of polyphenols *in vitro* on islets or cultured insulin-producing cells against oxidative challengers. It was also shown that polyphenols from olive leaves protected INS-1 cells from H_2O_2 toxicity (Cumaoglu et al. 2011). Cells pre-incubated with whole leaf extract or individual polyphenol compound oleuropein, followed by peroxide treatment, showed a lower percentage of necrotic and apoptotic death compared to untreated controls. Polyphenols stimulated activity of catalase, which resulted in a lower level of cellular ROS, along with improvement in insulin production. In this study it was suggested that polyphenols act through a redox-modulating mechanism rather than through direct free radical scavenging. Supplementation of cultured media with polyphenols from green tea increased recovery rates of isolated human and nonhuman primate islets. Polyphenols from tea extracts preserved islets by increasing the level of anti-apoptotic Bcl-2 and decreasing level of pro-apoptotic BAX (Zhang et al. 2004).

In animal models of both T1D and T2D, polyphenols of different origin were reported to lower blood glucose levels (Al-Awwadi et al. 2004; Su, Hung, and Chen 2006; Ciocoiu et al. 2009; Dixit and Kar 2010; Ong et al. 2011). The hypoglycemic effect can be caused by multiple mechanisms. Polyphenols can decrease glucose absorption from intestine as they inhibit amylase, a polysaccharide-hydrolyzing enzyme (Ong et al. 2011). On the other hand, some studies show that polyphenols enhance glucose uptake by muscle cells via increasing expression of glucose transporter Glut4 (Cao et al. 2007; Ong et al. 2011). The third possible mechanism of lowering blood glucose by these compounds could be an inhibition of gluconeogenesis in liver by inhibiting glucose-6-phosphatase, a key enzyme in this process (Ong et al. 2011). Lowering blood glucose itself alleviates excessive metabolic burden on islets and undoubtedly plays a positive role in preserving their mass and function, a notion which was confirmed by (Coskun et al. 2005; Hahm, Park, and Son 2011).

Administration of polyphenols to diabetic animals resulted in a lower level of markers of oxidative stress as judged by lower levels of products of lipid peroxidation in pancreatic homogenates (Coskun et al. 2005), kidneys (Lee, Wang, et al. 2009) and blood plasma (Ciocoiu et al. 2009; Hininger-Favier et al. 2009) of STZ-rats. This may be a result of direct antioxidant activity of polyphenols, as well as a result of their ability to upregulate expression of antioxidant-defense enzymes: glutathione peroxidase (GSHPx) superoxide dismutase and catalase activities (Ciocoiu et al. 2009; Lee, Wang, et al. 2009; Dixit and Kar 2010). It is well known that oxidative stress plays a substantial role in the destruction of beta-cells by infiltrated self macrophages and lymphocytes. The fact that administration of polyphenols to NOD mice, a model for autoimmune diabetes, significantly decreased incidence of the disease is evidence of a direct redox modulation by polyphenols (Zunino, Storms, and Stephensen 2007).

Numerous publications were devoted to study effects of polyphenols on a number of health-related functions. However, it should be noted that the effective therapeutic dose of natural polyphenols is rather high varying from 15 to 500mg/kg of animal body weight. This translates into 1 to 20g of polyphenols a day for a human patient of 65 kg of weight; the amount seems to be unfeasible to get as a part of regular diet or acute therapeutic treatment. However, studies on the effect of polyphenol-rich diets with much lower doses on human patients mostly of T2D report improvement in several metabolic responses, like blood glucose and lipid content, insulin sensitivity and markers of oxidative stress (Banini et al. 2006; Dembinska-Kiec et al. 2008; Stote and Baer 2008; Zunino 2009; Fenercioglu et al. 2010).

6. Concluding remarks

According to the current hypothesis, the lack of anti-ROS defense capacity in pancreatic beta-cells is related to the signaling role of oxidants in glucose sensing and insulin release. In normal physiological conditions the levels of oxidants are low and mitochondria play an essential role in the chain of signaling events by releasing ROS in a controlled manner. Redox reactions in beta-cells are in fine balance, but this balance can be destroyed by persistent hyperglycemia and hyperlipidemia. In conditions of diabetes beta-cells are imposed to a burden of free radicals not considered in evolution and, therefore, antioxidants administered in a proper way can alleviate the oxidative burden and offset the destruction of beta-cells.

7. References

Acharya, J. D., and S. S. Ghaskadbi. 2010. Islets and their antioxidant defense. *Islets* 2 (4):225-35.

Afanas'ev, I. B., E. A. Ostrachovitch, N. E. Abramova, and L. G. Korkina. 1995. Different antioxidant activities of bioflavonoid rutin in normal and iron-overloading rats. *Biochem Pharmacol* 50 (5):627-35.

Affourtit, C., M. Jastroch, and M. D. Brand. 2011. Uncoupling protein-2 attenuates glucose-stimulated insulin secretion in INS-1E insulinoma cells by lowering mitochondrial reactive oxygen species. *Free Radic Biol Med* 50 (5):609-16.

Al-Awwadi, N., J. Azay, P. Poucheret, G. Cassanas, M. Krosniak, C. Auger, F. Gasc, J. M. Rouanet, G. Cros, and P. L. Teissedre. 2004. Antidiabetic activity of red wine polyphenolic extract, ethanol, or both in streptozotocin-treated rats. *J Agric Food Chem* 52 (4):1008-16.

Arimboor, R., M. Rangan, S. G. Aravind, and C. Arumughan. 2011. Tetrahydroamentoflavone (THA) from Semecarpus anacardium as a potent inhibitor of xanthine oxidase. *J Ethnopharmacol* 133 (3):1117-20.

Armann, B., M. S. Hanson, E. Hatch, A. Steffen, and L. A. Fernandez. 2007. Quantification of basal and stimulated ROS levels as predictors of islet potency and function. *Am J Transplant* 7 (1):38-47.

Arner, E. S., J. Nordberg, and A. Holmgren. 1996. Efficient reduction of lipoamide and lipoic acid by mammalian thioredoxin reductase. *Biochem Biophys Res Commun* 225 (1):268-74.

Banday, A. A., A. Marwaha, L. S. Tallam, and M. F. Lokhandwala. 2005. Tempol reduces oxidative stress, improves insulin sensitivity, decreases renal dopamine D1 receptor hyperphosphorylation, and restores D1 receptor-G-protein coupling and function in obese Zucker rats. *Diabetes* 54 (7):2219-26.

Banini, A. E., L. C. Boyd, J. C. Allen, H. G. Allen, and D. L. Sauls. 2006. Muscadine grape products intake, diet and blood constituents of non-diabetic and type 2 diabetic subjects. *Nutrition* 22 (11-12):1137-45.

Batinic-Haberle, I., Z. Rajic, A. Tovmasyan, J. S. Reboucas, X. Ye, K. W. Leong, M. W. Dewhirst, Z. Vujaskovic, L. Benov, and I. Spasojevic. 2011. Diverse functions of cationic Mn(III) N-substituted pyridylporphyrins, recognized as SOD mimics. *Free Radic Biol Med* 51 (5):1035-53.

Benov, L., and I. Batinic-Haberle. 2005. A manganese porphyrin suppresses oxidative stress and extends the life span of streptozotocin-diabetic rats. *Free Radic Res* 39 (1):81-8.

Bindokas, V. P., A. Kuznetsov, S. Sreenan, K. S. Polonsky, M. W. Roe, and L. H. Philipson. 2003. Visualizing superoxide production in normal and diabetic rat islets of Langerhans. *J Biol Chem* 278 (11):9796-801.

Bottino, R., A. N. Balamurugan, S. Bertera, M. Pietropaolo, M. Trucco, and J. D. Piganelli. 2002. Preservation of human islet cell functional mass by anti-oxidative action of a novel SOD mimic compound. *Diabetes* 51 (8):2561-7.

Bottino, R., A. N. Balamurugan, H. Tse, C. Thirunavukkarasu, X. Ge, J. Profozich, M. Milton, A. Ziegenfuss, M. Trucco, and J. D. Piganelli. 2004. Response of human islets to isolation stress and the effect of antioxidant treatment. *Diabetes* 53 (10):2559-68.

Brown, J. E., H. Khodr, R. C. Hider, and C. A. Rice-Evans. 1998. Structural dependence of flavonoid interactions with Cu2+ ions: implications for their antioxidant properties. *Biochem J* 330 (Pt 3):1173-8.

Brownlee, M. 2003. A radical explanation for glucose-induced beta cell dysfunction. *J Clin Invest* 112 (12):1788-90.

Burkart, V., T. Koike, H. H. Brenner, Y. Imai, and H. Kolb. 1993. Dihydrolipoic acid protects pancreatic islet cells from inflammatory attack. *Agents Actions* 38 (1-2):60-5.

Cao, H., I. Hininger-Favier, M. A. Kelly, R. Benaraba, H. D. Dawson, S. Coves, A. M. Roussel, and R. A. Anderson. 2007. Green tea polyphenol extract regulates the expression of genes involved in glucose uptake and insulin signaling in rats fed a high fructose diet. *J Agric Food Chem* 55 (15):6372-8.

Ciocoiu, M., A. Miron, L. Mares, D. Tutunaru, C. Pohaci, M. Groza, and M. Badescu. 2009. The effects of Sambucus nigra polyphenols on oxidative stress and metabolic disorders in experimental diabetes mellitus. *J Physiol Biochem* 65 (3):297-304.

Coskun, O., M. Kanter, A. Korkmaz, and S. Oter. 2005. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. *Pharmacol Res* 51 (2):117-23.

Cumaoglu, A., L. Rackova, M. Stefek, M. Kartal, P. Maechler, and C. Karasu. 2011. Effects of olive leaf polyphenols against HO toxicity in insulin secreting beta-cells. *Acta Biochim Pol* 58 (1):45-50.

Czubkowski, P., P. Socha, and J. Pawlowska. 2011. Oxidative stress in liver transplant recipients. *Ann Transplant* 16 (1):99-108.

Darville, M. I., and D. L. Eizirik. 1998. Regulation by cytokines of the inducible nitric oxide synthase promoter in insulin-producing cells. *Diabetologia* 41 (9):1101-8.

De Rosa, S., P. Cirillo, A. Paglia, L. Sasso, V. Di Palma, and M. Chiariello. 2010. Reactive oxygen species and antioxidants in the pathophysiology of cardiovascular disease: does the actual knowledge justify a clinical approach? *Curr Vasc Pharmacol* 8 (2):259-75.

Dembinska-Kiec, A., O. Mykkanen, B. Kiec-Wilk, and H. Mykkanen. 2008. Antioxidant phytochemicals against type 2 diabetes. *Br J Nutr* 99 E Suppl 1:ES109-17.

Dixit, Y., and A. Kar. 2010. Protective role of three vegetable peels in alloxan induced diabetes mellitus in male mice. *Plant Foods Hum Nutr* 65 (3):284-9.

Faust, A., V. Burkart, H. Ulrich, C. H. Weischer, and H. Kolb. 1994. Effect of lipoic acid on cyclophosphamide-induced diabetes and insulitis in non-obese diabetic mice. *Int J Immunopharmacol* 16 (1):61-6.

Favre, D., G. Niederhauser, D. Fahmi, V. Plaisance, S. Brajkovic, N. Beeler, F. Allagnat, J. A. Haefliger, R. Regazzi, G. Waeber, and A. Abderrahmani. 2011. Role for inducible cAMP early repressor in promoting pancreatic beta cell dysfunction evoked by oxidative stress in human and rat islets. *Diabetologia* 54 (9):2337-46.

Fenercioglu, A. K., T. Saler, E. Genc, H. Sabuncu, and Y. Altuntas. 2010. The effects of polyphenol-containing antioxidants on oxidative stress and lipid peroxidation in Type 2 diabetes mellitus without complications. *J Endocrinol Invest* 33 (2):118-24.

Fridlyand, L. E., and L. H. Philipson. 2004. Does the glucose-dependent insulin secretion mechanism itself cause oxidative stress in pancreatic beta-cells? *Diabetes* 53 (8):1942-8.

Genova, M. L., M. M. Pich, A. Biondi, A. Bernacchia, A. Falasca, C. Bovina, G. Formiggini, G. Parenti Castelli, and G. Lenaz. 2003. Mitochondrial production of oxygen radical species and the role of Coenzyme Q as an antioxidant. *Exp Biol Med (Maywood)* 228 (5):506-13.

Gimeno, R. E., M. Dembski, X. Weng, N. Deng, A. W. Shyjan, C. J. Gimeno, F. Iris, S. J. Ellis, E. A. Woolf, and L. A. Tartaglia. 1997. Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. *Diabetes* 46 (5):900-6.

Graciano, M. F., L. R. Santos, R. Curi, and A. R. Carpinelli. 2011. NAD(P)H oxidase participates in the palmitate-induced superoxide production and insulin secretion by rat pancreatic islets. *J Cell Physiol* 226 (4):1110-7.

Grankvist, K., S. L. Marklund, and I. B. Taljedal. 1981. CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. *Biochem J* 199 (2):393-8.

Grankvist, K., S. Marklund, J. Sehlin, and I. B. Taljedal. 1979. Superoxide dismutase, catalase and scavengers of hydroxyl radical protect against the toxic action of alloxan on pancreatic islet cells in vitro. *Biochem J* 182 (1):17-25.

Grivennikova, V. G., and A. D. Vinogradov. 2006. Generation of superoxide by the mitochondrial Complex I. *Biochim Biophys Acta* 1757 (5-6):553-61.

Hahm, S. W., J. Park, and Y. S. Son. 2011. Opuntia humifusa stems lower blood glucose and cholesterol levels in streptozotocin-induced diabetic rats. *Nutr Res* 31 (6):479-87.

Halliwell, B and Gutteridge, J.M.C. 2001. *Free Radicals in Biology and Medicine*. Oxford: University Press.

Haramaki, N., D. Han, G. J. Handelman, H. J. Tritschler, and L. Packer. 1997. Cytosolic and mitochondrial systems for NADH- and NADPH-dependent reduction of alpha-lipoic acid. *Free Radic Biol Med* 22 (3):535-42.

Hininger-Favier, I., R. Benaraba, S. Coves, R. A. Anderson, and A. M. Roussel. 2009. Green tea extract decreases oxidative stress and improves insulin sensitivity in an animal model of insulin resistance, the fructose-fed rat. *J Am Coll Nutr* 28 (4):355-61.

Hodnick, W. F., D. L. Duval, and R. S. Pardini. 1994. Inhibition of mitochondrial respiration and cyanide-stimulated generation of reactive oxygen species by selected flavonoids. *Biochem Pharmacol* 47 (3):573-80.

Hodnick, W. F., F. S. Kung, W. J. Roettger, C. W. Bohmont, and R. S. Pardini. 1986. Inhibition of mitochondrial respiration and production of toxic oxygen radicals by flavonoids. A structure-activity study. *Biochem Pharmacol* 35 (14):2345-57.

Hodnick, W. F., E. B. Milosavljevic, J. H. Nelson, and R. S. Pardini. 1988. Electrochemistry of flavonoids. Relationships between redox potentials, inhibition of mitochondrial respiration, and production of oxygen radicals by flavonoids. *Biochem Pharmacol* 37 (13):2607-11.

Jegatheeswaran, S., and A. K. Siriwardena. 2011. Experimental and clinical evidence for modification of hepatic ischaemia-reperfusion injury by N-acetylcysteine during major liver surgery. *HPB (Oxford)* 13 (2):71-8.

Kaneto, H., G. Xu, K. H. Song, K. Suzuma, S. Bonner-Weir, A. Sharma, and G. C. Weir. 2001. Activation of the hexosamine pathway leads to deterioration of pancreatic beta-cell function through the induction of oxidative stress. *J Biol Chem* 276 (33):31099-104.

Khaldi, M. Z., H. Elouil, Y. Guiot, J. C. Henquin, and J. C. Jonas. 2006. Antioxidants N-acetyl-L-cysteine and manganese(III)tetrakis (4-benzoic acid)porphyrin do not prevent beta-cell dysfunction in rat islets cultured in high glucose for 1 wk. *Am J Physiol Endocrinol Metab* 291 (1):E137-46.

Konarkowska, B., J. F. Aitken, J. Kistler, S. Zhang, and G. J. Cooper. 2005. Thiol reducing compounds prevent human amylin-evoked cytotoxicity. *FEBS J* 272 (19):4949-59.

Korkina, L. G., and I. B. Afanas'ev. 1997. Antioxidant and chelating properties of flavonoids. *Adv Pharmacol* 38:151-63.

Kutlu, B., A. K. Cardozo, M. I. Darville, M. Kruhoffer, N. Magnusson, T. Orntoft, and D. L. Eizirik. 2003. Discovery of gene networks regulating cytokine-induced dysfunction and apoptosis in insulin-producing INS-1 cells. *Diabetes* 52 (11):2701-19.

Lacraz, G., F. Figeac, J. Movassat, N. Kassis, and B. Portha. 2010. Diabetic GK/Par rat beta-cells are spontaneously protected against H2O2-triggered apoptosis. A cAMP-dependent adaptive response. *Am J Physiol Endocrinol Metab* 298 (1):E17-27.

Lee, B. W., S. J. Kwon, H. Y. Chae, J. G. Kang, C. S. Kim, S. J. Lee, H. J. Yoo, J. H. Kim, K. S. Park, and S. H. Ihm. 2009. Dose-related cytoprotective effect of alpha-lipoic acid on hydrogen peroxide-induced oxidative stress to pancreatic beta cells. *Free Radic Res* 43 (1):68-77.

Lee, W. C., C. J. Wang, Y. H. Chen, J. D. Hsu, S. Y. Cheng, H. C. Chen, and H. J. Lee. 2009. Polyphenol extracts from Hibiscus sabdariffa Linnaeus attenuate nephropathy in experimental type 1 diabetes. *J Agric Food Chem* 57 (6):2206-10.

Leloup, C., C. Tourrel-Cuzin, C. Magnan, M. Karaca, J. Castel, L. Carneiro, A. L. Colombani, A. Ktorza, L. Casteilla, and L. Penicaud. 2009. Mitochondrial reactive oxygen species are obligatory signals for glucose-induced insulin secretion. *Diabetes* 58 (3):673-81.

Lenzen, S., J. Drinkgern, and M. Tiedge. 1996. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radic Biol Med* 20 (3):463-6.

Liu, Y., G. Fiskum, and D. Schubert. 2002. Generation of reactive oxygen species by the mitochondrial electron transport chain. *J Neurochem* 80 (5):780-7.

MacDonald, M. J., R. D. Husain, S. Hoffmann-Benning, and T. R. Baker. 2004. Immunochemical identification of coenzyme Q0-dihydrolipoamide adducts in the E2 components of the alpha-ketoglutarate and pyruvate dehydrogenase complexes partially explains the cellular toxicity of coenzyme Q0. *J Biol Chem* 279 (26):27278-85.

Maechler, P., and C. B. Wollheim. 2001. Mitochondrial function in normal and diabetic beta-cells. *Nature* 414 (6865):807-12.

Magnitsky, S., L. Toulokhonova, T. Yano, V. D. Sled, C. Hagerhall, V. G. Grivennikova, D. S. Burbaev, A. D. Vinogradov, and T. Ohnishi. 2002. EPR characterization of ubisemiquinones and iron-sulfur cluster N2, central components of the energy coupling in the NADH-ubiquinone oxidoreductase (complex I) in situ. *J Bioenerg Biomembr* 34 (3):193-208.

Michalska, M., G. Wolf, R. Walther, and P. Newsholme. 2010. Effects of pharmacological inhibition of NADPH oxidase or iNOS on pro-inflammatory cytokine, palmitic acid or H2O2-induced mouse islet or clonal pancreatic beta-cell dysfunction. *Biosci Rep* 30 (6):445-53.

Moriscot, C., S. Candel, V. Sauret, J. Kerr-Conte, M. J. Richard, M. C. Favrot, and P. Y. Benhamou. 2007. MnTMPyP, a metalloporphyrin-based superoxide dismutase/ catalase mimetic, protects INS-1 cells and human pancreatic islets from an in vitro oxidative challenge. *Diabetes Metab* 33 (1):44-53.

Nakada, S., T. Ishikawa, Y. Yamamoto, Y. Kaneko, and K. Nakayama. 2003. Constitutive nitric oxide synthases in rat pancreatic islets: direct imaging of glucose-induced nitric oxide production in beta-cells. *Pflugers Arch* 447 (3):305-11.

Nelson, D.L. and Cox, M.M 2005. *Lehninger Principles of Biochemistry*. Forth Edition ed. New York, NY: W.H. Freeman and Co.

Newsholme, P., E. P. Haber, S. M. Hirabara, E. L. Rebelato, J. Procopio, D. Morgan, H. C. Oliveira-Emilio, A. R. Carpinelli, and R. Curi. 2007. Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. *J Physiol* 583 (Pt 1):9-24.

Newsholme, P., D. Morgan, E. Rebelato, H. C. Oliveira-Emilio, J. Procopio, R. Curi, and A. Carpinelli. 2009. Insights into the critical role of NADPH oxidase(s) in the normal and dysregulated pancreatic beta cell. *Diabetologia* 52 (12):2489-98.

Nicholls, D.G. , and F.J Ferguson. 2002. Bioenergetics 3. Academic Press.

Ong, K. W., A. Hsu, L. Song, D. Huang, and B. K. Tan. 2011. Polyphenols-rich Vernonia amygdalina shows anti-diabetic effects in streptozotocin-induced diabetic rats. *J Ethnopharmacol* 133 (2):598-607.

Oprescu, A. I., G. Bikopoulos, A. Naassan, E. M. Allister, C. Tang, E. Park, H. Uchino, G. F. Lewis, I. G. Fantus, M. Rozakis-Adcock, M. B. Wheeler, and A. Giacca. 2007. Free fatty acid-induced reduction in glucose-stimulated insulin secretion: evidence for a role of oxidative stress in vitro and in vivo. *Diabetes* 56 (12):2927-37.

Packer, L., E. H. Witt, and H. J. Tritschler. 1995. alpha-Lipoic acid as a biological antioxidant. *Free Radic Biol Med* 19 (2):227-50.

Pandey, K. B., and S. I. Rizvi. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev* 2 (5):270-8.

Parasassi, T., R. Brunelli, G. Costa, M. De Spirito, E. Krasnowska, T. Lundeberg, E. Pittaluga, and F. Ursini. 2010. Thiol redox transitions in cell signaling: a lesson from N-acetylcysteine. *ScientificWorldJournal* 10:1192-202.

Patel, M., and B. J. Day. 1999. Metalloporphyrin class of therapeutic catalytic antioxidants. *Trends Pharmacol Sci* 20 (9):359-64.

Pi, J., Y. Bai, K. W. Daniel, D. Liu, O. Lyght, D. Edelstein, M. Brownlee, B. E. Corkey, and S. Collins. 2009. Persistent oxidative stress due to absence of uncoupling protein 2 associated with impaired pancreatic beta-cell function. *Endocrinology* 150 (7):3040-8.

Pietta, P. G. 2000. Flavonoids as antioxidants. *J Nat Prod* 63 (7):1035-42.

Pocernich, C. B., M. L. Bader Lange, R. Sultana, and D. A. Butterfield. 2011. Nutritional approaches to modulate oxidative stress in Alzheimer's disease. *Curr Alzheimer Res* 8 (5):452-69.

Ramudo, L., and M. A. Manso. 2010. N-acetylcysteine in acute pancreatitis. *World J Gastrointest Pharmacol Ther* 1 (1):21-6.

Ricquier, D., and F. Bouillaud. 2000. Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance. *J Physiol* 529 Pt 1:3-10.

Rigoulet, M., E. D. Yoboue, and A. Devin. 2011. Mitochondrial ROS generation and its regulation: mechanisms involved in H(2)O(2) signaling. *Antioxid Redox Signal* 14 (3):459-68.

Robertson, R. P., J. Harmon, P. O. Tran, Y. Tanaka, and H. Takahashi. 2003. Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes* 52 (3):581-7.

Sakai, K., K. Matsumoto, T. Nishikawa, M. Suefuji, K. Nakamaru, Y. Hirashima, J. Kawashima, T. Shirotani, K. Ichinose, M. Brownlee, and E. Araki. 2003. Mitochondrial reactive oxygen species reduce insulin secretion by pancreatic beta-cells. *Biochem Biophys Res Commun* 300 (1):216-22.

Sakuraba, H., H. Mizukami, N. Yagihashi, R. Wada, C. Hanyu, and S. Yagihashi. 2002. Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese Type II diabetic patients. *Diabetologia* 45 (1):85-96.

Santos, L. R., E. Rebelato, M. F. Graciano, F. Abdulkader, R. Curi, and A. R. Carpinelli. 2011. Oleic Acid Modulates Metabolic Substrate Channeling during Glucose-Stimulated Insulin Secretion via NAD(P)H Oxidase. *Endocrinology* 152 (10):3614-21.

Schroeder, M. M., R. J. Belloto, Jr., R. A. Hudson, and M. F. McInerney. 2005. Effects of antioxidants coenzyme Q10 and lipoic acid on interleukin-1 beta-mediated inhibition of glucose-stimulated insulin release from cultured mouse pancreatic islets. *Immunopharmacol Immunotoxicol* 27 (1):109-22.

Selivanov, V. A., T. V. Votyakova, V. N. Pivtoraiko, J. Zeak, T. Sukhomlin, M. Trucco, J. Roca, and M. Cascante. 2011. Reactive oxygen species production by forward and reverse electron fluxes in the mitochondrial respiratory chain. *PLoS Comput Biol* 7 (3):e1001115.

Selivanov, V. A., T. V. Votyakova, J. A. Zeak, M. Trucco, J. Roca, and M. Cascante. 2009. Bistability of mitochondrial respiration underlies paradoxical reactive oxygen species generation induced by anoxia. *PLoS Comput Biol* 5 (12):e1000619.

Selivanov, V. A., J. A. Zeak, J. Roca, M. Cascante, M. Trucco, and T. V. Votyakova. 2008. The role of external and matrix pH in mitochondrial reactive oxygen species generation. *J Biol Chem* 283 (43):29292-300.

Shen, W., K. Liu, C. Tian, L. Yang, X. Li, J. Ren, L. Packer, E. Head, E. Sharman, and J. Liu. 2008. Protective effects of R-alpha-lipoic acid and acetyl-L-carnitine in MIN6 and isolated rat islet cells chronically exposed to oleic acid. *J Cell Biochem* 104 (4):1232-43.

Sklavos, M. M., S. Bertera, H. M. Tse, R. Bottino, J. He, J. N. Beilke, M. G. Coulombe, R. G. Gill, J. D. Crapo, M. Trucco, and J. D. Piganelli. 2010. Redox modulation protects islets from transplant-related injury. *Diabetes* 59 (7):1731-8.

Skulachev, V. P., V. N. Anisimov, Y. N. Antonenko, L. E. Bakeeva, B. V. Chernyak, V. P. Erichev, O. F. Filenko, N. I. Kalinina, V. I. Kapelko, N. G. Kolosova, B. P. Kopnin, G. A. Korshunova, M. R. Lichinitser, L. A. Obukhova, E. G. Pasyukova, O. I. Pisarenko, V. A. Roginsky, E. K. Ruuge, Senin, II, Severina, II, M. V. Skulachev, I. M. Spivak, V. N. Tashlitsky, V. A. Tkachuk, M. Y. Vyssokikh, L. S. Yaguzhinsky, and D. B. Zorov. 2009. An attempt to prevent senescence: a mitochondrial approach. *Biochim Biophys Acta* 1787 (5):437-61.

Song, K. H., W. J. Lee, J. M. Koh, H. S. Kim, J. Y. Youn, H. S. Park, E. H. Koh, M. S. Kim, J. H. Youn, K. U. Lee, and J. Y. Park. 2005. alpha-Lipoic acid prevents diabetes mellitus in diabetes-prone obese rats. *Biochem Biophys Res Commun* 326 (1):197-202.

Soule, B. P., F. Hyodo, K. Matsumoto, N. L. Simone, J. A. Cook, M. C. Krishna, and J. B. Mitchell. 2007. The chemistry and biology of nitroxide compounds. *Free Radic Biol Med* 42 (11):1632-50.

Starkov, A. A., and G. Fiskum. 2003. Regulation of brain mitochondrial H2O2 production by membrane potential and NAD(P)H redox state. *J Neurochem* 86 (5):1101-7.

Starkov, A. A., B. M. Polster, and G. Fiskum. 2002. Regulation of hydrogen peroxide production by brain mitochondria by calcium and Bax. *J Neurochem* 83 (1):220-8.

Stote, K. S., and D. J. Baer. 2008. Tea consumption may improve biomarkers of insulin sensitivity and risk factors for diabetes. *J Nutr* 138 (8):1584S-1588S.

Su, H. C., L. M. Hung, and J. K. Chen. 2006. Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. *Am J Physiol Endocrinol Metab* 290 (6):E1339-46.

Takatori, A., Y. Ishii, S. Itagaki, S. Kyuwa, and Y. Yoshikawa. 2004. Amelioration of the beta-cell dysfunction in diabetic APA hamsters by antioxidants and AGE inhibitor treatments. *Diabetes Metab Res Rev* 20 (3):211-8.

Tanaka, Y., C. E. Gleason, P. O. Tran, J. S. Harmon, and R. P. Robertson. 1999. Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. *Proc Natl Acad Sci U S A* 96 (19):10857-62.

Tanaka, Y., P. O. Tran, J. Harmon, and R. P. Robertson. 2002. A role for glutathione peroxidase in protecting pancreatic beta cells against oxidative stress in a model of glucose toxicity. *Proc Natl Acad Sci U S A* 99 (19):12363-8.

Tang, C., P. Han, A. I. Oprescu, S. C. Lee, A. V. Gyulkhandanyan, G. N. Chan, M. B. Wheeler, and A. Giacca. 2007. Evidence for a role of superoxide generation in glucose-induced beta-cell dysfunction in vivo. *Diabetes* 56 (11):2722-31.

Targonsky, E. D., F. Dai, V. Koshkin, G. T. Karaman, A. V. Gyulkhandanyan, Y. Zhang, C. B. Chan, and M. B. Wheeler. 2006. alpha-lipoic acid regulates AMP-activated protein kinase and inhibits insulin secretion from beta cells. *Diabetologia* 49 (7):1587-98.

Tiedge, M., S. Lortz, J. Drinkgern, and S. Lenzen. 1997. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes* 46 (11):1733-42.

Tsubouchi, H., T. Inoguchi, M. Inuo, M. Kakimoto, T. Sonta, N. Sonoda, S. Sasaki, K. Kobayashi, H. Sumimoto, and H. Nawata. 2005. Sulfonylurea as well as elevated glucose levels stimulate reactive oxygen species production in the pancreatic beta-cell line, MIN6-a role of NAD(P)H oxidase in beta-cells. *Biochem Biophys Res Commun* 326 (1):60-5.

Turrens, J. F. 2003. Mitochondrial formation of reactive oxygen species. *J Physiol* 552 (Pt 2):335-44.

Uchizono, Y., R. Takeya, M. Iwase, N. Sasaki, M. Oku, H. Imoto, M. Iida, and H. Sumimoto. 2006. Expression of isoforms of NADPH oxidase components in rat pancreatic islets. *Life Sci* 80 (2):133-9.

Ursini, F., M. Maiorino, P. Morazzoni, A. Roveri, and G. Pifferi. 1994. A novel antioxidant flavonoid (IdB 1031) affecting molecular mechanisms of cellular activation. *Free Radic Biol Med* 16 (5):547-53.

Vinogradov, A. D. 2008. NADH/NAD+ interaction with NADH: ubiquinone oxidoreductase (complex I). *Biochim Biophys Acta* 1777 (7-8):729-34.

Votyakova, T. V., and I. J. Reynolds. 2001. DeltaPsi(m)-Dependent and -independent production of reactive oxygen species by rat brain mitochondria. *J Neurochem* 79 (2):266-77.

Wang, X., H. Li, D. De Leo, W. Guo, V. Koshkin, I. G. Fantus, A. Giacca, C. B. Chan, S. Der, and M. B. Wheeler. 2004. Gene and protein kinase expression profiling of reactive oxygen species-associated lipotoxicity in the pancreatic beta-cell line MIN6. *Diabetes* 53 (1):129-40.

Welsh, N., B. Margulis, L. A. Borg, H. J. Wiklund, J. Saldeen, M. Flodstrom, M. A. Mello, A. Andersson, D. G. Pipeleers, C. Hellerstrom, and et al. 1995. Differences in the expression of heat-shock proteins and antioxidant enzymes between human and rodent pancreatic islets: implications for the pathogenesis of insulin-dependent diabetes mellitus. *Mol Med* 1 (7):806-20.

Wilcox, C. S. 2010. Effects of tempol and redox-cycling nitroxides in models of oxidative stress. *Pharmacol Ther* 126 (2):119-45.

Wilcox, C. S., and A. Pearlman. 2008. Chemistry and antihypertensive effects of tempol and other nitroxides. *Pharmacol Rev* 60 (4):418-69.

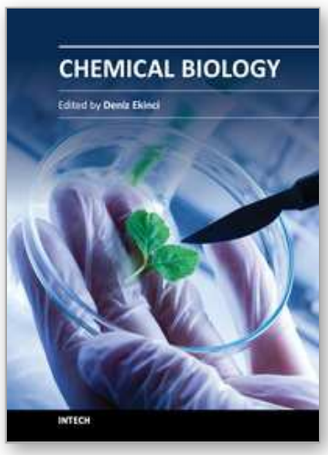
Zhang, G., S. Matsumoto, S. H. Hyon, S. A. Qualley, L. Upshaw, D. M. Strong, and J. A. Reems. 2004. Polyphenol, an extract of green tea, increases culture recovery rates of isolated islets from nonhuman primate pancreata and marginal grade human pancreata. *Cell Transplant* 13 (2):145-52.

Zhang, Z., J. Jiang, P. Yu, X. Zeng, J. W. Larrick, and Y. Wang. 2009. Hypoglycemic and beta cell protective effects of andrographolide analogue for diabetes treatment. *J Transl Med* 7:62.

Zraika, S., K. Aston-Mourney, D. R. Laybutt, M. Kebede, M. E. Dunlop, J. Proietto, and S. Andrikopoulos. 2006. The influence of genetic background on the induction of oxidative stress and impaired insulin secretion in mouse islets. *Diabetologia* 49 (6):1254-63.

Zunino, S. 2009. Type 2 diabetes and glycemic response to grapes or grape products. *J Nutr* 139 (9):1794S-800S.

Zunino, S. J., D. H. Storms, and C. B. Stephensen. 2007. Diets rich in polyphenols and vitamin A inhibit the development of type I autoimmune diabetes in nonobese diabetic mice. *J Nutr* 137 (5):1216-21.



Chemical Biology

Edited by Prof. Deniz Ekinci

ISBN 978-953-51-0049-2
Hard cover, 444 pages
Publisher InTech
Published online 17, February, 2012
Published in print edition February, 2012

Chemical biology utilizes chemical principles to modulate systems to either investigate the underlying biology or create new function. Over recent years, chemical biology has received particular attention of many scientists in the life sciences from botany to medicine. This book contains an overview focusing on the research area of protein purification, enzymology, vitamins, antioxidants, biotransformation, gene delivery, signaling, regulation and organization. Particular emphasis is devoted to both theoretical and experimental aspects. The textbook is written by international scientists with expertise in synthetic chemistry, protein biochemistry, enzymology, molecular biology, drug discovery and genetics many of which are active chemical, biochemical and biomedical research. The textbook is expected to enhance the knowledge of scientists in the complexities of chemical and biological approaches and stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of chemical biology.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen