We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Oxidative Stress in Diabetes Mellitus and the Role Of Vitamins with Antioxidant Actions

Maria-Luisa Lazo-de-la-Vega-Monroy and
Cristina Fernández-Mejía

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/51788

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects of insulin action, insulin secretion or both [1]. Diabetes has taken place as one of the most important diseases worldwide, reaching epidemic proportions. Global estimates predict that the proportion of adult population with diabetes will increase 69% for the year 2030 [2].

Hyperglycemia in the course of diabetes usually leads to the development of microvascular complications, and diabetic patients are more prone to accelerated atherosclerotic macrovascular disease. These complications account for premature mortality and most of the social and economical burden in the long term of diabetes [3].

Increasing evidence suggests that oxidative stress plays a role in the pathogenesis of diabetes mellitus and its complications [4]. Hyperglycemia increases oxidative stress, which contributes to the impairment of the main processes that fail during diabetes, insulin action and insulin secretion. In addition, antioxidant mechanisms are diminished in diabetic patients, which may further augment oxidative stress [5, 6]. Several studies have addressed the possible participation of dietary antioxidants, such as vitamins, in ameliorating the diabetic state and retarding the development of diabetes complications [7, 8].

The aim of this chapter is to revise the current knowledge of the role of oxidative stress in the pathogenesis of diabetes mellitus and its complications, and to discuss the existing evidence of the effects of vitamins as antioxidant therapy for this disease.

2. Oxidative stress

At the beginning of life, the organisms obtained their energy (ATP) by anoxygenic photosinthesis, for which oxygen was toxic. Most of the metabolic pathways were developed during this anaerobic stage of life, in which oxygen came later. Cyanobacteria started producing oxygen from photosynthesis, which raised the atmospheric oxygen, and favored those organisms which have evolved into eukaryotic cells with mitochondria, able to use oxygen for a more efficient energy production [9].

Whenever a cell's internal environment is perturbed by infections, disease, toxins or nutritional imbalance, mitochondria diverts electron flow away from itself, forming reactive oxygen species (ROS) and reactive nitrogen species (RNS), thus lowering oxygen consumption. This "oxidative shielding" acts as a defense mechanism for either decreasing cellular uptake of toxic pathogens or chemicals from the environment, or to kill the cell by apoptosis and thus avoid the spreading to neighboring cells [9]. Therefore, ROS formation is a physiological response to stress.

The term "oxidative stress" has been used to define a state in which ROS and RNS reach excessive levels, either by excess production or insufficient removal. Being highly reactive molecules, the pathological consequence of ROS and RNS excess is damage to proteins, lipids and DNA [10]. Consistent with the primary role of ROS and RNS formation, this oxidative stress damage may lead to physiological dysfunction, cell death, pathologies such as diabetes and cancer, and aging of the organism [11].

2.1. ROS and RNS production

ROS and RNS are highly reactive molecules, which can be free radicals such as superoxide ($^{\bullet}O_2^{\,-}$), hydroxyl ($^{\bullet}OH$), peroxyl ($^{\bullet}RO_2$), hydroperoxyl ($^{\bullet}HRO_2^{\,-}$), nitric oxide ($^{\bullet}NO$) and nitrogen dioxide ($^{\bullet}NO_2^{\,-}$), or nonradicals such as hydrogen peroxide (H_2O_2), hydrochlorous acid (HOCl), peroxynitrite ($ONOO^-$), nitrous oxide (HNO_2), and alkyl peroxynitrates (RONOO). Most of the studies regarding diabetes and its complications have addressed the role of superoxide ($^{\bullet}O_2^{\,-}$), nitric oxide ($^{\bullet}NO$), and peroxynitrite ($ONOO^-$) in this disease. There are basically two pathways for $^{\bullet}O_2^{\,-}$ production: NADPH oxidases and mitochondrial function, while $^{\bullet}NO$ and $ONOO^-$ are produced by the Nitric Oxide Synthase pathway [10].

2.1.1. NADPH oxidases

Oxidases are enzymes which catalyze redox reactions involving molecular oxygen (O_2). Superoxide is generated by oxidases via one-electron reduction of oxygen and the oxidation of their substrates. Several oxidases exist in the body, such as xantine oxidase, glucose oxidase, monoamine oxidase, cytochrome P450 oxidase, and NADPH oxidases.

NADP in the cell exists in its reduced (NADPH) and oxidized (NADP+) forms. NADPH supplies reducing power in reactions for biosynthesis, and it also serves as electron donor substrate for the NADPH oxidase. This enzyme is a membrane-bound electron transport complex which pumps electrons from NADPH in the cytosol across biological membranes

and into intracellular and extracellular compartments, such as nucleus, endoplasmic reticu‐lum, endosome, phagosome, mitochondria and extracellular space. It is the only enzyme whose primary function is generating superoxide and/or hydrogen peroxide, mainly for preventing the transfer of pathogens and for cellular bactericidal function[12, 13].

2.1.2. Mitochondrial electron transport chain

Mitochondrion is the site of eukaryotic oxidative metabolism. It contains the enzymes need‐ed for converting pyruvate into Acetyl-CoA, the citric acid cycle (also known as the Krebs cycle) and for fatty acid oxidation. Additionally, it performs the electron transport and oxi‐dative phosphorylation. Substrate (amino acid, fatty acid and carbohydrate) oxidation in the citric acid cycle release electrons, which are transferred to the coenzymes NAD+ and FAD to form NADH and FADH2. These electrons then pass into the mitochondrial electron-trans‐port chain, a system of linked electron carrier proteins comprised by Complexes I, II, III and IV. Complex I, III, and IV drive the exit of protons from the mitochondrial matrix, producing a proton gradient across the inner mitochondrial membrane. The free energy stored in this electrochemical gradient drives the condensation of ADP with inorganic phosphate in order to form ATP by oxidative phosphorylation. Along this electron transport, molecular oxygen is the final electron acceptor, which will be then reduced to H_2O [14, 15]. However, between 0.4 and 4% of all oxygen consumed will be converted into superoxide anion [16]. There is also a normal threshold for protonic potential above which electron transfer is inhibited at complex III, causing the electrons to go back to complex II where there are transferred to molecular oxygen prematurely and not to complex IV as it naturally occurs. Therefore, the endproduct of this transfer is superoxide [17].

Mitochondria play an important role in the maintenance of cellular redox status, acting as a redox sink and limiting NADPH oxidase activity. However, when the proton potential threshold is surpassed, mitochondria is also a significant source of ROS, which may further stimulate NADPH oxidases, creating a vicious cycle of ROS production [18]. When mito‐chondria cannot further extract oxygen, cell and tissue oxygen levels rise, decreasing the tis‐sue extraction of oxygen from the blood. This results in tissue vascularity reduction, which may be associated with peripheral vascular disease and, in time, chronic tissue hypoxia and ischemia [9].

2.1.3. NO and RNS production

Nitric oxide •NO is produced by the enzyme nitric oxide synthase (NOS), of which there are three isoforms: neural (nNOS or NOS-I) expressed in neurons, inducible (iNOS or NOS-II) expressed in smooth muscle of bold vessels, hepatocites, macrophagues and neuroendocirne tissue, and endothelial (eNOS or NOS-III) expressed constitutively in endothelial cells. iNOS and eNOS can be stimulated by the redox state in the cell, cytokines, hormones and nu‐trients [19, 20]. NOS catalyze the oxidation of the terminal guanidine nitrogen of the L–argi‐nine, in presence of oxygen and NADPH, to yield L-citruline and •NO [21].

Once produced and released, •NO can diffuse freely through membranes or act on different cellular targets. •NO participates as mediator of several physiological effects such as vasore-laxation, macrophague activation, gene expression and apoptosis. Usually, •NO is considered as a vasculoprotective molecule. However, one of its multiple effects is also protein nitrosilation at the thiol groups and RNS generation such as peroxynitrite ($ONOO^-$), as •NO easily reacts with $•O_2^-$. Therefore, the amount of $•O_2^-$ determines whether •NO acts as a protective or harmful molecule [10, 22].

2.2. Antioxidant defenses in the organism

As a small part the oxygen consumed for aerobic processes will be converted into superoxide anion [16], which will have to be scavenged or converted into less reactive (and harmful) molecules. The main enzymes that regulate this process are Superoxide dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Catalase (Figure 1). When ROS overproduction or chronic hyperglycemia occurs, the activity of these enzymes is insufficient, leading to more ROS and RNS formation and activation oxidative stress pathways.
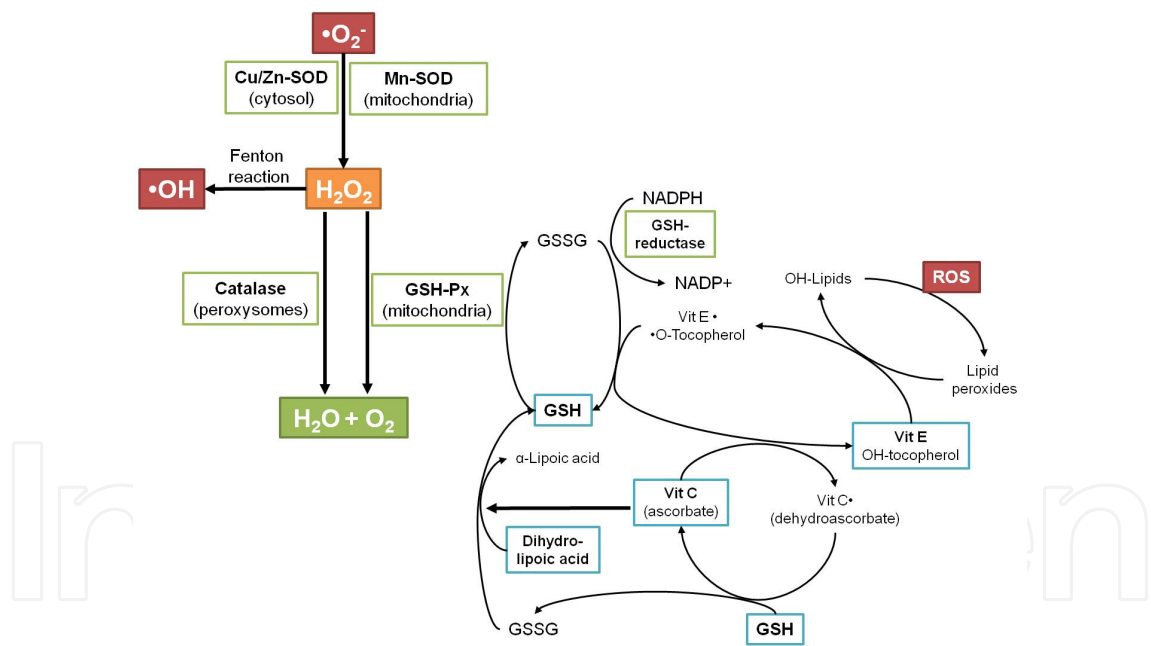

Figure 1. Antioxidant defenses in the organism.

SOD is considered a first-line defense against ROS. This enzyme is present in nearly all cells, and converts $•O_2^-$ into H_2O_2. Mitochondrial and bacterial SOD contain Mn, while cytosolic SOD is a dimer containing Cu and Zn. As the H_2O_2 may still react with other ROS, it needs to be degraded by either one of the other two antioxidant enzymes, GSH-Px or catalase [10, 12].

GSH peroxidase is located in the mitochondria. It catalyzes degradation of H_2O_2 by reduction, where two gluthathione (GSH) molecules are oxidized to glutathione disulfide (GSSG).

Regeneration of GSH by GSH-reductase, requires NADPH, which is oxidized to NADP+. Catalase, on the other hand, is localized primarily in peroxisomes, and so it detoxifies the H_2O_2 that diffuses from the mitochondria to the cytosol, converting it into water and molecular oxygen [10, 12].

There are also nonenzymatic antioxidant mechanisms, which mostly help regenerate GSSG back into GSH. Antioxidant vitamins such as A, C, E and alpha-lipoic acid are among these mechanisms. Although all these antioxidant defenses work together to eliminate H_2O_2 (and thus superoxide) from the cell, in the presence of reduced transition metals (Cu, Fe), H_2O_2 can be transformed into ·OH, which is a highly reactive ROS, by the Fenton reaction [10, 23].

2.3. Metabolic and signaling pathways involved in oxidative stress in diabetes

There are several molecular pathways involved in ROS formation and ROS induced damage. Here we will review the ones that have been related to oxidative stress in diabetes. Not surprisingly, most of them are related to glucose and/or lipid metabolism.

2.3.1. Glucose oxidation and GAPDH

In order to generate energy, glucose needs to be first oxidized inside the cells by glycolysis. In this process, once glucose enters the cells, it is phosphorylated to form glucose-6-phosphate, a reaction mediated by hexocinases. Glucose-6-P is then converted to Fructose-6-P by phosphoglucoisomerase, which can undergo two fates: the pentose phosphate pathway, where reduction of $NADP^+$ to NADPH occurs, or to continue glycolysis to yield Gliceraldehyde-3-P. Glyceraldehyde-3-P dehydrogenase (GAPDH) phosphorylates this product and glycolysis is further completed until its end product pyruvate, which enters the Krebs cycle and mitochondrial metabolism (Figure 2).

It has been proposed that hyperglycemia-induced mitochondrial superoxide production activates damaging pathways by inhibiting glyderaldehyde-3-phosphate dehydrogenase (GAPDH) [4, 24], an enzyme that normally translocates in and out of the nucleus [25, 26]. ROS inhibit glyderaldehyde-3-phosphate dehydrogenase through a mechanism involving the activation of enzyme poly-ADP-ribose polymerase-1 (PARP-1). This enzyme is involved in DNA repair and apoptotic pathways. ROS cause strand breaks in nuclear DNA which activates PARP-1. PARP-1 activation results in inhibition of glyderaldehyde-3-phosphate dehydrogenase by poly-ADP-ribosylation [27]. This results in increased levels of all the glycolytic intermediates upstream of GAPDH. Accumulation of glyceraldehyde 3-phosphate activates two major pathways involved in hyperglycemia-complications: a) It activates the AGE pathway deriving glyceraldehyde phosphate and dihydroxyacetone phosphate to the nonenzymatic synthesis of methylglyoxal. b) Increased glyceraldehyde 3-phosphate favors diacylglycerol production which activates PKC pathway. Further upstream, levels of the glycolytic metabolite fructose 6-phosphate increase, which then increases flux through the hexosamine pathway, where fructose 6-phosphate is converted by the enzyme glutamine-fructose-6-phosphate amidotransferase (GFAT) to UDP–N-Acetylglucos-

amine. Finally, inhibition of GAPDH favors the accumulation of the first glycolytic metabolite, glucose. This increases its flux through the polyol pathway, consuming NADPH in the process [24].

2.3.2. The polyol pathway

The family of aldo-keto reductase enzymes catalyzes the reduction of a wide variety of carbonyl compounds to their respective alcohols. These reactions utilize nicotinic acid adenine dinucleotide phosphate (NADPH). Aldo-keto reductase has a low affinity (high Km) for glucose, and at the normal glucose concentrations, metabolism of glucose by this pathway is a very small percentage of total glucose metabolism. However, in a hyperglycemic environment, increased intracellular glucose results in its increased enzymatic conversion to the polyalcohol sorbitol, with concomitant decreases in NADPH [4] (Figure 2). Since NADPH is a cofactor required to regenerate reduced glutathione, an antioxidant mechanism, and this compound is an important scavenger of reactive oxygen species (ROS), this could induce or exacerbate intracellular oxidative stress [24]. Moreover, sorbitol is oxidated to fructose by sorbitol dehydrogenase, which can lead to PKC activation via the increased NADH/NAD+ ratio [4]. Although this mechanism does not produce ROS in a direct way, it takes part in the redox imbalance causing oxidative stress.

2.3.3. Hexosamine pathway

When glucose levels are within normal range, a relatively low amount of fructose-6-P is drived away from glycolysis. If intracellular glucose rises, excess fructose-6-phosphate is diverted from glycolysis to provide substrate for the rate-limiting enzyme of this pathway, GFAT. This enzyme converts fructose 6-phosphate to glucosamine 6-phosphate, which is then converted to UDP-NAcetylglucosamine, which is essential for making the glycosyl chains of proteins and lipids. Specific O-Glucosamine-N-Acetyl transferases use this metabolite for post-translational modification of specific serine and threonine residues on cytoplasmic and nuclear proteins [24, 28].

2.3.4. Diacylglycerol formation and PKC activation

The Protein Kinase C (PKC) family comprises at least eleven isoforms of serine/threonine kinases, which participate in signaling pathways activated by phosphatidyl serine, Calcium and Diacylglycerol (DAG). DAG levels are elevated chronically in the hyperglycemic or diabetic environment due to an increase in the glycolytic intermediate dihydroxyacetone phosphate (figure 2). This intermediate is reduced to glycerol-3-phosphate, which, conjugated with fatty acids, increases de novo synthesis of DAG [29]. Evidence suggests that the enhanced activity of PKC isoforms could arise from inhibition of the glycolytic enzyme glyceraldehide-3-phosphate dehydrogenase by increased ROS intracellular levels [4, 24]. Other studies suggest that enhanced activity of PKC isoforms could also result from the interaction between AGEs and their extracellular receptors [30]. PKC isoforms constitute a wide range of cellular signals, including activation of NADPH oxidase and NF-κB, resulting in excessive ROS production. They also increase vascular permeability, stabilize

vascular endothelial growth factor (VEGF) mRNA expression and increase leukocyte-endothelium interaction [11].

2.3.5. Glyceraldehyde autoxidation

Accumulation of glyceraldehyde 3-phosphate, besides activating the AGE formation and the PKC pathway, it can oxidize itself. This autoxidation generates H_2O_2, which further contributes to oxidative stress [31].

2.3.6. Advanced glycation end-products (AGEs)

Intracellular hyperglycaemia is the primary initiating event in the formation of both intracellular and extracellular AGEs [32]. AGEs can arise from intracellular auto-oxidation of glucose to glyoxal, decomposition of the Amadori product (glucose-derived 1-amino-1-deoxyfructose lysine adducts) to 3-deoxyglucosone (perhaps accelerated by an amadoriase), and nonenzymatic phosphate elimination from glyceraldehyde phosphate and dihydroxyacetone phosphate to form methylglyoxal. These reactive intracellular dicarbonyl glyoxal, methylglyoxal and 3-deoxyglucosone react with amino groups of intracellular and extracellular proteins to form AGEs [4]. Intracellular production of AGE precursors can damage cells by three general mechanisms: 1) Intracellular proteins modified by AGEs have altered function, 2) Extracellular matrix components modified by AGE precursors interact abnormally with other matrix components and with matrix receptors (integrins) that are expressed on the surface of cells, and 3) Plasma proteins modified by AGE precursors bind to AGE receptors (such as RAGE and AGE-R1,2 and 3) on cells such as macrophages, vascular endothelial cells and vascular smooth muscle cells. AGE receptors binding induces the production of ROS, which in turn activates PKC. It also activates NF-κB and NADPH oxidase, and disturbs MAPK signaling [31].

2.3.7. Stress-sensitive signaling pathways

In addition to direct damage of biomolecules in the cells, oxidative stress is also involved in activation of several stress-sensitive signaling pathways, which can result in inflammation, cytokine release, and even apoptosis. Among these pathways we find the transcription factor NF-κB, which together with PARP acts as a transcriptional coactivator of inflammation molecules such as iNOS, intracellular adhesion molecule-1 (ICAM-I), and histocompatibility complex class II [33]. p38 MAPK pathway and c-Jun Nterminal kinase (JNK) (also known as stress-activated protein kinase (SAPK) participate in cellular responses to stress due to osmotic shock, cytokines and UV light, playing a role in cellular proliferation, apoptosis, and inflammatory responses [33]. Jak/STAT is another important signaling pathway, which initiates and mediates cellular responses to cytokines such as interferons and interleukins [33].
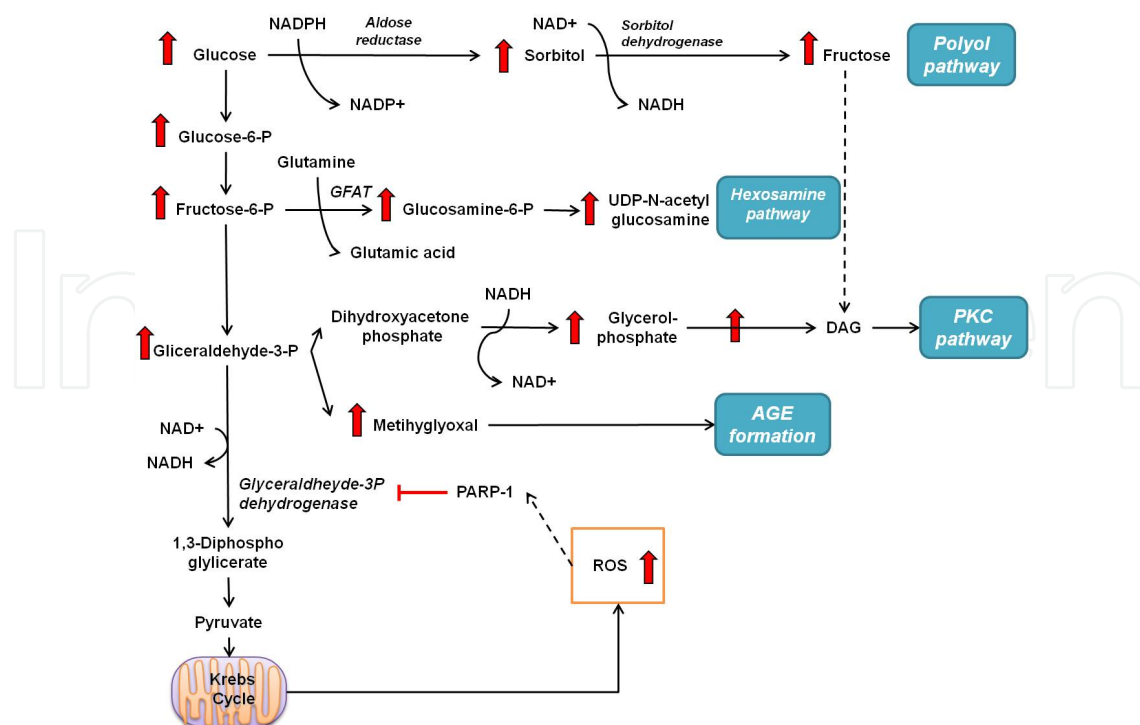
Figure 2. Oxidative stress-related pathways derived from glucose metabolism.

2.4. ROS induced damages

Being highly reactive species, ROS may modify and damage nucleic acids, proteins, lipids and carbohydrates, finally leading to cell damage. Among the motifs that can react with ROS we have the metal ligand from metalloproteases and Fe from oxihemoglobin. $^{\bullet}O_2^{\ -}$ can also modify and inhibit catalases, while $^{\bullet}OH$ can bind to the histidine residue from SOS causing its inhibition. ROS react mostly with insaturated and sulfur containing molecules, thus, proteins with high contents of tryptophan, tyrosine, phenylalanine, histidine, methionine and cysteine can suffer ROS modifications. Finally, ROS may also break peptidic bonds after oxidation of proline residues by $^{\bullet}O_2^{\ -}$ or $^{\bullet}OH$ [31].

ROS and RNS may also modify fatty acids, lipoproteins, and phospholipids, a process termed lipid peroxidation, where $^{\bullet}OH$ and $^{\bullet}O_2^{\ -}$ form hydroperoxide lipids. Hydroperoxyde products cause severe damage to plasma membranes, or they can diffuse to other cells in the organisms and cause vascular permeability and inflammation by binding to (oxidized low-density lipoprotein) LOX receptors, and apoptosis [31].

H_2O_2 in cells can function as a signaling molecule leading to cellular proliferation or can result in cell death. At low concentrations, H_2O_2 serves as a second messenger to activate NF-κB and various kinases (p38 MAPK, ERK, PI3K, Akt, JAK2, STAT). H_2O_2 at slightly higher concentrations can induce the release of cytochrome c and apoptosis-inducing factor (AIF) from mitochondria into the cytosol where they trigger the activation of caspase, leading to cell death by apoptosis [12].

3. Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, caused by a defect on insulin production, insulin action or both [1]. There are two main types of diabetes: type 1 and type 2 diabetes.

Type 1 diabetes is due to an autoimmune destruction of the insulin producing pancreatic beta-cells, which usually leads to absolute insulin deficiency. Patients with type 1 diabetes require insulin for survival. This type of diabetes accounts for 5-10% of the total cases of diabetes worldwide. Type 2 diabetes represents approximately 90% of the total diabetes cases, and it is characterized by impairment in insulin action and/or abnormal insulin secretion [1].

The origins of type 2 diabetes are multifactorial. Obesity, age, ethnic origin and familiar history of diabetes are among the factors that contribute to its development. Even though a strong genetic component has been recognized, genotype only establishes the conditions for the individual to be more or less prone to environmental effects and lifestyle factors [34].

Type 2 diabetes develops when insulin secretion or insulin action fails. The impairment of insulin actions is known as insulin resistance, presented as a suppression or retard in metabolic responses of the muscle, liver and adipose tissue to insulin action. This failure is located at the signaling pathways held after insulin binding to its specific receptor [35]. Chronic insulin resistance leads to hyperglycemia.

When the beta cells cannot secrete enough insulin in response to the metabolic demand caused by insulin resistance, frank diabetes type 2 occurs. This failure in the beta cell may be due to an acquired secretory dysfunction and/or a decrease in beta-cell mass [36]. All type 2 diabetic patients have some defect in the ability of beta cells to produce or secrete insulin [37].

3.1. Insulin action and insulin resistance

Once secreted to the portal circulation, insulin is transported to peripheral tissues, on which it will exert mainly anabolic actions [38]. Insulin starts its action by binding to insulin receptor, a transmembrane protein belonging to protein tyrosine kinase activity receptors superfamily, which can autophosphorylate. This initiates a series of events involving protein and membrane lipid phosphorylation, coupling proteins and cytoskeleton activity [39] [40]. The three main signaling pathways activated in response to insulin receptor phosphorylation are 1) PI3K 2)MAPK, and 3) Cb1. These pathways act in a concerted way to translate the signal of insulin receptor into biological actions in target organs, such as glucose transport by transporting GLUT4 vesicles to the membrane, protein, lipid and glycogen synthesis, mitosis and gene expression [40] (Figure 3).

As protein phosphorylation activates these signaling pathways, dephosphorylation inhibits them. Different phosphatases such as protein-tyrosine phosphatase 1B (PTP1B), Phosphatase and tensin homolog (PTEN), SH2-containing tyrosine- protein phosphatase (SHO2), and suppressor of cytokine signaling 3 (SOCS-3) dephosphorylate and shut down insulin signaling [35]. Any alteration in the insulin pathway, being inefficient phosphorylation or

increment in phosphatase acticity, causes impairment in insulin action. This is the molecular mechanism leading to insulin resistance.
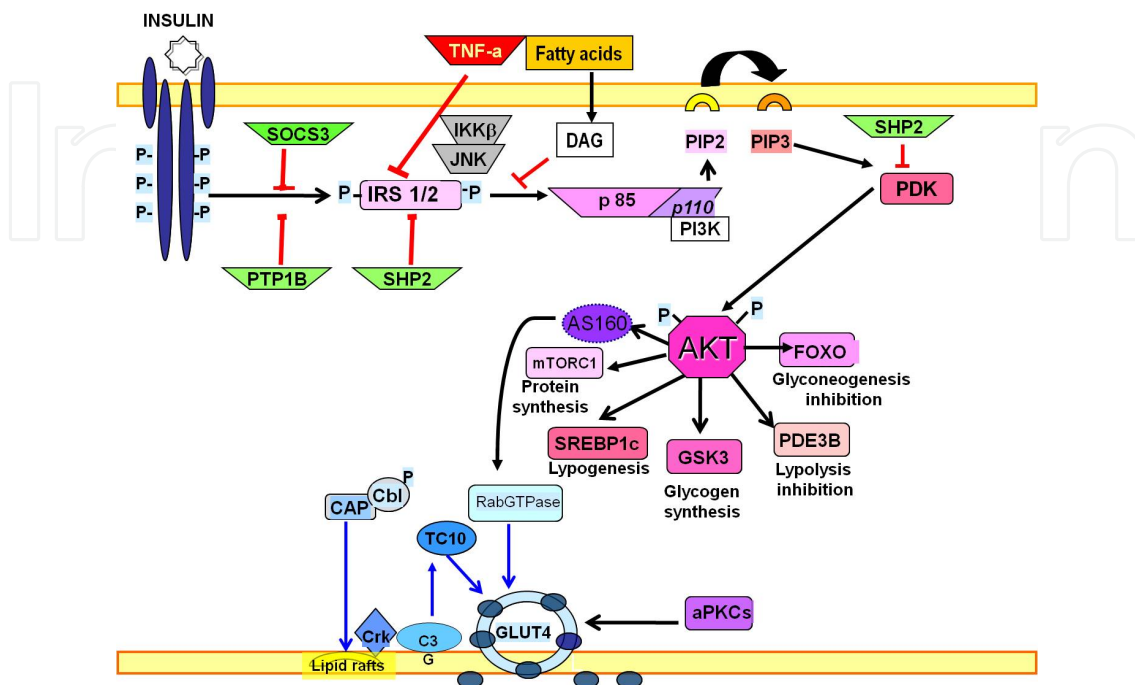

Figure 3. Molecular mechanisms of insulin signaling.

3.2. Insulin secretion

Beta-cells in the endocrine pancreas are responsible for secreting insulin in response to rises in blood nutrient levels during the postprandial state. Glucose is the most important nutrient for insulin secretion. The process by which glucose promotes insulin secretion requires glucose sensing and metabolism by the beta-cell, a process called glucose-stimulated insulin secretion (Figure 4). In the first phase of insulin secretion, glucose enters the cell by glucose transporters (GLUT2 in rodents, GLUT1 in humans). Glucose is then phosphorylated to form glucose-6-phosphate by glucokinase [41]. The generation of ATP by glycolysis, the Krebs cycle and the respiratory chain closes the ATP-sensitive K+ channel (KATP) [42], allowing sodium (Na+) entry without balance. These two events depolarize the membrane and open voltage-dependent T-type calcium (Ca2+) and sodium (Na+) channels. Na+ and Ca2+ entry further depolarizes the membrane and voltage-dependent calcium channels open. This activation increases intracellular Ca2+ ([Ca2+]i) [43], which leads to fusion of insulin-containing secretory granules with the plasma membrane and the first phase insulin secretion [44, 45].
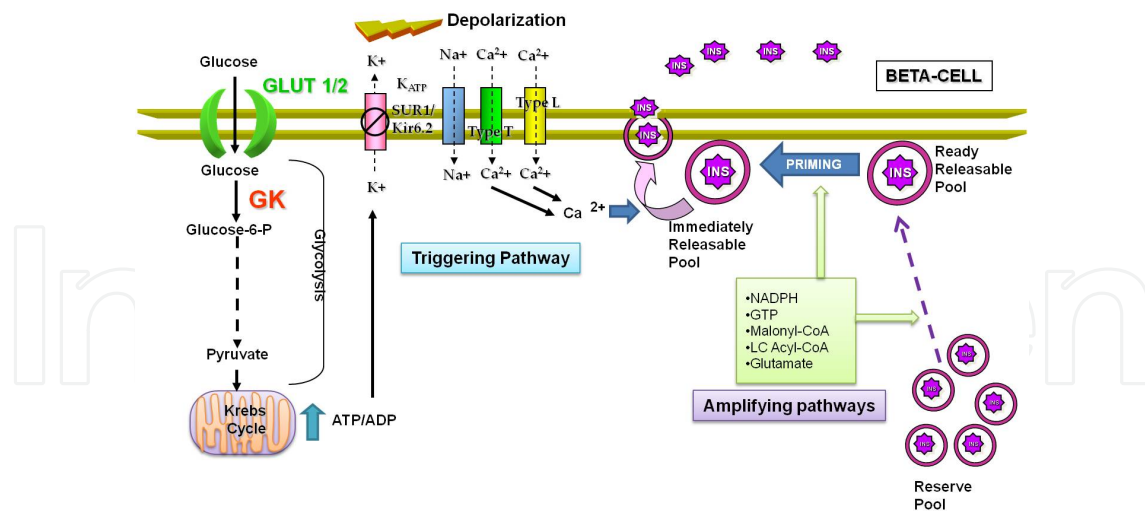
Figure 4. Mechanisms of biphasic glucose-stimulated insulin secretion.

Besides increasing ATP/ADP ratio, glucose metabolism in the beta cell can generate a series of metabolic coupling signals that can initiate and sustain a second insulin secretion phase. Some of these coupling factors participate in mitocondrial metabolism and anaplerosis, constituting cycles involving NADPH, pyruvate, malate, citrate, isocitrate, Acyl-CoA and glutamate [46]. Diverse signaling pathways can also contribute to glucose-induced insulin secretion such as CaMKII [47-49], PKA [50, 51], PKC [51, 52] y PKG [53, 54]. Most secretagogues and potentiators of insulin secretion, such as nutrients, hormones and neurotransmitters, use these pathways to modulate insulin secretion.

4. Oxidative stress in diabetes mellitus

Hyperglycemia and free fatty acid intake are among the causes for oxidative stress conditions [23]. Hence, it may not be surprising that diabetic subjects tend to have more oxidative cell and organism environments than healthy subjects, i.e. an increase in ROS generation [5, 55, 56]. Moreover, diabetic patients present a decrease in antioxidant defenses. The antioxidant enzyme levels are affected by diabetes, which further increase oxidative stress [5, 6].

Oxidative stress has been proposed as a major participant in the patophysiology of diabetic complications [27]. Nevertheless, regarding diabetes onset and development, oxidative stress has also shown to affect the two major mechanisms failing during diabetes: insulin resistance and insulin secretion.

4.1. Oxidative stress processes in insulin resistance

ROS and RNS affect the insulin signaling cascade [5]. As with other ROS effects, low doses play a physiological role in insulin signaling. After insulin stimulation of its receptor in adipocytes, H_2O_2 is produced via NADPH oxidase, which by inhibits PTP1B catalytic activity, thus increasing tyrosine phosphorylation [57].

However, oxidative stress caused by hyperglycemia in diabetes may impair insulin signaling, leading to insulin resistance. Although no mechanisms have been completely established, several responses to ROS excess in the insulin signaling have been proposed.

Disturbs in cellular redistribution of insulin signaling components may alter the insulin cascade, a process mediated by NF-κB [58]. A decrease in GLUT4 gene transcription and increase in GLUT1 (insulin independent glucose transporter) has also been observed, as well as increases in phosphorylation of IRS protein in an insulin receptor-independent fashion (perhaps by the stress kinases). Altogether, hyperglycemia and insulin resistance may also lead to altered mitochondrial function, and insulin action impairment by cytokines in response to metabolic stress [59, 60]. An increase in the hexosamine pathway has also been linked to insulin resistance. Moreover, it has been proposed that this pathway acts as a cellular sensor for the glucose excess. From that point of view, insulin resistance may be a protective mechanism from the glucose excess entrance [28].

4.2. Oxidative stress processes in insulin secretion

Pancreatic beta-cells are especially sensitive to ROS and RNS, because their natural enzymatic antioxidant defenses are lower compared to other tissues such as liver. Moreover, they lack the ability to adapt their low enzyme activity levels in response to stress such as high glucose or high oxygen [61]. Glucose enters to the beta-cell in an insulin independent fashion, because besides providing energy, glucose sensing in the beta-cell is crucial for insulin secretion. It has been suggested that hyperglycemia can generate chronic oxidative stress by the glucose oxidation pathway [62], leading to an excess in mitochondrial superoxide production, which further activates uncoupling protein-2 (UCP-2). This protein lowers ATP/ADP relationship through proton leak in the beta-cell, which reduces insulin secretion [63].

ROS also increase the stress signaling pathways in the beta cells, such as NF-κB activity, which potentially leading to beta-cell apoptosis [64], and the JNK pathway which has been related to suppression of insulin gene expression, possibly by reduction of PDX-1 DNA binding activity, a major regulator of insulin expression [65]. It has also been shown that the activation of the hexosamine pathway in beta-cells leads to suppression of PDX-1 binding to the insulin and other genes involved in insulin secretion, perhaps contributing to the beta-cell dysfunction present in diabetes mellitus [66].

As in other cell types, NO in beta-cells has physiologic roles. NO may regulate glucokinase activity by s-nitrosilation [67] in the beta-cell, and possibly increase insulin secretion. However, NO excess and concomitant NRS may cause apoptosis through caspase-3 activation and decrease in ATP levels [68].

Besides ROS hyperproduction, excess mitochondrial metabolism resulting form hyperglycemia in the beta-cell may also alter mitochondrial shape, volume and behavior, uncoupling K-ATP channels from mitochondrial activity and thus altering glucose-induced insulin secretion [69].

5. Diabetic complications

Hyperglycemia, is the responsible of the development of diabetes complications as well. Hyperglycemia damage is produced in cells in which glucose uptake is independent of insulin, which, similarly to what happens in beta-cells, explains that the cause of the complications resides inside the cells [4]. Prolonged exposure to high glucose levels, genetic determinants of susceptibility and accelerating factors such as hypertension and dyslipidemia participate in the development of diabetic complications. Moreover, the development and progression of damage is proportional to hyperglycemia, which makes the lowering of glucose levels the most important goal for preventing complications and treating diabetes.

The main tissues affected by diabetes complications at the microvasculature levels are retina, renal glomerulus, and peripheral nerves. Diabetes is also associated with accelerated atherosclerotic disease affecting arteries that supply the heart, brain, and lower extremities. In addition, diabetic cardiomyopathy is a major diabetic complication [24].

5.1. Oxidative stress in diabetic complications

Oxidative stress plays a pivotal role in the development of diabetes complications, both at the microvascular and macrovascular levels. Results derived from two decades of diabetes complications investigation point towards mitochondrial superoxide overproduction as the main cause of metabolic abnormalities of diabetes. Thus, all of the above reviewed pathways are involved in microvasculature and macrovasculature hyperglycemic damage [24].

5.2. Microvascular complications

Diabetic retinopathy: Diabetic retinopathy appears in most patients after 10 to 15 years after diabetes onset. Background retinopathy presents small hemorrhages in the middle layers of the retina, appearing as "dots". Lipid deposition occurs at the margins of the hemorraghe, and microaneurisms (small vascular dilatations) and edema may appear. Proliferative retinopathy occurs when new blood vessels on the surface of the retina cause vitreous hemorrhage, and eventually, blindness. As the cells of the retina contain high amounts of aldo-ketoreductase, they have high susceptibility to increase the polyol pathway in the presence of excess glucose, with concomitant decreases in NADPH [4]. Sorbitol produced in this process increases osmotic stress, which has been linked to microaneurysm formation, thickening of the basement membranes and loss of pericytes. It is also thought that retina cells are damaged by glycoproteins, particularly form AGEs. Additionally, ROS by themselves may damage the cells. Importantly, VEGF, growth hormone and TGF-beta increases during diabetes may be the cause of proliferation of blood vessels [70].

Diabetic nephropaty: this complication causes glomerular basement membrane thickness, microaneurism formation, and mesangial nodule formation, all which are reflected in proteinuria and, in the end, renal insufficiency. The mechanisms for injury also involve the increased polyol pathway and AGE formation. AGE binding to its receptors has been proven to play a role as well in renal damage, fibrosis and inflammation associated with diabetic

nephropaty. This actions of AGE also potentiate oxidative stress, while synergizing with rennin-angiotensin system activation, which leads to a vicious cycle causing kidney failure. As mentioned, diabetic patients, and particularly those with nephropaty, have lowered antioxidant defenses. Moreover, AGE receptors are significantly increased [71].

Diabetic neuropathy: Diabetic neuropathy is defined as the presence of symptoms and/or signs of peripheral nerve dysfunction in diabetic patients after exclusion of other causes. Peripheral neuropathy in diabetes may manifest in several different forms, including sensory, focal/multifocal, and autonomic neuropathies. [72]. Mechanisms of nerve injury are less known but likely related also to the polyol pathway, AGE formation and ROS themselves [70]. Oxidized proteins and lipoproteins also interact with receptors in the membrane of neurons, initiating inflammatory signaling mechanisms which further produce ROS, damaging cellular components and leading to neuronal injury [73].

5.3. Macrovascular complications

The central pathological mechanism in macrovascular complications is atherosclerotic disease. Atherosclerosis occurs as a result of chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system. This damages cause accumulation of oxidized lipids from LDL particles in the endothelial wall of arteries, whose rupture leads to acute vascular infarction. Additionally, platelet adhesion and hypercoagulability also occurs in type 2 diabetes, increasing the risk of vascular occlusion [70]. It has been proposed that increased superoxide production is the central and major mediator of endothelial tissue damage, causing direct inactivation of two antiatherosclerotic enzymes, endothelial nitric oxide synthase and prostacyclin synthase and that the activation of oxidative stress pathways is involved in the pathogenesis of complications [24].

Endothelial cells also contain high amounts of aldo-keto reductase, and are thus prone to increased polyol pathway activation. Moreover, a large body of evidence supports hypothesis that hyperglycemia or diabetes leads to vascular diacylglycerol accumulation and subsequent PKC activation, causing a variety of cardiovascular defects [29]. PKC activation has been associated with vascular alterations such as increases in permeability, contractility, extracellular matrix synthesis, cell growth and apoptosis, angiogenesis, leukocyte adhesion, and cytokine activation and inhibition [29]. Hyperglycemia-induced activation of PKC has also been implicated in the overexpression of the fibrinolytic inhibitor, plasminogen activator inhibitor-1 (PAI-1) [74]. In smooth muscle PKC hyperactivity is associated with decreased NO production [75] and has been shown to inhibit insulin-stimulated expression of eNOs in endothelial cells.

In arterial endothelial cells O-glucosamine-acylation participates in vascular complications interfering with the action of Akt/PKB, a critical insulin signaling protein, on eNOS [76]. GFAT activity is associated with increased transcription of transforming growth factor (TGF) alpha and beta and PAI-1, factors involved in the proliferation of vascular smooth-muscle and endothelial cells. This effect appears to be mediated by O-glucosamine-acylation of the transcription factor, Sp1 [77]. Increased TGF-beta and PAI-1 are associated with capillary and vascular occlusion by mechanisms associated with collagen and fibronectin expres-

sion causing capillary occlusion, in the case of TGF-beta, and decreased fibrinolysis in the case of PAI-1. O-GlcNAcylation impairs cardiomyocyte calcium cycling decreasing sarcoplasmic reticulum calcium ATPase 2a (Serca 2a) [78-80].
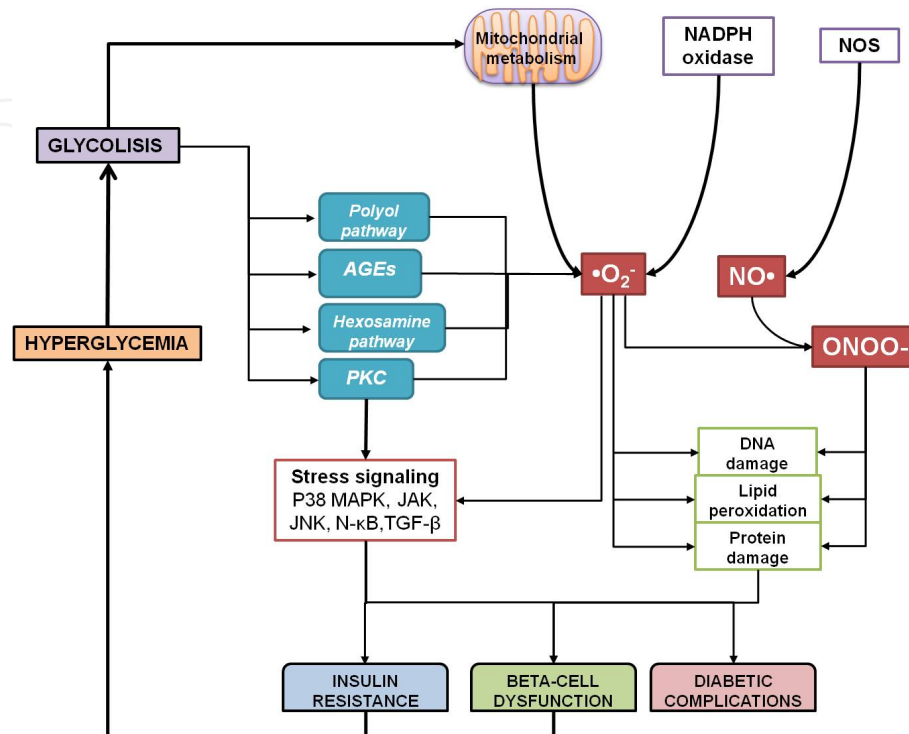

Figure 5. Oxidative stress pathways in diabetes mellitus.

6. Antioxidant vitamins and diabetes mellitus

As mentioned above, vitamins C, E, and A constitute the non enzymatic defense against oxidative stress, by regenerating endogenous antioxidants (Figure 1). Vitamin C has a role in scavenging ROS and RNS by becoming oxidated itself. The oxidized products of vitamin C, ascorbic radical and dehydroascorbic radical are regenerated by glutathione, NADH or NADPH. In addition, vitamin C can reduce the oxidized forms of vitamin E and glutathione [81]. Vitamin E is a fat-soluble vitamin which may interact with lipid hydroperoxides and scavenge them. It also participates, together with vitamin C, in gluthatione regeneration by interaction with lipoic acid [23]. Vitamin A has a plethora of cellular actions. Besides modulating gene expression, cell growth and differentiation, this vitamin may also act as antioxidant, although the mechanisms of action in this role are not fully deciphered. The antioxidant potential of carotenoids (vitamin A) depends on their distinct membrane-lipid interactions, while some carotenoids can decrease lipid peroxidation, others can stimulate it [82].

Since oxidative stress is present during the progression of diabetes and its complications, amelioration of oxidative status, mainly by increasing antioxidant non-enzymatic defenses, has been largely proposed and studied. Several clinical observational trials have particularly studied the correlation between vitamin E status in plasma and/or diet, and markers of oxidation, inflammation, type 2 diabetes incidence, and diabetic complications. Although inverse association has been found for vitamin E in some studies [7, 83, 84], the association found in other study disappeared after adjustment for cardiovascular risk factors such as obesity, smoking, and hypertension [85], or have observed no beneficial effect at all [7, 8]. Such contrasting results have also been reported for studies looking association of vitamin A and C consumption and amelioration of diabetes status and/or complications [7, 8, 81, 86].

On the other hand, in interventional trials with vitamin supplementation, the effects of vitamins E, C and A, alone or in diverse combinations, have yielded barely any promising result. There appears to be no beneficial effect of vitamin supplementation on diabetes or macrovascular complications [7, 8, 81]. Some of these studies have even evidenced associations between vitamin supplementation and an increased incidence of stroke [7]. Likewise, supplementation with antioxidant vitamins can even block beneficial ROS production during exercise, inhibiting the health-promoting effects of exercise in humans [87].

Paradoxically, in spite of the solid evidence of increased oxidative stress in diabetes, and the well established actions of vitamins as antioxidants, the association studies between antioxidant vitamin status and its beneficial effects in diabetes has no consistent results at all. What is more, interventional studies have failed in demonstrating a favorable effect of vitamin supplementation, discouraging its use as antioxidant therapy for diabetes.

Several reasons have been suggested for these contradictory observations. First, as vitamins may be easily oxidized, a vitamin may have antioxidant or oxidant properties, depending on the presence of other vitamins and the oxidative state in the cells i.e., if the oxidized form of a vitamin is not correctly reversed into the reduced form. Additionally, some vitamins may also activate oxidative stress pathways and further increase the oxidative stress, such as the activation of PKC by retinoids [88].

Vitamin doses may also be part of the problem, as the effect of vitamins depends on dietary concentrations and/or supplement intake. The wide variety of doses reached with diet and supplements, and the lack of an established "pharmacological" dose of vitamins, makes it difficult to ascertain the true net effect of vitamin status or supplementation needed to generate beneficial effects. As well, the required dose for antioxidant effects versus the required for the vitamin's role in the body may differ, which, together with vitamin's bioavailability and its interaction with other vitamins, are caveats for assessing and finding vitamins' effects, if any [7, 88].

Finally, the antioxidant effects of vitamins may not be sufficient to scavenge the great amount of ROS present in diabetes. Certainly, glucose levels have been correlated to the presence and severity of the complications. However once hyperglycemia has established, the incidence of complications after tight glycemic control remains the same. This effect has been termed glycemic memory, and is the cause for accumulative damage ren-

dering diabetic complications. Considering that hyperglycemia is the main cause of oxidative stress in diabetes, in a similar way, the chronic undesirable effects that occur by ROS production may generate a vicious cycle difficult to break, in which ROS damage exacerbates the diabetic state, increasing glucose levels, which will further induce more oxidative imbalance [24].

7. Conclusions

Diabetes mellitus has reached epidemic proportions in the last decade, becoming one of the most important diseases worldwide. Several studies indicate oxidative stress is present in the dysfunction of insulin action and secretion that occur during diabetes, as well as in the development of diabetic complications. Nevertheless, oxidative stress is not the primary cause of diabetes, but rather a consequence of nutrient excess, given that oxidative stress is a natural response to stress, in this case, to glucose and/or lipid overload.

Vitamins such as E, C and A with antioxidant properties constitute the physiological non-enzymatic defense against oxidative stress. However, the evidence in favor of the use of vitamin supplementation as antioxidant therapy remains uncertain. Although some beneficial effects have been proven in observational studies, the results of interventional trials are still ineffective. Perhaps more studies on the physiopathology of oxidative stress and the role of vitamins in it, as well as standardizing vitamin dosage and assessing their undesirable effects are needed in order to determine a clear participation of vitamin supplementation in amelioration of the oxidative balance. More studies addressing the possibility of targeting directly at the enzymes and mechanisms involved in ROS production and not by antioxidants are needed as well.

Given that it is mostly dietary vitamin intake which has shown an association with ameliorating the diabetic state, and that oxidative stress is a response to excess of nutrients, it seems that attending the cause of excessive ROS production represents the best therapeutic option. Thus, adequate dietary interventions that reduce hyperglycemia, and increases in oxygen consumption (i.e. improve mitochondrial function) by exercise remain the primary choices for diabetes treatment and prevention of its complications.

Acknowledgements

Author details

Maria-Luisa Lazo-de-la-Vega-Monroy[*] and Cristina Fernández-Mejía

Unidad de Genética de la Nutrición, Departamento de Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México/ Instituto Nacional de Pediatría, México

References

[1] ADA. (2009). Diagnosis and classification of diabetes mellitus. *Diabetes care*, 32(1), 62-7.

[2] Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract*, 87(1), 4-14.

[3] King, H., Aubert, R. E., & Herman, W. H. (1998). Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care*, 21(9), 1414-1431.

[4] Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature*, 414(6865), 813-820.

[5] Rains, J. L., & Jain, S. K. (2011). Oxidative stress, insulin signaling, and diabetes. *Free Radic Biol Med*, 50(5), 567-575.

[6] Maritim, A. C., Sanders, R. A., & Watkins, J. B. (2003). Diabetes, oxidative stress, and antioxidants: A review. *Journal of Biochemical and Molecular Toxicology*, 17(1), 24.

[7] Sheikh-Ali, M., Chehade, J. M., & Mooradian, A. D. (2011). The antioxidant paradox in diabetes mellitus. *Am J Ther*, 18(3), 266-278.

[8] Cuerda, C., Luengo, L. M., Valero, Vidal. A., Burgos, R., Calvo, F. L., et al. (2011). Antioxidants and diabetes mellitus: review of the evidence]. *Nutr Hosp*, 26(1), 68-78.

[9] Naviaux, R. K. (2012). Oxidative Shielding or Oxidative Stress? *J Pharmacol Exp Ther*.

[10] Johansen, J. S., Harris, A. K., Rychly, D. J., & Ergul, A. (2005). Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardiovasc Diabetol*, 4(1), 5.

[11] Ceriello, A. (2006). Oxidative stress and diabetes-associated complications. *Endocr Pract*, 12(1), 60-62.

[12] Fisher, A. B., Zhang, Q. Â. Â., Geoffrey, J. L., & Steven, D.S. (2006). Nadph and nadph oxidase. *Encyclopedia of Respiratory Medicine.*, Oxford, Academic Press, 77.

[13] Drummond, G. R., Selemidis, S., Griendling, K. K., & Sobey, C. G. (2011). Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. *Nat Rev Drug Discov*, 10(6), 453-471.

[14] Maechler, P., & Wollheim, C. B. (2001). Mitochondrial function in normal and diabetic beta-cells. *Nature*, 414(6865), 807-812.

[15] Giacco, F., & Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circ Res*, 107(9), 1058-1070.

[16] Boveris, A. (1984). Determination of the production of superoxide radicals and hydrogen peroxide in mitochondria. *Methods Enzymol*, 105, 429-435.

[17] Korshunov, S. S., Skulachev, V. P., & Starkov, A. A. (1997). High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett*, 416(1), 15-18.

[18] Dikalov, S. Cross talk between mitochondria and NADPH oxidases. *Free Radical Biology and Medicine*, 51(7), 1289.

[19] Schmidt, H. H., Lohmann, S. M., & Walter, U. (1993). The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. *Biochim Biophys Acta*, 1178(2), 153-175.

[20] Hobbs, A. J., & Ignarro, L. J. (1996). Nitric oxide-cyclic GMP signal transduction system. *Methods Enzymol*, 269, 134-148.

[21] Murad, F. (1999). Cellular signaling with nitric oxide and cyclic GMP. *Braz J Med Biol Res*, 32(11), 1317-1327.

[22] Mc Donald, L. J., & Murad, F. (1995). Nitric oxide and cGMP signaling. *Adv Pharmacol*, 34, 263-275.

[23] Evans, J. L., Goldfine, I. D., Maddux, B. A., & Grodsky, G. M. (2002). Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev*, 23(5), 599-622.

[24] Giacco, F., & Brownlee, M. Oxidative stress and diabetic complications. *Circ Res*, 107(9), 1058-1070.

[25] Mazzola, J. L., & Sirover, M. A. (2003). Subcellular localization of human glyceraldehyde-3-phosphate dehydrogenase is independent of its glycolytic function. *Biochim Biophys Acta*, 1622(1), 50-56.

[26] Tristan, C., Shahani, N., Sedlak, T. W., & Sawa, A. The diverse functions of GAPDH: views from different subcellular compartments. *Cell Signal*, 23(2), 317-323.

[27] Brownlee, M. (2005). The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*, 54(6), 1615-1625.

[28] Buse, M. G. (2006). Hexosamines, insulin resistance, and the complications of diabetes: current status. *Am J Physiol Endocrinol Metab*, 290(1), 1-8.

[29] Geraldes, P., & King, G. L. Activation of protein kinase C isoforms and its impact on diabetic complications. *Circ Res*, 106(8), 1319-1331.

[30] Scivittaro, V., Ganz, M. B., & Weiss, M. F. (2000). AGEs induce oxidative stress and activate protein kinase C-beta(II) in neonatal mesangial cells. *Am J Physiol Renal Physiol*, 278(4), 676-683.

[31] Camacho-Ruiz, A., & Esteban-Méndex, M. Diabetes y radicales libres. *In: Morales-González JA, Madrigal-Santillán EO, Nava-Chapa G, Durante-Montiel I, Jongitud-Falcón A, Esquivel-Soto J. 2010Editors. Diabetes. 2nd ed. Pachuca, Hidalgo, México.: Universidad Autónoma del Estado de Hidalgo.*

[32] Degenhardt, T. P., Thorpe, S. R., & Baynes, J. W. (1998). Chemical modification of proteins by methylglyoxal. *Cell Mol Biol (Noisy-le-grand)*, 44(7), 1139-1145.

[33] Gomperts, B. D., Kramer, I. M., & Tatham, P. E. R. (2003). Signal Transduction. London, UK, Elsevier Academic Press.

[34] Permutt, M. A., Wasson, J., & Cox, N. (2005). Genetic epidemiology of diabetes. J Clin Invest , 115(6), 1431-9.

[35] Zick, Y. (2004). Uncoupling insulin signalling by serine/threonine phosphorylation: a molecular basis for insulin resistance. *Biochem Soc Trans*, 32(5), 812-816.

[36] Weir, G. C., Laybutt, D. R., Kaneto, H., Bonner-Weir, S., & Sharma, A. (2001). Beta-cell adaptation and decompensation during the progression of diabetes. *Diabetes*, 50(1), 154-159.

[37] Leahy, J. L., Hirsch, I. B., Peterson, K. A., & Schneider, D. (2010). Targeting beta-cell function early in the course of therapy for type 2 diabetes mellitus. *J Clin Endocrinol Metab*, 95(9), 4206-4216.

[38] Lazo-de-la-Vega-Monroy, M. L., & Fernandez-Mejia, C. Bases moleculares de la diabetes tipo 2. *In: Morales-González JA, Madrigal-Santillán EO, Nava-Chapa G, Durante-Montiel I, Jongitud-Falcón A, Esquivel-Soto J. 2010Editors. Diabetes. 2nd ed. Pachuca, Hidalgo, México:Universidad Autónoma del Estado de Hidalgo.*

[39] Bjornholm, M., & Zierath, J. R. (2005). Insulin signal transduction in human skeletal muscle: identifying the defects in Type II diabetes. *Biochem Soc Trans*, 33(2), 354-357.

[40] Withers, D. J., Gutierrez, J. S., Towery, H., Burks, D. J., Ren, J. M., Previs, S., et al. (1998). Disruption of IRS-2 causes type 2 diabetes in mice. *Nature*, 391(6670), 900-904.

[41] Matschinsky, F. M. (1996). Banting Lecture 1995. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. *Diabetes*, 45(2), 223-241.

[42] Aguilar-Bryan, L., Clement, J. P., Gonzalez, G., Kunjilwar, K., Babenko, A., & Bryan, J. (1998). Toward understanding the assembly and structure of KATP channels. *Physiol Rev*, 78(1), 227-245.

[43] Hiriart, M., & Aguilar-Bryan, L. (2008). Channel regulation of glucose sensing in the pancreatic beta-cell. *Am J Physiol Endocrinol Metab*, 295(6), 1298-1306.

[44] Rorsman, P., & Renstrom, E. (2003). Insulin granule dynamics in pancreatic beta cells. *Diabetologia*, 46(8), 1029-1045.

[45] Straub, S. G., & Sharp, G. W. (2002). Glucose-stimulated signaling pathways in biphasic insulin secretion. *Diabetes Metab Res Rev*, 18(6), 451-463.

[46] Jitrapakdee, S., Wutthisathapornchai, A., Wallace, J. C., & Mac Donald., M.J. (2010). Regulation of insulin secretion: role of mitochondrial signalling. *Diabetologia*, 53(6), 1019-1032.

[47] Krueger, K. A., Bhatt, H., Landt, M., & Easom, R. A. (1997). Calcium-stimulated phosphorylation of MAP-2 in pancreatic betaTC3-cells is mediated by Ca2+/calmodulin-dependent kinase II. *J Biol Chem*, 272(43), 27464-27469.

[48] Nielander, H. B., Onofri, F., Valtorta, F., Schiavo, G., Montecucco, C., Greengard, P., et al. (1995). Phosphorylation of VAMP/synaptobrevin in synaptic vesicles by endogenous protein kinases. *J Neurochem*, 65(4), 1712-1720.

[49] Easom, R. A. (1999). CaM kinase II: a protein kinase with extraordinary talents germane to insulin exocytosis. *Diabetes*, 48(4), 675-684.

[50] Sharp, G. W. (1979). The adenylate cyclase-cyclic AMP system in islets of Langerhans and its role in the control of insulin release. *Diabetologia*, 16(5), 287-296.

[51] Jones, P. M., & Persaud, S. J. (1998). Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic beta-cells. *Endocr Rev*, 19(4), 429-461.

[52] Doyle, M. E., & Egan, J. M. (2003). Pharmacological agents that directly modulate insulin secretion. *Pharmacol Rev*, 55(1), 105-131.

[53] Laychock, S.G., Modica, M.E., & Cavanaugh, C.T. (1991). L-arginine stimulates cyclic guanosine 3',5'-monophosphate formation in rat islets of Langerhans and RINm5F insulinoma cells: evidence for L-arginine:nitric oxide synthase.(6), *Endocrinology*, 129(6), 3043-3052.

[54] Russell, M. A., & Morgan, N. (2010). Expression and functional roles of guanylate cyclase isoforms in BRIN-BD11 beta-cells. *Islets*, 2(6), 23-31.

[55] Guzik, T. J., Mussa, S., Gastaldi, D., Sadowski, J., Ratnatunga, C., Pillai, R., et al. (2002). Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. *Circulation*, 105(14), 1656-1662.

[56] Ceriello, A., Mercuri, F., Quagliaro, L., Assaloni, R., Motz, E., Tonutti, L., et al. (2001). Detection of nitrotyrosine in the diabetic plasma: evidence of oxidative stress. *Diabetologia*, 44(7), 834-838.

[57] Mahadev, K., Motoshima, H., Wu, X., Ruddy, J. M., Arnold, R. S., Cheng, G., et al. (2004). The NAD(P)H oxidase homolog Nox4 modulates insulin-stimulated genera-

tion of H2O2 and plays an integral role in insulin signal transduction. *Mol Cell Biol*, 24(5), 1844-1854.

[58] Ogihara, T., Asano, T., Katagiri, H., Sakoda, H., Anai, M., Shojima, N., et al. (2004). Oxidative stress induces insulin resistance by activating the nuclear factor-kappa B pathway and disrupting normal subcellular distribution of phosphatidylinositol 3-kinase. *Diabetologia*, 47(5), 794-805.

[59] Eriksson, J. W. (2007). Metabolic stress in insulin's target cells leads to ROS accumulation- a hypothetical common pathway causing insulin resistance. *FEBS Lett*, 581(19), 3734-3742.

[60] Bloch-Damti, A., & Bashan, N. (2005). Proposed mechanisms for the induction of insulin resistance by oxidative stress. *Antioxid Redox Signal*, 7(11-12), 1553-1567.

[61] Tiedge, M., Lortz, S., Drinkgern, J., & Lenzen, S. (1997). Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes*, 46(11), 1733-1742.

[62] Robertson, R. P., Harmon, J., Tran, P. O., Tanaka, Y., & Takahashi, H. (2003). Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes*, 52(3), 581-587.

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

[64] Rhodes, C. J. (2005). Type 2 diabetes-a matter of beta-cell life and death? *Science*, 307(5708), 380-384.

[65] Kaneto, H., Matsuoka, T. A., Nakatani, Y., Kawamori, D., Matsuhisa, M., & Yamasaki, Y. (2005). Oxidative stress and the JNK pathway in diabetes. *Curr Diabetes Rev*, 1(1), 65-72.

[66] Kaneto, H., Xu, G., Song, K. H., Suzuma, K., Bonner-Weir, S., Sharma, A., et al. (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), 31099-31104.

[67] Rizzo, M. A., & Piston, D. W. (2003). Regulation of beta cell glucokinase by S-nitrosylation and association with nitric oxide synthase. *J Cell Biol*, 161(2), 243-248.

[68] Tejedo, J., Bernabe, J. C., Ramirez, R., Sobrino, F., & Bedoya, F. J. (1999). NO induces a cGMP-independent release of cytochrome c from mitochondria which precedes caspase 3 activation in insulin producing RINm5F cells. *FEBS Lett*, 459(2), 238-243.

[69] Drews, G., Krippeit-Drews, P., & Dufer, M. (2010). Oxidative stress and beta-cell dysfunction. *Pflugers Arch*, 460(4), 703-718.

[70] Fowler, M. J. (2008). Microvascular and Macrovascular Complications of Diabetes. *Clinical Diabetes*, 26(2), 77-782.

[71] Thomas, M. C. (2011). Advanced glycation end products. *Contrib Nephrol*, 170, 66-74.

[72] ADA. (2012). Standards of Medical Care in Diabetes. *Diabetes Care*, 170(1), 11-63.

[73] Vincent, A. M., Callaghan, B. C., Smith, A. L., & Feldman, E.L. Diabetic neuropathy: cellular mechanisms as therapeutic targets. *Nat Rev Neurol*, 7(10), 573.

[74] Feener, E. P., Xia, P., Inoguchi, T., Shiba, T., Kunisaki, M., & King, G. L. (1996). Role of protein kinase C in glucose- and angiotensin II-induced plasminogen activator inhibitor expression. *Contrib Nephrol*, 118, 180-187.

[75] Ganz, M. B., & Seftel, A. (2000). Glucose-induced changes in protein kinase C and nitric oxide are prevented by vitamin E. *Am J Physiol Endocrinol Metab*, 278(1), 146-152.

[76] Akimoto, Y., Kreppel, L. K., Hirano, H., & Hart, G. W. (2001). Hyperglycemia and the O-GlcNAc transferase in rat aortic smooth muscle cells: elevated expression and altered patterns of O-GlcNAcylation. *Arch Biochem Biophys*, 389(2), 166-175.

[77] Du, X. L., Edelstein, D., Rossetti, L., Fantus, I. G., Goldberg, H., Ziyadeh, F., et al. (2000). Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Natl Acad Sci USA*, 97(22), 12222-12226.

[78] Clark, R. J., Mc Donough, P. M., Swanson, E., Trost, S. U., Suzuki, M., Fukuda, M., et al. (2003). Diabetes and the accompanying hyperglycemia impairs cardiomyocyte calcium cycling through increased nuclear O-GlcNAcylation. *J Biol Chem*, 278(45), 44230-44237.

[79] Pang, Y., Bounelis, P., Chatham, J. C., & Marchase, R. B. (2004). Hexosamine pathway is responsible for inhibition by diabetes of phenylephrine-induced inotropy. *Diabetes*, 53(4), 1074-1081.

[80] Liu, J., Pang, Y., Chang, T., Bounelis, P., Chatham, J. C., & Marchase, R. B. (2006). Increased hexosamine biosynthesis and protein O-GlcNAc levels associated with myocardial protection against calcium paradox and ischemia. *J Mol Cell Cardiol*, 40(2), 303-312.

[81] Garcia-Bailo, B., El -Sohemy, A., Haddad, P. S., Arora, P., Benzaied, F., Karmali, M., et al. (2011). Vitamins D, C, and E in the prevention of type 2 diabetes mellitus: modulation of inflammation and oxidative stress. *Biologics*, 5, 7-19.

[82] Mc Nulty, H., Jacob, R. F., & Mason, R. P. (2008). Biologic activity of carotenoids related to distinct membrane physicochemical interactions. *Am J Cardiol*, 101(10A), 20-29.

[83] Salonen, J. T., Nyyssonen, K., Tuomainen, T. P., Maenpaa, P. H., Korpela, H., Kaplan, G. A., et al. (1995). Increased risk of non-insulin dependent diabetes mellitus at low plasma vitamin E concentrations: a four year follow up study in men. *BMJ*, 311(7013), 1124-1127.

[84] Mayer-Davis, E. J., Costacou, T., King, I., Zaccaro, D. J., & Bell, R. A. (2002). Plasma and dietary vitamin E in relation to incidence of type 2 diabetes: The Insulin Resistance and Atherosclerosis Study (IRAS). *Diabetes Care*, 25(12), 2172-2177.

[85] Reunanen, A., Knekt, P., Aaran, R. K., & Aromaa, A. (1998). Serum antioxidants and risk of non-insulin dependent diabetes mellitus. *Eur J Clin Nutr*, 52(2), 89-93.

[86] Sinclair, A. J., Taylor, P. B., Lunec, J., Girling, A. J., & Barnett, A. H. (1994). Low plasma ascorbate levels in patients with type 2 diabetes mellitus consuming adequate dietary vitamin C. *Diabet Med*, 11(9), 893-898.

[87] Ristow, M., Zarse, K., Oberbach, A., Kloting, N., Birringer, M., Kiehntopf, M., et al. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci USA*, 106(21), 8665-8670.

[88] Chertow, B. (2004). Advances in diabetes for the millennium: vitamins and oxidant stress in diabetes and its complications. *MedGenMed*, 6(3), 4.