Oxidative Stress in Diabetes Mellitus: Is There a Role for Hypoglycemic Drugs and/or Antioxidants?

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1. Introduction

The most recent statistics indicate that the global prevalence of diabetes mellitus, estimated as 366 million in 2011, will increase to 522 million by 2030 (Whiting et al., 2011). Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or insulin action (ADA, 2011). There are basically two types of diabetes mellitus: type 1 and type 2 diabetes mellitus. Type 1 diabetes mellitus, an autoimmune disease, is characterized by the loss of pancreatic β-cells resulting in absolute insulin deficiency. It accounts for about 5-10 % of all newly diagnosed diabetes mellitus (ADA, 2011). On the other hand, type 2 diabetes mellitus is characterized by insulin resistance and β cell dysfunction. It remains the most common form of diabetes mellitus and constitutes about 90-95 % of all diabetes cases (ADA, 2011). In spite of the availability of different classes of oral hypoglycemic drugs, the incidence of microvascular complications (nephropathy, retinopathy and neuropathy) and macrovascular complications atherosclerosis, coronary artery disease, peripheral arterial disease and stroke continues to rise unabated in diabetic patients, even with treatment (Roglic and Unwin, 2010).

Pharmacological agents with different mechanisms of action are often combined to achieve optimal glycemic control (Turner et al., 1999). However, despite the use of multiple drugs, a lot of diabetic patients do not achieve the optimal glycemic goal (Turner et al., 1999). The Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) have demonstrated that intensive treatment of hyperglycemia reduces risk of developing microvascular and macrovascular complications (DCCT, 1993, UKPDS, 1998). However, recent findings indicate that intensive treatment of hyperglycemia is associated with higher incidence of weight gain, hypoglycemia and mortality than conventional therapy (Ismail-Beigi et al., 2010). These findings suggest that intensive therapy is not only detrimental, but limited and may not be the best. Besides, these findings may suggest that it is high time "hyperglycemia alone" was made the "culprit" in the management of diabetes mellitus. Generally, hypoglycemic drugs are incapable to prevent

pancreas degeneration, worsening of glycemic control and diabetic complications (DCCT, 1993, UKPDS, 1998, Turner et al., 1999, Ball et al., 2000). All these have been linked to increased oxidative stress in diabetes mellitus (Figueroa-Romero et al., 2008, Giacco and Brownlee, 2010). The aim of this chapter is to shed more light on the prospective of managing diabetes mellitus more effectively by targeting both "hyperglycemia and oxidative stress simultaneously". The data presented in this chapter convincingly suggest that the current management of diabetes mellitus may be improved upon by targeting hyperglycemia and oxidative stress as two potential therapeutic targets in diabetes mellitus.

2. General overview of oxidative stress

Considering the aim of this chapter, the general concept of oxidative stress will be discussed only in brief. Oxidative stress can defined as an "imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage" (Sies, 1991). According to Halliwell, "oxidative stress refers to a serious imbalance between reactive species production and antioxidant defenses" (Halliwell and Gutteridge, 2007). It occurs due to an increased generation and/or reduced elimination of reactive species (RS) by the antioxidant defense system. Oxidative stress is usually associated with oxidative damage, which is defined as "the biomolecular damage caused by attack of RS upon the constituents of living organisms" (Halliwell and Gutteridge, 2007). Most of the biologically relevant RS are either reactive oxygen species (ROS) or reactive nitrogen species (RNS). ROS include free radicals such as superoxide ($O_2^{\bullet-}$) and hydroxyl ($^{\bullet}OH$), and non-free radicals such as hydrogen peroxide (H_2O_2). Reactive nitrogen species include free radicals such as nitric oxide ($^{\bullet}NO$) and nitrogen dioxide ($NO_2^{\bullet-}$), and non-free radicals such as peroxynitrite ($OONO^-$) (Sies, 1991, Halliwell and Gutteridge, 2007). The generation of RS by aerobic organisms may occur as by products of metabolism (e.g. during operation of electron transfer chains), intentionally (e.g. during inflammation), or as a result of accidents of chemistry (such as the autoxidation of unstable biomolecules, e.g. dopamine) (Halliwell, 2011). Of all the RS, significant roles of $O_2^{\bullet-}$, $^{\bullet}NO$, and $OONO^-$ have been implicated in diabetic cardiovascular complications (Johansen et al., 2005). In order to prevent oxidative damage, it is important that excess RS is eliminated from the cells. Oxidative damage to cellular components impairs cellular functions. Besides their toxicities, ROS are also required in certain conditions and for physiological functions. For instance, during inflammation, the phagocytes release ROS which kill invading bacteria. ROS generated during mild or moderate exercise constitute part of the mechanism of exercise- or training-induced adaptation (Sachdev and Davies, 2008).

The ability of cells or tissues to withstand oxidative stress is largely dependent on the efficiency of the overall antioxidant defense system to scavenge excess RS, without compromising the physiological roles of ROS (Halliwell, 2011). The antioxidant defense system consists of endogenously-synthesized antioxidants which include antioxidant enzymes, glutathione, vitamins, small molecules and micronutrients (Sies, 1991, Halliwell and Gutteridge, 2007). An antioxidant is defined as "any substance that delays, prevents or removes oxidative damage to a target molecule" (Halliwell and Gutteridge, 2007). Antioxidant enzymes are enzymes which scavenge or eliminate a variety of RS including those generated during biological processes (Sies, 1991, Halliwell and Gutteridge, 2007). The main antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT) and glutathione

peroxidase (GPx) (Halliwell, 2011). SOD maintains the cellular levels of $O_2^{\bullet-}$ within the physiological concentrations by converting $O_2^{\bullet-}$ to H_2O_2, a more stable ROS (Halliwell and Gutteridge, 2007, Fukai and Ushio-Fukai, 2011). CAT metabolizes H_2O_2 to O_2 and H_2O (Halliwell and Gutteridge, 2007). CAT exerts two enzymatic activities, depending on the concentrations of its substrate (H_2O_2) (Scibior and Czeczot, 2006). It elicits a catalytic function at high concentrations of H_2O_2, whereas it produces a peroxidatic effect at lower concentrations of H_2O_2 (Scibior and Czeczot, 2006). GPx enzymatically reduces H_2O_2 to O_2 and H_2O using a hydrogen donor, glutathione (GSH) which is oxidized to glutathione disulfide (GSSG) (Lubos et al., 2011). Unlike CAT, GPx has a broader spectrum of substrates, detoxifying organic hydroperoxides and lipid peroxides (Lubos et al., 2011). However, CAT compared to GPx has a higher K_M for H_2O_2 and thus can protect against a higher concentration of H_2O_2 (Halliwell and Gutteridge, 2007).

The other antioxidant enzymes include glutathione reductase (GR), glutathione S-transferase (GST), peroxiredoxin, thioredoxin and thioredoxinreductase (Andreyev et al., 2005). GR scavenges $O_2^{\bullet-}$ and $^{\bullet}OH$ non-enzymatically or by serving as an electron donor to certain enzymes involved in the metabolism of ROS (Andreyev et al., 2005, Slonchak and Obolens'ka, 2009). GR helps to regenerate GSH via reduction of oxidized glutathione (GSSG) (Slonchak and Obolens'ka, 2009). GST comprises a family of multifunctional phase II biotransformation enzymes with a broad spectrum for a variety of substrates including epoxides, carcinogens, mutagens, 4-hydroxy-2-nonenal and malondialdehyde (MDA) (Andreyev et al., 2005, Slonchak and Obolens'ka, 2009). It catalyzes the conjugation of many electrophilic compounds with GSH (Andreyev et al., 2005). These enzymes work co-operatively together in order to scavenge RS and xenobiotics and thereby protect cells against oxidative damage (Halliwell, 2011). Generally, antioxidant enzymes differ from one another in terms of structure, tissue distribution, co-factor requirement, function, substrate specificity and affinity. The uniqueness of the antioxidant defense system lies in its capability to maintain the RS at certain steady-state levels thereby create and maintain a balance between the beneficial and injurious effects of RS (Lushchak, 2010). More detailed information on oxidative stress including free radicals, ROS, RNS, antioxidant enzymes, antioxidants, antioxidant defense system, and markers of oxidative stress can be obtained from following references (Sies, 1991, Johansen et al., 2005, Halliwell and Gutteridge, 2007, Halliwell, 2011).

3. Oxidative stress and its sources in diabetes mellitus

Under normal conditions and in most diseases including diabetes mellitus, mitochondria are the main source of RS and oxidative stress. Under physiological conditions, e.g. during mitochondrial oxidative metabolism, the bulk of oxygen (O_2) utilized is reduced to H_2O, while less than 2% of O_2 consumed is converted to $O_2^{\bullet-}$ (Brand, 2010). $O_2^{\bullet-}$ is an important ROS because it may be converted to other RS including H_2O_2, OH and $ONOO^-$ (Andreyev et al., 2005). Within physiological conditions, the body is protected from the detrimental effects of these free radicals by a network of antioxidant defense system. However, this defense system becomes impaired in diabetes mellitus and is further exacerbated by chronic hyperglycemia which generates ROS, resulting in oxidative stress. Some of the various sources of ROS and oxidative stress in diabetes mellitus include:

3.1 Hyperglycemia

Chronic hyperglycemia is the hallmark of diabetes mellitus. Evidence implicates mitochondrial generation of $O_2^{\bullet-}$ as a significant source of ROS (Andreyev et al., 2005). With persistent hyperglycemia, disproportionate amounts of glucose are delivered to the cells. This results in enhanced glucose flux through glycolysis and the tricarboxylic acid (TCA) cycle (Drews et al., 2010). This leads to an overdrive of the mitochondrial electron transport chain, which generates greater amounts of $O_2^{\bullet-}$ more than mitochondrial SOD can dismutase (Wiernsperger, 2003, Brand, 2010). This tilts the normal delicate balance between mitochondrial ROS production and mitochondrial ROS degradation in favor of mitochondrial ROS generation, and oxidative stress ensues (Brand, 2010). Evidence indicates that hyperglycemia-induced excessive mitochondrial $O_2^{\bullet-}$ production plays an important role in generating other RS in diabetes mellitus (Nishikawa et al., 2000b). Furthermore, glucose autooxidation produces ROS in the presence of transition metal ions (Johansen et al., 2005). Elevated glucose in diabetes may also react with lipids, resulting in the generation of RS (Johansen et al., 2005). Through non-enzymatic glycation reaction, glucose can react with proteins to produce several intermediate products such as Amadori and Sciff base products before generating advanced glycosylation endproducts (AGEs) (Johansen et al., 2005). Evidence indicates ROS are generated at each step of these reactions. Moreover, with excessive glucose in diabetes, it has been shown that glucose is diverted to other pathways such as sorbitol and hexosamine pathways where glucose is metabolized and ROS are generated (Johansen et al., 2005, Figueroa-Romero et al., 2008, Giacco and Brownlee, 2010).

3.2 Impaired antioxidant defense system

Impaired antioxidant defense system, such as reduced levels of endogenous antioxidants, reduced/enhanced antioxidant enzyme activities and increased levels of oxidative stress markers such as MDA, is very common in diabetes mellitus (Maritim et al., 2003, Johansen et al., 2005, Rahimi et al., 2005, Erejuwa et al., 2010a). The insufficient scavenging of RS as a result of impaired antioxidant defense system in diabetes may contribute to increased oxidative damage. The mechanisms for the impaired antioxidant defenses in diabetes mellitus remain poorly understood. It may be due to non-enzymatic glycation of these enzymes by hyperglycemia and thereby impairs their individual functions. Furthermore, evidence indicates that the antioxidant enzymes produce optimal protection when they function together (Michiels et al., 1994). Thus, glycation of any of these antioxidant enzymes may impair the efficiency of the entire antioxidant defense system or network. For instance, if SOD activity is impaired, this may result in $O_2^{\bullet-}$ build-up. On the other hand, if SOD activity is up-regulated, this may result in increased levels of H_2O_2. In either case, this may affect the activity of CAT or GPx. Similarly, if GR activity is impaired, there may be increased steady-state GSSG levels which will prevent regeneration of GSH, an endogenous antioxidant.

3.3 Increased activity of ROS-generating enzymes

The activities of many ROS-generating enzymes such as cyclooxygenase, xanthine oxidase, lipoxygenases, myeloperoxidase, NADPH oxidases and eNOS are augmented in diabetes (Wiernsperger, 2003). Besides, evidence implicates a role of endothelial NO synthase (eNOS). The eNOS produces NO which scavenges $O_2^{\bullet-}$ in non-diabetic subjects. However,

the eNOS has been found to be uncoupled in diabetic blood vessels where it produces $O_2\bullet^-$ instead of $\bullet NO$ (Wiernsperger, 2003). Hyperglycemia might play an important role in the upregulation and uncoupling of ROS-generating enzymes.

3.4 Hyperinsulinemia

Insulin resistance characterized by hyperinsulinemia is frequent in the majority of the individuals with type 2 diabetic mellitus. Insulin induces the release of H_2O_2 when activating its receptors (Wiernsperger, 2003). Even though H_2O_2 is a non-free radical, it is membrane permeable and can diffuse to other sites, different from its site of production (Sies, 1991, Halliwell and Gutteridge, 2007). Chronic hyperinsulinemia together with the impaired antioxidant defenses in diabetes will lead to inefficient scavenging of H_2O_2. In the presence of transition metals such as copper and iron, H_2O_2 undergoes Fenton reaction to generate $\bullet OH$ which is implicated in the initiation and propagation of lipid peroxidation. Thus, hyperinsulinemia via H_2O_2 formation may increase RS and contribute to oxidative stress and damage in diabetes mellitus. Besides, insulin stimulates the release of many neurotransmitters, via activation of sympathetic nervous system. Many of these neurotransmitters are known to generate ROS and induce oxidative stress (Wiernsperger, 2003).

3.5 Insulin deficiency

Insulin deficiency is frequently observed in the diabetic patients. In some type 2 diabetic patients, insulin deficiency may be so severe such that the injection of exogenous insulin is required to control hyperglycemia (Turner et al., 1999, Cook et al., 2005). Insulin deficiency augments the activity of fatty acyl coenzyme A oxidase. Fatty acyl coenzyme A oxidase is an enzyme which is responsible for the oxidation of fatty acids, resulting in the increased generation of H_2O_2 (Schonfeld et al., 2009). H_2O_2 is well recognized for its role in exerting deleterious effects on cellular components such as proteins, nucleic acid and lipids including polyunsaturated fatty acids (PUFAs) (Sies, 1991, Halliwell and Gutteridge, 2007). These injurious effects of H_2O_2 can be mediated directly or indirectly through $\bullet OH$ formation or reaction with transition metals (such as copper or iron) to form toxic aldehydes, which are highly susceptible to free radical attack (Sies, 1991, Halliwell and Gutteridge, 2007). This sets up a chain reaction which further propagates the formation of more free radicals or RS and thereby contributes to or exacerbate oxidative stress.

3.6 Other sources of ROS and oxidative stress in diabetes mellitus

Diabetes mellitus is characterized by lipid abnormalities such as elevated LDL and cholesterol (ADA, 2011). These abnormalities are further exacerbated by the increased oxidizing environment which enhances the formation of oxidized LDLs (oxLDLs), glycated LDL and oxysterols (formed from the oxidation of cholesterol) (Johansen et al., 2005). These oxidized lipid products bind to specific receptor proteins or activate inflammatory proteins which generate ROS (Johansen et al., 2005). The import of oxLDLs in the vascular wall is the main mechanism by which ROS and oxidative stress induce atherosclerosis (Sies, 1991, Wiernsperger, 2003, Halliwell and Gutteridge, 2007). Evidence indicates that the levels of certain pro-oxidants such as ferritin and homocysteine are elevated in diabetes (Penckofer et

al., 2002). Free iron can increase ROS generation and the oxidation of LDL cholesterol. Similarly, homocysteine can generate ROS in the presence of transition metals which may enhance the oxidation of LDL cholesterol (Penckofer et al., 2002). Another source of ROS in diabetes mellitus is leptin. Elevated levels of leptin are associated with insulin resistance and diabetes mellitus. Evidence implicates a role of leptin in inducing ROS and oxidative stress in aortic endothelial cells in a dose-dependent manner while it produces additive effects with those of glucose (Wiernsperger, 2003). Other potential sources of ROS include aging, menopause, diet and physical activity (Penckofer et al., 2002). Increased ROS is reported in older people. Menopause may also enhance ROS production in older women. The levels of oestrogen, an antioxidant which decreases the oxidation of LDL cholesterol, usually decline during menopause (Penckofer et al., 2002). Aging and menopause may exacerbate oxidative stress since a large number of type 2 diabetics are older men and women. Consumption of foods rich in carbohydrates and fats, as opposed to antioxidant-enriched diets, may also enhance ROS formation. Sedentary lifestyle may predispose to increased ROS generation. This is corroborated by evidence which indicates mild or moderate, but not strenuous, exercise induces antioxidant defenses (Sachdev and Davies, 2008).

4. Role of oxidative stress in the pathogenesis of diabetes mellitus

Evidence implicates the role of oxidative stress in the different stages of the development of diabetes mellitus, starting from the pre-diabetes state, impaired glucose tolerance, postprandial hyperglycemia, mild diabetes and finally to overt diabetes mellitus (Ceriello et al., 1998). Loss of β-cell function, resulting from impaired secretory capacity and increased apoptosis, is a main occurrence in the pathogenesis of both types of diabetes (Drews et al., 2010). Besides β-cell dysfunction, insulin resistance is also a major characteristic feature of type 2 diabetes mellitus (Evans et al., 2003). Oxidative stress plays an important role in the pathogenesis of both β-cell dysfunction and insulin resistance (Evans et al., 2003, Drews et al., 2010).

4.1 Role of oxidative stress in β-cell dysfunction

The β-cells express low level of antioxidant enzymes such as SOD, CAT and GPx and thereby increase their susceptibility to oxidative stress (Tiedge et al., 1997). Increased mitochondrial ROS production in the β-cells results from enhanced glucose or fatty acid flux through glycolysis and the TCA cycle (Drews et al., 2010). This generates excess $O_2^{\bullet-}$ which gives rise to other ROS and RNS. The insufficiency of antioxidant enzymes to scavenge these ROS leads to oxidative stress. Besides mitochondria, NADPH oxidases, nitric oxide synthases, and phagocytes are other key sources of ROS in the β-cells (Drews et al., 2010). In type 1 diabetes mellitus, evidence implicates the role of ROS in impaired β-cell function caused by autoimmune reactions, cytokines and inflammatory proteins (Drews et al., 2010). Similarly, in type 2 diabetes mellitus, the role of ROS is implicated in the β-cell dysfunction as well as insulin resistance (Drews et al., 2010). The pancreas is highly susceptible to oxidative stress as evidenced by studies which show that H_2O_2 impairs insulin secretion in pancreatic β-cells (Maechler et al., 1999) and products of oxidative stress inhibit glucose-stimulated insulin secretion (Miwa et al., 2000). Other evidence shows that overexpression of antioxidant enzymes in islets or transgenic mice and antioxidants such as N-acetyl-L-cysteine (NAC) protect against ROS-induced β-cell toxicity (Tiedge et al., 1998, Drews et al.,

2010). Research within the last decade has recognized the role of glucotoxicity as the main causal determinant of β-cell dysfunction (Drews et al., 2010). The role of glucotoxicity in β-cell dysfunction is demonstrated by studies which indicate increased glucose concentrations impair insulin release in non-diabetic subjects (Marchetti et al., 2008). In contrast, improved glycemic control results in improved insulin secretion in patients with type 2 diabetes (Marchetti et al., 2008). The fact that antioxidants reduce or prevent the toxicities of elevated glucose on the expression of insulin mRNA, insulin content and secretion lends support to the role of oxidative stress in mediating the toxic effects of glucotoxicity (Tanaka et al., 1999). Besides glucotoxicity, lipotoxicity may also play a role in impaired β-cell function. Free fatty acids have been shown to uncouple mitochondrial oxidative phosphorylation and increase ROS formation in rat pancreatic islets (Carlsson et al., 1999), deplete pancreatic β-cell insulin content (Bollheimer et al., 1998) and inhibit glucose-induced insulin secretion and biosynthesis (Zhou and Grill, 1994). Both glucotoxicity and lipotoxicity may be involved in β-cell dysfunction in diabetes mellitus (Marchetti et al., 2008). The role of oxidative stress is also implicated in β-cell deficit, as well as increased apoptosis observed in humans with type 2 diabetes (Drews et al., 2010).

4.2 Role of oxidative stress in insulin resistance

Insulin resistance precedes the onset of diabetes mellitus and is influenced by factors such as genetic make-up and environmental factors including increased calorie intake, sedentary lifestyle, obesity, pregnancy and abnormally elevated levels of certain hormones (Evans et al., 2003). The role of oxidative stress is implicated in the pathogenesis of insulin resistance (Kim et al., 2006). Both pyruvate and fatty acids can serve as energy substrate in muscle and adipose tissue. Pyruvate is derived from glucose and other sugars, while fatty acids originate from fats. Once transported across the inner mitochondrial membrane, these fuel substrates are converted to acetyl CoA by mitochondrial enzymes. During citric acid cycle, oxidation of the carbon atoms of the acetyl groups in acetyl CoA generates CO_2. Besides CO_2 formation, the citric acid cycle generates high-energy electrons which are carried by NADH and $FADH_2$. The increased uptake of energy substrate in the muscle and adipose tissue enhances citric acid cycle activity. This in turn generates mitochondrial NADH more than required. This leads to an overdrive of the oxidative phosphorylation which increases the mitochondrial transmembrane proton gradient. These high-energy electrons are then transferred to O_2 leading to production of $O_2^{\bullet-}$ which is further converted to other ROS. This sets the stage for oxidative stress (Talior et al., 2003). A recent study showed that the skeletal muscle of high-fat diet-induced insulin-resistant rats liberated more mitochondrial H_2O_2 and had impaired ability to maintain normal redox balance compared to the data obtained in the skeletal muscle of the insulin-sensitive control rats (Anderson et al., 2009). Similar findings were observed in insulin-resistant, morbidly obese human subjects (Anderson et al., 2009). This study provides strong evidence in support of a role of mitochondrial ROS production and oxidative stress in the pathogenesis of insulin resistance.

Various mechanisms by which oxidative stress contributes to insulin resistance have been identified. These include oxidative stress-impaired insulin-induced GLUT4 translocation in adipocytes (Rudich et al., 1998), oxidative stress-induced impairment in insulin stimulation of protein kinase B and glucose transport in adipocytes (Rudich et al., 1999), oxidative stress-induced interactions between the PI3-kinase-dependent signaling pathway and

activation of p38 MAPK (Kim et al., 2006), and interruption of insulin-induced cellular redistribution of insulin receptor substrate-1 and phosphatidylinositol 3-kinase in adipocytes (Tirosh et al., 1999). Other studies indicate that oxidative stress can directly induce considerable insulin resistance in skeletal muscle by interfering with insulin signaling, glucose uptake and glycogen synthesis (Dokken et al., 2008). This oxidative stress-induced insulin resistance is mediated in part through reduced insulin-modulated suppression of glycogen synthase kinase-3 (GSK-3beta) (Dokken *et al.*, 2008; Henriksen, 2010). Evidence has also implicated a role of inducible nitric oxide synthase and $^{\bullet}$NO donor in the degradation of insulin receptor substrate-1. Increased S-nitrosylation of certain molecules or pathways involved in insulin signaling such as insulin receptor, insulin receptor substrate-1, and protein kinase B/Akt in skeletal muscle have also been reported to play an important role in insulin resistance (Carvalho-Filho et al., 2009).

In order to protect against increased glucose-induced oxidative stress (Talior et al., 2003), cells tend to respond by limiting more energy fuel or substrates from gaining access into the cells. Cells may utilize various mechanisms, including inhibition of α-ketoglutarate dehydrogenase, to limit the amount of NADH available for the oxidative phosphorylation. Therefore, to limit insulin-dependent nutrient uptake into the cells, the insulin receptors become less sensitive to the action of insulin. This marks the onset of insulin resistance - a phenomenon whereby normal amounts of insulin can no longer activate the glucose transport system in insulin-sensitive tissues such as skeletal muscle and adipose tissue. This results in enhanced steady-state glucose levels in the blood. It is suggested that, in this setting, insulin resistance may be a compensatory mechanism employed by the cells to prevent further uptake of insulin-stimulated glucose and fatty acids (Hoehn et al., 2009). This compensatory mechanism may result in reduced formation and accumulation of RS leading to reduced oxidative stress and damage. With persistently increased blood glucose levels, hyperglycemia ensues leading to overt type 2 diabetes mellitus.

4.3 Could antioxidants play a role in preventing diabetes mellitus and/or its progression?

In view of evidence which implicates a role of oxidative stress in β-cell dysfunction and insulin resistance, the question that arises is: could antioxidants play a role in preventing diabetes mellitus and/or its progression? As discussed earlier, there is a possibility for such a role of antioxidants. Antioxidants such as vitamin C, vitamin E, β-carotene, α-lipoic acids and honey have been shown to ameliorate hyperglycemia through increased β-cell mass and insulin secretion. However, at the moment, the evidence to recommend or prescribe antioxidants for the prevention of diabetes mellitus is very weak. Instead, efforts should be made to create and increase the awareness on (1) the role of ROS and oxidative stress in the pathogenesis and/or progression of diabetes mellitus and (2) the importance of increased consumption of antioxidant-enriched diets including fruits and vegetables, as opposed to increased calorie intake. The recommendation for increased consumption of fruits and vegetables is very important because even in the developed countries, majority of the population do not meet these requirements. The importance of exercise should also not be left out. These could be achieved by incorporating into educational curricula - at all levels of education. In addition, those who are already diabetic should be enlightened on the importance of maintaining good glycemic control in order to prevent or delay the progression or complications of diabetes mellitus. Diabetic patients also need to be informed

on the need for adherence to dietary regimens and compliance to prescribed medications. These recommendations could also be an important component of training received by healthcare providers.

5. Role of oxidative stress in the complications of diabetes mellitus

There is strong evidence implicating a role of oxidative stress in diabetic nephropathy, retinopathy and neuropathy which constitute the microvascular complications (Figueroa-Romero et al., 2008, Giacco and Brownlee, 2010, Brownlee, 2005). Similarly, a role of oxidative stress is implicated in the macrovascular complications (coronary artery disease, peripheral arterial disease and cerebrovascular disease) (Giacco and Brownlee, 2010, Brownlee, 2005). This section highlights the different mechanisms by which hyperglycemia causes diabetic complications.

5.1 Hyperglycemia-enhanced polyol pathway

The polyol pathway comprises two enzymes: aldose reductase (AR) and sorbitol dehydrogenase (SDH) (Brownlee, 2005). AR reduces a broad spectrum of substrates such as glucose, galactose, methylglyoxal, glucosone, deoxyglucosone and lipid-derived aldehydes (Petrash, 2004). Under euglycemic conditions, glucose is not reduced by AR. The bulk of glucose is normally phosphorylated by hexokinase to produce glucose-6-phosphate, a substrate for glycolysis and pentose phosphate pathway. However, under hyperglycemic conditions, AR reduces glucose to sorbitol (Brownlee, 2005). Sorbitol is oxidized to fructose by SDH (Giacco and Brownlee, 2010). With chronic hyperglycemia, the AR pathway becomes enhanced leading to increased formation of sorbitol. As a result of activated AR pathway, there is increased consumption of NADPH (as an obligate co-factor) by AR (Figueroa-Romero et al., 2008). The GR also requires NADPH, as co-factor, for the regeneration of GSH, an endogenous scavenger of ROS. Therefore, increased utilization of NADPH caused by enhanced activity of AR reduces intracellular concentration of GSH. Reduced levels of GSH will impair the activity of GPx which utilizes GSH as a hydrogen donor (Halliwell and Gutteridge, 2007). Taken together, decreased NADPH impairs GR activity, reduces GSH level and impairs GPx activity. This impairs the antioxidant defense network and increases cellular susceptibility to oxidative stress. NO• has been shown to inhibit AR activity in diabetes (Chandra et al., 2002). In view of evidence which indicates ROS can decrease NO• bioavailability, therefore, increased oxidative stress in diabetes will further augment AR activity. Thus, hyperglycemia-enhanced polyol pathway will exacerbate oxidative stress in diabetes mellitus.

Besides, the increased levels of sorbitol caused by enhanced AR activity increase the osmolality of intracellular milieu. Sorbitol, myoinositol, glycerophosphorylcholine, betaine and taurine are physiological osmolytes which help to maintain homeostasis in cells such as renal medullary cells (Yancey and Burg, 1989). Thus, as a compensatory mechanism, this leads to efflux of intracellular osmolytes (Figueroa-Romero et al., 2008). Some of these osmolytes e.g. myoinositol play a vital role in signaling transduction, while others such as taurine are endogenous antioxidants (Figueroa-Romero et al., 2008). Hence, increased sorbitol formation reduces endogenous antioxidants and other important osmolytes. This further impairs cellular function and increases intracellular susceptibility to oxidative stress. In contrast, the SDH pathway generates fructose from sorbitol. Similar to AR pathway,

chronic hyperglycemia increases the activity of SDH which results in the formation of high amounts of fructose (Figueroa-Romero et al., 2008). Increased SDH activity result in an increased NADH:NAD$^+$ ratio, which may inhibit oxidation of triose phosphates (Nishikawa et al., 2000a). Accumulation of triose phosphates increases *de novo* synthesis of diacylglycerols (DAG) which activate protein kinase C. Increased levels of triose phosphate may also increase generation of methylglyoxal, a potent AGE-precursor. Besides, increased fructose levels may enhance glycation and further contribute to reduced levels of NADPH, impaired antioxidant defenses and formation of AGEs.

5.2 Hyperglycemia-enhanced formation of advanced glycation end products

Advanced glycation end products (AGEs) are adducts formed non-enzymatically by the reaction between reducing carbohydrates and proteins, DNA, or lipids (Ahmed, 2005). AGEs also include products that are formed non-enzymatically from the reaction between AGE precursors, glycated sugars or oxidized products of fatty acid (in arterial endothelial cells) and protein (Giacco and Brownlee, 2010). They are produced through three major pathways: (1) convertion of glucose to glyoxal; (2) degradation of Amadori products to 3-deoxyglucosone; and (3) conversion of glyceraldehyde-3-phosphate to methylglyoxal (Nishikawa et al., 2000a, Ahmed, 2005, Figueroa-Romero et al., 2008). In diabetes mellitus, the levels of AGEs become elevated as a result of chronic hyperglycemia (Duran-Jimenez et al., 2009). The formation of AGEs occurs in different stages. During these stages of AGE formation, a number of highly reactive intermediates and cross-linkers, which enhance the binding affinity of AGEs to proteins, are also formed (Ahmed, 2005). AGEs can inhibit the antiproliferative effects of nitric oxide (Maritim et al., 2003). AGEs bind to and modify intracellular proteins thereby altering their functions (Giacco and Brownlee, 2010). In the vasculature, AGEs interact with cell surface protein or extracellular matrix components resulting in the formation of cross-linked proteins which enhance stiffening within the arterial vessel (Ahmed, 2005). AGEs can also modify plasma proteins which in turn activate receptor for advanced glycation end products (RAGE) on cells such as macrophages, vascular endothelial and smooth muscle cells (Giacco and Brownlee, 2010). AGEs can bind directly to and activate the receptors for AGEs.(Griesmacher *et al.*, 1995, Ahmed, 2005).

The binding of AGEs or AGE-modified plasma proteins to RAGE induces the release of ROS (Nishikawa et al., 2000a; Ahmed, 2005; Giacco and Brownlee, 2010). The ROS activate the expression of several genes and proteins that are involved in inflammatory cascade and implicated in the pathogenesis of diabetic cardiovascular disease. These genes and proteins include nuclear factor kappa β, tumor necrosis factor α, interleukin-1 and granulocyte-macrophage colony-stimulating factor (Nishikawa et al., 2000a, Ahmed, 2005, Giacco and Brownlee, 2010).

5.3 Hyperglycemia-activated protein kinase C pathway

Protein kinase C (PKC) is an enzyme that modulates the functions of other proteins through their phosphorylation. PKC is activated by the elevated level of DAG, derived from enhanced formation of triose phosphate via hyperglycemia (Giacco and Brownlee, 2010). Hyperglycemia may also increase DAG content through phosphatidylcholine hydrolysis (Nishikawa et al., 2000a). In diabetes, elevated levels of triose phosphate occur through increased *de novo* synthesis due to inhibition of glycolytic enzyme glyceraldehyde phosphate

dehydrogenase (GAPDH) by increased ROS (Giacco and Brownlee, 2010). Besides DAG, evidence indicates that AGEs can also activate PKC pathway to increase the expression of vascular endothelial growth factor (VEGF) (Ahmed, 2005). Similarly, increased ROS levels in vascular endothelial cells may also enhance PKC pathway (Nishikawa et al., 2000a). Enhanced PKC activity induces several cytokines and protein signals including plasminogen activator inhibitor (PAI-1), NF-κB, NAD(P)H oxidases, endothelin-1, transforming growth factor β (TGF-β) and extracellular matrix (ECM) (Nishikawa et al., 2000a). These pathological alterations have been implicated in basement membrane thickening, vasoconstriction, altered capillary permeability, hypoxia and activation of angiogenesis (Nishikawa et al., 2000a, Figueroa-Romero et al., 2008).

5.4 Hyperglycemia-enhanced hexosamine pathway

Evidence has implicated the role of hexosamine pathway in the toxic or adverse effects of hyperglycemia in diabetes mellitus (Schleicher and Weigert, 2000). Under physiological conditions, a small quantity of fructose-6 phosphate derived from glycolysis is diverted to the hexosamine pathway. Glutamine: fructose-6 phosphate amidotransferase (GFAT) then converts fructose-6 phosphate to glucosamine-6 phosphate, which is converted to uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc) (Schleicher and Weigert, 2000). The enzyme O-GlcNAc transferase then utilizes UDP-GlcNAc as a substrate, fixing O-GlcNAC to protein residues of transcription factors such as Sp1 and thus modify their expression (Figueroa-Romero et al., 2008). Similar to the other pathways, with chronic hyperglycemia, the hexosamine pathway becomes enhanced (Figueroa-Romero et al., 2008, Schleicher and Weigert, 2000). This leads to increased formation of UDP-GlcNAc and increased activity of O-GlcNAc transferase, with consequent alterations in gene expression (Figueroa-Romero et al., 2008). This pathway is implicated in the hyperglycemia-mediated increases in the transcription of TGF-α and TGF-$β_1$. Over-expression of these transcription factors such as TGF-$β_1$ is known to activate the proliferation of collagen matrix, basement membrane thickening and inhibition of mesangial cell mitogenesis, thus contributing to microvascular complications such as nephropathy (Schleicher and Weigert, 2000).

5.5 Hyperglycemia-activated Poly-ADP Ribose Polymerase (PARP) pathway

Poly(ADP-ribose) polymerase (PARP) is a family of enzymes that detect single- and double-stranded DNA and repair damaged DNA (Virag and Szabo, 2002). Activation of PARP is a direct cellular response to metabolic- or chemical-induced DNA damage. Once PARP senses and identifies a damaged DNA, it binds to the DNA and forms homodimers and catalyzes the cleavage of nicotinamide adenine dinucleotide (NAD^+) into nicotinamide and ADP-ribose (Virag and Szabo, 2002). It then uses ADP-ribose to synthesize a poly(ADP-ribose) chain (PAR) which serves as a signal for several DNA repairing enzymes such as DNA ligase III and DNA polymerase beta (polβ). In hyperglycemic environment, there is increased ROS formation leading to oxidative damage which activates PARP. As a result of NAD^+ utilization, this depletes cellular NAD^+ stores and induces a progressive ATP depletion. This further increases the vulnerability of cells to oxidative stress and damage as well as cell death (Figueroa-Romero et al., 2008). Available evidence suggests that PARP's catalytic activity may cause altered gene expression, increased oxidative stress and diversion of glycolytic intermediates to other pathogenic pathways (Figueroa-Romero et al.,

2008). A recent study demonstrated the beneficial effects of PARP inhibition in diabetic complications (Lupachyk et al., 2011).

5.6 Hyperglycemia-induced mitochondrial $O_2^{\bullet-}$ overproduction

Recent data indicates that hyperglycemia-induced mitochondrial $O_2^{\bullet-}$ overproduction is the sole underlying mechanism (directly or indirectly) by which hyperglycemia induces cellular damage (Giacco and Brownlee, 2010). During mitochondrial oxidative phosphorylation, $O_2^{\bullet-}$ is generated due to leakage of electrons from electron transport chain (ETC) on molecular oxygen. In euglycemic environment, about 0.2–2% of O_2 utilized by the mitochondria is reduced to $O_2^{\bullet-}$ (Bashan et al., 2009, Brand, 2010). The antioxidant defense network maintains the mitochondrial level of ROS within physiological concentrations (Andreyev et al., 2005, Brand, 2010). However, in hyperglycemic environment, enhanced glucose flux through glycolysis and TCA causes an overdrive of the mitochondrial ETC resulting in mitochondrial dysfunction and increased ROS formation (Bashan *et al.*, 2009; Brand, 2010). Elevated levels of ROS lead to oxidative stress and damage. Oxidative damage to DNA can activate Poly(ADP-ribose) polymerase (PARP) pathway (Wei, 1998).

Besides this pathway, evidence suggests that hyperglycemia, via $O_2^{\bullet-}$ overproduction , inhibits G6PDH. G6PDH is the rate-limiting enzyme of the pentose phosphate pathway necessary for generating reducing equivalents to the antioxidant defense system (Brand, 2010). Inhibition of G6PDH leads to increased levels of glycolytic intermediates resulting in increased flux into these pathways. For instance, glyceraldehyde 3-phosphate can non-enzymatically be converted to methylglyoxal which activates AGEs pathway (Giacco and Brownlee, 2010). Similarly, glyceraldehyde 3-phosphate is a precursor of DAG which can activate PKC pathway. Increased levels of fructose 6-phosphate will increase flux through the hexosamine pathway (Giacco and Brownlee, 2010). Furthermore, reduced levels of G6PDH result in increased glucose concentrations which further enhances flux through the polyol pathway. The aldose reductase pathway becomes intensified leading to increased formation of sorbitol. This sets up a chain reaction that continuously activates one pathway or the other generating ROS which may cause DNA damage. This causes activation of PARP pathway. Thus, virtually all these pathways, namely polyol pathway, formation of AGEs pathway, PKC pathway, hexosamine pathway, and poly-ADP ribose polymerase (PARP) pathway, can be activated by hyperglycemia-induced mitochondrial $O_2^{\bullet-}$ overproduction.

6. Interrelation among glycemic control, oxidative stress and diabetic complications

So far, this chapter has identified the various sources of oxidative stress in diabetes mellitus. It has also presented evidence that indicates hyperglycemia enhances formation of ROS in diabetes mellitus. It has also shown that hyperglycemia-induced oxidative stress plays an important role in the activation of several pathogenic pathways implicated in the pathogenesis of diabetic complications. Generally, the mechanisms by which hyperglycemia causes cellular damage can be classified into two groups (Nishikawa et al., 2000a). The first group of mechanisms entails constant acute fluctuations in cellular metabolism which are reversible following restoration of euglycemia. The second group of mechanisms involves cumulative changes in long-lived macromolecules which are irreversible even after euglycemia is restored (Nishikawa et al., 2000a). Studies have demonstrated the beneficial

effects of reduced hyperglycemia or glycemic control on the risk of developing diabetic complications (DCCT, 1993, UKPDS, 1998). Nevertheless, recent findings suggest that treatment of chronic hyperglycemia to achieve optimal glycemic goal in diabetic patients is limited and detrimental (Ismail-Beigi et al., 2010). It is recommended that the consequences (higher mortality rate, hypoglycemia and weight gain) should be weighed against the benefits of intensive therapy (Ismail-Beigi et al., 2010). Besides, available evidence indicates that achieving and/or maintaining optimal glycemic control in diabetic patients is difficult (Turner et al., 1999, Cook et al., 2005). The difficulty in maintaining optimal glycemic control is attributed to deterioration of pancreatic β-cell function, which is linked to hyperglycemia-induced oxidative stress (Drews et al., 2010). Interestingly, even in diabetic patients given pancreatic transplants, diabetic complications such as nephropathy continued to deteriorate at least five years after their diabetes had been cured (Fioretto et al., 1998). This contradicts evidence that links hyperglycemia to diabetic complications. Therefore, this section attempts to explain the interrelation among glycemic control, oxidative stress and diabetic complications and possible role of hypoglycemic drugs (and/or insulin) and antioxidants in the management of diabetic complications by answering the following questions:

1. Does reduced or intensive therapy of hyperglycemia prevent induction or development of oxidative stress?
2. Does reduced or intensive therapy of hyperglycemia prevent diabetic complications?
3. Why does reduced or intensive therapy of hyperglycemia not prevent diabetic complications?
4. Could there be a role of antioxidants in the management of diabetic complications?
5. Could there be a role of hypoglycemic drugs (and/or insulin) and antioxidants in the management of diabetic complications?

6.1 Does reduced or intensive therapy of hyperglycemia completely restore or ameliorate oxidative stress?

Hyperglycemia induces oxidative stress in diabetes mellitus. However, does evidence indicate that reduced or intensive treatment of hyperglycemia completely prevent development of oxidative stress? This section aims to answer this important question by presenting both experimental and clinical data. In diabetic rats, after two months of poor glycemic control, re-institution of good glycemic control for seven additional months partially reduced the elevated caspase-3 activity, levels of NF-kβ, lipid peroxides and nitric oxides with no beneficial effect on nitrotyrosine formation. In contrast, after six months of poor glycemic control, re-institution of good glycemic control for seven additional months demonstrated no significant effects on the elevated caspase-3 activity, NF-kβ, and oxidative stress parameters (Kowluru, 2003, Kowluru *et al.*, 2004). In another follow up study, after six months of poor glycemic control, normalization of hyperglycemia for another 6 months also had no significant effect on retinal nitrotyrosine levels, neither did oxidative stress parameters improve (Kowluru et al., 2007). Other studies have also shown that reduced hyperglycemia does not completely restore redox status (Rahimi et al., 2005, Erejuwa et al., 2010a, Erejuwa et al., 2011b).

Evidence suggests that proteins (collagen) are likely to be glycated irrespective of blood glucose levels (Monnier et al., 1999). In patients with type 2 diabetes mellitus, insulin treatment only partially improved oxidative stress parameters (Seghrouchni et al., 2002). This is evidenced by the elevated levels of thiobarbituric acid reactive substances and reduced erythrocyte GSH (Seghrouchni et al., 2002). In type 2 diabetic patients, treatment

with gliclazide for 12 weeks ameliorated oxidative stress better than did glibenclamide (Fava et al., 2002). Available data from DCCT and Epidemiology of Diabetic Complications and Interventions (EDIC) Trial indicated that type 1 diabetic patients in the intensive therapy group still had increased levels of protein glycation products and AGEs despite intensive treatment (Genuth et al., 2005). However, protein glycation and AGE formation were less compared with those in the conventional treatment group (Genuth et al., 2005).

The data highlighted in this section reveal that reduced or intensive therapy of hyperglycemia does not completely prevent induction of oxidative stress. In other words, once hyperglycemia (either via mitochondrial $O_2^{\bullet-}$ overproduction or other mechanisms) activates any of these mechanistic pathways (especially the polyol pathway), intensive therapy or even normalization of hyperglycemia (which is even difficult with the current hypoglycemic drugs) would have limited effects on oxidative stress. Once oxidative stress is induced in diabetes mellitus, it can enhance the generation of ROS and initiate redox-chain reactions which may activate various inflammatory proteins and signaling pathways including all the abovementioned mechanistic pathways. Even if normal glucose level is restored, these inflammatory proteins and signaling pathways on their own could cause oxidative stress and/or sustain the oxidative stress originally or previously induced by hyperglycemia. Thus, with or without hyperglycemia, the resulting ROS and oxidative stress secondary to hyperglycemia would suffice to activate some of these pathways and set the stage for oxidative stress or exacerbate the already developed or existing oxidative stress. Besides, evidence implicates a role of oxidative stress in many neurodegenerative disorders such as hypertension, cancer and Alzheimer's disease. All of these disorders are characterized by euglycemia. Therefore, it means or suggests that oxidative stress can be an entirely independent phenomenon which can exist even with normalization of hyperglycemia.

6.2 Does reduced or intensive therapy of hyperglycemia prevent diabetic complications?

Data from animal and human studies indicate that intensive therapy may delay the progression of diabetic complications, but does not prevent diabetic complications. A study that investigated the effect of improved glycemic control on the progression of retinopathy in diabetic dogs found that diabetic dogs with 5-year poor glycemic control developed diabetic retinopathy while those with 5-year good glycemic control had no diabetic retinopathy (Engerman and Kern, 1987). The third group comprised diabetic dogs with 2.5-year poor glycemic control. It was observed that these diabetic dogs did not develop diabetic retinopathy. However, the dogs later developed diabetic retinopathy despite 2.5-year good glycemic control (Engerman and Kern, 1987). The study further revealed that the extent of pathology of diabetic retinopathy in the third group (with 2.5-year poor glycemic control + 2.5-year good glycemic control) was similar to that of the dogs with 5-year poor glycemic control (Engerman and Kern, 1987). Similarly, in sucrose-fed diabetic rats, cure of diabetes via islet transplantation at 12 weeks (but only at 6 weeks after the confirmation of diabetes) did not prevent lesions or progression of diabetic retinopathy (Hammes et al., 1993). Kowluru and colleagues (2007) also reported that after 6 months of poor glycemic control in rats, normalization of hyperglycemia for 6 months had no effect on the lesions or pathology of diabetic retinopathy.

In type I diabetic patients whose diabetes had been cured (through pancreas transplantation) but still had diabetic nephropathy, the thickness of glomerular and tubular basement membranes was still similar at 5 years versus baseline values. In contrast, it was significantly reduced only by the 10th year versus baseline values. The study further showed that while mesangial fractional volume had increased by the 5th year, it significantly decreased by the 10th year (Fioretto et al., 1998). In the DCCT, due to the considerable benefits of intensive therapy, patients in the conventional therapy group were transitioned to intensive therapy group (DCCT, 1993). A long-term follow-up study under the EDIC Trial (DCCT/EDIC, 2002, DCCT/EDIC, 2003, Pop-Busui et al., 2009) showed that patients who were formerly in the intensive therapy group had lower incidence of diabetic microvascular complications than the patients who were originally in the conventional treatment group, despite 8 years of similar and normalized glycemic control in the EDIC trial (DCCT/EDIC, 2002). It was further reported that the conventionally treated-DCCT patients who were transitioned to intensive therapy group in the EDIC trial still had greater incidence of macrovascular pathology or complications (Nathan et al., 2003, Nathan et al., 2005, Cleary et al., 2006, Patel et al., 2008). Evidence from clinical trials also suggests that the incidence or development of diabetic complications is more likely to depend on the intensity of oxidative stress than on the level of glycemic control (Monnier et al., 1999, Genuth et al., 2005). Besides, evidence indicates that the degree of insulin resistance correlates with the onset of diabetic complications, independently of glycemic levels (Chillaron et al., 2009). In type 2 diabetic patients, it was reported that gliclazide treatment delayed the progression of diabetic nephropathy only, whereas it produced no significant effect on the development or progression of retinopathy or macrovascular complications (Patel et al., 2008). These findings are very important because gliclazide is one of the few hypoglycemic agents with an antioxidant effect (Fava et al., 2002).

It is evident from the different aforementioned hyperglycemia-induced mechanistic pathways and the data (both experimental and clinical) presented in this section that the pathogenesis of diabetic complications is a complex process. It involves oxidative stress and ROS-activated pathways including several inflammatory proteins, cytokines and growth factors. It is clear that hyperglycemia exerts long-term injurious effects in patients with type 1 and type 2 diabetes mellitus. Similar long-lasting detrimental effects of hyperglycemia also occur in animals (dogs). In both animals and humans with diabetes, glycemic control only delays the development or progression of diabetic complications. It does not prevent or completely restore diabetic complications. This is understandable in view of the fact that these diabetic complications are not primary effects of hyperglycemia but are its sequelae (secondary effects). In fact, in few cases in which normalization of hyperglycemia or cure of diabetes mellitus do prevent diabetic complications, it has to be initiated at a very early stage of the disease and maintained for several years. Overall, these findings clearly show that intensive therapy or normalization of hyperglycemia does not prevent diabetic complications.

6.3 Why does reduced or intensive therapy of hyperglycemia not prevent diabetic complications?

Investigators have introduced different phrases to describe this concept or observation in which reduced or intensive therapy of hyperglycemia does not prevent diabetic complications. Some of these phrases include ''glycemic memory'', "hyperglycemic memory'', "metabolic memory'' or "lasting memory''. Glycemic memory is a term that refers to the development of diabetic complications during post-hyperglycemic normoglycemia

(Nishikawa et al., 2000a). It is a phenomenon whereby early glycemic milieu or environment is remembered in many target organs such as heart, eye, nerve and kidney. Evidence suggests that oxidative stress may contribute to the inability of reduced or intensive treatment of hyperglycemia to prevent diabetic complications (Ceriello et al., 2009). It is proposed that glycemic memory could involve two stages: induction and perpetuation (Nishikawa et al., 2000a). During induction, hyperglycemia generates increased ROS levels. This may result from increased production of reducing equivalents formed from overdrive of the mitochondrial ETC. As a result of increased ROS, cellular dysfunction and mutations in mitochondrial DNA may occur (Wei, 1998). On the other hand, perpetuation, which is glycemic memory itself, could occur because ROS-induced mutated mitochondrial DNA would encode defective ETC subunits (Nishikawa et al., 2000a). This hypothesis of defective mitochondrial DNA subunits seems valid. Two years after this hypothesis, a study demonstrated that methylglyoxal modified mitochondrial proteins causing disturbances in mitochondria in kidney of rats (Rosca *et al.*, 2002). The study further showed that methylglyoxal produced an inhibitory effect on the tricarboxylic acid (TCA) cycle and the electron respiratory chain in kidney of rats. In another related study, it was shown that methylglyoxal-modified mitochondria considerably augmented $O_2^{\bullet-}$ production,, independently of the level of hyperglycemia, and were characterized by oxidative damage (Rosca *et al.*, 2005).

Furthermore, Ihnat and colleagues (2007) also provided evidence in support of the role of oxidative stress in mediating the hyperglycemic memory (Ihnat et al., 2007). The authors showed that in human endothelial and ARPE-19 retinal cells, the levels of protein kinase C-beta, NAD(P)H oxidase subunit p47phox, BCL-2-associated X protein, 3-nitrotyrosine, ffibronectin and poly (ADP-ribose) remained elevated for 1 week after the levels of glucose had normalized. The study showed that inhibition of ROS production using antioxidant alpha-lipoic acid prevented the induction of these glucose-induced oxidative stress markers (Ihnat et al., 2007). Similar findings were also reported in aortic endothelial cells both *in vitro* and in non-diabetic mice (El-Osta et al., 2008). The study showed that short-term hyperglycemic spikes produced long-lasting effects on vascular cells (El-Osta et al., 2008). This suggests that transient spikes of hyperglycemia may be an HbA1c-independent risk factor for diabetic complications (El-Osta et al., 2008). A recent study also indicated that elevated glucose levels caused continual changes in cell viability and apoptosis-related gene expressions even after recovery of normoglycemia (Wei et al., 2011). The changes were associated with increased ROS production (Wei et al., 2011). Besides, evidence implicates the role of protein glycation and formation of AGEs in metabolic memory (Genuth et al., 2005, Ceriello et al., 2009). AGEs may reduce NADPH and impair antioxidant system which in turn can generate more ROS (Ahmed, 2005). Data from the DCCT suggest that the tendency of collagen to be glycated is less dependent on the level of blood glucose (Monnier et al., 1999). It was also demonstrated that both conventional and intensive therapies did not prevent protein glycation and formation of AGEs (Genuth et al., 2005). The data further revealed that the incidences of retinopathy and nephropathy were significantly associated with the levels of protein glycation and AGEs (Genuth et al., 2005). This phenomenon termed "glycemic memory" has also been corroborated in several other studies (Nathan et al., 2005, Holman et al., 2008).

Recent findings indicate that elevated oscillating glucose concentrations may contribute to increased risk for cardiovascular diabetic complications (Ceriello et al., 2008). As

demonstrated by Ceriello and colleagues (2008), using a euinsulinemic hyperglycemic clamp, glucose at 5, 10, and 15 mmol/l was given in rising steps as a single "spike", and oscillating between basal and high levels over 24-hour in non-diabetic subjects and type 2 diabetic patients. The authors reported that glucose at 10 and 15 mmol/l led to a concentration-dependent fasting blood glucose-independent impaired endothelial function and increased intensity of oxidative stress in both non-diabetic subjects and type 2 diabetic patients (Ceriello et al., 2008). The study also revealed that oscillating glucose concentrations between 5 and 15 mmol/l every 6 hour for 24 hours caused further significant impairments in endothelial function and induced oxidative stress compared with continuous 10 or 15 mmol/l glucose (Ceriello et al., 2008). These findings are very relevant because the current management of diabetes mellitus (including intensive therapy) does not control postprandial hyperglycemia. Besides, both endothelial function and oxidative stress are implicated in the pathogenesis of diabetic micro- and macrovascular complications. Oxidative stress also plays an important role in endothelial dysfunction (Sies, 1991, Halliwell and Gutteridge, 2007). This study is also remarkable because it shows that oscillating levels of glucose result in endothelial dysfunction and induced oxidative stress even in non-diabetic subjects (euglycemia). These postprandial hyperglycemia fluctuations may contribute to the continuous deterioration of diabetic complications, despite restoration of euglycemia. Therefore, postprandial hyperglycemia-induced endothelial dysfunction and oxidative stress may explain the mechanism of glycemic or hyperglycemic memory.

The findings indicate that ROS-induced mutated DNA mitochondria may continuously generate RS (Nishikawa et al., 2000a). The data also reveal that AGEs can modify or glycate protein content of mitochondrial TCA cycle and the electron respiratory chain (Ceriello, 2009, Ceriello et al., 2009). Irrespective of the level of glycemic control, all these will generate RS and trigger cellular injury. Hence, it does suggest that oxidative stress in diabetes mellitus can exist as an independent entity. As explained previously, oxidative stress is also implicated in other disorders characterized by euglycemia. Since hyperglycemia enhances the pathogenic pathways of diabetic complications via oxidative stress, therefore, whether hyperglycemia is normalized or the recommended optimal glycemic goal ≤ 6.5% HbA1c is achieved and/or maintained, the already existing oxidative stress can activate these same mechanistic pathways and thus propagate glycemic memory (induce diabetic complications).

6.4 Could there be a role of antioxidants in the management of diabetic complications?

Since oxidative stress is implicated in the pathogenesis of diabetes and its complications, there ought to be a place for antioxidants in the treatment of diabetes mellitus. However, previous clinical trials using antioxidants have yielded both promise and inconsistent results. Even though the data from the large scale clinical trials are inconclusive, it is noteworthy that many of those clinical trials were characterized by inappropriate study designs or several limitations (Johansen et al., 2005, Penckofer et al., 2002, Wierzba, 2005, Robinson et al., 2006, Willcox et al., 2008). These include: trials did not address specific diabetic populations; some studies included both healthy and unhealthy subjects; no data establishing the occurrence of oxidative stress in the patients before treatment and comparing such data with those obtained after treatment; the short duration of treatment; most of the trials were performed with vitamins A, C and E without consideration for other

antioxidants; the use of vitamin E supplementation without the concurrent use of vitamin C (Johansen et al., 2005, Penckofer et al., 2002, Wierzba, 2005, Robinson et al., 2006, Willcox et al., 2008). Other limitations include: some trials are gender-specific (comprising either men or women); lack of pharmacokinetic data of these antioxidants, before and after treatment, so as to ascertain if these antioxidants reached the target cells/tissues in adequate concentrations; no data to show that effects of different doses of each antioxidant were investigated so as to obtain and select optimal dose; the suppression of gamma-tocopherol by alpha-tocopherol; vitamins inappropriately administered relative to meal ingestion; and poor patient compliance are some of the issues that may contribute to the failure of antioxidants in clinical studies (Johansen et al., 2005, Penckofer et al., 2002, Wierzba, 2005, Robinson et al., 2006, Willcox et al., 2008). Some endpoints that were not directly related to oxidative stress such as mortality were used in some trials (Johansen et al., 2005). Hence, limited research and findings are available on the effects of antioxidants in diabetic patients. However, available evidences in small or medium sample-sized diabetic studies, both experimental and clinical, suggest antioxidants might play a role in the management of diabetes mellitus.

In diabetic rats with neuropathy, α-lipoic acid supplementation ameliorated oxidative stress parameters and improved lesions of diabetic neuropathy such as conduction velocity of the digital nerve, deficits in nerve conduction and nerve blood flow (Coppey et al., 2001). Evidence suggests that antioxidants can inhibit some of the pathways of diabetic complications such as protein kinase C-signaled increases in TGF-β in mesangial cells (Scott and King, 2004). Similarly, antioxidant treatment prevented the elevation in the levels of protein kinase C-beta, NAD(P)H oxidase subunit p47phox, BCL-2-associated X protein, 3-nitrotyrosine, fibronectin and poly (ADP) ribose in the retina of diabetic rats (Ihnat et al., 2007). In the kidney of diabetic rats, honey supplementation considerably reduced hyperglycemia, attenuated antioxidant enzymes, ameliorated oxidative stress markers and reduced mesangial matrix expansion and glomerular basement membrane thickness (Erejuwa et al., 2010a, Erejuwa et al., 2010c).

In patients with type 1 diabetes, supplementation with vitamins E and/or C combination ameliorated oxidative stress and improved endothelium-dependent vasorelaxation (Johansen et al., 2005, Rahimi et al., 2005). A study found that supplementation with combined chromium (Cr) and vitamins C and E ameliorated oxidative stress, reduced fasting blood glucose, HbA1c and insulin resistance in type 2 diabetes (Lai, 2008). Similarly, a recent study reported that vitamin E supplementation significantly reduced malondialdehyde (MDA) levels and increased the concentrations of GSH and vitamin E in type 1 diabetic patients (Gupta et al., 2011). The study also found a negative correlation between oxidative stress marker (MDA) and antioxidants (vitamin E and GSH) and a positive correlation between exogenously administered antioxidant (vitamin E) and endogenous antioxidant (GSH) (Gupta et al., 2011). However, the study showed that vitamin E supplementation in type 1 diabetic patients did not produce significant effects in metabolic parameters (Gupta et al., 2011). In patients with type 1 diabetes mellitus, vitamins C and E supplementation ameliorated oxidative stress markers, improved vascular dysfunction, retinal blood flow and creatinine clearance (Scott and King, 2004). These studies indicate that antioxidants could play a role in the management of diabetes mellitus. However, considering that diabetes mellitus is a disorder with multiple etiology and metabolic derangements, antioxidant supplementation alone is likely to be less effective.

This is corroborated by findings of Gupta and co-workers (2011). That could also explain the failure of antioxidants in clinical trials.

6.5 Could there be a role of hypoglycemic drugs (and/or insulin) and antioxidants in the management of diabetic complications?

A closer look at the various mechanistic pathways implicated in the pathogenesis of diabetic complications indicates that hyperglycemia-enhanced polyol pathway (via depletion of intracellular NADPH) and $O_2^{\bullet-}$ inhibition of G6PDH (via hyperglycemia-enhanced $O_2^{\bullet-}$ overproduction) would play a major role in impairing antioxidant defenses. This will increase intracellular susceptibility to oxidative stress during diabetes. As regards evidence, very few studies have investigated the effects of combined hypoglycemic agents and antioxidants in diabetic rodents or patients. Interestingly, all these studies found beneficial effects of combination of these two agents in both animals and human with diabetes mellitus. A study that investigated the effects of 4-week insulin and/or antioxidant (vitamin E and C) supplementation in diabetic rats found that the antioxidant treatment improved some of the oxidative stress parameters whereas insulin treatment prevented weight loss and ameliorated the activities and expression of antioxidant enzymes. In contrast, the combination of insulin and antioxidants resulted in normalization of all measurements including oxidative stress parameters (Sindhu et al., 2004).

Similarly, comparison of the effects of glibenclamide alone or combined with honey in pancreas of diabetic rats indicated that, even though glibenclamide reduced hyperglycemia, it only partially ameliorated oxidative stress parameters (most of the data were insignificant) (Erejuwa et al., 2011b). However, the combination of glibenclamide and honey significantly reduced hyperglycemia and ameliorated oxidative stress parameters in pancreas of diabetic rats (Erejuwa et al., 2011b). A similar study also showed that a combination of glibenclamide and metformin produced a limited antioxidant effect compared to when they were combined with honey in pancreas of diabetic rats (Erejuwa et al., 2010b). In the kidney of diabetic rats treated with metformin and/or glibenclamide, impaired antioxidant defenses were reported. In contrast, metformin and/or glibenclamide combined with honey significantly ameliorated oxidative stress parameters and restored the activities of antioxidant enzymes in the kidney of diabetic rats (Erejuwa et al., 2011a).

In type 1 and type 2 diabetic patients, a study found that while optimal glycemic control reduced the levels of MDA and increased the levels of GSH and vitamin E, it did not normalize the oxidative stress parameters (Chugh et al., 1999). However, after 4 weeks of vitamin E supplementation, the levels of oxidative stress markers were further reduced while those of endogenous antioxidants were increased compared to the optimal control values (without antioxidant treatment) (Chugh et al., 1999). A similar beneficial effect of vitamin E supplementation and optimal glycemic control was also reported in type 2 diabetic patients (Sharma et al., 2000). A number of other studies have also shown that antioxidants reduce glucose levels, improve insulin secretion and insulin resistance during diabetes (Penckofer et al., 2002). Moreover, a study found that in type 1 diabetic patients, normalization of glucose levels did not ameliorate hyperglycemia-induced endothelial dysfunction (Ceriello et al., 2007). The authors reported that, neither insulin nor vitamin C was able to ameliorate oxidative stress or normalize endothelial dysfunction (Ceriello et al., 2007). On the contrary, combination of insulin and vitamin C significantly decrease the

intensity of oxidative stress and normalized endothelial dysfunction in type 1 diabetic patients (Ceriello et al., 2007).

A critical analysis of the mechanistic pathways of hyperglycemia-induced diabetic complications indicates that they are all characterized by increased formation of ROS and impaired antioxidant defense network, which would further exacerbate oxidative stress and damage. In other words, these pathways begin and end with oxidative stress. Besides, evidence indicates that postprandial hyperglycemic fluctuations can cause endothelial dysfunction and induce oxidative stress in diabetic subjects and even in euglycemic subjects. Hence, findings from these studies clearly indicate that it is oxidative stress all over in diabetes mellitus and its complications. Moreover, the data from experimental and clinical studies indicate that there is a role for co-administration of hypoglycemic drugs or insulin and antioxidants in diabetes mellitus. It is possible that normalization of hyperglycemia may be achieved with hypoglycemic drugs, insulin, their combinations or even via pancreatic transplant. However, in patients with diabetic complications, whether euglycemia is achieved and/or maintained, oxidative stress (and oxidative stress-induced sequelae) becomes an independent entity. Thus, oxidative stress, as a possible independent entity, in diabetes mellitus necessitates antioxidant therapy. On account of these observations, findings and data, there seems little doubt that antioxidant therapy or other therapeutic intervention of oxidative stress in combination with hypoglycemic drugs or insulin should result in better management of diabetes mellitus. This should also prevent or reduce ROS-linked diabetic complications.

6.6 Other potential beneficial effects of hypoglycemic drugs (and/or insulin) and antioxidants in diabetes mellitus

Diabetes mellitus is characterized by impairments in renal and hepatic function as well as impaired metabolism of glucose, lipid and protein. Lipid abnormalities and induction of oxidative stress enhance the oxidation and glycation of low-density lipoproteins (LDLs), thereby exacerbate endothelial dysfunction (Penckofer et al., 2002). Studies have shown that antioxidants and some hypoglycemic drugs can prevent oxidation of LDL (Fava et al., 2002, Maritim et al., 2003, Rahimi et al., 2005). Besides, evidence indicates that antioxidants can ameliorate lipid abnormalities (Rahimi et al., 2005, Erejuwa et al., 2011d). The beneficial effects of antioxidants on glycemic control (blood glucose, fructosamine and glycosylated hemoglobin) in diabetes have also been documented (Rahimi et al., 2005, Lai, 2008, Erejuwa et al., 2010a; 2011d). Furthermore, antioxidants improve C-peptide and insulin levels as well as insulin resistance in diabetes mellitus (Rahimi et al., 2005, Lai, 2008, Erejuwa et al., 2011d). Co-administration of hypoglycemic drugs (glibenclamide or metformin) and antioxidant (honey) considerably improved glycemic control and lipid parameters in diabetic rats more than the effects produced by individual hypoglycemic drug (Erejuwa et al., 2011d). It is worth mentioning that other non-antioxidant constituents of honey, such as fructose and oligosaccharides, might contribute to this improved glycemic control and lipid parameters. In addition, antioxidants ameliorated and improved impaired renal function which has been documented in diabetes mellitus (Slyvka et al., 2009, Erejuwa et al., 2011d) or in combination with hypoglycemic drugs may produce synergism (Erejuwa et al., 2011d).

In the duodenum and jejunum of diabetic rats, a number of alterations in the brush border membrane (BBM) fluidity, non-enzymatic glycation, oxidative stress and damage have been

reported (Bhor and Sivakami, 2003). Similarly, increased protein glycation and lipid peroxidation might exacerbate diabetes-related alterations in BBM fluidity (Watala and Winocour, 1992). Therefore, antioxidants may ameliorate intestinal oxidative stress and improve BBM fluidity, thereby promote healing and enhance gastrointestinal tract health in diabetes mellitus. This might impact positively on glycemic control. Antioxidants may also augment bioavailability of essential macronutrients or co-administered hypoglycemic drugs (Faria et al., 2009). Furthermore, antioxidants may help to ameliorate liver damage and hepatic oxidative stress which are common in diabetes mellitus (Dias et al., 2005, Erejuwa et al., 2012b). Considering the role of liver in glucose homeostasis and the fact that some hypoglycemic agents (e.g. glibenclamide) mediate their effects via liver, these hepatic effects of antioxidants combined with those of hypoglycemic drugs may enhance liver functions and contribute to improved glycemic control. Moreover, evidence suggests that the use of antioxidants is associated with reduced weight gain (Razquin et al., 2009, Erejuwa et al., 2012a), therefore, co-administration of antioxidants and hypoglycemic agents, especially glibenclamide, may be beneficial in type 2 diabetic patients, majority of whom are obese. Majority of diabetic patients end up developing hypertension which further increases the risk of developing diabetic complications including cardiovascular events. Interestingly, oxidative stress is also implicated in the pathogenesis and/or complications of hypertension. Therefore, a combination of hypoglycemic drugs and antioxidants, via improved glycemic control and amelioration of oxidative stress, may help to prevent or delay the development of hypertension and diabetic complications (Erejuwa et al., 2011c, 2012a). Besides, the combination of both agents may help to minimize the adverse effects or toxicities of hypoglycemic agents. The use of antioxidants may necessitate lower doses of hypoglycemic agents to achieve the same therapeutic effect, thereby limiting the side or adverse effects of these drugs.

7. Conclusions

These studies indicate that hyperglycemia exerts long-term injurious effects in patients with type 1 and type 2 diabetes mellitus. Similar long-lasting detrimental effects of hyperglycemia also occur in diabetic animals. In both animals and humans with diabetes, glycemic control only delays the development or progression of diabetic complications. It does not prevent or completely restore diabetic complications. In few cases in which good glycemic control or cure of diabetes does prevent diabetic complications, it has to be initiated at a very early stage of the disease and maintained for several years. This is probably impracticable in the larger diabetic population. Evidence indicates it is not easy to achieve and/or maintain glycemic control in many diabetic patients close to the physiological range commonly observed in their healthy counterparts. Together with recent findings which demonstrate the deleterious effect of intensive therapy of hyperglycemia, it can be inferred that any therapeutic option that target hyperglycemia alone is not only limited and ineffective but may also be detrimental in diabetic patients. In view of the alarming rate of global prevalence of diabetes mellitus and the associated complications, morbidity and mortality, there is an urgent need for a better or new therapeutic management. While efforts are being made by researchers and scientists to unravel the main cause(s) of diabetes mellitus, it is high time clinicians, physicians and diabetologists began to look for an alternative and/or a complementary therapy to the current management of diabetes mellitus.

At the moment, one of such options or alternatives is the prospective of managing diabetes mellitus by targeting both hyperglycemia and oxidative stress simultaneously. As the data presented in this chapter have revealed, co-administration of oral hypoglycemic drugs (and/or insulin) and antioxidants might prove to be a better therapy in the management of diabetes mellitus. This is because, with the combination, hypoglycemic drugs (and/or insulin) will target hyperglycemia to improve glycemic control and reduce hyperglycemia-enhanced ROS production. In addition, administration of antioxidants will help to scavenge or eliminate RS including those generated in the various pathways highlighted in this chapter. Besides, many antioxidants have hypoglycemic effect which may also contribute to improved glycemic control. The co-administration of these two agents will help to minimize the level of oxidative stress in the vasculature and other targets of diabetic complications such as kidney (reducing diabetic nephropathy), retina (reducing diabetic retinopathy), nerves (reducing diabetic neuropathy) and heart (reducing diabetic cardiomyopathy). Moreover, evidence has shown that the pancreas and the liver are also target of oxidative stress in diabetes mellitus. Hence, co-administration of antioxidants will help to ameliorate oxidative stress in these tissues and organs (pancreas and liver) which play key roles in glucose homeostasis in diabetes mellitus.

In addition to improved glycemic control and amelioration of oxidative stress, evidence suggests that co-administration of hypoglycemic drugs and antioxidants may exert other beneficial effects on gastrointestinal tract, lipid profile, renal function and others which will contribute to better management of diabetic patients. Considering the limitations with antioxidants, coupled with the latest advances in our understanding of the various mechanisms involved in ROS formation, other interventions such as inhibition of the mitochondrial ROS overproduction, developing mitochondria-targeted antioxidants, blockage of hyperglycemia-induced mechanistic pathways are viable therapeutic options. This chapter has shown that the prospective of managing diabetes mellitus more effectively by targeting both hyperglycemia and oxidative stress simultaneously holds much promise. This new therapeutic option is worth investigating in patients with diabetes mellitus. Hence, both small and large, well designed, randomized clinical trials that examine the effect of combination of hypoglycemic drugs (and/or insulin) and specific antioxidants in patients with type 1 or type 2 diabetes mellitus are recommended. This may revolutionize the management of diabetes mellitus, at least in the interim, while attempts are being made to discover its main cause(s) and develop more potent and effective antidiabetic drugs.

8. Dedication

This chapter is dedicated to the memory of my dad, Educator Sephaniah Adeyemi Erejuwa, who battled diabetes mellitus and later succumbed to its complications. It is also dedicated to millions of people globally who are suffering from this disorder and its complications

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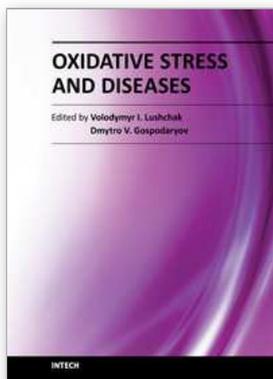
Oxidative Stress and Diseases

Edited by Dr. Volodymyr Lushchak

The development of hypothesis of oxidative stress in the 1980s stimulated the interest of biological and biomedical sciences that extends to this day. The contributions in this book provide the reader with the knowledge accumulated to date on the involvement of reactive oxygen species in different pathologies in humans and animals. The chapters are organized into sections based on specific groups of pathologies such as cardiovascular diseases, diabetes, cancer, neuronal, hormonal, and systemic ones. A special section highlights potential of antioxidants to protect organisms against deleterious effects of reactive species. This book should appeal to many researchers, who should find its information useful for advancing their fields.

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