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# Biochemical Evaluation of Oxidative Stress in Type 1 Diabetes

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/52836>

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## 1. Introduction

### 1.1. Type 1 diabetes mellitus

Diabetes mellitus is considered to be one of the most common chronic diseases worldwide, and recognized as one of the leading causes of morbidity and mortality (American Diabetes Association, 2010). It has been reported that the prevalence of diabetes mellitus will increase from 6% to over 10% in the next decade (Rosen et al., 2001). According to the World Health Organization in 2000, a total of 171 million people in all age groups worldwide (2.8% of the global population) have been affected by diabetes mellitus, and the number of persons is expected to increase to 366 million (4.4% of the global population) by 2030 (Wild et al., 2004).

Type 1 diabetes mellitus accounts for 5-10% of all diagnosed cases of diabetes mellitus, and exhibits hyperglycemia as its hallmark. It is caused by pancreatic  $\beta$ -islet cell failure with resulting insulin deficiency mortality and risk factors may be autoimmune, genetic, or environmental (American Diabetes Association, 2004). Type 1 diabetes mellitus is an autoimmune disorder involving immune-mediated recognition of islet  $\beta$ -cells by auto-reactive T cells. This subsequently leads to the liberation of pro-inflammatory cytokines and reactive oxygen species. There is destruction of pancreatic  $\beta$ -cells in the islets of Langerhans and loss of insulin secretion (Delmastro & Piganelli, 2011). The Jun kinase pathway is also activated by the pro-inflammatory cytokines, and there is evidence that oxidative stress is involved in  $\beta$ -cell destruction (Kaneto et al., 2007). The loss of  $\beta$ -cell mass consequential to the activation of pro-apoptotic signaling events is increasingly recognized as a causal and committed stage in the development of type 1 diabetes mellitus (Watson & Loweth, 2009).

Moreover, pancreatic  $\beta$ -cells are sensitive to cytotoxic damage caused by reactive oxygen species as gene expression and activity of antioxidant enzymes such as glutathione peroxidase activity is decreased in these cells (Lenzen et al., 1996).

Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a central role in the onset of diabetes mellitus as well as in the development of vascular and neurologic complications of the disease (Niedowicz & Daleke, 2005). Studies advancing the role of oxidative stress in vascular endothelial cells proposed that oxidative stress mediate the diversion of glycolytic intermediates into pathological pathways (Rolo & Palmeira, 2006; Turk, 2010). Oxidative stress is increased in diabetes mellitus owing to an increase in the production of oxygen free radicals and a deficiency in antioxidant defense mechanisms. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins (Rodríguez-Janeiro et al., 2010). Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance (Ceriello, 2006).

This review will explore recent evidence in the literature of the use of biomarkers to assess oxidative stress which is recognized as a significant mediator in the development of macrovascular or cardiovascular complication in type 1 diabetes mellitus, as well as the potential for prevention of complications through the use of antioxidants. There is also a search for other biomarker of oxidative stress which might be clinically useful in patients with diabetes mellitus. Such a biomarker could potentially indicate the severity of disease, identify those at increased risk of complications and monitor response to treatment.

## 2. Oxidative stress and beta-cell destruction

Impairment in the oxidant/antioxidant equilibrium creates a condition known as oxidative stress. There is a complex interaction between antioxidants and oxidants such as reactive oxygen species, which modulates the generation of oxidative stress. Oxidative stress takes place in a cellular system when the generation of reactive oxygen species increases and overwhelms the body's antioxidant capacity and defenses (Baynes, 1991). If the free radicals are not removed by the cellular antioxidants, they may attack and damage lipids, carbohydrates, proteins and nucleic acids (Baynes & Thorpe, 1999).

Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases (Maritim et al., 2003; Isabella et al., 2006). There is growing evidence that have connected oxidative stress to a variety of pathological conditions, including cancer, cardiovascular diseases, chronic inflammatory disease, post-ischaemic organ injury, diabetes mellitus, xenobiotic/drug toxicity, and rheumatoid arthritis (El Faramawy & Rizk, 2011; Samanthi et al., 2011). In recent years, much attention has been focused on the role of oxidative stress. It has been reported that oxidative stress participates in the progression and pathogenesis of secondary diabetic complications. This includes impairment of

insulin action and elevation of the complication incidence (Ceriello, 2006). Furthermore, there is evidence for the role of reactive oxygen species and oxidative stress in the development of type 1 diabetic complications including retinopathy, nephropathy, neuropathy, and accelerated coronary artery disease (Phillips et al., 2004; Niedowicz & Daleke, 2005).

It has also been reported that oxidative stress induced by reactive oxygen and nitrogen species is critically involved in the impairment of  $\beta$ -cell function, and thus play a role in the pathology of type 1 diabetes mellitus (West, 2000). Islet  $\beta$ -cells are highly susceptible to oxidative stress because of their reduced levels of endogenous antioxidants (Azevedo-Martins et al., 2003; Kajikawa et al., 2002). With decreased antioxidant capacity,  $\beta$ -cells are extremely sensitive towards oxidative stress. Cell metabolism and potassium (adenosine-5'-triphosphate) channels in  $\beta$ -cells are important targets for reactive oxygen species and other oxidants. The alterations of potassium (adenosine-5'-triphosphate) channel activity by the oxidants, is crucial for oxidant-induced dysfunction as genetic ablation of potassium (adenosine-5'-triphosphate) channels attenuates the effects of oxidative stress on  $\beta$ -cell function (Drews, 2010).

Oxidative stress may reduce insulin sensitivity and damage the  $\beta$ -cells within the pancreas. The reactive oxygen species produced by oxidative stress can penetrate through cell membranes and cause damage to the  $\beta$ -cells of pancreas (Chen et al., 2005; Lepore et al., 2004). Reactive oxygen species produced from free fatty acids can cause mitochondrial deoxyribonucleic acid damage and impaired pancreatic  $\beta$ -cell function (Rachek et al., 2006). Mitochondrial and nitrogen oxides ( $\text{NO}_x$ )-derived reactive oxygen species have been implicated in  $\beta$ -cell destruction and subsequently type 1 diabetes mellitus. Furthermore, increased glucose can cause rapid induction of the Krebs cycle within the  $\beta$ -cell mitochondria, leading to augmented reactive oxygen species production (Newsholme et al., 2007). The superoxide leaked from the mitochondria can contribute to the formation of hydrogen peroxide which may play a role in uncoupling glucose metabolism from insulin secretion (Maechler et al., 1999).

### 3. Oxidative stress induced by hyperglycaemia in type 1 diabetes

#### 3.1. Pathways involved in the production of oxidants

There are multiple sources of reactive oxygen species production in diabetes including those of non-mitochondrial and mitochondrial origins. Reactive oxygen species accelerates four important molecular mechanisms that are involved in oxidative tissue damage induced by hyperglycemia. These four pathways are increased advanced glycation end product, increased hexosamine pathway flux, activation of protein kinase C, and increased polyol pathway flux (also known as the sorbitol-aldose reductase pathway) (Rolo & Palmeira, 2006).

In the polyol pathway, the two enzymes aldose reductase and sorbitol dehydrogenase cause reactive oxygen species production. Glucose is reduced to sorbitol through the use of reduced nicotinamide adenine dinucleotide phosphate, a reaction catalyzed by aldose reductase. This pathway metabolizes 30 - 35% of the glucose present during hyperglycemia. The

available reduced nicotinamide adenine dinucleotide phosphate is depleted resulting in the reduction of glutathione regeneration and nitric oxide synthase activity (Ramana et al., 2003). The oxidation of sorbitol to fructose with the concomitant production of reduced nicotinamide adenine dinucleotide is catalyzed by sorbitol dehydrogenase. The reduced nicotinamide adenine dinucleotide phosphate may be used by nicotinamide adenine dinucleotide phosphate oxidases to generate superoxide anion (Moore & Roberts, 1998). Vitamin C supplementation has been found to be effective in reducing sorbitol accumulation in the red blood cells of diabetic patients. In a study conducted by Cunningham et al. (1994) who investigated the effect of two different doses of vitamin C supplements (100 and 600 mg) during a 58 day trial on young adults with type 1 diabetes mellitus, vitamin C supplementation at either dose within 30 days normalized sorbitol levels.

Glucose at high concentrations undergoes non-enzymatic reactions with primary amino groups of proteins to form glycated residues called Amadori products. These early glycation products undergo further complex reactions, such as rearrangement, dehydration, and condensation, to become irreversibly cross-linked, heterogeneous fluorescent derivatives called advanced glycation end products (Thornalley, 2002). The advanced glycation end products binds to a cell surface receptor known as receptor for advanced glycation end product. As a result of interaction of advanced glycation end products, with receptor for advanced end product, there is the induction of the synthesis of reactive oxygen species via a mechanism which involves localization of pro-oxidant molecules at the cell surface (Yan et al., 1994) and the participation of activated nicotinamide adenine dinucleotide phosphate oxidase (Wautier et al., 2001). The reactive aldehydes methylglyoxal and glyoxal are produced from enzymatic and non-enzymatic degradation of glucose, lipid and protein catabolism, and lipid peroxidation. These aldehydes form advanced glycation end products with proteins that are implicated in diabetic complications. Han et al. (2007) assessed plasma methylglyoxal and glyoxal using a novel liquid chromatography-mass spectrophotometry method in 56 young patients (6 - 22 years) with type 1 diabetes mellitus without complications. They found that mean plasma methylglyoxal and glyoxal levels were higher in the diabetic patients compared with their non-diabetic counterparts. They suggest that increased plasma methylglyoxal and glyoxal levels give an indication of future diabetic complications and emphasized the need for aggressive management (Han et al., 2007).

It has been shown that through receptor for advanced glycation end products mediated effects, advanced glycation end product induces reactive oxygen species production possibly through an nicotinamide adenine dinucleotide phosphate oxidase, and the subsequent expression of inflammatory mediators and activation of redox-sensitive transcription factors (Wautier et al., 2001; Schmidt et al., 1996). Furthermore, advanced glycation end products, binding to receptor for advanced glycation end product activate protein kinase C- $\alpha$ -mediated activation of nuclear factor- $\kappa$ B (NF $\kappa$ B) and nicotinamide adenine dinucleotide phosphate oxidase. This may cause the generation of mitochondrial reactive oxygen species and induce the production of various inflammatory cytokines further aggravating oxidative stress (Simm et al., 1997).



Advanced glycation end product in high concentration in body is toxic and can modify the structure of intracellular proteins especially those involved in gene transcription, and can cause damage to biological membranes and the endothelium. It may diffuse to the extracellular space and directly modify extracellular proteins such as laminin and fibronectin to disturb signaling between the matrix and cells that act via receptor for advanced glycation end products, which is present on many vascular cells (Bierhaus et al. 1998). In addition, advanced glycation end products can modify blood proteins such as albumin, causing them to bind to advanced glycation end product receptors on macrophages/mesangial cells and increase the production of growth factors and proinflammatory cytokines (Brownlee, 2005). Kostolanská et al. (2009) observed significantly higher glycated hemoglobin, serum advanced glycation end products and advanced oxidation protein products concentrations in 81 patients with type 1 diabetes mellitus compared with controls. They suggest that the measurement of glycated hemoglobin, serum advanced glycation end products and advanced oxidation protein products may be useful to predict the risk of development of diabetic complications (Kostolanská et al., 2009).

Antioxidants or antibodies against receptor for advanced glycation end product prevent both oxidative stress and the downstream signaling pathways that can be activated by ligation of receptor for advanced glycation end product. Advanced glycation end product-mediated reaction oxygen species production is implicated in diabetic vascular complications and in blood vessel endothelial activation (Cameron & Cotter, 1999; Mullarkey et al., 1990). The formation and accumulation of advanced glycation end products have been involved in the development and progression of diabetic micro- and macroangiopathy. The advanced glycation end product-receptor for advanced glycation end product interaction produces oxidative stress and subsequently evokes thrombosis and vascular inflammation, thereby playing an important role in diabetic vascular complications (Yamagishi, 2009; Niiya et al., 2006). In a recent study, median levels of malondialdehyde and increased plasma levels of soluble receptor for advanced glycation end product were found in 42 type 1 diabetic patients during the early years after diagnosis (0-10 years). These findings suggest that increased plasma levels of soluble receptor for advanced glycation end product in type 1 diabetes may provide protection against cell damage and may be sufficient to eliminate excessive circulating malondialdehyde during early years after disease onset (Reis et al., 2012).

## **4. Free radicals formed during oxidative stress**

### **4.1. Reactive oxygen species in type 1 diabetes**

Reactive oxygen species consist of oxygen free radicals such as superoxide anion ( $O_2^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radical ( $\bullet OH$ ), singlet oxygen, nitric oxide, and peroxytrite (Chong et al., 2005). Most of these free radicals are produced at low concentrations during normal physiological conditions in the body and are scavenged by endogenous enzymatic and non-enzymatic antioxidant systems that include superoxide dismutase, glutathione peroxidase, catalase, and small molecule substances such as vitamins C and E.

Reactive oxygen species induced tissue injury as well as they are involved in signaling pathways and gene expression (Ha & Lee, 2000). Excess generation of reactive oxygen species such as superoxide anion, hydrogen peroxide, hydroxyl radical and reactive nitrogen species such as nitric oxide oxidize target cellular proteins, nucleic acids, or membrane lipids and damage their cellular structure and function (Brownlee, 2001). There is also evidence that reactive oxygen species also regulate the expression of genes encoding for proteins involved in immune response, inflammation and cell death (Ho & Bray, 1999).

Hydroxyl radicals, hydrogen peroxide, and superoxide anion are byproducts of xanthine oxidase. Xanthine oxidase and xanthine dehydrogenase catalyze the conversion of hypoxanthine to xanthine and then to uric acid, with the former reducing oxygen as an electron acceptor while the latter can reduce either oxygen or nicotinamide adenine dinucleotide (NAD<sup>+</sup>) (Fatehi-Hassanabad et al., 2010). Superoxide anion is also produced by nicotinamide adenine dinucleotide phosphate oxidases and cytochrome P450, and is the most commonly occurring oxygen free radical that produces hydrogen peroxide by dismutation. This is achieved via the Haber-Weiss reaction in the presence of ferrous iron by copper (Cu)-superoxide dismutase or manganese (Mn)-superoxide dismutase. Mitochondrial superoxide anion is produced from excess reduced nicotinamide adenine dinucleotide produced in the Krebs cycle (Fubini & Hubbard, 2003). Elevated free or non-esterified fatty acids in type 1 diabetic patients enter the Krebs cycle causing the production of acetyl-CoA to subsequently excess reduced nicotinamide adenine dinucleotide (Steinberg & Baron, 2002). The superoxide anion undergo dismutation to hydrogen peroxide, which if not degraded by catalase or glutathione peroxidase, and in the presence of transition metals, can lead to production of hydroxyl radical, the most active oxygen free radical. Hydroxyl radical alternatively may be formed through an interaction between superoxide anion and nitric oxide (Fubini & Hubbard, 2003; Wolff, 1993).

Superoxide anion can also react with nitric oxide to form the reactive peroxynitrite radicals (Hogg & Kalyanaraman, 1998). Excess production of superoxide anion by the mitochondrial electron transport chain, induced by hyperglycaemia has been reported to have a role in triggering protein kinase C, hexosamine and polyol pathway fluxes, and advanced glycation end product formation pathways which are involved in the pathogenesis of diabetic complications (Nishikawa et al., 2000; Brownlee, 2001). In a study conducted by Hsu et al. (2006), plasma superoxide anion (determined by a chemiluminescent assay) gave photoemission which was considerably higher in 47 type 1 diabetic children than those in controls. The findings confirm the presence of oxidative stress in children with type 1 diabetes mellitus (Hsu et al., 2006).

#### **4.2. Reactive nitrogen species in type 1 diabetes**

Nitric oxide is an important regulator of endothelial function and the impairment of its activity is determinant of the endothelial dysfunction (Ignarro, 2002). It is an important vascular target for ROS and is produced by constitutive and inducible nitric oxide synthases. These enzymes oxidize L-arginine to citrulline in the presence of biopterin, reduced nicotinamide adenine dinucleotide phosphate, and oxygen (Alp & Channon, 2004). Constitutive

endothelial nitric oxide synthase contains reductase and oxygenase domains that are connected by a calmodulin-binding region and requires cofactor groups such as heme, flavin mononucleotide, flavin adenine dinucleotide, tetrahydrobiopterin, and  $\text{Ca}^{2+}$ -calmodulin for activation (Gorren & Mayer, 2002; Andrew & Mayer, 1999). If there is none or insufficient L-arginine, the endothelial nitric oxide synthase produce superoxide instead of nitric oxide and this is referred to as the uncoupled state of nitric oxide synthase (Channon, 2004).

Oxidative stress decreases the bioavailability of endothelium-derived nitric oxide in diabetic patients. In a 3-year longitudinal study involving 37 patients with recent-onset (less than 2 years) type 1 diabetes, oxidative stress was evident by elevated malondialdehyde excretion and serum  $\text{NO}_x$  (nitrate and nitrite) (Hoeldtke et al., 2011). In a latter study,  $\text{NO}_x$  was also higher in 99 female subjects with uncomplicated type 1 diabetes (duration disease <10 years) compared with 44 sex-matched controls (Pitocco et al., 2009). Mylona Karayanni et al. (2006) examined possible correlation between oxidative stress parameters and adhesion molecules derived from endothelial/platelet activation, P-selectin and tetranectin in a group of juveniles with type 1 diabetes mellitus. Significantly elevated  $\text{NO}_x$  and lipid hydroperoxide levels, elevated tetranectin and P-selectin plasma levels, and lower glutathione peroxidase activity were found in the diabetic children compared with healthy controls. Based on these findings the authors suggested that decreased anti-oxidative protection from overproduction of lipid hydroperoxide and  $\text{NO}_x$  overproduction is present in juveniles with type 1 diabetes mellitus. There is also a parallel endothelial/platelet activation which contributes to the vascular complications of type 1 diabetes mellitus (Mylona-Karayanni et al., 2006).

Nitric oxide can react with superoxide to form peroxynitrite which in turn oxidizes tetrahydrobiopterin and causes further uncoupling of nitric oxide formation (Yung et al., 2003). In diabetes mellitus, elevated glucose may cause an increase in the expression of both reduced nicotinamide adenine dinucleotide phosphate and of inducible nitric oxide synthase via the activation of NF- $\kappa$ B, (Spitaler & Graier, 2002). The upregulated inducible nitric oxide synthase will synthesize the superoxide anion instead of nitric oxide, leading to oxidative and nitrosative stress (Llorens & Nava, 2003). The stable protein adduct, nitrotyrosine, is a marker of peroxynitrite (Ischiropoulos, 1998) and nitrogen dioxide (Prutz et al., 1985). Moreover, increased oxidative and nitrosative stress activates poly(ADP-ribose) polymerase-1, which substrate, nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) as well as slows the rate of glycolysis, electron transport, and adenosine triphosphate formation (Pacher & Szabó, 2006).

The formation of peroxynitrite can further lead to the generation of peroxynitrous acid. The spontaneous decomposition of peroxynitrous acid results in the formation of hydroxyl radicals that can cause endothelial damage (Elliott et al., 1993; Beckman & Koppenol, 1996) thereby reduces the efficacy the endothelium-derived vasodilator system that participates in the general homeostasis of the vasculature (Benz et al., 2002). Overproduction of both nitric oxide and superoxide anion has been reported in response to hyperglycemia (Cosentino et al., 1997; Cериello et al., 2002), and nitric oxide may work through peroxynitrite to directly alter cellular structure and function (Pfeiffer et al., 2001). Increased nitric oxide levels have been reported in both saliva and plasma of diabetic patients in comparison to healthy subjects (Astaneie et al., 2005).



## 5. Enzymatics and non-enzymatic antioxidants

### 5.1. Intracellular enzymes activity in type 1 diabetes

A number of natural antioxidants are present in the body to scavenge oxygen free radicals and prevent oxidative damage to biological membranes. Antioxidant defense mechanisms involve both non-enzymatic and enzymatic strategies. One group of these antioxidants is intracellular enzymes such as manganese superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferases. These enzymes represent a protective mechanism against the damage caused by the oxidative stress and most of these enzymes are polymorphic (Fang et al., 2002; Mates et al., 1999).

Superoxide dismutase is considered a primary enzyme since it is involved in the direct elimination of reactive oxygen synthase (Halliwell, 1994). Isoforms of superoxide dismutase are Cu/Zn-superoxide dismutase which is found in both the cytoplasm and the nucleus, and Mn-superoxide dismutase that is present in the mitochondria. The latter can be released into extracellular space (Reiter et al., 2000). Cu/Zn-superoxide dismutase over-expression inhibits oxidized low density lipoprotein which is can elevate deoxyribonucleic acid binding activity of activator protein-1 and NF- $\kappa$ B (Yung et al., 2006). Superoxide dismutase catalyzes the conversion of superoxide anion radicals produced in the body to hydrogen peroxide. This decreases the possibility of superoxide anion interacting with nitric oxide to form reactive peroxynitrite (Reiter et al., 2000). Low Cu/Zn-superoxide dismutase is a potential early marker of susceptibility to diabetic vascular disease. Suys et al. (2007) found that erythrocyte superoxide dismutase activity and Cu/Zn-superoxide dismutase were higher in type 1 diabetic subjects and was positively associated with flow-mediated dilatation. Based on these findings the authors suggest that higher circulating Cu/Zn-superoxide dismutase could protect type 1 diabetic children and adolescents against endothelial dysfunction (Suys et al., 2007). Furthermore, Reznick and colleagues analyzed both serum and salivary superoxide dismutase activity in 20 patients with type 1 diabetes mellitus. A significant association was found between the level of glycemic control as indicated by the glycated hemoglobin values and an increase in both salivary and serum superoxide dismutase activity (Reznick et al., 2006). On the contrary, in a study which assessed correlations between increase of oxidative stress and the development of microalbuminuria in 87 type 1 diabetic patients (44 with normal urinary protein excretion, and 43 with microalbuminuria), there was a decreased in activity of superoxide dismutase. This was associated with an increased microalbuminuria in type 1 diabetic patients (Artenie et al., 2005).

Selenium-dependent glutathione peroxidase works in conjunction with superoxide dismutase in protecting cell proteins and membranes against oxidative damage. In the literature, glutathione peroxidase response to diabetes has been conflicting. Diabetics have been reported to be associated with increased glutathione peroxidase activity in 90 pregnant women with type 1 diabetes mellitus (Djordjevic et al., 2004) and in young diabetic patients (Ndahimana et al., 1996). On the other hand, decreased glutathione peroxidase activity was reported in the early stages of type 1 diabetes in children and adolescents (Dominguez et al., 1998) or unchanged in type 1 diabetic patients with early retina degenerative lesions (Faure

et al., 1995). The low glutathione peroxidase activity could be directly explained by either low glutathione content or enzyme inactivation under severe oxidative stress (Faure et al., 1995). However, some authors found no differences between glutathione peroxidase activity of type 1 diabetic patients and control subjects (Jain et al., 1994; Murakami et al., 1993; Majchrzak et al., 2001).

Catalase, located in peroxisomes, decomposes hydrogen peroxide to water and oxygen (Winterbourn & Metodiewa, 1994). In addition, glutathione peroxidase in the mitochondria and the lysosomes also catalyses the conversion of hydrogen peroxide to water and oxygen (Yung et al., 2006). A significant increase in the catalase activity in lymphocytes was found in 40 children with type 1 diabetes during all phases (at the beginning of diabetes, in remission period and in the later chronic course) compared with the control group. The highest catalase activity occurs in the early course of disease followed by a linear decrease and the lowest activity in chronic course (Zivić, 2008). Conversely Dave and colleagues (2007) reported significant decreased glutathione peroxidase, catalase and glutathione, and significant increase in thiobarbituric acid reactive substances concentration in type 1 diabetic patients with and without nephropathy compared with normal healthy individuals (Dave et al., 2007).

## 5.2. Non-enzymatic antioxidant levels in type 1 diabetes

In addition to enzymatic antioxidants, the major natural antioxidants, most of which are derived from dietary sources are vitamin A, vitamin C or ascorbic acid, vitamin E and carotenoids. Water-soluble vitamin C and fat-soluble vitamin E together make up the antioxidant system for mammalian cells (Engler et al., 2003). Vitamins A, C, and E are obtained from the diet and function to directly detoxify free radicals. Vitamin C forms the first line of defense against plasma lipid peroxidation is considered the most important antioxidant in plasma (Frei et al., 1990). Vitamin C under certain conditions may foster toxicity by generating pro-oxidants, and is also engaged in the recycling processes which involved the generation of reduced forms of the vitamins. In the processes of regeneration,  $\alpha$ -tocopherol is reconstituted when ascorbic acid recycles the tocopherol radical; dihydroascorbic acid, which is formed, is recycled by glutathione (Weber, 1997).

Vitamin E involves all tocopherol and tocotrienol derivatives that comprise the major lipophilic exogenous antioxidant in tissues (Di Mambro et al., 2003). Vitamin E, a component of the total peroxyl radical-trapping antioxidant system reacts directly with superoxide and peroxyl radicals, and singlet oxygen and in so doing protects membranes from lipid peroxidation (Weber & Bendich, 1997). In a study by Gupta et al. 2011 that evaluated the oxidative stress in 20 type 1 diabetic children, reduced glutathione and vitamin E levels were decreased and malondialdehyde levels were elevated compared with controls. After supplementation with vitamin E (600 mg/daily for three months) there was a significant decrease in malondialdehyde levels and significant increase in glutathione and vitamin E. The findings indicate that vitamin E ameliorates oxidative stress in type 1 diabetes mellitus patients and improves antioxidant defense system. In a latter study high-dose vitamin E supplementation (1200 mg/day) reduces markers of oxidative stress and improves antioxidant defense

in young patients with type 1 diabetes mellitus. However vitamin E supplementation did not decreased albumin excretion rate in these patients (Giannini et al., 2007).

$\alpha$ -Tocopherol is very effective in lipid peroxidation inhibition and is the primary *in vivo* chain-breaking, lipid-soluble antioxidant in human serum. A reduction in serum  $\alpha$ -tocopherol could be attributed to its consumption while scavenging free radicals in lipoproteins or biomembranes (Frei, 1994). In the Pittsburgh Epidemiology of Diabetes Complications Study cohort, a 10-year prospective study of childhood-onset type 1 diabetes,  $\alpha$ -tocopherol or  $\gamma$ -tocopherol did not showed protection against incident coronary artery disease overall. However, high  $\alpha$ -tocopherol levels among patients with renal disease and in those using vitamin supplements were associated with lower coronary artery disease risk in type 1 diabetes (Costacou et al., 2006). All the antioxidants work in a synergistic manner with each other and against different types of free radicals. This is shown in the way in which vitamin E suppresses the propagation of lipid peroxidation, and vitamin C working with vitamin E inhibits hydroperoxide formation (Laight et al., 2000).

Glutathione functions as a direct free-radical scavenger, and as a co-substrate for glutathione peroxidase activity (Meister & Anderson, 1983). Glutathione, a tri-peptide present in millimolar concentrations is the most prevalent low-molecular weight peptide antioxidant in cells. Reduced glutathione normally plays the role of a direct intracellular free-radical scavenger through interaction with free radicals and is the substrate of many xenobiotic elimination reactions (Gregus et al., 1996). It is also involved in other cellular functions such as the elimination of hydrogen peroxide, detoxification processes such as protection of the sulfhydryl group of cysteine in proteins, and regeneration of oxidized vitamin E (Lu, 1999). In 30 children with type 1 diabetes at onset, there was a significant reduction in all glutathione forms (total, reduced, oxidized, and protein-bound glutathione). This indicates that there is glutathione depletion upon early onset of type 1 diabetes mellitus (Pastore et al., 2012). In another study, Likidlilid et al. (2007) compared the glutathione level, and glutathione peroxidase activity in 20 type 1 diabetic patients (with fasting glucose > 140 mg/dL) and a normal healthy group. They found that the level of red cell reduced glutathione was significantly lower in type 1 diabetic patients but red cell glutathione peroxidase activity was significantly increased. The decrease of red cell glutathione may be due to its higher rate of consumption, increasing glutathione peroxidase activity or a reduction of pentose phosphate pathway, stimulated by insulin, resulting in lowered glutathione recycle (Likidlilid et al., 2007). In a recent study, reduced glutathione and vitamin E levels were decreased and malondialdehyde levels were higher in 20 type 1 diabetic children compared with healthy controls. After supplementation with vitamin E (600 mg/daily for three months), there was a significant decrease in malondialdehyde levels and significant increase in glutathione and vitamin E levels. This shows that vitamin E ameliorates oxidative stress in type 1 diabetic patients and improves antioxidant defense system (Gupta et al., 2011).

Other nonenzymatic antioxidants include  $\alpha$ -lipoic acid, mixed carotenoids, coenzyme Q<sub>10</sub>, several bioflavonoids, antioxidant minerals (copper, zinc, manganese and selenium), and the cofactors (folic acid, vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>).  $\beta$ -carotene is a lipid soluble and chain-breaking antioxidant that effectively quenches singlet oxygen and inhibits lipid peroxida-

tion. At low physiological oxygen pressures, it exhibits effective radical-trapping antioxidant behaviour (Frei, 1994). Coenzyme Q<sub>10</sub> has been found to have a very important role in mitochondrial bioenergetics. It is an electron carrier-proton translocator in the respiratory chain and potent antioxidant which works by directly scavenging radicals or indirectly by regenerating vitamin E. In a study by Menke and colleagues (2008), plasma concentrations of coenzyme Q<sub>10</sub> in 39 children with type 1 diabetes mellitus were higher than in healthy children. The findings suggest that elevated plasma concentration and the intracellular redox capacity of coenzyme Q<sub>10</sub> in diabetic children may contribute to the body's self-protection during a state of enhanced oxidative stress (Menke et al., 2008). In another study, Salardi and colleagues (2004) determine whether serum hydroperoxides as oxidative markers and vitamin E and coenzyme Q<sub>10</sub> as indexes of antioxidant capacity could be related to metabolic control in 75 unselected children, adolescents, and young adults with type 1 diabetes. Vitamin E and coenzyme Q<sub>10</sub> were not significantly different from age-matched control subjects. However, there were significant positive correlations between coenzyme Q<sub>10</sub> and glycated hemoglobin, and vitamin E and glycated hemoglobin. It was also observed that diabetic patients with poor metabolic control and complications had elevated vitamin E levels and coenzyme Q<sub>10</sub> levels (Salardi et al., 2004).

Small molecules that have antioxidant capacity such as glutathione and uric acid are synthesized or produced within the body (Engler et al., 2003). A study by Maxwell et al. (1997) found significantly reduced total serum antioxidant status in 28 patients with type 1 diabetes mellitus as attributed by lower uric acid and vitamin C levels. Furthermore, multiple regression analysis showed that uric acid, vitamin E and vitamin C were the main contributors to serum total antioxidant activity.

## 6. Markers of oxidative stress in type 1 diabetes

### 6.1. Biomarkers of lipid peroxidation in type 1 diabetes

Oxidative stress and its contribution to low-density lipoprotein oxidation have been implicated in the pathogenesis of vascular diabetic complications. Diabetes produces disturbances of lipid profiles, especially an increased susceptibility to lipid peroxidation, which is responsible for increased incidence of atherosclerosis, a major complication of diabetes mellitus (Siu & To, 2002). Polyunsaturated fatty acids with multiple bonds and lipoproteins in the plasma membrane are very susceptible to attack by reactive oxygen species (Esterbauer & Schaur, 1991). The hydroxyl radicals extract a hydrogen atom from one of the carbon atoms in the polyunsaturated fatty acid and lipoproteins, initiating a free radical chain reaction which leads to lipid peroxidation. This characterized by membrane protein damage through subsequent free radical attacks (Halliwell, 1995). Lipid peroxidation can produce advanced products of oxidation, such as aldehydes, alkanes and isoprostanes (Moore & Roberts, 1998). Elevation of lipid peroxidation negatively affects membrane function causing reduced membrane fluidity and changing the activity of membrane bound enzymes and receptors (Acworth et al., 1997).



In diabetes mellitus, persistence of hyperglycemia was reported to cause increased production of oxidative parameters of lipid peroxidation including malondialdehyde. In a study by Firoozrai and colleagues (2007), malondialdehyde levels were significantly elevated in diabetic patients. The level of malondialdehyde was positively correlated with duration of diabetes and glycated hemoglobin and negatively with ferric reducing ability of plasma (Firoozrai et al., 2007). In a latter study that investigated the effect of glycemic control on oxidative stress and the lipid profile of pediatric type 1 diabetes mellitus patients, total cholesterol, low density lipoprotein-cholesterol, apolipoprotein A, apolipoprotein B, and malondialdehyde levels were significantly elevated compared with controls. In addition, serum malondialdehyde levels and malondialdehyde/low density lipoprotein-cholesterol index were significantly elevated in metabolically poorly controlled in relation to metabolically well-controlled diabetic patients. Based on these findings the authors suggested that type 1 diabetic children, especially those who are metabolically poorly controlled are at high risk of atherosclerosis and vascular complications of diabetes mellitus, and that there is a significant relationship between the lipid profile and oxidative stress (Erciyas et al., 2004).

Isoprostanes are prostaglandin-like compounds formed through peroxidation of arachidonic acid, and have been used extensively as biomarkers of lipid peroxidation as a risk factor for atherosclerosis and other diseases (Roberts & Marrow, 2000). Oxidative stress parameters such as advanced oxidation protein products, total peroxyl radical-trapping antioxidant parameter, and F2-isoprostanes (8-epi-prostaglandin-F2: 8-isoPGF2 $\alpha$ ) were not significantly different in 27 pre-pubertal patients with type 1 diabetes mellitus (with less than 5 years of disease) compared with controls (Gleisner et al., 2006). In another study, Flores and colleagues (2004) evaluated the effect of the normalization of blood glucose levels on urinary F2-isoprostanes at the onset of type 1 diabetes in 14 patients. There was a statistically significant reduction in F2-isoprostanes after insulin therapy (after 16 weeks) which was accompanied by a significant reduction in glycated hemoglobin (Flores et al., 2004).

Lipid hydroperoxides are potentially atherogenic and are degraded by enzymes such as paraoxonase-1 and lipoprotein-associated phospholipase A<sub>2</sub> (Van Lenten et al., 2001; Macphee et al., 2005). Paraoxonase-1 is an enzyme associated with high density lipoprotein surface and the antioxidant effect of the latter is partially related to paraoxonase. This enzyme is able to hydrolyze lipid hydroperoxides and to delay or inhibit the initiation of oxidation of lipoproteins induced by metal ions (Watson et al., 1995). It has been suggested that individuals with low paraoxonase-1 activity may have a greater risk of developing diseases such as diabetes mellitus in which oxidative damage and lipid peroxidation are involved, compared with those with high paraoxonase-1 activity (Durrington et al., 2001; Nourooz-Zadehet al., 1995).

Wegner et al. (2011) reported that 80 type 1 diabetic patients had lower paraoxonase-1 arylesterase activity and higher lipid hydroperoxide levels, and that there was a negative correlation between paraoxonase-1 arylesterase activity and lipid hydroperoxide levels. In a latter study, paraoxonase-1 activity was reduced in patients with type 1 diabetes mellitus with retinopathy, confirming that oxidative stress could play a role in pathogenesis of diabetic retinopathy (Nowak et al., 2010). A similar finding of lower high density lipoprotein-paraoxonase-1 activity in 31 type 1 diabetic patients compared with the same number of sex-



and age-matched healthy subjects was reported by Ferretti et al. (2004). These findings confirm a linkage between paraoxonase-1 activity and lipid peroxidation of lipoproteins and suggest that the ability of high density lipoprotein to protect erythrocyte membranes might be related to the paraoxonase-1 activity (Ferretti et al., 2004). The low paraoxonase-1 arylesterase activity suggests insufficient high density lipoprotein capacity to protect against lipid oxidation in patients with type 1 diabetes (Wegner et al., 2011). It is also hypothesized that the lower high density lipoprotein protective action against membrane peroxidation and decrease paraoxonase-1 activity in diabetic patients could contribute to acceleration of arteriosclerosis in patients with type 1 diabetes mellitus (Ferretti et al., 2004). Furthermore, there are several studies linking diabetes and even postprandial hyperglycemia with increased low density lipoprotein oxidative susceptibility (Ceriello, 2000). Decreased insulin in diabetes mellitus increases the activity of fatty acyl coenzyme A oxidase, which initiates  $\beta$ -oxidation of fatty acids, resulting in lipid peroxidation (Horie et al., 2006).

## 6.2. Biomarkers of protein peroxidation and oxidative damage to DNA in type 1 diabetes

High plasma glucose concentrations can increase the levels of glycation and oxidative damage to cellular and plasma proteins in diabetes mellitus. Glycation of proteins is a complex series of reactions where early-stage reactions leads to the formation of the early glycation adduct, fructosyl-lysine and  $\text{NH}_2$ -terminal fructosyl-amino acids, and later-stage reactions form advanced glycation end products (Thornalley, 2002). The oxidation of proteins produces nitrotyrosine and protein carbonyl derivatives and nitrotyrosine (Adams et al., 2001). The oxidized or nitrosylated products of free radical attack have reduced biological activity, leading to loss of cell signaling, energy metabolism, transport, and other major cellular functions. These altered oxidized products also are targeted for proteosome degradation, further reducing cellular function. There is also cell death through necrotic or apoptotic mechanisms as a result of the accumulation of cellular injury (Rosen et al., 2001).

Carbonyl group formation is considered an early and stable marker for protein oxidation in the body. Diabetes mellitus is associated with carbonyl stress where there is an increase of reactive carbonyl compounds caused by their enhanced formation and/or decreased degradation or excretion (Miyata et al., 1999.) This leads to the formation of advanced glycation end products such as pentosidin and carboxymethyllysine and advanced oxidation protein products, and damage to a number of biologically important compounds (Miyata et al. 1999; Witko-Sarsat et al., 1996). Telci et al. (2000) examined the influence of oxidative stress on oxidative protein damage in 51 young type 1 diabetic patients clinically free of complications and 48 healthy normolipidaemic age-matched controls. The levels of plasma carbonyl and plasma lipid hydroperoxide were increased in adolescent and young adult type 1 diabetic patients compared with controls.

Modifications in endothelial cell function are proposed to play an important role in atherogenesis. These perturbations include increased permeability to circulating lipoproteins particularly low density lipoprotein, increased retention of these lipoproteins, the loss of endothelial cell-directed vasodilatation, and the increased expression of intercellular cell adhesion molecule-1 and vascular cell adhesion molecule-1 (Ross, 1999). Koitka et al. (2004) re-

ported evidence of endothelial dysfunction in patients with type 1 diabetes. In another study of 45 type 1 diabetic children, there was significantly lower peak brachial artery flow-mediated dilation response and increased carotid artery intima-media thickness. This suggests that altered endothelium function in children with type 1 diabetes may predispose them to the development of early atherosclerosis (Jarvisalo et al., 2004). Furthermore, in a double-blind, placebo-controlled, randomized study of 41 young subjects with type I diabetes mellitus, vitamin E supplementation (1,000 IU for three months) had a positive effect on the endothelial function as evident by improved endothelial vasodilator function in both the conduit and resistance vessels (Skyrme-Jones, 2000).

In addition to lipids and proteins, reactive oxygen species reacts with deoxyribonucleic acid resulting in various products, such as 8-hydroxydeoxyguanosine, that is excrete in urine owing to deoxyribonucleic acid repair processes. Urinary 8-hydroxydeoxyguanosine has been proposed as an indicator of oxidative damage to deoxyribonucleic acid. Goodarzi and colleagues (2010) evaluated the relationship between oxidative damage to deoxyribonucleic acid and protein glycation in 32 patients with type 1 diabetes. There were elevated levels of urinary 8-hydroxydeoxyguanosine, glycated hemoglobin, plasma malondialdehyde, and glycated serum protein in 32 patients with type 1 diabetes. There was a significant correlation between urinary 8-hydroxydeoxyguanosine and glycated hemoglobin. The findings indicate that that deoxyribonucleic acid is associated to glycemic control level (Goodarzi et al., 2010). In a study which investigated whether advanced glycation end product production and oxidative stress are augmented in young patients with type 1 diabetes at early clinical stages of the disease, advanced glycation end products, pentosidine, and 8-hydroxydeoxyguanosine and acrolein-lysine were significantly higher in the patients with type 1 diabetes compared with healthy control subjects (Tsukahara et al., 2003).

### **6.3. Biomarkers of oxidative stress present in breath**

Oxidative stress has been implicated in the major complications of diabetes mellitus, including retinopathy, nephropathy, neuropathy and accelerated coronary artery disease (Ceriello & Morocutti, 2000; Androne et al., 2000; Mackness et al., 2002). There is a clinical need for markers of oxidative stress which could potentially identify diabetic patients at increased risk for these complications. The introduction of breath microassays has enhanced the detection of oxidative stress because reactive oxygen species oxidize polyunsaturated fatty acids in membranes to alkanes such as ethane and pentane. These are excreted in the breath as volatile organic compounds (Kneepkens & Lepage, 1994). Another marker of oxidative stress is the breath methylated alkane contour, comprising a three-dimensional display of C4 to C20 alkanes and monomethylated alkanes in the breath (Phillips et al., 2004). Phillips et al. (2004) reported significantly increased volatile organic compounds and breath methylated alkane contour in the breath of type 1 diabetic patients which was independent of glycemic as they did with blood glucose concentration or with glycation hemoglobin levels.

## 7. Conclusion

This review presented convincing experimental and clinical evidence that the aetiology of oxidative stress in diabetes mellitus arises from a number of mechanisms that includes excessive reactive oxygen species production from the peroxidation of lipids, auto-oxidation of glucose, glycation of proteins, and glycation of antioxidative enzymes, which limit their capacity to detoxify oxygen radicals. There is also evidence that supports the role of hyperglycemia in producing oxidative stress and, eventually, severe endothelial dysfunction in blood vessels of individuals with type 1 diabetes mellitus. The induction of oxidative stress is a key process in the onset and development of diabetic complications, but the precise mechanisms has not been fully elucidated. A number of biomarkers of oxidative stress have been studied in type 1 diabetic patients such as malondialdehyde, F2-isoprostanes, advanced glycation end product and nitrotyrosine. The introduction of breath microassays has enhanced the detection of oxidative stress.

Type 1 diabetic patients have been found to have decreased amounts and efficiency of antioxidant defenses (both enzymatic and non-enzymatic) due to increased consumption of distinct antioxidant components (e.g. intracellular glutathione) or to primarily low levels of antioxidant substances (flavonoids, carotenoids, vitamin E and C). This review also presents small clinical studies that have demonstrated improvements in a variety of oxidative stress biomarkers in type 1 diabetic patients who have received vitamin A, C or E supplements. However, the findings of key prospective randomized controlled antioxidant clinical trials have failed to demonstrate a significant benefit, in the prevention of cardiovascular events. There is a need for continued investigation of the association between reactive oxygen species, type 1 diabetes mellitus and its complications in order to clarify the molecular mechanisms by which increased oxidative stress accelerates the development of diabetic complications. This will have implication for the prevention and development of therapeutic choices for type 1 diabetic patients.

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