Review Article

THE PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF DIABETES ON THE BALANCE BETWEEN OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE SYSTEM

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SUMMARY : The number of reports on the effects of diabetes is still increasing because the diabetes is one of the major disease of industrialized and non-industrialized societies. Because at least 30 million people throughout the world suffer from diabetes, there is currently great interest in the potential contribution of increased oxidative stress to the pathogenesis of diabetes as well as its complications. Thus, the goal of this review was to explain the behaviors of the free radicals and antioxidant enzymes against diabetes. Hyperglycemia is a widely known cause of enhanced free radical concentrations and decreased antioxidant defense system, this mechanism may occur via at least four different routes: increased glycolysis; intercellular activation of sorbitol (polyol) pathway; auto-oxidation of glucose and non-enzymatic protein glycation. In addition, this overview not only to investigate the genesis of free radicals in hyper/hypo-insulinemia, but also to elucidate the inverse relationship between insulin action and free radicals. Increased free radicals in diabetes may be caused the pathogenesis of atherogenesis, atherosclerosis and the degeneration in the biological macromolecules; proteins and lipoproteins, and other degenerative disorders. Furthermore, this overview was extended to show the effect of streptozotocin on diabetes, antioxidant enzymes and oxidative stress. In general, the hypothesis was that, the imbalance of generation and scavenging of free radicals play an important role in determining tissue damage associated with diabetes. However, this argument is still ambiguous because of the difficulties of the direct observation of the active oxygen species in biological systems due to their short life time.

Key Words: Diabetes, Oxidative stress, Antioxidant system, hypo/hyperglycemia, Atherogenesis, Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous metabolic disorder characterized by hyperglycaemia resulting from defective insulin secretion, resistance to insulin action or both (1). Type 1 diabetes is the consequence of an autoimmune-mediated destruction of pancreatic β-cells, leading to insulin deficiency. Patients require insulin treatment for survival. Type 2 diabetes is characterized by insulin resistance and relative, rather than absolute, insulin deficiency. Type 2 diabetes usually occurs in obese individuals and is associated with hypertension and dyslipidaemia. The capacity of nutrients to stimulate insulin release from the pancreatic β-cell reflects their capacity to augment oxidative fluxes in the islet cells (2). Also, oxidant stress associated with insulin resistance and non-insulin-dependent *diabetes mellitus* (3,4) contributes to poor insulin action (5-7). Thus, the treatment aims to reduce insulin resistance (diet, exercise and drug therapy) and to stimulate insulin secretion.

In DM, oxidative stress seems mainly to be due to an increased production of free radicals and/or a sharp reduction of antioxidant defenses (8-14). Oxygen-derived free

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radicals have been implicated in the pathophysiology of various disease states, including diabetes mellitus (13). It is well known that superoxide anion is the primary radical formed by the reduction of molecular oxygen that may lead to secondary radicals or reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radical (15,16). Also, Jang *et al.* (17) found that increased oxidative stress has been suggested to be involved in the pathogenesis and progression of diabetic tissue damage. On the other hand, there is evidence that diabetes induces changes in the activities of antioxidant enzymes in various tissues (9). *Diabetes mellitus* is characterized by increased generation of glycoxidation products associated with the advanced oxidative stress (18). The presence of higher glucose or glycated protein concentration enhances lipid peroxidation (19) and reversely, lipid peroxides may increase the extent of advanced glycation end-products (20).

Oxidative stress in DM was thought to be a result of free radicals generated during autoxidation of glucose (21). Increased levels of ROS in type 2 DM was implicated to contribute to a hypercoagulable state (22), and, most recently, evidence was provided for the accumulation of oxidation products prior to the development of diabetes (23). The causes of enhanced free radical production are hyperglycemia (24) and hyperinsulinemia (25). Thus, in the following, the various mechanisms of ROS against DM will be explained:

Mechanisms of oxygen free radicals production by hyperglycemia

Hyperglycemia is a widely known cause of enhanced plasma free radical concentrations (24). Free radical production caused by hyperglycemia may occur via at least four different routes: i) increased glycolysis (26); ii) intercellular activation of sorbitol (polyol) pathway (27); iii) autooxidation of glucose (28) and iv) non-enzymatic protein glycation (29).

Increased Glycolysis

Hyperglycemia seems to enhance non-oxidative metabolism (glucose conversion to lactate) through increasing glucose-6-phosphate (G6P) (26). Increased glucose metabolism to lactate is associated with an increase in NADH/NAD^+ ratio (27). Under this condition of markedly accelerated glycolysis, oxidation of glyceraldehydes 3-phosphate (GAP) to 1,3-biphosphoglycerate (1,3-DPG) by glyceraldehyde 3-phosphate dehydrogenase appears to become the rate limiting step in glycolysis (30), this reaction is coupled to reduction of NAD^+ to NADH. In the cytosol, NADH is oxidized to NAD^+ by lactate dehydrogenase (LDH), coupled to reduction of pyruvate to lactate. Thus, the increase in the ratio of NADH/NAD^+ will reflect increased lactate/pyruvate ratio (27). The mechanism by which an increased rate of glycolysis increases free cytosolic NADH / NAD^+ ratio (redox imbalance) appears to result from a disequilibrium between the rate of oxidation of GAP to 1,3-DPG and the rate of reduction of pyruvate (30). This result indicates that, the increased glycolysis as a consequence of hyperglycemia is closely related to an increase in NADH / NAD^+ ratio due to impaired oxidation of NADH to NAD^+.

Increased activity of sorbital pathway

The increased glucose flux via sorbitol pathway (a pathway of a minor significant under normal glycemic condition) which leads to the accumulation of both sorbitol and fructose is thought to be one of the main metabolic disturbances related to diabetic hyperglycemia (31). In this pathway, glucose is reduced to sorbitol by aldose reductase (AR), coupled with oxidation of NADH/NAD^+. Sorbitol is then oxidized to fructose coupled with reduction of NAD+ to NADH by sorbitol dehydrogenase (SDH) (32).

Previous studies suggested several hypothesis for tissue injury caused by increased sorbitol pathway activity: 1) the decreased availability of NADPH (required for maintenance of reduced glutathione) which is oxidized to $NADP^+$ through reduction of glucose to sorbitol by aldose reductase (33). Furthermore, the competition between aldose reductase and glutathione reductase for NADPH cofactor depletes reduced glutathione (31). Attention has been focused on this GSH depletion, because it can play a role in increased oxygen free radicals production, which is thought to lead to oxidative tissue damage in diabetes (34); 2) increased NADH/NAD^+ ratio, which is related to accelerated oxidation of sorbitol to fructose by NAD^+-dependent sorbitol dehydrogenase (35). Williamson *et al.* (27) and Ceriello *et al.* (37) reported that NADH produced in the cytosol by oxidation of sorbitol to fructose can remain there temporarly but for a long run it has to be transported into the mitochondria to be oxidized by respi-

ratory chain causing generation of superoxide radical and other oxygen reactive species derived from it. Thus, an increase in the cytosolic NADH may be accompanied by increased load of mitochondria NADH, which in turn leads to increased oxygen radicals generation.

Glucose auto-oxidation

Glucose can be auto-oxidized in a cell-free system under physiological conditions via enediol tautomer formation which generates hydrogen peroxide; reactive intermediate such as hydroxyl and superoxide radicals, and ketoaldehydes (36,38). Transition metals such as iron are believed to be of crucial importance in the cascade of these reactions, as they catalyze auto-oxidation of glucose (38). In this review, several studies have reported that glucose auto-oxidation can actually occur and could be responsible for increased oxygen radicals in diabetes (39-41).

Non-enzymatic protein glycation (glycosylation)

Non-enzymatic glycation is a spontaneous chemical reaction between glucose and the amino groups of proteins in which reversible Shift bases and more stable Amadori products are formed (42). Advanced glycation end products (AGEs) are then produced by auto-oxidation of Amadori product (The Diabetes Control and Complications Trials Research Group, 1993). AGEs elicit their cellular effects by binding to specific cellular receptors (43-46), one of which, RAGEs (receptor for AGEs), has been identified on endothelial cells (47-49), monocytes / macrophages, mesangial cells, neurons and smooth muscle cells. Interaction of AGEs with endothelial surface RAGEs generates intracellular oxidative stress and therapy modulates cellular functions, even in the presence of intact antioxidant mechanisms (50-53). This process is probably enhanced and amplified when antioxidant defense mechanisms are reduced (54).

Mechanisms of oxygen free radicals production by hyper-insulinemia

Decline in physical fitness, increase in body fatness and upper body fat distribution are frequently associated with hyperinsulinemia and insulin resistance (55). Several lines of evidence seem to indicate the relationship between hyperinsulinemia and free radical production. Krieger-Brauer and Kather (56) reported that in intact human fat cells, exposure to insulin leads to a time-and dose-dependent accumulation of hydrogen peroxidase in the suspension medium. This effect, which has been related to the presence of a membrane-bound NADPH oxidase was found to persist after cell disruption and not to require ATP indicating that the receptor kinase step was bypassed. In addition, increased insulin concentration in rats following intraperitoneal injection of dextrose has been found to be associated with increased free radical production (57).

Since fasting hyperinsulinemia is considered to be a halmark of insulin resistance (55), a relationship between insulin resistance and plasma free radical concentration can not be excluded (58-59). The genesis of free radical concentration in insulin resistant conditions (25) might be due to:

An insulin-mediated overdrive of the sympathetic nervous system activities

Hyperinsulinemia is responsible for the overdrive of the sympathetic nervous system (60). In diabetic animals, catecholamines may increase free radical production through induction of the metabolic rate and auto-oxidation (61).

Elevation in plasma non-esterified fatty acid concentration

Insulin resistance has been shown to be associated with elevated fasting plasma non-esterified fatty acid (NEFA) concentration (55,62). Recently, Toborek and Henning (63) showed that fatty acids cause an increase in oxidative stress in cultured endothelial cells and an initial decrease in reduced glutathione concentrations after 6h of exposure to the incubation medium.

Traverse *et al.* (64) and Betteridge (65) reported that the imbalance of generation and scavenging of free radicals play an important role in determining tissue damage associated with diabetes. Lipid peroxidation is the primary cellular damage resulting from free radical reactions. Also, significant changes in cellular lipid structures are generally occurring in diabetic states (66,67). In these states, the structure changes are oxidative in nature due to peroxidation of the lipids, that defined as peroxidative deterioration of unsaturated fatty acids of cellular membrane phospho-

lipids, via intermediate radical reactions (68,69), with a result of producing hydroperoxides. The net effect of these combined reactions is the generation of highly toxic peroxyl radicals (ROO^-) which generate new lipid hydroperoxides because of their close proximity in biomembranes to other lipids (65,70,71).

Mechanisms of oxygen free radicals production by hypo-insulinemia

Hypoinsulinemia increases the activity of fatty acyl-CoA oxidase that indicates 3-oxidation of fatty acids resulting in increased production of H_2O_2 (72). Kakkar *et al.* (73) and Tatsuki *et al.* (74) recorded significant increase of erythrocytic and pancreatic catalase (CAT) activity in streptozotocin diabetic rats and ascribed this increase to the accentuated oxidative stress in diabetes. However, Matkovics *et al.* (75) demonstrated a significant decrease of CAT activity in erythrocyte hemolysates of streptozotocin diabetic rats.

Changes in antioxidant enzyme activities due to diabetes

Several studies examined the tissue levels of the enzymatic antioxidant defenses in diabetes with varying results. Piper *et al.* (76) demonstrated that, in experimental diabetes the activity of catalase was increased in vascular tissues with absence of any significant changes in the activity of the other major antioxidant enzymes (superoxide dismutase and glutathione peroxidase). In addition, Wohaieb and Godin (77) showed increased activities of catalase and superoxide dismutase (SOD) in the pancreas of diabetic rats, while the liver showed a generalized decrease in the activities of catalase, SOD and glutathione peroxidase (GSH-Px). In the previous study, the increase in the activities of both CAT and SOD occurred in the tissue with the lowest antioxidant enzymatic activities (pancreas) before onset of diabetes, suggesting a compensatory response to an increase in endogenous oxidant radicals in pancreas by diabetes. A decrease in the concentration of reduced glutathione (GSH) has been observed in erythrocytes from diabetic subjects, as a result of decreases in activities of the enzymes involved in GSH synthesis (such as γ-glutamycystein synthetase) or in the transport rate of oxidized glutathione (GSSG) from erythrocytes (78) and enhanced sorbitol pathway (31). In addition, a decrease in the activity of glutathione reductase (GSSG-R) which acts to reduce GSSG to GSH, has also been reported (79). Kazuhiro *et al.* (80) and Matkovics *et al.* (75) elucidated that GSSG-R activity decreased in erythrocyte hemolysates of streptozotocin diabetic rats and attributed this decrease to the enzyme glycation by the uncontrolled hyperglycemia (81). Also, Jos *et al.* (82) and Dominguez *et al.* (83) reported a significant decrease of erythrocyte GSH-Px activity in diabetic children and adolescents compared with control subjects. They attributed this decrease to a decline in blood glutathione content in those diabetics, since GSH is a substrate and cofactor for this enzyme. Therefore, low GSH content indicates low GSH-Px activity, which may produce increased oxidative stress propensity. Moreover, enzyme inactivation either through glycation process (84) or under conditions of increased oxidative stress might also contribute to low GSH-Px activity (85).

Oxidative stress in diabetes

Both radical and non-radical oxidants can induce lipid peroxidation particularly of those lipoproteins that contain unsaturated fatty acids. A product of the reaction between a superoxide anion and nitric oxide, known as peroxynitrite, is a particularly powerful oxidant of low-density lipoproteins (LDLs) (86). The evidence for oxidative damage in diabetes has been reported as far back as 1979 by Sato *et al.* (87). The authors reported that the average level of lipid peroxides in plasma is higher in diabetic patients than in normal people and the diabetic patients with angiopathy had higher lipid peroxide levels than other diabetic patients. Further suggestion is that the high levels of lipid peroxide in plasma may cause an increase in lipid peroxide levels in the intima of the blood vessel, which may then initiate atherosclerosis. Recent studies have found increased and similar *in vitro* oxidizability of LDL fractions of plasma from diabetic patients and identified autoantibodies against oxidatively modified LDL in type I diabetic patients again suggesting that LDL oxidation occurs *in vivo* in diabetes (88).

Lipid peroxidation has been implicated in the pathogenesis of many degenerative disorders (89) including naturally occurring (90) and chemically induced diabetes mellitus (91,92). Consequently, mechanisms in the formation of lipid hydroperoxides and biologically active metabo-

lites, together with their effect on cellular structure and function are becoming of increasing importance to the study of diabetogenesis (93).

Lipid hydroperoxides (LHP) produced from a variety of long-chain polyunsaturated fatty acid precursors via intermediate radical reactions, involve oxygen and metal cations (iron and copper). The net result of these combined reactions is the generation of highly reactive and cytotoxic lipid radicals, which generate new LHP because of their close proximity in biomembranes to other lipids. Extracellularly, lipid hydroperoxides are transported in the systemic circulation by low- and high-density lipoproteins (90). When released locally, LHP produce structural damage (94) Peroxidative regulation occurs through intervention by lipid and water-soluble antioxidants, as well as by specific antioxidant enzymes, i.e., dioxide (1-) dismutase, peroxidase and catalase.

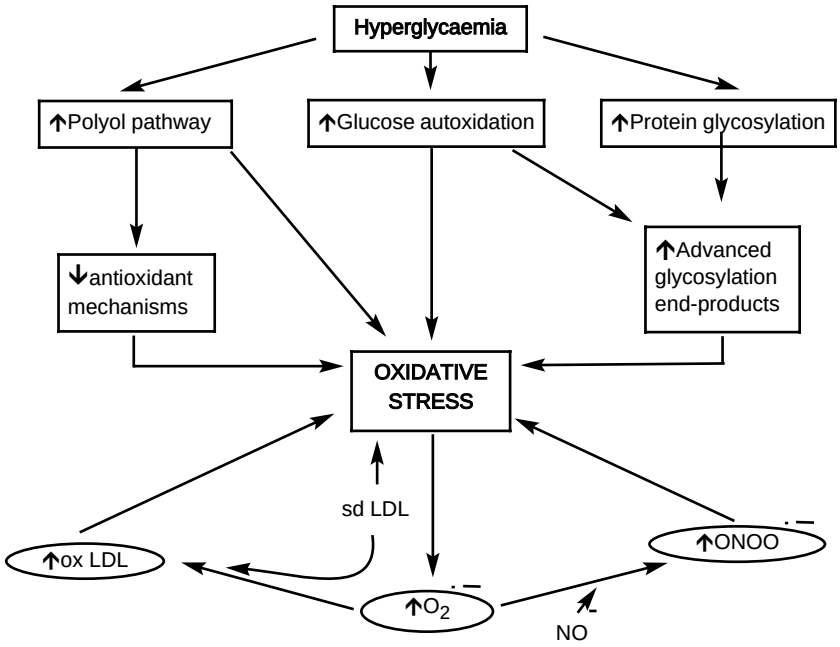
The formation of LHP and their metabolites are important in clinical medicine because they alter membrane structure and function, especially in the retinal portion of eye which is very sensitive to oxidative stress. For example, a steady decline is observed in the electroretinogram not only in the streptozotocin (STZ) model (95) but also when synthetic LHP is injected into the vitreous of experimental animals (96). These changes are irreversible.

Moreover, support for the concept of increased oxidative stress (increased generation of free radicals) in diabetes is derived principally from *in vitro* experiments (13,97). The primary causal factor is hyperglycaemia and this operates via several mechanisms (Figure 1), although the individual contribution of each mechanism to hyperoxidative stress remains undefined, as does also the dose response relationship between hyperglycaemia and overall oxidative stress in diabetes. Glycoxidation of glucose generates reactive oxygen species, such as superoxide, hydrogen peroxide and hydroxyl radical (13). These accelerate the formation of advanced glycosylation end-products (AGEs) which in turn generate more free radicals (13,42). Increased cellular uptake of glucose stimulates protein kinase C activity (98) which, amongst other effects, activates peroxidase enzymes and the cyclo- oxygenase (COX) pathway (98,99), with resultant overproduction of oxidative molecules. By elevating endothelial cell calcium, hyperglycaemia also stimulates the synthesis of NO (100,101), but in the presence of superoxide, nitric oxide (NO) is converted into the highly potent oxidant molecule peroxynitrite ($ONOO^-$) (102). Antioxidant defences may also be impaired in diabetes, thereby contributing to net oxidative stress. Decreased tissue concentrations of antioxidants, such as vitamin E, SOD and CAT, have also been demonstrated *in vitro* (77). Although there are extreme difficulties in measuring free radicals *in vivo*, some support for the notion of increased oxidative stress in diabetes and its association with poor metabolic control and coronary heart disease has been derived from observations in patients with *diabetes mellitus* (103,104). Increased oxidative stress may provide a plausible pathobiological basis for the direct association between hyperglycaemia and increased cardiovascular persuasive risk in *diabetes mellitus* (105,106). In spite of recent persuasive evidence (107), definitive clinical proof for the role of oxidative stress in the pathogenesis of atherosclerosis in both diabetic and non diabetic subjects remains outstanding.

In addition, there were an inverse relationship between insulin action and oxidative stress or hypofibrinolysis. Insulin resistance and increased oxidative stress have been observed in obese Type 2 diabetic patients (108). The relationship between insulin action and oxidative stress was therefore suggested (25). This finding of an inverse relationship between plasma malondialdehyde concentration and glucose disposal rate during hyperinsulinemic clamp is in agreement with this suggestion. A decrease of oxidative stress could therefore improve insulin action in subjects with insulin resistance. Drugs acting like scavengers of oxygen radicals are promissing tool in the treatment of patients with increased oxidative stress.

On the other hand, lipid peroxide levels in plasma of diabetic patients have been found to be significantly higher than in healthy individuals (109). Furthermore, Sato *et al.* (87) reported an increase in thiobarbituric acid reaction in these patients especially in poorly controlled diabetic and diabetica with angiopathy. This elevation has been considered as a cause of organ or tissue degeneration. Significantly higher values of thiobarbituric acid - reactive substances (TBARS), which provide an indirect measurement of lipid peroxidation and decreased erythrocyte antioxidant enzyme activities, have been observed in serum of adult diabetic patients (84,110), heart, pancreas

Figure 1 : Pathogenesis of hyperoxidative stress in non-insulin dependent diabetes. In boxes are shown mechanisms that are directly related to hyperglycaemia. In circles are some mechanisms that result from the reaction of free radicals (e.g. superoxide 02) with lipoproteins (e.g. small, dense low- density lipoprotein, sd LDL) and nitric oxide (NO), ox LDL, oxidized LDL- ONOO, peroxynitrite.



and blood of Streptozotocin diabetic rats (73). On other instance, TBARS is considered as an indicator of free radical production. An increase in TBARS level in liver may therefore be due to increased oxidative stress that might promote DNA and protein alterations (28) including changes in the enzyme activities implicated in lipid metabolism and free radicals scavenging process (111).

Also, increased oxidative stress in *diabetes mellitus* may be a reason for such decrease in erythrocytes count. Hyperglycemia can burden the cells with extra free radicals (112). This, coupled with reduced GSH content secondary to its increased utilization in diabetic erythrocytes (81) can cause peroxidative breakdown of phospholipids fatty acids in the erythrocytes membrane. This is supported by the fact that erythrocytes of diabetic patients are more susceptible to lipid peroxidation when treated with hydrogen peroxide *in vitro* (113,114).

In addition, the decrease in hematocrit (PCV) percentage may be attributed to the reduction in the total red blood cell count and the failure in blood osmoregulation and plasma osmolarity (115,116).

The relation between atherogenesis process and oxidative stress

Recent observations have focused attention on additional mechanisms that may be relevant to atherogenesis both in patients with type 2 diabetes and in the obese. Patients with type 2 diabetes and/or obesity have an increase in oxidative stress and inflammation. Increased oxidative stress in type 2 diabetes is indicated by an increase in ROS generation by circulating mononuclear cells, increased lipid peroxidation (117), protein carbonylation (118), nitro-tyrosine formation (119), and DNA damage (120). More recently, increased oxidative stress also was demonstrated in the obese, as reflected in increased lipid peroxidation, protein carbonylation, and ortho-tyrosine and meta-tyrosine formation. These changes reversed after caloric restriction to 1,000 calories/day for 4 weeks, as did ROS generation by leukocytes (121). Similarly, glucose and macronutrient intake set up a state of oxidative stress and inflammation (122,123). Thus, there is a close link between type 2 diabetes and macronutrient intake, oxidative stress, inflammation, and obesity.

Oxidative modification of macromolecules (Proteins and lipoproteins)

Oxidative damage to biologically important macromolecules may occur by means of nonradical oxidants such as hydrogen peroxide, hypochlorous acid or singlet oxygen, and by radical oxygen species like superoxide anion and hydroxyl radicals. These oxidants attack double bonds in unsaturated fatty acids resulting in the formation of lipid peroxides. Study of lipid peroxidation is, however, hampered by instability of the peroxidation products and the complexity of assays (124). Oxygen radicals, particularly the very aggressive hydroxyl radical, can also oxidize apolipoproteins and other plasma proteins, the products of which are much more stable than lipid peroxides.

The decrease in the total proteins concentration in serum of diabetic rats may be ascribed to

1) decreased amino acids uptake (125),

2) greatly decreased concentration of variety of essential amino acids (126),

3) increased conversion rate of glycogenic amino acids to $C0_2$ and H_20 (127) and

4) reduction in protein synthesis secondary to a decreased amount and availability of mRNA (128). Furthermore, Wanke and Wong (129) attributed the decrease of albumin concentration in experimental diabetes to the presence of inhibitor(s) of albumin promotor activity in the liver.

Relation between streptozotocin (STZ) administration and ROS

Streptozotocin administration damages pancreatic beta cells and results in diabetes in experimental animals. The mechanisms by which STZ induces diabetes are not clearly understood. Oxygen free radicals, including hydroxyl radicals, have been suggested to be involved in the toxic action of STZ (130-132). STZ is a chemically unstable molecule that accumulates in pancreatic beta cells (133) and produces toxic radicals during its decay. Highly reactive carbonium radicals originating from the decay of STZ molecules might increase the production of oxygen free radicals (130). These highly reactive radicals exert direct or indirect toxic effects on islet endothelium (134) and mediate fragmentation of nuclear DNA in beta cells (131,135). It is also found that STZ, at low dose, damages pancreatic beta cells by eliciting non-specific islet inflammation with infiltration by mononuclear cells (136). Nitric oxide generated by STZ has been proposed to be involved in the damage of pancreatic beta cells (137,138).

Moreover, Matkovics *et al.* (75) speculated that intraperitoneal injection of streptozotocin into rats induced a significant decrease in erythrocytes SOD activity. The exact nature of the decrease in SOD activity might be explained by:

1) a direct response to the increased formation of active oxygen species such as superoxide and hydroxyl radicals (67);

2) an irreversible inactivation of SOD by its product hydrogen peroxide because the exposure of intact erythrocytes to H_2O_2 resulted in the inactivation of endogenous CuZn - SOD in a concentration-dependent manner (139,140);

3) an increase in the non-enzymatic glycation of SOD (83,84) and

4) the impaired zinc status described in diabetes (141), since zinc is a part of the catalytic site of CuZn-SOD (67). Thus, it seems that diabetes promotes a decrease in SOD activity. However, Young *et al.* (11) indicated that insulin treatment of Streptozotocin diabetic rats reduced generation of free radicals by glucose auto-oxidation.

In conclusion, this review improved the disturbance in the concentration of oxidative stress and antioxidant defense system in diabetic rats; generally, increased oxidative stress with decrease in the antioxidant enzymes. Further studies are required to determine if these beneficial effects result in changes of diabetes complications only or not (inherited program modulate them).

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