

## Review article

# DIABETES, OXIDATIVE STRESS AND PHYSICAL EXERCISE

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### ABSTRACT

Oxidative stress, an imbalance between the generation of reactive oxygen species and antioxidant defense capacity of the body, is closely associated with aging and a number of diseases including cancer, cardiovascular diseases, diabetes and diabetic complications. Several mechanisms may cause oxidative insult in diabetes, although their exact contributions are not entirely clear. Accumulating evidence points to many interrelated mechanisms that increase production of reactive oxygen and nitrogen species or decrease antioxidant protection in diabetic patients. In modern medicine, regular physical exercise is an important tool in the prevention and treatment of diseases including diabetes. Although acute exhaustive exercise increases oxidative stress, exercise training has been shown to up regulate antioxidant protection. This review aims to summarize the mechanisms of increased oxidative stress in diabetes and with respect to acute and chronic exercise.

**KEY WORDS:** Diabetes, physical activity, antioxidants, reactive oxygen species.

### DİYABET, OKSİDATİF STRES VE FİZİKSEL EGZERSİZ

#### ÖZET

Oksidatif stres oksidan oluşumu ve antioksidan savunma arasındaki dengenin oksidanlar yönünde bozulması durumudur. Oksidatif stres; yaşlanma, kanser, kalp hastalıkları, diyabet ve diyabetin komplikasyonları başta olmak üzere pek çok patolojik tablonun ve de yaşlanmanın patogenezi ile yakın ilişkiindedir. Diyabette oksidatif stres pek çok mekanizmaya bağlı olarak artabilmektedir, ancak bu mekanizmaların kesin katkısı tam olarak ispatlanabilmiş değildir. Çok sayıdaki deneysel bulgular artan reaktif oksijen ve nitrojen türlerinin oluşumunun ve zayıflayan antioksidan savunmanın bu karmaşık mekanizmaların temelini oluşturduğunu göstermektedir. Düzenli fiziksel aktivite modern tıpta, diyabet de dahil olmak üzere pek çok hastalıkta tedavi ve koruyucu amaçlı olarak kullanılmaktadır. Her ne kadar akut fiziksel egzersiz oksidatif stressi artırsa da, düzenli egzersiz programları antioksidan savunmayı kuvvetlendirmektedir. Bu derlemede diyabette artmış olan oksidatif stres nedenlerini, akut egzersiz ve düzenli fiziksel aktivite yönlerinden özetlemeye çalıştık.

**ANAHTAR KELİMELER:** Diyabet, fiziksel aktivite, antioksidan, reaktif oksijen.

### INTRODUCTION

During moderate exercise oxygen consumption increases by 8-10 folds, and oxygen flux through the muscle may increase by 90-100 folds. Even moderate exercise may increase free radical production and overwhelm antioxidant defenses, resulting in oxidative insult (Sen and Packer, 2000).

It was first shown in 1978 by Dillard et al (Dillard et al., 1978) that in humans, even a moderate intensity of exercise increased the content of pentane,

a lipid peroxidation byproduct, in expired air. 1982 Davies et al. for the first time provided the direct evidence using electron paramagnetic resonance spectroscopy. In rats exhaustive treadmill exercise increased the free radical concentration by 2- to 3-fold of skeletal muscle and liver (Davies et al., 1982).

Further studies of our group and several other groups demonstrated that strenuous exercise induces oxidative stress as measured by oxidative damage of lipids, proteins and even the genetic material (Sen et al., 1994a; 2000; Goldfarb et al., 1996; Tiidus et al.,

1996; Khanna et al., 1999; Ji, 1999; Atalay and Sen, 1999; Sen, 1999; Atalay et al., 2000; Selamoglu et al., 2000). On the other hand, exercise training - both endurance and interval type - appears to induce antioxidant protection and decrease oxidative insult. Thus regular physical exercise protects against exercise induced oxidative stress (Atalay et al., 1996a; 1996b; Powers et al., 1997; 1999; Khanna et al., 1999; Sen, 1999).

Diabetes mellitus (DM) is a syndrome characterized by abnormal insulin secretion, derangement in carbohydrate and lipid metabolism, and is diagnosed by the presence of hyperglycemia. Diabetes is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to development of nephropathy, neuropathy and retinopathy (Zimmet et al., 1997). The prevalence of type 2 DM among adults varies from less than 5% to over 40% depending on the population in question (Zimmet et al., 1997). Due to increasing obesity, sedentariness and dietary habits in both Western and developing countries, the prevalence of type 2 DM is growing at an exponential rate (Zimmet and Lefebvre, 1996; 1998). Type 1 DM is less common.

Increased oxidative stress as measured by indices of lipid peroxidation and protein oxidation has been shown to be increased in both insulin dependent diabetes (IDDM), and non-insulin dependent (NIDDM) (Sato et al., 1979; Velazquez et al., 1991; Collier et al., 1992; MacRury et al., 1993; Neri et al., 1994; Yaqoob et al., 1994; Griesmacher et al., 1995; Niskanen et al., 1995; Laaksonen et al., 1996; Santini et al., 1997; Laaksonen and Sen, 2000; Cederberg et al., 2001), even in patients without complications. Increased oxidized low density lipoprotein (LDL) or susceptibility to oxidation has also been shown in diabetes (Collier et al., 1992; Neri et al., 1994; Yaqoob et al., 1994; Griesmacher et al., 1995; Laaksonen et al., 1996; Santini et al., 1997).

Despite strong experimental evidence indicating that oxidative stress may determine the onset and progression of late-diabetes complications (Baynes, 1991; Van Dam et al., 1995; Giugliano et al., 1996), controversy exists about whether the increased oxidative stress is merely associative rather than causal in DM. This is partly because measurement of oxidative stress is usually based on indirect and nonspecific measurement of products of reactive oxygen species, and partly because most clinical studies in DM patients have been cross-sectional (Laaksonen and Sen, 2000).

The mechanisms behind the apparent increased oxidative stress in diabetes are not entirely clear. Accumulating evidence points to a number of inter-

related mechanisms (Lyons, 1993; Cameron and Cotter, 1993; Tesfamariam, 1994; Cameron et al., 1996), increasing production of free radicals such as superoxide (Nath et al., 1984; Ceriello et al., 1991; Wolff et al., 1991; Dandona et al., 1996) or decreasing antioxidant status (Asayama et al., 1993; Tsai et al., 1994; Ceriello et al., 1997; Santini et al., 1997). These mechanisms include glycoxidation (Hunt et al., 1990; Wolff et al., 1991) and formation of advanced glycation products (AGE) (Lyons, 1993; Schleicher et al., 1997), activation of the polyol pathway (Cameron et al., 1996; Cameron and Cotter, 1993; Grunewald et al., 1993; Kashiwagi et al., 1994; De Mattia et al., 1994; Kashiwagi et al., 1996) and altered cell26 and glutathione redox status (Grunewald et al., 1993; Kashiwagi et al., 1994; 1996; De Mattia et al., 1994) and ascorbate metabolism (Sinclair et al., 1991) antioxidant enzyme inactivation (Arai et al., 1987; Blakytyn and Harding, 1992; Kawamura et al., 1992), and perturbations in nitric oxide and prostaglandin metabolism (Tesfamariam, 1994; Maejima et al., 2001).

Large prospective studies (Lakka et al., 1994; Paffenbarger et al., 1994) suggest that regular exercise and physical fitness as measured by maximal oxygen consumption have protective effect on cardiovascular diseases and mortality. Diabetic patients were not studied, however, and the mechanisms by which exercise lowers cardiovascular mortality remained unclear. Exercise as a tool of preventive medicine has been widely recommended, also for diabetic patients (American Diabetes Association, 1998). Regular exercise can strengthen antioxidant defenses and may reduce oxidative stress at rest and after acute exercise (Sen et al., 1994b; Sen, 1995; Kim et al., 1996). However, the relative benefits or risks of acute and chronic exercise in relation to oxidative stress in groups with increased susceptibility to oxidative stress such as diabetic patients are not known enough. Laaksonen et al. (1996) recently found increased oxidative stress as measured by plasma thiobarbituric acid reactive substances (TBARS) at rest and after exercise in young men with type 1 DM. Physical fitness as measured by maximal oxygen consumption ( $VO_{2\text{ max}}$ ), however, was strongly inversely correlated with plasma TBARS in the diabetic men only, suggesting a protective effect of fitness against oxidative stress.

## **MECHANISMS FOR INCREASED OXIDATIVE STRESS IN DIABETES**

### **Advanced glycation endproducts**

Advanced glycation or glycosylation endproducts (AGEs) are the products of glycation and oxidation (glycooxidation), which are increased with age, and at an accelerated rate in diabetes mellitus (Sell et al., 1992; Dyer et al., 1993).

In vitro studies have suggested that glycation itself may result in production of superoxide (Jones et al., 1987; Sakurai and Tsuchiya, 1988). Oxidation has been hypothesized to result in generation of superoxide, H<sub>2</sub>O<sub>2</sub> and through transition metal catalysis, hydroxyl radicals (Wolff et al., 1991). Catalase and other antioxidants decrease cross linking and AGE formation (Elgawish et al., 1996; Schleicher et al., 1997).

### Alterations in glutathione metabolism

Tissue glutathione plays a central role in antioxidant defense (Sen and Hanninen, 1994; Meister, 1995). Reduced glutathione detoxifies reactive oxygen species such as hydrogen peroxide and lipid peroxides directly or in a glutathione peroxidase (GPX) catalyzed mechanism. Glutathione also regenerates the major aqueous and lipid phase antioxidants, ascorbate and  $\alpha$ -tocopherol. Glutathione reductase (GRD) catalyzes the NADPH dependent reduction of oxidized glutathione, serving to maintain intracellular glutathione stores and a favorable redox status. Glutathione-S-transferase (GST) catalyzes the reaction between the -SH group and potential alkylating agents, rendering them more water soluble and suitable for transport out of the cell. GST can also use peroxides as a substrate (Mannervik and Danielson, 1988).

### Glutathione homeostasis

Type 2 diabetic patients had decreased erythrocyte GSH and increased GSSG levels (De Mattia et al., 1994; Jain and McVie, 1994). Blood GSH was significantly decreased in different phases of type 2 DM such as: glucose intolerance and early hyperglycemia (Vijayalingam et al., 1996), within two years of diagnosis and before development of complications (Sundaram et al., 1996) and in poor glycemic control (Peuchant et al., 1997). Red cells from type 2 DM patients had decreased GSH levels, impaired gamma-glutamyl transferase activity and impaired thiol transport (Yoshida et al., 1995). Treatment with an anti-diabetic agent for 6 months corrected these changes.

Thornalley et al. (1996) found an inverse correlation between erythrocyte GSH levels and the presence of DM complications in type 1 and 2 DM patients. However, most studies have also found decreased blood or red cell glutathione levels in type 2 DM patients. Less firm conclusions can be drawn in

type 1 DM patients. It has to be clarified whether the levels are decreased in patients without complications and whether patients with complications have even lower levels. The pathophysiological significance of decreased glutathione levels in DM remains to be shown.

### Glutathione dependent enzymes

Walter et al. (1991) found no difference in whole blood GRD activity in type 1 and type 2 DM patients compared to non-diabetic control patients. Muruganandam et al. (1992) also found normal red cell GRD enzyme kinetics in type 1 DM patients. On the other hand, blood GRD activity was lower in children with type 1 DM compared to healthy children (Stahlberg and Hietanen, 1991).

A large number of studies have shown that red blood cell, whole blood and leukocyte, glutathione peroxidase (GPX) activity was similar in type 1 and type 2 DM patients compared to control groups (Walter et al., 1991; Leonard et al., 1995; Akkus et al., 1996). On the other hand, erythrocyte GPX activity was also impaired in Asian diabetic patients (Tho and Candlish, 1987). In type 1 DM plasma selenium levels were normal, but red cell selenium content and GPX activity were decreased (Osterode et al., 1996).

Normal red cell GST enzyme kinetics were found in type 1 DM patients (Muruganandam et al., 1992). GST activity has been reported to be decreased in heart and liver (McDermott et al., 1994).

Changes in glutathione dependent enzymes in experimental diabetic models have been contradictory. Most studies show tissue and time dependent changes in enzyme activity. Even taking these factors into account, no consensus can be found among studies about the impact of DM on glutathione dependent enzyme activity. Changes in glutathione dependent enzymes in diabetic patients are also inconsistent. Differences in results cannot be completely explained by study methodology.

### Impairment of superoxide dismutase and catalase activity

Superoxide dismutase (SOD) and catalase are also major antioxidant enzymes. SOD exists in three different isoforms. Cu,Zn-SOD is mostly in the cytosol and dismutates superoxide to hydrogen peroxide. Extracellular (EC) SOD is found in the plasma and extracellular space. Mn-SOD is located in mitochondria. Catalase is a hydrogen peroxide decomposing enzyme mainly localized to peroxisomes or microperoxisomes. Superoxide may react with other reactive oxygen species such as nitric oxide to

form highly toxic species such as peroxynitrite, in addition to direct toxic effects (Tsfamariam, 1994). Peroxynitrite reacts with the tyrosine residues in proteins resulting with the nitrotyrosine production in plasma proteins, which is considered as an indirect evidence of peroxynitrite production and increased oxidative stress. Although nitrotyrosine was not detectable in the plasma of healthy controls, nitrotyrosine was found in the plasma of all type 2 diabetic patients examined. Consistent with these results, plasma nitrotyrosine values were correlated with plasma glucose concentrations (Ceriello et al., 2001). Furthermore, exposure of endothelial cells to high glucose leads to augmented production of superoxide anion, which may quench nitric oxide. Decreased nitric oxide levels result with impaired endothelial functions, vasodilation and delayed cell replication (Giugliano et al., 1996).

Alternatively, superoxide can be dismutated to much more reactive hydrogen peroxide, which through the Fenton reaction can then lead to highly toxic hydroxyl radical formation (Wolff et al., 1991). Decreased activity of cytoplasmic Cu,Zn-SOD and especially mitochondrial (Mn-) SOD in diabetic neutrophils was found. Consequently superoxide levels as estimated indirectly by cytochrome c reduction were elevated in neutrophils from diabetic patients as a result of decreased SOD activity (Nath et al., 1984). Major reason for the decreased SOD activity is the glycosylation of Cu,Zn-SOD which has been shown to lead to enzyme inactivation both in vivo and in vitro (Arai et al., 1987). Also Cu,Zn-SOD cleavage and release of  $\text{Cu}^{++}$  in vitro resulted in transition metal catalyzed ROS formation (Kaneto et al., 1996). Erythrocyte Cu,Zn-SOD activity correlated inversely with indices of glycemic control in DM patients, however (Tho et al., 1988). Red cell Cu,Zn/SOD activity has also been found to be decreased in DM patients (Arai et al., 1987), (Kawamura et al., 1992). Glycation may decrease cell-associated EC-SOD, which could predispose to oxidative damage. Jennings et al. (Jennings et al., 1991) found decreased red cell Cu,Zn-SOD activity in type 1 DM patients with retinopathy compared to type 1 DM patients without microvascular complications and non-diabetic control subjects. However, there are reports disagreeing with these findings. Red cell Cu,Zn-SOD activity was similar in type 1 and 2 DM patients compared to normal subjects (Tho and Candlish, 1987), (Walter et al., 1991), (Leonard et al., 1995; Faure et al., 1995), irrespective of microvascular complications (Walter et al., 1991). Leukocyte SOD activity was similar between type 2 DM patients and healthy control subjects, despite increased lipid peroxidation and decreased ascorbate levels (Akkus et

al., 1996). Furthermore, increased red cell SOD activity and serum MDA levels were reported in patients of type 1 DM with normo- microalbuminuria and retinopathy compared to healthy subjects (Yaqoob et al., 1994; Skrha et al., 1994).

Red cell superoxide and catalase activities were decreased in 105 subjects with impaired glucose tolerance (IGT) and early hyperglycemia and also in type 2 DM patients (Vijayalingam et al., 1996). However, in another study red cell catalase and SOD activities were normal in 26 type 2 DM patients in poor glycemic control (Peuchant et al., 1997). EC-SOD activity was found to be similar in type 1 DM patients (Adachi et al., 1996), despite somewhat higher plasma EC-SOD levels (MacRury et al., 1993; Adachi et al., 1996).

The wide variability among studies does not allow conclusions to be drawn as to whether SOD isoform or catalase enzyme activities are abnormal in diabetic patients. Again, differences in methodology or study design do not completely explain the conflicting findings among studies.

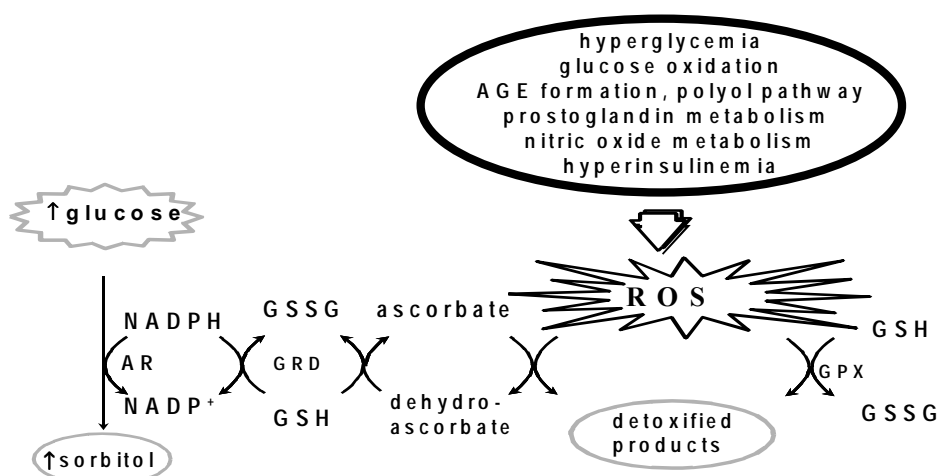
### The polyol pathway

Hyperglycemia induces the polyol pathway, resulting in induction of aldose reductase and production of sorbitol (Figure 1). Importance of the polyol pathway may vary among tissues. Induction of oxidative stress may occur through many different mechanisms, including depletion of NADPH and consequent disturbance of glutathione and nitric oxide metabolism.

Mean red cell GSH and NADPH levels and NADPH/NADP<sup>+</sup> and GSH/GSSG ratios were decreased in 18 type 2 diabetic patients compared to 16 non-diabetic control subjects (De Mattia et al., 1994; Bravi et al., 1997). One week of treatment with the aldose reductase inhibitor Tolrestat improved the NADPH and GSH levels in those patients whose NADPH levels were depressed ( $n=8$ ). Thus in at least a subset of type 2 DM patients activation of the polyol pathway appears to deplete erythrocyte NADPH and GSH. Similarly in a recent study aldose reductase inhibitor sorbinil restored nerve concentrations of antioxidants reduced glutathione (GSH) and ascorbate, and normalized diabetes-induced lipid peroxidation in streptozotocin-diabetic rats (Obrosova et al., 2002).

## LIPID PEROXIDATION AND PROTEIN OXIDATION IN DIABETES MELLITUS

### Lipid peroxidation in diabetic patients



**Figure 1.** Mechanisms for increased oxidative stress in diabetes mellitus. ROS; reactive oxygen species, GSH; reduced glutathione, GSSG; oxidized glutathione, GRD; glutathione reductase, GPX; glutathione peroxidase, AR; aldose reductase (modified from Laaksonen and Sen, 2000).

Lipid peroxidation end-products very commonly detected by the measurement of thiobarbituric acid reactive substances (TBARS). This assay has, however, been criticised for the lack of specificity. Lipid peroxidation as measured by lipid hydroperoxides (Hermes-Lima et al., 1995) have been shown to correlate closely with TBARS data in tissue samples. With proper caution, TBARS measurement may provide meaningful information (Draper et al., 1993).

Use of TBARS as an index of lipid peroxidation was pioneered by Yagi et al. (1976), whose group also showed increased plasma TBARS levels in DM (Sato et al., 1979) consistent with other's results (Noberasco et al., 1991; Altomare et al., 1992; Gallou et al., 1993; Jain and McVie, 1994; Gugliucci et al., 1994; Nourooz-Zadeh et al., 1995; Ozben et al., 1995; Nacitarhan et al., 1995; Freitas et al., 1997). Similarly, increased plasma peroxide concentrations were reported in type 1 and type 2 DM patients (Walter et al., 1991; Faure et al., 1993). Diabetic red blood cells (RBC)s were shown to be more susceptible to lipid peroxidation as measured by TBARS in rats and humans (Godin et al., 1988; Fujiwara et al., 1989). Oxidizability of plasma as measured by lipid hydroperoxides was greater in DM group, although baseline levels were similar in subjects with normal glucose tolerance, impaired glucose tolerance, and type 2 DM (Haffner et al., 1995). Furthermore, plasma

TBARS level was significantly increased in type 2 DM with the duration of disease and development of complications (Sundaram et al., 1996).

Liposomes constructed from red cell membranes of DM patients were highly sensitive to superoxide induced lipid peroxidation (Urano et al., 1991). SOD and vitamin E inhibited lipid peroxidation. MDA levels showed a significant correlation with glycosylated Hb. LDL lipid peroxidation was increased in 19 poorly controlled diabetic patients compared to age and gender matched subjects (Watala and Winocour, 1992).

The formation of conjugated dienes reflect early events of lipid peroxidation (Ahotupa et al., 1998). Spectrophotometric assay of conjugated dienes, however, does not provide information on hydroperoxides in samples. Serum levels of a conjugated diene isomer of linoleic acid were higher in DM patients with microalbuminuria than control subjects (Collier et al., 1992).

Plasma TBARS were elevated in women but not men in a study investigating lipid peroxidation in 56 young adult type 1 DM and 56 matched non-diabetic control subjects (Evans and Orchard, 1994). Similarly a recent report by Marra et al. (2002) showed that higher lipid peroxidation measured as lipid hydroperoxide, total conjugated diene coupled with lower total plasma antioxidant capacity at the early stage of type 1 diabetes, especially in women, which

may suggest the increased susceptibility of diabetic women to cardiovascular complications. Furthermore lipid peroxidation was increased and ascorbate levels were decreased in leukocytes from 53 type 2 DM patients compared to 34 age matched control subjects (Akkus et al., 1996). Serum MDA levels were higher in 20 patients with newly diagnosed type 2 DM than in matched controls (Armstrong et al., 1996). RBC free and total MDA levels were elevated in 26 poorly controlled type 2 DM patients (Peuchant et al., 1997). After three days of euglycemia maintained by constant insulin and glucose infusion, free MDA significantly decreased.

The vitamin E/lipid peroxide ratio was a major determinant of LDL susceptibility to oxidation. MDA levels were higher in DM patients compared to control subjects. Furthermore, LDL peroxidation was tightly correlated to the extent of LDL glycation. In men, TBARS was correlated with triglyceride levels and HbA<sub>1c</sub>, but not in women. Dietary treatment decreased HbA<sub>1c</sub> and MDA levels significantly. Lipid hydroperoxides and conjugated dienes were elevated in 72 patients with well controlled type 1 DM without complications, independent of metabolic control or diabetes duration (Santini et al., 1997). Plasma TBARS but not oxysterols were higher in 14 normo-lipidemic DM patients than in control subjects (Mol et al., 1997). Plasma lipid hydroperoxide levels were substantially higher in 41 type 2 diabetic patients compared to 87 control subjects (Nourooz Zadeh et al., 1997). Plasma lipid hydroperoxide levels were similar in diabetic patients with or without complications as well as in smokers and non-smokers. Plasma lipid peroxide levels, LPS-stimulated monocyte production of TNF-alpha and monocyte adhesion to endothelial cells were enhanced in 8 poorly controlled type 2 DM patients on glyburide therapy compared to 8 healthy subjects (Desfaits et al., 1998). Gliclazide administration reversed these abnormalities.

On the other hand, no difference in serum conjugated diene levels between otherwise healthy diabetic patients and healthy control subjects was noted (MacRury et al., 1993; Sinclair et al., 1992; Jennings et al., 1991), although conjugated diene levels were increased in 26 diabetic patients with micro-angiopathy complication (Jennings et al., 1991). TBARS levels in both poorly and well controlled type 2 DM patients did not differ from control subjects, whereas hydroxyl radical formation was elevated in DM patients (Ghiselli et al., 1992).

Plasma TBARS levels were similar in type 1 DM and type 2 DM patients as in control subjects (Neri et al., 1994; Leonard et al., 1995; Zoppini et al.,

1996). However, MDA was elevated in DM patients with micro-vascular complications compared to DM patients without complications and matched healthy subjects (Neri et al., 1994).

Most published studies have found increased lipid peroxidation in both type 1 and type 2 DM patients. Conflicting results have also been found, however, and they cannot be explained simply based on study design or methodology. It is less clear whether lipid peroxidation is increased in DM even before development of micro- and macrovascular disease. A causal role for lipid peroxidation in the development of diabetic macro- and microvascular complications is far from established.

Niskanen et al. (1995) showed for the first time that plasma TBARS were elevated in 22 patients with impaired glucose tolerance. After 10 years follow up fasting insulin and glucose levels were predictive of plasma TBARS levels in multiple regression analyses, suggesting a role for insulin resistance in inducing oxidative stress. Supporting these findings, lipid peroxidation was elevated in 105 subjects with IGT and early hyperglycemia and also in type 2 DM patients (Vijayalingam et al., 1996). On the other hand, baseline lipid hydroperoxide levels were similar in 75 subjects with normal glucose tolerance, impaired glucose tolerance, and type 2 DM (Haffner et al., 1995).

Although results to date on the role of insulin resistance as a mechanism for increased oxidative stress are intriguing, studies are surprisingly few. Given the attention focused on insulin resistance in the pathogenesis of DM and cardiovascular disease in general, future studies should also address the role of insulin resistance in oxidative stress.

### **Susceptibility of LDL cholesterol to oxidation**

Incubation of LDL cholesterol with glucose at concentrations seen in the diabetic state increased susceptibility of LDL to oxidation as measured by TBARS and conjugated diene formation, electrophoretic mobility and degradation by macrophages (Kawamura et al., 1994; Bowie et al., 1993). LDL and RBC membranes isolated from type 1 and type 2 DM patients were much more susceptible to oxidation than LDL from normal subjects (Bowie et al., 1993; Rabini et al., 1994). Furthermore susceptibility of LDL to oxidation was strongly correlated with degree of LDL glycosylation (Bowie et al., 1993). Plasma TRAP (total peroxy radical trapping potential) was lower and susceptibility of LDL to oxidation as measured by the lag phase of conjugated diene formation after initiation of LDL

oxidation by the addition of copper was greater in poorly controlled type 1 diabetic subjects than in normal control subjects (Tsai et al., 1994).

In contrast, there was no difference between type 1 diabetic patients and non-diabetic subjects in the susceptibility of LDL and VLDL cholesterol to oxidation in a number of studies (Gugliucci et al., 1994; O'Brien et al., 1995; Jenkins et al., 1996; Mol et al., 1997). Although, there was no difference between the groups for LDL vitamin E content, LDL fatty acid composition in cholesterol esters or triglycerides, LDL glycation was elevated in the type 1 DM subjects (O'Brien et al., 1995).

Most studies have found increased susceptibility of LDL cholesterol to oxidation in DM patients, although some well-designed studies have had conflicting results. Studies carried out to date do not allow firm conclusions to be drawn about whether LDL is more susceptible to oxidation in DM patients without complications than in healthy subjects, or about what effect complications and glycemic control have on the susceptibility of LDL to oxidation.

#### **Autoantibodies to oxidized cholesterol**

Type 1 and type 2 DM patients had significantly higher antibody ratio (calculated as the ratio of antibodies against modified versus native LDL) than control subjects for Cu<sup>++</sup>-oxidized LDL and malondialdehyde-modified LDL (Bellomo et al., 1995; Festa et al. 1998; Griffin et al., 1997).

In contrast, in early diagnosed or 10 years follow up type 1 DM patients, levels of serum autoantibodies to oxidized LDL cholesterol or malondialdehyde-modified LDL were similar compared to healthy control subjects (Uusitupa et al., 1996; Mironova et al., 1997; Korpinen et al., 1997). Furthermore, in a study performed among DM patients with normo- and macroalbuminuria with a long duration of diabetes and healthy subjects, antibody levels against malondialdehyde-modified LDL did not differ among normoalbuminuric DM, albuminuric DM and control subjects (Korpinen et al., 1997). In a very recent study, increased ratios of oxidized LDL antibodies were detected in type 2 diabetics only with macrovascular disease (Hsu et al., 2002).

No clear consensus has been found concerning the presence of increased oxidized LDL antibodies for LDL cholesterol oxidizability or especially for indices of plasma or serum lipid peroxidation in DM patients. Although interesting results linking oxidized LDL antibodies to carotid atherosclerosis in the general population have been published (Salonen et al., 1992), similar conclusions cannot be drawn from studies in

diabetic patients. Whether this is an argument against increased oxidative stress or its role in the pathogenesis of atherosclerosis in DM or against the use of oxidized LDL autoantibodies as a marker of lipid peroxidation in DM remains unclear.

#### **Protein Oxidation in diabetic patients**

Proteins are an important target for oxidative challenge. Reactive oxygen species modify amino acid side chains of proteins such as arginine, lysine, threonine and proline residues to form protein carbonyls. They can be readily measured by the reaction with 2,4-dinitrophenyl hydrazine using spectrophotometric, immunohistochemical and radioactive counting methods. Protein carbonyl content is the most widely used marker of oxidative modification of proteins and suggested to be a reliable marker of oxidative stress (Chevion et al., 2000). Elevated protein carbonyl levels were detected both in type 1 and type 2 and also in experimental diabetes (Dominguez et al., 1998; Cakatay et al., 2000; Telci et al., 2000; Jang et al., 2000; Cederberg et al., 2001). Furthermore, protein carbonyl content is well correlated with the complications of diabetes (Altomare et al., 1997).

In addition to lipid and protein oxidation, oxidative damage of DNA has been reported in diabetic patients. Type 1 and type 2 DM patients have significantly higher levels of 8-hydroxydeoxyguanosine, indicator of oxidative damage of DNA, in mononuclear cells (Dandona et al., 1996). These changes might contribute to atherogenesis in DM and to the microangiopathic complications of the disease.

#### **EXERCISE, PHYSICAL FITNESS AND OXIDATIVE STRESS IN DIABETES MELLITUS**

Oxidative stress is implicated in the accelerated atherosclerosis and microvascular complications of diabetes mellitus. Furthermore, physical exercise may acutely induce oxidative damage, although regular training appears to enhance antioxidant defenses, and in some animal studies, it has decreased lipid peroxidation.

Exercise is a major therapeutic modality in the treatment of DM (American Diabetes Association, 1998; Laaksonen et al., 2000). To maximize the benefits of exercise, it is important to understand the effect of acute and long term physical exercise on oxidative stress and antioxidant defenses in diabetes. With these goals in mind, we recruited 9 otherwise healthy type 1 DM and 13 control men aged 20-30 y (Laaksonen et al., 1996; Atalay et al., 1997). The

subjects rode for 40 min on a bicycle ergometer at 60% of their  $VO_{2\text{ max}}$  after a five min warm up. Blood samples were drawn at rest and immediately after exercise. We used as measures of oxidative stress plasma TBARS, and in response to exercise changes in GSSG levels and the GSSG/TGSH (total glutathione) ratio. For indices of antioxidant defenses, blood TGSH and GSSG levels and red cell GPX, GRD, GST, superoxide and catalase activities were measured.

Red cell GRD activity at rest was 15% higher in the diabetic group ( $P < 0.05$ ). However, erythrocyte Cu,Zn-SOD and catalase activities at rest were significantly lower in the diabetic group. Acute exercise increased erythrocyte Se-GPX activity modestly in the control group, but not in the IDDM group. Post-exercise Se-GPX activity was significantly higher in the control group compared to the IDDM group. Although acute exercise did not significantly affect GRD activity because of the higher resting values, post-exercise GRD activity was also higher in the IDDM group compared to the control group. Erythrocyte GST, Cu,Zn-SOD and catalase activities were similar in control and DM group after exercise (Atalay et al., 1997).

We found increased plasma TBARS in the diabetic men both at rest and after exercise, showing for the first time increased exercise induced oxidative stress in DM (Laaksonen and Sen, 2000). These results also support previous studies suggesting that type 1 DM patients have increased lipid peroxidation even in the absence of complications. Decreased Cu,Zn-SOD activity coupled with increased superoxide production (Nath et al., 1984; Ceriello et al., 1991; Wolff et al., 1991; Dandona et al., 1996) could exacerbate oxidative stress, especially if not compensated with increased catalase or Se-GPX activity. Superoxide may react with other reactive oxygen species such as nitric oxide to form highly toxic species such as peroxynitrite, in addition to direct toxic effects (Tsfamariam, 1994). Alternatively, superoxide can be dismutated to the much more reactive hydrogen peroxide, which through the Fenton reaction can then lead to highly toxic hydroxyl radical formation (Wolff et al., 1991). Thus decreased catalase activity could also contribute to the increased oxidative stress found in the type 1 DM subjects. Increased glucose (Yadav et al. 1994) and hydrogen peroxide levels (Ou and Wolff, 1994) have also been shown to inactivate catalase. As reviewed above, decreased red cell SOD and catalase activity have often, but not always, been found in DM patients.

Increased blood TGSH levels in the DM men could represent an adaptive response to increased

oxidative stress, mediated possibly in part through increased red cell GRD activity. Most other studies have found either decreased or unchanged glutathione levels in DM patients. Relatively few studies have examined glutathione levels in type 1 patients. Frequently, older patients have complications, or have been poorly described with respect to presence of diabetic complications or glycemic control. In the study by Di Simplicio et al. (1995), however, type 1 DM patients without complications appeared to have increased platelet GSH.

The strongly negative association between plasma TBARS and  $VO_{2\text{ max}}$  suggests that good physical fitness may have a protective role against oxidative stress. The intriguing question - can lipid peroxidation be decreased through regular training in diabetes - is thus raised. If so, this may have far-reaching clinical implications, and the role of oxidative stress in the development of diabetic micro- and macrovascular complications needs to be firmly established.

In a recent study in streptozotocin-induced experimental diabetic rats, our group showed that endurance training decreased lipid peroxidation measured by TBARS level in vastus lateralis muscle and increased glutathione peroxidase in red gastrocnemius muscle (Gul et al., 2002). However, endurance training increased conjugated dienes and decreased glutathione peroxidase activity in heart. Consistent with these results, decreased levels of cardiac antioxidants have been previously observed in endurance trained healthy rats (Kihlstrom et al., 1989). Acute exhaustive exercise induced oxidative stress measured as increased TBARS level in liver and increased dienes in heart. Increased TBARS levels in liver of untrained diabetic rats after acute exhaustive exercise are in agreement with our previous study carried out in normal rats (Khanna et al., 1999). These results suggest that despite the adverse effects in heart, endurance training appears to up-regulate glutathione dependent antioxidant defense in skeletal muscle in experimental DM.

## CONCLUSION

Diabetes mellitus is associated with a markedly increased mortality from coronary heart disease, not explainable by traditional risk factors. Although data are not yet conclusive, oxidative stress has been increasingly implicated in the pathogenesis of diabetic micro- and macrovascular disease. Some evidence also supports a role of physical fitness in decreasing lipid peroxidation. If regular physical exercise can be shown to have a protective effect against oxidative



stress in DM, this may have direct impact on the use of physical exercise as a safe therapeutic modality in diabetes.

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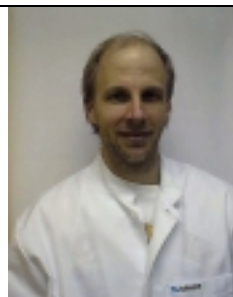
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