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Disturbances of micronutrient and antioxidant status in diabetes

BY J. J. STRAIN

Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA, Northern Ireland

Oxidants or reactive oxygen species (ROS) have been implicated in the pathology of a number of human diseases including insulin-dependent diabetes mellitus (IDDM) and non-isulin-dependent diabetes mellitus (NIDDM). Sato *et al.* (1979) were the first to report increased levels of plasma thiobarbituric acid-reactive substances (TBARS), a putative measure of ROS-induced lipid peroxidative damage, in IDDM and NIDDM patients. Subsequent studies have confirmed this observation of increased lipid peroxides in blood from NIDDM (Kaji *et al.* 1985; Uzel *et al.* 1987) and IDDM (Jongkind *et al.* 1989) patients and in various tissues from rats with streptozotocin (STZ)- or alloxan-induced diabetes (Higuichi, 1982; Karpen *et al.* 1982; Matkovics *et al.* 1982).

The possibility that the long-term complications of diabetes are associated with increased ROS-mediated damage is also supported by the work of Sato *et al.* (1979) who found higher plasma TBARS in diabetic patients with angiopathy compared with those without angiopathy. Moreover, increased lipid peroxide levels were also found in lenses of both diabetic patients (Costagliola *et al.* 1988) and STZ-induced diabetic rats (Yeh & Ashton, 1990).

The elevated levels of lipid peroxides in diabetes could result from the hyperglycaemic state as there is a significant relationship between erythrocyte membrane lipid peroxidation and hyperglycaemia in diabetic patients (Jain *et al.* 1989) and plasma lipid peroxides are higher in poorly-controlled compared with well-controlled diabetic patients (Sato *et al.* 1979). Jain *et al.* (1990) found that the increase in TBARS in erythrocytes from STZ-induced diabetic rats could be prevented in those rats in which hyperglycaemia was controlled by insulin treatment. Further indications that complications of diabetes associated with elevated levels of apparently oxidative changes to proteins and lipids are reviewed by Baynes (1991). It has been suggested that increased generation of ROS may arise from transition-metal catalysed autoxidation of glucose and other small autoxidizable molecules (Hunt & Wolff, 1990) or oxidation of glycated proteins (Gillery *et al.* 1989; Mullarkey *et al.* 1990). These mechanisms may explain the increased lipid peroxidation associated with hyperglycaemia.

Alternatively, increased ROS-mediated lipid peroxidation in diabetes may result from disturbances in antioxidant defence. There is now ample evidence of substantial and complex antioxidant enzyme alterations in experimental and clinical diabetes (Godin *et al.* 1988), although results from different laboratories are not consistent (Oberley, 1988; Asayama *et al.* 1989). Some of the inconsistent observations of antioxidant enzymes in diabetes could arise from *inter alia* effects of starvation (Wohaieb & Godin, 1987) or ageing (Cand & Verdetti, 1989) on these enzymes.

NIACIN AND RIBOFLAVIN

A role for ROS in the aetiology of diabetes has been proposed by Okamoto (1983) in a model for β cell damage. This model postulates a common final pathway for the toxic

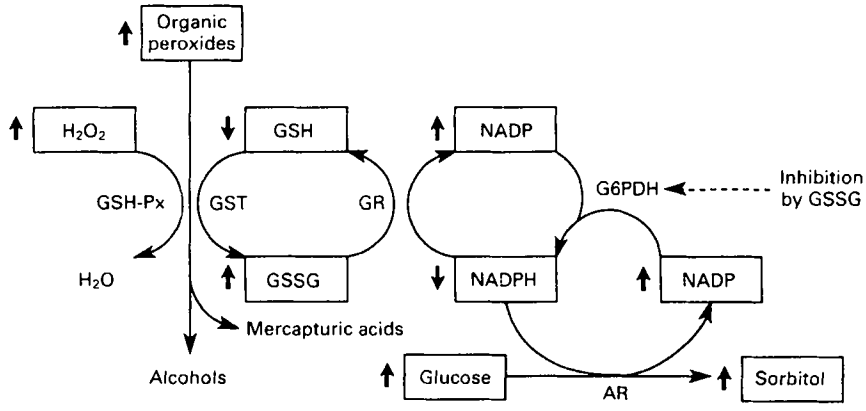

Fig. 1. Effect of competition between aldose reductase (*EC* 1.1.1.21; (AR)) and glutathione reductase (*EC* 1.6.4.2; (GR)) for NADPH. G6PDH, glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49); GSH, reduced glutathione; GSH-Px, glutathione peroxidase (*EC* 1.11.1.9); GSSG, oxidized glutathione; GST, glutathione-S-transferase (*EC* 2.5.1.18).

effect of agents such as alloxan, STZ, radiation, viruses, and inflammatory tissue damage through the involvement of ROS in breakage of nuclear DNA. The DNA damage initiates the repair process in which the enzyme poly (ADP-ribose) synthetase (*EC* 6.5.1.2) becomes activated. Cellular NAD is used as a source of ADP-ribose for DNA repair and this results in a sharp fall in intracellular levels of NAD. The combined effect of the non-physiological levels of NAD and ROS-mediated mitochondrial damage is considered to result in a fall in ATP levels and subsequently a fall in insulin release (Hellerstrom *et al.* 1986). Administration of nicotinamide or poly (ADP-ribose) synthetase inhibitors can maintain β cell function after exposure to diabetogenic agents alloxan and STZ (Okamoto, 1983).

Reasons why β cells of the pancreas should be most vulnerable to these agents are presently unclear. Oberley (1988) has suggested that uptake, metabolism, and anti-oxidant status of the β cell may all be involved in the selective toxicity of alloxan, with the most conclusive evidence favouring a major role for ROS. The oxidative basis of the selective toxicity of STZ for the β cell, however, is less clear as an alkylation mechanism appears to be involved in the DNA strand breaks. Nevertheless it is probable that increased levels of ROS are involved in STZ action through an interference with glutathione (GSH) metabolism (Oberley, 1988). The central role of GSH and its relationship with the phosphorylated form of NAD and with the polyol pathway in diabetes is given in Fig. 1. Aldose reductase (*EC* 1.1.1.21) inhibitors which can inhibit depletion of GSH can prevent lipid peroxidation of rat lens resulting from insulin deficiency (Yeh & Ashton, 1990).

Abnormalities in GSH metabolism are a feature of diabetes; for example, Murakami *et al.* (1989) found that the decrease in GSH in erythrocytes of diabetic patients was brought about by impaired GSH synthesis and that the increase in oxidized GSH (GSSG) was brought about by the decreased transport of GSSG through the erythrocyte membrane together with a decrease in glutathione reductase (*EC* 1.6.4.2; GR) activity. Gebre-Medhin *et al.* (1982), however, have reported increased GR activity in IDDM children. Perhaps inconsistencies in GR activity reported in the literature can be

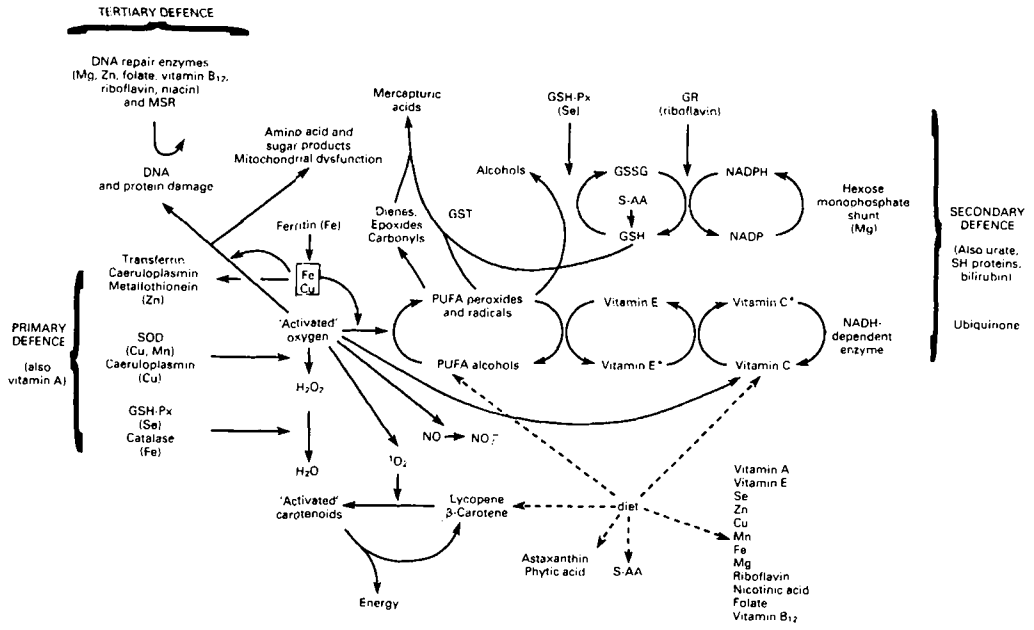

Fig. 2. Antioxidant defence system (after Strain *et al.* 1991). GR, glutathione reductase (*EC* 1.6.4.2); GSH, reduced glutathione; GSH-Px, glutathione peroxidase (*EC* 1.11.1.9); GSSG, oxidized glutathione; GST, glutathione-S-transferase (*EC* 2.5.1.18); MSR, methionine sulphoxide reductase (*EC* 1.8.4.5); PUFA, polyunsaturated fatty acids; S-AA, sulphur amino acids; SH-proteins, sulphydryl proteins; SOD, superoxide dismutase (*EC* 1.15.1.1); Fe Cu, transition metal-catalysed oxidant damage to biomolecules.

explained by differences in riboflavin (a co-factor needed for GR activity) status. For example Cole *et al.* (1976) have demonstrated decreased GR activities in IDDM children which could be restored by dietary riboflavin supplementation.

Whatever the precise nature of the toxic effects of alloxan or STZ, β cells appear to be defective in handling ROS compared with most other body tissues. Antioxidant agents such as the antioxidant enzymes can prevent or ameliorate the toxic effects of alloxan or STZ on β cells (Okamoto, 1983; Oberley, 1988).

Two important animal models for IDDM are the non-obese diabetic mouse (NOD) and the BB rat. In both these models spontaneous diabetes develops via immunological mechanisms. Diabetes can be prevented by treatment of animals with nicotinic acid or desferrioxamine, or both (Nomikos *et al.* 1986) and can be ameliorated with the cholesterol-lowering drug of known antioxidant properties, Probucol (Drash *et al.* 1988).

Fig. 2 shows the role of niacin, riboflavin, and other micronutrients in antioxidant defence. Obviously many of these nutrients have myriad biological roles distinct from antioxidant defence. For conceptual convenience antioxidant defences have been classified as a primary defence which is concerned with limiting the production of new ROS through the activity of such enzymes as superoxide dismutase (*EC* 1.15.1.1; SOD), glutathione peroxidase (*EC* 1.11.1.9; GSH-Px), catalase (*EC* 1.11.1.6) and caeruloplasmin (*EC* 1.16.3.1). Further protection is provided by caeruloplasmin, transferrin, lactoferrin, and other metal-binding proteins which act as primary antioxidants by limiting the availability of free ferrous or cuprous ions and, hence, decreasing formation

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of the hydroxyl radical, regarded as the most highly reactive of the primary oxidants (Halliwell, 1987).

Secondary antioxidants trap ROS directly to prevent amplification of radical formation and, thus, act as disrupters of free-radical chain reactions. Further antioxidant defence, the tertiary defence system, includes enzymes capable of repairing previous oxidant damage to important biomolecules (Fig. 2).

IRON

The use of the Fe-chelator desferrioxamine to prevent inflammatory tissue damage in NOD mice (Nomikos *et al.* 1986) indicates that Fe might be catalysing the formation of hydroxyl radicals via the Fenton reaction (see Strain *et al.* 1991) in β cells. Thus, conditions of Fe overload in various tissues, including the pancreas, might precipitate the destruction of the β cell leading to the onset of diabetes via a ROS mechanism. Indeed there are strong links between increased Fe status and diabetes. Overt diabetes occurs in about 60% of patients with idiopathic haemochromatosis (Powell, 1985). While the prevalence of idiopathic haemochromatosis in the general population is 2-5/1000, the prevalence of previously unrecognized idiopathic haemochromatosis among diabetic patients was 9·6/1000 (Phelps *et al.* 1989). Insulin resistance is markedly improved after depletion of body Fe stores by phlebotomy, resulting in lower insulin requirements in those patients with IDDM and improvement in glucose tolerance in about 50% of patients with NIDDM (Stremmel *et al.* 1987).

Glucose intolerance and overt diabetes are also observed in secondary forms of Fe-loading disease. Merkel *et al.* (1988) suggested that insulin resistance and increased insulin secretion develop in older children with thalassaemia treated with long-term hypertransfusion therapy before the development of diabetes. Apart from impaired insulin secretion caused by selective deposition of Fe in β cells of the pancreas, other possible mechanisms which may explain these findings include the development of insulin resistance due to Fe accumulation in the liver and muscle. It is tempting to speculate that the increased Fe status in obese women (Fricker *et al.* 1990) and those with greater body mass index (Micozzi *et al.* 1989) might give increased risk of NIDDM. Although associated with increased serum triacylglycerols, Fe deficiency does not seem to have a significant effect on glucose homeostasis (Mertz, 1982).

Diabetes is also associated with abnormalities in Fe metabolism. In experimental STZ-induced diabetic rats, Fe content was increased in liver, kidney and femur (Johnson & Evans, 1984) and in muscle but was decreased in the duodenum compared with control rats fed on equivalent quantities of Fe (Failla & Kiser, 1981). The acute diabetogenic action of STZ in rats results in greater hepatic Fe and lower serum Fe levels (McDermott *et al.* 1991). These defects in Fe metabolism may result in decreased Fe transfer to the fetus during late gestation in diabetic mothers and may partially explain the abnormal Fe distribution in infants of diabetic mothers (Georgieff *et al.* 1990).

COPPER

Like Fe, Cu can catalyse ROS formation in in vitro systems and much of the primary antioxidant defence is involved in ensuring that Cu is unavailable for such reactions (Fig. 2). Unlike Fe, however, no satisfactory explanation has been proposed for the liberation

of free cuprous ions in vivo (cf. superoxide liberation of ferrous species from ferritin, Biemond *et al.* 1988). In contrast the evidence for an important role for Cu in antioxidant defences through maintaining caeruloplasmin and Cu,Zn-SOD activities has accumulated since the pioneering work of Paynter (1980) who found that dietary Cu and manganese deficiencies, or both, were associated with increased susceptibility of rat heart mitochondria to oxidant damage as measured by TBARS. Direct evidence of increased lipid peroxidation in tissues from Cu-deficient rats have been provided by other workers (Balevska *et al.* 1981; Lynch & Strain, 1989; Wachnik *et al.* 1989; Strain & Lynch, 1990) while Lawrence & Jenkinson (1987) have found that Cu deficiency in the rat resulted in increased carbon tetrachloride-induced lipid peroxidation as indicated by expired ethane. Similarly Saari *et al.* (1990*a*) observed enhanced ethane production in Cu-deficient rats. Moreover the cardiovascular defects of Cu deficiency can be ameliorated by dietary supplementation with the exogenous antioxidants, t-butylhydroquinone (Johnson & Saari, 1989) and dimethyl sulphoxide (Saari *et al.* 1990*b*).

Historically one of the earliest recognized signs of Cu deficiency, was the impaired glucose tolerance in animals observed by Keil & Nelson (1934). More recently it was demonstrated that the diabetogenic effects of Cu deficiency were dependent on the type of carbohydrate fed to rats with fructose being more diabetogenic than glucose (Fields *et al.* 1984*a*). Although the extent of lipid peroxidation and other signs of Cu deficiency in the rat are also exacerbated by dietary fructose compared with glucose or starch (Fields *et al.* 1984*b*) it is unclear how impairment of antioxidant defence might be related to signs of Cu deficiency such as increased blood glucose, glucosuria, decreased insulin secretion and insulin-receptor bonding, lipogenesis and epididymal fat, intestinal hexose uptake, liver and diaphragm glycogen, and hepatic glucose-6-phosphatase (*EC* 3.1.3.9) activity as well as increases in serum triacylglycerols and cholesterol and hepatic glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49) activity (see Sorenson, 1989). Cu deficiency can also increase the severity of STZ-induced diabetes (Cohen *et al.* 1982) and the susceptibility of the exocrine pancreas to oxidative damage (Dubick *et al.* 1989).

There is additional evidence from human studies of a link between Cu deficiency and diabetes. Decreased glucose tolerance was observed in two men during experimental Cu depletion (0·78 mg/d) but improved with Cu repletion (6 mg/d) beyond the glucose tolerance observed before the initiation of depletion (Klevay *et al.* 1986).

There also appears to be altered Cu metabolism in diabetes. In general STZ-induced diabetes increases Cu content associated with metallothionein of rat liver and kidney tissues (Uriu-Hare *et al.* 1988). The near normal hepatic Cu concentration found in the diabetic obese animal, however, is probably due to the opposing influences of the obese and diabetic conditions on the hepatic concentration of Cu (Donaldson *et al.* 1987). Nevertheless treatment with Cu,Zn-SOD and SOD mimetic Cu complexes can inhibit STZ- or alloxan-induced diabetes (Sorenson, 1989). It is possible, therefore, that increased Cu,Zn-SOD activity in the pancreatic β cell offers greater protection against ROS.

Even though there appears to be higher Cu concentrations in some tissues of the diabetic rat, the Cu may not be biologically available. For example the formation of the highly stable polyol–Cu complex (Hamalainen & Makinen, 1989) may increase Cu requirements in diabetes. Rat tissue levels of sorbitol are elevated by fructose consumption compared with starch or glucose consumption and Cu deficiency can lead to even higher levels of this polyol (Fields *et al.* 1989). The disruption of GSH metabolism in

STZ-induced diabetes may also compromise the role of Cu in antioxidant defence. Recent in vitro work indicates that a Cu–GSH complex is required to transfer Cu to the holoform of Cu,Zn-SOD (Ciriolo *et al.* 1990) and this requirement could explain the earlier work of Loven *et al.* (1986) who suggested a link between GSH metabolism and Cu,Zn-SOD in STZ-induced diabetes. Moreover, the glycation of Cu,Zn-SOD and its inactivation in diabetes (Taniguchi *et al.* 1989) may further impair antioxidant defences.

Recently Fields *et al.* (1991) have found that deferoxamine can ameliorate the pathology of Cu deficiency most probably by decreasing body Fe overload. It is well known that Cu deficiency can disrupt Fe transport and metabolism and it is possible that some of the anti-diabetic action of dietary Cu may be due to the alleviation of impaired mobilization of stored Fe.

In general serum Cu and caeruloplasmin levels are increased in IDDM and NIDDM patients (Mooradian & Morley, 1987). It is probable that these increases reflect the greater inflammatory conditions in diabetes as caeruloplasmin is an acute phase reactant (DiSilvestro, 1990). Jones *et al.* (1988) have concluded that the increased caeruloplasmin–ferroxidase (*EC* 1.16.3.1) activity and Fe-bonding proteins in diabetic serum may be a response to oxidative stress. Certainly increased blood Cu levels do not necessarily reflect increased body Cu status (DiSilvestro, 1990). Sjogren *et al.* (1986) have found that the level of Cu in striated muscle was significantly lower in IDDM patients compared with healthy controls even though much higher levels of plasma Cu were observed in these patients compared with controls.

ZINC

High concentrations or doses of Zn are known to have antioxidant-like effects in in vitro systems and in vivo. Similarly, there is some evidence to suggest an increased oxidative stress in Zn deficiency but the precise role of Zn as an antioxidant has still to be elucidated (Bray & Bettger, 1990). Two possible mechanisms are the protection of sulphydryl groups against oxidation and the inhibition of transition metal-catalysed production of ROS through induction of metallothionein (Fig. 2). The enzymic activity of Cu,Zn-SOD, however, is unaffected by Zn status (Taylor *et al.* 1988).

Crystalline insulin contains Zn but the effect of Zn deficiency on impaired glucose tolerance or insulin secretion is controversial and remains unproven (Mooradian & Morley, 1987). It has been suggested that the insulin-like effects of Zn in adipocytes involve the ability of Zn to modulate peroxide generation (May & Contoreggi, 1982), but any effects of Zn on insulin secretion are biphasic with higher concentrations impairing insulin secretion (Mooradian & Morley, 1987). It is probable that the latter arises from the well known antagonistic effect of Zn supplements on Cu status.

Lower tissue levels of Zn have been reported in genetically obese (*db/db* and *ob/ob*) mice and (SHR-corpulent and obese Zucker) rats (Failla & Micahelis, 1984; Kennedy *et al.* 1986; Donaldson *et al.* 1987). However, at least for the *db/db* mouse, when results were expressed on an ash weight basis Zn levels were found to be normal and the low femur Zn concentrations seen in *db/db* mice appear to be primarily due to a generalized decrease in their bone mineral content (Donaldson *et al.* 1988). Chemically-induced diabetes also has little effect on tissue Zn concentrations (Levine *et al.* 1983).

Continuous intravenous infusion of glucose over 7 d in dogs greatly increased urinary Zn, and hyperzincuria is a consistent observation among NIDDM (Kinlaw *et al.* 1983)

and IDDM (Sjogren *et al.* 1986) patients. In the study of Sjogren *et al.* (1986) hyperzincuria was accompanied by large decreases in plasma Zn levels. Yet the level of Zn in muscle and erythrocytes was unaltered. As with Cu, plasma Zn is a poor indicator of Zn status and the low plasma levels of IDDM patients are probably explained by increased inflammatory stress in these patients. It is possible that hyperzincuria reflects the higher turnover of lean body tissue in diabetes mellitus.

MANGANESE

Mn is an essential component of the antioxidant enzyme Mn-SOD found in cell mitochondria (Fig. 2). Activity of this enzyme is influenced by Mn status (Paynter, 1980). Other metalloenzymes containing Mn are the gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (*EC* 4.1.1.32), and pyruvate carboxylase (*EC* 6.4.1.1).

Mn deficiency in second-generation rats results in impaired gluconeogenesis in the neonatal period, abnormal glucose tolerance, low plasma insulin levels, low insulin output from the pancreas and depressed insulin synthesis (Baly *et al.* 1985). Acute Mn toxicity can also affect glucose homeostasis in the rat (Keen *et al.* 1985). The latter workers observed a rise in plasma glucose and glucagon and a decrease in plasma insulin concentration. These findings complement earlier work where Mn deficiency in guinea-pigs can cause impaired glucose utilization which can be reversed by Mn supplementation (Everson & Shrader, 1968) and intra-uterine Mn deficiency results in atrophy of islet cells (Shrader & Everson, 1968).

A number of studies have shown greatly elevated hepatic Mn levels in rats with STZ-induced diabetes (Failla & Kiser, 1981; Bond *et al.* 1983) and excessive accumulation of Mn was found in fetuses of diabetic rat dams (Eriksson, 1984; Uriu-Hare *et al.* 1985). Elevated activities of arginase (*EC* 3.5.3.1), a Mn-dependent enzyme, was also found (Bond *et al.* 1983) and this may explain the increased rates of hepatic amino acid metabolism and urea synthesis which characterize insulin deficiency. These observations of increased Mn status in animal models of diabetes are also consistent with the findings of Loven *et al.* (1982, 1983) who reported increased tissue Mn-SOD activity in experimental diabetes. Results of blood analyses of diabetic patients are inconsistent and both low and high Mn levels have been reported (see Mooradian & Morley, 1987).

SELENIUM AND VITAMIN E

The selenoenzyme GSH-Px protects against tissue damage caused by both hydrogen peroxide and lipid peroxides (Fig. 2). In the latter chain-breaking role the Se-dependent enzyme has a close metabolic relationship with the major lipid-soluble antioxidant, vitamin E.

The activity of GSH-Px is increased in rat erythrocytes and kidney in both alloxan- and STZ-induced diabetes but, as with the other antioxidant enzymes, observed changes were *inter alia* tissue dependent (Dohi *et al.* 1988; Godin *et al.* 1988). Changes in activities of the non-Se-dependent glutathione-S-transferases (*EC* 2.5.1.18; GST) have also been reported (Murray & Zalunzy, 1989) in experimental diabetes. In human studies there appears to be no consistent changes in blood GSH-Px activity in either IDDM or NIDDM (see Oberley, 1988).

Although much work has centred on GSH and the GSH-requiring enzymes in diabetes, there is little information on Se status. There is one report of a higher mean serum Se level in IDDM children compared with healthy controls (Gebre-Medhin *et al.* 1984). Further evidence for disturbances of Se metabolism in diabetes is provided by Dohi *et al.* (1988) who found increased serum Se levels in STZ-induced diabetic rats.

Asayama *et al.* (1986) studied the effect of Se deficiency and vitamin E deficiency on insulin secretory reserve and free-radical-scavenging systems in pancreatic islet cells. Glucose intolerance developed only in those rats with the combined Se and vitamin E deficiencies. Apart from the expected decrease in GSH-Px activities in various tissues with Se deficiency, Mn-SOD concentrations in islets were significantly lower than control levels in response to vitamin E or Se deficiencies. The combined deficiency appeared to have an additive effect on Mn-SOD levels and it was suggested that decreases in Mn-SOD reflected decreased mitochondrial activity. These workers also confirmed the earlier observations of Grankvist *et al.* (1981) and Malaisse *et al.* (1982) on the low antioxidant status of pancreatic islet cells compared with other tissues. Levels of Cu,Zn-SOD and total SOD and especially GSH-Px in islets were the lowest among studied tissues.

A combined deficiency of vitamin E and Se has also been shown to enhance the sensitivity of rats to STZ-induced diabetes (Slonim *et al.* 1983). Vitamin supplementation can protect rats from the diabetogenic action of alloxan and STZ (Slonim *et al.* 1983) and can decrease the incidence of diabetes in the spontaneously diabetic BB rats (Behrens *et al.* 1986). Platelet (Karpen *et al.* 1982) and hepatic (Higuichi, 1982) vitamin E levels are known to be depressed in STZ-induced diabetes. Furthermore diabetes-prone BB rats before the onset of diabetes have lower levels of vitamin E in adrenal gland, thymus and pancreas but not in plasma and other tissues compared with control BB rats (Behrens & Madere, 1991). Work from the latter laboratory has indicated increased accumulation of tocopherols in serum and some other tissues from the diabetes-prone BB rats after the onset of diabetes (Behrens *et al.* 1984).

Some clinical studies have shown higher vitamin E concentrations in plasma platelets and adipose tissue of diabetic patients compared with normal controls (see Mooradian & Morley, 1987). Vandewoude *et al.* (1987), however, have argued that apparent increases in plasma vitamin E may result from altered plasma lipid transport capacity in diabetes. They found no differences in lipid-standardized plasma vitamin E (vitamin E:cholesterol ratio) levels among IDDM or NIDDM patients compared with respective age- and sex-matched controls.

Not all studies have shown increased platelet vitamin E levels in clinical diabetes. Karpen *et al.* (1984) and Watanabe *et al.* (1984) have found evidence for vitamin E-deficient platelets in diabetic patients. Thus, Gisinger *et al.* (1990) have proposed the therapeutic potential of vitamin E to alter platelet function in diabetes. Reports of studies to test the efficacy of vitamin E indicate that platelet activity and eicosanoid production can be normalized by vitamin E supplementation in experimental diabetes (Karpen *et al.* 1982; Gilbert *et al.* 1983) and in diabetic patients (Collette *et al.* 1988; Gisinger *et al.* 1988). A recent study has also demonstrated that vitamin E administration may decrease oxidative protein glycosylation in diabetic subjects independently of changes in plasma glucose (Ceriello *et al.* 1991). Increased non-enzymic protein glycosylation is another abnormality which occurs in diabetes and which may be involved in the long-term tissue complications of diabetes (Kennedy & Baynes, 1984).

ASCORBIC ACID

Ascorbic acid (AA) is a key component of antioxidant defence (Fig. 2) and is consumed most rapidly under oxidative stress (Niki *et al.* 1988). There is a well-established synergistic relationship between AA and vitamin E in the inhibition of lipid peroxidation in in vitro systems (Niki, 1987). The vitamin C (ascorbyl) radical formed by the reduction of vitamin E radical may be reduced back to AA by an NADH-dependent system. The ascorbyl radical is an intermediate in the reversible two-step oxidation process of AA to form dehydroascorbate (DHAA) and the latter oxidation product can be reduced back to AA with GSH and GR (Niki, 1991).

Numerous studies have suggested major disturbances of vitamin C metabolism in experimentally-induced diabetes (Zebrowski & Bhatnagar, 1979; Yew, 1983), diabetes-prone rats (Behrens & Madere, 1991) and in diabetic patients (Yue *et al.* 1990; Cunningham *et al.* 1991; Sinclair *et al.* 1991). In general there seems to be an impaired tissue AA storage in both NIDDM (Chen *et al.* 1983) and IDDM (Cunningham *et al.* 1991), although not all are in agreement (Schorah *et al.* 1988). In the study of Cunningham *et al.* (1991) after allowing for differences in dietary vitamin C intake, there was a mean storage AA deficit in mononuclear leucocytes (an indicator of tissue vitamin C status) of 50% in adults with IDDM compared with non-diabetics. There also appear to be increased serum DHAA:AA ratios and decreased serum AA in NIDDM patients compared with age-matched controls (Sinclair *et al.* 1991). These findings suggest that there is an increased oxidative stress and that vitamin C uptake into tissues is impaired in diabetes mellitus. In vitro studies have shown that active transport of both AA and DHAA appear to be decreased by hyperglycaemia and insulin deficiency; DHAA and AA competing for uptake with glucose (Padh *et al.* 1985; Cunningham, 1988). Inhibition of uptake and, thus, recycling of ascorbyl radical and DHAA by NADH and GSH systems, may lead to increased formation of diketogluconic acid, a breakdown product of AA. Tolrestat, an aldose reductase inhibitor (Fig. 1), has been shown to elevate plasma AA in diabetic rats (Yue *et al.* 1989), while AA supplementation of diabetic patients lowered erythrocyte sorbitol levels (Vinson *et al.* 1989). Mechanisms for these effects are unknown at present but it is possible that there is an intimate relationship between the antioxidant GSH systems and vitamin C metabolism. Vitamin C has also been implicated in long-term complications of diabetes through effects of AA deficiency on collagen abnormalities (McLennan *et al.* 1988), immune function (Pecoraro & Chen, 1987) and platelet activation (Sarji *et al.* 1979).

CONCLUSIONS

There is now strong evidence for a role for ROS in the development of experimentally-induced diabetes and support for a disturbed oxidant–antioxidant balance as a predisposing factor in animal models is growing. Disturbances of micronutrient status may be a predisposing factor but how these observations in animal models relate to the aetiology of IDDM or NIDDM is presently unclear.

There is also some evidence for a role for ROS in diabetic complications in animal models and in diabetic patients. Whatever the precise role of oxidant damage in diabetes, a substantial body of evidence has accumulated to indicate major disturbances of antioxidant and micronutrient status in the diabetic state.

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