

CONSEQUENCES OF THE DIABETIC STATUS ON THE OXIDANT/ANTIOXIDANT BALANCE

D. BONNEFONT-ROUSSELOT (1, 2), J.P. BASTARD (3), M.C. JAUDON (1), J. DELATTRE (2)


SUMMARY - It has been shown that elevated extra- and intra-cellular glucose concentrations result in an oxidative stress, which is defined as an imbalance between prooxidants and antioxidants. Several mechanisms seem to be involved in the genesis of this oxidative stress, which has been reported both in experimental diabetes in animals and in type 1 and type 2 diabetic patients: glucose autoxidation, protein glycation and formation of advanced glycation endproducts, and the polyol pathway. Reciprocally, oxidative stress is involved in the origin of type 1 diabetes, especially via the apoptosis of pancreatic beta-cells, as well as insulin resistance in type 2 diabetes. Glucose control plays an important role in the prooxidant/antioxidant balance. Macromolecules such as molecules of extracellular matrix, lipoproteins and deoxyribonucleic acid are also damaged by free radicals in diabetes mellitus. A supplementation with antioxidants has been proposed as a complementary treatment, and some antidiabetic agents may by themselves have antioxidant properties independently of their role on glucose control. The aim of this paper was to review the consequences of the diabetic status on the oxidant/antioxidant balance.

Key-words: diabetes, glycation, lipid peroxidation, oxidative stress, antioxidants.

RÉSUMÉ - Conséquences de l'état diabétique sur la balance oxydants/anti-oxydants.

Des concentrations élevées de glucose extra- ou intra-cellulaires induisent un stress oxydant, défini comme un déséquilibre entre pro-oxydants et anti-oxydants. Plusieurs mécanismes semblent impliqués dans la genèse de ce stress oxydant, comme il a pu être montré dans le diabète expérimental chez l'animal et dans les diabètes de type 1 et de type 2 chez les patients: autoxydation du glucose, glycation des protéines et formation des produits de glycation avancés, et voie des polyols. Inversement, le stress oxydant peut être à l'origine du diabète de type 1, en particulier par un mécanisme d'apoptose des cellules bêta pancréatiques, ou de l'insulinorésistance dans le diabète de type 2. L'équilibre glycémique joue un rôle très important dans la balance prooxydant/antioxydant. Les macromolécules telles que les molécules de la matrice extracellulaire, les lipoprotéines et l'acide desoxyribonucléique sont aussi les cibles des radicaux libres dans le diabète sucré. Une supplémentation par des anti-oxydants a été proposée comme traitement complémentaire, et certains médicaments antidiabétiques peuvent avoir par eux-mêmes des propriétés anti-oxydantes indépendamment de leur rôle sur l'équilibre glycémique. Le but de cet article est de passer en revue les conséquences de l'état diabétique sur la balance oxydants/anti-oxydants.

Mots-clés : diabète, glycation, peroxydation lipidique, stress oxydant, antioxydants.

 : D. Bonnefont-Rousselot, Laboratoire de Biochimie, Hôpital de la Salpêtrière, 47 boulevard de l'Hôpital, 75651 Paris Cedex 13, France.
E-mail: dominique.rousselot@psl.ap-hop-paris.fr

Received : April 3, 2000

(1) Laboratoire de Biochimie, Hôpital de la Salpêtrière, 47 boulevard de l'Hôpital, 75651 Paris Cedex 13, France.

(2) Laboratoire de Biochimie Métabolique et Clinique, UFR des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75270 Paris Cedex 06, France.

(3) Laboratoire de Biochimie, Hôpital Tenon, 4 rue de Chine, 75020 Paris, France.

Oxidative stress is caused by a relative overload of oxidants, i.e., reactive oxygen species. This impairs cellular functions and contributes to the pathophysiology of many diseases. Evidence has accumulated suggesting that diabetic patients are under oxidative stress and that complications of diabetes seem to be partially mediated by oxidative stress [1-3]. Thus, it has been shown that oxygenated free radicals are able to alter vascular function and to disturb cellular homeostasis, especially by inhibiting synthesis and action of nitric oxide (NO[•]) and by activating NFκB in endothelial cells. Moreover, tight interrelations have been demonstrated between oxygenated free radicals and advanced protein glycation, since advanced glycation endproducts (AGEs) are themselves able to produce free radicals and to be involved in diabetes complications. Indeed, recent evidence supports the fact that AGEs induce endothelial dysfunction via a receptor specific pathway [4]. Free radicals and AGEs are thus likely to be involved in some complications of diabetes, especially in the pathogenesis of diabetic nephropathy. Recent investigation demonstrated that glycoxidation products (N-(carboxymethyl)lysine, pentosidine), a subclass of AGEs which requires both glycation and oxidative stress for their formation, accumulate in expanded mesangial and nodular lesions in diabetic nephropathy [5-8]. The diabetic retinopathy risk also correlates with intracellular concentrations of the glycoxidation product N-(carboxymethyl)lysine [9]. After a general survey of the interrelations between elevated glucose concentrations and oxidative stress, this review will deal with the involvement of oxidative stress in the origin of diabetes, the oxidation of macromolecules and the markers of oxidative stress, and finally the antioxidant treatments in diabetes.

■ ELEVATED GLUCOSE CONCENTRATIONS AND OXIDATIVE STRESS

Elevated extra- and intra-cellular glucose concentrations result in an oxidative stress [10]. A relationship between glucose concentration and oxidative stress has been shown in cultured cells. Thus, endothelial cells from bovine aorta incubated with 30 mM glucose lead to an increased production of oxygenated free radicals and an enhanced concentration of thiobarbituric acid-reactive substances (TBARS) in cells [11]. Moreover, the increased free radical production is associated with a concomitant increase in intracellular AGE formation [11]. Antioxidants such as α-tocopherol, desferroxamine or dimethylsulfoxide inhibit both production of free radicals and AGE formation [11]. In addition, high glucose concentrations can lead to an enhancement of both activity and

mRNA of antioxidant enzymes (Cu, Zn-superoxide dismutase, catalase, glutathione peroxidase) [12]. This overexpression of antioxidant enzymes could constitute a response to a glucose-induced oxidative stress. Several mechanisms seem to be involved in the development of an oxidative stress in the presence of elevated glucose concentrations, namely glucose autooxidation, protein glycation, AGE formation and the polyol pathway (Fig. 1).

Glucose autooxidation – In the presence of transition metals, glucose leads to an ene-diol radical anion; this latter radical reduces molecular oxygen, resulting in the formation of superoxide anions (O₂^{•-}), with a concomitant production of a carbonyl compound. Superoxide anion can disproportionate into hydrogen peroxide, which in the presence of transition metals produces extremely reactive hydroxyl radicals (•OH) [13, 14].

Protein glycation and advanced glycation endproducts (AGEs) – Protein glycation results from the formation of a covalent binding between the aldehydic glucose function and the free amino groups of proteins. In the presence of transition metals (such as copper, iron), glycated proteins can give an electron to the molecular oxygen, leading to oxygenated free radicals. This property has been shown for the first time by Gillery *et al.* [15] and has been further confirmed by others [16], even in the absence of transition metal ions [17, 18]. When the protein half-life is longer than ten weeks, glycated proteins undergo irreversible modifications leading to Maillard products or AGEs [19, 20]. As glycated proteins, AGEs are also able to produce oxygenated free radicals via complex biochemical mechanisms [21]. Yan *et al.* [22] showed that interaction of AGEs with endothelial cells leads to an oxidative stress by a receptor-mediated process. Wautier *et al.* [4] demonstrated that AGEs on the surface of diabetic erythrocytes bind to the vessel wall via a specific receptor, inducing an oxidative stress in

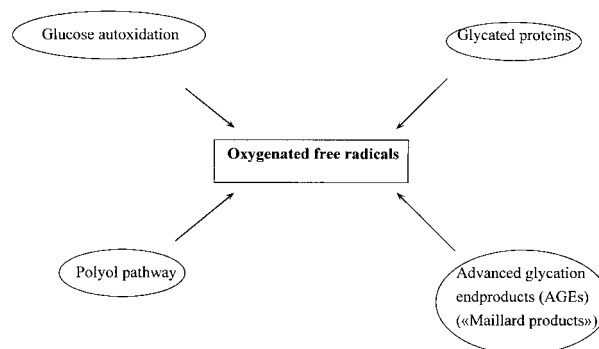


FIG. 1. Mechanisms involved in glucose-induced oxidative stress.

the endothelium. This generation of intracellular oxidative stress results in the activation of the transcription factor NF κ B which translocates into the nucleus and induces the expression of genes regulated by NF κ B [23]. Since these original observations, several interrelations have been shown between oxidative stress and AGEs. Thus, reactive oxygen species can in turn accelerate the AGE formation. New definitions rose, such as glycooxidation which has been proposed by Baynes [24]. Glycooxidation refers to AGE formation through an oxidative pathway. During these complex reactions, protein modification is generally due to compounds with a carbonyl or a dicarbonyl function. Thus, a "carbonyl stress" hypothesis has been proposed [5, 24, 25]. Among these carbonyl compounds, N ϵ -(carboxymethyl)lysine (CML) in proteins could bind redox active transition metal ions such as Cu²⁺, which could facilitate free radical production [26]. CML accumulation may thus result in a deleterious vicious cycle since CML formation itself is catalyzed by lipoxidation and glycooxidation [26]. Increased levels of CML have been observed in serum from children and adolescents with type 1 diabetes, and interestingly this increase preceded the development of micro- and macrovascular complications [27]. Among the intermediates occurring in the cross-linking of proteins by glucose, a special attention should be paid to the alpha-dicarbonyl compound called methylglyoxal [28, 29]. This compound, which is formed in early glycation, is a precursor of advanced glycation adducts. Short periods of hyperglycemia (as observed in impaired glucose tolerance) may be sufficient to increase methylglyoxal concentration *in vivo* [30]. Methylglyoxal can react with collagen, thereby interfering with crucial cell-matrix interactions, especially via the loss of specific arginine residues [31] involved in integrin-mediated attachment [32]. Methylglyoxal is physiologically detoxified by the cytosolic glutathione-dependent glyoxalase system [33]. Aberrations in the expression of the human glyoxalase have been reported in diabetes [34]. Impairment of the detoxification via glyoxalase contributes to cytotoxicity and to chronic pathogenesis associated with diabetes mellitus [35]. It is noteworthy that degradation of oxidized proteins can also be impaired in diabetes, as shown by Portero-Otin *et al.* [35] in the cytosol of kidney and liver of rats with streptozotocin-induced diabetes. Nevertheless, a serine protease that preferentially degrades oxidized and glycated proteins has been characterized in human erythrocyte cytosol [36]. This enzyme is adherent to oxidized membranes and is responsible for degradation of proteins modified by oxidation and glycation [36].

The polyol pathway – Several studies have shown that glucose at abnormally high intracellular concentration is preferentially metabolized via the polyol pathway. Glucose is reduced by the aldose reductase into sorbitol which is oxidized to fructose by the sorbitol dehy-

drogenase. NADPH is required for the activity of aldose reductase. Therefore, an enhancement of the polyol pathway results in an intracellular depletion of NADPH [38]. Antioxidant enzymes such as glutathione reductase which regenerates reduced glutathione need NADPH. Thus, an intracellular depletion of this cofactor, by decreasing the activity of glutathione reductase, decreases the intracellular content of reduced glutathione, which constitutes an important factor for the protection towards oxygenated free radical-induced damages [39, 40]. Intracellular depletion of NADPH also leads to another deleterious effect, that is a decreased NO[•] synthesis, since NADPH is cofactor of the NO-synthase which synthesizes NO[•] from L-arginine. Moreover, NO[•] metabolism can also be altered by an abnormal production of superoxide anions, which is a consequence of an elevated intracellular glucose concentration. Superoxide anions are involved in the physiological inactivation of NO[•]; they react with NO[•] to form peroxynitrite ([•]OONO) which is a potential oxidizing agent due to its decomposition into nitrogen dioxide (NO₂) and [•]OH [39]. It has also been hypothesized that proteins such as collagen, which contain AGEs, could scavenge NO[•] in endothelium and thus limit its diffusion into smooth muscle cells [41]. An elevated intracellular glucose concentration could thus result in several abnormalities of the NO[•] metabolism, which could be involved in some vascular complications of diabetes.

■ ROLE OF OXIDATIVE STRESS IN THE ORIGIN OF TYPE 1 DIABETES OR OF INSULIN RESISTANCE IN TYPE 2 DIABETES

Nitric oxide has been suggested to be involved in autoimmune pancreatic beta-cell destruction in insulin-dependent diabetes mellitus [42, 43]. Apoptosis of pancreatic beta-cells could also result from the effects of aldose reductase (role of the polyol pathway in the redox imbalance), as shown in a pancreatic beta-cell line transfected with rat aldose reductase cDNA [44]. In contrast, thioredoxin, a redox-active protein, has been shown to protect cells from oxidative stress and apoptosis, and lead to a lower incidence of diabetes in nonobese diabetic transgenic mice that overexpress thioredoxin [45]. Insulin resistance may also be associated with an intracellular production of free radicals, which in turn could be responsible for a deterioration of insulin action, thus leading to a vicious circle [46]. Thus, 3T3-L1 adipocytes exposed to low micromolar hydrogen peroxide concentrations display impaired insulin-stimulated GLUT4 translocation from internal membrane pools to the plasma membrane [47]. The mechanism involved would be an impairment of the cellular redistribution of the normal insulin-stimulated insulin receptor substrate-1 and

phosphatidylinositol 3-kinase between the cytosol and the internal membrane [48]. Hydrogen peroxide at low concentrations would be a potent inhibitor of insulin signaling and thus may be involved in the development of insulin resistance [49].

■ OXIDATION OF MACROMOLECULES IN DIABETES

Oxidation of extracellular matrix molecules – Extracellular matrix plays a key role in many physiological functions and its abnormalities could be involved in the pathogenesis of diabetic complications. Indeed, the glomerular filtration barrier consists of matrix components including collagen and noncollagen molecules such as laminin and proteoglycans. An increased permeability of the filtration barrier in diabetes may be attributed to an alteration of matrix molecules [50, 51]. Involvement of glycation and oxidation processes is highly suggested in modifications of extracellular matrix molecules in diabetes. Thus, matrix glycation interferes with normal cell-matrix interactions and intracellular signaling that can potentially result in differential gene expression contributing to the changes seen in diabetic nephropathy [52]. In addition, glycation of extracellular matrix components may contribute to altered interaction of human mononuclear cells with collagen I, as shown *in vitro* by Menon *et al.* [53] who reported in this interaction the involvement of specific, high affinity cell surface molecules. Moreover, as described above, glucose under oxidative conditions can react with proteins to form AGEs. AGE-modified proteins bind to cell surface receptors and other AGE binding proteins whose several types have been identified [54]. Cell activation in response to AGE-modified proteins is associated with increased expression of extracellular matrix proteins, vascular adhesion molecules, cytokines and growth factors [54]. In diabetic rats, the presence of AGEs has been shown by immunocytochemistry in the renal extracellular matrix, and could thereby participate in the pathogenesis of renal complications [55]. It has been hypothesized that AGEs could induce crosslinking of collagen, as recently shown *in vitro* [56]. The extent of AGE-induced crosslinking is dependent on the duration of incubation with AGEs and is not prevented by radical scavengers or EDTA, whereas glucose-induced crosslinking of collagen is prevented by radical scavengers. This shows that AGEs can directly induce crosslinking of collagen, by a pathway that involves an amino-carbonyl interaction and that is independent of oxidative conditions [56]. Another factor which plays a role in extracellular matrix regulation is the metabolism of ascorbic acid, which is known to be abnormal in diabetes. Indeed, ascorbic acid has a stimulatory effect on the sulphate

incorporation into cell and matrix proteoglycans, and glucose is able to inhibit this action [57]. Thus this would be of particular importance in the physiopathology of diabetes. In addition, modification of the production of extracellular matrix components occurs in diabetes. Accumulation of type IV collagen and laminin has thus been observed in the mesangium of the glomerule within two years from the onset of diabetes in a model of insulin-dependent diabetes in baboons [58]. This results in a thickening of basement membrane and enlargement of the mesangium. Among the factors which are involved in the regulation of the production of extracellular matrix molecules, transforming growth factor- β (TGF- β) could play an especially important role [59]. Indeed, high glucose concentration increases TGF- β mRNA and protein level in cultured proximal tubular cells and glomerular epithelial and mesangial cells, whereas neutralizing anti-TGF- β antibodies prevents the stimulation of matrix synthesis in renal cells [59]. In a similar way, treatment of streptozotocin-diabetic mice with neutralizing monoclonal antibodies directed against TGF- β reduces glomerular hypertrophy and the increase in extracellular matrix mRNA levels [59].

Oxidation of lipoproteins – Diabetic patients have an increased risk of cardiovascular disease (2- to 4-fold as high as non diabetic subjects). Given the oxidative theory of atherosclerosis, the question of an increased *in vitro* oxidizability of low density lipoproteins (LDLs) in diabetic patients has been addressed. Data related to this oxidizability show some discrepancies. Thus, an increased susceptibility of LDLs to *in vitro* oxidation has been reported, both in type 1 and type 2 diabetes [60-62]. However, others have not confirmed this observation and showed either a similar [63, 64] or even a decreased [65] oxidizability of LDLs isolated from diabetic patients. Such discrepancies could also be due to the heterogeneity of diabetic populations. These studies should take into account the glycemic control and the vascular complications. Jenkins *et al.* [64] have evaluated the LDL oxidizability of 15 diabetic patients whose type 1 diabetes was well controlled and who did not have any vascular complications. No change of the oxidizability of their LDL has been observed, in comparison with LDL from normoglycemic subjects. An increased oxidizability of LDLs could appear associated with a severe hyperglycemia or vascular complications [66]. Thus, the influence of the severity of the hyperglycemia on the hyperoxidizability of LDLs and very low density lipoproteins (VLDLs) has been recently confirmed in diabetic subjects with frequent hyperketonemia [67]. Thus glycemic control is very important in order to avoid an increased susceptibility of LDLs to oxidation in diabetic patients [68, 69]. It has been reported that the amount of partially oxidized LDLs in plasma was significantly correlated with insulin resistance and its metabolic consequences [70]. Several studies showed

that glycation of apolipoproteins, especially of apolipoprotein B, was enhanced in diabetic subjects. Since glycation and lipoxidation are two tightly related phenomena, the question whether glycation could influence the susceptibility to oxidation has been addressed. *In vitro* incubation of LDLs with glucose leads to an increased oxidizability of these LDLs [71, 72]. However, no relationship between glycation and LDL oxidation has been demonstrated *in vivo* in diabetic patients [73]. Nevertheless, it has been shown in non-human primates that diabetes-induced glycation of LDLs increases their binding to arterial proteoglycans compared to LDLs of non-diabetic animals [74]. This may result in an increased retention of LDLs in the arterial intima and thus be one mechanism for increased atherogenicity of diabetic LDLs. Moreover, LDL oxidation generates new epitopes and leads to the formation of antibodies raised against oxidized LDLs. The presence of anti-oxidized LDLs antibodies in plasma has been proposed as a predictive factor for the progression of carotid atherosclerosis. Such antibodies have been found both in plasma and in atherosclerotic plaques of diabetic patients [75]. It has been reported that serum of type 1 diabetes patients contained both antibodies to oxidized LDLs and antigen-antibody complexes (oxidized LDL-containing immune complexes) [76]. Presence of those complexes may be a risk factor for the development of macrovascular disease in these patients. In addition, circulating oxidized LDL-containing immune complexes seem to interfere with the assay of free oxidized LDL antibodies [76]. An enhancement of the plasma concentration of antibodies raised against oxidized LDLs has also been observed in type 2 diabetic subjects [77].

Oxidation of deoxyribonucleic acid (DNA) – As other biomolecules (proteins, lipoproteins...), DNA exhibits free radical-induced damages. In animals as well as in humans, aging results in an increased content of oxidized bases in DNA [78]. The same observation has been reported in diabetic patients, by using two separated methodologies. The first one consists in measuring the 8-hydroxydeoxyguanosine (8OHdG) content in lymphocytes from type 1 and type 2 diabetic patients by high performance liquid chromatography (HPLC). 8OHdG is a marker of DNA oxidation. It has been shown that the 8OHdG is markedly enhanced in the DNA of both diabetic groups as compared to controls. This is paralleled by an increased production of oxygenated free radicals in these lymphocytes stimulated by N-formylmethionylleucylphenylalanine [79]. The second approach was the Comet assay, which allows the determination of DNA breakages. It has been shown that these damages were more frequent in a diabetic population than in a control normoglycemic population [80]. It is noteworthy that high glucose concentrations can also induce mutations in mitochondrial DNA *in vitro* [81].

■ OXIDATIVE STRESS MARKERS AND DIABETES

Three kinds of oxidative stress markers are classically used to evaluate an oxidative stress. They are markers of lipid peroxidation, plasma total antioxidant status and specific antioxidant defense systems (*Table I*).

Markers of lipid peroxidation – Free radical measurement is difficult given their high reactivity, their very short half-life and their low concentration. Therefore, indirect markers are commonly used to evaluate secondary products of lipid peroxidation such as TBARS, hydroperoxides and F₂-isoprostanes. TBARS assay is easy to perform and usually used but it lacks specificity [82]. Most studies report elevated plasma TBARS levels in type 1 or type 2 diabetic patients compared to a normoglycemic population [83, 84]. The question was addressed whether the diabetes type, the glycemic balance or the presence of cardiovascular complications could modulate the TBARS level. Griesmacher *et al.* [84] showed that type 2 diabetic patients exhibited significantly higher plasma TBARS levels than type 1 diabetic patients. Diabetic subjects with a good glycemic control (HbA1c < 6.5%) have plasma TBARS concentrations lower than those with a worse control (HbA1c > 6.5%). In type 1 diabetic patients, plasma TBARS concentrations are higher in patients with vascular complications [85]. Finally, it has been reported that type 2 diabetic patients with angiopathy exhibited higher TBARS values than those without angiopathy [84]. An enhanced lipid peroxidation has also been shown in erythrocyte and leukocyte membranes of diabetic patients [86, 87]. Another marker of lipid peroxidation, namely hydroperoxides, exhibits elevated values in both types of diabetes [88, 89]. F₂-isoprostanes have recently been proposed as markers of lipid peroxidation in diabetic patients. They consist of a series of prostaglandin F₂-like compounds formed during peroxidation of arachidonic acid by a mechanism independent of the cyclooxygenase pathway [90]. Plasma levels of one of them, the 8-epi-PGF₂α, are increased in subjects with non-insulin dependent diabetes mellitus [91]. Plasma levels of 8-epi-PGF₂α are also elevated approximately 5-fold in obese Zucker rats (a model of insulin resistance) relatively to age-matched, insulin-sensitive lean Zucker rats, and supplementation with vitamin E reduced 8-epi-PGF₂α levels and reversed glucose-stimulated hyperinsulinemia in the obese Zucker rats [92]. Urinary levels of 8-epi-PGF₂α are higher in type 1 and type 2 diabetic patients than in control subjects, and they are lowered by vitamin E supplementation [93].

Plasma total antioxidant status – A lot of compounds in plasma exhibit an antioxidant effect. They are present at different concentrations. They can be divided into three groups:

TABLE I. Some markers of oxidative stress in diabetes.

Marker	Variation (medium)	Type of diabetes	Reference
TBARS	↑ (plasma)	Type 2	Gallou et al. [83]
	↑ (plasma)	Type 1	Greismacher et al. [84]
	↑ (plasma)	Type 1	Ruiz et al. [85]
	↑ (erythrocytes)	Type 2	Vijayalingam et al. [86]
	↑ (leukocytes)	Type 2	Akkus et al. [87]
Hydroperoxides	↑ (plasma)	Type 2	Nourooz-Zadeh et al. [89]
	↑ (plasma)	Type 1	Santini et al. [88]
8-epi-PGF ₂ α	↑ (plasma)	Type 2	Gopaul et al. [91]
Total antioxidant status	↓ (plasma)	Type 1	Tsai et al. [96]
	↓ (plasma)	Type 2	Ceriello et al. [97]
	↓ (plasma)	Types 1 and 2	Maxwell et al. [98]
	↓ (plasma)	Type 2	Opara et al. [99]
Vitamin E	↑ (plasma)	Type 1	Tsai et al. [96]
	→ (plasma)	Type 2	Ceriello et al. [97]
	→ (plasma)	Types 1 and 2	Maxwell et al. [98]
	↓ (platelets)	Type 1	Kunisaki et al. [105]
	→ (platelets)	Types 1 and 2	Caye-Vaugien et al. [106]
Vitamin C	→ (plasma)	Type 1	Tsai et al. [96]
	→ (plasma)	Type 2	Ceriello et al. [97]
	↓ (plasma)	Types 1 and 2	Maxwell et al. [98]

TBARS: thiobarbituric acid-reactive substances. 8-epi-PGF₂α: 8-epi-prostaglandin F₂α. ↑: increased; → unchanged; ↓ decreased.

– metal chelators, such as transferrine and ceruleo-plasmine, which inhibit the initiation phase of oxygenated free radical production;

– free radical scavengers, such as vitamin E, vitamin C, reduced glutathione, uric acid, bilirubin, serumalbumin, which act on the propagation phase of lipid peroxidation;

– antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase. Nevertheless, these enzymes exhibit a poor activity in plasma.

Methods have been developed to measure the total antioxidant status or the total peroxy radical trapping antioxidant parameter in plasma [94, 95]. Most studies conducted in type 1 or type 2 diabetic patients showed a significant decrease of the plasma total antioxidant status [96-99]. This status is lowered during an oral glucose tolerance test in normal and non-insulin-dependent diabetic subjects, so that even an acute

hyperglycemia can induce an oxidative stress [100]. It is noteworthy that one study reported no decrease of plasma antioxidant activity in subjects at increased risk for type 1 diabetes (risk assessed by the presence of type 1 diabetes-associated autoantibodies) [101]. Moreover, the latter study suggested that the clinical onset of diabetes was not preceded by signs of increased systemic oxidative stress evaluated by plasma antioxidant capacity.

Antioxidant defense systems – Among the nonenzymatic antioxidant defense systems, most studies in experimental as well as in human diabetes have focused on vitamin E, liposoluble antioxidant carried by lipoproteins in plasma [102]. In streptozotocin-induced diabetes in rat, an enhanced vitamin E concentration has been found in the plasma, liver and heart [102]. Investigation conducted in diabetic patients led to heterogeneous results. Indeed, unchanged,

elevated or decreased plasma vitamin E concentrations have been reported, independently of the type of diabetes [96, 97, 103, 104]. Such discrepancies could be explained by the fact that the circulating lipid level was not always taken into account to interpret the plasma vitamin E concentration. Heterogeneous results have also been reported in platelets and erythrocytes. Thus, Kunisaki *et al.* [105] reported low vitamin E levels in platelets from type 1 diabetic subjects, whereas Caye-Vaugien *et al.* [106] found unchanged vitamin E concentrations in platelets and erythrocytes of type 1 and type 2 diabetic patients. Vitamin C has also been studied in diabetes. It plays a major role in regenerating vitamin E from the α -tocopheroxyl radical [107]. According to some authors, vitamin C plasma concentrations are unchanged in type 1 and type 2 diabetic patients [96, 97, 103]. In contrast, Asayama *et al.* [108] reported elevated vitamin C levels in type 1 diabetic subjects, whereas others [109-111] described markedly lowered values in diabetes. In fact, a recent study shows that, after adjustment for several important covariates (such as dietary intake of vitamin C, physical activity, number of cigarettes smoked during the 5 days preceding examination), serum vitamin C concentrations do not differ significantly in patients with newly diagnosed diabetes from those in subjects without diabetes [112]. Finally, cellular reduced glutathione level has been suggested to be lowered in animal diabetes models as well as in diabetic patients [113]. In a similar way, intraplatelet content of reduced glutathione is significantly lower in diabetes patients with high glycosylated hemoglobin than in those with low glycosylated hemoglobin [114].

It is noteworthy that, besides these classical antioxidants, metals play also an important role in diabetes. Thus, zinc deficiency has been commonly described [115-120]. Serum zinc deficiency is associated with hyperzincuria. Interestingly, it has been reported that a low groundwater content of zinc may be associated with later development of childhood onset diabetes [121]. Nevertheless, zinc supplementation in non-insulin dependent diabetic subjects has been shown to worsen their glucose intolerance [116] and had no effect on LDL oxidizability [120]. In contrast, zinc supplementation appears to be more efficient in insulin-dependent diabetes, since it corrects zinc deficiency and decreases lipid peroxidation [122]. However, some authors reported no significant difference in plasma zinc levels between diabetic patients and healthy controls [123, 124]. Manganese seems also involved in the oxidative stress status in diabetes. Thus, a manganese deficiency in streptozotocin-induced diabetes rats can lead to markedly decreased activities of manganese-SOD (Mn-SOD) in kidney and heart and of Cu, Zn-SOD in kidney [125]. This is associated with an increased lipid peroxidability of erythrocytes and a depletion of reduced glutathione in liver and kidney [125]. Urinary excretion of manga-

nese is higher in diabetic patients than in control subjects; moreover, diabetic patients who were non treated with insulin or those with liver disorders excreted significantly more manganese than diabetic patients without such disorders [126]. With regard to selenium, it appears that serum or plasma selenium concentrations measured in patients with diabetes mellitus were significantly lower than those determined in healthy subjects [124, 127], whereas urine selenium concentrations were not significantly different between these two groups [127]. A negative correlation between the plasma contents of selenium and glycosylated hemoglobin has even been reported [124]. In addition, reduced selenium concentration in red blood cells of type 1 diabetic patients could contribute to their impaired hemorheology [104]. It is of note that sodium selenate administration resulted in an improvement in glucose tolerance in streptozotocin diabetic rats, associated with a normalization of heart function by contrast with non-treated diabetic rats [128]. Moreover, selenium supplementation (and more efficiently selenium + vitamin E supplementation) could play a beneficial role in experimental diabetes, in controlling oxidative stress, especially in liver [129] and in platelets [130]. With regard to the enzymatic antioxidant defenses, discrepancies again appeared in the results of the studies [102]. Human extracellular superoxide dismutase (EC-SOD) can undergo a glycation, which is associated with a decrease in its affinity for heparin but without affecting its enzymic activity. The primary glycation sites on EC-SOD have been identified as two lysine residues present in the heparin-binding domain in the carboxyterminal end [131]. In diabetes, the proportion of glycosylated EC-SOD is markedly higher than in healthy subjects, so that the proportion of EC-SOD present on the surface of the endothelium (bound to heparan sulphate proteoglycan) may be decreased, enhancing the susceptibility of cells to superoxide radicals produced in the extracellular space [132]. Besides, erythrocyte Cu, Zn-SOD activity has been found unchanged, elevated or decreased in type 1 diabetic patients, whereas it was unchanged or lowered in type 2 diabetic subjects [102]. Increased glycosylated Cu, Zn-SOD has been reported in erythrocytes of patients with type 1 diabetes and this enzyme is less active than the non-glycosylated fraction [133]. Glycation of Cu, Zn-SOD mainly occurred in patients with a poor glycemic control [134]. Glycation of human erythrocyte Cu, Zn-SOD leads to inactivation of the enzyme, due to the glycation of two lysine residues probably located in an active site liganding loop [135, 136]. Inactivation is accompanied by a loss of antigenicity [137]. The glycation reaction further results in site-specific and random fragmentation of human Cu, Zn-SOD [138]. Nevertheless, these observations contrast with those of Strange *et al.* [139] and Ruiz *et al.* [85] who observed an unaltered erythrocyte activity of Cu, Zn-SOD in type 1 diabetes.

Plasma glutathione peroxidase (GSH-Px) activity has been reported to be elevated in both types of diabetes [102, 140]. Erythrocyte GSH-Px activity is unchanged [102, 139], decreased in type 1 diabetes [85, 102, 104] or enhanced in type 2 diabetes [102, 110]. The rise in GSH-Px activity observed for example in diabetic patients with retinopathy may be a compensatory mechanism to prevent tissue damage [110]. This enhanced activity has also been reported in retina pericytes of diabetic patients [141]. Nevertheless, others have found a decreased GSH-Px activity in diabetic patients with retinopathy [142, 143]. The site of glycation of GSH-Px has been identified in the bovine enzyme and is located at approximately 15 Å from the active site selenocysteine [144]. Glycation of GSH-Px seems to be responsible for a decrease in affinity of this enzyme, which could contribute to the hyperaggregability of the diabetic platelet due to the impairment of glutathione peroxidase [114]. Evaluation of the percentage of glycated GSH-Px in platelets can be evaluated by a combination of boronate affinity chromatography and ELISA methodology [145].

Thus, both for nonenzymatic and enzymatic systems, results show a great discrepancy, probably in relation with the heterogeneity of the diabetic populations, the glycemic control, the age of the patients and the possible presence of vascular complications. A special interest should be mentioned for the role of the glycemic equilibrium on the prooxidant/antioxidant balance. Indeed, metabolic disturbances and oxidative stress seem to be tightly related, an improved glycemic control being associated with a lowering of the prooxidant status [146]. For example, in poorly equilibrated insulin-dependent diabetic patients such as ketotic patients, lipid peroxidation is enhanced in plasma, as assessed by malondialdehyde and lipid hydroperoxides [119]. This is associated with low plasma zinc concentrations. After continuous insulin treatment, the patients reach a stable glycemic state and the above parameters in parallel approach reference values, which indicates the beneficial effect of insulin perfusion on the antioxidant/oxidant balance [119]. Nevertheless, total normalization of the parameters of oxidative stress appears not to be reached by glycemic control alone, indicating continued oxidant injury despite optimal control of the diabetes [147].

■ ANTIOXIDANT TREATMENTS AND DIABETES

Given the involvement of oxidative stress in diabetes complications, supplementation with antioxidants could be of interest, by allowing a delay in the appearance or in the development of vascular complications [1]. Some information is available on the effects of treatments with classical antioxidants such as vitamin E, vitamin C or lipoic acid. Treatment of streptozotocin-induced diabetes pregnant rats with vi-

tamin E resulted in a decrease of plasma lipid peroxidation in comparison with untreated animals, associated with higher cell glutathione content and SOD activity, thus suggesting that vitamin E supplementation could in part reduce the imbalance between oxidants and antioxidants [148]. It is noteworthy that vitamin E supplementation in streptozotocin-induced diabetic rats protects against lipid peroxidation and contributes to avoid elevation of plasma glucose levels, whereas vitamin E deficiency leads to increased hepatic TBARS levels in these diabetic rats [148]. A vitamin E supplementation in diabetic patients results in an improvement of the insulin effect and a better glycemic control [147, 150, 151], by reducing glucose, hemoglobin A1c and fructosamin values, by decreasing plasma lipid peroxidation and LDL oxidizability [61, 152]. However, one study reports that vitamin E could deteriorate insulin action and worsen the hypofibrinolysis in obese type 2 diabetic patients [153]. In addition, an antioxidant treatment using vitamin E, vitamin C and N-acetylcysteine in diabetic mice can preserve beta-cell function [154]. Thus, this antioxidant treatment preserves the amounts of insulin content and insulin mRNA, and expression of a beta-cell-specific transcription factor is more clearly visible in the nuclei of islets cells after this treatment. A supplementation with α -tocopherol nicotinate in type 2 diabetic patients with retinopathy resulted in a decreased lipid peroxidation in erythrocyte membranes, with an improvement of the blood rheological properties [155]. Clinical investigation has been carried out to assess the effects of lipoic acid in the treatment of neuropathy in diabetic subjects. A multicenter double blind study in 328 type 2 diabetic patients showed an improvement of some clinical features of neuropathy after a 3-week treatment with α -lipoic acid [156].

Since the advanced protein glycation and oxidative stress could result in a lot of metabolic disorders in diabetes [157, 158], the design of molecules which can inhibit AGE formation and "scavenge" free radicals could be of great interest. Aminoguanidine (also called pimagedine) is a nucleophilic hydrazine which reacts with early protein glycation products, such as Amadori products. It inhibits the formation of AGEs such as pentosidine [159] which lead to intra- and inter-molecular bindings in proteins with long half-life such as collagen [160]. Aminoguanidine and beta-resorcyline aminoguanidine reduce the formation of AGEs, inhibit malondialdehyde formation in erythrocytes incubated with hydrogen peroxide, and thus may have therapeutic potential [161]. *In vivo*, a treatment with aminoguanidine in animals inhibits the development and the progression of the main diabetes complications such as retinopathy, nephropathy and neuropathy [162]. Renal AGE levels, as assessed by fluorescence or by radioimmunoassay in rats, which were increased after three weeks of diabetes compared to nondiabetic control animals, were attenuated by

aminoguanidine therapy [163]. In addition, it has been reported that aminoguanidine, which is structurally related to N-nitro-L-arginine, can prevent experimental diabetes-induced vascular changes which could be mediated by nitric oxide production [164, 165]. More recently, specific antioxidant properties of aminoguanidine have been demonstrated. Philis-Tsimikas *et al.* [166] reported an antioxidant effect of aminoguanidine at high concentrations on the LDL peroxidation initiated by copper ions, which has also been shown by others [167]. A scavenging effect of aminoguanidine towards $\cdot\text{OH}$ and peroxy ($\text{RO}_2\cdot$) radicals has been demonstrated [168, 169]. Endogenous vitamin E and β -carotene have also been shown to be protected by this antioxidant upon oxidation of LDLs by $\cdot\text{OH}/\text{O}_2\cdot^-$ free radicals [170]. When rat retina cells are submitted to an oxidative stress in the presence of aminoguanidine, both oxygenated free radical production and lipid peroxidation product formation are inhibited. Moreover, oxidative stress-induced apoptosis of these cells is abolished [171]. All these effects are dose-dependent. To investigate the *in vivo* effect of aminoguanidine on lipid peroxidation in diabetes, aminoguanidine has been given for nine weeks to streptozotocin-induced diabetic rats and lipid peroxidation has been measured in plasma (lipid hydroperoxides) and red blood cells (membrane malondialdehyde and thiobarbituric acid-reactive substances) [172]. Lipid peroxidation was significantly lower in aminoguanidine-treated rats than in untreated rats. The results of this study thus suggest that aminoguanidine may have an additional beneficial effect as an antioxidant against lipid peroxidation in a prevention trial for diabetic vascular complications. Another experimental study performed in streptozotocin-induced diabetes in rats showed that aminoguanidine was able to decrease erythrocyte malondialdehyde level [173]. If diabetes complications are partly related to oxidative stress and to AGEs, a molecule such as aminoguanidine would thus be of great interest. Separate multicenter clinical trials of aminoguanidine in type 1 and 2 diabetes are in progress to monitor the effect of treatment on the amount of proteinuria, progression of renal insufficiency, and the course of retinopathy [174]. Thus, a randomized, double-blind, placebo-controlled trial comparing two dose levels of aminoguanidine with placebo on the progression of the diabetic nephropathy has been conducted in 599 type 2 diabetic patients with renal disease from 84 centers in the United States and the Canada [175]. Follow-up was to be two years after the date of randomization of the final enrolled patient, so that the trial ended in March 1998. The results of this study would contribute to determine whether aminoguanidine would slow the progression of diabetic renal disease.

Apart from the classical antioxidant treatment, specific antagonists of the AGE receptors are an interesting way of research. To date, hydroimidazolones ap-

pear to be the most likely candidates to pharmacologically inhibit AGE receptor-mediated cell activation and thus to especially prevent vascular complications of diabetes [54]. In addition, a new class of compounds is under development: they are potent inhibitors of glycation and AGE formation, such as aryl (and heterocyclic) ureido, and aryl (and heterocyclic) carboxamido phenoxy isobutyric acids and related molecules [176]. More upstream, novel drugs are able to inhibit the conversion of Amadori compounds (ketoamins resulting from the rearrangement of Schiff bases) to AGEs [177]. These drugs are called "Amadorins" whose first member is pyridoxamin. Its therapeutic potential is currently being investigated in different animal models.

Finally, oral antidiabetic agents themselves can have a potential antioxidant activity. Thus, in high fructose-fed rats (a diet that leads to insulin resistance), metformin is able to improve erythrocyte antioxidant activities (Cu, Zn-SOD, GSH-Px) [178]. It also increases blood glutathione level that is classically low in diabetic animals [178-180], independently of its effect on insulin activity. In addition, metformin is able to prevent collagen glycation in a canine diabetic model [181]. Due to the fact that guanidino compounds can block dicarbonyl groups [182], the effects of the diamino biguanide metformin have been investigated on the methylglyoxal formation in type 2 diabetes. Indeed, metformin reduces the levels of methylglyoxal (above mentioned as a reactive alpha-dicarbonyl thought to contribute to diabetic complications as a precursor of AGEs) [183]. Thus, metformin treatment can prevent diabetic complications not only by lowering plasma glucose, but also by inhibiting AGE formation [184]. Thiazolidinediones are other antidiabetic drugs which are able to possess antioxidant properties. As an example, troglitazone has a combined chemical structure of thiazolidinedione and a-tocopherol-like structure (chroman ring). It has been shown to inhibit galactose-induced cataractogenesis in rat lens culture and lipid peroxide formation associated with this process [185]. In a similar way, troglitazone is able to inhibit Cu^{2+} -induced oxidation of LDLs and the subsequent uptake and degradation of these LDLs by macrophages, by acting as an aqueous-phase antioxidant in addition to its effect on glucose homeostasis [186]. The antioxidant activity of troglitazone seems also involved in the inhibition of the expression of adhesion molecules such as intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin on human umbilical vein endothelial cells (HUVECs) induced for example by oxidized LDLs [187]. This reduction in expression is paralleled by a significant fall in NF- κ B translocation [187]. Similarly, sulfonilureas can exhibit antioxidant activity. Thus, gliclazide decreases LDL oxidation and monocyte adhesion to endothelial cells, suggesting a beneficial effect of this

drug in the prevention of atherosclerosis associated with type 2 diabetes [188]. Treatment with gliclazide also induces a decrease in plasma lipid peroxides and an enhancement of erythrocyte Cu, Zn-SOD activity, this effect resulting from a free radical scavenging activity independent of glycemic control [189].

CONCLUSION

Possible sources of oxidative stress in diabetes include an increased production of radical oxygen species, especially from glycation or lipoxidation processes, and decreased enzymatic or nonenzymatic antioxidant defense systems. Improvement of glycemic control seems to be a beneficial factor to decrease oxidative stress in diabetic patients. Prevention of AGE formation by drugs such as aminoguanidine may help to delay the development of diabetic complications (kidney, eye, blood vessel and nerve damage). Apart from classical antioxidants (vitamin E, vitamin C, acid lipoic) used to decrease oxidative stress, oral antidiabetic agents themselves can exhibit an antioxidant activity independent of their action on glycemic control, which confers them a high therapeutic potential.

REFERENCES

- Hayoz D, Ziegler T, Brunner HR, Ruiz J. Diabetes mellitus and vascular lesions. *Metabolism*, 1998, 47, 16-19.
- Rösen P, Du X, Tschöpe D. Role of oxygen derived radicals for vascular dysfunction in the diabetic heart: prevention with α -tocopherol? *Mol Cell Biochem*, 1998, 188, 103-111.
- Szaleczky E, Prechl J, Feher J, Somogyi A. Alterations in enzymatic antioxidant defence in diabetes mellitus – a rationale approach. *Postgrad Med J*, 1999, 75, 13-17.
- Wautier JL, Wautier MP, Schmidt AM, et al. Advanced glycation end products (AGEs) on the surface of diabetic erythrocytes bind to the vessel via a specific receptor inducing oxidant stress in the vasculature: a link between surface-associated AGEs and diabetic complications. *Proc Natl Acad Sci USA*, 1994, 91, 7742-7746.
- Suzuki D, Miyata T, Saotome N, et al. Immunohistochemical evidence for an increased oxidative stress and carbonyl modification in proteins in diabetic glomerular lesions. *J Am Soc Nephrol*, 1999, 10, 822-832.
- Horie K, Miyata T, Maeda K, et al. Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. *J Clin Invest*, 1997, 100, 2995-3004.
- Schleicher ED, Wagner E, Nerlich AG. Increased accumulation of the glycoxidation product N^ε-(carboxymethyl)lysine in human tissues in diabetes and aging. *J Clin Invest*, 1997, 99, 457-468.
- Suzuki D, Miyata T. Carbonyl stress in the pathogenesis of diabetic nephropathy. *Int Med*, 1999, 38, 309-314.
- Hammes HP, Brownlee M, Lin J, Schleicher E, Bretzel RG. Diabetic retinopathy risk correlates with intracellular concentrations of the glycoxidation product N^ε-(carboxymethyl)lysine independently of glycohaemoglobin concentrations. *Diabetologia*, 1999, 42, 603-607.
- Giugliano D, Ceriello A. Oxidative stress and diabetic vascular complications. *Diabetes Care*, 1996, 19, 257-267.
- Giardino I, Edelstein D, Brownlee M. Bcl-2 expression antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation end products in bovine endothelial cells. *J Clin Invest*, 1996, 97, 1422-1428.
- Ceriello A, dello Russo P, Amstad P, Cerutti P. High glucose induces antioxidant enzymes in human endothelial cells in culture. Evidence linking hyperglycemia and oxidative stress. *Diabetes*, 1996, 45, 471-477.
- Hunt JV, Dean RT, Wolff SP. Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and aging. *Biochem J*, 1988, 256, 205-212.
- Hunt JV, Smith CCT, Wolff SP. Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. *Diabetes*, 1990, 39, 1420-1424.
- Gillery P, Monboisse JC, Maquart FX, Borel JP. Glycation of proteins as a source of superoxide. *Diabetes Metab*, 1988, 14, 25-30.
- Sakurai T, Tsuchiya S. Superoxide production from non-enzymatically glycosylated proteins. *FEBS Lett*, 1988, 236, 406-410.
- Ortwerth BJ, James H, Simpson G, Linetsky M. The generation of superoxide anions in glycation reactions with sugars, osones, and 3-deoxyosones. *Biochem Biophys Res Commun*, 1998, 245, 161-165.
- Mossine VV, Linetsky M, Glinsky GV, Ortwerth BJ, Feather MS. Superoxide free radical generation by Amadori compounds: the role of acyclic forms and metal ions. *Chem Res Toxicol*, 1999, 12, 230-236.
- Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *New Engl J Med*, 1988, 318, 1315-1321.
- Trivin F, Chevenne D, Hauteceuvre M. Produits de Maillard et complications chroniques du diabète sucré. *Ann Biol Clin*, 1999, 57, 445-454.
- Mullarkey CJ, Edelstein D, Brownlee M. Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. *Biochem Biophys Res Commun*, 1990, 173, 932-939.
- Yan SD, Schmidt AM, Anderson GM, et al. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem*, 1994, 269, 9889-9897.
- Bierhaus A, Chevion S, Chevion M, et al. Advanced glycation end product-induced activation of NF κ B is suppressed by α -lipoic acid in cultured endothelial cells. *Diabetes*, 1997, 46, 1481-1490.
- Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes*, 1991, 40, 405-412.
- Myiata T, Inagi R, Asahi K, et al. Generation of protein carbonyls by glycoxidation and lipoxidation reactions with autoxidation products of ascorbic acid and polyunsaturated fatty acids. *FEBS Lett*, 1998, 437, 24-28.
- Saxena AK, Saxena P, Wu X, Obrenovich M, Weiss MF, Monnier VM. Protein aging by carboxymethylation of lysines generates sites for divalent metal and redox active copper binding: relevance to diseases of glycoxidative stress. *Biochem Biophys Res Commun*, 1999, 260, 332-338.
- Berg TJ, Clausen JT, Torjesen PA, Dahl-Jorgensen K, Bangstad HJ, Hanssen KF. The advanced glycation end product N^ε-methyllysine is increased in serum from children and adolescents with type 1 diabetes. *Diabetes Care*, 1998, 21, 1997-2002.
- Chellan P, Nagaraj RH. Protein crosslinking by the Maillard reaction: dicarbonyl-derived imidazolium crosslinks in aging and diabetes. *Arch Biochem Biophys*, 1999, 368, 98-104.
- Lederer MO, Klaiber RG. Cross-linking of proteins by Maillard processes: characterization and detection of lysine-arginine crosslinks derived from glyoxal and methylglyoxal. *Bioorg Med Chem*, 1999, 7, 2499-2507.
- Thornalley PJ, Langborg A, Minhas HS. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem J*, 1999, 344, 109-116.
- Oya T, Hattori N, Mizuno Y, et al. Methylglyoxal modification of protein. Chemical and immunochemical characterization of methylglyoxal-arginine adducts. *J Biol Chem*, 1999, 274, 18492-18502.
- Paul RG, Bailey AJ. The effect of advanced glycation end-product formation upon cell-matrix interactions. *Int J Biochem Cell Biol*, 1999, 31, 653-660.
- Thornalley PJ. Glutathione-dependent detoxification of alpha-oxoaldehydes by the glyoxalase system: involvement in disease mechanisms and antiproliferative activity of glyoxalase I inhibitors. *Chem Biol Interact*, 1998, 111, 137-151.

- 34 Ranganathan S, Ciaccio PJ, Walsh ES, Tew KD. Genomic sequence of human glyoxalase-I: analysis of promoter activity and its regulation. *Gene*, 1999, 240, 149-155.
- 35 Abordo EA, Minhas HS, Thornalley PJ. Accumulation of alpha-oxoaldehydes during oxidative stress: a role in cytotoxicity. *Biochem Pharmacol*, 1999, 58, 641-648.
- 36 Portero-Otin M, Pamplona R, Ruiz MC, Cabiscol E, Prat J, Bellmunt MJ. Diabetes induces an impairment in the proteolytic activity against oxidized proteins and a heterogeneous effect in nonenzymatic protein modification in the cytosol of rat liver and kidney. *Diabetes*, 1999, 48, 2215-2220.
- 37 Fujino T, Tada T, Beppu M, Kikugawa K. Purification and characterization of a serine protease in erythrocyte cytosol that is adherent to oxidized membranes and preferentially degrades proteins modified by oxidation and glycation. *J Biochem*, 1998, 124, 1077-1085.
- 38 Lee AY, Chung SS. Contributions of polyol pathway to oxidative stress in diabetic cataract. *Faseb J*, 1999, 13, 23-30.
- 39 Tesfamariam B. Free radicals in diabetic endothelial cell dysfunction. *Free Radic Biol Med*, 1994, 16, 383-391.
- 40 Greene DA, Stevens MJ. The sorbitol-osmotic and sorbitol redox hypotheses. In: Le Roith D, Taylor SI, Olefsky JM, eds. "Diabetes Mellitus". Philadelphia: Lippincott-Raven Publishers, 1996.
- 41 Bucala R, Tracey KJ, Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilation in experimental diabetes. *J Clin Invest*, 1991, 87, 432-438.
- 42 Corbett JA, McDaniel ML. Does nitric oxide mediate autoimmune destruction of beta-cells? Possible therapeutic interventions in IDDM. *Diabetes*, 1992, 41, 897-903.
- 43 Kolb H, Kolb-Bachofen V. Nitric oxide: a pathogenic factor in autoimmunity. *Immunol Today*, 1992, 13, 157-160.
- 44 Hamaoka R, Fujii J, Miyagawa J, et al. Overexpression of the aldose reductase gene induces apoptosis in pancreatic beta-cells by causing a redox imbalance. *J Biochem*, 1999, 126, 41-47.
- 45 Hotta M, Tashiro F, Ikegami H, et al. Pancreatic beta-cell specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes. *J Exp Med*, 1998, 188, 1445-1451.
- 46 Ceriello A. Oxidative stress and glycemic regulation. *Metabolism*, 2000, 49, 27-29.
- 47 Rudich A, Tirosch A, Potashnik R, Hemi R, Kannety H, Bashan N. Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. *Diabetes*, 1998, 47, 1562-1569.
- 48 Tirosch A, Potashnik R, Bashan N, Rudich A. Oxidative stress disrupts insulin-induced cellular redistribution of insulin receptor substrate-1 and phosphatidylinositol 3-kinase in 3T3-L1 adipocytes. A putative cellular mechanism for impaired protein kinase B activation and GLUT4 translocation. *J Biol Chem*, 1999, 274, 10595-10562.
- 49 Hansen LL, Ikeda Y, Olsen GS, Busch AK, Mosthaf L. Insulin signaling is inhibited by micromolar concentrations of H₂O₂. Evidence for a role of H₂O₂ in tumor necrosis factor alpha-mediated insulin resistance. *J Biol Chem*, 1999, 274, 25078-25084.
- 50 Brown DM, Charonis AS, Furcht LT, et al. An overview of role of matrix components. *Diabetes Care*, 1991, 14, 157-159.
- 51 Gilbert RE, Cox A, Dziadek M, Cooper ME, Jerums G. Extracellular matrix and its interaction in the diabetic kidney: a molecular biological approach. *J Diabetes Complications*, 1995, 9, 252-254.
- 52 Krishnamurti U, Rondeau E, Sraer JD, Michael AF, Tsilibary EC. Alterations in human glomerular epithelial cells interacting with nonenzymatically glycosylated matrix. *J Biol Chem*, 1997, 272, 27966-27970.
- 53 Menon RP, Sudhakaran PR. Enhanced adhesion of human mononuclear cells to nonenzymatically glycosylated collagen I. *Mol Cell Biochem*, 1995, 148, 115-121.
- 54 Thornalley PJ. Cell activation by glycosylated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs. *Cell Mol Biol*, 1998, 44, 1013-1023.
- 55 Bendayan M. Immunocytochemical detection of advanced glycosylated end products in rat renal tissue as a function of age and diabetes. *Kidney Int*, 1998, 54, 438-447.
- 56 Sajithlal GB, Chithra P, Chandrakasan G. Advanced glycation end products induce crosslinking of collagen in vitro. *Biochim Biophys Acta*, 1998, 1407, 215-224.
- 57 McAuliffe AV, Fischer EJ, McLennan SV, Yue DK, Turtle JR. High glucose inhibits effect of ascorbic acid on [35S] sulphate incorporation in mesangial cell and matrix proteoglycan. *Diabetes Res Clin Pract*, 1997, 37, 101-108.
- 58 Heffernan S, James V, Zilkens R, et al. Changes of extracellular matrix in a baboon (*Papio hamadryas*) model of insulin dependent diabetes: studies using electron microscopy and X-ray diffraction techniques. *Diabetes Res Clin Pract*, 1996, 34, 65-72.
- 59 Hoffman BB, Sharma K, Ziyadeh FN. Potential role of TGF-beta in diabetic nephropathy. *Miner Electrolyte Metab*, 1998, 24, 190-196.
- 60 Beaudoux JL, Guillausseau PJ, Peynet J, et al. Enhanced susceptibility of low-density lipoprotein to *in vitro* oxidation in type I and type 2 diabetic patients. *Clin Chim Acta*, 1995, 239, 131-141.
- 61 Anderson JW, Gowri MS, Turner J, et al. Antioxidant supplementation effects on low-density lipoprotein oxidation for individuals with type 2 diabetes mellitus. *J Am Coll Nutr*, 1999, 18, 451-461.
- 62 Tan KC, Ai VH, Chow WS, Chau MT, Leong L, Lam KS. Influence of low density lipoprotein (LDL) subfraction profile and LDL oxidation on endothelium-dependent and independent vasodilation in patients with type 2 diabetes. *J Clin Endocrinol Metab*, 1999, 84, 3212-3216.
- 63 Gugliucci A, Menini T, Stahl AJC. Susceptibility to copper-enhanced autooxidation of VLDL+LDL fractions from diabetic patients. *Biochem Mol Biol Int*, 1994, 32, 139-147.
- 64 Jenkins AJ, Klein RL, Chassereau CN, Hermayer KL, Lopes-Virella MF. LDL from patients with well-controlled IDDM is not more susceptible to *in vitro* oxidation. *Diabetes*, 1996, 45, 762-767.
- 65 Taus M, Ferretti G, Curatola G, Dousset N, Solera ML, Valdiguié P. Lower susceptibility of low density lipoprotein to *in vitro* oxidation in diabetic patients. *Biochem Int*, 1992, 28, 835-842.
- 66 Picard S, Talussot C, Serusclat A, Ambrosio N, Berthezene F. Minimally oxidized LDL as estimated by a new method increase in plasma of type 2 diabetic patients with atherosclerosis or nephropathy. *Diabetes Metab*, 1996, 22, 25-30.
- 67 Jain SK, Mc Vie R, Jaramillo JJ, Chen Y. Hyperketonemia (acetoacetate) increases the oxidizability of LDL+VLDL in type I diabetic patients. *Free Radic Biol Med*, 1998, 24, 175-181.
- 68 Leonhardt W, Hanefeld M, Muller G, et al. Impact of concentrations of glycosylated hemoglobin, alpha-tocopherol, copper, and manganese on oxidation of low-density lipoproteins in patients with type I diabetes, type II diabetes and control subjects. *Clin Chim Acta*, 1996, 254, 173-186.
- 69 Oranje WA, Rondas-Colbers GJ, Swennen GN, Jansen H, Wolfenbuttel BH. Lack of effect on LDL oxidation and antioxidant status after improvement of metabolic control in type 2 diabetes. *Diabetes Care*, 1999, 22, 2083-2084.
- 70 Carantoni M, Abbasi F, Warmerdam F, et al. Relationship between insulin resistance and partially oxidized LDL particles in healthy, nondiabetic volunteers. *Arterioscler Thromb Vasc Biol*, 1998, 18, 762-767.
- 71 Kawamura M, Heinecke JW, Chait A. Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide-dependent pathway. *J Clin Invest*, 1994, 94, 771-778.
- 72 Galle J, Schneider R, Winner B, et al. Glyc-oxidized LDL impair endothelial function more potently than oxidized LDL: role of enhanced oxidative stress. *Atherosclerosis*, 1998, 138, 65-77.
- 73 Lyons TJ, Jenkins AJ. Lipoprotein glycation and its metabolic consequences. *Curr Opin Lipidol*, 1997, 8, 174-180.
- 74 Edwards II, Wagner JD, Litwak KN, Rudel LL, Cefalu WT. Glycation of plasma low density lipoproteins increases interaction with arterial proteoglycans. *Diabetes Res Clin Pract*, 1999, 46, 9-18.
- 75 Lopes-Virella MF, Virella G. Cytokines, modified lipoproteins, and arteriosclerosis in diabetes. *Diabetes*, 1996, 45, S40-S44.
- 76 Lopes-Virella MF, Virella G, Orchard TJ, et al. Antibodies to oxidized LDL and LDL-containing immune complexes as risk factors for coronary artery disease in diabetes mellitus. *Clin Immunol*, 1999, 90, 165-172.
- 77 Bellomo G, Moggi E, Poli M, Agosta F, Bollati P, Finardi G. Autoantibodies against oxidatively modified low-density lipoproteins in NIDDM. *Diabetes*, 1995, 44, 60-66.

- 78 Delattre J, Bonnefont-Rousselot D. Oxidative stress, free radicals and aging. In: Galteau MM, Delwaide P, Siest G, Henny J, eds. "Biologie Prospective". Compte-rendus du 9^{ème} Colloque International de Biologie Prospective de Pont-à-Mousson. Paris: John Libbey Eurotext, 1997, 197-203.
- 79 Dandona P, Thusu K, Cook S, et al. Oxidative damage to DNA in diabetes mellitus. *Lancet*, 1996, 347, 444-445.
- 80 Collins AR, Raslova K, Somorovska M, et al. DNA damage in diabetes: correlation with a clinical marker. *Free Radic Biol Med*, 1998, 25, 373-377.
- 81 Fukagawa NK, Li M, Liang P, Russell JC, Sobel BE, Absher PM. Aging and high concentrations of glucose potentiate injury to mitochondrial DNA. *Free Radic Biol Med*, 1999, 27, 1437-1443.
- 82 Lefevre G, Beljean-Leymarie M, Beyerle F, et al. Évaluation de la peroxydation lipidique par le dosage des substances réagissant avec l'acide thiobarbiturique. *Ann Biol Clin*, 1998, 56, 305-319.
- 83 Gallou G, Ruelland A, Campion L, et al. Increase in thiobarbituric acid reactive substances and vascular complications in type 2 diabetes mellitus. *Diabetes Metab*, 1994, 20, 258-264.
- 84 Griesmacher A, Kindhauser M, Andert SE, et al. Enhanced serum levels of thiobarbituric acid-reactive substances in diabetes mellitus. *Am J Med*, 1995, 98, 469-475.
- 85 Ruiz C, Alegria A, Barbera R, Farre R, Lagarda MJ. Lipid peroxidation and antioxidant enzyme activities in patients with type 1 diabetes mellitus. *Scand J Clin Lab Invest*, 1999, 59, 99-105.
- 86 Vijayalingam S, Parthiban A, Shanmugasundaram K, Mohan V. Abnormal antioxidant status in impaired glucose tolerance and noninsulin-dependent diabetes mellitus. *Diabet Med*, 1996, 13, 715-719.
- 87 Akkus I, Kalak S, Vural H, et al. Leukocyte lipid peroxidation, superoxide dismutase, glutathione peroxidase and serum and leukocyte vitamin C levels of patients with type II diabetes mellitus. *Clin Chim Acta*, 1996, 244, 221-227.
- 88 Santini SA, Marra G, Giardina B, et al. Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. *Diabetes*, 1997, 46, 1853-1858.
- 89 Nourooz-Zadeh J, Tajaddini-Sarmadi J, McCarthy S, Betteridge DJ, Wolff SP. Elevated levels of authentic plasma hydroperoxides in NIDDM. *Diabetes*, 1995, 44, 1054-1058.
- 90 Morrow JD, Hill KE, Burk RF, Nammour TH, Badr KF, Roberts LJ. F₂-isoprostanes consist of a series of prostaglandin F₂-like compounds formed during peroxidation of arachidonic acid by a mechanism independent of the cyclooxygenase pathway. *Proc Natl Acad Sci USA*, 1990, 87, 9383-9387.
- 91 Gopaul NK, Ånggård EE, Mallet AI, Betteridge DJ, Wolff SP, Nourooz-Zadeh J. Plasma 8-epi-PGF₂α levels are elevated in individuals with NIDDM. *FEBS Lett*, 1995, 368, 225-229.
- 92 Laight DW, Desai KM, Gopaul NK, Ånggård EE, Carrier MJ. F₂-isoprostane evidence of oxidant stress in the insulin resistant, obese Zucker rat: effects of vitamin E. *Eur J Pharmacol*, 1999, 377, 89-92.
- 93 Davì G, Mezzetti A, Vitacolonna E, et al. In vivo formation of 8-epi-prostaglandin-F₂α in diabetes mellitus. Effects of tight metabolic control and vitamin E supplementation. *Diabetes*, 1997, 46 (suppl. 1), 113A.
- 94 Childs RE, Badsk WG. The steady-state kinetics of peroxidase with 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulphonic acid) as chromogen. *Biochem J*, 1975, 145, 93-103.
- 95 Brasseur L, Théron P, Legrand A. Pouvoir antioxydant total du plasma. *Act Pharm Biol Clin*, 1995, 8, 239-244.
- 96 Tsai EC, Hirsch IB, Brunzell JD, Chait A. Reduced plasma peroxy radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. *Diabetes*, 1994, 43, 1010-1014.
- 97 Cerello A, Bortolotti N, Pirisi M, et al. Total plasma antioxidant capacity predicts thrombosis-prone status in NIDDM patients. *Diabetes Care*, 1997, 20, 1589-1593.
- 98 Maxwell SRJ, Thomason H, Sandler D, et al. Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. *Eur J Clin Invest*, 1997, 27, 484-490.
- 99 Opara EC, Abdel-Rahman E, Soliman S, et al. Depletion of total antioxidant capacity in type 2 diabetes. *Metabolism*, 1999, 48, 1414-1417.
- 100 Cerello A, Bortolotti N, Crescentini A, et al. Antioxidant defences are reduced during the oral glucose tolerance test in normal and non-insulin-dependent diabetic subjects. *Eur J Clin Invest*, 1998, 28, 329-333.
- 101 Leinonen JS, Alho H, Harmoinen A, Lehtimäki T, Knip M. Unaltered antioxidant activity of plasma in subjects at increased risk for IDDM. *Free Radic Res*, 1998, 29, 159-164.
- 102 Tribe RM, Poston L. Oxidative stress and lipids in diabetes: a role in endothelium vasodilator dysfunction? *Vasc Med*, 1996, 1, 195-206.
- 103 Hughes K, Choo M, Kuperan P, Ong CN, AW TC. Cardiovascular risk factors in non-diabetic controls: a population-based survey among Asians in Singapore. *Atherosclerosis*, 1998, 136, 25-31.
- 104 Osterode W, Holler C, Ulberth F. Nutritional antioxidants, red cell membrane fluidity and blood viscosity in type 1 (insulin dependent) diabetes mellitus. *Diabet Med*, 1996, 13, 1044-1050.
- 105 Kunisaki M, Umeda F, Inoguchi T, Watanabe J, Nawata H. Effects of vitamin E administration in diabetes mellitus. *Diabetes Res*, 1990, 14, 37-42.
- 106 Caye-Vaugien C, Krempf M, Lamarche P, Charbonnel B, Pieri J. Determination of alpha-tocopherol in plasma, platelets and erythrocytes of type I and type II diabetic patients by higher performance liquid chromatography. *Int J Vitam Nutr Res*, 1990, 60, 324-330.
- 107 Kagan VE, Serbinova EA, Forte T, Scita G, Packer L. Recycling of vitamin E in human low density lipoproteins. *J Lipid Res*, 1992, 33, 385-397.
- 108 Asayama K, Uchida N, Nakane T, et al. Antioxidants in serum of children with insulin-dependent diabetes mellitus. *Free Radic Biol Med*, 1993, 15, 597-602.
- 109 Armstrong AM, Chestnutt JE, Gorley MJ, Young IS. The effect of dietary treatment on lipid peroxidation and antioxidant status in newly diagnosed non-insulin dependent diabetes. *Free Radic Biol Med*, 1996, 21, 719-726.
- 110 Rema M, Mohan V, Bhaskar A, Shanmugasundaram KR. Does oxidant stress play a role in diabetic retinopathy? *Indian J Ophthalmol*, 1995, 43, 17-21.
- 111 Will JC, Byers T. Does diabetes mellitus increase the requirement for vitamin C? *Nutr Rev*, 1996, 54, 193-202.
- 112 Will JC, Ford ES, Bowman BA. Serum vitamin C concentrations and diabetes: findings from the third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr*, 1999, 70, 49-52.
- 113 Jain SK, Mc Vie R. Effect of glycemic control, race (white versus black) and duration of diabetes on reduced glutathione content in erythrocytes of diabetic patients. *Metabolism*, 1994, 43, 306-309.
- 114 Muruganandam A, Drouillard C, Thibert RJ, Cheung RM, Draisey TF, Mutus B. Glutathione metabolic enzyme activities in diabetic platelets as a function of glycemic control. *Thromb Res*, 1992, 67, 385-397.
- 115 Sjogren A, Floren CH, Nilsson A. Magnesium, potassium and zinc deficiency in subjects with type II diabetes mellitus. *Acta Med Scand*, 1988, 224, 461-466.
- 116 Raz I, Karsai D, Katz M. The influence of zinc supplementation on glucose homeostasis in NIDDM. *Diabetes Res*, 1989, 11, 73-79.
- 117 Raz I, Havivi E. Trace elements in blood cells of diabetic subjects. *Diabetes Res*, 1989, 10, 21-24.
- 118 Faure P, Roussel A, Coudray C, Richard MJ, Halimi S, Favier A. Zinc and insulin sensitivity. *Biol Trace Elem Res*, 1992, 32, 305-310.
- 119 Faure P, Corticelli P, Richard MJ, et al. Lipid peroxidation and trace element status in diabetic ketotic patients: influence of insulin therapy. *Clin Chem*, 1993, 39, 789-793.
- 120 Blostein-Fujii A, DiSilvestro RA, Frid D, Katz C, Malarkey W. Short-term zinc supplementation in women with non-insulin-dependent diabetes mellitus: effects on plasma 5'-nucleotidase activities, insulin-like growth factor I concentrations, and lipoprotein oxidation rates in vitro. *Am J Clin Nutr*, 1997, 66, 639-642.
- 121 Haglund B, Ryckenberg K, Selinus O, Dahlquist G. Evidence of a relationship between childhood-onset type I diabetes and low groundwater concentration of zinc. *Diabetes Care*, 1996, 19, 873-875.
- 122 Faure P, Benhamou PY, Perard A, Halimi S, Roussel AM. Lipid peroxidation in insulin-dependent diabetic patients with early retina degenerative lesions: effects of an oral zinc supplementation. *Eur J Clin Nutr*, 1995, 49, 282-288.

- 123 Zargar AH, Shah NA, Masoodi SR, *et al.* Copper, zinc, and magnesium levels in non-insulin dependent diabetes mellitus. *Postgrad Med J*, 1998, 74, 665-668.
- 124 Ruiz C, Alegria A, Barbera R, Farre R, Lagarda J. Selenium, zinc and copper in plasma of patients with type I diabetes mellitus in different metabolic states. *J Trace Elem Med Biol*, 1998, 12, 91-95.
- 125 Thompson KH, Godin DV, Lee M. Tissue antioxidant status in streptozotocin-induced diabetes in rats. Effects of dietary manganese deficiency. *Biol Trace Elem Res*, 1992, 35, 213-224.
- 126 El-Yazigi A, Hannan N, Raines DA. Urinary excretion of chromium, copper, and manganese in diabetes mellitus and associated disorders. *Diabetes Res*, 1991, 18, 129-134.
- 127 Navarro-Alarcon M, Lopez-G de la Serrana H, Perez-Valero V, Lopez-Martinez C. Serum and urine selenium concentrations as indicators of body status in patients with diabetes mellitus. *Sci Total Environ*, 1999, 228, 79-85.
- 128 Battell ML, Delgatty HL, McNeill JH. Sodium selenate corrects glucose tolerance and heart function in STZ diabetic rats. *Mol Cell Biochem*, 1998, 179, 27-34.
- 129 Douillet C, Bost M, Accominotti M, Borson-Chazot F, Ciavatti M. Effect of selenium and vitamin E supplements on tissue lipids, peroxides, and fatty acid distribution in experimental diabetes. *Lipids*, 1998, 33, 393-399.
- 130 Douillet C, Bost M, Accominotti M, Borson-Chazot F, Ciavatti M. In vitro and in vivo effects of selenium and selenium with vitamin E on platelet functions in diabetic rats. Relationship to platelet sorbitol and fatty acid distribution. *Biol Trace Elem Res*, 1996, 55, 263-277.
- 131 Adachi T, Ohta H, Hayashi K, Hirano K, Marklund SL. The site of nonenzymic glycation of human extracellular-superoxide dismutase in vitro. *Free Radic Biol Med*, 1992, 13, 205-210.
- 132 Adachi T, Ohta H, Hirano K, Hayashi K, Marklund SL. Non-enzymic glycation of human extracellular superoxide dismutase. *Biochem J*, 1991, 279, 263-267.
- 133 Kawamura N, Ookawara T, Suzuki K, Konishi K, Mino M, Taniguchi N. Increased glycated Cu, Zn-superoxide dismutase levels in erythrocytes of patients with insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab*, 1992, 74, 1352-1354.
- 134 Arai K, Iizuka S, Tada Y, Oikawa L, Taniguchi N. Increase in the glycosylated form of the erythrocyte Cu, Zn-superoxide dismutase in diabetes and close association of the non-enzymatic glycosylation with the enzyme activity. *Biochim Biophys Acta*, 1987, 924, 292-296.
- 135 Sakurai T, Matsuyama M, Tsuchiya S. Glycation of erythrocyte superoxide dismutase reduces its activity. *Chem Pharm Bull*, 1987, 35, 302-307.
- 136 Arai K, Maguchi S, Fujii S, Ishibashi H, Oikawa K, Taniguchi N. Glycation and inactivation of human Cu, Zn-superoxide dismutase. Identification of the in vitro glycosylated sites. *J Biol Chem*, 1987, 262, 16969-16972.
- 137 Yan H, Harding JJ. Glycation-induced inactivation and loss of antigenicity of catalase and superoxide dismutase. *Biochem J*, 1997, 328, 599-605.
- 138 Ookawara T, Kawamura N, Kitagawa Y, Taniguchi N. Site-specific and random fragmentation of Cu, Zn-superoxide dismutase by glycation reaction. Implication of reactive oxygen species. *J Biol Chem*, 1992, 267, 18505-18510.
- 139 Strange RC, Jones P, Bicknell J, Scarpello J. Expression of Cu, Zn-superoxide dismutase and glutathione peroxidase in erythrocytes from diabetic and non-diabetic subjects. *Clin Chim Acta*, 1992, 207, 261-263.
- 140 Cser A, Sziklai-Laszlo I, Menzel H, Lombek I. Selenium status and lipoproteins in healthy and diabetic children. *J Trace Elem Electrolytes Health Dis*, 1993, 7, 205-210.
- 141 Li W, Yanoff M, Jian B, He Z. Altered mRNA levels of antioxidant enzymes in pre-apoptotic pericytes from human diabetic retinas. *Cell Mol Biol*, 1999, 45, 59-66.
- 142 Uzel N, Sivas A, Uysal M, Oz H. Erythrocyte lipid peroxidation and glutathione peroxidase activities in patients with diabetes mellitus. *Horm Metab Res*, 1987, 19, 89-90.
- 143 Kowluru RA, Kern TS, Engerman RL. Abnormalities of retinal metabolism in diabetes or experimental galactosemia. IV. Antioxidant defense system. *Free Radic Biol Med*, 1997, 22, 587-592.
- 144 Baldwin JS, Lee L, Leung TK, Muruganandam A, Mutus B. Identification of the site of non-enzymatic glycation of glutathione peroxidase: rationalization of the glycation-related catalytic alterations on the basis of three dimensional protein structure. *Biochim Biophys Acta*, 1995, 1247, 60-64.
- 145 Muruganandam A, Tannous M, Mutus B. ELISA for in vivo assessment of nonenzymatically glycosylated platelet glutathione peroxidase. *Clin Biochem*, 1994, 27, 293-298.
- 146 Wierusz-Wysocka B, Wysocki H, Byks H, Zozulinska D, Wykretowicz A, Kazmierczak M. Metabolic control quality and free radical activity in diabetic patients. *Diabetes Res Clin Pract*, 1995, 27, 193-197.
- 147 Sharma A, Kharb S, Chugh SN, Kakkar R, Singh GP. Evaluation of oxidative stress before and after control of glycemia and after vitamin E supplementation in diabetic patients. *Metabolism*, 2000, 49, 160-162.
- 148 Kinalski M, Sledziewski A, Telejko B, Zarzycki W, Kinalska I. Antioxidant therapy and streptozotocin-induced diabetes in pregnant rats. *Acta Diabetol*, 1999, 36, 113-117.
- 149 Vannucchi H, Araujo WF, Bernardes MM, Jordao Junior AA. Effect of different vitamin E levels on lipid peroxidation in streptozotocin-diabetic rats. *Int J Vitam Nutr Res*, 1999, 69, 250-254.
- 150 Jain SK, Mc Vie R, Jaramillo JJ, Palmer M, Smith T. Effect of modest vitamin E supplementation on blood glycosylated hemoglobin and triglyceride levels and red cell indices in type I diabetic patients. *J Am Coll Nutr*, 1996, 15, 458-461.
- 151 Paolisso G, d'Amore A, Galzerano D, *et al.* Daily vitamin E supplements improve metabolic control but not insulin secretion in elderly type II diabetic patients. *Diabetes Care*, 1993, 16, 1433-1437.
- 152 Jain SK, Mc Vie R, Jaramillo JJ, *et al.* Effects of modest vitamin E supplementation on lipid peroxidation products and other cardiovascular risk factors in diabetic patients. *Lipids*, 1996, 31, S87-S90.
- 153 Skrha J, Sindelka G, Kvasnicka J, Hilgertova J. Insulin action and fibrinolysis influenced by vitamin E in obese Type 2 diabetes mellitus. *Diabetes Res Clin Pract*, 1999, 44, 27-33.
- 154 Kaneto H, Kajimoto Y, Miyagawa J, *et al.* Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. *Diabetes*, 1999, 48, 2398-2406.
- 155 Chung TW, Hau Yu JJ, Liu DZ. Reducing lipid peroxidation stress of erythrocyte membrane by α -tocopherol nicotinate plays an important role in improving blood rheological properties in type 2 diabetic patients with retinopathy. *Diabet Med*, 1998, 15, 380-385.
- 156 Ziegler D, Hanefeld M, Ruhnau KJ, *et al.* and the Aladin Study group. Treatment of symptomatic diabetic peripheral neuropathy with the antioxidant α -lipoic acid. *Diabetologia*, 1995, 38, 1425-1433.
- 157 Kennedy AL, Lyons TJ. Glycation, oxidation and lipoxidation in the development of diabetic complications. *Metabolism*, 1997, 46, 14-21.
- 158 Brownlee M. Glycation products and the pathogenesis of diabetic complications. *Diabetes Care*, 1992, 15, 1835-1843.
- 159 Nilsson BO. Biological effects of aminoguanidine: an update. *Inflamm Res*, 1999, 48, 509-515.
- 160 Brownlee M, Vlassara H, Kooney A, Ulrich P, Cerami A. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science*, 1986, 232, 1629-1632.
- 161 Jakus V, Hrnčiarova M, Carsky J, Krahulec B, Rietbrock N. Inhibition of nonenzymatic protein glycation and lipid peroxidation by drugs with antioxidant activity. *Life Sci*, 1999, 65, 1991-1993.
- 162 Guillausseau PJ. Pharmacological prevention of diabetic microangiopathy: blocking the pathogenic mechanisms. Traitement préventif de la microangiopathie diabétique: bloquer les mécanismes pathogéniques. *Diabetes Metab*, 1994, 20, 219-228.
- 163 Youssef S, Nguyen DT, Soulis T, Panagiotopoulos S, Jerums G, Cooper ME. Effect of diabetes and aminoguanidine therapy on renal advanced glycation end-product binding. *Kidney Int*, 1999, 55, 907-916.
- 164 Corbett JA, Tilton RG, Chang K, *et al.* Aminoguanidine, a novel inhibitor of nitric oxide formation, fails to protect against insulinitis and hyperglycemia induced by multiple low dose streptozotocin injections in mice. *Autoimmunity*, 1993, 15, 311-314.
- 165 Joly GA, Ayres M, Chelly F, Kilbourn RG. Effects of N-G-methyl-L-arginine, N-G-nitro-L-arginine, and aminoguanidine on constitutive and inducible nitric oxide synthase in rat aorta. *Biochim Biophys Res Commun*, 1994, 199, 147-154.

- 166 Philis-Tsimikas A, Parthasarathy S, Picard S, Palinski W, Witztum JL. Aminoguanidine has both pro-oxidant and antioxidant activity toward LDL. *Arterioscler Thromb Vasc Biol*, 1995, 15, 367-376.
- 167 Picard S, Parthasarathy S, Fruebis J, Witztum JL. Aminoguanidine inhibits oxidative modification of low density lipoprotein and the subsequent increase in uptake by macrophage scavenger receptors. *Proc Natl Acad Sci USA*, 1992, 89, 6876-6880.
- 168 Lisfi D, Jore D, Bonnefont-Rousselot D, Delattre J, Belâaraj A, Gardès-Albert M. Rôle anti-oxydant de l'aminoguanidine soumise à l'action des radicaux libres $\cdot\text{OH}$ et $\text{O}_2^{\cdot-}$ produits par radiolyse continue de l'eau. *J Chim Phys*, 1997, 94, 283-288.
- 169 Courderot-Masuyer C, Dalloz F, Maupoil V, Rochette L. Antioxidant properties of aminoguanidine. *Fundam Clin Pharmacol*, 1999, 13, 535-540.
- 170 Lisfi D, Bonnefont-Rousselot D, Fernet M, Jore D, Delattre J, Gardès-Albert M. Endogenous vitamin E and β -carotene protection by aminoguanidine upon oxidation of human low density lipoproteins (LDLs) by $\cdot\text{OH}/\text{O}_2^{\cdot-}$ free radicals. *Radiat Res* 2000, 153, 497-507.
- 171 Giardino I, Fard AK, Hatchell DL, Browlee M. Aminoguanidine inhibits reactive oxygen species formation, lipid peroxidation and oxidant-induced apoptosis. *Diabetes*, 1998, 47, 1114-1120.
- 172 Ihm SH, Yoo HJ, Park SW, Ihm J. Effect of aminoguanidine on lipid peroxidation in streptozotocin-induced diabetic rats. *Metabolism*, 1999, 48, 1141-1145.
- 173 Kedziora-Kornatowska KZ, Luciak M, Blaszczyk J, Pawlak W. Effect of aminoguanidine on erythrocyte lipid peroxidation and activities of antioxidant enzymes in experimental diabetes. *Clin Chem Lab Med*, 1998, 36, 771-775.
- 174 Friedman EA. Advanced glycosylated end products and hyperglycemia in the pathogenesis of diabetic complications. *Diabetes Care*, 1999, 22, B65-B71.
- 175 Freedman BI, Wuertth JP, Cartwright K, et al. Design and baseline characteristics for the aminoguanidine Clinical Trial in Overt Type 2 Diabetic Nephropathy (ACTION II). *Control Clin Trials*, 1999, 20, 493-510.
- 176 Rahbar S, Kumar Yernini K, Scott S, Gonzales N, Lalezari I. Novel inhibitors of advanced glycation endproducts. *Biochem Biophys Res Commun*, 1999, 262, 651-656.
- 177 Khalifah RG, Baynes JW, Hudson BG. Amadorins: novel post-Amadori inhibitors of advanced glycation reactions. *Biochem Biophys Res Commun*, 1999, 257, 251-258.
- 178 Faure P, Rossini E, Wiernsperger N, Richard MJ, Favier A, Halimi S. An insulin sensitizer improves the free radical defense system potential and insulin sensitivity in high fructose-fed rats. *Diabetes*, 1999, 48, 353-357.
- 179 Ewis SA, Abdel-Rahman MS. Effect of metformin on glutathione and magnesium in normal and streptozotocin-induced diabetic rats. *J Appl Toxicol*, 1995, 15, 387-390.
- 180 Ewis SA, Abdel-Rahman MS. Influence of atenolol and/or metformin on glutathione and magnesium levels in diabetic rats. *J Appl Toxicol*, 1997, 17, 409-413.
- 181 Jyothirmayi GN, Soni BJ, Masurekar M, Lyons M, Regan TJ. Effects of metformin on collagen glycation and diastolic dysfunction in diabetic myocardium. *J Cardiovasc Pharmacol Ther*, 1998, 3, 319-326.
- 182 Ruggiero-Lopez D, Lecomte M, Moinet G, Patereau G, Lagarde M, Wiernsperger N. Reaction of metformin with dicarbonyl compounds. Possible implication in the inhibition of advanced glycation end product formation. *Biochem Pharmacol*, 1999, 58, 1765-1773.
- 183 Beisswenger PJ, Howell SK, Touchette AD, Lal S, Szwegold BS. Metformin reduces systemic methylglyoxal levels in type 2 diabetes. *Diabetes*, 1999, 48, 198-202.
- 184 Tanaka Y, Uchino H, Shimizu T, et al. Effect of metformin on advanced glycation endproduct formation and peripheral nerve function in streptozotocin-induced diabetic rats. *Eur J Pharmacol*, 1999, 376, 17-22.
- 185 Yokoyama T, Yoshida Y, Inoue T, Horikoshi H. Inhibition of galactose-induced cataractogenesis by troglitazone, a new antidiabetic drug with an antioxidant property, in rat lens culture. *J Ocul Pharmacol Ther*, 1999, 15, 73-83.
- 186 Crawford RS, Mudaliar SR, Henry RR, Chait A. Inhibition of LDL oxidation in vitro but not ex vivo by troglitazone. *Diabetes*, 1999, 48, 783-790.
- 187 Cominacini L, Garbin U, Pasini AF, et al. The expression of adhesion molecules on endothelial cells is inhibited by troglitazone through its antioxidant activity. *Cell Adhes Commun*, 1999, 7, 223-231.
- 188 Renier G, Desfaits AC, Serri O. Gliclazide decreases low-density lipoprotein oxidation and monocyte adhesion to the endothelium. *Metabolism*, 2000, 49, 17-22.
- 189 Jennings PE, Belch JJ. Free radical scavenging activity of sulfonylureas: a clinical assessment of the effect of gliclazide. *Metabolism*, 2000, 49, 23-26.