Effects of insulin treatment on endoneurial and systemic oxidative stress in relation to nerve conduction in streptozotocin-diabetic rats

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Abstract. As increased oxidative stress is probably a pathogenetic factor in the development of diabetic complications, we studied nerve function and endogenous antioxidants in plasma, erythrocytes and sciatic nerve of untreated and insulin-treated streptozotocin-diabetic rats. After 18 weeks, the diabetes-induced sciatic nerve conduction velocity deficits were approximately 65% improved by insulin ($P < 0.001$). Plasma superoxide dismutase was significantly reduced in diabetes ($P < 0.01$); smaller decreases in plasma catalase and glutathione levels were observed. These changes were corrected by insulin treatment. In erythrocytes, decreased superoxide dismutase ($P < 0.05$) and increased total glutathione levels ($P < 0.05$) were found. All effects of diabetes, including a rise in plasma malonyldialdehyde ($P < 0.05$), were partially reversed by insulin treatment. In nervous tissue, diabetes caused increased catalase activity, uninfluenced by insulin ($P < 0.05$). Nerve superoxide dismutase and glutathione did not change. The data suggest that, in diabetes, changes in systemic rather than endoneurial oxidative stress lead to nerve dysfunction.

Keywords. Catalase, experimental diabetes, glutathione, nerve conduction, oxidative stress, superoxide dismutase.

Introduction

Reactive oxygen species (ROS) have been proposed as a potential causal factor for the pathogenesis of diabetic complications, including neuropathy [1,2]. Several authors have shown that experimental hyperglycaemia leads to increased lipid peroxidation in different organs [3–5], reflecting a rise in ROS production. In addition to animal studies, increased lipid peroxidation has also been found in plasma and erythrocytes from diabetic patients, especially those

with microangiopathy [6,7]. Several mechanisms have been proposed to explain the increased oxidative stress in diabetes. ROS are probably formed as a result of the transition of glucose into enediols in the presence of catalytically active metal ions, a mechanism that has been referred to as glucose auto-oxidation [8]. Enhanced protein glycation, as a consequence of chronic hyperglycaemia, also induces increased presence of ROS, especially hydroxyl radicals ($\bullet\text{OH}$). This mechanism is generally known as auto-oxidative glycosylation [1,9]. Both auto-oxidative glycosylation and glucose auto-oxidation are generally considered to be responsible for increased oxidative damage to proteins and lipids in diabetes, but the interaction between these mechanisms has not yet been clarified. A third factor that may be of importance is the decreased endogenous ROS-scavenging capacity that has been observed in both clinical and experimental hyperglycaemia [2].

Subsequently, antioxidant drug treatment has been studied to determine its protective capacity in diabetic complications. Our group demonstrated that chronic administration of reduced glutathione (GSH) partially protects against the development of experimental diabetic neuropathy [10]. In two studies, Cameron *et al.* [11,12] showed that the antioxidant drugs butylated hydroxytoluene and probucol prevented the appearance of reduced nerve conduction velocity in diabetic rats and, recently, they also reported a protective effect of *N*-acetylcysteine [13].

Only a few studies have been performed to investigate the defence mechanisms against oxidative stress in the peripheral nerve. Romero *et al.* [14,15] observed that sciatic nerve GSH concentrations and glutathione peroxidase and glutathione reductase activity are low compared with brain and liver tissue, whereas superoxide dismutase was relatively high. Low & Nickander [16] observed reduced superoxide dismutase levels and increased amounts of conjugated dienes in sciatic nerve from diabetic rats. These changes were reversed by insulin treatment. The same group also recently observed decreased sciatic nerve GSH

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concentrations in diabetes, which could be corrected with antioxidant treatment using α -lipoic acid [17].

The pathogenesis of diabetic nerve dysfunction is associated with both endoneurial metabolic changes [18,19] and reduced endoneurial microcirculation [20–22]. As increased oxidative stress in diabetes may lead to changes in the oxidative state of the nerve and to GSH depletion [17], direct metabolic effects of antioxidant compounds may be responsible for the observed improvement in nerve conduction velocity. However, antioxidant treatment has also been shown to ameliorate sciatic nerve nutritive blood flow [12]. The present study was performed to try to distinguish between the direct harmful effects of hyperglycaemia-induced ROS production on nerve tissue and the effects of increased systemic oxidative stress on nerve function in experimentally hyperglycaemic rats. As we hypothesized that ROS are principally responsible for the reduction of nerve microcirculation leading to secondary nerve conduction deficits, we investigated the influence of hyperglycaemia and insulin treatment on endogenous antioxidant levels of both the sciatic nerve and erythrocytes and plasma in the streptozotocin-diabetic rat. Consequently, we compared the changes that were observed with plasma lipid peroxidation and nerve conduction velocity.

Materials and methods

Animal procedures

All animal experiments were approved by the University ethical commission for animal experiments. Male Wistar rats (initial weight ± 250 g) were housed on sawdust in Macrolon cages, two rats per cage, and maintained on a 12 h/12 h light–dark cycle. All animals received food and water *ad libitum*. Twenty-four animals were given 40 mg kg^{-1} streptozotocin (Serva, Heidelberg, Germany) intravenously (i.v.) and eight animals were used as non-diabetic controls (group C). Diabetes was confirmed after 48 h by measuring whole blood glucose levels with a glucose oxidase method (Minilab; Bayer, Munich, Germany). After 4 weeks, the diabetic rats were divided into two groups. Insulin treatment was started in group DI, while group DU was left untreated.

Insulin was administered by subcutaneous insulin pellets (Møllegaard, Ejby, Denmark), which deliver 2 IU of insulin per pellet daily. Treatment was started with 1.5 pellets. An extra pellet was implanted when blood glucose levels exceeded 15 mmol L^{-1} . Blood glucose was measured weekly.

Motor and sensory nerve conduction velocity (MNCV and SNCV) were measured at the left hind paw in the sciatic nerve, as described by De Koning & Gispen [23]. Measurements were performed under general anaesthesia (0.175 mL of Hypnorm containing 10 mg mL^{-1} fentanyl and 0.2 mg mL^{-1} fentanyl citrate, Janssen Pharmaceutica, Tilburg, The Netherlands) at weeks 4, 6, 8, 10, 12, 14, 16 and 18. All

measurements were performed by the same examiner (PSVD). The body temperature of the animals was kept at a constant temperature of $36\text{--}37^\circ\text{C}$.

All animals were decapitated at week 18, and blood was subsequently collected in heparinized tubes, separated into plasma and red blood cells and processed immediately. Both sciatic nerves were located, removed, desheathed and stored in liquid nitrogen until homogenization and further processing. Nerve homogenization was performed on ice in a 50 mmol L^{-1} sodium phosphate buffer (pH 7.5) on the same day.

Biochemical procedures

The following biochemical measurements were performed: in plasma malonyldialdehyde (MDA), superoxide dismutase (SOD), catalase and total glutathione; in red blood cells malonyldialdehyde, superoxide dismutase, catalase, total glutathione, GSH and oxidized glutathione (GSSG); in sciatic nerve homogenate SOD, catalase and total glutathione.

Malonyldialdehyde assay. Plasma and erythrocytes were deproteinized with trichloroacetic acid (TCA) before MDA measurement. MDA was measured as thiobarbituric acid reactive substance, according to Aust [24].

Superoxide dismutase assay. No pretreatment of samples was performed. SOD activity was determined by measuring the delay in cytochrome *c* reduction in the presence of hypoxanthine and xanthine oxidase [25].

Catalase assay. No pretreatment of samples was performed. Catalase activity was determined by measuring oxygen production from hydrogen peroxide (H_2O_2) according to Meerhof & Roos [26].

Total glutathione assay. Erythrocyte lysate, sciatic nerve and plasma were deproteinized in a TCA/EDTA solution. The supernatant was extracted with diethylether. Total glutathione concentrations were determined according to Tietze [27] using 5,5-dithiobis(2-nitrobenzoic acid) (3-carboxy-4-nitrophenyl) disulphide (DTNB), NADPH and glutathione reductase.

Reduced glutathione assay. Erythrocyte lysate was deproteinized with a 0.04 mol L^{-1} sulphuric acid and a 0.3 mol L^{-1} tungstic acid solution. Total sulphydryl concentrations, representing GSH, were measured in a 96-well microtitre plate with DTNB [28].

Oxidized glutathione assay. Samples were deproteinized with 30% HClO_4 . GSSG was measured with DTNB after alkylation of GSH with NEM according to Redegeld *et al.* [29].

Protein assay. Protein concentrations in sciatic

Figure 1. Changes in mean weight ($\pm$ SEM) for control animals (Δ , $n = 8$), untreated (DU, $\blacksquare$, $n = 11$) and insulin-treated (DI, $\bullet$, $n = 8$) diabetic rats. Insulin treatment was started at week 4. The curves for the control animals and the untreated diabetics are significantly different ($P < 0.001$). The curve for the insulin-treated animals is significantly different from the controls from week 0, and from the untreated diabetics from week 4 ($P < 0.001$).

nerve were measured in a 96-well microtitre plate at 595 nm, according to Bradford [30].

Glycated haemoglobin: HbA1c was measured using a standard high-performance liquid chromatography technique [31].

Statistical analysis

The initial effect of diabetes at week 4 was calculated by the unpaired Student's *t*-test, comparing all diabetic animals with group C. To evaluate weight and nerve conduction velocity curves, analysis of variance for repeated measurements (ANOVAR) was used. For all biochemical data, the effects of diabetes vs. the non-diabetic condition were analysed in group DU and group C using Student's *t*-test. In case of non-equality of variances according to Levene's test, the *P*-value for inequality of variances was used. To evaluate the effect of insulin treatment, the three groups were

analysed using analysis of variance (ANOVA) with a *post hoc* Bonferroni test. If inequality of variances was observed, data were log transformed before analysis. In one case (plasma superoxide dismutase), as log or reciprocal transformation did not lead to equal variances, a non-parametric test (Kruskal–Wallis, followed by Mann–Whitney *U*) was used.

Results

Four animals in group DI and one in group DU died during the study. The data obtained from these animals were excluded in the final analysis. Insulin treatment, which was started at week 4, immediately led to an increase in weight (Fig. 1, $P < 0.001$). HbA1c levels in group DU were significantly increased compared with group DI and group C. Group DI had higher glucose and HbA1c levels than group C (Table 1, $P < 0.05$).

Figure 2. Changes in motor nerve conduction velocity (MNCV, mean $\pm$ SEM) for control animals (Δ , $n = 8$), untreated (DU, $\blacksquare$, $n = 11$) and insulin-treated (DI, $\bullet$, $n = 8$) diabetic rats. At week 4, mean MNCV in all diabetic animals was reduced in comparison with controls ($P < 0.05$). The curves for the control animals and group DU are significantly different ($P < 0.001$). The curve for group DI is significantly different from the controls ($P < 0.01$) and from group DU ($P < 0.001$) from week 4.

Figure 3. Changes in sensory nerve conduction velocity (SNCV, mean $\pm$ SEM) for control animals (Δ , $n = 8$), untreated (DU, $\blacksquare$, $n = 11$) and insulin-treated (DI, $\bullet$, $n = 8$) diabetic rats. At week 4, the difference in mean SNCV between all diabetic animals and controls was not significant ($P = 0.056$). The curves for the control animals and group DU are significantly different ($P < 0.001$). The curve for group DI is significantly different from the controls ($P < 0.01$) and from group DU ($P < 0.001$) from week 4.

Table 1. Mean body weight, glucose and HbA1c for the different groups of animals

	Weight (g)	Glucose (mmol $^{-1}$)	HbA1c (%)
C ($n = 8$)	492 $\pm$ 9	6.5 $\pm$ 0.5	3.0 $\pm$ 0.2
DU ($n = 11$)	200 $\pm$ 14	29.1 $\pm$ 0.6	11.3 $\pm$ 0.6
DI ($n = 8$)	415 $\pm$ 15	14.5 $\pm$ 1.2	5.5 $\pm$ 0.6

Glucose and glycated haemoglobin (HbA1c) in control (C), untreated diabetic (DU) and insulin-treated diabetic (DI) rats 18 weeks after streptozotocin injection and 14 weeks after the start of insulin treatment. Data are group means $\pm$ SEM. Statistical analysis for weight, glucose and HbA1: Student's *t*-test group DU vs. C ($P < 0.001$; ANOVA $F < 0.001$, *post hoc* Bonferroni ($P < 0.05$) significant for DI vs. C and DI vs DU (statistical analysis of HbA1 data was performed after log transformation because of inequality of variances).

Nerve conduction

Mean MNCV was significantly reduced in all diabetic animals at week 4 in comparison with group C (Fig. 2; diabetics 50.0 ± 1.1 vs. controls 53.3 ± 0.7 m s^{-1} , $P < 0.05$, mean $\pm$ SEM). Mean SNCV in all diabetic animals at week 4 was reduced in comparison with control animals, but this difference did not reach significance (Fig. 3; diabetics 52.8 ± 0.9 vs. controls 55.8 ± 1.1 m s^{-1} , $P = 0.056$). The difference in SNCV between group DU and group C was highly significant at week 6 (Fig. 3; group DU 46.8 ± 0.6 and group C 60.5 ± 1.1 m s^{-1} , $P < 0.001$). The maximum difference between group DU and group C was 12.1 m s^{-1} for MNCV and 13.7 m s^{-1} for SNCV at week 10. Group DI, which started insulin treatment at week 4, showed important increases in MNCV and SNCV from week 6 onwards compared with group DU but, until week 18, the difference between group DI and group C remained approximately $3\text{--}4 \text{ m s}^{-1}$. All three curves were significantly different for both MNCV and SNCV when differences were calculated using ANOVA (DU vs. C and vs. DI $P < 0.001$; DI vs. C $P < 0.01$).

Antioxidant activity and lipid peroxidation

Plasma SOD was significantly decreased ($P < 0.01$) in group DU compared with group C. Plasma catalase and total glutathione were reduced in group DU, but these differences did not reach significant levels. Plasma MDA levels were significantly increased in group DU compared with group C ($P < 0.05$). After insulin treatment, plasma SOD was completely corrected ($P < 0.01$), catalase and total glutathione increased, whereas plasma MDA in group DI remained intermediate (Table 2).

A small but significant reduction in erythrocyte SOD was observed in group DU compared with group C ($P < 0.05$). Increased total glutathione levels ($P < 0.05$), combined with a trend towards elevated GSH and GSSG levels were found in group DU compared with group C. The observed increase in erythrocyte MDA in group DU was not statistically significant. Red blood cell catalase did not change. Insulin treatment partly corrected all changes induced

by diabetes, but without reaching the levels observed in the control animals (Table 3).

In sciatic nerve homogenate, the only effect of diabetes was an increase in catalase ($P < 0.05$) in group DU vs. group C. SOD and total glutathione were not influenced by diabetes (Table 4).

Discussion

Many authors have associated hyperglycaemia-induced changes in the oxidative state with diabetic complications [1,2,32]. Increased oxidative stress and lipid peroxidation have been observed in diabetic patients and animals, and they may be reflected by changes in the scavenging capacity of red blood cells and plasma. In the present study, we investigated the role of antioxidant capacity in the development of nerve dysfunction in experimental diabetes and the effects of insulin treatment. As the toxic effects of ROS on the peripheral nerve can be either direct or mediated by changes in nerve microcirculation, we studied nervous as well as systemic changes in antioxidant capacity.

The present data show that untreated diabetes did not affect the endogenous antioxidants SOD or total glutathione in the sciatic nerve, but caused a significant increase in the H_2O_2 scavenger catalase, which was not corrected by insulin treatment. The changes in systemic antioxidant levels were more important, showing a significant decrease in plasma and erythrocyte SOD as well as an increase in erythrocyte total glutathione levels. Although not statistically significant, similar decreases were observed in plasma total glutathione and catalase, as well as an increase in red blood cell GSH. All diabetes-induced changes in plasma and erythrocytes, as well as the observed nerve conduction velocity deficit, were partially reversed by insulin treatment.

Most experimental drugs that protect the diabetic nerve against reduced conduction velocity also have a beneficial effect on sciatic nerve microcirculation. Antioxidant treatment with both probucol and α -tocopherol was found to improve nerve conduction and nerve blood flow in diabetic animals [12,33]. Probucol treatment also increased endoneurial

Table 2. Antioxidant and MDA concentrations in plasma

	SOD ($\mu\text{mol L}^{-1}$)	Catalase (mg L^{-1})	Total glutathione ($\mu\text{mol L}^{-1}$)	MDA ($\mu\text{mol L}^{-1}$)
C ($n = 8$)	4.18 ± 0.11	2.33 ± 0.17	7.0 ± 1.1	0.46 ± 0.11
DU ($n = 11$)	2.42 ± 0.44	1.50 ± 0.43	5.2 ± 0.5	0.87 ± 0.10
DI ($n = 8$)	4.63 ± 0.12	1.81 ± 0.33	7.3 ± 1.3	0.62 ± 0.05

Superoxide dismutase (SOD), catalase, total glutathione and malonyldialdehyde (MDA) concentrations in plasma from control (C), untreated diabetic (DU) and insulin-treated diabetic (DI) rats. All data are group means $\pm$ SEM. Statistical analysis: SOD showed inequality of variances, also after log or reciprocal transformation; Kruskal-Wallis one-way ANOVA all groups $P = 0.0013$, Mann-Whitney U -test group DU vs. C and DU vs. DI $P < 0.01$; MDA Student's t -test group C vs. DU $P < 0.05$, ANOVA all groups $F = 0.3824$.

Table 3. Antioxidant and MDA concentrations in red blood cells

	SOD ($\mu\text{mol g}^{-1}$ Hb)	Catalase (mg g^{-1} Hb)	Total glutathione ($\mu\text{mol g}^{-1}$ Hb)	GSH ($\mu\text{mol g}^{-1}$ Hb)	GSSG (nmol g^{-1} Hb)	MDA (nmol g^{-1} Hb)
C ($n = 8$)	0.96 ± 0.04	12.4 ± 0.3	4.4 ± 0.2	3.0 ± 0.2	48 ± 4	9.8 ± 2.2
DU ($n = 11$)	0.84 ± 0.03	11.9 ± 0.3	5.9 ± 0.6	3.8 ± 0.3	60 ± 6	13.3 ± 1.4
DI ($n = 8$)	0.92 ± 0.04	11.5 ± 0.3	4.6 ± 0.3	3.1 ± 0.2	50 ± 7	10.7 ± 1.8

Superoxide dismutase (SOD), catalase, total, reduced (GSH) and oxidized (GSSG) glutathione and malonyldialdehyde (MDA) concentrations in red blood cells from control (C), untreated diabetic (DU) and insulin-treated (DI) diabetic animals. All data are group means $\pm$ SEM. Statistical analysis: Superoxide dismutase Student's *t*-test group C. vs. DU $P < 0.05$, ANOVA $F = 0.0655$; total glutathione Student's *t*-test group C vs. DU $P < 0.05$, ANOVA all groups $F = 0.1320$ (after log transformation because of inequality of variances); GSH Student's *t*-test group C vs. DU $P = 0.053$, ANOVA all groups $F = 0.0671$.

oxygen tension in diabetic rats and showed similar effects in non-diabetic rats pretreated with the pro-oxidant primaquin [12]. Nagamatsu *et al.* [17] demonstrated that antioxidant treatment of diabetic rats with α -lipoic acid also leads to an increase in nerve blood flow. Similar observations were made by Cameron & Cotter [34] using the iron chelator deferoxamine. The present study shows that the changes induced in the oxidative state of the animal by diabetes are not principally found in the sciatic nerve, but rather in plasma and red blood cells. This may indicate that the main beneficial effect of antioxidants may be related to increased nerve micro-circulation and improved capillary function rather than a direct antioxidative effect on the peripheral nerve.

ROS scavenger measurements in the sciatic nerve showed an increase in catalase, but no change in SOD or glutathione in diabetic animals. Catalase concentrations in sciatic nerve in diabetes have not been reported before, but data from other tissues are conflicting, as increased, decreased as well as unchanged levels have been observed in diabetic liver, kidney, heart and pancreas [2,5,35,36]. Catalase levels were shown to be elevated in arterial tissue from diabetic rats [37]. No significant change in catalase concentration has been found in central nervous tissue [5,38]. In the present study, nerve catalase concentrations varied more in insulin-treated animals than in the controls or in those without treatment. We could not show a correlation between nerve catalase levels and nerve conduction or glycated haemoglobin within

the insulin-treated group. The mechanism behind changes in catalase remains unclear. Enzyme induction owing to the increased presence of H_2O_2 may play a role, but needs further study. *In vitro* studies showed an increase in catalase mRNA production in cell cultures that were incubated with H_2O_2 [39].

Low & Nickander [16] observed a reduction in Cu,Zn-SOD concentrations in diabetic nerve after 1 month of diabetes, which was partly corrected by insulin treatment. The same group observed a decrease in sciatic nerve GSH after 1 month of diabetes, but this effect had disappeared 2 months later [17,40]. The present results show that, after a longer duration of diabetes of 18 weeks, no differences are observed in sciatic nerve total glutathione. As we measured total glutathione instead of GSH, modifications in the endoneurial GSH/GSSG balance are not detected by this technique. Nevertheless, most endoneurial glutathione is present in the reduced form [41], and the total glutathione that was detected is probably representative of the amount of GSH present. The small increase in SOD levels after insulin treatment may reflect an anabolic effect of hyperinsulinaemia on protein synthesis rather than the result of improved metabolic regulation, as glucose and HbA1c levels in insulin-treated rats were still significantly elevated compared with the control animals.

Previously, we reported a beneficial effect of GSH supplementation on the nerve conduction velocity of diabetic rats, suggesting that increased oxidative stress in diabetes is associated with a glutathione deficiency

Table 4. Antioxidant concentrations in sciatic nerve

	SOD ($\mu\text{mol g}^{-1}$ protein)	Catalase (mg g^{-1} protein)	Total glutathione ($\mu\text{mol g}^{-1}$ protein)
C ($n = 8$)	1.50 ± 0.11	2.0 ± 0.1	18.9 ± 1.4
DU ($n = 11$)	1.35 ± 0.11	2.7 ± 0.2	17.6 ± 1.1
DI ($n = 8$)	1.73 ± 0.10	2.7 ± 0.4	22.4 ± 3.3

Superoxide dismutase (SOD), catalase and total glutathione concentrations in sciatic nerve from control (C), untreated diabetic (DU) and insulin-treated diabetic (DI) animals. All data are group means $\pm$ SEM. Statistical analysis: catalase Student's *t*-test group C vs. group DU $P < 0.05$, ANOVA all groups $F = 0.1625$.

in nervous tissue [10]. However, as sciatic nerve total glutathione levels were not influenced by diabetes or insulin treatment in this study, it remains to be established whether the beneficial effect of treatment with GSH on nerve conduction is caused by improvement of nerve blood flow or capillary function rather than by a change in the antioxidant capacity of the sciatic nerve itself. The absence of changes in the oxidative state of the diabetic nerve could be reflected by the unchanged endoneurial MDA levels that we reported previously [41]. However, the observation by Nagamatsu *et al.* [17] that GSH is decreased in the sciatic nerve in experimental diabetes at 1 month of diabetes suggests that a change in endoneurial GSH/GSSG balance may occur early after streptozotocin injection, but that this change does not persist.

We observed a significant increase in both total and reduced glutathione concentrations in erythrocytes. Mukherjee *et al.* [5] have reported decreased red blood cell GSH concentrations in rats that had been diabetic for 2–5 weeks. Murakami *et al.* [42] showed decreased GSH and elevated GSSG levels, as well as reduced gamma-glutamylcysteine synthetase and glutathione reductase activity in erythrocytes from diabetic patients. In two other studies [43,44], GSH depletion in red blood cells from diabetic patients was found. In these studies, reduced concentrations of NADPH in diabetes, either as a consequence of increased polyol pathway flux or as a result of attenuated glucose-6-phosphate dehydrogenase activity, were suggested to lead to decreased reduction of GSSG to GSH. However, these studies were performed in human subjects with moderate hyperglycaemia. The present data were obtained from streptozotocin-diabetic rats with extreme hyperglycaemia, which might lead to increased NADPH production by increased flux of glucose through the hexose monophosphate shunt, and could thus be responsible for the small rise in GSH concentration. Nevertheless, the balance between hexose monophosphate shunt activity and polyol pathway flux in diabetes, and its effects on NADPH levels, has not yet been fully clarified.

Erythrocyte SOD activity was reduced in diabetic animals and partially corrected by insulin treatment. These findings confirm the studies by Loven *et al.* [45] and Matkovic *et al.* [38] and are probably related to increased glycation of the enzyme [46]. If the enzymatic activity of SOD is attenuated, less superoxide $O_2^{\cdot-}$ is reduced to H_2O_2 , and more $O_2^{\cdot-}$ is available for reduction of iron to Fe^{2+} , which may increase hydroxyl radical formation. In plasma an important decrease in SOD and a partial reduction in catalase and total glutathione were found in diabetic rats, with a correcting effect of insulin treatment. Together with the diabetes-induced increase in plasma MDA, these data confirm that elevated oxidative stress is present in animals with experimental diabetes. Plasma levels cannot be directly extrapolated to the local situation in neural tissue, such as sciatic nerve. However, increased amounts of plasma MDA and decreased

antioxidant levels may be associated with endothelial structure and function, and the present findings possibly reflect a change in the oxidative state of endothelium. In the past, important changes in the structure of endoneurial capillaries have been reported [22,47–49]. Furthermore, diabetes has been shown to lead to reduced endothelial antioxidant capacity [50]. As endothelial function is essential to the maintenance of transport of oxygen and nutrients to the peripheral nerve, an increase in systemic oxidative stress associated with reduced endothelial ROS-scavenging activity could be responsible for endothelial damage and secondary nerve dysfunction.

In conclusion, long-term experimental hyperglycaemia leads to increased systemic oxidative stress, associated with changes in erythrocyte and plasma ROS scavengers, but no decrease in sciatic nerve glutathione or superoxide dismutase levels was observed. The changes in the oxidative state in diabetes are associated with reduced nerve blood flow and secondary nerve dysfunction. Structural and functional endothelial changes, possibly induced by ROS, may be partially responsible for the development of diabetic neuropathy. Further studies should thus focus on the link between oxidative stress and endothelial function in relation to nerve microcirculation in experimental hyperglycaemia.

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