Effect of PUFA-rich plant oil on risk factors of STZ-induced diabetes in Wistar rats

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Objective: This study has been focused on the effect of an n-6 polyunsaturated fatty acid (PUFA) rich plant oil on oxidation and glycooxidation stress markers as well as on antioxidant enzyme activities in male Wistar rats with streptozotocin-induced diabetes.

Methods: The non-diabetic and diabetic groups of Wistar rats were administered plant oil at concentrations of 100 and 500 mg/kg body weight and controls without plant oil. The parameters of glycaemic control, lipid profile, total antioxidant status, antioxidant enzyme activities, together with oxidative and glycooxidative stress markers were measured in the blood.

Results: The intake of the plant oil did not significantly influence the parameters of glycaemic control and significantly increased the levels of all lipid profile parameters in the diabetic rats. Plant oil administration significantly decreased the total antioxidant status and glutathione peroxidase activity and the activity of Cu/Zn superoxide dismutase was significantly increased. The plant oil also increased the levels of lipoperoxides and advanced the glycation end products.

Discussion: These results suggest that the plant oil with high concentrations of n-6 PUFA – linoleic acid, acts prooxidatively when administered to the rats.

Keywords: Diabetes mellitus, Rats, Plant oil, N-6 PUFA

Introduction

Diabetes is a metabolic disease associated with increased oxidative stress and reactive oxygen species (ROS) production. Oxidative stress plays a crucial role in the development of diabetic complications.¹ The primary factor leading to pathophysiological alterations in human or animal tissues is a permanent exposure to high levels of blood glucose.² Chronic hyperglycaemia causes increased ROS production via glucose autooxidation and non-enzymatic protein glycation.³ Free radicals affect the biomolecules such as lipids and proteins. Non-enzymatic lipid oxidation and glycation leads to the formation of lipid peroxide (LP), malondialdehyde (MDA), and advanced glycation end products (AGEs).⁴

To protect the molecules from ROS, the cells have an antioxidant defence system which consists of Cu/Zn superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and paraoxonase-1 (PON1).^{5,6}

Antioxidant enzyme activities as well as total antioxidant status (TAS) can be moderated by polyunsaturated fatty acids (PUFAs).⁷ There are lots of studies supporting the beneficial effects of n-3 PUFAs in preventing diabetic complications, such as increased blood pressure, hypertension, arrhythmias, and other cardiovascular diseases.^{1,7,8} However, there has not been sufficient amount of research looking at the influence of omega-6 PUFAs on diabetes. Omega-6 PUFAs are characterized by the presence of at least two carbon-carbon double bonds with the first bond at the sixth carbon from the methyl end. This double bond can participate in ROS formation and leads to oxidative stress. It is believed that n-6 PUFAs play a role in the production of inflammatory mediators and oxidative stress markers.^{3,9} There is a lack of information about the link between n-6 PUFA and proinflammatory and prooxidative responses.^{10–12}

However, there are also inconsistencies relating to the effects of n-6 PUFAs on lipid profiles. While some studies support a beneficial effects of n-6 PUFAs on the blood lipid profile, others have shown mixed results.^{11,12} In some studies, diets rich in n-6 PUFAs were shown to reduce low-density lipoprotein

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cholesterol (LDL-chol)¹¹ while having a positive effect on high-density lipoprotein cholesterol (HDL-chol).¹³ A significant inverse association has been observed between n-6 PUFAs and serum triacylglycerides (TAG)¹⁴ while other studies have not shown significant effects of n-6 PUFAs on serum HDL-chol and LDL-chol.¹²

Linoleic acid (LA, 18:2) is abundant in plant oils and seeds¹¹ and is a dietary n-6 PUFA that cannot be synthesized by humans.¹⁰ In order to gain more information about the n-6 PUFA effect on diabetes, we examined the influence of a plant oil with a significant content of the n-6 PUFA LA, on glucose homeostasis, lipid profile, antioxidant enzyme activities, TAS of plasma, and markers of oxidative stress in a group of streptozotocin (STZ)-induced diabetic rats.

Materials and methods

Animals and study design

Sixty adult male Wistar rats were randomly selected into the following groups: the non-diabetic control group (C, 8 rats) without oil intake; the non-diabetic rats treated daily with plant oil with a concentration of 100 mg/kg body weight (BW) (CC1, 8 rats); the non-diabetic rats treated daily with plant oil with a concentration of 500 mg/kg BW (CC2, 8 rats); the diabetic control rats without oil intake (D, 12 rats); the diabetic rats treated daily with plant oil with a concentration of 100 mg/kg BW (DC1, 12 rats), and the diabetic rats treated daily with plant oil with a concentration of 500 mg/kg BW (DC2, 12 rats). The rats were bred in groups of four to six in polypropylene cages and they had free access to food and water daily. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH publication number 85-23, revised 1996), as well as with the guidelines formulated by the European Community for the Use of Experimental Animals (L358-86/609/EEC).

Diabetes was induced by an intravenous injection of STZ (Sigma-Aldrich, Taufkirchen, Germany) in a single dose of 45 mg/kg BW. STZ was dissolved in 0.1 mol/l citrate buffer (pH 4.5) and injected into the tail vein. Control animals received an equal amount of citrate buffer. Diagnosis of diabetes was confirmed by monitoring glucose concentration in the blood from the tail vein; animals with glucose plasma concentration above 15 mmol/l were considered diabetic. Body weight was determined prior to and then three times after STZ treatment until the termination of the experiment. Rats were not treated with insulin or with any other hypoglycaemic agents. The duration of treatment was 7 weeks and then the animals were sacrificed by intramuscular injection of thiopental (60 mg/kg BW).

Characterization of plant oil

The plant oil (kindly provided by Prof. Wagner, Vienna University) used in our experiments had the following composition: 64.5% LA – C18:2 (9, 12), 15.6% oleic acid – C18:1 (9), 10.4% linolenic acid – C18:3 (9, 12, 15), 7.0% palmitic acid – C16:0, and 2.5% stearic acid – C18:0.

Blood collection

Blood was collected from the vena porte of anaesthetized rats into test tubes containing heparin centrifuged (1200 g, 10 minutes) to separate plasma from the blood cells and the plasma samples were immediately aliquoted and stored at –80°C until use. The haemolysate solution was prepared from the isolated red blood cells. Erythrocytes were washed three times with physiological solution, and then 0.5 ml of erythrocytes were haemolysed in 1.5 ml cold distilled water. Haemolysate solutions were stored at –20°C and used for the determination of haemoglobin (Hb) concentration and antioxidant enzyme activities.

Determination of lipid profile markers and parameters of glycaemic control

The fasting plasma concentration of glucose, fructosamine, total cholesterol (TCH), HDL-chol, very low-density lipoprotein cholesterol (VLDL-chol), and TAGs were determined using a Hitachi 911 Analyzer (Roche Diagnostics, Rotkreuz, Switzerland). LDL-chol was calculated by the Friedewald equation.

Determination of Hb

Hb levels were determined according to the Drabkin method¹⁵ and expressed in g/l.

Determination of Cu/Zn SOD activity in erythrocytes

Cu/Zn SOD activity was determined by commercial kit (Fluka, Buchs, Switzerland), using bovine Cu/Zn SOD as a standard (Sigma-Aldrich, Taufkirchen, Germany). Cu/Zn SOD activity was expressed in U/mg Hb.

Determination of GPx activity in erythrocytes

GPx activity was determined indirectly using a commercial kit (Sigma-Aldrich, St. Louis, USA). The change in absorbance was monitored spectrophotometrically at 340 nm. The activity of GPx was expressed in U/mg Hb.

Determination of CAT activity in erythrocytes

Catalase activity was determined according to Aebi¹⁶ and is based on a change of hydrogen peroxide absorbance over time at a wavelength of 240 nm. Catalase activity was calculated by using the formula:

$$A[\text{kat}] = \frac{dA/\text{minute} \times DF}{43.6 \times V} \times 16.7 \times 10^{-3}$$

where dA/minute is the absorbance change per 1 minute, DF is the dilution factor, V is the volume of the sample, the molar absorption coefficient of hydrogen peroxide is $43\,600\text{ mol/l/cm}$, and 16.7×10^{-3} is a conversion factor of U activity units to katal. CAT activity was expressed in U/mg Hb.

Determination of the arylesterase and lactonase activities of PON1

Arylesterase and lactonase activities of PON1 were assayed in plasma samples spectrophotometrically at a wavelength of 270 nm.^{17,18}

Sample preparation for the determination of PON1 arylesterase activity: plasma samples were centrifuged (2500 g, 5 minutes) and the supernatant was diluted 600 times by Tris-HCl solution (1 mmol/l CaCl_2 in 50 mmol/l Tris, pH = 8.0). Then, 10 mmol/l phenylacetate solution was added to the diluted supernatant and the absorbance was measured at 0 and 2 minutes.

Sample preparation for the determination of PON1 lactonase activity: plasma sample was diluted with Tris-HCl solution (1 mmol/l CaCl_2 in 50 mmol/l Tris, pH 7.5) in a ratio of 1:5. Then, 2 mmol/l dihydrocoumarin (DHC) was added with final DHC concentration of 1 mmol/l. The absorbance was measured at 1 and 5 minutes.

The arylesterase and lactonase activities of PON1 were expressed as $\mu\text{mol/minute/ml} = \text{U/ml plasma}$.

Determination of blood plasma TAS

TAS was determined by the Trolox equivalent antioxidant capacity method. The absorbance was measured spectrophotometrically at a wavelength of 734 nm. The plasma antioxidant status was determined from the analytical curve for Trolox as a standard and expressed in mmol Trolox/l of plasma.

Determination of lipoperoxides in plasma

Lipoperoxides (LP) in plasma were determined as described by El-Saadani *et al.*¹⁹ Determination is based on the ability of peroxide to oxidize iodide (I^-) to iodine (I_2). Iodine in the reaction mixture then reacts with excess iodide to form I_3 with the absorption maximum at 365 nm at which was spectrophotometrically measured. LP concentrations were calculated by the formula:

$$c[\text{nmol/ml}] = \frac{A_{\text{SA}}}{\epsilon(I_3)} \times DF$$

where A_{SA} is the absorbance of the sample, $\epsilon(I_3)$ is the molar absorption coefficient for $\text{I}_3 = 24\,600\text{ mol/l/cm}$, and DF is the dilution factor.

Determination of AGEs in plasma

Determination of AGEs was based on the spectrofluorimetric method of Henle *et al.*²⁰ Plasma samples were centrifuged at 3000 g, for 5 minutes at

4°C. The samples were then diluted to 1:40 with phosphate-buffered saline pH 7.4 and fluorescence intensity was recorded at the emission maximum ($\sim 370\text{ nm}$) upon excitation at 465 nm. Fluorescence intensity was expressed in arbitrary units (AU)/g protein.

Determination of protein concentrations

Protein concentrations were determined using the Lowry method.²¹ Bovine serum albumin was used as a standard. Protein concentrations were expressed as mg/ml of plasma.

Determination of protein carbonyls in plasma

Protein carbonyls (PCs) were measured by enzyme-linked immunosorbent assay according to Buss *et al.*²² The absorbances were read at 492 nm and expressed in nmol/mg protein.

Determination of glutathione level in plasma

Glutathione (GSH) level was measured by high-performance liquid chromatography (HPLC) according to Garaiova *et al.*²³ GSH levels were expressed in $\mu\text{mol/l}$.

MDA determination

MDA level was determined by HPLC using a LaChrom, Hitachi, FL Detector L-7480 (Merck, Darmstadt, Germany). 1,1,3,3-Tetraethoxypropane was used as the standard.

The reaction mixture contained 50 μl sample or standard, 250 μl phosphoric acid (1.22 mol/l), 450 μl distilled water, and 250 μl thiobarbituric acid (0.046 mol/l) was incubated at 95°C for 60 minutes. On cooling the reaction mixture, 360 μl HPLC-grade methanol and 40 μl NaOH (1 mol/l) were added to 200 μl sample or standard reaction mixture and centrifuged for 5 minutes at 9500 g. Ten microlitres of the supernatant was used for further HPLC analysis. Mobile phase: 40% (v/v) methanol in 25 mmol/l phosphate buffer, pH 6.5; flow rate: 0.8 ml/minute; column: Lichrocart 250-4 Lichrospher 100 RP-18 (5 μm); fluorescence detector: FD 532/552 nm. The MDA concentration was expressed in μmol of MDA per litre of plasma.

Statistical analysis

Data are presented as median with an interquartile range (Q1–Q3, 25–75%). For statistical analysis, we employed the statistical programme StatsDirect® 2.3.7 (Stats Direct Sales, Sale, Cheshire M33 3UY, UK). The Box–Whisker test was used to determine any deviation from normality. For statistical comparison between the control and diabetic groups, the Mann–Whitney test was used. The remaining results were evaluated using the non-parametric Kruskal–Wallis test, and for *post hoc* pairwise comparisons, the Conover–Inman test was used.

Correlations between the variables were determined by regression analysis. Statistical significance of correlations was evaluated by Spearman's rank correlation.

Results

Effect of plant oil on parameters of glycaemic control

Treatment with STZ resulted in significantly ($P < 0.001$) increased plasma levels of glucose and fructosamine (Table 1) in the diabetic rats compared to the non-diabetic rats. Oil intake had no effect on these levels for either the non-diabetic or diabetic groups (Table 1).

Effect of plant oil on lipid profile

When the diabetic rats (D) were compared with the non-diabetic rats, TCH, TAG, LDL-chol, HDL-chol, and VLDL-chol levels were higher than those in the non-diabetic rats by 50% for TCH ($P < 0.001$), 93% for TAG ($P < 0.001$), 13% for LDL-chol, 90% for HDL-chol ($P < 0.001$), and 50% for VLDL-chol ($P < 0.05$) (Table 1).

The plant oil supplement did not have any effect on the lipid profiles of the non-diabetic rats in Groups CC1 or CC2 (Table 1). Comparison of the diabetic rats supplemented with the plant oil (Groups DC1 and DC2) with the control diabetic rats (D) indicated a dose response to the oil supplementation. At 500 mg/kg BW, all lipid parameters were significantly ($P < 0.05$) higher than those for the control rats (Table 1) while at 100 mg/kg BW, only LDL-chol ($P < 0.05$) and VLDL-chol ($P < 0.05$) were significantly elevated (Table 1).

Effect of plant oil on antioxidant enzyme activities, GSH level, and TAS of plasma

GPx activity and the GSH level were significantly decreased ($P < 0.001$, $P < 0.05$) in the diabetic rats compared with the control group. However, there were no significant changes in SOD, the CAT activity, the lactonase activity of PON1, or the TAS level in the plasma (Table 2).

In the non-diabetic group, supplementation with the plant oil affected the antioxidant status of the rats. At 100 mg/kg BW intake (CC1), the SOD levels (Fig. 1A) were significantly ($P < 0.001$) higher than in the controls but GPx ($P < 0.001$) and the arylesterase activity of PON1 ($P < 0.05$) were significantly lower (Table 2) suggesting a dose effect. In the CC2 group, TAS ($P < 0.05$) was also significantly lower than in the control group.

In the diabetic control group (D), GSH level ($P < 0.05$), GPx ($P < 0.001$), and the arylesterase activity of PON1 ($P < 0.05$) were significantly lower than in the non-diabetic control rats (C).

Table 1 Influence of plant oil on parameters of glycaemic control and the lipid profile of plasma

Parameters	C	CC1	CC2	D	DC1	DC2
Glucose (mmol/l)	10.67 (9.64–11.35)	11.69 (9.69–13.16)	11.28 (10.53–11.99)	42.39** (23.28–45.96)	40.14 (38.45–48.37)	43.88 (42.23–47.68)
Fructosamine (μmol/l)	20.5 (19.75–22.0)	24.5 (22.75–26.5)	21.0 (18.5–24.5)	164.0** (153–178.5)	165.0 (148.25–177)	174.0 (164–200.5)
Total cholesterol (mmol/l)	1.52 (1.37–1.73)	1.65 (1.49–1.7)	1.7 (1.52–1.7)	2.28** (2.17–2.45)	2.52 (2.16–2.59)	2.67* (2.54–2.8)
Triacylglyceride (mmol/l)	0.85 (0.72–0.88)	0.84 (0.76–0.96)	0.96 (0.58–0.89)	1.64** (1.18–2.14)	2.11 (1.58–2.94)	3.26* (2.8–4.42)
LDL-chol (mmol/l)	0.15 (0.12–0.18)	0.17 (0.17–0.2)	0.17 (0.14–0.2)	0.17 (0.16–0.18)	0.20* (0.18–0.25)	0.28* (0.2–0.33)
HDL-chol (mmol/l)	0.52 (0.47–0.57)	0.51 (0.49–0.56)	0.59 (0.45–0.54)	0.99** (0.92–1.01)	1.05 (0.84–1.06)	1.14* (1.09–1.17)
VLDL-chol (mmol/l)	0.38 (0.41–0.33)	0.41 (0.44–0.34)	0.37 (0.75–0.3)	0.57* (0.81–0.53)	0.89* (1.34–0.72)	1.48* (2.0–1.28)

Values are expressed as median with an interquartile range (Q1–Q3; 25–75%). Significance was estimated by the Kruskal–Wallis test and *post hoc* pairwise comparison of the Conover–Imman test. We compared the CC1, CC2, D groups to the C group: * $P < 0.05$; ** $P < 0.001$ and the DC1 and DC2 groups to the D group: * $P < 0.05$; ** $P < 0.001$. C, the control group without oil administration; CC1, the non-diabetic group treated daily with plant oil with a concentration of 100 mg/kg BW; CC2, the non-diabetic group treated daily with plant oil with a concentration of 500 mg/kg BW; D, the diabetic group without oil administration; DC1, the diabetic group treated daily with plant oil with a concentration of 100 mg/kg BW; DC2, the diabetic group treated daily with plant oil with a concentration of 500 mg/kg BW.

Table 2 Influence of plant oil on antioxidant enzyme activities, GSH level, and TAS of plasma

Parameters	C	CC1	CC2	D	DC1	DC2
Cu/Zn SOD (U/mg Hb)	45.27 (44.03–47.26)	98.41** (96.2–119.46)	115.06** (111.17–121.11)	45.09 (43.64–48.83)	59.36*** (57.45–64.41)	60.11*** (57.79–61.31)
CAT (U/mg Hb)	0.166 (0.163–0.177)	0.162 (0.156–0.176)	0.161 (0.146–0.176)	0.164 (0.144–0.169)	0.164 (0.145–0.178)	0.121* (0.099–0.137)
GPx U/mg Hb)	0.631 (0.6–0.673)	0.217** (0.202–0.217)	0.097** (0.067–0.129)	0.279** (0.251–0.304)	0.041*** (0.039–0.445)	0.052*** (0.051–0.059)
Arylesterase activity of PON1 (U/ml)	147.18 (138.31–165.79)	123.15* (117.11–128.61)	113.7* (108.72–130.56)	122.27* (106.49–130.82)	101.0 (87.19–117.6)	117.19 (106.34–133.43)
Lactonase activity of PON1 (U/ml)	29.43 (26.14–31.84)	29.84 (29.2–32.7)	29.17 (28.19–30.59)	25.88 (25.52–31.64)	23.47 (20.46–27.23)	23.3 (19.59–27.22)
TAS (mmol/l)	1.34 (0.69–1.45)	1.51 (1.39–1.62)	0.85* (0.63–1.05)	1.57 (1.32–2.02)	0.49*** (0.43–0.69)	0.44*** (0.34–0.54)
GSH (μmol/l)	49.21 (40.42–49.65)	37.4 (24.83–43.4)	40.2 (36.75–46.18)	30.02* (25.14–32.07)	19.24*** (13.97–19.92)	20.06* (19.05–20.81)

Values are expressed as median with an interquartile range (Q1–Q3; 25–75%). Significance was estimated by the Kruskal–Wallis test and *post hoc* pairwise comparison of the Conover–Inman test. We compared the CC1, CC2, D groups to the C group: * $P < 0.05$; ** $P < 0.001$ and the DC1 and DC2 groups to the D group: *** $P < 0.05$; **** $P < 0.001$. C, the control group without oil administration; CC1, the non-diabetic group treated daily with plant oil with a concentration of 100 mg/kg BW; CC2, the non-diabetic group treated daily with plant oil with a concentration of 500 mg/kg BW; D, the diabetic group without oil administration; DC1, the diabetic group treated daily with plant oil with a concentration of 100 mg/kg BW; DC2, the diabetic group treated daily with plant oil with a concentration of 500 mg/kg BW. CAT, catalase; Cu/Zn SOD, Cu/Zn superoxide dismutase; GPx, glutathione peroxidase; PON1, paraoxonase-1; TAS, total antioxidant status.

Supplementing the diabetic rats with the plant oil caused significant increases ($P < 0.001$) in the SOD levels and in Group DC1, there were significant decreases in the GPx ($P < 0.001$), TAS ($P < 0.001$), and GSH ($P < 0.001$) levels. In the DC2 group, significant decreases in CAT ($P < 0.05$), GPx ($P < 0.001$), TAS ($P < 0.001$), and GSH ($P < 0.05$) were observed (Table 2).

Effect of plant oil on selected oxidative and glycooxidative stress markers

In the diabetic rats, significant increases in the levels of oxidative and glycooxidative stress markers were observed: LP by 162% ($P < 0.05$); MDA by 226% ($P < 0.001$); PC by 35% ($P < 0.001$); AGEs by 67% ($P < 0.001$) (Table 3).

The plant oil supplement had a significant effect on the LP level ($P < 0.05$) and the AGEs, MDA, and PC levels ($P < 0.001$) of the non-diabetic rats in Group CC1. In the CC2 group, AGEs, MDA, and PC levels were also significantly ($P < 0.001$) higher than in the control group (Table 3). Supplementing the diabetic rats with the plant oil did not significantly influence the levels of PC and MDA. A significant decrease ($P < 0.05$) in the AGEs level was observed in the DC1 group. In the DC2 group, significant increases in both the LP ($P < 0.05$) and AGEs ($P < 0.001$) levels were observed (Table 3).

Discussion

Many clinical trials have been engaged in the study of n-6 PUFA effects on diabetes.^{7,24,25} N-6 PUFAs are well known for their proinflammatory and prooxidative effects. In this study, we confirmed that daily supplementation of n-6 PUFA to rats over a period of 7 weeks resulted in increased levels of oxidative damage of lipids (LP, MDA) and proteins (PC) as well as glycooxidative damage of lipids and proteins (AGEs). The prooxidative effects of n-6 PUFAs¹¹ are known and are related to the double bonds present in LA, which is the major component of the administered plant oil, which are prone to oxidation.²⁶ Hyperglycaemia contributes to the elevated generation of free radicals, as well as to oxidative stress. Increased free radical level causes oxidation and damage of biomolecules such as lipids and proteins resulting in LP, MDA, PC, and AGEs formation and positive correlations between hyperglycaemia and markers of oxidative stress were found in the case of AGEs ($r = 0.88$, $n = 7$, $P = 0.0119$) in the diabetic group DC2.

The plant oil influenced antioxidant enzyme activities with respect to the progression of diabetes over the 7 weeks in rats. Under oxidative stress and long-term diabetes, the antioxidant enzyme activities are changed.²⁶ GPx was the only enzyme with decreased activity in the diabetic rats in Group D to the

Figure 1 Influence of plant oil on activities of Cu/Zn SOD (A) and selenium-dependent GPx (B) in erythrocytes. Values are expressed as median with an interquartile range (Q1–Q3; 25–75%). Significance was estimated by the Kruskal–Wallis test and *post hoc* pairwise comparison of the Conover–Inman test. We compared the CC1, CC2, D groups to the C group: * $P < 0.05$; ** $P < 0.001$ and the DC1 and DC2 groups to the D group: * $P < 0.05$; ** $P < 0.001$. C, the control group without oil administration; CC1, the non-diabetic group treated daily with plant oil with a concentration of 100 mg/kg BW; CC2, the non-diabetic group treated daily with plant oil with a concentration of 500 mg/kg BW; D, the diabetic group without oil administration; DC1, the diabetic group treated daily with plant oil with a concentration of 100 mg/kg BW; DC2, the diabetic group treated daily with plant oil with a concentration of 500 mg/kg BW.

control group. However, Cu/Zn SOD and CAT activities in the diabetic rats in Group D were similar to the non-diabetic control rats (Group C). We did not show any correlation of antioxidant enzymes with AGEs, suggesting that Cu/Zn SOD and CAT were not probably glycated and therefore their activities are not changed. However, it is possible that the decline in GPx activity may be due to the low glutathione reductase (GR) activity and the GSH level which have been observed during diabetes.^{26,27} Impaired GSH defence in the diabetic rats may also contribute to high levels of MDA,²⁸ although a negative correlation of MDA with GSH was observed in the non-diabetic group CC1.

High oxidative stress is also linked to an H_2O_2 level increase. Increased production of H_2O_2 results in a decrease in Cu/Zn SOD activity.^{27–29} We did not observe any decrease in Cu/Zn SOD activity in this study, but there may be insufficient H_2O_2 accumulated to exert an inhibitory effect on Cu/Zn SOD. Normally, H_2O_2 is further metabolized to H_2O and O_2 by CAT but this activity was not changed in the Group D diabetic rats compared to the control rats in Group C.

We observed that the plant oil significantly decreased the antioxidant enzyme activities, especially GPx, and CAT. GPx activity in both the diabetic and the non-diabetic groups was significantly reduced after oil intake. Our results are in accordance with Chung *et al.*³⁰ We also observed a simultaneous GSH level decrease, which may indicate reduced activity of GR. Decreased GR activity could also be the consequence of NADPH consumption in the sorbitol pathway, since NADPH is a cofactor for aldose reductase as well as for GR.^{31,32} During times of hyperglycaemia and high oxidative stress, GPx can be glycated and inhibited by structural alteration affecting the active

site.³³ Reduced GPx activity has been observed in diabetic patients, possibly as a result of increased lipid peroxidation³⁴ and in this study, reduced GPx activity was observed in both the diabetic and the non-diabetic groups, although this correlation was not significant.

The only enzyme with elevated activity after oil intake for both the non-diabetic and the diabetic rats was Cu/Zn SOD. This activation can be caused by an increased superoxide ion generation under oxidative stress conditions. However, there could be an alternative explanation for this increase in Cu/Zn SOD. PUFAs may act at gene level³⁵ and may elevate the gene expression of Cu/Zn SOD.³⁶ An elevated Cu/Zn SOD activity has also been observed in the kidneys of STZ-induced diabetic rats.²⁸ An increased Cu/Zn SOD activity may also elevate H_2O_2 generation which is a substrate for CAT. Therefore, we expected an elevated CAT activity. However, we observed a lower CAT activity after oil intake, which can be caused by H_2O_2 consumption in reactions such as the generation of free radicals but it could also be that the CAT becomes inhibited and is unable to scavenge high levels of H_2O_2 .²⁸

There was no significant reduction in PON1 activity in the diabetic rats. Reduced PON1 activity may be linked to high levels of LP and MDA which inhibit PON1⁵ and this is supported by the negative correlations observed between PON1 with LP ($r = -0.815$, $n = 5$, $P = 0.0417$) and with MDA ($r = -0.514$, $n = 10$, $P = 0.044$) in Group DC2 rats.

HDL-chol is able to protect LDL-chol from lipid peroxidation due to the antioxidative activity of PON1.⁵ However, we observed no significant correlation of PON1 with HDL-chol in the diabetic group, although we detected a marginally significant correlation ($r = 0.788$, $n = 8$, $P = 0.066$) in Group

Table 3 Influence of plant oil on parameters of oxidative and glycooxidative stress in plasma

Parameters	C	CC1	CC2	D	DC1	DC2
LP (nmol/ml)	39.35 (37.77–42.48)	63.94* (63.49–70.43)	41.73 (38.16–44.86)	103.29* (83.05–178.3)	127.73 (125.87–193.47)	368.6 [■] (268.77–455.35)
MDA (μmol/l)	0.035 (0.031–0.048)	0.086** (0.069–0.092)	0.066** (0.059–0.075)	0.114** (0.109–0.132)	0.15 (0.12–0.18)	0.13 (0.12–0.16)
PC (nmol/mg protein)	0.34 (0.33–0.35)	0.44** (0.39–0.46)	0.42** (0.41–0.45)	0.46** (0.43–0.49)	0.49 (0.44–0.52)	0.5 (0.45–0.52)
AGEs (AU/g protein)	36.33 (33.01–37.42)	49.52** (48.48–51.77)	47.99** (45.35–53.9)	60.78** (58.71–61.82)	69.37 [■] (62.49–77.74)	90.45 [■] (88.7–99.73)

Values are expressed as median with an interquartile range (Q1–Q3; 25–75%). Significance was estimated by the Kruskal–Wallis test and *post hoc* pairwise comparison of the Conover–Iman test. We compared the CC1, CC2, D groups to the C group: * $P < 0.05$; ** $P < 0.001$ and the DC1 and DC2 groups to the D group: [■] $P < 0.05$; [■] $P < 0.001$. C, the control group without oil administration; CC1, the non-diabetic group treated daily with plant oil with a concentration of 100 mg/kg BW; CC2, the non-diabetic group treated daily with plant oil with a concentration of 500 mg/kg BW; D, the diabetic group without oil administration; DC1, the diabetic group treated daily with plant oil with a concentration of 100 mg/kg BW; DC2, the diabetic group treated daily with plant oil with a concentration of 500 mg/kg BW. AGEs, advanced glycation end products; PC, protein carbonyl; LP, lipoperoxide; MDA, malondialdehyde.

D. Under hyperglycaemic conditions, it is feasible that PON1 and thus HDL-chol can be glycated which is supported by the negative correlation between PON1 and the glucose level ($r = -0.815$, $n = 5$, $P = 0.0331$). HDL proteins damaged by glycation may not be able to bind to PON1, which could result in a reduced PON1 activity. PON1 activity can also be decreased by high levels of LDL-chol which oxidizes and thus inhibits PON1 under high oxidative stress conditions and also elevates the AGE levels.⁵ This hypothesis is supported by the trend towards a significant negative correlation of LDL-chol with PON1 ($r = -0.561$, $n = 8$, $P = 0.0694$) in the DC2 diabetic group as well as by the positive correlation between LDL-chol and AGEs ($r = 0.822$, $n = 11$, $P = 0.0052$) in the DC1 diabetic group. Our results are in accordance with Rasic-Milutinovic *et al.*,⁵ who reported lower PON1 activity in the diabetic patients after n-6 PUFA intake.

Significantly increased plasma TAG levels ($P < 0.05$) after oil intake in Group DC2 diabetic rats could be due to insufficient bonding between PUFAs and peroxisome proliferator-activated receptor α (PPAR α). This leads to a blockade of the PUFA inhibitory effect on lipogenesis resulting in an increased TAG formation as seen in other studies^{35,37} showing that PUFAs (mainly the n-3 PUFAs) increase the transcription of genes involved in β -oxidation, such as carnitine palmitoyltransferase-1 and acylCoA oxidase, by the bonding between PUFAs and PPAR α . The effects of n-6 PUFAs at the gene level require further research. Significantly increased TCH level ($P < 0.05$) was observed in the Group DC2 diabetic rats. Significantly elevated LDL-chol level ($P = 0.0059$) and VLDL-chol level ($P = 0.0012$) were seen in the DC1 diabetic rats. However, it is surprising that the levels of TCH and LDL-chol are both increased together with the HDL-chol level ($P < 0.05$) which contrasts with many clinical trials.³⁸ This difference may be related to differences between human and rat metabolism. Unlike humans, in rats 60% of the cholesterol is transported as HDL-chol.³⁹ Such a difference may explain the simultaneously elevated levels of TCH and HDL-chol in the diabetic rats in Groups DC1 and DC2. We also observed positive correlations of TCH with HDL-chol (DC1 group: $r = 0.89$, $n = 12$, $P < 0.05$; DC2 group: $r = 0.89$, $n = 7$, $P < 0.05$).

Increased hyperglycaemia is linked to a higher oxidative stress and lowered antioxidant enzyme activities leading to a decreased TAS of plasma in the diabetic rats ($P < 0.001$) (Fig. 2). Higher levels of oxidative stress are also associated with an elevated generation of markers of lipid and protein oxidative damage as a consequence of decreased TAS. This was confirmed by the negative correlations of TAS with LP

Figure 2 Influence of plant oil on levels of LPs (A) and advanced glycooxidation end-products (B) in plasma. Values are expressed as median with an interquartile range (Q1–Q3; 25–75%). Significance was estimated by the Kruskal–Wallis test and *post hoc* pairwise comparison of the Conover–Inman test. We compared the CC1, CC2, D groups to the C group: * $P < 0.05$; ** $P < 0.001$ and the DC1 and DC2 groups to the D group: * $P < 0.05$; ** $P < 0.001$. C, the control group without oil administration; CC1, the non-diabetic group treated daily with plant oil with a concentration of 100 mg/kg BW; CC2, the non-diabetic group treated daily with plant oil with a concentration of 500 mg/kg BW; D, the diabetic group without oil administration; DC1, the diabetic group treated daily with plant oil with a concentration of 100 mg/kg BW; DC2, the diabetic group treated daily with plant oil with a concentration of 500 mg/kg BW.

($r = -0.656$, $n = 6$, $P = 0.016$) and AGEs ($r = -0.963$, $n = 6$, $P = 0.0083$) in the Group DC2 diabetic rats.

In summary, we conclude that supplementation with an n-6 PUFA plant oil with a significant content of LA, might be acting prooxidatively, leading to the generation of oxidative stress in rats.

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