Troglitazone increases the resistance of low density lipoprotein to oxidation in healthy volunteers

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Summary The oxidative modification of low density lipoprotein is of importance in atherogenesis. Antioxidant supplementation has been shown, in published work, to increase low density lipoprotein resistance to oxidation in both healthy subjects and diabetic subjects; in animal studies a contemporary reduction in atherogenesis has been demonstrated. Troglitazone is a novel oral antidiabetic drug which has similarities in structure with vitamin E. The present study assessed the effect of troglitazone 400 mg twice daily for 2 weeks on the resistance of low density lipoprotein to oxidation in healthy male subjects. Ten subjects received troglitazone and ten received placebo in a randomised, placebo-controlled, parallel-group design. The lag phase (a measure of the resistance of low density lipoprotein to oxidation) was determined by measurement of fluorescence development during copper-catalysed oxidative modification of low density lipoprotein. The lag phase was increased by 27% ($p < 0.001$) at week 1 and by 24% ($p < 0.001$) at week 2 in the troglitazone treated group compared with the placebo group. A number of variables known to influence the resistance of low density lipoprotein to oxidation were measured. They included macronutrient consumption, plasma and lipoprotein

lipid profile, alpha-tocopherol, beta-carotene levels in low density lipoprotein, low density lipoprotein particle size, mono and polyunsaturated fatty acid content of low density lipoprotein and pre-formed low density lipoprotein hydroperoxide levels in low density lipoprotein. Troglitazone was associated with a significant reduction in the amount of pre-formed low density lipoprotein lipid hydroperoxides. At weeks 1 and 2, the low density lipoprotein hydroperoxide content was 17% ($p < 0.05$) and 18% ($p < 0.05$) lower in the troglitazone group compared to placebo, respectively. In summary the increase in lag phase duration in the troglitazone group appeared to be due to the compound's activity as an antioxidant and to its ability to reduce the amount of pre-formed low density lipoprotein lipid hydroperoxides. This antioxidant activity could provide considerable benefit to diabetic patients where atherosclerosis accounts for the majority of total mortality. [Diabetologia (1997) 40: 1211–1218]

Keywords LDL, LDL oxidation, antioxidant, lipid hydroperoxides, atherosclerosis, non-insulin-dependent diabetes mellitus.

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Abbreviations. BCA, Bicinchoninic acid; CAD, coronary artery disease; EDTA ethylenediaminetetraacetic acid; HDL, high density lipoprotein; HPLC, high performance liquid chromatography; 15-HETE, 15 hydroxyecosatetraenoic acid; LDL, low density lipoprotein; NIDDM, non-insulin-dependent diabetes (NIDDM); PUFAs, polyunsaturated fatty acids; VLDL, very low density lipoprotein.

Troglitazone, a thiazolidine-2-dione derivative, is a novel oral anti-diabetic drug under development for the treatment of non-insulin-dependent diabetes (NIDDM). It has been reported to enhance insulin sensitivity [1], increase the conversion of glucose to glycogen in rat HepG2 cells [1], reduce hepatic gluconeogenic enzyme activity in rats and mice [2] and to improve metabolic control in NIDDM patients [3, 4].

There is a much increased risk of coronary artery disease (CAD) in diabetes and around 60% of the

total mortality in diabetes is due to atherosclerosis and subsequent complications [5]. The reasons for this are not fully understood. However it is known that certain post-secretory modifications in the structure of lipoproteins, including the oxidative modifications of low density lipoprotein (LDL), render them more atherogenic [6]. LDL oxidation occurs within the artery wall; it is inhibited in plasma where many defence systems such as antioxidants and metal ion binding proteins exist [6]. Since oxidised LDL cannot be obtained from whole blood, considerable research has involved measurement of the susceptibility to in vitro oxidation of isolated LDL particles [7, 8]. This is increased in patient groups with CAD [9, 10] and at risk of developing CAD [11, 12]. Antioxidant supplementation has been shown to reduce the progression of atherosclerotic lesions in Watanabe hyperlipidaemic rabbits [13] and to increase the resistance of LDL to oxidation in both healthy subjects [14, 15] and diabetic subjects [16]. Two large-scale studies have shown that supplementation with high doses of vitamin E was associated with significantly decreased risk of CAD [17, 18]. Troglitazone has similarities in structure with vitamin E. In vitro studies [19, 20], in which the compound was added to pooled plasma, indicated that the compound possessed antioxidant activity, probably acting as a free radical scavenger. The present study compares the effect of troglitazone with that of placebo, administered to healthy volunteers, on the susceptibility of LDL to copper-mediated oxidation. The study also analyses data for variables which are known to influence the susceptibility of LDL to oxidation, and which may aid interpretation of results. These include the LDL fatty acid composition, alpha-tocopherol and beta-carotene concentrations, hydroperoxide concentration and LDL particle size.

Materials and methods

Subjects. Twenty healthy male subjects aged between 21 and 45 years, with a body mass index ranging between 20 and 29 kg/m², a fasting plasma glucose of <5.5 mmol/l and fasting plasma total cholesterol and triglyceride levels of <5.81 and <1.80 mmol/l respectively, participated in the study. The subjects were free from significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, haematological, metabolic and psychiatric disease as determined by history, physical examination and laboratory screen. Subjects who had received a regular course of medication during the 4 weeks prior to the study, cholesterol lowering medications or vitamin supplementation during the previous 3 months, or probucol during the previous 12 months were excluded from the study. Subjects who smoked more than 5 cigarettes a day or who regularly drank more than 4 units of alcohol per day or who drank excessive coffee were excluded from the study. Subjects received a full verbal and written explanation of the nature and purposes of the study and each gave written informed consent prior to participation in the study. The study was conducted in accordance with the Declaration of Helsinki, and its revisions.

Safety evaluations. Subjects underwent a medical examination which included history, physical examination, 12-lead ECG and laboratory safety screens during the 3 weeks prior to the first dose.

Further blood and urine samples for laboratory safety tests were obtained pre-dose 1 (day 1), pre-dose 29 (day 15) and 2 weeks after the last dose (day 29).

Treatment details. This was a double-blind, randomised, placebo-controlled, parallel group study, in which 10 subjects received troglitazone 400 mg (as 2 × 200 mg tablets) and 10 received matching placebo, twice daily for 14.5 days.

Subjects were randomly assigned to one of two groups to receive treatment according to the randomisation code. Doses were taken with 100 ml water, at the start of breakfast and evening dinner at approximately 12 h intervals.

During the study period, from 1 week prior to the first dose until 2 weeks after the last dose, the subjects were required to: 1) adhere strictly to their usual diet and lifestyle, avoiding excess; 2) not consume more than 4 cups of caffeinated coffee per day; 3) avoid strenuous exercise which was not part of their usual routine; 4) not smoke for 24 h before any blood sample in the study period; 5) not consume more than 4 units of alcohol per day; 6) not take any other medication.

Food intake. The quantity and type of food consumed throughout the 5 week study period was recorded in a food diary. Total calories consumed per week, lipid, protein and carbohydrate consumption (%), total calories/week, were determined as previously described [21]; subjects used a conversion table to convert "portions" of various foods into weight in grams.

Methods. Subjects fasted from 21.30 hours on the evenings prior to blood sampling. Blood samples (5 ml into lithium heparin for plasma troglitazone assay and 60 ml into EDTA 1 mg/ml) were obtained by venepuncture between 08.00–08.45 hours (i.e. 12 h ± 45 min after the previous dose) on the following occasions: pre-dose 1 (day 1), pre-dose 15 (day 8), pre-dose 29 (day 15), 2 weeks post study (day 29). Additional samples were obtained at 2 h post doses 1 and 29. The tubes were kept on ice and centrifuged at 2000 rev/min for 20 min at 4°C with in approximately 15 min of collection. The plasma was processed for the separation of lipoprotein within 5 h.

The following variables were measured at each time point: plasma, high density lipoprotein (HDL), LDL and very low density lipoprotein (VLDL) cholesterol and triglyceride; susceptibility of LDL to in vitro copper-mediated oxidative modification; alpha-tocopherol and beta-carotene content of LDL; LDL hydroperoxides as 15-hydroxyecosatetraenoic acid (15-HETE); LDL mono (oleic acid) and polyunsaturated (linoleic, linolenic, arachidonic, eicosapentaenoic acids) fatty acids; LDL particle size; plasma and LDL troglitazone concentrations.

Lipoprotein separation. Lipoproteins were isolated by sequential ultracentrifugation in NaBr solutions [22] containing 1 mg/ml EDTA and stored at 4°C. LDL was stored under nitrogen at 4°C in a sterile, dark environment and used within 1 day. Cholesterol and triglyceride levels in plasma and lipoprotein fractions were determined by Technicon autoanalyzer II (Technicon, Instruments Corp., Tarrytown, N.Y., USA) methodology. Protein was measured by the Pierce BCA protein assay reagent [23].

LDL oxidation. The method for LDL oxidation with Cu⁺⁺ and for the evaluation of its susceptibility to oxidation, i.e. evaluation of the length of the lag phase, was based on

Table 1. Lag phase (min) during multiple oral administration of troglitazone 400 mg twice daily or placebo: statistical analysis

Occasion	Troglitazone adjusted mean ^a (n = 10)	Placebo adjusted mean ^a (n = 9)	Ratio (T/P)	95 % confidence interval	p-value
2 h post-dose 1 (day 1)	81.6	74.3	1.10	(1.01, 1.19)	0.030
Pre-dose 15 (day 8)	96.2	75.8	1.27	(1.22, 1.32)	< 0.001
Pre-dose 29 (day 15)	97.9	79.2	1.24	(1.13, 1.35)	< 0.001
2 h post-dose 29 (day 15)	93.7	74.9	1.25	(1.15, 1.36)	< 0.001
2 weeks post study (day 29)	80.5	74.8	1.08	(1.00, 1.16)	0.056

^a Geometric mean adjusted for pre-dose values

development of fluorescence during copper-catalysed LDL oxidative modification as described [8].

Alpha-tocopherol and beta-carotene measurement. LDL alpha-tocopherol was measured by an HPLC method as previously described [24]. Beta-carotene was evaluated from the absorbance of LDL lipid extract at 450 nm using an extinction coefficient of 2590 mmol/l · cm⁻¹.

LDL hydroperoxide assay. For LDL hydroperoxide assay, aliquots of LDL were frozen at -80°C immediately after LDL was separated by ultracentrifugation. LDL hydroperoxides were measured as 15-HETE by a radioimmunoassay method using a commercially available kit (PerSeptive Diagnostics, Cambridge, Mass., USA). Prior to measurement 15-HETE was extracted as previously reported [25]. The sensitivity of the method was about 2.5 pg/0.1 ml. The intra-assay variation was less than 4 %.

LDL fatty acid content. LDL fatty acid content was analysed by gas-liquid chromatography [26]. Analysis was performed on a Hewlett-Packard 5890 II with a flame ionisation detector.

LDL particle composition and size. LDL size was estimated indirectly from the ratio of core (cholesteryl ester and triglyceride) to surface (protein, free cholesterol and phospholipid) components [27, 28]. Free cholesterol and phospholipids were measured by commercially available enzymatic reagents (Boehringer-Mannheim, Mannheim, Germany). Cholesteryl ester was derived from total-free cholesterol.

Troglitazone concentration. Troglitazone was extracted from plasma samples with a mixture of ethyl acetate and hexane and the extract analysed by liquid chromatography with ultra violet detection. Troglitazone was analysed in LDL in a similar manner except that solvent precipitation was used rather than solvent extraction. The lower limit of quantification for the assay was 100 ng/ml in plasma and 10 ng/ml in LDL.

Statistical analysis. Parameters were analysed separately using analysis of covariance allowing for effects due to treatment and including the relevant pre-dose 1 value as covariate. All parameters, except for calorie intake per week and the proportion consumed as lipid, carbohydrate or protein and body weight, were log transformed prior to analysis in order to improve the assumptions of homogeneity of variance and normality of distribution. For each parameter that was log transformed an estimate of the ratio of the adjusted geometric means for troglitazone relative to placebo was obtained together with a 95 % confidence interval and was tested for significance.

Results

Twenty subjects completed this study with no withdrawals. All adverse events reported were of a minor nature and none could be attributed with certainty to the administration of study treatments.

In one subject in the placebo group the plasma triglyceride level, although within the normal range at screening, was elevated at day 1 (376 mg/dl, pre-dose 1) decreasing thereafter. All data for this subject have been omitted from the calculation of means and from the statistical analysis.

Body weight and food intake. Body weight did not change during the study. Neither the total calorie consumption nor the percentage of calories consumed as lipid, or carbohydrate, changed significantly during the study. A statistically significant difference between the treatment groups was observed for the percentage of calories consumed as protein during week 2 such that consumption was 12 % lower ($p = 0.017$) in the troglitazone group.

Plasma and LDL troglitazone concentrations. Median plasma trough concentrations of troglitazone pre-dose 15 and pre-dose 29 were 496 ng/ml (range 254–850) and 420 ng/ml (range 208–565) respectively; at 2 h post-doses 1 and 29 they were 1715 ng/ml (range 1010–2490) and 1985 ng/ml (range 1120–2540). The amount of troglitazone incorporated into LDL was small, with median values of 32 ng/mg LDL protein (range 19–85) and 32 ng/mg LDL protein (range 10–48) pre-dose 15 and pre-dose 29 respectively. Values of troglitazone in the 2 h post-dose 1 and 29 samples were higher than those found pre-dose. Median values were 125 ng/mg LDL protein (range 65–174) and 159 ng/mg LDL protein (range 79–213) respectively.

Lag phase. The geometric mean lag phase for the placebo group pre-dose 1 was 83.1 min (range 70.5–106.2 min) and for the troglitazone group was 76.4 min (range 66.9–93.4 min). Adjusted mean values and the statistical analysis of lag phase are shown in Table 1. The duration of the lag phase (i.e. the resistance to LDL to oxidation) increased significantly during troglitazone administration. The lag phase

Fig 1. Geometric mean lag phase for the oxidative modification of LDL from healthy subjects against time

Fig 2. Ratio (%) of adjusted means for troglitazone 400 mg twice daily and placebo at each time point for lag phase (upper section) and pre-formed LDL lipid hydroperoxides (lower section)

was 27% (CI 22%, 32%; $p < 0.001$) and 24% (CI 13%, 35%; $p < 0.001$) higher in the troglitazone group compared to the placebo group at pre-dose 15 (week 1) and pre-dose 29 (week 2), respectively. The geometric mean lag phase for the oxidative modification of LDL from healthy subjects against time is represented in Figure 1. Troglitazone also increased the duration of the lag phase in the 2 h post-dose samples (Table 1). The lag phase was 10 and 25% higher in the troglitazone group compared to the placebo group post-doses 1 and 29, respectively. Both increases were statistically significant.

LDL pre-formed lipid hydroperoxides. Adjusted mean values and the statistical analysis of LDL hydroperoxides are shown in Table 2. Troglitazone significantly reduced the amount of LDL lipid hydroperoxides. At weeks 1 and 2 the pre-dose LDL lipid hydroperoxides content was 17% (CI 5%, 28%; $p < 0.05$) and 18% (CI 2%, 32%; $p < 0.05$) lower in the troglitazone group compared to placebo, respectively. Figure 2 shows the ratio (%) of adjusted means for troglitazone 400 mg twice daily and placebo at each time point for lag phase (upper section) and

pre-formed LDL lipid hydroperoxides (lower section). Interestingly the highest values of lag phase matched the lowest concentrations of pre-formed LDL hydroperoxides.

Other parameters. There were no changes in plasma and lipoprotein cholesterol. Plasma and VLDL triglyceride levels in the fasting samples obtained pre-dose 15, pre-dose 29 and 2 weeks post study did not change significantly during the study. Non-fasting plasma and VLDL triglyceride concentrations at 2 h post-dose 1 were 23 and 22% lower in the troglitazone group compared to the placebo ($p = 0.002$ and $p = 0.014$, respectively). These effects were not seen at 2 h post-dose 29. Adjusted mean values and the statistical analysis of plasma triglycerides are shown in Table 3. LDL and HDL triglyceride levels did not change during the study. No statistically significant differences were observed between the two treatment groups for levels of alpha-tocopherol and beta-carotene. There were no changes in LDL particle size. Adjusted mean values and the statistical analysis of LDL size are shown in Table 4. There were no changes in polyunsaturated fatty acids (PUFAs) measured: linoleic, linolenic, arachidonic and eicosapentaenoic acid. A statistically significant difference between the treatment groups was observed pre-dose 15 with oleic acid 21% higher in the troglitazone group compared to the placebo group ($p = 0.049$). However the ratio of LDL oleic acid content to linoleic acid content did not significantly change during the study. Adjusted mean values and the statistical analysis for oleic acid and for the major LDL PUFAs (linoleic and arachidonic acid) are shown in Table 5.

Discussion

In view of the difficulties in identifying oxidised lipoprotein in plasma, many workers have attempted to analyse the oxidation susceptibility of isolated LDL particles [7, 8]. There is growing evidence of a relationship between susceptibility of LDL to oxidation and atherosclerotic risk [9–12]. Furthermore the reduced progression of atherosclerosis in antioxidant-treated animals is associated with decreased susceptibility of LDL to oxidation [29]. A novel strategy in the prevention of atherosclerosis could therefore be to prevent the deleterious effect of oxidised LDL by reducing the oxidation rate of LDL. In the present study the administration of troglitazone 400 mg twice daily for 2 weeks to healthy subjects increased the lag phase by approximately 24%. This effect was evident at both 1 and 2 weeks of treatment. Our data are consistent with other studies in which an increase of LDL antioxidants, by incubation or oral supplementation, always resulted in a strict proportional increase in the length of the lag phase [14, 20, 15, 30]. Oxidative

Table 2. LDL lipid hydroperoxides (pg/mg LDL protein) during multiple oral administration of troglitazone 400 mg twice daily or placebo: statistical analysis

Occasion	Troglitazone adjusted mean ^a (n = 10)	Placebo adjusted mean ^a (n = 9)	Ratio (T/P)	95 % confidence interval	p-value
2 h post-dose 1 (day 1)	1010	1168	0.86	(0.78, 0.96)	< 0.009
Pre-dose 15 (day 8)	905	1093	0.83	(0.72, 0.95)	< 0.013
Pre-dose 29 (day 15)	865	1059	0.82	(0.68, 0.98)	< 0.036
2 h post-dose 29 (day 15)	916	1141	0.80	(0.69, 0.94)	< 0.001
2 weeks post study (day 29)	948	1046	0.91	(0.78, 1.06)	< 0.197

^a Geometric mean adjusted for pre-dose values**Table 3.** Plasma triglycerides (mmol/l) during multiple oral administration of troglitazone 400 mg twice daily or placebo

Occasion	Troglitazone adjusted mean ^a (n = 10)	Placebo adjusted mean ^a (n = 9)	Ratio (T/P)	95 % confidence interval	p-value
2 h post-dose 1 (day 1)	0.89	1.16	0.77	(0.66, 0.89)	< 0.002
Pre-dose 15 (day 8)	0.90	0.89	1.01	(0.81, 1.25)	< 0.983
Pre-dose 29 (day 15)	0.98	0.94	1.04	(0.80, 1.39)	< 0.709
2 h post-dose 29 (day 15)	0.92	1.09	0.84	(0.66, 1.10)	< 0.204
2 weeks post study (day 29)	0.85	0.99	0.86	(0.59, 1.23)	< 0.363

^a Geometric mean adjusted for pre-dose values**Table 4.** LDL size (ratio of core to surface LDL components) during multiple oral administration of troglitazone 400 mg twice daily or placebo

Occasion	Troglitazone adjusted mean ^a (n = 10)	Placebo adjusted mean ^a (n = 9)	Ratio (T/P)	95 % confidence interval	p-value
2 h post-dose 1 (day- 1)	0.694	0.676	1.03	(0.99, 1.06)	< 0.120
Pre-dose 15 (day 8)	0.714	0.694	1.03	(0.99, 1.07)	< 0.175
Pre-dose 29 (day 15)	0.703	0.730	0.96	(0.91, 1.02)	< 0.182
2 h post-dose 29 (day 15)	0.698	0.701	1.00	(0.97, 1.03)	< 0.801
2 weeks post study (day 29)	0.697	0.693	1.01	(0.98, 1.03)	< 0.659

^a Geometric mean adjusted for pre-dose values

modification begins only when LDL has been largely depleted of its antioxidants [31], which suggests that the lag phase is mainly determined by the antioxidant content. On a molar basis, the major antioxidant in LDL is vitamin E, a chain breaking antioxidant. Nevertheless, vitamin E levels in LDL do not correlate with the duration of lag phase in non-supplemented subjects [14]. Vitamin E is therefore important, but not the only variable which determines the resistance of LDL to oxidation.

Prospective epidemiological studies have shown that phenotype B with small dense LDL is associated with an increased risk of CAD [32]. De Graaf et al. [33] separated LDL of normal subjects in three sub-fractions (LDL1: very light, LDL2: light, LDL3: dense) and assessed their oxidation resistance by measuring the diene compared to time profile in copper stimulated oxidation. They reported that dense LDL fractions (LDL2, LDL3) had a reduced lag phase (-20%) and contained more conjugated diene after 4 h oxidation, probably due to their higher

content in PUFAs. These results were also confirmed in patients with familial hypertriglyceridaemia, insulin-dependent and non-insulin-dependent diabetes and with coronary heart disease [28]. Furthermore it is generally believed that the amount of PUFAs and oleic acid in the LDL could be important for its susceptibility to oxidation [11, 34]. The first step in the LDL lipid peroxidation, in fact, is the formation of monohydroperoxy derivatives of fatty acids and linoleic and arachidonic acids have been demonstrated to be more susceptible to forming monohydroperoxy derivatives than other fatty acids [35]. Actually Esterbauer et al. [31] proposed a scheme in which the role of the copper in the oxidative process of LDL is to catalyse the conversion of trace amounts of pre-formed lipid hydroperoxides to alkoxy and peroxy radicals, which in turn start another round of lipid peroxidation. More recently Yoshida and Niki [36] reported that the rate of copper-catalysed oxidation of methyl linoleate, was proportional to the half power of the initial preformed peroxide concentration.

Table 5. LDL oleic, linoleic and arachidonic acid ($\mu\text{g}/\text{mg}$ LDL protein) during multiple oral administration of troglitazone 400 mg twice daily or placebo

Occasion	Troglitazone adjusted mean ^a (<i>n</i> = 10)	Placebo adjusted mean ^a (<i>n</i> = 9)	Ratio (T/P)	95 % confidence interval	<i>p</i> -value
<i>LDL oleic acid</i>					
2 h post-dose 1 (day 1)	182.4	186.0	0.98	(0.80, 1.20)	< 0.837
Pre-dose 15 (day 8)	197.8	163.6	1.21	(1.00, 1.46)	< 0.049
Pre-dose 29 (day 15)	169.7	169.1	1.00	(0.81, 1.24)	< 0.973
2 h post-dose 29 (day 15)	188.6	161.3	1.17	(0.98, 1.39)	< 0.076
2 weeks post study (day 29)	164.0	163.9	1.0	(0.85, 1.17)	< 0.994
<i>LDL linoleic acid</i>					
2 h post-dose 1 (day 1)	279.7	283.0	0.89	(0.81, 1.19)	< 0.839
Pre-dose 15 (day 8)	271.1	264.3	1.03	(0.89, 1.18)	< 0.696
Pre-dose 29 (day 15)	247.6	281.5	0.88	(0.77, 1.00)	< 0.063
2 h post-dose 29 (day 15)	276.9	280.7	0.99	(0.86, 1.13)	< 0.833
2 weeks post study (day 29)	258.8	262.6	0.99	(0.86, 1.13)	< 0.824
<i>LDL arachidonic acid</i>					
2 h post-dose 1 (day 1)	82.7	77.4	1.07	(0.86, 1.33)	< 0.522
Pre-dose 15 (day 8)	77.5	66.5	1.16	(0.91, 1.49)	< 0.204
Pre-dose 29 (day 15)	68.9	71.5	0.96	(0.78, 1.19)	< 0.713
2 h post-dose 29 (day 15)	77.5	72.0	1.08	(0.87, 1.33)	< 0.474
2 weeks post study (day 29)	81.1	72.2	1.12	(0.88, 1.44)	< 0.331

^a Geometric mean adjusted for pre-dose values

Moreover it has been demonstrated that Ebselen, a synthetic Se-containing compound possessing peroxidase activity, totally prevented the oxidative modification of LDL by Cu^{++} [37].

A number of variables known to influence the resistance of LDL to oxidation were therefore measured in this study. There were no consistently significant differences between the treatment groups in total calories consumed as lipid protein or carbohydrate. Alpha-tocopherol and beta-carotene levels in LDL did not alter significantly during the study period. As for the lipid profile, there were no changes in plasma and lipoprotein cholesterol. Fasting triglyceride values did not change during the study. Non-fasting plasma triglyceride at 2 h post-dose 1 (but not at 2 h post-dose 29) were 23 % lower in the troglitazone group compared to the placebo. This is an interesting finding that implies that troglitazone may have as yet unexplored therapeutic effects on altered postprandial triglyceridemic response, a condition associated with increased progression of coronary artery lesions [38]. The likelihood that postprandial triglycerides may be affected by a drug which has blood glucose lowering effect and in particular an effect on fatty acid metabolism through its effect on insulin action should be considered. Unfortunately in this study we did not measure pre and postprandial blood glucose or insulin. The present data therefore allow no definitive conclusion on the matter and this point requires further study. Considering triglyceride is thought to be very important in influencing LDL size [39], a change in particle size was expected at 2 h post-dose 1. LDL particle size, however, did not change during the study possibly because of the small triglyceride

changes. We can reasonably conclude therefore that the change in lag phase at 2 h post-dose 1 is unlikely to be due to alteration of LDL particle size.

There were fluctuations in the amounts of oleic acid and PUFAs, but these fluctuations were small and unlikely to have influenced the resistance of LDL to oxidation. Consistently there were no changes in the ratio of LDL oleic acid content to linoleic acid content which is considered one of the most important determinants of the susceptibility of LDL to oxidation [34].

At variance with these variables, the administration of troglitazone 400 mg twice daily for 2 weeks significantly reduced the preformed LDL lipid hydroperoxides at pre-dose 15 and 29 without any significant change in oleic or PUFA composition. Recent studies demonstrated that native LDL contains preformed lipid hydroperoxides [40–42] and that they were stable in plasma [43]. The increase in lag phase induced by troglitazone administration may, therefore, be due not only to its radical scavenging activity but also to its ability to reduce trace amounts of preformed lipid hydroperoxides in LDL. The results are consistent with the classic kinetic model proposed by Niki for lipid peroxidation [44], where the length of the lag phase is directly related to the amount of antioxidants and inversely related to the initiation rate and therefore to the amount of preformed lipid hydroperoxides and to the products formed from their decomposition by Cu^{++} . Open questions are why LDL contains hydroperoxides and how troglitazone can reduce their concentrations.

The results of this study also show that the increase in lag phase observed pre-dose was similar to that

observed at peak troglitazone concentrations (2 h post-dose), suggesting that the increase in resistance of LDL to oxidation was maintained for the duration of the dosing interval. It has to be emphasized, however, that the pre and post-troglitazone LDL levels were different, the latter being higher than the former. Even if on the basis of the present results we cannot draw any definitive conclusion on this point, a contamination of LDL fraction from the elevated troglitazone plasma concentration at 2 h post-dose 1, could be a likely explanation. This hypothesis is supported by our previous in vitro study showing a direct highly significant relationship between the length of lag phase and the troglitazone LDL concentration [20].

In conclusion the administration of troglitazone 400 mg twice daily for 2 weeks increased the resistance of LDL to copper-mediated oxidation by approximately 24 %. The increase in lag phase duration in the troglitazone group appeared to be due to the compound's activity as an antioxidant and to its ability to reduce the amount of pre-formed LDL lipid hydroperoxides.

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