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Inhibition of rat lipoprotein oxidation after tetradecylthioacetic acid feeding

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Abstract

We have previously shown that tetradecylthioacetic acid (TTA), a sulfur containing saturated fatty acid analogue, inhibits the oxidative modification of human low-density lipoprotein (LDL) *in vitro*. The oxidative modification of LDL is believed to be a crucial step in the progression of atherosclerosis. In the present study, we investigated the effect of TTA oral administration on the susceptibility of rat lipoprotein to undergo oxidative modification *ex vivo*. Lipoprotein resistance to copper-induced oxidation was highly improved after TTA administration to rats. Conjugated dienes produced after 150 min of lipoprotein oxidation were dramatically lowered in the TTA treated rats compared to controls. Malondialdehyde and lipid peroxides production by oxidation was highly limited. These effects were independent of any Vitamin E effects. More than 50% relative reduction in polyunsaturated fatty acids of the n-3 family, and more than 30% relative increase in 18:1n-9 fatty acid in the triacylglycerol (TAG)-rich lipoprotein were observed. TAG-rich lipoprotein lipids of TTA fed rats were decreased with more than 50% reduction in TAG. The data reported in this paper indicate a potent *in vivo* antioxidant capability of TTA that beside its hypolipidemic effect might be of importance in relation to the development of atherosclerosis.

Keywords: Atherosclerosis; Tetradecylthioacetic acid; Rat lipoproteins; Fatty acids; Lipoprotein oxidation; Thia fatty acid analogues

1. Introduction

Several lines of evidence indicate that the development of atherosclerosis is related to free radical processes, lipid peroxidation, and oxidative modification of low-density lipoprotein (LDL) [1,2]. Some studies have shown that administration of antioxidants significantly reduces the development of atherosclerosis plaque in the animal model [3,4]. In addition, it was shown that incubation with oxidatively modified lipoproteins increases matrix metalloproteinases (MMPs) expression in both vascular cells [5] and macrophages [6], and antioxidants can decrease expression and activity of MMPs produced by macrophage-derived foam cells of rabbit experimental lesions [7]. Hence, oxidized LDL and antioxidants might modulate plaque stability in opposite directions. The influence of dietary fatty acids and antioxidants on the resistance of LDL to oxidation is therefore, of relevance not only to the early progression of atherosclerosis but also to the subsequent acute events and plaque rupture.

Tetradecylthioacetic acid (TTA) (CH_3–$(CH_2)_{13}$–S–CH_2–COOH) is a saturated fatty acid analogue in which a sulfur atom is inserted in the third position from the carboxylic end in the carbon chain, blocking it for β-oxidation. When administered to rats, this fatty acid analogue has profound effect on lipid metabolism including increasing fatty acid oxidation and decreased plasma lipids [8,9]. Earlier, we reported that TTA inhibited the oxidative modification of human LDL *in vitro*, and that this effect was due to both metal ion binding and free radical scavenging [10]. It is therefore, of interest to see if TTA has the same effect *in vivo* as *in vitro*.

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Abbreviations: TTA, tetradecylthioacetic acid; LDL, low-density lipoprotein; TAG, triacylglycerol; VLDL, very low-density lipoprotein; MDA, malondialdehyde; LPO, lipid peroxides; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; HDL, high-density lipoprotein; BHT, butylated hydroxytoluene.

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Fatty acids with one or no double bonds are more resistant to peroxidation by reactive oxygen species than polyunsaturated fatty acids [11]. Moreover, chain length and degree of unsaturation of lipoprotein fatty acids play an important role in the oxidative modification of lipoprotein particles [12,13]. We have suspended TTA in different oils enriched with different fatty acids to compare the effect of TTA with that of fatty acids with known effects on the oxidation of lipoproteins. Furthermore, we wanted to investigate how different dietary fatty acids might influence the effect of TTA on the oxidative modification of lipoproteins. We used rape seed oil that is enriched with 18:1n-9, a fatty acid which is considered protective against lipoprotein oxidation [12,14]. Soy bean oil is enriched with 18:2n-6, and it has been reported that the LDL content of 18:2n-6 strongly correlates with either the rate or extent of oxidation [12]. Fish oil is rich in polyunsaturated fatty acids (PUFAs), which might render the lipoproteins more vulnerable to oxidation [12,15].

In rat, the main lipoprotein is high-density lipoprotein (HDL), and LDL is found in relatively small amounts [16]. Both LDL and very low-density lipoprotein (VLDL) can be oxidized [17,18], and combined together, they contain enough lipids to evaluate effects on the oxidizability, therefore, we investigated the effect of TTA on the *ex vivo* oxidation of triacylglycerol (TAG)-rich lipoprotein fraction, i.e. the VLDL + LDL fraction.

2. Materials and methods

TTA was synthesized as previously described [19]. Soy bean oil and rape seed oil (II) were commercially available from Mills, and Bauck KG, respectively. Rape seed oil (I) was a gift from Karlshamns AB (pub). Fish oil was a gift from Hordafór AS. All these oils were used as solvents for TTA. The fatty acid composition of these oils is shown in Table 1. All other chemicals were obtained from common commercial sources and were of either reagent or HPLC grade.

2.1. Animals and diets

Adult pathogen free male albino rats of the Wistar strain, weighing 180–200 g, were obtained from Møllegaard Breeding Laboratory. They were housed in pairs in metal wire cages and maintained on a 12 hr cycle of light and dark at $20 \pm 3^{\circ}$. The rats had free access to standard rat pellet food and water. They were acclimatized under these conditions for 1 week before the experiment started. TTA was suspended in the different oils and was administered to rats daily (150 mg/kg/day) for 10 days by gastric intubation. At the end of the experiment, rats were anesthetized with Hypnorm Dormicum (fluanisone-fenatanylmidazolam, 0.2 mL 100 g^{-1} body weight) (F. Hoffman-La Roche AG) given subcutanously to rats, cardiac puncture was performed and blood samples were collected in EDTA vacutainer tubes. The protocol was approved by the Norwegian State Commission for Animal Experimentation.

Table 1
The fatty acid composition (wt.%) of the oils used as solvents of TTA

Fatty acid	Fish oil	Soy bean oil	Rape seed oil (I)	Rape seed oil (II)
14:0	5.57	0.09	nd	nd
16:0	13.31	11.02	4.31	4.58
18:0	2.27	3.34	1.60	1.59
16:1n-7	5.99	nd	nd	nd
18:1n-9	13.98	21.30	58.43	59.48
18:1n-7	2.83	1.52	3.06	3.09
18:2n-6	3.98	55.50	20.13	19.17
18:3n-3	1.13	5.90	10.46	10.14
20:1n-9	6.93	nd	0.98	1.01
18:4n-3	2.12	nd	nd	nd
20:4n-6	7.96	nd	nd	nd
20:5n-3	8.69	nd	nd	nd
22:5n-3	3.08	nd	nd	nd
22:6n-3	13.10	nd	nd	nd
Minor FAs	9.06	1.33	1.03	0.94

Rape seed oil (I) is raw rape seed oil, while Rape seed oil (II) is refined and commercially available. Minor FAs: minor fatty acids. Fish oil, refined and bleached, was from salmon fish. nd: not detected. Other fatty acids found in the fish oil include 20:1n-11 and 20:4n-3. Erucic acid (22:1n-9) found in rape seed oils was less than 1%.

2.2. Isolation of TAG-rich lipoprotein fraction

Plasma was obtained by centrifugation at 3000 rpm for 15 min. Plasma TAG-rich lipoprotein fractions were prepared by ultracentrifugation in a Beckman (L-80) ultracentrifuge using T 50.4 rotor. Briefly, 3 mL plasma were centrifuged at a density of 1.063 *g*/mL for 19 hr at 40,000 rpm and 15°. The tubes were sliced, and the floating fraction in the top 1 mL of each tube was harvested by aspiration. The fractions were then dialyzed extensively against 150 mM sodium chloride, 16 mM sodium phosphate and 4 mM potassium phosphate, pH 7.4, bubbled with nitrogen.

Protein was determined by the method described by Bradford [20] using the commercially available Bio-Rad Protein assay (Bio-Rad laboratory), with bovine serum albumin as a standard. The absorbance was read on a LKB Ultrolab System 2074 Absorptiometer at 590 nm.

2.3. Oxidation of the TAG-rich lipoprotein fractions

The oxidation was initiated as described previously [21]. Basically, 25 μg lipoprotein–protein were placed in 1 mL quartz cuvette to which phosphate buffered solution at pH 7.4 was added. The oxidation was initiated by adding freshly prepared $CuCl_2 \cdot 2H_2O$ solution to a final concentration of 5 μM. Oxidation was performed at 37° in a single-beam UV–Vis recording spectrophotometer (Shimadzu UV-2501 PC), with a capacity of measuring six samples simultaneously. Absorbance was recorded every

2 min up to 2.5 hr. The initial absorbance was set to an arbitrary value and then the increase was recorded every 2 min for 150 min. The absorption data were stored on a personal computer and then used to graph the diene curve by subtracting from all data the initial absorbance. The kinetics of the reaction were analyzed as described elsewhere [22]. Upon the completion of the oxidation an aliquot (200 μL) was taken and stored at −80°, and later analyzed for malondialdehyde (MDA) content. To the rest, 200 μM EDTA and 40 μM butylated hydroxytoluene (BHT) (final concentrations) were added and stored at 4° until lipid peroxides (LPO) measurement was performed, not later than 10 days.

2.4. Measurement of lipoprotein lipids

Lipoprotein TAG and cholesterol were measured according to Technicon method no. S4A-0324L90, and S4A-0305L90, respectively. Phospholipids were measured using the enzymatic kit of bioMérieux.

2.5. Measurement of lipid peroxides

Aliquots of the TAG-rich lipoprotein fractions before and after oxidation were analyzed for their lipid peroxides content by a colorimetric method as described previously [10]. Cumene hydroperoxide was used as standard.

2.6. Measurement of malondialdehyde

Aliquot (100 μL) of the TAG-rich lipoprotein fraction, before and after oxidation were analyzed for their malondialdehyde content by high-performance liquid chromatography (HPLC) method as described elsewhere [23].

2.7. Determination of fatty acid composition

The fatty acid composition of the oils and TAG-rich fractions was measured as follows. Lipids were transesterified with 14% BF_3-methanol [24]. After alkaline hydrolysis of methyl esters [25], cholesterol and non-saponifiable material were removed and free fatty acids were methylated with diazomethane [26]. Fatty acids were determined on GC 8000 Top gas chromatograph (CE Instruments), equipped with a flame ionization detector, programmable temperature of vaporisation injector and AS 800 autosampler using a SP 2340 fused silica capillary column (60 m × 0.25 mm × 0.20 μm) (Supelco). Chromatographic conditions were as reported earlier [27]. Identification of chromatographic peaks was performed by means of known standards (Larodan Fine Chemicals) and confirmed by GC/MS analysis (GCQ, Finnigan MAT). Quantification was made with Chrom-Card A/D 1.0 chromatography station (CE Instruments) based on heneicosanoic acid as an internal standard, and weight percentage was calculated.

2.8. Measurement of Vitamin E

The TAG-rich lipoprotein fractions were analyzed for Vitamin E (α-tocopherol) by HPLC as described previously [23].

2.9. Statistical analysis

All data are presented as mean ± SD. Differences between the treatment groups are analyzed by non-paired *t* test. Correlation testing was evaluated with Pearson's test for correlation. Results are considered as statistically significant different when $P < 0.05$.

3. Results

3.1. The effect of TTA on the different lipids in the TAG-rich lipoprotein fraction

The effect of TTA on the level of the different lipids in the TAG-rich fraction is shown in Table 2. In agreement with previous findings where TTA reduced plasma VLDL lipids after administered to rats [28], the lipids in the TAG-rich lipoprotein fraction were reduced significantly in the TTA treated rats. The TAG was reduced by approximately 50% irrespective of the oil used to dissolve TTA. Cholesterol and phospholipids levels were also reduced in the TTA fed group. It is noteworthy that fish oil had a tendency

Table 2
TAG-rich fraction lipids content

Diet	Triacylglycerol (mmol/L plasma)	Cholesterol (mmol/L plasma)	Phospholipids (mmol/L plasma)
Fish oil	1.13 ± 0.40	0.18 ± 0.02	0.25 ± 0.06
Fish oil + TTA	0.60 ± 0.07*	0.04 ± 0.05*	0.10 ± 0.02*
Soy bean oil	1.22 ± 0.50	0.15 ± 0.08	0.24 ± 0.12
Soy bean oil + TTA	0.54 ± 0.16*	0.01 ± 0.01*	0.08 ± 0.03*
Rape seed oil (I)	1.23 ± 0.38	0.17 ± 0.06	0.23 ± 0.06
Rape seed oil (I) + TTA	0.66 ± 0.26*	0.04 ± 0.03*	0.11 ± 0.04*
Rape seed oil (II)	1.46 ± 0.25	0.15 ± 0.03	0.26 ± 0.03
Rape seed oil (II) + TTA	0.56 ± 0.16*	0.03 ± 0.03*	0.09 ± 0.02*

Data are mean ± SD of four values obtained from four rats.
* $P < 0.05$ compared to the corresponding control group.

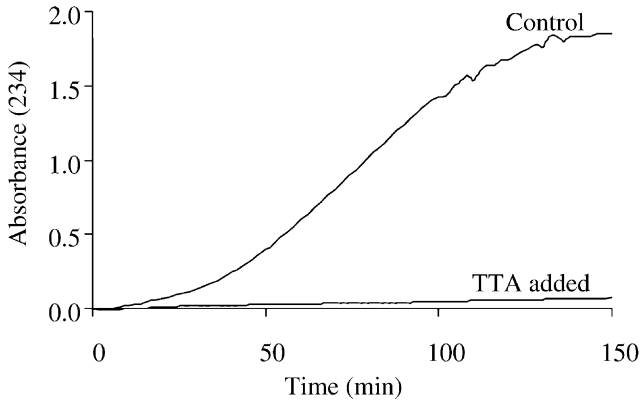

Fig. 1. Effect of TTA feeding to rats on formation of conjugated dienes in TAG-rich lipoprotein fraction during copper-induced oxidation. The data show results from one representative experiment.

to reduce TAG, while rape seed oil (II) had a tendency to increase TAG compared to soy bean oil.

3.2. The effect of TTA on oxidation of the TAG-rich lipoprotein fraction

Lipoproteins were oxidized as described in Section 2. The oxidation was monitored spectrophotometricaly at 234 nm for the production of conjugated dienes. The kinetics of copper-induced lipoprotein oxidation were strikingly different between the TTA treated groups and the corresponding control groups (Fig. 1). The lag-time before onset of formation of conjugated dienes could not be defined in the TTA treated groups (Fig. 1). The diene production at the end of the oxidation period i.e. 150 min was significantly lowered in the TTA treated groups compared to the corresponding control groups (Table 3). In addition, both LPO (Fig. 2) and MDA (Fig. 3) concentrations were significantly reduced in the lipoprotein fractions of the TTA treated rats. This was observed both before and after oxidation. These results were consistent for all oils used. On the other hand, fish oil administration resulted in the shortest lag-time and $T_{1/2}$, while rape seed oils which are enriched with 18:1n-9 showed the longest lag-time and $T_{1/2}$ (Table 3).

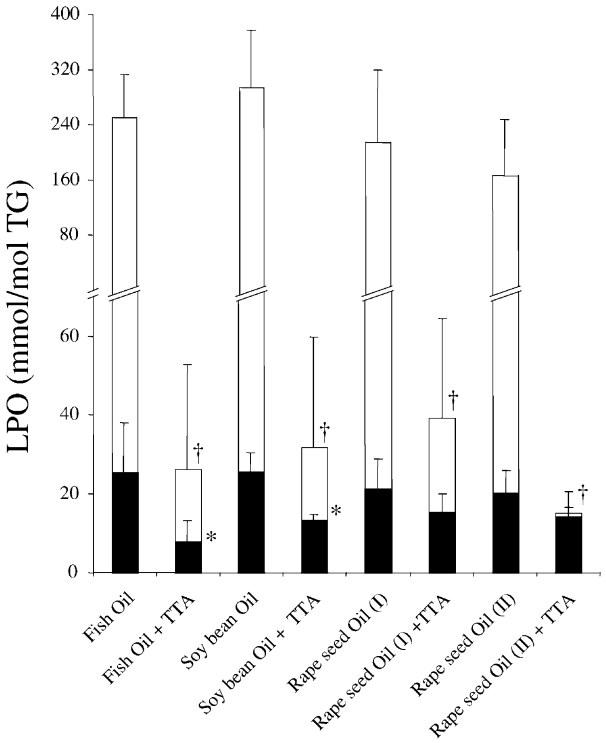

Fig. 2. The effect of TTA on the LPO content produced before and after oxidation of the TAG-rich fraction as described in the methods section. Filled bars represent the values before oxidation, while open bars represent additional LPO produced after oxidation. Data are mean ± SD of four values obtained from four rats. ∗: before oxidation, †: after oxidation $P < 0.05$ compared to the corresponding control group.

3.3. The effect of TTA on Vitamin E in the TAG-rich lipoprotein fraction

Fig. 4 shows the effect of TTA on TAG-rich lipoprotein fraction content of Vitamin E. There was no significant difference between the treated groups and the corresponding controls.

3.4. Lipoprotein fatty acid composition

The fatty acid composition of the TAG-rich lipoprotein fractions was analyzed (Table 4). The sum of the mono-

Table 3
The lag-time and half-time of the TAG-rich fraction oxidation by copper

Treatment	Half-time (min)	Lag-time (min)	Diene production at 150 min (nmol)
Fish oil	67 ± 11	31 ± 3	56.31 ± 16.21
Fish oil + TTA	ND	ND	2.88 ± 3.92*
Soy bean oil	73 ± 6	39 ± 3	57.40 ± 16.82
Soy bean oil + TTA	ND	ND	10.34 ± 14.51*
Rape seed oil (I)	112 ± 23	58 ± 15	40.36 ± 16.54
Rape seed oil (I) + TTA	ND	ND	4.80 ± 7.71*
Rape seed (II)	114 ± 39	69 ± 32	39.80 ± 22.61
Rape seed (II) + TTA	ND	ND	0.67 ± 1.34*

Data are mean ± SD of four rats. Half-time ($T_{1/2}$) is half the time needed to reach the maximum diene production, diene production was calculated using the molar absorptivity (ϵ) of conjugated dienes which is 29,500 L mol^{-1} cm^{-1}. ND: not defined.

* $P < 0.05$ compared to the corresponding control.

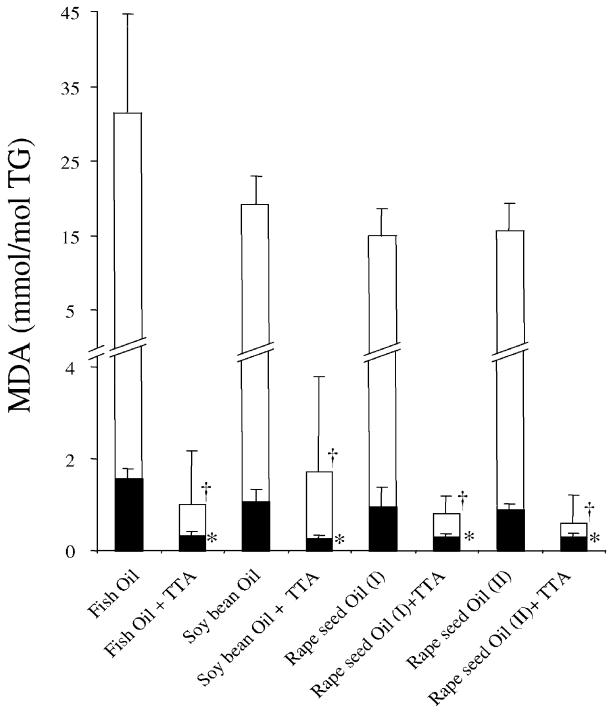

Fig. 3. The effect of TTA on the MDA content produced before and after oxidation of the TAG-rich fraction as described in the methods section. Filled bars represent the values before oxidation, while open bars represent additional MDA produced after oxidation. Data are mean ± SD of four values obtained from four rats. ∗: before oxidation; †: after oxidation $P < 0.05$ compared to the corresponding control group.

unsaturated fatty acids (MUFAs) increased significantly and the sum of the PUFAs, on the other hand, decreased significantly regardless of the oil in which TTA was dissolved. The reduction in PUFAs was accounted for by reduction in both n-3 and n-6 fatty acids. Both 18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3 from the n-3 family, and 18:2n-6 from the n-6 family decreased significantly. The sum of the saturated fatty acids did not change significantly after TTA feeding. The increase in MUFAs was mainly due to an increase in the 18:1n-9. It is noteworthy that the Δ-9 desaturated form of TTA was found in a higher concentration than TTA itself.

Rape seed oils (I & II) are enriched with 18:1n-9 fatty acid and it was seen that rape seed oil fed rats increased their lipoprotein content of 18:1n-9. Nevertheless, TTA feeding further increased this monounsaturated fatty acid. Soy bean oil, on the other hand, is enriched with 18:2n-6, and soy bean oil fed rats had an increased lipoprotein content of 18:2n-6, nonetheless, TTA feeding decreased this fatty acid.

Fig. 5 shows a significant positive correlation between lipoprotein PUFA content and both MDA (Fig. 5A), and LPO (Fig. 5B). On the other hand, Fig. 6 shows a significant negative correlation between lipoproteins MUFA content and both MDA (Fig. 6A), and LPO (Fig. 6B). This indicates that the change of the fatty acid composition (Table 4) in the lipoproteins of the TTA fed rats has contributed to the increased oxidation resistance (Table 3 and Figs. 1–3).

4. Discussion

The present investigation demonstrates for the first time that lipoprotein fractions isolated from TTA fed rats had significantly less LPO and MDA compared to rats fed different oils without TTA (Figs. 2 and 3). This indicate a significantly reduced oxidation level of these lipoproteins

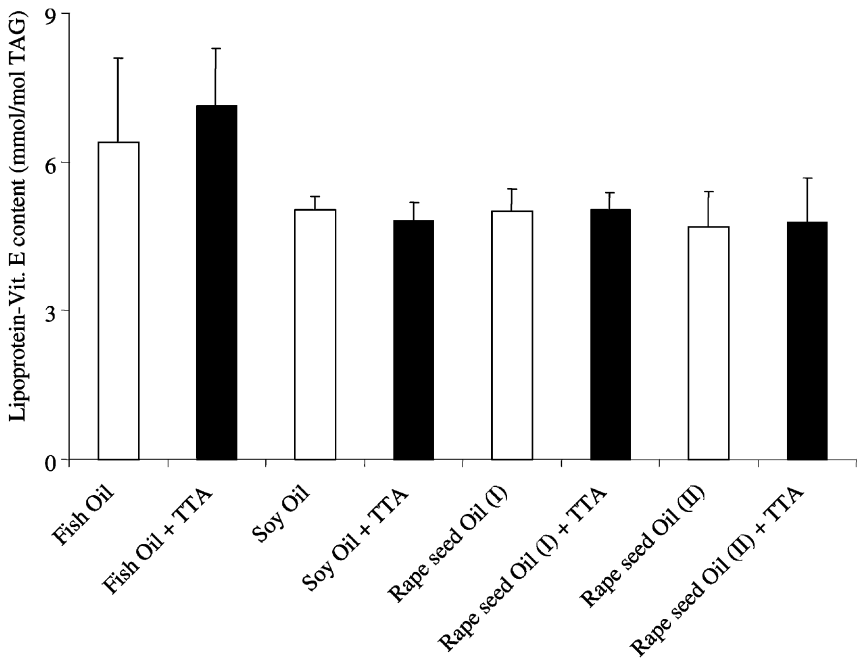

Fig. 4. Concentration of Vitamin E in the lipoprotein fractions. Data are mean ± SD of four readings obtained from four fractions of four rats.

Table 4
The fatty acid composition (wt.%) of the TAG-rich lipoprotein fractions

Fatty acid	Fish oil	Fish oil + TTA	Soy bean oil	Soy bean oil + TTA	Rape seed oil (I)	Rape seed oil (I) + TTA	Rape seed oil (II)	Rape seed oil (II) + TTA
16:0	22.1 ± 1.8	20.7 ± 1.4	21.1 ± 0.8	20.1 ± 0.8	19.6 ± 2.9	20.4 ± 1.5	21.3 ± 1.2	19.5 ± 0.9
18:0	3.8 ± 0.1	4.5 ± 0.6	4.2 ± 0.3	4.3 ± 0.1	3.9 ± 0.2	4.2 ± 0.1	3.7 ± 0.1	4.5 ± 0.2*
16:1n-7	2.9 ± 0.7	1.9 ± 0.5	1.7 ± 0.8	1.6 ± 0.1	2.0 ± 0.9	1.9 ± 0.6	2.4 ± 0.5	1.3 ± 0.2*
18:1n-9	20.0 ± 1.6	33.0 ± 2.8*	18.8 ± 1.1	29.2 ± 2.3*	22.9 ± 0.4	29.8 ± 1.4*	23.4 ± 1.4	31.6 ± 1.7*
18:1n-7	3.4 ± 0.8	2.6 ± 0.2	2.4 ± 0.5	2.0 ± 0.2	2.7 ± 0.6	2.4 ± 0.5	3.0 ± 0.4	2.1 ± 0.1*
TTA:1n-8	0.0 ± 0.0	1.7 ± 0.2*	0.0 ± 0.0	1.4 ± 0.1*	0.0 ± 0.0	1.3 ± 0.2*	0.0 ± 0.0	1.6 ± 0.4*
18:2n-6	27.1 ± 1.8	21.8 ± 1.7*	33.3 ± 2.1	27.0 ± 2.3*	29.1 ± 2.1	24.7 ± 1.8*	27.8 ± 2.1	23.2 ± 2.9*
18:3n-3	1.2 ± 0.0	0.8 ± 0.1*	1.3 ± 0.1	1.1 ± 0.2	1.8 ± 0.3	1.1 ± 0.1*	1.7 ± 0.1	1.0 ± 0.3*
20:4n-6	2.4 ± 0.7	2.8 ± 0.1	4.5 ± 0.8	4.2 ± 0.5	4.0 ± 1.3	3.9 ± 0.3	3.5 ± 0.4	4.6 ± 0.8*
20:5n-3	2.8 ± 1.3	0.9 ± 0.1*	1.0 ± 0.2	0.5 ± 0.1*	1.5 ± 0.6	0.7 ± 0.1*	1.2 ± 0.3	0.5 ± 0.2*
22:5n-3	2.6 ± 0.5	0.4 ± 0.2*	1.2 ± 0.3	0.3 ± 0.1*	1.7 ± 0.3	0.5 ± 0.2*	1.7 ± 0.4	0.4 ± 0.2*
22:6n-3	6.0 ± 0.4	2.3 ± 0.9*	4.1 ± 0.6	1.4 ± 0.5*	4.3 ± 0.5	2.1 ± 0.6*	4.2 ± 0.5	2.0 ± 0.8*
TTA	0.0 ± 0.0	0.7 ± 0.1*	0.0 ± 0.0	0.7 ± 0.1*	0.0 ± 0.0	0.8 ± 0.1*	0.0 ± 0.0	1.1 ± 0.2*
PUFA n-3	12.7 ± 2.2	4.5 ± 1.2*	7.8 ± 0.9	3.5 ± 0.9*	9.6 ± 1.2	4.5 ± 0.9*	8.9 ± 1.0	4.0 ± 1.0*
PUFA n-6	30.4 ± 2.6	25.8 ± 1.6*	39.8 ± 2.5	32.5 ± 2.8*	34.9 ± 3.0	29.9 ± 1.8*	33.0 ± 2.4	29.4 ± 1.8*
SFA.	27.9 ± 1.8	27.1 ± 0.6	27.6 ± 0.7	26.9 ± 1.3	25.8 ± 2.6	27.1 ± 1.6	27.2 ± 1.1	26.5 ± 0.9
MUFA	28.5 ± 3.1	39.8 ± 2.4*	24.6 ± 2.0	36.3 ± 2.4*	29.6 ± 1.6	37.5 ± 0.8*	30.8 ± 2.2	38.8 ± 1.6*

The percentage values for individual fatty acids contained in each group do not add up to 100% because only the most prevalent fatty acids are listed. PUFA n-3 is the sum of n-3 fatty acids. PUFA n-6 is the sum of n-6 fatty acids. SFA is the sum of saturated fatty acids. MUFA is the sum of monounsaturated fatty acids. Data are mean ± SD of four rats (*t*-test).

* $P < 0.05$ compared to the corresponding control group.

in vivo. In addition, these lipoproteins were more resistant to copper-induce oxidation compared to corresponding controls, with dramatically changed lipoprotein oxidation kinetics (Fig. 1). After oxidation both conjugated dienes, LPO and MDA were markedly lower in lipoproteins from TTA fed rats. (Table 3 and Figs. 2 and 3). These data indicate an antioxidant protective effect of TTA on plasma lipoproteins.

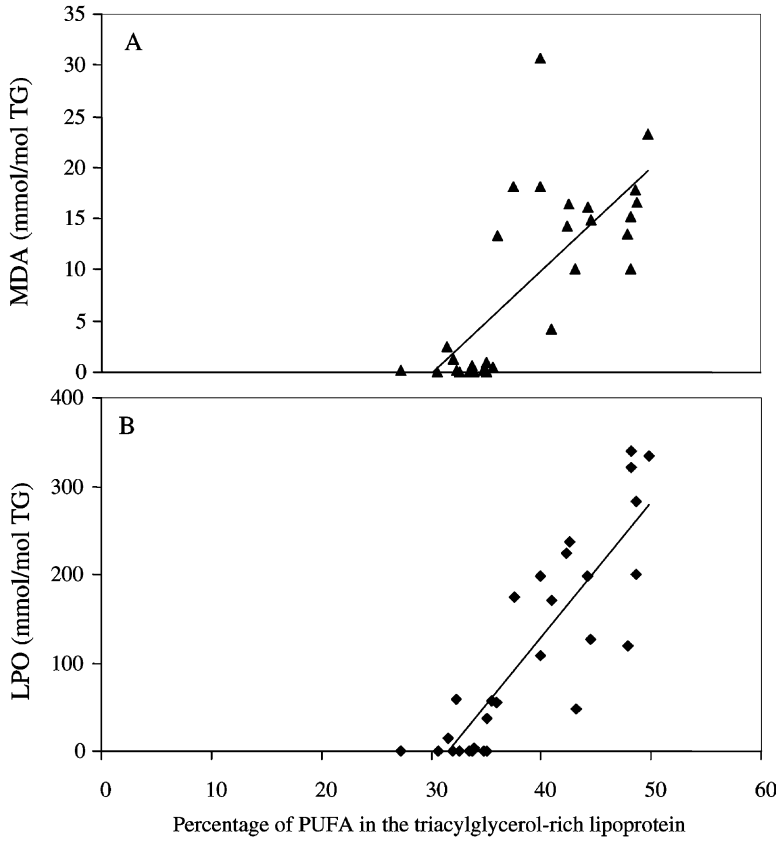

Fig. 5. Correlation between the percentage of PUFA-content in the TAG-rich lipoprotein and both MDA (A), $R^2 = 0.5534$, $P < 0.0001$, and LPO (B), $R^2 = 0.7512$, $P < 0.0001$, produced during oxidation.

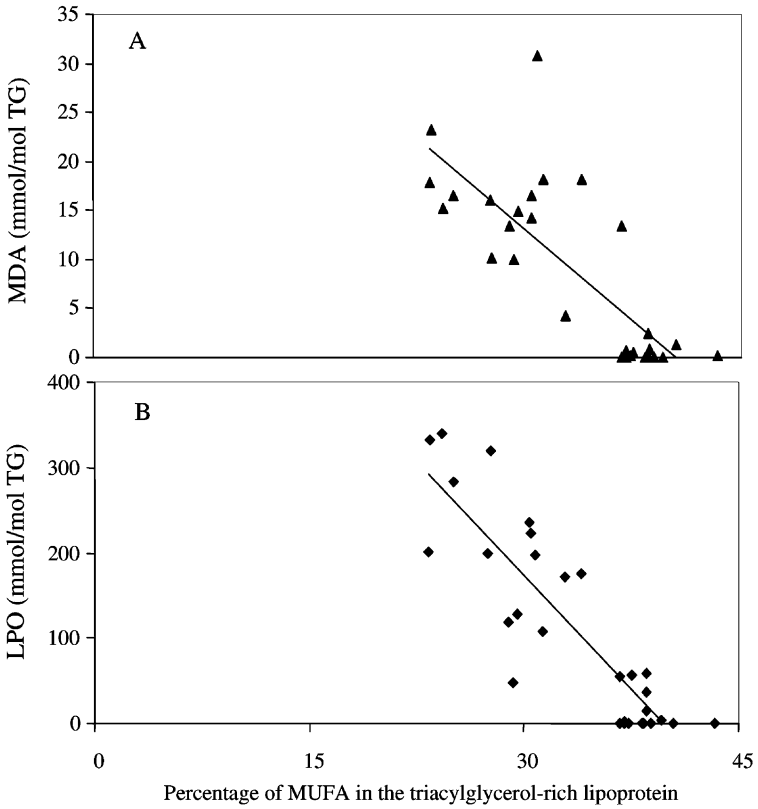

Fig. 6. Correlation between the percentage of PUFA-content in the TAG-rich lipoprotein and both MDA (A), $R^2 = 0.6314$, $P < 0.0001$, and LPO (B), $R^2 = 0.7646$, $P < 0.0001$, produced during oxidation.

The changes in lipoprotein fatty acid composition have probably contributed to the increased oxidation resistance observed after TTA feeding. The sum of MUFAs increased (more than 25%), due mainly to 18:1n-9, while the sum of PUFAs decreased (more than 50% in PUFA n-3 and 10% in PUFA n-6) in the lipoprotein fractions of the TTA fed rats (Table 4). It has been reported that Linoleic acid (18:2n-6) enriched lipoproteins exhibit high oxidizability [12]. This fatty acid was decreased significantly in TAG-rich lipoprotein after TTA feeding (Table 4).

TTA has previously been reported to be a good substrate for Δ-9 desaturase, and both the activity and gene expression of the enzyme were up regulated after TTA feeding [29]. This may explain the increased content of the 18:1n-9 fatty acid in lipoprotein fractions from TTA fed rats. The Δ-9 desaturated metabolite of TTA was present in slightly higher amounts than TTA itself in lipoprotein fractions from TTA treated rats (Table 4). Whether this metabolite has any antioxidant effects or if it affects the oxidizability of lipoproteins remains to be determined.

Earlier, we showed that TTA possess intrinsic antioxidant properties that prevented both metal ion and non-metal ion mediated oxidation of human LDL *in vitro* [10]. In addition, TTA prevented the oxidation of 2-deoxyguanosine induced by ascorbic acid [10], and interacted with the superoxide radical [31]. These results indicate free radical scavenging effects. The concentrations of TTA tested in these studies were 5–20 μM.

In the present study, we present evidence for an indirect antioxidant effect through reduced total particles and reduced PUFAs and increased MUFAs (Table 4). The concentration of TTA was 5–10 μM when assaying lipoproteins from TTA fed rats for copper-induce lipid peroxidation *in vitro*, and the concentration of desaturated TTA was 11–15 μM (data not shown). These concentrations are within the range that exhibited antioxidant effects in the former *in vitro* studies [10,31]. Therefore, a direct antioxidant effect of TTA as demonstrated in the previous studies [10,31] can not be excluded.

The observed decrease in oxidized lipoproteins could, however, simply be due to reduced levels of plasma lipids. In the present study, an approximately 50% decrease in TAG lipoprotein content was observed in addition to reduction in cholesterol and phospholipids. Nevertheless, there was a significant decrease in both LPO and MDA content per mol TAG. This indicates that the reduced rate of lipid peroxidation is probably not related to the lipid lowering effect of TTA. Moreover, fish oil consumption is associated with reduced serum concentrations of TAG and VLDL [30], and our data indicate a similar effects (Table 2), nonetheless, in rats fed fish oil there was a tendency of enhanced the oxidation i.e. shortened lag-time and $T_{1/2}$ (Table 3).

Another factor that might influence the resistance to oxidation is endogenous antioxidants in the particles [32]. Vitamin E (α-tocopherol) is the major lipid-soluble chain

breaking antioxidant in plasma lipoproteins [33]. Vitamin E is transported in plasma only in lipoproteins, and its concentration is related to the concentration of plasma lipids [34]. In this study, lipoprotein Vitamin E absolute concentrations were reduced in the TTA treated rats compared to the corresponding controls (data not shown). However, when lipoprotein Vitamin E concentration was calculated per mol TAG there was no significant difference between TTA fed rats and their corresponding control groups (Fig. 4).

Using copper ions to induce lipoprotein oxidation is of biological relevance since its *in vivo* involvement in atherosclerosis has been reported in several studies [35–37].

The validity of our results is supported by several findings. First, *in vitro* experiments have provided similar results [10,31]. Second, all oils used as solvents for TTA in this study gave consistent results. Third, all parameters measured, i.e. conjugated dienes, MDA and LPO, indicate that TTA has potent antioxidant properties.

In conclusion, this study suggests that TTA markedly enhance lipoprotein resistance to oxidative challenge. This enhancement can be due to an intrinsic antioxidant effect of TTA and probably its metabolites [10,31], and/or favorable changes in the fatty acid composition of the lipoprotein particles. The reduction in lipoprotein lipids has probably little effect if any on the increased oxidation resistance.

The bio-pharmacological effect of TTA reported in this paper may be of interest in relation to atherosclerosis progression. However, care should be taken in extrapolating results from rats to humans. Further human studies are in progress to explore the human benefits of this compound in relation to heart disease in man.

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