Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions

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Abstract In order to investigate the influence of fatty acid pattern and antioxidants other than vitamin E on lipid peroxidation and antioxidant levels of plasma very low density and low density lipoproteins (VLDL + LDL), the effects of three diets (equalized for vitamin E) containing soybean oil, olive oil, or an oleate-rich mixture of triglycerides (triolein) were studied in rats. A significantly lower concentration of thiobarbituric acid-reactive substances (TBA-RS) in plasma and lipoproteins was found after the olive oil diet (soybean oil, 3.7 ± 0.4 nmol/ml; triolein, 2.1 ± 0.5 nmol/ml; olive oil, 1.5 ± 0.3 nmol/ml, in plasma) (soybean oil, 0.99 ± 0.16 nmol/ml; triolein, 0.96 ± 0.13 nmol/ml; olive oil, 0.38 ± 0.12 nmol/ml, in the VLDL + LDL fraction). Furthermore, the results from in vitro copper-induced lipid peroxidation, expressed in terms of conjugated dienes, lipid hydroperoxides, and TBA-RS content, showed that VLDL + LDL particles from olive olive oil-fed rats were remarkably resistant to oxidative modification. The results suggest that the fatty acid unsaturation of dietary oils is not the only determining factor of the antioxidant capacity of lipoproteins in this animal model. The maximal protection observed after the olive oil diet may be explained by the presence of other unidentified antioxidants in addition to vitamin E, derived from oil intake. Therefore, the optimal balance between the content of unsaturated fatty acids and natural antioxidants in dietary oils appears to be of major importance.—**Scaccini, C., M. Nardini, M. D'Aquino, V. Gentili, M. Di Felice, and G. Tomassi.** Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions. *J. Lipid Res.* 1992. **33:** 627–633.

Supplementary key words olive oil • soybean oil • triolein • dietary fatty acids • antioxidants

Oxidative damages, including those associated with lipid peroxidation, are generally believed to be a significant factor in many pathological processes (1).

In the pathogenesis of atherosclerosis, evidence is increasing that lipoprotein peroxidation may be involved (2). When low density lipoproteins (LDL) are modified in vitro (3) by peroxidation, they are taken up by a scavenger receptor on the surface of monocytes and macrophages (4, 5); this uptake might lead to the formation of fatty streaks, the first step in the formation of an atherosclerotic plaque (6).

There is evidence, by immunocytochemical methods, that oxidized LDL is generated in vivo and it has been demonstrated that proteins with malondialdehyde-modified lysine residues are present in atherosclerotic lesions of rabbit aortas (2, 7). Avogaro, Bittolo-Bon, and Cazzolato (8) found that an oxidatively modified LDL subfraction was present in normal human subjects (5–20% of total LDL).

Therefore, the influence of dietary fatty acids and antioxidants on the resistance of LDL to oxidation is of relevance in the regulation of atherosclerosis. Parthasarathy and coworkers (9) demonstrated that LDL isolated from plasma of rabbits fed an oleate-rich variant sunflower oil were remarkably more resistant to transition-metal-induced oxidation than LDL particles rich in polyunsaturated fatty acids. Moreover, Jessup et al. (10) suggested that unidentified endogenous antioxidants may also be significant in the prevention of oxidative modifications of LDL.

In order to investigate this point, natural soybean oil, extra virgin olive oil, and an oleate-rich mixture of triglycerides, mimicking olive oil (triolein, 75% oleic acid), were used for the preparation of synthetic diets for experiment in rats.

Since both LDL and VLDL have been found to undergo lipid peroxidation (11, 12) and since rats have a relatively small amount of LDL (13) we chose to investigate the in vivo and in vitro oxidation of the lipoprotein fraction containing both LDL and VLDL.

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; SO, soybean oil; OO, olive oil; TO, triolein; TBA-RS, thiobarbituric acid-reactive substances; TRAP, total(peroxyl) radical-trapping antioxidant parameter; HPLC, high performance liquid chromatography.

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TABLE 1. Fatty acid composition of the experimental diets

Fatty Acid	Soybean Oil	Olive Oil	Triolein
		% of total fatty acids	
12:0		0.2	0.5
14.0	0.2	0.2	2.2
16:0	10.9	12.0	4.9
18:0	3.7	1.9	1.6
18.1	21.2	75.2	73.7
18:2	54.1	7.6	10.1
18:3	7.2	0.5	0.1
20:0	0.3	0.4	
20:1	0.4	0.2	1.1
20:4	0.6	0.6	0.4
22:0	0.4	0.1	
24:0	0.3	0.1	0.3

Values are means of two determinations.

METHODS

Diet and animals

Thirty male Wistar rats (initial weight 68 ± 6 g) were individually housed in wire-bottom stainless-steel cages under controlled lighting. The animals were randomly divided into three groups of ten and fed, for 6 weeks, experimental diets supplemented with 15% (w/w) soybean oil (SO), olive oil (OO), or a oleate-rich mixture of triglycerides (triolein, 75% oleic acid from Fluka), mimicking olive oil in fatty acid composition (TO). The diet composition (w/w) was: 20% casein, 40% rice starch, 15% fat, 0.3% dl-methionine, 17% sucrose, 3% fiber, 3.5% salt mixture (AIN 76), 1% vitamin mixture (AIN 76), and 0.2% choline chloride. Diets were prepared weekly and stored at 4°C under nitrogen.

Samples of diets were analyzed for fatty acids composition by gas-liquid chromatography (14) (**Table 1**). Oils were analyzed for vitamin E content according to Carpenter (15). The dietary vitamin E was adjusted to 82 IU/kg of diet and total tocopherol content was measured according to McMurray, Blauchflower, and Rice (16) on the same day as preparation of the diet.

Preparation of lipoproteins

Lipoprotein fractions were isolated by sequential ultracentrifugation in a Beckman T-100 benchtop Ultracentrifuge, using a T-100.3 rotor, according to Havel, Eder, and Bragdon (17). A 3-ml centrifuge tube was used for 2 ml plasma (containing 1 mg/ml EDTA Na_2). Plasma density was adjusted to 1.055 g/ml (18) with a high density salt solution (containing NaCl, KBr, and EDTA Na_2). After centrifugation for 6 h at 100,000 rpm, VLDL + LDL particles were removed using a tube slicer.

Plasma and lipoprotein analyses

Cholesterol, phospholipid, triglyceride, and uric acid concentrations were determined using reagent kits from Boehringer Mannheim. Vitamin E was determined according to Bieri, Tolliver, and Catignani (19). Plasma carotenoids were determined according to Stacewicz-Sapuntzakis et al. (20). Ascorbic acid was measured by HPLC according to the method of Farber et al. (21). The plasma diene-conjugated derivative of linoleic acid [18:2 (9, 11)] was determined by HPLC according to Iversen, Cawood, and Dormandy (22). Thiobarbituric acid-reactive substance (TBA-RS) was measured in plasma according to Yagi (23) and in the lipoprotein fraction according to Maseki et al. (24); in both determinations, 0.01% BHT was added to the TBA reagent. Sulfhydryl group concentration was measured by the spectrophotometric method described by Ellman (25). Plasma and lipoprotein fatty acids were extracted according to Mueller and Binz (26) and derivatized, using MeOH-HCl as reagent (27).

Total (peroxyl) radical-trapping antioxidant parameter (TRAP) was calculated according to Wayner et al. (28) on the basis of the molar concentration of vitamin E, urate, ascorbate, and sulfhydryl groups in plasma. The individual stoichiometric factor (n) for urate, ascorbate, and sulfhydryl groups was determined, as described by Wayner et al. (28) by the oxygen electrode method, using a Clark electrode (YSI) with a Gilson 5/6 oxigraph.

Oxidation of lipoproteins

Pools of five freshly prepared VLDL + LDL samples for each dietary group were dialyzed against a 200-fold volume of phosphate-buffered saline (PBS, 0.01 M phosphate, 0.15 M NaCl, pH 7.4) at 4°C in the dark for 24 h; the dialysis buffer was changed twice during this time period.

Suitable volumes from the dialyzed solutions were diluted with PBS to obtain a final lipoprotein concentra-

TABLE 2. Plasma fatty acid composition

Fatty Acid	Soybean Oil	Olive Oil	Triolein
		% of total fatty acids	
14:0	4.6 ± 0.8	1.9 ± 0.1	2.2 ± 0.4
16:0	19.7 ± 0.9	20.2 ± 0.5	21.2 ± 1.2
16:1	0.9 ± 0.1	1.1 ± 0.1	1.4 ± 0.2
18:0	15.7 ± 0.7	14.1 ± 0.5	13.5 ± 0.8
18:1	10.6 ± 1.7	22.9 ± 0.5	22.7 ± 0.6
18:2	19.3 ± 1.2	5.4 ± 0.4	5.0 ± 0.4
18:3	2.2 ± 0.3	0.7 ± 0.3	0.4 ± 0.1
20:0	1.1 ± 0.2	0.7 ± 0.2	0.8 ± 0.1
20:1	0.5 ± 0.3	0.9 ± 0.2	0.8 ± 0.3
20:3	0.7 ± 0.1	4.5 ± 0.3	4.5 ± 0.5
20:4	15.6 ± 1.2	15.3 ± 1.4	16.0 ± 1.1
20:5	1.2 ± 0.1	2.8 ± 0.8	2.9 ± 0.5
22:0	0.8 ± 0.4	1.0 ± 0.1	1.3 ± 0.3
22:4	0.4 ± 0.1	0.9 ± 0.1	0.6 ± 0.1
22:5	0.9 ± 0.5	1.5 ± 0.4	2.0 ± 0.8
22:6	2.2 ± 0.2	2.4 ± 0.2	1.5 ± 0.2

Values are given as mean ± SD.

TABLE 3. Effects of dietary oils on various plasma parameters

Parameter	Soybean Oil	Olive Oil	Triolein	*P*
Triglycerides (mg/100 ml)	129.2 ± 43.5[a,b]	149.0 ± 40.5[a]	107.5 ± 49.9[a,b]	0.172
Total cholesterol (mg/100 ml)	71.2 ± 11.7[a]	87.4 ± 10.5[b]	70.1 ± 10.5[a]	0.006
TBA-RS (nmol/ml)	3.7 ± 0.4[a]	1.5 ± 0.3[b]	2.1 ± 0.5[c]	<0.001
Vitamin E (μg/ml)	6.3 ± 1.3[a,b]	6.7 ± 1.3[a]	5.2 ± 0.8[b]	0.024
Ascorbic acid (μg/ml)	9.4 ± 2.0	10.4 ± 3.4	10.8 ± 3.7	0.563
Uric acid (mg/100 ml)	1.1 ± 0.1[a]	1.8 ± 0.7[b]	1.4 ± 0.4[a,b]	0.013
Sulfhydryl groups (nmol/ml)	260 ± 31	281 ± 49	263 ± 30	0.474
$TRAP_{calc}$* (μM)	372 ± 40[a]	485 ± 106[b]	424 ± 69[a,b]	0.029

Values with different superscripts are significantly different by Anova (Scheffe F-test); mean ± SD.
*Stoichiometric factors used for calculation: vitamin E = 2, urate = 2.25; ascorbate = 1.41; sulfhydryl groups = 0.45.

tion of 0.5 mg/ml. Lipoprotein concentration was calculated from the mass of protein and individual lipids.

Oxidation was initiated by the addition of freshly prepared $CuCl_2$ (5 μM final concentration) at 37°C (29). The kinetics of conjugated diene formation was followed by continuously monitoring absorption at 234 nm, using a Beckman DU 70 spectrophotometer, thermostated at 37°C. Lipid hydroperoxides were estimated iodometrically at different time points, according to El-Saadani et al. (30). TBA-RS was measured according to Maseki et al. (24). Vitamin E concentration was measured according to Bieri et al. (19).

Statistical analysis

Data are presented as mean ± standard deviation. Statistical analysis was performed using a one-factor analysis of variance and Scheffe's method for multiple comparisons. Differences with $P < 0.05$ were considered statistically significant.

RESULTS

There was no significant difference in food intake, weight gain, final weight, and relative liver weight (g/100 g of body weight) among rats fed different diets (data not shown).

The plasma fatty acid composition in the three groups (**Table 2**) reflected dietary fatty acids. Linoleic acid was the major component (19.3%) of the plasma fatty acids in the SO group, and oleic acid in the OO and TO groups (22.9% and 22.7%, respectively); arachidonic acid and docosahexaenoic acid, the main precursors of malondialdehyde in mammalian tissues (31), were similar in the three groups.

Concentration of various plasma components in rats fed the various experimental diets are reported in **Table 3**. OO-fed rats were more resistant to in vivo lipid peroxidation: plasma TBA-RS was significantly lower ($P < 0.001$) in the OO-fed group: 1.5 ± 0.3 nmol/ml versus 3.7 ± 0.4 (SO) and 2.1 ± 0.5 nmol/ml (TO). Plasma levels of vitamin E (OO, 6.7 ± 1.3 μg/ml vs. SO, 6.3 ± 1.3 μg/ml, and TO, 5.2 ± 0.8 μg/ml, $P = 0.024$) and uric acid (OO, 1.8 ± 0.7 mg/dl vs. SO, 1.1 ± 0.1 mg/dl, and TO, 1.4 ± 0.4 mg/dl, $P = 0.013$) were significantly different among the three dietary groups. Vitamin C (OO, 10.4 ± 3.4 μg/ml; SO, 9.4 ± 2.0; TO, 10.8 ± 3.7) and -SH group levels (OO, 281 ± 49 nmol/ml; SO, 260 ± 31 nmol/ml; 263 ± 30 nmol/ml) were similar in the three groups.

The calculated TRAP were significantly higher ($P < 0.03$) in OO-fed rats than in the SO-fed group; this may be due to the higher contribution of plasma urate to the calculation of TRAP. OO and TO groups showed similar TRAP values, in spite of the different plasma levels of TBA-RS.

The plasma diene-conjugated derivative of linoleic acid [18:2 (9, 11)] was not quantified since its concentration was too low to be detected by the method used. We tried to measure the plasma levels of carotenoids (α- and β-carotene, cryptoxanthine, lutein, zeaxanthin, and lycopene) in the three experimental groups, but the concentrations were also too low to be detected (<1 nmol/ml plasma).

TABLE 4. VLDL + LDL fatty acid composition

Fatty Acid	Soybean Oil	Olive Oil	Triolein
	% of total fatty acids		
14:0	1.0	1.0	0.7
16:0	19.7	22.9	19.9
16:1	6.6	3.2	1.8
18:0	5.9	7.2	8.0
18:1	15.5	42.4	44.7
18:2	31.9	8.3	8.8
18:3	3.1	0.4	0.9
20:4	9.3	8.1	8.8
20:5	0.8	0.4	0.4
22:5	1.0	1.1	1.3
22:6	3.6	1.3	1.0

Values are means of two determinations.

TABLE 5. Effect of dietary oils on lipoprotein parameters

VLDL + LDL	Soybean Oil	Olive Oil	Triolein	P
Cholesterol (mg/100 ml)	10.8 ± 4.8	12.8 ± 2.5	9.9 ± 3.3	0.581
TBA-RS (nmol/ml)	0.99 ± 0.16[a]	0.38 ± 0.12[b]	0.96 ± 0.13[a]	<0.001
Vitamin E (μg/ml)	1.8 ± 0.8	1.7 ± 0.7	1.6 ± 0.5	0.817

Values with different superscripts are significantly different by Anova (Scheffe F-test); mean ± SD. Results are means ± SD; six rats for each dietary group.

The fatty acid composition of VLDL + LDL fractions is reported in **Table 4**. OO and TO lipoprotein fractions were very similar with respect to their fatty acid composition, oleic acid being the major component (42.4% and 44.7%, respectively). Linoleic acid represented 31.9% of fatty acids in the SO fraction.

The distribution of total cholesterol, vitamin E, and TBA-RS in the VLDL + LDL fraction is shown in **Table 5**. The lipoprotein fraction of OO-fed rats exhibited a markedly lower content of lipid peroxidation products (as monitored by thiobarbituric acid reactivity), when compared to the other two groups of animals (OO, 0.38 ± 0.12 nmol/ml vs. SO, 0.99 ± 0.16 nmol/ml; and TO, 0.96 ± 0.13 nmol/ml; $P < 0.001$). No significant differences among the three dietary groups were found in vitamin E and cholesterol levels (Table 5).

When the VLDL + LDL fractions from the three dietary groups were subjected to in vitro copper-induced oxidation (5 μM $CuCl_2$ in PBS at 37°C), marked differences were observed in the susceptibility to lipid peroxidation, as indicated by the rate of diene conjugation (absorption at 234 nm) (**Fig. 1**). As expected, the lipoprotein fraction from rats fed the SO diet was readily oxidized, as seen by the short lag phase (less than 30 min) and by the steep increase in the rate of formation of conjugate dienes. On the other hand, the lipoprotein fraction from rats fed the OO diet was remarkably resistant to lipid peroxidation, as seen by the length of the lag phase (more than 4 h) and by the slower rate of conjugated diene formation. VLDL + LDL of TO-fed rats showed a higher rate of lipid peroxidation and a shorter lag phase compared to the OO-fed rats.

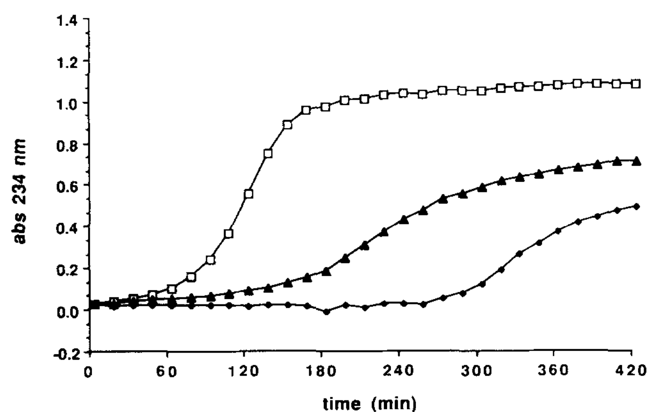

Fig. 1 Rate of formation of conjugated dienes. Two pools of five freshly prepared VLDL + LDL samples (0.5 mg/ml) for each dietary group were oxidized in PBS containing 5 μM Cu^{2+}, at 37°C; absorbance was measured continuously at 234 nm. The zero-time levels were subtracted from the values shown and each point represents the mean of duplicate determinations. VLDL + LDL from rats fed for 6 weeks on a diet of 15% (w/w) soybean oil, SO (□), olive oil, OO (♦), and an oleate-rich mixture of triglycerides, TO (▲).

The rate of generation of lipid hydroperoxides (**Fig. 2**) and TBA-RS (**Fig. 3**) in VLDL + LDL from the three dietary groups confirmed the behavior observed for conjugated diene formation. In both cases, the VLDL + LDL fraction from OO-fed animals showed a high resistance to peroxidation, compared to both SO-fed rats (as expected), and to TO-fed animals.

The vitamin E contents of the three lipoprotein fractions measured at time zero were comparable: SO, 2.9 μg/mg lipoprotein; OO, 2.9 μg/mg lipoprotein; TO, 2.6 μg/mg lipoprotein.

DISCUSSION

Recent reports (10, 32-35) have suggested that vitamin E is not the only antioxidant responsible for the resistance of LDL to oxidation, indicating that other natural endogenous antioxidants, mainly from dietary intake, could be involved in the prevention of oxidative modification.

Recently, Parthasarathy et al. (9) demonstrated that LDL particles isolated from plasma of rabbits fed a high-oleate variant sunflower oil were remarkably more resistant to oxidative modification than those isolated from plasma of rabbits fed conventional sunflower oil, suggesting that the introduction of monounsaturated fatty acids in place of polyunsaturated fatty acids protects LDL against oxidative modification by simply reducing the number of polyunsaturated fatty acids available as targets for peroxidation.

Other experiments with isolated and perfused rat hearts have shown that the susceptibility to hydroperoxide-induced oxidative stress (measured by ultra-weak chemiluminescence emission and eicosanoid release) can be modulated by dietary fats differing in unsaturated fatty acid and antioxidant content (36).

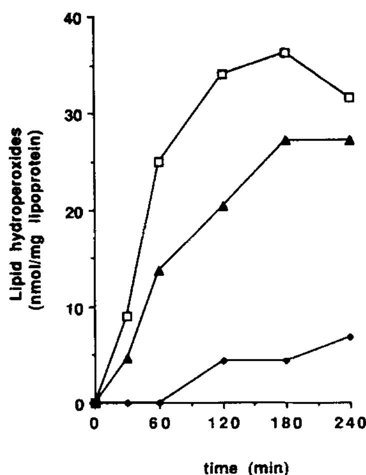

Fig. 2. Production of lipid hydroperoxides during Cu^{2+}-stimulated oxidation of rat VLDL + LDL fraction. Two pools of five freshly prepared VLDL + LDL samples (0.5 mg/ml) for each dietary group were oxidized in PBS containing 5 μM Cu^{2+}, at 37°C. Peroxides were determined iodometrically (31). The zero-time levels were subtracted from the values shown and each point represents the mean of duplicate determinations. VLDL + LDL from rats fed for 6 weeks on a diet of 15% (w/w) soybean oil, SO (□), olive oil, OO (♦), and an oleate-rich mixture of triglycerides, TO (▲).

In order to evaluate the effect of fatty acid unsaturation and the contribution of antioxidants (other than vitamin E) contained in olive oil (37–40) on the oxidative stability of plasma and lipoproteins, we compared a conventional soybean oil diet (54% linoleic acid) with an olive oil diet (75% oleic acid) and a diet containing an oleate-rich synthetic mixture of triglycerides (74% oleic acid). All diets had the same vitamin E content.

The measurement of lipid peroxidation in plasma, expressed as TBA-RS, showed that SO-fed animals had (significantly) the highest TBA-RS levels. Nevertheless, in the TO group the plasma TBA-RS was higher than in the OO group, in spite of similar plasma fatty acid composition. Urate, protein sulfhydryl groups, ascorbate, and vitamin E have been reported to account for most of the peroxyl radical-trapping antioxidant activity in plasma (28). In our study, the interaction among these various plasma antioxidants may only partially explain the different TBA-RS plasma levels found in the three groups. In fact, the TRAP values calculated for the OO and TO groups were not significantly different, indicating that the observed TBA-RS levels were not fully accounted for by those antioxidants entered into the calculation (urate, ascorbate, -SH, and vitamin E).

The observed difference in TBA-RS plasma levels between OO and TO groups may be related to the reported presence in olive oil of antioxidants other than vitamin E (37–40). Among these, β-carotene has been proposed to act as plasma antioxidant (41). The level of plasma carotenoids is determined by the efficiency of the cleavage of carotenes. As reported in the literature, rat intestine has a very efficient cleavage enzyme, which results in very low plasma carotenoid levels (42). In our experiments, plasma β-carotene, as well as the other carotenoids, was undetectable in all experimental groups. Moreover, the plasma vitamin A contents of OO-fed rats (379 ± 64 μg/ml) and TO-fed rats (344 ± 53 μg/ml) were not significantly different. Therefore, the higher antioxidant potential showed by olive oil fed animals cannot be ascribed to a different content in β-carotene or vitamin A.

Furthermore, our results confirm the reported lack of correlation between TBA-RS and vitamin E in LDL (10, 32–35). In fact, in the LDL + VLDL fraction, TBA-RS concentration was independent of the α-tocopherol content, which was identical in the three experimental groups.

The results from the in vitro copper-induced peroxidation of VLDL + LDL particles, expressed in terms of conjugated dienes, lipid hydroperoxides, and TBA-RS, strengthen the hypothesis of the presence in VLDL + LDL of OO-fed rats of other antioxidants effective in protection against lipid peroxidation. In fact, the VLDL + LDL fraction from OO-fed animals was much more resistant to oxidative modification not only compared to SO-fed rats (as expected) but also to TO-fed rats, although the initial vitamin E content was the same in the three experimental groups.

Our results indicate that the resistance to lipid peroxidation of lipoprotein fractions is modulated by both

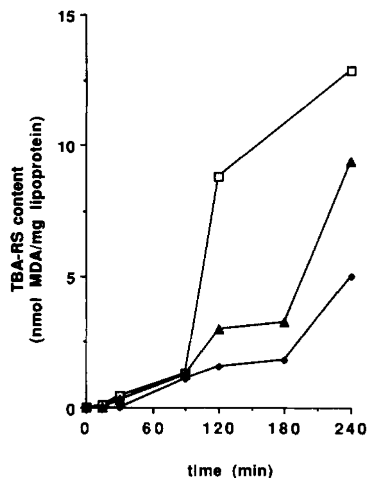

Fig. 3 Production of TBA-RS during Cu^{2+}-stimulated oxidation of rat VLDL + LDL fraction. Two pools of five freshly prepared VLDL + LDL samples (0.5 mg/ml) for each dietary group were oxidized in PBS containing 5 μM Cu^{2+}, at 37°C. TBA-RS was determined according to Maseki et al. (25). The zero-time levels were subtracted from the values shown and each point represents the mean of duplicate determinations. VLDL + LDL from rats fed for 6 weeks a diet of 15% (w/w) soybean oil, SO (□), olive oil, OO (♦), and an oleate-rich mixture of triglycerides, TO (▲).

dietary fatty acid composition and antioxidant content. The use of monounsaturated fats in the diet, rather than polyunsaturated fats, generates lipoprotein particles markedly resistant to oxidative modification. On the other hand, the dietary contribution of antioxidant compounds affects the overall resistance of lipoproteins to lipid peroxidation. Our experiments indicate that olive oil contains some natural components, in addition to well-known tocopherols and carotenoids, with antioxidant action in vivo, the nature of which needs further investigation.

The authors thank Mrs. Pierina Rami and Mrs. Rita Rami for their skillful assistance. Supported by the National Research Council (Italy), Special Project RAISA, Subproject 4, Paper n. 302.

Manuscript received 26 June 1991, in revised from 21 November 1991, and in re-revised form 10 February 1992.

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