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Solid Monounsaturated Diet Lowers LDL Unsaturation Trait and Oxidisability in Hypercholesterolemic (Type IIb) Patients

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Lowering high cholesterol concentration decreases the probability of atherosclerotic-related pathology onset. MUFA and PUFA decrease total plasma and LDL cholesterol but PUFA may increase the susceptibility of LDL to undergo oxidative modifications thus becoming more atherogenetic. Olive oil, the predominant fat source in Mediterranean diet, may combine the advantages of both lowering cholesterol level and decreasing LDL susceptibility to oxidation. We studied the effects of feeding MUFA vs PUFA enriched diet on LDL composition and feature in hypercholesterolemic (IIb) patients. Antioxidant values remained constant during the study while LDL fatty acids composition reflected the dietary intake: MUFA concentration increased 11% whereas PUFA decreased 10% after olive oil diet ($p < 0.05$). PUFA/MUFA ratio and the unsaturation index were lower at the end of MUFA-enriched diet. The challenge, *in vitro*, of oleate-enriched LDL with Cu^{2+} yielded to lower lag-phase ($p < 0.05$) in diene conjugated production; the same LDL gave lower lipid hydroperoxide contents after exposition to AAPH. We conclude that oleate-enriched LDL and with lower PUFA content were more resistant to oxidative modifications, as measured by different peroxidation indexes. This feature acquired with the diet may be an useful tool for lowering LDL oxidation and indirectly their atherogenicity.

Keywords: Soy oil, virgin olive oil, LDL oxidation, antioxidants, conjugated dienes, hypercholesterolemia

INTRODUCTION

Atherosclerosis and coronary heart diseases (CHD) are multifactor disorders associated to a number of causes such as dyslipidemia, hypertension, diabetes and obesity.[1] Among such agents, cholesterol concentration carried by LDL is one of the most relevant.[2,3] Thus, the incidence of arteriosclerosis and its various complications appears to be directly related to the level of this specific class of plasma lipid, as reported in several epidemiological studies;[4] in fact, lowering high cholesterol level decreases the probability of atherosclerotic-related pathology onset.

Decades of research have clearly demonstrated that diet has a strong influence on lipids and lipoprotein plasma levels, being dietary fats the

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most directly implicated factors.[5,6] The investigations regarding the relationships between plasma cholesterol level and the quantity/quality of dietary fats, revealed the raising cholesterol action of saturated fatty acids (SFA). This class of lipids was beneficially substituted by monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) for decreasing total plasma cholesterol and LDL-cholesterol (LDL-C) concentrations.[7,8]

In spite of the ability of PUFA enriched diet to reduce plasma cholesterol level, the degree of fatty acids unsaturation influences the susceptibility of LDL to undergo oxidative modifications, making these particles more prone to peroxidation and in principle more atherogenetic.

When MUFA substitute SFA, they can reduce total and LDL-C concentration without affecting HDL cholesterol (HDL-C), which in turn is efficiently lowered by PUFA. In addition, MUFA lead to favourable changes in the lipid profile of lipoproteins, generating LDL particles more resistant to oxidative modifications.[9]

According to the above findings the interest has been recently focused on the benefits of the Mediterranean diet (MEDDT) on hyperlipidemia and other established cardiovascular risk factors.[10] MEDDT is characterised by low SFA content and concomitant abundance of MUFA intake whose predominant source is olive oil. Furthermore, high MUFA intake may combine the advantages of both lowering cholesterol level and decreasing LDL susceptibility to oxidation. This aspect could be crucial because lipoprotein oxidation is now widely accepted as a contributing factor to atherosclerosis pathogenesis.[11]

Lipid oxidation leads to a chain reaction and to various kinds of aldehydes as final products. The interaction of these compounds with apolipoprotein B is probably critical in generating a form that is taken up, via the scavenger receptor, by macrophages present into the subendothelial space from bloodstream. Scavenger receptor leads to unlimited uptake of LDL underwent oxidative modifications (ox-LDL) by macrophages that finally become lipid loaded foam cells. These latter contribute to the formation of fatty streaks and subsequently to plaque formation.[12]

The purpose of the present study was to evaluate the effects of feeding with MUFA vs PUFA enriched diets on plasma and LDL lipid profile and antioxidant contents in mild hypercholesterolemic subjects. In addition, we studied the changes on plasma and LDL fatty acids composition at the end of each diet period. LDL accumulation of conjugated dienes (CD) and the increased hydroperoxides (HP) after an exogenous stimuli were detected as oxidative modification parameters.

MATERIALS AND METHODS

Subjects

Thirteen unrelated subjects (6 women, 7 men) aged 47–59 years, showing a IIb phenotype by Fredrickson classification, took part in the study. The patients and their relatives had been followed routinely in the GC Descovich Atherosclerosis Research Center of Bologna University: all had IIb phenotype for at least 3 consecutive controls (every 2 months). None of them were taking any medication or drugs at the time of the experiment. The study was approved by the Local Ethical Committee. The experimental protocol was explained in detail and informed consent was obtained from each patients.

Experimental Design

The study was divided in three consecutive diet periods. The first period lasted 4 weeks during which all subjects received a linoleic rich diet. The second and the third periods, each one 4 week long, consisted of an oleate rich diet.

Diets

Two different diets were designed and consisted of solid-food meals. The diets were isocaloric;

TABLE I Mean daily intake of energy and nutrients of subjects during the test diets (data are mean ± SD)

	Diets	
	Soy oil	Virgin olive oil
Energy (kcal)	1750 ± 308	1756 ± 296
Proteins (%)	15 ± 1	15 ± 2
Lipids (%)	30 ± 5	30 ± 5
Saturated fatty acids (%)	5 ± 1	5 ± 1
Monounsaturated fatty acids (%)	7 ± 2	19 ± 3
Polyunsaturated fatty acids (%)	16 ± 2	4 ± 1
Polyunsaturated/ Monounsaturated	1.5 ± 0.3	0.38 ± 0.01
Cholesterol (mg)	114 ± 23	110 ± 20
Carbohydrates (%)	58 ± 3	58 ± 4
Dietary fiber (g)	31 ± 6	31 ± 6

the protein, dietary fibre, cholesterol and carbohydrate contents were maintained constant. Each diet varied only in the percentage of MUFA or PUFA (Table I). Participants were required to replace most of the usual fat intake with soy oil (SO) during the first 4 weeks and virgin olive oil (VO) for the rest of the study period. Subjects were informed individually by a dietitian and taught to identify and quantify fat intake by means of simplified food tables. They were instructed in methods of using the oils in their diets; both oils were used as frying-oil, as cooking oil and for dressing during each assigned period. Although patients prepared their own meal at home, food intake were carefully monitored by daily food record throughout the duration of the study; the records were reviewed weekly by a dietitian to ensure adherence to the recommended food guidelines.[13]

Blood Sampling and Laboratory Analysis

Blood samples were taken before the start of the study (BASELINE) and at the end of each diet test period. The laboratory analysis were performed on samples collected after 12 h overnight fasting, without stasis in the sitting position; blood (20 ml) was drown into EDTA-containing tubes (1 mg/ml). Plasma was isolated by centrifugation at 1000× *g*, for 10 min. Plasma total cholesterol, HDL cholesterol (after LDL and VLDL precipitation by dextran sulphate magnesium) and triacylglycerols were measured using enzymatic kits (Boelrringer Mamiheim GmbH) adapted on autoanalyser ACP 5040 (Eppendorf).[14,15] Quality control was carried out by the World Health Organization Lipid Reference Centre in Prague according to the procedure adopted for Lipid Clinics participating in the WHO-ERICA Projects[16] (precision and accuracy ± 3% for total cholesterol and ± 4% for triglycerides). Plasma apolipoproteins A-I and B were measured by immunoturbidimetry (Turbitimer, Behring). LDL triglycerides and cholesterol were determined on isolated LDL using enzymatic kits (Boehringer Marinheim GmbH) adapted on autoanalyser ACP 5040 (Eppendorf).[14]

LDL Isolation

LDL was isolated immediately after separation of fresh plasma by density gradient ultracentifugation.[17] Plasma (5 ml) was adjusted to a density of 1.3 g/ml with solid KBr. Five milliliters of this solution were placed in polyallomer ultracentrifuge tubes (18.5 ml) and layered under KBr solution (density 1.006 g/ml). The tubes were centrifuged in a Sorvall 65 V13 vertical rotor at 65 000 rpm for 90 min at 10°C in a Kontron ultracentrifuge (Centrikon T 2070). The LDL samples were dialysed at 4°C overnight in PBS buffer with chelating resin (Cheelex 100, Sigma) at pH 7.4. The protein content was measured by the method of Lowry *et al.*[18] using bovine serum albumin as standard.

LDL Oxidation

Oxidation of LDL was determined as the CD production by continuously monitoring the increase in absorbance at 234 nm according to the method of Esterbauer.[19] Freshly prepared LDL (50 μg prot/ml) were incubated with 5 μM of $CuSO_4$ at 37°C in a Beckman DU64 Spectrophotometer. Absorbance at 234 nm was

automatically recorded at each 5 min interval for 180 min. The resistance of LDL to undergo lipid peroxidation (i.e. lag phase), the rate of lipid peroxidation that rapidly accelerates when the antioxidant have been consumed (i.e., maximal propagation rate (CD rate)), and the diene peak (i.e. maximal diene concentration (CDmax)), were evaluated.[19]

Ferrous-oxide xylenol orange method (FOX2) was used for determining HP, i.e., HP levels were assayed according to the principle of the rapid peroxide-mediated oxidation of Fe^{2+} to Fe^{3+} under acid conditions[20] slightly modified[21] using tryphenylphosphine (TPP), an agent that avoids artifactual colour generation in samples which contain substantial quantities of loosely available iron. Briefly, LDL samples (0.1 mg) were incubated in the presence of 2 mM 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) at 37°C for 30 min, with and without 1 mM TPP. FOX2 reagent was added to the samples in a water shaking bath. After centrifugation ($2000 \times g$ for 5 min) the supernatants were monitored spectrophotometrically at 560 nm.

Vitamin E (Vit. E) and Coenzyme Q_{10}(CoQ_{10}) Determination

Plasma and lipoprotein Vit. E and total CoQ_{10} levels were determined as previously described.[22] The extract was analysed by reversed-phase HPLC (Beckman Gold System) using an ODS column 150×4.6 mm; the mobile phase was methanol–ethanol (60:40, v/v) and the flow rate was 1 ml/min. The UV detector was set at 292 nm for Vit. E and 275 nm for CoQ_{10}. The calibration curves were done with known concentrations of the two analites.

Fatty Acids Composition

Plasma and LDL fatty acids composition were determined by capillary gas chromatography on a HRGC 5300 Mega Series chromatograph (Carlo Erba Instrument) equipped with CIP Sil 88, 50 m × 0.25 mm column after trans esterification process as described by Lepage and Roy.[23]

Statistics

All variables were confirmed normal in distribution in this study group. Data are expressed as mean ± standard deviation. Statistical evaluation of data was made by one way analysis of variance (ANOVA); when significant main differences were detected ($p < 0.05$), Duncan's multiple range test was used for a *post hoc* comparison. Relationship between variables were examined using linear regression analysis with Pearson's correlation coefficient. All tests were performed using a PC Statistical Package for Social Sciences (SPSS) programme.

RESULTS

The study was undertaken among free-living individuals who were given detailed and specific dietary advice as well as free supply of the test oils. Seven day dietary records meticulously kept throughout the study showed a high level of compliance with both diets. Mean body weights did not differ significantly among dietary periods.

Plasma Lipids, Apolipoproteins and Antioxidants Values

There were no significant differences in total LDL and HDL cholesterol, as well as in plasma triacylglycerols, between the baseline and the fixed two diets. Plasma apolipoprotein (Apo) B concentration was 12% less ($p < 0.05$) after the linoleic acid diet (113 ± 15 mg/dl) than at baseline (129 ± 17 mg/dl). Plasma ApoA-I levels were not different after both diets and at baseline. A positive correlation was found between Apo A-I vs total cholesterol ($r = 0.7$; $p < 0.02$) (Figure 1) during the diet periods but not at baseline. Liposoluble antioxidant patterns remained unchanged.

Plasma Fatty Acids Content

Plasma fatty acids composition (Table II) showed a significantly higher level of total SFA ($p < 0.05$), at the end of the linoleate diet with respect to baseline and the first 4 weeks of oleate rich diet. In particular, palmitic acid (16:0) was 24% lower after the VO diet with respect to SO diet ($p < 0.05$) and 10% lower with respect to baseline ($p < 0.05$).

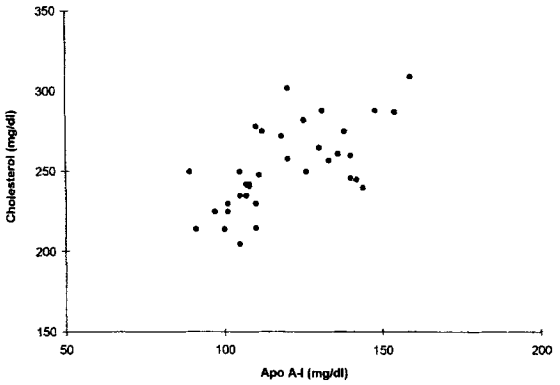

FIGURE 1 Correlation between Apo A-I vs total cholesterol ($r = 0.7$; $p < 0.02$).

Stearic acid (18:0) had an 20% increase during the SO diet.

LDL Lipids and Antioxidant Values

The analysis of the LDL compositional parameters evidenced an increase of triacylglycerols content at the end of 8 weeks of VO diet from baseline (28 ± 8 and 20 ± 6 mg/dl respectively, $p < 0.05$). No significant differences were found in protein and cholesterol concentrations among the diet periods and baseline. CoQ_{10} and Vit. E level remained constant during the study (Table III).

LDL Fatty Acids

The changes in the LDL fatty acids composition reflected the dietary intake of the subjects during the course of the study. As shown in Table IV oleic acid was higher after VO diet ($p < 0.05$) than both after the SO diet (−17%) and at baseline (8%). MUFA concentration increased 11% whereas PUFA decreased 10% after VO diet

TABLE II Effects of the diets on plasma fatty acid pattern (fatty acid amount is expressed in % of total fatty acids) (Data are mean ± SD)

	Baseline	Diets			Statistical analysis
		Soy oil (4 weeks)	Virgin olive oil (4 weeks)	Virgin olive oil (8 weeks)	
14:0	0.9 ± 0.3	0.8 ± 0.3	0.6 ± 0.2	1.1 ± 0.4	—
16:0	19 ± 2 (b)	21 ± 4 (c)	16 ± 2 (a)	21 ± 2 (d)	a vs b, c, d $p < 0.05$
18:0	7.4 ± 0.9	8.3 ± 1.7 (b)	7.2 ± 0.8	6.6 ± 0.5 (a)	a vs b $p < 0.05$
16:1n-7	1.7 ± 0.9	1.9 ± 1	1.7 ± 0.9	2 ± 0.9	—
16:1n-9	0.5 ± 0.09	0.4 ± 0.09	0.3 ± 0.07	0.4 ± 0.1	—
18:1n-9	22 ± 4	22 ± 4	23 ± 4	21 ± 2	—
24:1	2.3 ± 0.6	2.6 ± 0.7	2.4 ± 0.4	1.8 ± 0.7	—
18:2n-6	25 ± 4	25 ± 6	24 ± 3	25 ± 3	—
20:3n-6	1.6 ± 0.4	1.1 ± 0.5	1.3 ± 0.5	1.6 ± 0.3	—
20:4n-6	8.5 ± 2	7.2 ± 2	8.5 ± 2	6.5 ± 1	—
Saturated fatty acids	32 ± 2 (b)	36 ± 5 (a)	30 ± 2 (c)	33 ± 2	a vs b, c $p < 0.05$
Monounsaturated fatty acids	29 ± 5	30 ± 6	29 ± 4	29 ± 2	—
Polyunsaturated fatty acids	39 ± 7	34 ± 10	39 ± 6	37 ± 3	—
18:1/18:2	0.8 ± 0.3	0.9 ± 0.4	0.9 ± 0.3	0.9 ± 0.1	—
PUFA/MUFA	1.3 ± 0.5	1.2 ± 0.6	1.3 ± 0.3	1.3 ± 0.2	—
Unsaturation index*	130 ± 15	121 ± 21	123 ± 9	120 ± 2	—

*UI is defined as $\sum$ mol% of each fatty acid × number of double bonds of the same fatty acid.

TABLE III Effects of the diets on LDL chemical composition (data are mean ± SD)

	Baseline	Diets			Statistical analysis
		Soy oil (4 weeks)	Virgin olive oil (4 weeks)	Virgin olive oil (8 weeks)	
Cholesterol (mg/dl)	141 ± 24	151 ± 26	160 ± 16	155 ± 16	—
Triglycerides (mg/dl)	20 ± 6 (b)	26 ± 5	25 ± 8	28 ± 8 (a)	a vs b $p < 0.05$
Coenzyme Q10 (μg/mg chol)	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	—
Vitamin E (μg/mg chol)	4.9 ± 1	5.2 ± 1	4.5 ± 1	5.2 ± 1	—

TABLE IV Effects of the diets on LDL fatty acid composition (fatty acid amount is expressed in % of total fatty acids) (data are mean ± SD)

	Baseline	Diets			Statistical analysis
		Soy oil (4 weeks)	Virgin olive oil (4 weeks)	Virgin olive oil (8 weeks)	
14:0	0.7 ± 0.3	0.5 ± 0.3	0.5 ± 0.2	0.9 ± 0.4	—
16:0	16 ± 2	15 ± 2	15 ± 2	19 ± 2	—
18:0	6.3 ± 0.7	6 ± 0.3	6 ± 0.6	5.3 ± 0.3	—
16:1n-7	1.6 ± 0.8	1.4 ± 0.7	1.7 ± 1	1.9 ± 1	—
16:1n-9	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	—
18:1n-9	18.5 ± 1 (b)	16.5 ± 0.5 (a)	20 ± 1 (c)	19 ± 0.7 (d)	a vs b, c, d $p < 0.05$
24:1	2.8 ± 0.1	3 ± 0.1	2.7 ± 0.1	2.2 ± 0.2	—
18:2n-6	29 ± 13	32 ± 4	29 ± 3	30 ± 3	—
20:3n-6	1.6 ± 0.4	1.7 ± 0.4	1.7 ± 0.3	2 ± 0.4	—
20:4n-6	9.8 ± 2	9.2 ± 1	9 ± 1	7.4 ± 1.6	—
Saturated fatty acids	27 ± 0.7 (b)	28 ± 0.7 (c)	28 ± 1(d)	31 ± 0.4 (a)	a vs b, c, d $p < 0.05$
Monounsaturated fatty acids	25 ± 0.5 (b)	23 ± 0.8 (a)	26 ± 0.8 (c)	25 ± 0.7 (d)	a vs b, c, d $p < 0.05$
Polyunsaturated fatty acids	46 ± 1(b)	48 ± 1(c)	45 ± 1.6	43 ± 0.8 (a)	a vs b, c $p < 0.05$
18:1/18:2	0.6 ± 0.03	0.5 ± 0.03 (a)	0.7 ± 0.03 (b)	0.7 ± 0.03 (c)	a vs b, c $p < 0.05$
PUFA/MUFA	1.8 ± 0.07 (b)	2.1 ± 0.1(a)	1.8 ± 0.1(c)	1.7 ± 0.08 (d)	a vs b, c, d $p < 0.05$
Unsaturation index*	140 ± 3 (a)	136 ± 2 (b)	127 ± 4 (c)	128 ± 1 (d)	a vs b, c, d $p < 0.05$

*UI is defined as $\sum$ mol% of each fatty acid × number of double bonds of the same fatty acid.

compared with SO diet ($p < 0.05$). The PUFA/MUFA ratio and the unsaturation index (UI) (defined as $\sum$ mol% of each fatty acid × number of double bonds of the same fatty acid) were significantly lower at the end of the VO diet than at baseline and after SO diet ($p < 0.05$). The unsaturated fatty acid content after 8 weeks of VO diet was lower than at baseline and at the end of SO diet ($p < 0.05$). PUFA n-3 and PUFA n-6 level were significantly higher during PUFA enriched diet than during the other diet periods; the 18:1/18:2 ratio decreased at the end of SO diet with respect to VO diet ($p < 0.05$).

LDL Oxidation

When LDL samples were subjected to oxidative modification *in vitro* and accumulation of conjugated dienes was estimated (Figure 2), a significant difference was seen in the duration of the lag-phase among dietary period and baseline. The LDL after 8 weeks of VO diet were more resistant to oxidative modification as shown by the longer lag-phase (55 ± 9 min) with respect to baseline (47 ± 8 min; $p < 0.05$) and even more after SO diet (43 ± 8 min; $p < 0.05$). No differences were found in the CD maximal propagation rate and CD

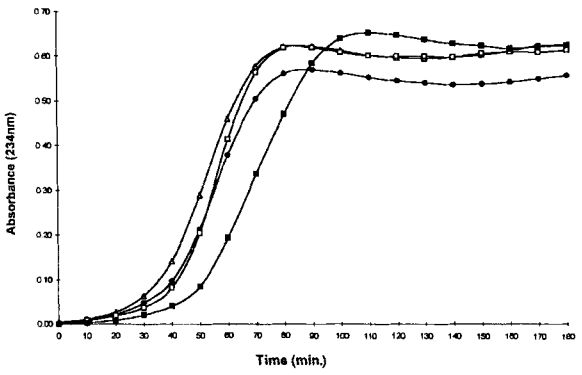

FIGURE 2 Conjugated dienes formation during Cu^{2+} oxidation of LDL after soy oil diet (4 weeks) (●), virgin olive oil [4 weeks (□); 8 weeks (■)] and at recruitment (△).

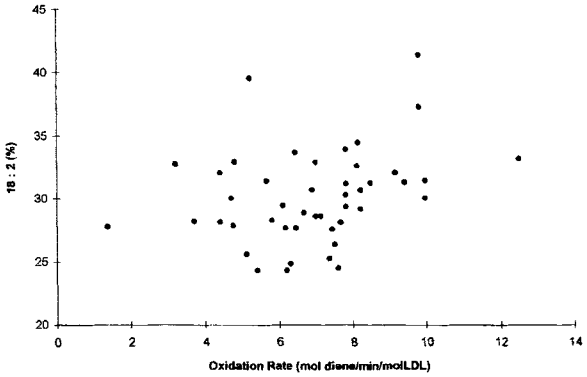

FIGURE 3 Correlation between 18:2 content and the oxidation rate in LDL ($r = 0.3$; $p < 0.05$).

maximal concentrations among dietary periods and baseline.

Among the several couples of variables and parameters tested, five of them resulted to correlate with high statistical significance and deep biochemical meaning. The rate of LDL oxidation significantly correlated with the 18:2 content ($r = 0.3$, $p < 0.05$) (Figure 3) or the percent of total PUFA ($r = 0.4$, $p < 0.01$) (Figure 4). The rate of LDL oxidation also correlated with the PUFA/MUFA ratio ($r = 0.4$, $p < 0.01$) (Figure 5). The lag-phase before initiation of CD formation was strongly correlated with the 18:1 levels ($r = 0.4$, $p < 0.01$) (Figure 6).

The reduced susceptibility of LDL after VO diet (either after 4 or 8 weeks) was confirmed by concomitant measurement of lipid HP

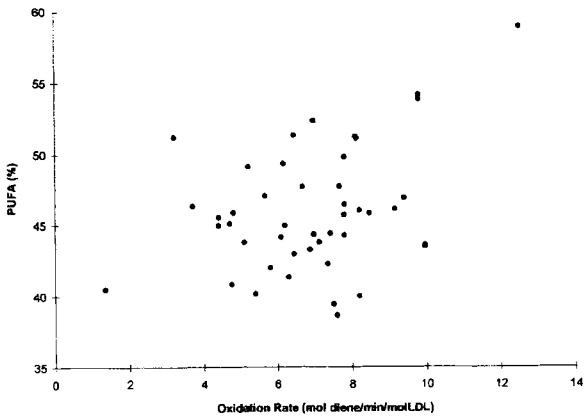

FIGURE 4 Correlation between the percent of total PUFA and the oxidation rate ($r = 0.4$; $p < 0.01$).

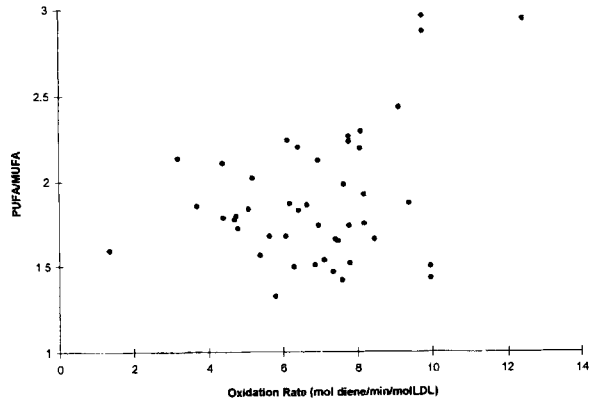

FIGURE 5 Correlation between the PUFA/MUFA ratio and the oxidation rate ($r = 0.04$; $p < 0.01$).

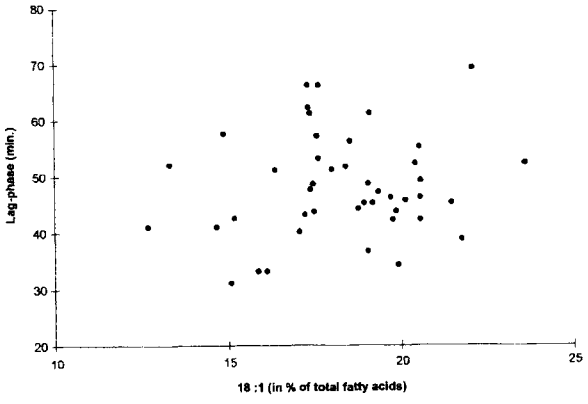

FIGURE 6 Correlation between the Lag-phase of conjugated dienes formation by Cu^{2+} and 18:1 content in LDL ($r = 0.4$; $p < 0.01$).

TABLE V Indexes of LDL oxidisability after the two diets and at baseline (data are mean ± SD)

	Baseline	Diets			Statistical analysis
		Soy oil (4 weeks)	Virgin olive oil (8 weeks)	Virgin olive oil (8 weeks)	
Lag-phase (min)	47 ± 8 (b)	43 ± 8 (c)	43 ± 6 (d)	55 ± 9 (a)	a vs b, c, d $p < 0.05$
Oxidation rate (mol diene/min/mol LDL)	6.5 ± 1	7 ± 2	7.6 ± 2	6.7 ± 2	—
CD (mol diene/mol LDL)	281 ± 54	290 ± 36	282 ± 45	290 ± 75	—
HP (nmol/mg LDL prot)					
30 min	37 ± 26	30 ± 24	14 ± 13	33 ± 15	—
60 min	69 ± 31	69 ± 43	37 ± 20	53 ± 15	—
90 min	101 ± 49	92 ± 65	72 ± 26	79 ± 19	—
120 min	151 ± 62 (b)	170 ± 90 (c)	103 ± 42	91 ± 21(a)	a vs b, c $p < 0.05$
180 min	224 ± 100 (b)	198 ± 82	166 ± 30	138 ± 20 (a)	a vs b $p < 0.05$

Measurement of CD was performed in LDL after Cu^{2+}-induced oxidation. LDL were 50 μg prot/ml, Cu^{2+} was 5 μM, temperature 37°C and absorbance was continuously measured at 234 nm. Measurement of HP was performed in LDL (0.1 mg) after AAPH (2 mM) induced oxidation monitoring the absorbance change at 560 nm after fixed times. See text for more details.

(Table V). These lipoproteins had lower increment of HP, in comparison with LDL obtained after SO diet and at baseline. The HP content of LDL after VO diet were always the lowest; nevertheless the statistical significance was reached after 120 min (91 ± 21 MUFA vs 170 ± 90 PUFA and 151 ± 62 BASELINE, $p < 0.05$) and 180 min (138 ± 20 MUFA vs 198 ± 82 PUFA and 224 ± 100 BASELINE, $p < 0.05$) from the oxidative insult.

DISCUSSION

It has been well documented that hypercholesterolemia is both a common and important risk factor for CHD. In general, guidelines on the treatment of hypercholesterolemic people recommended to lower cholesterol levels initially with a diet and subsequently with medications.[2] The present study, in which a practical solid-food diet was used, was undertaken to examine, in mildly hypercholesterolemic patients, the effects of linoleic or olive oil supplementation, on plasma and LDL lipids content and the influence of diet on fatty acids composition and on oxidative parameters. We found that the different diets did not affect plasma and LDL cholesterol as well as triglycerides concentration. An unexpected increment of plasma SFA was found after SO diet. In this study, similarly to that reported by Chang *et al.*,[23] we could not observe the hypocholesterolemic effect of a high monounsaturated diet. It is to underline that also the diet rich in PUFA did not change plasma lipids, as we could expect, and as it has been already reported.[25,26] Even though the diet failed in affecting LDL-C levels, neither HDL-C concentrations were modified, but remained the same during the course of the study. The very modest response to both diets does not obviously reflect inadequate compliance. In fact patients were provided the oils to be consumed at home, they were followed by a dietitian and fatty acids change of LDL confirmed a satisfactory intake. The results from the seven days dietary record clearly reflected a good adherence of the patients to the recommendations and the food prescriptions during each diet period. It is possible that the relative failure of the two diets to lower total and LDL cholesterol might be due to a variability in response within the subjects, but above all it must be taken into account that the dietary recommendations were not so far different from the usual intake of Italian population in order to expect a marked differences on the lipid plasma profile as it occurs in case of typical

subjects from Western countries. Other variables could affect the degree of the diets response.

Our failure to achieve the cholesterol changes previously predicted by some investigators[27,28] but that have not been observed by others,[24,29] could be also attributed to the different kinds of diets employed (i.e. solid vs liquid diet) or to fats proceeding from different sources (e.g., conventional oils, genetically modified oils, margarine or butter).

The increase of LDL triglycerides at the end of 8 weeks of VO diet perhaps reflects the mobilisation of a larger amount of VLDL particles from the liver to the blood under condition of high MUFA intake as reported by Chang and Huang.[24]

The fact that LDL fatty acids composition can be modified thus reflecting the fatty acids composition of the diet, is clearly demonstrated from the analysis of the lipoprotein fatty acid pattern at the end of each diet. Four weeks of solid food diet rich in linoleic or oleic acids markedly increased in all participants the LDL content of the respective fatty acids and a series of related parameters such as UI or 18:1/18:2 ratio resulted changed despite the short duration of the study. Nevertheless, the most important fact regarded the deep changes occurred in LDL features: LDL enriched in oleic acid and with lower PUFA content were more resistant to oxidative modifications, as measured by different peroxidation indexes. First of all the length of the "lag-phase" was longer in oleate-enriched LDL. Several authors reported[30,31] that PUFA oxidation in LDL is preceded by a loss of antioxidants, in particular Vit. E and CoQ_{10}, which are the main determinants of the oxidation resistance of the particles. On the other hand data from studies both *in vitro* and *in vivo*[32,33] showed that they are not the only parameters influencing the initiation phase of peroxidation; preformed HP, different PUFA content, cholesterol level must be also taken into account. In fact, the group of Parthasarathy[9,29,34] reiteratedly found that LDL fatty acid composition may affect the initiation phase of peroxidation. However, a part of the effect on the lag-phase could be also ascribed to some peculiar antioxidants typical of virgin olive oil that patients took during the treatment. In fact, virgin olive oil is particularly rich in antioxidant substances (hydroxytyrosol, oleuropein) that had been demonstrated to inhibit LDL oxidation *in vitro*.[35,36]

Since we found that the Vit. E and CoQ_{10} levels in plasma and in LDL particles remained unchanged during the course of the study, as it occurred for cholesterol content, we may conclude that LDL proneness to undergo oxidative modifications is mainly the result of compositional change due to the enrichment from the different diets of the relative fats.

Such indication was also confirmed by the results of several correlations; in fact, parameters connected with increasing peroxidation (such as the rate of LDL peroxidation) positively correlated with PUFA, PUFA/MUFA and 18:2 while the lag-phase correlated with 18:1 content (i.e., indicating a higher protection). Moreover, when the tendency toward oxidative stress was measured as the increase in HP content, LDL from SO diet promoted significantly higher HP formation than did LDL isolated after VO diet. The above are in accordance with the findings reported by Reaven *et al.*[34] in which the subjects were fed a liquid diet providing all the dietary fats in a fixed ratio. Our study demonstrates that under more physiological and realistic dietary conditions, the fatty acids modifications occurred as a consequence of the diets, directly affect the tendency of peroxide formation. This is an important fact since free radicals and LDL modification are considered to be relevant pathoetiological factors in the development of atherosclerosis.

We have previously demonstrated that experimental diet, whose unique fat source was olive oil, deeply affected rat liver microsomal and mitochondrial membrane composition as well as their peroxidation susceptibility;[37,38] the present investigation suggests that similar results can be obtained during short clinical dietary treatment even though the extent of the changes induced are

less evident than in animal model study. This is due to several reason. (i) the relative short duration of the diet, (ii) the presence of other fats source in the food for human nutrition, (iii) the use of free living population instead of controlled experimental animals, (iv) the use of solid instead of liquid diets, (v) the use of synthetic fatty acid mixture.

The possibility of dietary manipulation of LDL composition is an useful tool for lowering their probability to undergo oxidative modification, that in other words represents an important indirect method for diminishing the atherogenic feature of such structures.

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References

[1] Anderson, K.M., Odell, P.M., Wilson, P.W.F. and Kannel, W.B. (1990) Cardiovascular disease risk profile. *American Heart Journal*, **121**, 283–298.

[2] National Cholesterol Education Program. Adult treatment panel II. (1994) *Circulation*, **89**, 1336–1356.

[3] Lipids Research Clinics Program: The lipid research clinics coronary prevention trial results. I. Reduction in incidence of coronary heart disease. (1984) *Journal of American Medical Association*, **251**, 352–364.

[4] Castelli, W.P., Garrison, R.J., Wilson, P.W.F., Abbot, R.D., Kalousdian, S. and Kannel, W.B. (1986) Incidence of coronary heart disease and lipoprotein cholesterol levels: the Framingham Study. *Journal of American Medical Association*, **256**, 2835–2838.

[5] Hegstedt, D.M., McGandy, R.B., Myers, M.L. and Stare, F.T. (1965) Quantitative effects of dietary fat on serum cholesterol in man. *American Journal of Cliniical Nutrition*, **17**, 281–295.

[6] Grundy, S.M. and Denke, M.A. (1990) Dietary influences on serum lipids and lipoproteins. *Journal of Lipid Research*, **31**, 1149–1172.

[7] Mattson, F.H. and Grundy, S.M. (1985) Comparison of the effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *Journal of Lipid Research*, **26**, 194–202.

[8] Mensink, R.P. and Katan, M.B. (1989) Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. *New England Journal of Medicine*, **321**, 436–441.

[9] Parthasarathy, S., Khoo, J.C., Miller, E., Barnett, J., Witztum, J.L. and Steinberg, D. (1990) Low density lipoprotein rich in oleic acid is protected against oxidative modification: implications for dietary prevention of atherosclerosis. *Proceedings of the National Academy of Science of USA*, **87**, 3894–3898.

[10] International Consensus Statement: "Olive Oil and the Mediterranean Diet: Implications for Health in Europe". Meeting convened by the European Commission at the Italian National Research Council, Rome, 11 April 1997.

[11] Witzun, J.L. and Steinberg, D. (1991) Role of oxidised low density lipoprotein in atherogenesis. *Journal of Clinical Investigations*, **88**, 1785–1792.

[12] Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C. and Witztum, J.L. (1989) Beyond cholesterol. Modification of low-density lipoprotein that increase its atherogenity. *New England Journal of Medicine*, **320**, 915–924.

[13] Mann, J. (1993) Diseases of the heart and circulation: the role of dietary factors in dietology and management. In *Human Nutrition and Dietetics* (eds. J.S. Garrow and W.P.T. James), Churchill Livingstone, Edinburgh, pp. 619–650.

[14] Roeschlau, P., Bernt, E. and Gruber, W. (1974) Enzymatic determination of total cholesterol in serum. *Journal of Clinical Chemistry and Clinical Biochemistry*, **12**, 226–227.

[15] Warnick, G.R., Benderson, J. and Albers, J.J. (1982) Dextran sulphate-Mg precipitation procedure for quantification of high-density lipoprotein cholesterol. *Clinical Chemistry*, **28**, 1379–1382.

[16] WHO-ERICA Research Group. (1988) The CHD risk map of Europe. The 1st report of the WHO-ERICA Project. *European Heart Journal*, **1**, 1–36.

[17] Chung, B.H., Segrest, J.P., Ray, M.J., Brunzell, J.D., Hokanson, J.E., Krauss, R.M., Beaudrie, K. and Cone, J.T. (1986) Single vertical spin density gradient ultracentrifigation. *Methods in Enzymology*, **128**, 181–209.

[18] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measured with the folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265–275.

[19] Esterbauer, H., Striegi, G., Puhl, H. and Rothender, M. (1989) Continuosly monitoring of *in vitro* oxidation of human low density lipoprotein. *Free Radical Research Communications*, **6**, 67–75.

[20] Jiang, Z., Hunt, J.V. and Wolff, S.P. (1992) Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. *Analytical Biochemistry*, **202**, 384–389.

[21] Neurooz-Zadeh, J., Tajaddini-Sarmadi, J. and Wolff, S. (1994) Measurement of plasma hydroperoxide concentrations by the ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine. *Analytical Biochemistry*, **220**, 403–409.

[22] Grossi, G., Bargossi, A.M., Fiorella, P.L., Piazzi, S., Battino, M. and Bianchi, G.P. (1992) Improved high-performance liquid chromatographic method for the determination of Coenzyme Q_{10} in plasma. *Journal of Chromatography*, **593**, 217–226.

[23] Lepage, G. and Roy, C.C. (1986) Direct transesterification of all classes of lipid in one-step reaction. *Journal of Lipid Research*, **27**, 114–120.

[24] Chang, N.W. and Huang, P.C. (1990) Effects of dietary monounsaturated fatty acids on plasma lipids in humans. *Journal of Lipid Research*, **31**, 2141–2147.

[25] Howard, B.V., Hannah, J.S., Heiser, C.C., Jablonski, K.A., Paidi, M.C., Alarif, L., Robbins, D.C. and Howard, W.J. (1995) Polyunsaturated fatty acids result in a greater cholesterol lowering and less triacylglycerol elevation than do monounsaturated fatty acids in a dose–response comparison in a multiracial study group. *American Journal of Clinical Nutrition*, **62**, 392–402.

[26] Mensik, R.P. and Katan, M.B. (1992) Effects of dietary fatty acids on serum lipids and lipoproteins. *Arteriosclerosis and Thrombosis*, **12**, 911–918.

[27] Ginsberg, H., Barr, S., Gilbert, A., Karmally, W., Deckelbaum, R., Kaplan, K., Ramakrishnan, R., Holleran, R. and Dell, R. (1990) Reduction of plasma cholesterol levels in normal men on an American Heart Association step I diet or a step I diet added monounsaturated fat. *New England Journal of Medicine*, **322**, 574–579.

[28] Berry, E., Eisenberg, S., Haratz, D., Friedlander, Y., Norman, Y., Kaufmann, N. and Stein, Y. (1991) Effects of diets rich in monounsaturated fatty acids on plasma lipoprotein – the Jerusalem Nutrition Study: high MUFAs vs high PUFAs. *American Journal of Clinical Nutrition*, **53**, 899–907.

[29] Reaven, P., Parthasarathy, S., Grasse, B., Miller, E., Alzman, F., Mattson, F., Khoo, J., Steinberg, D. and Witzum, J. (1991) Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. *American Journal of Clinical Nutrition*, **54**, 701–706.

[30] Esterbauer, H., Gebicki, J., Puhl, H. and Jurgens, G. (1992) The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology & Medicine*, **13**, 341–390.

[31] Stocker, R., Bowry, V. and Frei, B. (1991) Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does α-tocopherol. *Proceedings of the National Academy of Science of USA*, **88**, 1646–1650.

[32] Kontush, A., Hubner, C., Finck, B., Kohlschutter, A. and Beisiegel, U. (1994) Low density lipoprotein oxidizability by copper correlates to its initial ubiquinol-10 and polyunsaturated fatty acids content. *FEBS Letters*, **341**, 69–73.

[33] Croft, K.D., Williams, P., Dimmitt, S., Abu-Amsha, R. and Beilin, L.J. (1995) Oxidation of low-density lipoproteins: effect of antioxidant content, fatty acid composition and intrinsic phospholipase activity on susceptibility to metal ion-induced oxidation. *Biochimica et Biophysica Acta*, **1254**, 250–256.

[34] Reaven, P., Parthasarathy, S., Grasse, B., Miller, E., Steinberg, D. and Witzum, J. (1993). Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in midly hypercholesterolemic subjects. *Journal of Clinical Investigations*, **91**, 668–676.

[35] Visioli, F. and Galli, C. (1994) Oleuropein protects low density lipoprotein from oxidation. *Life Sciences*, **55**, 1965–1971.

[36] Visioli, F., Bellomo, G., Montedoro, G.F. and Galli, C. (1995) Low density lipoprotein oxidation is inhibited *in vitro* by olive oil constituents. *Atherosclerosis*, **117**, 25–32.

[37] Huertas, J.R., Battino, M., Barzanti, V., Maranesi, M., Parenti-Castelli, G., Littarru, G.P., Turchetto, E., Mataix, F.J. and Lenaz, G. (1992) Mitochondrial and microsomial cholesterol mobilization after oxidative stress induced by adriamycin in rats fed with dietary olive and corn oil. *Life Science*, **50**, 2111–2118.

[38] Mataix, J., Quiles, J.L., Huertas, J.R., Battino, M. and Mañas, M. (1998) Tissue specific interactions of exercise, dietary fatty acids and vitamin E in lipid peroxidation. *Free Radical Biology & Medicine*, **24**, 511–521.