



Comparison of an oleic acid enriched-diet vs NCEP-I diet on LDL susceptibility to oxidative modifications

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Objective: The objective of this trial was to compare the effect on the susceptibility of plasma Low Density Lipoprotein (LDL) to oxidative modifications of consumption of two oleic rich diets, prepared with two different plant oils, virgin olive oil (OL)¹ and refined high monounsaturated fatty acids (MUFA sunflower oil (SU)), with the susceptibility of plasma LDL to oxidation after an National Cholesterol Education Program step 1 (NCEP-I) phase diet.

Design: A randomized crossover design.

Subjects and interventions: Twenty-two healthy normolipidemic young males consumed an NCEP-I diet for a 4-week period. Subjects were then assigned to two diets each of 4-weeks duration. Group one was placed on an olive oil enriched diet (40% fat, 22% MUFA) followed by a 4-week period of a MUFA diet enriched in sunflower oil (40% fat, 22% MUFA). In group two, the order of the diets was reversed.

Results: Both MUFA diets induced a decrease in saturated (14:0, 16:0, and 18:0) and an increase in monounsaturated and polyunsaturated *n*-6 (18:2, 20:3, and 20:5) plasma LDL-phospholipid fatty acids, compared to the NCEP-I diet ($P < 0.01$). No significant differences in lag times were observed between the olive oil and the NCEP-I diet periods. However there was a greater inhibition time ($P < 0.001$) when subjects consumed the MUFA rich sunflower oil diet compared to the NCEP-I diet. These differences were probably related to the relative enrichment of plasma LDL particles in α -tocopherol due to the high vitamin E content of the MUFA-rich sunflower oil. Indeed, the α -tocopherol content was positively correlated with lag time ($r = 0.338$; $P < 0.008$).

Conclusion: Our findings suggest that changes in plasma LDL α -tocopherol content with practical solid-food diets can decrease its susceptibility to oxidation.

Sponsorship: This work has been supported by grants from the Investigaciones de la Seguridad Social (FIS 92/0182, to Francisco Pérez Jiménez); and from Koype Co, Andújar, Jaén, Spain.

Descriptors: oxidized low density lipoproteins; α -tocopherol; phospholipid; monounsaturated fatty acids; low fat diets; fatty acids

European Journal of Clinical Nutrition (2000) 54, 61–67

Introduction

Coronary heart disease (CHD) due to atherosclerosis remains the most prevalent cause of death and disability in Europe and North America. The dramatic geographic differences in the incidence of this disease and the marked shifts in CHD rates observed in migrant populations emphasize the importance of environmental factors in the development of CHD.

Growing evidence suggests that oxidative modification of plasma low density lipoproteins (LDL) enhances their

atherogenic properties (Berliner & Heinecke, 1996; Grundy 1995; Jialal & Devaraj 1996a, Luck & Fruchart, 1991; Penn & Chisolm, 1994; Rosenfeld, 1991; Steinberg *et al*, 1989; Williams & Tabas, 1995). Oxidized LDL could promote atherosclerosis in several ways: by its cytotoxicity (Hughes *et al*, 1994; Thorne *et al*, 1996), its chemotactic effect on monocytes (Cushing *et al*, 1990), its inhibitory effect on macrophage motility, its effects on the vascular tone (Chin *et al*, 1992); Liao *et al*, 1995), its thrombogenic effects (Drake *et al*, 1991; Latron *et al*, 1991) and its uptake by the macrophage scavenger receptor mechanism, the later leading to stimulation of cholesterol esterification and foam cell formation (Maor *et al*, 1995). Furthermore, several lines of evidence support the *in vivo* existence of oxidized LDL (Bui *et al*, 1996; Hammer *et al*, 1995, Itabe *et al*, 1994; Ylä-Herttuala *et al*, 1994).

The first target of peroxidation are the polyunsaturated fatty acids within the LDL (Esterbauer *et al*, 1987). This process is inhibited by chain-breaking antioxidants such as vitamin E commonly present within plasma LDL particles (Bays & Dujovne, 1993). Hence lowering the content of antioxidant and/or increasing the amount of polyunsaturated fatty acids in plasma LDL should render these lipoproteins more prone to oxidative modifications (Esterbauer *et al*, 1987; Jialal & Devaraj, 1996b). Studies in

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Contributors: P Gómez and MD Escalante, *in vitro* experiments of oxidation of LDL. A Martín and F López Segura the determination of α -tocopherol. J López Miranda, selection of the populations, the control of the adherence of the diets and different biochemical determinations. A Blanco and F Fuentes the determinations of fatty acid composition and the control of the adherence of the diets. JM Ordovás provided advice during all the stages of the work and participated actively in data analysis and manuscript preparation. F Pérez-Jiménez is the group leader and contributed to the experimental design and data analysis. P Castro contributed to the experimental design, the control of adherence of the diets, data analysis and preparation of the manuscript.

Received 18 March 1998; revised 18 March 1999; accepted 30 March 1999

humans and animals support this hypothesis and show that a diet rich in polyunsaturated fatty acids increases the susceptibility of plasma LDL to oxidation compared with a diet high in monounsaturated fatty acids (MUFA) (Berry *et al*, 1991; Bonamone *et al*, 1992; Fraser, 1994; Fuller & Jialal, 1994; Parthasarathy *et al*, 1990; Reaven *et al*, 1991; Reaven & Witztum, 1993b; Reaven *et al*, 1994; Suzukawa *et al*, 1995; Thomas *et al*, 1994). In addition, *in vivo* supplementation with α -tocopherol (Jialal & Grundy, 1992; Reaven & Witztum, 1993b) but not with β -carotene (Jialal *et al*, 1995; Princen *et al*, 1992; Reaven *et al*, 1993c) increased the oxidation resistance of LDL.

The Mediterranean region has been characterized by a low prevalence of CHD, despite a fat intake that, at present, accounts for 35 to 40% of total calories (Moreiras-Varela, 1989). It has been postulated that this apparent protection may be due to the high proportion of MUFA present in the diet of these countries (Keys *et al*, 1986). Although the traditional source of dietary MUFA in these countries is olive oil, other sources are increasingly available, that is, low erucic acid rapeseed oil (canola oil), and oleic acid-rich variants of sunflower or safflower oil. While the fatty acid composition of these oils is quite similar, they vary considerably in terms of plant sterols and other non-nutrients. Virgin olive oil is frequently consumed in Mediterranean countries, while other MUFA enriched vegetable oils need to be refined. This technique removes some unsaponifiable materials although α -tocopherol is added to the oil during the preparation process. This is specially important in view of the evidence that some of these compounds, such as tocopherols (Jialal & Grundy 1992; Jialal *et al*, 1995; Princen *et al*, 1992; Roaven & Witztum, 1993b; Reaven *et al*, 1993c), may have antioxidant effects.

In the present study, we have compared the effects on the susceptibility of plasma LDL to oxidative modification of two high fat diets, similar in fatty acid composition but prepared using two different plant oils: crude olive oil, and refined high MUFA sunflower oil. Our aim was to examine whether these diets had a significant effect on the oxidative modification of LDL, independently of their fatty acid composition. Furthermore, since the two diets most commonly recommended to decrease plasma cholesterol are low fat diets (The Expert Panel, 1998), we compared these high fat, high MUFA diets, (40% fat, with 22% MUFA) to a low-fat high-carbohydrate diet (30% fat, 12% MUFA) recommended by the National Cholesterol Education Program (NCEP).

Methods

Subjects and protocol

Twenty-two healthy normolipemic (total cholesterol under 5.7 mmol/l) young men (mean age, 23 ± 0.4 y) were recruited from the medical students at the University of Cordoba, Spain. None of the subjects had any history of disease and none of them were on any type of medication or vitamins for 6 months before entry. Daily physical activity and a weekly dietary record were used to calculate their individual energy requirements. Students did not receive monetary compensation for their participation in the study and informed consent was obtained from all participants.

Mean Body Mass Index (BMI) [$\text{wt}(\text{kg})/\text{ht}^2(\text{m}^2)$] was 24.3 ± 0.5 at the beginning of the study, and it remained constant throughout the experimental period. Subjects were

encouraged to maintain their regular physical activity and life style habits, and were asked to record in a personal diary any event that could affect the outcome of the study (academic stress, smoking, and consumption of alcohol and other foods).

The study design (Figure 1) included an initial 4-week period during which all students consumed an National Cholesterol Education Program Step 1 (NCEP-I) diet, in which 30% of total calories were consumed as fat, with < 10% saturated fat (SAT), 12% MUFA and 8% polyunsaturated fatty acids (PUFA), with 285 mg of cholesterol per day. Then, two groups of 11 subjects each were assigned to two 4-week periods, following a crossover randomized design. Group one followed a 22% MUFA-olive oil diet for 4-weeks, followed by a 4-week period of a MUFA-sunflower oil diet. In group two, the order of the diets was reversed. Both diets contained 15% protein, 45% carbohydrate and 40% fat (10% SAT, 8% PUFA and 22% MUFA). Cholesterol intake was not a factor in this design and was kept constant at 285–300 mg per day during all study periods. Assignment of each volunteer to the sequence of diets was done randomly. Energy intake was adjusted as needed to maintain the initial body weight. Body weight was measured twice a week. Two investigators supervised the feeding of subjects and were blinded to the changes in serum-chemistry values. The other investigators supervised or performed all laboratory analyses and were blinded to the dietary assignments.

Diets

Composition of the experimental diets was calculated using the USDA food composition tables or the Spanish food composition tables for certain local foodstuffs. Fourteen menus, prepared using conventional mixed solid foods, were rotated during the experimental period. They included fish, veal, pork, chicken, ham, cheese, legumes, rice, pasta or vegetables. In addition, they consumed a daily fixed amount of fruits, bread, jam, whole milk and green salad. Virgin olive oil (*Olea Europea*), crude and nonrefined, and sunflower (*Helianthus annuus*) oil, rich in oleic acid (C18:1) were used for cooking, salad dressing and as spreads, during the MUFA periods. The fatty acid content of the oils was analyzed by gas liquid chromatography. Olive oil (Extra Sublime, Koype Co., Andujar, Jaen, Spain) contained 12.43% saturated fatty acids, 78.83% monounsaturated fatty acids, 7.99% *n*-6 polyunsaturated fatty acids and 0.75% *n*-3 polyunsaturated fatty acids. The sunflower oil (Vip-aceite, Koype Co.) consisted of 9.58% saturates, 78.97% monounsaturated, 11.4% *n*-6 polyunsaturated, 0.05% *n*-3 polyunsaturated, as shown in Table 1. The oil used in the preparation of the meals was the only difference between diet periods. Other non-fatty acid components of the oils were analyzed and are presented in Table 2.

All meals were prepared in the kitchen of the hospital and consumed in the dining room every afternoon (14.00h) and evening (21.00h) under the supervision of a dietician.

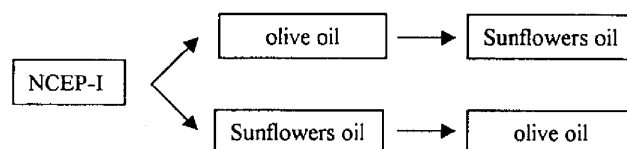


Figure 1 Diagram of the dietary period.

Table 1 Fatty acid composition (gr/100gr fatty acids) of both the high-oleic oils used to prepare the diets

Fatty acids	Olive oil	Sunflower oil
Saturated		
14:0 (myristic)	0.01	0.05
16:0 (palmitic)	8.44	4.06
17:0 (margaric)	0.15	0.06
18:0 (stearic)	3.28	4.09
20:0 (araquic)	0.44	0.33
22:0 (behenic)	0.11	0.99
Total saturated	12.43	9.58
Monounsaturated		
16:1 (palmitoleic)	0.54	0.17
17:1 (margaroleic)	0.24	0.09
18:1 (oleic)	77.56	78.46
20:1 (gadoleic)	0.49	0.25
Total monounsaturated	78.83	78.97
Polyunsaturated		
18:2 (linoleic)	7.99	11.40
18:3 (linolenic)	0.75	0.05
Total polyunsaturated	8.74	11.45

Table 2 Composition of nonglyceride contents in the high-oleic oils

	Olive oil	Sunflower oil
Total sterols (mg/Kg)	1500	3500
Delta-7-avenasterol (%)	< 0.5	5
Beta-sitosterol (%)	94	63
Campesterol (%)	3.2	9
Delta-7-stigmasterol (%)	< 0.5	16
Stigmasterol (%)	1	7
Tocopherols (mg/Kg)	15	50
Squalene (mg/Kg)	5000	300

Breakfast (08.00h) and an afternoon coffee-break (18.00h) were taken in the Medical School dining room, and included coffee, whole milk and toasted bread, dressed with margarine and marmalade in the NCEP-I diet period and with olive oil or sunflower oil in the other two diet periods. Duplicate samples of each diet period were collected, homogenized and stored under 70°C. Dietary protein, fat and carbohydrates were analyzed in the homogenate.

The study protocol was approved by the Human Investigation Review Committee at the University Hospital Reina Sofia.

Blood sampling and isolation of lipoproteins

Fasting blood samples were collected in Ethylene Diamine Tetraacetic Acid (EDTA) containing (1g/L) tubes at the end of each dietary period. Plasma was separated by low-speed centrifugation at 1500 × g at 4°C for 30min within one hour of venepuncture. Plasma very low density lipoproteins (VLDL) (d < 1.006 Kg/L) and LDL (d < 1.063 Kg/L) were separated by ultracentrifugation (Havel *et al*, 1955) using a Beckman (Palo Alto, CA) model LE-70 ultracentrifuge and a type NVT65 rotor. Proteins in LDL fraction were determined by the method of Lowry *et al* (1951) and cholesterol was assayed by enzymatic procedures (Boehringer Mannheim, FRG). To minimize interassay variation, LDL aliquots were stored at -80°C and analyzed at the end of the study.

Vitamin E concentration

Vitamin E concentration was measured by high-performance liquid chromatography according to a modification

of the methodology described by Kaplan *et al* (1987). Briefly, vitamin E acetate was prepared in 100% ethanol at 58µmol/L as an extraction internal standard and for standard curve preparation. Actual concentration of vitamin E was determined by measuring the absorbance of prepared solutions and the calculated concentration from known spectral data. LDL samples were extracted with petroleum ether and dried with nitrogen. The extract was reconstituted in a mobile phase, which consisted of acetonitrile, chloroform, 2-propanol and water (78:16:3.5:2.5, vol/vol/vol/vol), and was run at a flow rate of 2mL/min. The chromatographic analysis was performed on a 5nm particle C-18 column. Calculations were determined from a standard curve of peak area ratios of sample to internal standard.

Fatty acid composition

LDL lipids were extracted by the method of Rose & Oklander (1965). Cholesterol esters and phospholipids were separated by thin layer chromatography and the fatty acids in each fraction were transmethylated and analyzed (Ruiz-Gutierrez *et al*, 1993).

Oxidation of LDL

The formation of conjugated dienes was measured by incubating 100µg LDL protein with 5µmol/L CuSO₄ in 1.0mL PBS medium. The absorbance at 234nm was measured continuously in a spectrophotometer as previously described (Esterbauer *et al*, 1989). Results are expressed as the duration of the lag time before propagation of the oxidation reaction determined by the absolute increase in absorbance above the initial value.

Statistical analysis

The data were analyzed by one-way within-subjects (repeated measures) analysis of variance (ANOVA) to assess the effects of the different diet period. When significant effects were detected (*P* < 0.05), the Tukey honest significance difference test was used for post hoc comparison (CSS, Statsoft, Inc, Tulsa OK). All data are presented in the text and tables as mean ± s.e.m.

Results

All subjects tolerated the three experimental diets without complaints, except one subject who developed diarrhoea and anorexia and was excluded from the study. BMI did not change significantly during the three phases (24.3 ± 0.5). Diet composition was analyzed in duplicate portions of the meals and the results are shown in Table 3. A lower than calculated percentage of saturated fat was found during the stabilization period (NCEP-I phase), 6.8 vs 10%. No significant differences were noted for monounsaturated and polyunsaturated fatty acids (Table 3). Fatty acid composition in the cholesterol esters of plasma LDL was analyzed during each diet period (Table 4). Significant enrichment in the oleic and linoleic acid content of LDL cholesterol esters was observed during the olive and sunflower oil periods. In addition, there was a significant decrease in palmitic and stearic acid during both MUFA periods compared with the NCEP diet period (Table 4). The fatty acid composition of plasma LDL-phospholipids is shown in Table 5. When subjects consumed MUFA diets, saturated plasma LDL-phospholipids fatty acids were lower (14:0, 16:0 and 18:0) and monounsaturated and polyunsaturated plasma

Table 3 Mean daily intake in the three experimental diets

	NCEP-I diet	Olive oil diet	Sunflower oil diet
Energy (MJ)	10.8±0.54	11.1±0.53	11.3±0.50
Protein (% of total energy intake)			
Calculated	15	15	15
Analyzed	14.3	14.7	14.7
Fat (% of total energy intake)			
Calculated	30	40	40
Analyzed	29.7	39.6	39.7
Saturated			
Calculated	10	10	10
Analyzed	6.8	9.8	9.0
Monounsaturated			
Calculated	12	22	22
Analyzed	13.8	25.3	25.8
Polyunsaturated			
Calculated	8	8	8
Analyzed	9.1	4.5	4.9
Carbohydrates (% of total energy intake)			
Calculated	55	45	45
Analyzed	56.0	45.7	45.6
Cholesterol			
Calculated	285	285	285
α -tocopherol (mg)	12.2	12.4	13.1

LDL-phospholipids fatty acids [18:2 (*n*-6), 20:3 (*n*-6), and 20:5 (*n*-3)] were higher than when they consumed the NCEP-I diet.

Table 6 shows mean plasma lipid and apolipoprotein concentrations obtained from the two plasma samples collected for each of the experimental periods. When compared with the NCEP-I diet, the MUFA-olive oil diet resulted in significant increases in total cholesterol (6%, 0.25 mmol/L; *P* = 0.001), LDL cholesterol (6%,

Table 4 Fatty acid composition of plasma LDL-cholesterol esters in men during the three dietary periods

Fatty acid	NCEP-I diet	Olive oil diet	Sunflower oil diet
14:0	1.3±0.1	0.8±0.1*	0.9±0.9†
16:0	18.7±1.0	14.0±0.5*	13.5±0.5†
16:1	0.4±0.1	0.1±0.0	0.4±0.1
18:0	3.3±0.3	2.0±0.2*	2.3±0.2†
18:1	21.1±1.1	24.5±0.6*	24.1±0.7†
18:2	36.8±2.6	43.1±1.4*	44.7±1.0†
18:3	0.3±0.1	0.2±0.0	0.2±0.0
20:4	1.0±0.2	0.7±0.1	0.9±0.3

*Significantly different (*P* < 0.05) between NCEP-I and olive oil diets.

†Significantly different (*P* < 0.05) between NCEP-I and sunflower oil diets.

Table 6 Plasma lipid, lipoprotein, and apolipoprotein concentrations at the end of each diet period¹

	NCEP-I diet	Olive oil diet	Sunflower oil diet
Cholesterol (mmol/L)			
Total	4.01±0.14 (4.32, 3.72)	4.26±0.16* (4.62, 3.90)	3.95±0.13† (4.23, 3.65)
VLDL	0.20±0.02 (0.23, 0.16)	0.19±0.02 (0.23, 0.15)	0.16±0.01* (0.19, 0.13)
IDL	0.02±0.00 (0.03, 0.01)	0.02±0.00 (0.03, 0.01)	0.02±0.00 (0.03, 0.01)
LDL	2.48±0.13 (2.72, 2.20)	2.64±0.15* (2.95, 2.31)	2.40±0.11† (2.63, 2.15)
HDL	1.31±0.15 (1.41, 1.18)	1.41±0.01* (1.54, 1.26)	1.37±0.05* (1.48, 1.25)
Triacylglycerol (mmol/L)	0.85±0.04 (0.95, 0.75)	0.85±0.04 (0.95, 0.75)	0.82±0.05 (0.93, 0.72)
Apo A-I (mg/L)	1.07±0.04 (1.16, 0.98)	1.17±0.04* (1.25, 1.08)	1.16±0.04* (1.25, 1.08)
Apo B (mg/L)	0.89±0.04 (0.98, 0.80)	0.93±0.05 (1.04, 0.82)	0.85±0.04*† (0.93, 0.76)
Total: HDL cholesterol	3.12±0.15	3.12±0.16	2.93±0.12*†
Apo B: apo A-I	0.85±0.06	0.81±0.06	0.70±0.04*†

¹ \bar{x} ±ESM; *n* = 21, 95% cis in parentheses.

*Significantly different from NCEP-I diet, *P* < 0.05.

†Significantly different from olive oil diet, *P* < 0.05.

0.16 mmol/L; *P* = 0.049), and a non-significant trend in apo B (4%, 0.04 g/L). No significant differences were observed during the MUFA-sunflower oil diet for total and LDL cholesterol when compared with the NCEP-I diet. However, there was a significant decrease in very-low-density-lipoprotein (VLDL), cholesterol (20%, 0.04 mmol/L; *P* = 0.046) and apo B (5%, 0.04 g/L; *P* = 0.047). Both MUFA-enriched diets resulted in increases in HDL cholesterol (olive-oil: 7%, 0.1 mmol/L, *P* = 0.02; sunflower-oil: 4%, 0.04 mmol/L, *P* = 0.042) and apo A-I (9%, *P* = 0.01) compared with the NCEP-I diet. When both MUFA diets were compared, a significantly higher concentration of total cholesterol (7%, 0.31 mmol/L, *P* = 0.0001), L:DL cholesterol (10%, 0.24 mmol/L; *P* = 0.01), and apo B (10%, 0.08 g/L; *P* = 0.01) were noted during the olive oil-rich diet. No significant differences in triacylglycerol content were observed in the different lipoprotein fractions.

The inhibition time (lag time) was significantly longer (*P* < 0.001) when subjects consumed the MUFA rich sunflower diet compared with the olive oil and NCEP diet (Table 7). In addition, this increase was observed in the two groups of volunteers established in the randomization process at the beginning of study. No significant differences were observed for the lag time measured between the olive oil and NCEP diet. In parallel to the inhibition time, the α -tocopherol content of plasma LDL was significantly higher (*P* < 0.05) when subjects were on the MUFA rich sunflower oil diet than during the NCEP diet. No differences were found in γ -tocopherol content of plasma LDL between the three diet periods. The α -tocopherol content of plasma LDL

Table 5 Fatty acid composition of plasma LDL-phospholipids in men during the three dietary periods

Fatty acid	NCEP-I diet	Olive oil diet	Sunflower oil diet
14:0	1.09±0.32	0.56±0.16*	0.61±0.20†
16:0	38.0±4.76	27.5±1.48*	26.6±1.47†
18:0	21.8±3.74	15.0±0.88*	15.8±0.77†
18:1 (<i>n</i> -9)	7.10±3.30	13.1±1.33*	13.5±0.97†
18:1 (<i>n</i> -7)	1.42±0.39	1.76±0.22*	1.62±0.14†
18:2 (<i>n</i> -6)	11.8±3.73	20.8±2.08*	19.8±1.76†
18:3 (<i>n</i> -3)	0.34±0.32	0.16±0.09*	0.21±0.17
20:4 (<i>n</i> -6)	5.45±2.02	8.89±1.27*	9.27±1.06†
20:5 (<i>n</i> -3)	0.74±0.31	0.90±0.34	0.89±0.34
22:6 (<i>n</i> -3)	3.11±1.10	3.51±1.07	3.83±0.53

*Significantly different (*P* < 0.01) between NCEP-I and olive oil diets.

†Significantly different (*P* < 0.01) between NCEP-I and sunflower oil diets.

Table 7 Effect of the three dietary periods on tocopherol concentrations in low density lipoproteins and susceptibility to oxidative modifications of low density lipoproteins¹

	NCEP-I diet	Olive oil diet	Sunflower diet
α -tocopherol (mg/mg cholesterol)	7.73 \pm 3.08	7.56 \pm 3.90	8.75 \pm 3.02 [†]
γ -tocopherol (mg/mg cholesterol)	0.28 \pm 0.15	0.28 \pm 0.16	0.35 \pm 0.34
Lag time (min)	68.78 \pm 12	72.41 \pm 11	85.61 \pm 14 ^{&#}
Conjugated dienes and the max point (AU ²)	1766 \pm 286	1680 \pm 249	1740 \pm 263

¹x \pm ESM; n = 21.

²Arbitrary units.

[†]Significantly different (P < 0.05) between NCEP-I and sunflower oil diets.

^{&#}Significantly different (P < 0.01) between both MUFA diets.

[&]Significantly different (P < 0.001) between NCEP-I and sunflower oil diets.

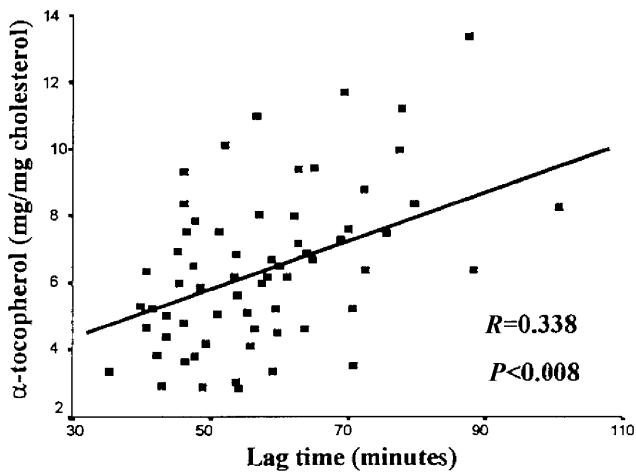


Figure 2 Correlation between the α -tocopherol content of plasma LDL and inhibition.

was positively correlated with the inhibition time ($r=0.338$; $P<0.008$) (Figure 2). In contrast, there was no significant correlation between the fatty acid composition of plasma LDL cholesterol esters and phospholipids and the lag time.

Discussion

The aim of this study was to evaluate using a crossover design, the effects of two MUFA rich-diets (40% fat, 22% MUFA, 10% SAT and 8% PUFA) using two different sources of oleic acid with different nonglyceride compounds, on the susceptibility to oxidative modification of plasma LDL. An NCEP-I diet was used as a reference. We studied these diets because the primary goal of dietary therapy for CHD is still to lower the content of saturated fatty acids in the diet. The question then arises as to what should replace these fat energy sources. It has been shown that both monounsaturated and polyunsaturated rich diets reduce total cholesterol levels when compared to saturated fat rich diets (Mata *et al*, 1992a; Mata *et al*, 1992b; Mensik & Katan 1989a). However, it is not clear whether the excess saturated fat should be replaced by carbohydrates

or by monounsaturated fat. In addition, a high fat diet with a 40% fat content, like the one used in our study, may be acceptable if the additional fat is in the form of monounsaturated fatty acids. In this respect, some studies have shown that the latter alternative results in a lipoprotein profile associated with a lower CHD risk (Grundy *et al*, 1986; Grundy *et al*, 1988; Mensink *et al*, 1989b; Mensink, 1992). Furthermore, the fatty acid composition of a diet is well known to influence the susceptibility of plasma LDL to oxidative modifications by varying the relative content of oleate and linoleate in these lipoproteins (Berry *et al*, 1991; Bonanome *et al*, 1992; Fraser & Jialal, 1994; Fuller & Jialal, 1994; Mata *et al*, 1996; Parthasarathy *et al*, 1990; Reaven *et al*, 1991; Reaven *et al*, 1993a).

Fraser & Jialal (1994), Reaven *et al*, (1991; 1993a) and Bonanome *et al*, (1992) reported that a lower tendency of plasma LDL particles to oxidize could be observed in subjects consuming a diet rich in oleic acid in comparison to a diet high in linoleic acid. Thus, these data lead to the hypothesis that diets rich in oleic acid, besides lowering plasma cholesterol levels as much as diets high in linoleic acid, might have the advantage of providing a protective effect against oxidative modification of plasma LDL (Esterbauer *et al*, 1987).

However, the precise mechanism responsible for the decreased susceptibility to peroxidation of LDL in subjects fed MUFA in comparison to a carbohydrate rich diet has not yet been identified. Olive oil is known to have a relatively high content of α -tocopherol and pharmacological supplementation with larger amounts of vitamin E has been shown to reduce LDL peroxidation (Jialal & Grundy, 1992; Jialal *et al*, 1995; Princen *et al*, 1992; Reaven & Witztum, 1993b; Reaven *et al*, 1993c).

In our study, both MUFA rich diets markedly increased the LDL cholesterol ester and phospholipid content of oleic and linoleic acid, and decreased their palmitic and stearic acid contents compared to an NCEP-I diet. In contrast, although the relative content of unsaturated fatty acid, more susceptible to peroxidation, was higher when following both MUFA diets than after the NCEP-I diet, no significant changes in inhibition time were observed between the high MUFA diets and the NCEP-I diets. Furthermore, the fact that diets rich in carbohydrate produce the same, as we observed in this study, or more (Berry *et al*, 1992) oxidative stress in plasma LDL than oleic acid suggests that olive oil may also contain other active antioxidants, such as polyphenols (Papadopoulos, 1991; Wiseman & Baskon, 1996). However, there was a greater inhibition time in the MUFA rich sunflower oil diet compared to the NCEP-I diet. These differences were probably related to the relative enrichment of these plasma LDL particles in α -tocopherol due to the high vitamin E content of the MUFA-rich sunflower oil. Indeed, the α -tocopherol in LDL was positively correlated with inhibition time. Although this positive correlation was not very strong, this observation is consistent with data that have recently appeared in the literature which show that a longer inhibition time can be observed in the plasma LDL of subjects supplemented with vitamin E in parallel to the increased plasma LDL α -tocopherol content (Esterbauer *et al*, 1992; Jialal & Grundy, 1992; Jialal *et al*, 1995; Princen *et al*, 1992; Reaven & Witztum, 1993b; Reaven *et al*, 1993c). Thus, our study demonstrates that the changes obtained in plasma LDL α -tocopherol content with practical solid-food diets can decrease its susceptibility to oxidation.

Furthermore, we did not observe any significant correlation between LDL cholesterol esters and phospholipid composition and the susceptibility of these particles to oxidative changes. In contrast, Reaven *et al* (1991) reported that LDL differences in 18:1 or 18:2 may influence lag time before conjugated-diene formation begins, as well as the peroxidation rate. However, because antioxidants are consumed first in the course of peroxidative reactions (Esterbauer *et al*, 1987; 1989), the impact of the fatty acid composition of LDL on the lipoprotein susceptibility to peroxidation could appear to be of less relevance than the protective effect provided by the antioxidant present within the lipoprotein itself (Esterbauer *et al*, 1987; 1989; 1992; Wiseman *et al*, 1996). In addition, the changes in peroxidation rates in plasma LDL, once depleted of antioxidant content, can be related to their fatty acid pattern. This hypothesis is also supported by many world-wide epidemiological studies that associate vegetable-rich or Mediterranean diets with a higher life expectancy, and antioxidants could be of prime importance in the beneficial effects of these diets (Gey *et al*, 1987, Gey & Puska, 1989; Gey *et al*, 1992; 1993; Riemersma *et al*, 1991). In addition, the plasma status of vitamin E has always been the strongest predictor of the up-to-six fold differences of CHD in European study populations (Gey *et al*, 1987; Gey & Puska, 1989; Gey *et al*, 1992).

In conclusion, in a population of young healthy males, the consumption of high MUFA diets, such as the 'Mediterranean' diet, resulted in no changes (olive oil diet) or in a decrease of plasma LDL susceptibility to oxidative modifications, due to its higher α -tocopherol content, compared to a low fat diet (NCEP step I). These findings suggest that oleic acid can be a suitable option as an energy source when replacing saturated fat in the diet. Furthermore, our study demonstrates that the changes obtained in plasma LDL α -tocopherol content with practical solid-food diets can decrease its susceptibility to oxidation.

Acknowledgements—We would like to thank all the subjects who participated in the study for their cooperation and enthusiasm. We also thank Jose Linars, Silvia Romero, José Luis Prada, Valentina Ruiz Gutierrez, Pilar Cañete Estrada, Carmen Rodriguez Panadero, Mercedes Lopez Pardo and the cooks of the 'Hospital Materno Infantil', for their excellent technical assistance.

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