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Effects of dietary saturated, monounsaturated and n-3 fatty acids on fasting lipoproteins, LDL size and post-prandial lipid metabolism in healthy subjects

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Abstract

Background: The influence of the quality of dietary fat on some aspects of lipid metabolism—i.e. lipoprotein concentrations, post-prandial lipids and LDL size—is not completely understood, especially in healthy individuals. *Objectives*: Aim of this study was to evaluate the effects of different types of dietary fat (monounsaturated vs. saturated fatty acids, and n-3 or placebo supplementation) on fasting lipoproteins, LDL size and post-prandial lipids in healthy people. *Design*: One hundred and sixty-two individuals were randomly assigned to follow two isoenergetic diets, one rich in saturated fatty acids (SFA diet) and the other in monounsaturated fatty acids (MUFA diet). Each group was further randomised to receive supplementation with fish oil (3.6 g/day) or placebo. *Results*: The type of diet significantly affected LDL cholesterol and triacylglycerol content, which was higher with the SFA diet and lower with the MUFA diet. The changes between the two diets were statistically significant for cholesterol ($P < 0.01$) and triacylglycerol ($P < 0.03$). VLDL cholesterol and triacylglycerol were significantly reduced and LDL cholesterol significantly increased by fish oil supplementation. Plasma triacylglycerol was significantly lower in those taking n-3 fatty acids, also 1 and 3 h after a test-meal. Neither type of diet nor n-3 supplementation affected LDL size. *Conclusions*: A moderate substitution of saturated fatty acids with monounsaturated fatty acids has beneficial effects on lipid metabolism also in healthy individuals. A moderate supplementation of long-chain n-3 fatty acids in healthy individuals reduces both fasting and post-prandial triacylglycerol concentrations but increases LDL cholesterol, irrespective of the type of diet.

Keywords: Diet; Saturated fatty acids; Monounsaturated fatty acids; n-3 Fatty acids; Lipid metabolism; LDL size

1. Introduction

The effects of changes in the quality of dietary fat on lipid metabolism have been extensively studied for many years, especially in high risk individuals [1–5]. However, most studies were of short duration and small. To overcome the problem of sample size, some authors have tried to draw more definite conclusions by performing meta-analyses of available studies, despite all the limitations of this kind of analysis [1]. As a matter of fact, there are still some unclear points in relation to both the effects of saturated versus monounsaturated fatty acids and those of n-3 fatty acids on plasma lipid levels. In particular, while the deleterious effects of saturated fat on LDL cholesterol are beyond any doubt [1–4], those on triacylglycerol and HDL metabolism are not univocal and an increase in HDL cholesterol concentrations with high saturated fat diets has been

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suggested on the basis of the results of meta-analyses [1,6]. Furthermore, there is evidence that a high saturated fat intake is associated, on the one hand, with an increase in LDL cholesterol and, on the other, with an increase in the proportion of the larger and less atherogenic LDL particles and LDL size [7–9]. These data have led some authors to question the real benefit of reducing saturated fat intake in the diet for the prevention of cardiovascular diseases, with possible negative consequences on the efforts to implement this kind of diet in high risk individuals, as well as in healthy people.

In relation to n-3 fatty acids, even if a large amount of studies has been carried out [5,10–12], few studies with an adequate sample size and duration have looked at the effects on lipoprotein fractions and in particular LDL size in healthy people.

Finally, in the last few years, much attention has been paid to post-prandial lipid levels as independent cardiovascular risk factor [13,14]. If this is true, the effects of dietary intervention should be considered also in relation to this as yet unclear point.

Therefore, our aim was to perform a study of adequate duration and sample size to evaluate more in depth some aspects of changes in dietary fat composition (monounsaturated vs. unsaturated fatty acids and supplementation of n-3 fatty acids vs. placebo) on fasting lipoproteins, LDL size and post-prandial lipid metabolism in healthy people.

2. Subjects and methods

2.1. Design of the study

The design of the study has been reported in detail previously [15]. It was a multicentre controlled dietary study performed in five different centres (Kuopio, Aarhus, Naples, Wollongong, and Uppsala), with a duration of 90 days. The participants were randomised to a diet containing a high proportion of saturated fatty acids (SFA diet) or monounsaturated fatty acids (MUFA diet). Within the two groups there was a second randomisation allotting the participants to supplementation with capsules containing long-chain n-3 fatty acids (3.6 g n-3 fatty acids/day containing 2.4 g of eicosapentaenoic and docosahexaenoic fatty acids, corresponding to three capsules twice daily of Pikasol, LUBE A/S, Denmark) or placebo capsules containing the same amount of olive oil. The test period was preceded by a 2-week 'stabilisation period' on the patients' habitual diets supplemented with placebo capsules.

Routine clinical examination, including an oral glucose tolerance test [16], were carried out during this period; participants performed a weighed 3-day dietary record to document pre-trial dietary habits. Tests and laboratory analyses were performed during days 0 and 1 before the start of the study and were repeated at days 89 and 90 at the end of the diet period. A postprandial evaluation of some parameters was performed at the end of the intervention period. Two additional 3-day dietary records were requested during the beginning of the second and third month of the treatment period.

2.2. Subjects

One hundred and sixty-two healthy Caucasian men and women (M = 86, F = 76, 30–32 from each centre), aged 30–65 years with normal or moderately increased body weight (BMI = 22–32 kg/m^2) were included (Table 1). The subjects were recruited through advertisements in local newspapers, from ongoing health surveys, or among university and hospital employees.

If premenopausal women were included care was taken to make all tests during the same time period of the menstrual cycle. Subjects with glucose intolerance according to the WHO criteria [16] were included but those with diabetes mellitus were excluded. Other reasons for exclusion were thyroid, liver and renal diseases, disabling diseases, specific eating habits due to cultural or religious reasons, very high habitual physical activity or alcohol intake (binge drinkers and subjects with a regular consumption of alcohol above 40 g/day). Participants were asked to maintain the degree of physical activity and alcohol intake, if any, unchanged throughout the study. The body weight had had to be fairly stable during the last 3 months before the study (body weight change less than 4 kg during this period). Persons using lipid lowering drugs, thiazide diuretics, beta blockers and corticosteroids were excluded and the participants were not to have taken preparations containing acetyl salicylic acid regularly

Table 1
Baseline parameters of the participants ($N = 162$; mean ± S.D.)

	SFA diet		MUFA diet	
	n-3	Placebo	n-3	Placebo
Age (years)	48 ± 8	49 ± 7	49 ± 7	49 ± 7
BMI (kg/m^2)	27 ± 3	26 ± 3	26 ± 3	26 ± 3
Sex (F/M)	15/26	17/25	17/22	18/22
LDL size (Å)	264.4 ± 6.3	266.7 ± 8.3	265.0 ± 8.0	265.4 ± 9.1
Lp (a) (U/l)	203.2 ± 212.2	208.6 ± 191.8	234.8 ± 271.2	274.9 ± 284.1
NEFA (mM/l)	0.56 ± 0.16	0.56 ± 0.24	0.55 ± 0.23	0.54 ± 0.17
LPL (mU/ml)	71.7 ± 28.1	77.6 ± 30.7	79.6 ± 33.1	77.4 ± 22.3
HL (mU/ml)	193.3 ± 79.2	203.1 ± 67.2	181.6 ± 67.4	172.5 ± 81.1

during the last month and not at all during the last week before the study. Moderate smokers were allowed to participate but were instructed not to change their smoking habits during the trial. All participants gave their informed consent to the study, which had been approved by the ethics committee at each centre.

2.3. Diets

All participants were instructed to eat isoenergetic diets with the same proportions of nutrients, including similar amount of total fat, but with a high proportion of either saturated (SFA diet) or monounsaturated (MUFA diet) fatty acids. The diets were calculated to contain 37% energy (E%) from fat with 17, 14 and 6E% of saturated, monounsaturated and polyunsaturated fatty acids, respectively, in the SFA diet and 8, 23 and 6E% of saturated, monounsaturated and polyunsaturated fatty acids, respectively, in the MUFA diet. All participants were instructed before the study by trained dieticians about how to prepare their meals. The participants met with dieticians at least twice during the first month of the study and at least once a month during the second and third month to assure a good compliance with the diet. All participants were supplied with edible fats to be used as spread, dressings, or for cooking. Bread, ice cream and ready made dishes, containing the appropriate types of fats, were prepared locally and distributed to the participants according to needs and preferences. Butter, margarine and oils to be used in the diets were all supplied by the same industry and distributed to the different centres. The SFA diet included butter (MD Foods, Denmark) and a table margarine (Van den Bergh Foods, Helsingborg, Sweden) containing 80% fat with 32% saturated, 17% monounsaturated and 26% polyunsaturated fatty acids. The MUFA diet included a spread (22% saturated, 50% monounsaturated and 8% polyunsaturated fatty acids, Van den Bergh Foods, Helsingborg, Sweden) and a fluid margarine (10% saturated, 59% monounsaturated and 8% polyunsaturated fatty acids, Carlshamns Mejeri, Karlshamns, Sweden) containing high proportions of oleic acid derived from high-oleic sunflower oil and negligible amounts of n-3 fatty acids. Olive oil was supplied by Carapelli, Eridania-Beghin-Say (Florence, Italy). The estimated proportion of trans-fatty acids was low and similar in both diets.

The nutrient composition of the diets was calculated from weighed 3-day dietary records (2 workdays, 1 day during the weekend). The intake during the test period was calculated as the mean value of the two 3-day dietary records performed during the second and third month of the study. The nutrient composition of the diets was calculated using local food databases, with inclusion of the appropriate margarine and oils used in the diets, and computerised calculation programs. In addition, the fatty acid composition of phospholipids and serum cholesterol esters was analysed in all participants before and at the end of the test periods to monitor dietary fat quality.

2.4. Clinical tests and laboratory analyses

Body weight was measured on a digital scale with an accuracy of 0.1 kg. Blood samples were drawn after a 12-h overnight fast, from an anticubital vein on days 0–1, and 89–90, respectively, before the start and at the end of the study. In four centres, at the end of the intervention blood samples for triacylglycerols and HDL cholesterol were drawn also before and after (1, 2, 3 h) a test meal consumed at noon, after the subjects had eaten their usual morning breakfast. The test meal contained ≈ 800 kcal and its composition resembled that of the diet followed by the subjects (rich in saturated for those on the SFA diet, and rich in monounsaturated fat for those on the MUFA diet). On the day the test meal was administered, the subjects took their habitual capsules (placebo or n-3 fatty acids).

The analyses of the laboratory tests were centralised to the research laboratory of one of the participating centres, with the exception of serum lipoproteins, which were measured locally on fresh samples; lipoprotein separation by ultracentrifugation was performed in three centres only. All blood serum and plasma samples were stored frozen at -70 °C and transported on dry ice to the laboratory where they were analysed.

2.5. Serum lipids and lipoproteins

Enzymatic colorimetric methods were used for the determination of cholesterol and triacylglycerols. Apolipoprotein B-containing lipoproteins were precipitated on serum with a sodium phosphotungstate and magnesium chloride solution [17] or with dextran sulphate and magnesium chloride [18], and HDL was assayed in the infranate. In three centres (Aarhus, Naples and Uppsala) VLDL were isolated by ultracentrifugation at a density of 1.006 g/ml [19], and LDL were calculated by the difference between bottom fraction ($d = 1.006$ g/ml) and HDL, obtained as previously described. Only recovery between 90 and 110% was accepted. The CV% between measurements was 1.1–2.2% for serum total cholesterol using two standards, and 2.4–3.8% for total triacylglycerols. The CV% for HDL-cholesterol was 1.2–1.2% (two standards).

2.5.1. Serum lipid fatty acid composition

The fatty acids in the serum lipid esters were separated by gas–liquid chromatography as described earlier [20].

2.5.2. Plasma NEFA

Plasma NEFA concentrations were measured by a standard enzymatic colorimetric method (Wako Chemicals, GMBH, Germany).

2.5.3. Lipoprotein (a)

Apolipoprotein (a) [Apo(a)] content in lipoprotein (a) [Lp(a)] was measured using the solid phase two-site immunoradiometric assay from Mercodia AB (Uppsala, Sweden). The concentration of the Apo (a) was transformed into Lp (a) by multiplying apo(a) concentrations by 0.7; the results are expressed in units per litre (U/l). Between-assay CV% were 3.5 and 3.2% at the Lp(a) concentrations of 138 and 3799 U/l, respectively. The assay is specific to Apo(a); apolipoprotein B has no measurable cross-reaction. Serum plasminogen up to 5 g/l gives no measurable cross-reaction in the assay.

2.6. LDL size

LDL particle size (diameter of the major LDL peak) was assessed on serum by non-denaturating polyacrylamide gradient gel electrophoresis using commercially available 2.5–16% gels (ISOLAB; Akron, OH, USA), with a minor modification of the method of McNamara et al. [21,22]. Briefly, samples were pre-stained with Sudan Black B lipid stain and scanned at 633 nm on a LKB Ultroscan XL laser densitometer (Pharmacia LKB Biotechnology, Piscataway, NY). Gels were calibrated using serum calibrators kindly provided by Donner Laboratories (Berkeley, CA), the sizes of which were confirmed by analytical ultracentrifugation. The coefficient of variation for control pools was 1.8–3.6%.

2.7. Lipase

Blood samples for the measurement of lipoprotein lipase (LPL) and hepatic lipase (HL) activities were collected at fasting in tubes containing EDTA-K3, 15 min after intravenous administration of heparin (100 IU/kg body weight). Plasma was immediately separated by centrifugation at 4 °C and stored at −20 °C. LPL and HL activities were evaluated according to Nilsson-Ehle and Ekman, using a (3H) trioleoylglycerol emulsion by di-oleoyl phosphatidyl choline as substrate, as previously described in detail [23].

2.8. Statistical method

Results for continuous variables are presented as mean and standard deviation. For variables with skewed distributions a logarithmic transformation was made before the statistical analysis. For the intention-to-treat population (defined as all randomised subjects who underwent at least one measurement during treatment) a confirmatory analysis was made: for each outcome variable the treatment effects were estimated from a statistical model in which treatment categories (SFA/MUFA, presence/absence of n-3 fatty acid), and their interaction were the factors analysed, whereas centre, age, gender and the baseline value of the outcome variable were the covariates [24]. The difference between treatment groups regarding adjusted means treatment effects are presented with p-values and 95% confidence intervals.

3. Results

The baseline parameters of subjects randomised to the four groups of intervention were very similar (Tables 1 and 2).

Compliance to dietary intervention was satisfactory, as shown in Table 3. Compliance to n-3 intake was evaluated by analysis of fatty acids in serum phospholipids and cholesterol esters: the proportion of 20:5 n-3 and 22:6 n-3 in serum phospholipids (those on cholesterol esters are similar) increased significantly only in the group randomised to take n-3 capsules (Table 4). In the groups on placebo there was a slight but significant decrease in both EPA and DHA. Body weight did not change during the intervention period (SFA diet with n-3: 80.7 ± 14.1 vs. 80.9 ± 14 kg; SFA diet without n-3: 76.6 ± 13.9 vs. 76.8 ± 13.1; MUFA diet with n-3: 75.6 ± 11.2 vs. 75.6 ± 11.1; MUFA diet without n-3: 76.0 ± 13.2 vs. 75.8 ± 13.3 kg, respectively, at the beginning and at the end of each period) (mean $\pm$ S.D.).

3.1. Effects on fasting lipoproteins

The effects on fasting serum lipids and LDL cholesterol, calculated by the Friedewald formula, were reported in the previous paper [14]. Here we report the effects of dietary intervention as well as n-3 supplementation on the concentrations of the serum lipoproteins isolated in 97 individuals (three centres, Aarhus, Naples and Uppsala). A change in fat quality (saturated vs. monounsaturated) affected the cholesterol and triacylglycerol content of the LDL fraction, which was significantly increased during the high SFA diet and significantly decreased with the high MUFA diet (Table 5). The differences between the two diets were statistically significant ($P = 0.0001$ for LDL cholesterol and 0.03 for LDL triacylglycerol). On the other hand, n-3 supplementation affected the VLDL fraction and LDL cholesterol but in an opposite way: both cholesterol and triacylglycerols of VLDL were significantly reduced by n-3 independently of the type of diet, while LDL cholesterol was significantly increased (Table 5). Moreover, during the high MUFA diet, n-3 supplementation completely cancelled the positive effect of this kind of diet on LDL (Table 5). No interaction between the

Table 2
Baseline lipoprotein concentrations (mean ± SD)

	SFA diet		MUFA diet	
	n-3 ($n = 25$)	Placebo ($n = 25$)	n-3 ($n = 24$)	Placebo ($n = 23$)
Cholesterol (mmol/l)	5.33 ± 0.90	5.35 ± 0.69	5.46 ± 1.01	5.51 ± 0.85
VLDL (mmol/l)	0.45 ± 0.20	0.36 ± 0.26	0.39 ± 0.34	0.37 ± 0.20
LDL (mmol/l)	3.67 ± 0.84	3.70 ± 0.63	3.73 ± 1.04	3.80 ± 0.72
HDL (mmol/l)	1.14 ± 0.20	1.22 ± 0.34	1.29 ± 0.28	1.31 ± 0.33
Triacylglycerol (mmol/l)	1.33 ± 0.57	1.15 ± 0.55	1.27 ± 0.69	1.14 ± 0.44
VLDL (mmol/l)	0.85 ± 0.46	0.68 ± 0.49	0.81 ± 0.66	0.71 ± 0.44
LDL (mmol/l)	0.32 ± 0.11	0.33 ± 0.09	0.32 ± 0.10	0.30 ± 0.12
HDL (mmol/l)	0.17 ± 0.08	0.15 ± 0.07	0.17 ± 0.07	0.16 ± 0.09

effects of dietary change and n-3 supplementation was present.

3.2. Effects on LDL size and other parameters

Neither the type of diet nor the n-3 supplementation affected LDL size, NEFA concentration or post-heparin HL activity (Table 6). LPL activity was significantly increased by n-3 supplementation (Table 6). However, there was no significant correlation between the changes in LPL activity and the decrease in VLDL TG obtained on n-3 supplementation ($r = -0.19$, NS). Lp(a) was significantly increased by the high MUFA diet (as reported in the previous paper) but was not influenced by n-3 supplementation (Table 6).

3.3. Effects on post-prandial lipids

In Fig. 1 the post-prandial triacylglycerol and HDL cholesterol values at the end of the study for the four groups of patients are presented: those taking n-3, independently of the type of diet, had significantly lower TG values before the test meal and this difference was maintained after the test meal ($P < 0.05$ at 1 and 3 h). The type of diet had no significant effect on the 3 h post-prandial triacylglycerol levels, even if the values during the high MUFA diet without n-3 were lower than with the high SFA diet without n-3 despite very slightly higher pre-prandial concentrations. HDL cholesterol values (Fig. 1) decreased during the post prandial period in all the groups; however, during the MUFA diet this decrease was slightly lower, with a difference of borderline significance 2 h after the test meal ($P = 0.06$).

Table 3
Dietary nutrient composition ($n = 162$) before and during the study (mean ± S.D.)

	SFA diet		MUFA diet	
	Before	During	Before	During
Energy (kcal)	2250 ± 550	2140 ± 500	2120 ± 500	2150 ± 450
Protein (E%)	15.6 ± 3.0	15.2 ± 2.5	15.8 ± 2.8	14.8 ± 2.3
Carbohydrate (E%)	45.8 ± 6.7	44.1 ± 5.2	47.3 ± 7.0	45.9 ± 4.2
Fat (E%)	33.7 ± 6.5	37.1 ± 4.1	33.3 ± 6.1	37.1 ± 4.2
SFA (E%)	13.5 ± 3.6	17.6 ± 2.5	13.3 ± 3.7	9.6 ± 1.8
MUFA (E%)	13.0 ± 3.7	13.1 ± 2.5	13.1 ± 3.2	21.2 ± 4.0
PUFA (E%)	4.8 ± 1.6	4.7 ± 1.5	4.7 ± 1.5	4.6 ± 0.8
Fiber (g/day)	23.8 ± 7.7	22.4 ± 6.6	23.0 ± 8.4	23.0 ± 8.4
Cholesterol (g/day)	316 ± 126	322 ± 91	310 ± 139	254 ± 80

4. Discussion

In relation to the effects of saturated and monounsaturated fatty acids on lipoprotein concentrations, this study shows that high SFA diets negatively influence the cholesterol and triacylglycerol content of LDL lipoproteins and that, on the contrary, high MUFA diets have beneficial effects on LDL cholesterol and triacylglycerols. These effects occur not only in high-risk individuals, as previously shown [1–4], but also in healthy people with normal lipid concentrations. Very recently a similar

Table 4
Effects of dietary intervention and n-3 supplementation on the eicosapentaenoic (EPA) and deicosaesaenoic (DHA) fatty acid content of serum phospholipids ($n = 162$)

	SFA diet			
	n-3		Placebo	
	Baseline	After 3 months	Baseline	After 3 months
EPA (%)	1.53 ± 0.14	6.19 ± 0.28[b]	1.47 ± 0.11	1.20 ± 0.08[a]
DHA (%)	4.67 ± 0.23	7.01 ± 0.18[b]	4.67 ± 0.15	4.29 ± 0.17[a]
	MUFA Diet			
	n-3		Placebo	
	Baseline	After 3 months	Baseline	After 3 months
EPA (%)	1.75 ± 0.28	5.77 ± 0.33[b]	1.54 ± 0.15	1.08 ± 0.09[a]
DHA (%)	4.71 ± 0.23	6.39 ± 0.23[b]	4.40 ± 0.18	4.13 ± 0.16[a]

[a] $P < 0.05$.
[b] $P < 0.001$ compared with baseline.

Table 5
Effects of dietary intervention and n-3 fatty acids supplementation on lipoprotein composition ($n = 97$) (mean ± S.E.M.)

Variables	SFA diet				MUFA diet				Treatment effects[b]. Statistical significance and 95% CI between brackets	
	n-3		Placebo		n-3		Placebo		Diet	n-3
	Δ[a]	P	Δ[a]	P	Δ[a]	P	Δ[a]	P		
Cholesterol (*Mmol/l*)										
VLDL	−0.19 ± 0.05	0.0003	−0.03 ± 0.05	0.94	−0.10 ± 0.05	0.18	0.05 ± 0.05	0.73	0.27 (−0.19 to 0.02)	0.01 (0.04 to 0.26)
LDL	0.52 ± 0.07	0.0001	0.27 ± 0.07	0.004	−0.06 ± 0.08	0.54	−0.21 ± 0.09	0.03	0.0001 (0.34 to 0.72)	0.003 (−0.38 to −0.01)
HDL	0.13 ± 0.04	0.001	0.03 ± 0.04	0.37	0.03 ± 0.04	0.68	0.10 ± 0.04	0.06	0.34 (−0.07 to 0.09)	0.60 (−0.10 to 0.06)
Triacylglycerol (*Mmol/l*)										
VLDL	−0.27 ± 0.06	0.001	−0.07 ± 0.06	0.82	−0.30 ± 0.06	0.0004	−0.12 ± 0.06	0.06	0.32 (−0.07 to 0.15)	0.01 (0.08 to 0.30)
LDL	0.02 ± 0.01	0.096	0.01 ± 0.01	0.73	−0.03 ± 0.01	0.01	0.01 ± 0.01	0.93	0.03 (0.002 to 0.06)	0.53 (−0.03 to 0.03)
HDL	−0.03 ± 0.01	0.003	−0.02 ± 0.01	0.09	−0.02 ± 0.01	0.12	−0.01 ± 0.06	0.81	0.09 (−0.03 to 0.0007)	0.09 (−0.002 to 0.02)

[a] Changes from baseline.
[b] Difference of changes from baseline between SFA and MUFA diets or n-3 supplementation and placebo.

Table 6
Effects of dietary intervention and n−3 fatty acids supplementation on other lipid parameters ($n = 162$; mean ± S.E.M.)

Variables	SFA diet				MUFA diet				Treatment effects[b]. Statistical significance and 95% CI between brackets	
	n-3		Placebo		n-3		Placebo		Diet	n-3
	Δ[a]	*P*	Δ[a]	*P*	Δ[a]	*P*	Δ[a]	*P*		
LDL size (Å)	−0.80 ± 0.75	0.29	−0.02 ± 0.76	0.97	−0.69 ± 0.83	0.41	−1.22 ± 0.75	0.11	0.49 (−1.01 to 2.09)	0.88 (−1.40 to 1.64)
Lp(a) (U/l)	0.06 ± 9.49	0.93	2.77 ± 9.38	0.58	31.65 ± 9.73	0.001	31.02 ± 9.62	0.05	0.02 (−49.1 to −10.8)	0.73 (−17.7 to 19.8)
NEFA (mM/l)	−0.07 ± 0.03	0.009	−0.06 ± 0.07	0.02	−0.07 ± 0.03	0.02	−0.01 ± 0.03	0.69	0.34 (−0.08 to 0.03)	0.20 (−0.02 to 0.09)
LPL (mU/ml)	11.13 ± 4.33	0.02	7.20 ± 4.19	0.25	10.67 ± 4.51	0.01	1.045 ± 4.55	0.82	0.46 (−5.51 to 12.12)	0.05 (−15.4 to 1.9)
HL (mU/ml)	−2.12 ± 7.28	0.77	−10.42 ± 7.06	0.14	1.10 ± 7.46	0.88	−3.64 ± 7.71	0.64	0.60 (−18.7 to 10.9)	0.46 (−19.9 to 9.1)

[a] Changes from baseline.
[b] Difference of changes from baseline between SFA and MUFA diets or n-3 supplementation and placebo.

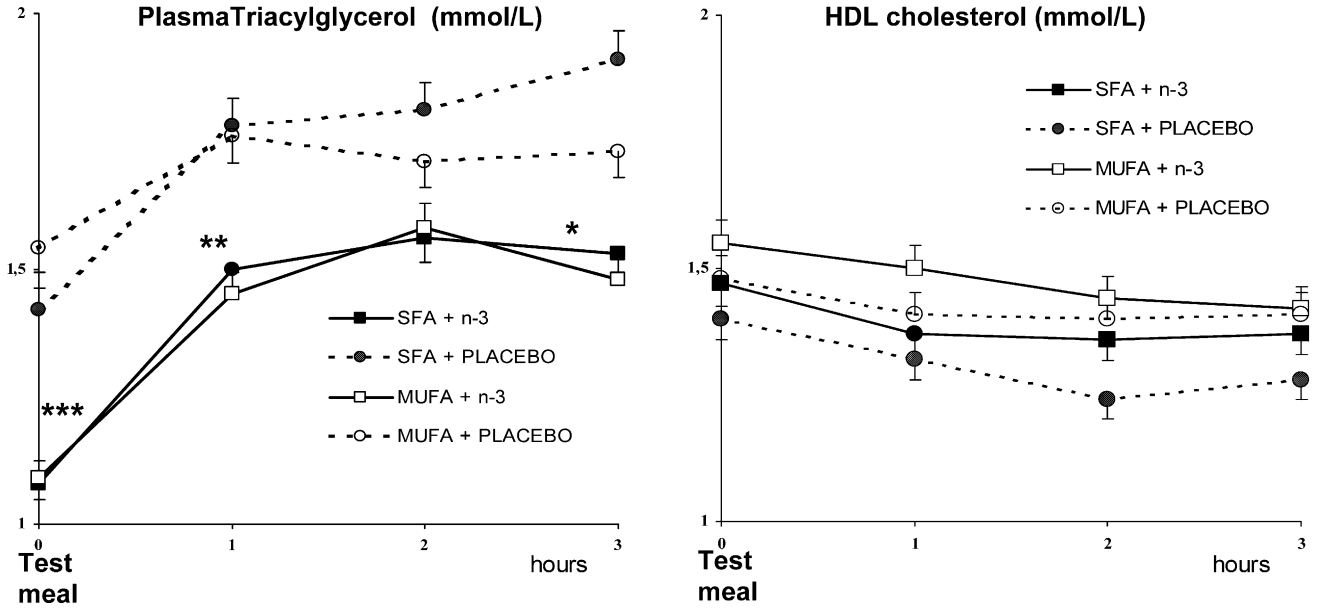

Fig. 1. Plasma triacylglycerol and HDL cholesterol values after a test meal, at the end of the two diets with or without n-3 supplementation ($N = 125$; mean ± S.E.M.; statistical significance for n-3 vs. placebo *, $P = 0.05$; **, $P = 0.04$; ***, $P = 0.006$).

effect on LDL cholesterol has been reported in free living young adults given for a short period of time two diets rich either in monounsaturated or n-6 polyunsaturated fat [25]. In our study, changes in the proportion of the SFA and MUFA have no effect on fasting VLDL and HDL concentrations. A decrease in triacylglycerol levels has been consistently reported with a reduction of saturated fatty acids in the diet, but most of the studies were made on hyperlipidemic patients and not healthy people [1,6,26]. Also the effects on HDL cholesterol levels of SFA are quite contradictory. Some data seem to suggest a slight increase in HDL cholesterol when using SFA compared with both MUFA and PUFA [1,6,27]. However, previous experiments with diets rich in MUFA or PUFA were based mainly on the use of hard margarine without controlling for the amount of trans fatty acids, which could explain the reduction of HDL found [28]. Our two diets were both characterised, instead, by similarly low levels of trans fatty acids. Therefore, on the basis of the data obtained in a large sample of healthy individuals with different background diets, we can conclude that replacing moderate amounts of saturated with monounsaturated fat, without any other dietary modification, is able to reduce by 15% both LDL cholesterol and LDL triacylglycerol without adverse effects on HDL. This conclusion is important, since some authors have questioned the real benefit of such modifications in the dietary prevention of cardiovascular diseases on the basis of the opposite effects of MUFA and SFA rich diets on LDL and HDL [1–4]. In addition, some cross-cultural, observational and intervention studies have shown that high SFA diets are associated with an increase in LDL cholesterol, but, at the same time, also with an increase in LDL size and in the largest LDL particles [7–9]. Therefore, the negative effects of high SFA diets on LDL concentrations could be minimised by this particular change in the distribution of LDL particles. Our data (obtained with an intervention of semi-long duration) confirm the adverse effects on LDL cholesterol concentration but not those on LDL size. The discrepancy between our results and those reported in the literature may be due to fact that our two diets were rigorously similar in terms of carbohydrate content. In any case, our results further support the validity of implementing low saturated fat diets in the prevention of cardiovascular diseases. To this respect, it is also important to underline the tendency of HDL cholesterol to decrease less in the post-prandial phase with a MUFA-rich diet in comparison with a SFA rich diet.

The only untoward effect of the high MUFA diet was the significant increase in Lp(a). Animal and human studies on this topic have given opposite results: as a matter of fact, it has been shown that replacing saturated with mono or polyunsaturated fatty acids induces in animals a reduction in Lp(a), while the opposite happens in humans [29]. A different trans-fatty acid content—which is known to influence Lp(a) [30]—could explain the results previously obtained in humans, but not ours, since our diets were very similar in terms of trans-fatty acid content. Whichever the mechanism underlying the increase in Lp(a) concentration with the MUFA diet, it is very unlikely that a 10% increase may be significant in terms of cardiovascular risk, also considering all the other beneficial effects of this kind of diet.

In relation to supplementation with moderate amounts of n-3 fatty acids, our study confirms that the most relevant effect of n-3 is to reduce significantly both VLDL cholesterol and triacylglycerol. This is true not only for patients with different metabolic abnormalities, as reported before [5,10–12], but also for healthy people, independently of the type of fatty acids—SFA or MUFA—present in the diet. Furthermore the hypotriglyceridemic effect of n-3 fatty acids is present also in the post-prandial period. At any rate, together with the reduction of VLDL, n-3 supplementation—as happens with other hypotriglyceridemic drugs—significantly increases LDL cholesterol also in healthy subjects also and not only in hyperlipidemic ones, as so far reported [5,10–12]. In particular, during the high MUFA diet, n-3 supplementation is able to abolish completely the positive effect of this kind of diet on LDL cholesterol. The data from a previous study on a very small sample of healthy individuals ($n = 6$) are different from ours, as they have shown no increase in LDL cholesterol on n-3 supplementation, although the results may have been affected by the shorter duration of the study (3 weeks) and the inadequate sample size. [31]. In any case, the opposite effect of n-3 fatty acids on VLDL and LDL concentrations in healthy subjects suggests that the supplementation of some foods with these fatty acids—which has been attempted in some countries—is questionable at least for what concerns lipid metabolism.

Supplementation with long-chain n-3 fatty acids significantly increased LPL activity, as also shown by some previous studies [32]. In any case, the lack of any significant relationship between the changes in LPL activity and the decrease in VLDL triacylglycerol on n-3 supplementation suggests that the main mechanism of action of these fatty acids on triacylglycerol metabolism is not at the LPL level but more on VLDL synthesis [33].

Another result of n-3 supplementation that needs to be commented upon is the lack of effect on LDL size, which is in line with data from a small group of diabetic patients with hypertriglyceridemia [34] and allows the observation to be extended also to healthy people. However, this is in contrast with a recent paper [35] reporting a significant increase in larger LDL and LDL size with fish oil in patients with combined hyperlipidemia. It is likely that the effect of n-3 on LDL size and distribution is different according to the preponderance of smaller LDL and may, therefore, be evident mainly in patients with the LDL pattern B (LDL size ≤ 255 Å), who were very few ($n = 15$) in our population. Our results do not completely rule out the occurrence of very small changes in the relative distribution of LDL subfractions, which our method of analysis may have not detected.

In conclusion, our study shows that replacing moderate amounts of saturated with monounsaturated fat in healthy subjects from different geographical areas of the western world provides beneficial effects on lipid metabolism, without any adverse influence on HDL cholesterol and LDL size. The only negative effects obtained with this diet relate to the significant increase in Lp(a) concentration. Moreover, a moderate supplementation with long-chain n-3 fatty acids in healthy subjects is able to reduce both fasting and post-prandial triacylglycerol levels, but at the same time increases LDL cholesterol irrespectively of the type of diet. Therefore, it is possible that the benefits of long-chain n-3 fatty acids on coronary mortality (DART and GISSI studies) [36,37] could be related more to the other n-3 effects—on cardiac arrhythmia, platelet aggregation, haemostatic variables, vascular reactivity—than to the ones on lipid metabolism.

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