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Dietary habits affect the susceptibility of low-density lipoprotein to oxidation

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Objective: To study, if there are differences in the fatty acid composition of low-density lipoprotein (LDL) in people eating three different long-standing habitual diets: vegetarian, high fish intake, or high saturated fat (milk fat) diet as a control group, and to study if these differences influence the oxidation susceptibility of LDL.
Design: Cross-sectional study using blood samples and a validated dietary frequency questionnaire with illustrations.
Setting: Helsinki University Central Hospital, Finland.
Subjects: The effect of three different types of long-standing diets of different fatty acid content (a strict vegetarian diet, $n = 11$; a high fish intake diet, $n = 9$; and a high saturated fat (milk fat) diet, controls, $n = 7$) on the serum and LDL fatty acid content, and on the susceptibility of LDL to oxidation *in vitro*, was studied in healthy normocholesterolemic volunteers who had been on these diets for years. Oxidation of LDL was carried out by using $CuSO_4$ as a pro-oxidant.
Results: There were no statistically significant differences in the serum lipids or lipoproteins, though the vegetarian group exhibited lowest mean values of total, high-density lipoprotein (HDL) and LDL cholesterol levels. Both the serum and LDL eicosapentaenoic, docosapentaenoic and docosahexaenoic acid proportions were highest in the fish and lowest in the vegetarian groups. Linoleic acid was highest among the vegetarians. In the fish group, the vitamin A concentration in serum was higher than in vegetarians and controls and β-carotene lower than in controls, but in α-tocopherol, or lycopene concentrations there were no statistically significant differences. The lag phase of LDL oxidation was shortest (116 min) in the fish group and longest (165 min) in the vegetarian group, and the control group was between them (129 min). The mean oxidation percentage after 2.5 h of copper-induced oxidation was highest (44%) in the fish group and lowest (22%) in the vegetarian group and intermediate (31%) in the control group.
Conclusion: Long-term dietary habits predict the fatty acid composition of serum and LDL, and influence the susceptibility of LDL to oxidation. In the fish group with the highest content of omega-3 fatty acids in LDL, the oxidation susceptibility of LDL was highest. In the vegetarian group with less omega-3 fatty acids in LDL, the LDL was more resistant to oxidation.
Sponsorship: Helsinki University Central Hospital.
Descriptors: oxidation; LDL; cholesterol; diet; fish;vegetarian; polyunsaturated fatty acids; saturated fatty acids; antioxidants

Introduction

Dietary fatty acids affect low-density lipoprotein (LDL) oxidation by changing its fatty acid composition (Berry *et al*, 1991; Reaven *et al*, 1993; Reaven, 1996). Of the fatty acids, polyunsaturated fatty acids are the most susceptible to oxidation (Bonanome *et al*, 1992; Reaven *et al*, 1993), and a diet enriched with linoleic acid increases LDL oxidation in both human (Berry *et al*, 1991; Reaven *et al*, 1991; Bonanome *et al*, 1992; Abbey *et al*, 1993; Corboy *et al*, 1993; Reaven *et al*, 1993; Reaven *et al*, 1994; Winklerhofer-Roob *et al*, 1995; Louheranta *et al*, 1996) and animal experiments (Thomas & Rudel, 1996). Lag time before the onset of the propagation phase of oxidation *in vitro* has been shown to be shortened (Abbey *et al*, 1993) or not altered (Reaven *et al*, 1991) by a linoleic acid rich diet.

Fish diet is rich in polyunsaturated long-chain omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), which are susceptible to oxidation (Gonzales *et al*, 1992) and supplementation of the diet with omega-3 fatty acids increases LDL oxidation in healthy subjects (Oostenburg *et al*, 1994; Suzukawa *et al*, 1995; Palozza *et al*, 1996), in hypertriglyceridemic patients (Hau *et al*, 1996), and in patients with non-insulin-dependent diabetes mellitus (McGrath *et al*, 1996). On the other hand, Bonanome *et al* (1996) in humans and Thomas & Rudel (1996) in non-human primates did not find any change due to supplementation with

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dietary omega-3 fatty acids in LDL susceptibility to oxidation. However, the fish oil diet in the latter study contained more monounsaturated fatty acids, which could partly explain the conflicting results. Monounsaturated fatty acids seem to protect LDL against oxidation, at least when they replace dietary polyunsaturated fatty acids (Berry *et al*, 1991; Bonanome *et al*, 1992; Corboy *et al*, 1993; Reaven *et al*, 1993; Reaven *et al*, 1994; O'Byrne *et al*, 1998; Schwab *et al*, 1998). Berry *et al* (1992) showed that LDL taken from subjects on a monounsaturated fatty acid enriched diet was less susceptible to oxidation than that from persons on a carbohydrate enriched diet.

According to animal studies, a diet rich in saturated fatty acids also reduced LDL oxidation in comparison with a diet rich in polyunsaturated fatty acids (Hennig *et al*, 1995) and the lag time seemed to be longer (Thomas & Rudel, 1996).

Most previous studies have consisted of supplementation with various types of fatty acids or short-term dietary interventions. The aim of the present study was to compare the susceptibility of plasma LDL to oxidation *ex vivo* in healthy volunteers who had for years been on one of the three different regular long-term diets of different fatty acid composition (a vegetarian diet, a high fish intake diet, or a high saturated fatty acid diet as a control diet).

Methods

Subjects

Altogether 27 healthy normocholesterolemic subjects gave their informed consent to participate in the study after a detailed interview on their habitual diet (which had lasted at least 2 y) and were recruited to the following groups:

(1) subjects who regularly (at least once a day) ate a great deal of fish ($n=9$);
(2) vegans or strict lacto-ovovegetarians ($n=11$);
(3) subjects on a diet with a high saturated fat intake ($n=7$).

Exclusion criteria were: serum total cholesterol >6.5 mmol/l, use of cholesterol-lowering or antioxidative drugs or dietary supplements and smoking.

Table 1 The sex, age, BMI, exercise habits and the serum lipid composition of the subjects in the fish, vegetarian and control groups[a]

	Fish n=9	*Vegetarian n=11*	*Controls n=7*
Male/Female	5/4	4/7	4/3
Age (y)	41±11.8	34±10.1	43±6.5
BMI (kg/m^2)	25.0±3.4^{v}	20.9±1.7^{c}	23.1±1.0
Exercise (times/wk)	3.1±2.4	3.4±2.5	3.4±2.4
Tot-chol (mmol/l)	5.05±0.85	4.13±0.89	5.15±0.60
HDL-chol (mmol/l)	1.39±0.22	1.22±0.29	1.43±0.43
LDL-chol (mmol/l)	3.47±0.88	2.69±0.67	3.32±0.69
VLDL-chol (mmol/l)	0.21±0.12	0.29±0.25	0.22±0.17
Tot-trigly (mmol/l)	1.04±0.30	1.16±0.53	1.01±0.48
HDL-trigly (mmol/l)	0.14±0.02	0.16±0.06	0.16±0.03
LDL-trigly (mmol/l)	0.30±0.05	0.30±0.06	0.31±0.04
VLDL-trigly (mmol/l)	0.59±0.29	0.71±0.48	0.49±0.26
LDL-chol/HDL-chol	2.56±0.75	2.32±0.86	2.52±0.85

BMI = body mass index; chol = cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; trigly = triglyceride; Tot = total. [a]Mean ± s.d. The Kruskall-Wallis test was used to compare the groups as regards to the parameters: In pairwise comparison, significant ($P<0.05$) differences were found in comparison to the vegetarian diet (v), and control diet (c).

Table 2 Daily food consumption (g/d) in the fish, vegetarian and control groups[a]

Food consumption	*Fish n=9*	*Vegetarian n=11*	*Controls n=7*
Cereal products	285±158	211±110	281±49
Vegetables	311±133	547±352	345±69
Fruit	240±92^{v}	498±309^{c}	210±106
Milk products	445±314^{v}	131±140^{c}	705±135
Meat	110±95^{v}	0±0^{c}	134±36
Fish	230±146^{v,c}	1±1^{c}	36±18
Eggs	31±31^{v}	3±16^{c}	18±4

[a]Mean ± s.d. The Kruskall-Wallis test was used to compare the groups as regards the parameters: In pairwise comparison, significant ($P<0.05$) differences were found in comparison to the vegetarian diet (v), and the control diet (c).

The sex, age, body mass index (BMI), exercise habits and serum lipids of the subjects are presented in Table 1, and the daily food consumption in Table 2.

Recording of the living habits and calculation of nutrient intake

A questionnaire on minor illnesses, use of medication and frequency of exercise (more than 30 min exercise resulting in breathlessness per week) was filled in by the subjects. The Nutrica programme with a Finnish database (Rastas *et al*, 1993) was used for calculation of the nutrient intake recorded by means of a frequency questionnaire accompanied by an illustrated book describing the quality of food and quantity of food portions (Pietinen *et al*, 1988a; 1988b).

Determination of serum lipids and lipoproteins

After 12 h (overnight) fasting the subjects gave 250 ml of blood into vacutainer EDTA (ethylenediamine tetraacetic acid) tubes. Plasma was separated by centrifugation. Cholesterol and triglycerides were measured using an enzymatic, colorimetric test (Unimate 7 Chol, Cobas Mira S, Roche, Basel Switzerland).

Oxidation of LDL in vitro

A rapid method was employed to isolate LDL from EDTA plasma for oxidation experiments. VLDL (very low-density lipoprotein) was separated by centrifugation in a Beckman Ti 50.4 rotor (269 000 g, 3.5 h, +15°C). Following the removal of the VLDL fraction by slicing of the centrifuge tubes, LDL was separated, using the same rotor (155 000 g, 18 h, +15°C), and isolated by slicing.

EDTA was removed by gel-filtration of LDL on Sephadex G-25 in phosphate buffered saline solution (PBS), and thereafter the LDL sample was divided into three equal aliquots (a, b and c) for the oxidation experiments: 60 mM EDTA was added to a final concentration of 1.2 mM to one aliquot part (part a, the native LDL). Copper mediated oxidation (Esterbauer *et al*, 1989) was begun in aliquots b and c. In short, gel-filtered LDL (100 μg protein/ml) in PBS (pH 7.4) was supplemented with $CuSO_4$ to a final concentration of 20 μM. In part b (the totally oxidized LDL), the diene absorption at 234 nm was recorded at +20.5°C with a Shimadzu UV-1202 spectrophotometer (Shimadzu Corp, Kyoto, Japan) equipped with a Shimadzu cell temperatures CPS controller (Shimadzu Corp, Tokyo, Japan). When diene formation reached its maximum the oxidation of this aliquot was stopped by adding EDTA as above. For part c (the partially oxidized LDL), the same

procedure was carried out but oxidation was stopped at 2.5 h by adding 60 mM EDTA. Oxidation experiments were carried out twice at 48 h intervals, and mean values were used for calculations. The protein content of LDL was analysed by the method of Lowry *et al* (1951).

Determination of esterified fatty acids of plasma and LDL
Aliquots of 250 µl of LDL fraction of 500 µl of plasma were transferred into test tubes for the fatty acid determination. An internal standard, trinonadecanate (100 mg/l, Labordan Chemicals, Malmö, Sweden) was added in 100 µl of methanol and mixed in. Next the samples were extracted with 1.9 ml of chloroform:methanol (1:1) and evaporated *in vacuo*. The lipid residue was dissolved in petroleum ether. Methyl derivatives of the esterified fatty acids were prepared by *trans*-esterification with sodium methanolate as described previously by Seppänen-Laakso *et al* (1990). PTV, Programmed Temperature Vaporization, split technique was used for sample introduction. The methyl derivatives were separated employing programmed temperature gradient on an OV-351 fused silica column (25 m, 0.32 mm i.d.) mounted on a Dani HR 3800 PTV gas chromatograph and detected with a FID, Flame Ionization Detector. A reference serum with certified fatty acid concentrations (Seronorm lipid Nycomed, Oslo, Norway) was used for quality control. The between-batch and within-batch imprecisions were not more than 2% for the fatty acid concentrations observed in the present study.

The weight percentage of each fatty acid was calculated in relation to sum of all fatty acids determined (as listed in Table 4).

The average degree of desaturation was calculated from the molar concentration of each fatty acid by multiplying it with the number of double bonds present in each fatty acid. The result was then divided by the molar concentration of the sum of all fatty acids determined to give average number of double bonds per fatty acid chain in the sample that is, degree of desaturation. The average chain length of the fatty acids in a sample was calculated in the same way taking the known chain length of each fatty acid instead of the number of double bonds.

Determination of serum antioxidants
250 µl of serum was mixed with 250 µl ethanol containing 1.5 g of butylated hydroxytoluene (BHT). The mixture was extracted with 1 ml of hexane, containing 250 mg BHT/l. 750 µl of the resulting extract was evaporated to dryness *in vacuo*. The dry residues were solved in 400 µl of CH_3CN:CH_2Cl_2:CH_3OH (7:2:1) and injected simultaneously into two HPLC, High Performance Liquid Chromatograp, systems employing one Gilson model 232 Bio automatic sample injector (Gilson Medical Electronics, Villiers-le-Bel, France). Each system consisted on one LKB Model 2248 pump (Pharmacia LKB, Uppsala, Sweden), Beckman System Gold Model 166 or 167 detector (Beckman Instruments Inc, Merwe, Galway, Ireland) and a Beckman Ultrasphere ODS column (150 × 4.6 mm). α-tocopherol was separated according to De Leenheer *et al* (1979) and the carotenoids according to Nellis & De Leenheer (1983). The complete baseline separation of the carotenoids was achieved at +30°C, while retinol and α-tocopherol were separated at ambient temperature. Retinol was detected at 324 nm, α-tocopherol at 292 nm and the carotenoids at 445 nm.

Statistical analysis
First, the Kruskal-Wallis test, which is a nonparametric one-way ANOVA, was used. Pairwise comparisons were performed using the Mann-Whitney U tests, and the *P*-values were Bonferroni-adjusted. These procedures are nonparametric *post-hoc* analyses. Spearman rank correlations were calculated to study the relationships between oxidation variables and fatty acid composition variables.

Ethics
The study protocol was approved by the Ethical Committee of the Helsinki University Central Hospital.

Results

The descriptions of the mean food consumption (Table 2) show that the recruiting of subjects on three different diets was successful: the differences between groups were as expected. The mean fish intake was 230 g/d in the fish group. The vegetarians ate no meat or fish and their mean milk consumption was lower than in the other two groups.

The nutrient intake reflected the food consumption, especially in the fish group, where the mean eicosapentaenoic acid (EPA) intake was 0.63 g/d (0.2E%) and the docosahexaenoic acid (DHA) intake 1.38 g/d (0.44E%). The mean intake of saturated fat in the vegetarian group was only half of that in the other two groups. There were no statistically significant differences in the vitamin C or vitamin E intake between the groups.

The serum or lipoprotein lipids did not significantly differ between the three dietary groups in the serum cholesterol, triglycerides or their subfractions, though the lowest mean values in the total, high-density lipoproteins (HDL) and LDL cholesterol were seen in the vegetarian group.

The lag phase of LDL oxidation was shortest (116 min) in the fish group (Figure 1, Table 3) and longest (165 min) in the vegetarian group, and intermediate in the control diet group (129 min). There were no statistically significant differences in the rate of oxidation or in the maximal oxidation. The oxidation percentage after 2.5 h $CuSO_4$

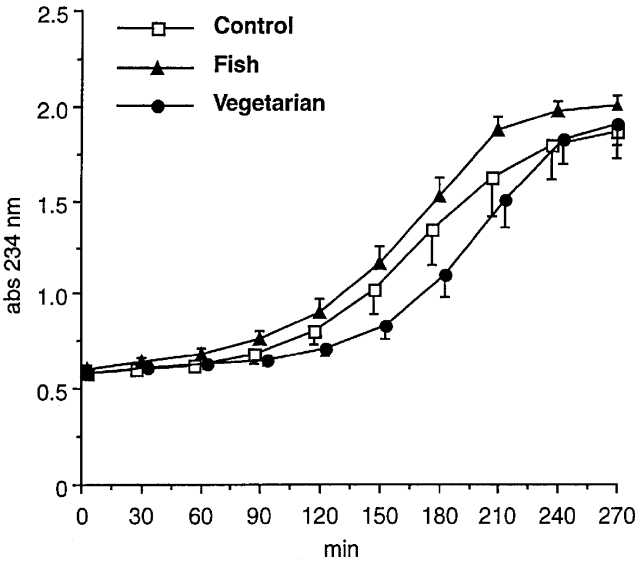

Figure 1 Time-oxidation curves of plasma low-density lipoprotein *in vitro* by $CuSO_4$ of subjects habitually living on fish, vegetarian or control diets. Mean ± s.e., $n = 7-11$ in each dietary group.

Table 3 Oxidation parameters: lag phase, oxidation percentage, maximal oxidation and the slope of the oxidation of LDL from the fish, vegetarian and control groups[a]

	Fish n = 8	*Vegetarian n = 10*	*Controls n = 6*
Lag phase (min)	116 ± 25.1[v]	165 ± 27.3	129 ± 39.2
Oxidation % (2.5 h)	44 ± 17.7[v]	22 ± 6.7	31 ± 12.6
Maximal oxidation	1.47 ± 0.09	1.52 ± 0.17	1.41 ± 0.20
Rate of oxidation	0.015 ± 0.0017	0.0018 ± 0.0031	0.015 ± 0.0039

[a]Mean ± s.d. The KrusKall-Wallis test was used to compare the groups as regards the parameters: In pairwise comparisons, significant ($P < 0.05$) differences were found in comparison to vegetarian diet (v).

treatment was highest (44%) in the fish group and lowest (22%) in the vegetarians, and between the two (31%) in the control group.

There EPA, DPA (docosapentaenoic acid) and DHA percentages of total fatty acids in LDL were significantly higher in the fish group than in the vegetarian group, and the percentage of linoleic acid lower. In the vegetarians the percentages of EPA and DPA (Table 4) were only about a half of those in the controls. The differences in the fatty acid composition of serum between the groups were similar to those observed in the LDL (data not shown).

Ther were no statistically significant differences in the degree of desaturation (number of double bonds per fatty acids): in the fish group the mean number of double bonds was 1.64 compared to 1.34 in the vegetarian group and 1.47 in the control group. The mean fatty acid chain length in LDL was similar in all three dietary groups (Table 4).

The concentration of vitamin A in serum was significantly higher and the concentration of β-carotene lower in the fish group as compared to the vegetarian and the control group (Table 5).

The length of the oxidation lag phase correlated statistically significantly positively with the oleic and linoleic acid proportion in LDL and negatively with EPA, DPA, DHA proportion as well as with the omega-3/omega-6 ratio, with the mean number of double bonds in the fatty acid chains in LDL and with the mean fatty acid chain length in LDL (Table 6, Figure 2).

Table 4 LDL fatty acid content (% of total LDL fatty acids), the fatty acid chain length and the number of double bonds in the fatty acid chain in the fish, vegetarian and control groups[a]

	Fish (n = 6) %	*Vegetarian (n = 9) %*	*Controls (n = 4) %*
Saturated			
Myristic acid	0.73 ± 0.19	0.74 ± 0.32	1.01 ± 0.25
Palmitic acid	21.20 ± 1.62	20.56 ± 1.96	20.92 ± 0.83
Stearic acid	6.86 ± 0.67	6.63 ± 0.75	7.55 ± 0.83
$\sum$	28.7 ± 1.79	27.93 ± 2.06	29.48 ± 0.92
Monounsaturated			
Palmitoleic acid	2.35 ± 1.15	1.78 ± 0.66	2.19 ± 0.83
Oleic acid	19.99 ± 2.98	20.43 ± 1.14	19.84 ± 1.65
$\sum$	22.34 ± 4.00	22.21 ± 1.11	22.03 ± 2.19
Omega-3 series			
α-linolenic acid	0.82 ± 0.16	0.87 ± 0.49	0.99 ± 0.22
Octadecatetraenoic acid	0.26 ± 0.09	0.30 ± 0.11	0.33 ± 0.07
Eicosapentaenoic acid	5.15 ± 3.51[v]	0.81 ± 0.42[c]	1.86 ± 0.54
Docosapentaenoic acid	0.78 ± 0.26[v]	0.44 ± 0.14	0.66 ± 0.06
Docosahexaenoic acid	4.93 ± 2.12[v]	1.68 ± 0.56[c]	3.27 ± 0.61
$\sum$	11.95 ± 5.81[v]	4.10 ± 1.22[c]	7.11 ± 0.87
Omega-6 series			
Linoleic acid	28.34 ± 4.73[v]	36.95 ± 2.86	32.89 ± 1.77
γ-Linolenic acid	0.38 ± 0.21	0.45 ± 0.19	0.31 ± 0.08
Eicosadienoic acid	0.32 ± 0.04	0.30 ± 0.07	0.34 ± 0.03
Dihomo-γ-linolenic acid	‘1.10 ± 0.37	1.60 ± 0.41	1.34 ± 1.18
Arachidonic acid	6.69 ± 1.79	6.30 ± 1.84	6.27 ± 0.51
$\sum$	36.83	45.60	41.15
Omega-6/omega-3	3.95 ± 3.28	12.77 ± 6.78	5.84 ± 0.55
Degree of desaturation[b]	1.64 ± 0.22	1.34 ± 0.06	1.47 ± 0.06
Chain length[d]	17.89 ± 0.18	17.66 ± 0.08	17.76 ± 0.06

LDL = low density lipoprotein. [a]Mean ± s.d. The Kruskall-Wallis test was used to compare the groups as regards to the parameters: In pairwise comparison significant ($P < 0.05$) differences were found in comparison to the vegetarian diet (v), and the control diet (c). [b]Number of double bonds per fatty acid residue. [d]Number of carbon atoms in fatty acid chain in LDL.

Table 5 Concentrations of antioxidant levels in serum in the fish, vegetarian and control groups[a]

	Fish n = 5	*Vegetarian n = 9*	*Controls n = 4*
Retinol (μmol/l)	2.4 ± 0.59[v,c]	1.75 ± 0.24	1.54 ± 0.21
α-Tocopherol (μmol/l)	31.2 ± 6.4	28.0 ± 6.3	32.1 ± 6.1
β-Carotene (μmol/l)	0.73 ± 0.35[c]	1.58 ± 0.65	2.20 ± 1.22
Lycopene (μmol/l)	0.41 ± 0.12	0.44 ± 0.25	0.62 ± 0.31

[a]Mean ± s.d. The Kruskall-Wallis test was used to compare the groups as regards the parameters: In pairwise comparison, significant ($P < 0.05$) differences were found in comparison to the vegetarian diet (v), and the control diet (c).

Table 6 Correlations (Spearman rank correlation *P*) between LDL fatty acid composition and oxidation parameters

LDL fatty acid parameter	*Lag phase*	*Rate of oxidation*	*Oxidation (%)*
Saturated			
Myristic acid	− 0.23	− 0.17	0.10
Palmitic acid	− 0.29	− 0.09	0.31
Stearic acid	− 0.28	− 0.18	0.19
$\sum$	− 0.29	− 0.15	0.26
Monounsaturated			
Palmitolenic acid	− 0.08	− 0.31	− 0.01
Oleic acid	0.54*	0.04	− 0.60*
$\sum$	0.53*	0.12	− 0.62*
Omega-3 series			
α-linolenic acid	− 0.02	− 0.08	− 0.14
Octadecatetraenoic acid	− 0.06	0.02	0.10
Eicosapentaenoic acid	− 0.88*	− 0.56*	0.79*
Docosapentaenoic acid	− 0.81*	− 0.55*	0.71*
Docosahexaenoic acid	− 0.69*	− 0.67*	0.59*
$\sum$	− 0.76*	− 0.66*	0.67*
Omega-6 series			
Linoleic acid	0.65*	0.56*	− 0.62*
γ-Linolenic acid	0.04	0.51*	0.01
Eicosadienoic acid	− 0.05	0.12	0.10
Dihomo-γ-linolenic acid	0.41	0.62*	− 0.34
Arachidonic acid	− 0.17	0.326	0.30
$\sum$	− 0.74*	− 0.22	0.66*
Omega 3/omega-6	0.74*	0.70*	− 0.66*
Double bonds/chain	− 0.49*	− 0.21	0.50*
Chain length	0.59*	0.54*	− 0.49*
Antioxidants			
Vitamin A	− 0.49*	− 0.08	0.59*
α-Tocopherol	− 0.61*	− 0.34	0.55*
β-Carotene	0.19	0.30	− 0.16
Lycopene	− 0.06	− 0.17	0.04

LDL = low density lipoprotein. *$P < 0.05$ Spearman rank correlation.

Discussion

The present study was designed to investigate whether a regular long-term high intake of fish and a strict vegetarian diet differ from a diet, rich in saturated fat in their effects on *in vitro* LDL oxidation resistance.

Our first aim was to confirm that a regular, long-standing diet, either vegetarian, high fish or the control Finnish diet

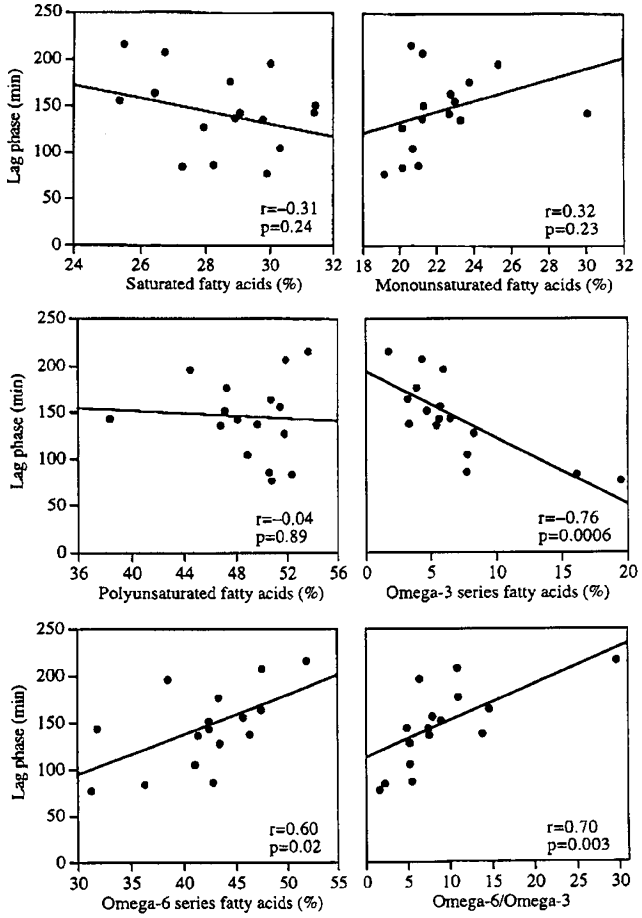

Figure 2 Linear regression analysis of the oxidation lag phase (min) versus the low-density lipoprotein concentration (% of total fatty acids) of saturated, monounsaturated and polyunsaturated, omega-3 series, omega-6 series fatty acids and omega-6/omega-3 ratio, $n = 7-11$ in each dietary group.

causes characteristic changes in the fatty acid composition of LDL. This was accomplished by showing that the serum and LDL fatty acid composition in different diet groups reflected the calculated nutrient intake. Next, we aimed to show that these alterations in fatty acid composition of LDL resulted in different susceptibilities to oxidation.

The recruitment of subjects was based on a dietary interview by a dietitian and was successful as regards to the food consumption. The mean daily intake of fish in the fish group was 230 g while it is about 28 g in the normal Finnish diet (Finravinto, 1997) which is similar to that observed in the control group of this study. The differences in the dietary habits were also reflected in the serum and LDL fatty acid composition, but not so clearly in the serum lipoproteins. The gender distribution in the groups was uneven, because of the overrepresentation of women in strict vegetarians in Finland which contributed to the low BMI in the vegetarian group.

There were statistically significant differences in the total serum cholesterol or HDL, LDL or VLDL (very low-density lipoprotein) cholesterol or in the LDL:HDL ratio, nor in the total serum triglycerides between the diet groups. However, when the total, LDL and HDL cholesterol of the vegetarian and the control group were pairwisely compared after ANOVA they were lower in the vegetarians in accord with a study on Finnish strict vegans (Rauma, 1996). In our study, the intake of fish was higher than in most earlier studies and the intake of EPA was higher and that of DHA lower compared to the studies on fish oil supplementation.

While most earlier studies on LDL oxidation involved supplementation of the diet with various fatty acids or consisted of short-term dietary interventions, our subjects consumed their habitual diet to which they had adhered many years before the study. In the supplementation studies, fish oil or EPA was added to the diet while in our study the subjects ate different kinds of fish, often salmon or other fat fish foods containing also cholesterol. Accordingly, cholesterol intake was higher in the fish group than in the vegetarian group. In the control group, the intake of milk products was high.

The LDL isolated from subjects in the fish group was characterized by markedly increased proportion of omega-3 fatty acids, mainly due to accumulation of EPA and DHA, to a decreased proportion of linoleic acid. The dietary intake was carefully recorded and analysed, and the lipid composition of both the serum and LDL were analysed.

The lag phase of LDL oxidation was shortest in the fish group, probably due to the higher proportion of long-chain polyunsaturated fatty acids, which are very susceptible to oxidation (Gonzales *et al*, 1992). The result is in accord with the study of Thomas & Rudel (1996) in non-human primates, in which the content of monounsaturated fatty acids also differed between the diets, and the study of Whitman *et al* (1994) in pigs in which the lag time was shorter in the high polyunsaturated fat diet compared to the low polyunsaturated. According to Esterbauer *et al* (1992 and 1995) the mean lag time of human LDL oxidation varies between 68 and 124 min. In our study, the lag phase in the strict vegetarians was longer probably due to the zero intake of EPA and DHA. The oxidation percentage of the LDL after 2.5 h oxidation was highest in the fish group and lowest in the vegetarians. These differences are in line with the differences in the long-chain fatty acid composition of LDL. The percentage of omega-3 fatty acids in LDL was negatively correlated with the rate of oxidation and the percentage of omega-6 positively correlated. This is most probably due to the fact that in omega-6 fatty acids there are more fatty acids with a higher number of double bonds than in the omega-3 fatty acids. This might also indicate the importance of the chain length because in omega-3 fatty acids there are more long-chain fatty acids than in omega-6 fatty acids, but could also indicate the importance of the site of double bonds in oxidation.

Conclusions

The results show that differences in long-term dietary habits (especially the fish diet and strict vegetarian diet) predicted changes in the fatty acid composition of serum and LDL. The increased content of omega-3 fatty acids in LDL derived from the fish diet group increased the oxidation susceptibility of LDL. The LDL oxidation resistance was greatest in the vegetarian group, which had the lowest proportion of unsaturated long chain fatty acids in LDL. These findings raise the question whether the increased *in vitro* oxidation susceptibility of LDL originating from the fish diet group causes increased atherogenicity *in vivo*. On the other hand omega-3 fatty acids induce reduced platelet activation and thromboxane production (Whitman *et al*. 1994), not necessarily resulting *in vivo* atheroclerosis.

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