Influence of long-chain polyunsaturated fatty acids on oxidation of low density lipoprotein

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Abstract:

Enrichment of low density lipoprotein (LDL) with long-chain fatty acids, such as eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) found in fish oil, is thought to increase its oxidative susceptibility although such an increase has not been clearly demonstrated. The purpose of this study was to determine the composition and fatty acid concentration of LDL obtained from postmenopausal women given a supplement of fish oil and relate these values to its oxidative susceptibility. Fish oil supplementation significantly increased LDL concentration of EPA (P = 0.0001) and DHA (P = 0.0001) and decreased that of linoleic acid P = 0.006). The concentration of free cholesterol, cholesterol ester, phospholipids and protein was unchanged while triglyceride concentration increased 8% (P = 0.02). Cu2+-mediated oxidation resulted in a shorter lag time, slower oxidation rate and similar concentrations of conjugated dienes of EPA/DHA-enriched LDL than EPA/DHA-unenriched LDL. Stepwise multiple regression indicated that the primary predictor of oxidative susceptibility of LDL was linoleic acid, even after enrichment with EPA and DHA. The oxidation rate of EPA/DHA-unenriched LDL correlated with the cholesteryl ester concentration (P = 0.003) while that of EPA/DHA-enriched correlated with the concentration of phospholipids (P = 0.03). These data suggest that EPA/DHA-enriched LDL have decreased oxidative susceptibility and that surface lipids may mediate its rate of oxidation.

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INTRODUCTION

Low density lipoprotein (LDL) is currently regarded as the most atherogenic of the plasma lipoproteins. Recent studies strongly suggest that the oxidation of LDL may produce a particle (oxLDL) far more atherogenic than native LDL. Much information has been generated to support the in vivo existence of oxLDL1[-5] and several ways in which it could promote atherosclerosis have been proposed.[6-10] The factors that modulate the oxidative susceptibility of the LDL particle are poorly understood but are likely to have broad implications in terms of strategies to intervene and decrease in vivo oxidation.

Among the factors that may influence the oxidative susceptibility of the LDL particle is its fatty acid content. Diets enriched in mono-unsaturated fatty acids have been shown to decrease oxidative susceptibility of LDL.' [1`4] On the other hand, since highly unsaturated fatty acids are preferentially oxidized in vitro,'" it is thought that EPA/DHA-enriched LDL will be more susceptible to oxidation. Studies to evaluate this issue have proved contradictory.

Some studies[19,20] have reported an increased oxidative susceptibility of EPA/DHA-enriched LDL. Harats et al[19] compared the effect of fish oil ingestion on peroxidation of LDL in smokers and nonsmokers and observed that in both groups the indices that suggested increased peroxidation, LDL thiobarbituric acid reactive substances (TBARS) and metabolism of LDL by macrophages, worsened. Hornstra et al[20] reported a shorter lag time in men supplemented with fish oil compared to an un-supplemented group. Other studies have reported no change[21-24] or decreased susceptibility to oxidization.[25-29]

Nenseter et a1[21] supplemented diets of humans with n-3 ethyl esters. They noted distinct enrichment in the EPA and DHA content of LDL cholesterol esters, triglycerides and phospholipids, but the susceptibility to lipid peroxidation in LDL assessed by measuring both total lipid peroxides and deradation of oxidatively modified LDL in macrophages was not different from a control group given corn oil. Frankel et a1[22] found that fish oil intake did not alter oxidative susceptibility when aldehydes unique to n-6 or n-3 fatty acids were used to monitor the extent of oxidation. Bonanome et a1[23] found no difference in hypertriglyceridemic hemodialyzed patients given n-3 fatty acids. Saito et a1[24] found that EPA ethyl esters rendered rabbit LDL less susceptible to oxidation. We recently found in postmenopausal women supplemented with fish oil a shortened lag time but slowed oxidation rate with no change in the production of conjugated dienes.[25] Similar results were reported by Hau et a1[26] in hypertriglyceridemic patients, Suzukawa et a1[27] in treated hypertensive subjects, Whitman et a1[28] in female Yucatan miniature swine fed an atherogenic diet and Thomas et a1[29] in cynomolgus monkeys.

It is difficult to sort out the influence of specific fatty acids on the oxidative susceptibility of LDL in these studies because often the fatty acid profile is not reported or, if reported, is given in relative units, i.e. relative weight percent or mole percent, so that as the amount of one fatty acid increases, that of another decreases. When the data are expressed this way, they do not relate meaningfully to indices that reflect oxidative susceptibility.

In addition, the susceptibility of LDL from postmenopausal women to oxidize has received limited evaluation. As they have an enhanced atherogenic-risk profile compared to premenopausal women, dietary modifications which may influence their cardiovascular risk need careful scrutiny.

Consequently, the purpose of this study was to determine the fatty acid concentration and composition of LDL obtained from postmenopausal women given a supplement of the long-chain polyunsaturated fatty acids EPA and DHA and relate these values to LDL oxidative susceptibility.

MATERIALS AND METHODS

Study design

This study was a subset of a larger human intervention study examining the interaction of supplements of α-tostudy examining the interaction of supplements of α-tocopheryl acetate and fish oil on in vitro and in vivo indices of lipid peroxidation. The subset was limited to samples from subjects obtained during the times when 15 g/d fish oil supplement was consumed without a-tocopheryl acetate supplementation.

Participants were recruited from the Oregon State University campus and surrounding community. They were healthy, normolipemic, nonsmoking, postmenopausal females. Approximately half of the women recruited used oral hormone replacement therapy; the other half did not. Women were considered postmenopausal if either they had been free of menstrual cycles for the preceding year or had used hormone replacement therapy for a comparable length of time. No subjects were taking medication such as corticosteroids, aspirin or nonsteroidal anti-inflammatory drugs. None were taking nutritional supplements except occasionally calcium; however, in no subject did combination of dietary and supplemental calcium intake exceed 1200 mg per day. In order to participate in the study, subjects had to be willing to refrain from the consumption of fish for the duration of the intervention. For the determination of the nutritional content of their habitual diets, subjects were instructed in the technique for keeping a 3-day (two weekdays and one weekend day) record of all foods and beverages consumed. The nutritional content of the subjects' diets was then evaluated from the 3-day diet records using Food Processor Plus v. 5.03 (ESHA, Salem, OR, USA). The study protocol was reviewed and approved by the institutional review board at Oregon State University and written consent was obtained from each participant.

The fish oil was obtained from the National Institute of Health's Fish Oil Test Material Program in sealed opaque containers containing 100 x 1 g capsules with tertiary butyl hydroquinone (TBHQ 2-(1,1-dimethylethyl)-1,4-benzenediol; 0.02%) added as an antioxidant but without the routine addition of vitamin E. TBHQ has been approved as a food grade, oil soluble antioxidant (CFR 21.121.1244, Federal Register, 1972). It is metabolized almost completely within a few days of ingestion and excreted in the urine 3^0 Because it is not stored in tissues, it does not function as an anti-oxidant in vivo. Consequently, it can be used to protect the oil from oxidation before it is consumed but does not prevent in vivo lipid peroxidation. The fatty acid profile of the fish oil was measured by gas chromatography as previously described[28] using tricosanoic acid (Nu-Chek Prep, Elysian, MN) as an internal standard.

Analytical procedures

Blood samples were collected into tubes containing Na_2EDTA (1 mg/mL) after an overnight fast of approximately 12 h. LDL was prepared by sequential ultracentrifugation[25] and appropriate aliquots

frozen at —80°C. The fatty acid profile of plasma and LDL was determined by gas chromatography as previously described.[31] LDL cholesterol was determined enzymatically by a modification of the method of Allain et a1.[32] Triglycerides were measured by a modification of the method of McGowan et a1.[33] Protein was determined by the method of Lowry et al [34] Free cholesterol was determined enzymatically using a product from Wako Pure Chemical Industry (Richmond, VA, USA). Cholesterol ester concentration was calculated in the following manner: (total cholesterol - free cholesterol) x 1.68. Phospholipids were measured enzymatically (Wako Pure Chemical Industry). The oxidation of LDL was measured using the continuous production of conjugated dienes as developed by Esterbauer et al.[35]

Statistical analysis

Summary statistics of the variables were calculated (mean and standard error) before and after the supplement of fish oil.[36] A paired t-test was used to determine significant differences between variables measured before and after the fish oil supplement.[36] Stepwise multiple regression was used to determine the relationships between the LDL content of α-tocopherol and selected fatty acids and content of α-tocopherol and selected fatty acids and the three indices associated with oxidative susceptibility (lag time, rate of formation of conjugated dienes and maximum production of conjugated dienes) for samples obtained before and after the fish oil supplement (SAS v6.11, 1996). The statistical model was:

$$Y = b_0 + b,[LLA] + b_2[EPA] + b_3[DHA] + b_4[\alpha\text{-tocopherol}] + e \text{ where}$$

Y = the dependent variable.
[LLA] = the concentration of linoleic acid in the LDL particle.
[EPA] = the concentration of EPA in the LDL particle. [DHA] = the concentration DHA in the LDL particle. [a-tocopherol] = the concentration of α-tocopherol in [α-tocopherol] = the concentration of α-tocopherol in the LDL particle .
b_0-13_4 = regression coefficients.
e = error term.

Independent variables were introduced using a stepwise procedure (SAS v.6.11, 1996). The regression equation that gave the minimum value of *P* but maximum value of R^2 was taken as the one that best described the relationship between the dependent and independent variables. All variables left in the model were significant at the 0.15 level or lower. The presence of significant interactive terms in the model generated by stepwise regression was evaluated using 2-way analysis of variance. For the multiple regressions performed, no significant interaction terms were found. Standardized regression coefficients for each term in the model were calculated to determine which term(s) was(were) the major contributors. Pearson's correlation coefficients were computed for the oxidative susceptibility and composition of the LDL particle.[36] The $P < 0.05$ level of significance was used to assess statistical significance.

Table 1 Characteristics of the subjects before supplementation with fish oil ($n = 42$)[a]

Characteristic	Value
Age (y)	58.4 ± 1.4
Weight (kg)	65.0 ± 1.4
BMI[b] (kg/m^2)	25.6 ± 0.4

'Data are given as means ± SEM; [6]BMI = body mass index.

RESULTS

The initial characteristics of the subjects are given in Table 1. Compliance was indicated by the anticipated drop in plasma triglycerides from 105.7 ± 6.8 mg/100 mL to 74.6 ± 5.9 mg/100 mL *(P* = 0.0001), increase in plasma EPA from 0.8 ± 0.1 to 7.2 ± 0.2 g/100 g fatty acids and DHA from 2.1 ± 0.1 to 5.2 ± 0.1 g/100 g fatty acids and return of empty supplement containers.

The subjects maintained their habitual diets throughout the study. This included a minimal intake of fish, making the supplement the major source of EPA and DHA. The average daily content of linolenic acid, EPA and DHA in the habitual diets was 0.8 + 0.4 g, 0.1 ± 0.1 g and 0.1 ± 0.2 g, respectively. The fatty acids supplied by the fish oil supplement have been reported previously.[25,37] Briefly, the women consumed 5.1 g saturated fatty acids, 3.5 g MUFA and 5.5 g PUFA of which 2.5 g was EPA and 1.8 g DHA each day. The average intake of macronutrients fell within the recommendations of the Dietary Guidelines with fat providing 29 ± 1% of the kcal, protein 17 ± 0.4%, and carbohydrate 56 ± 1%. Of the fat 10 ± 0.4% was supplied by MUFA, 7 ± 0.3% by PUFA and the remainder by saturated fatty acids. The habitual diet met or exceeded the appropriate RDA for every micronutrient.

The composition of the LDL particles is given in Figure 1. The only item that was changed by the fish oil supplement was the level of triglycerides. It increased 8% from

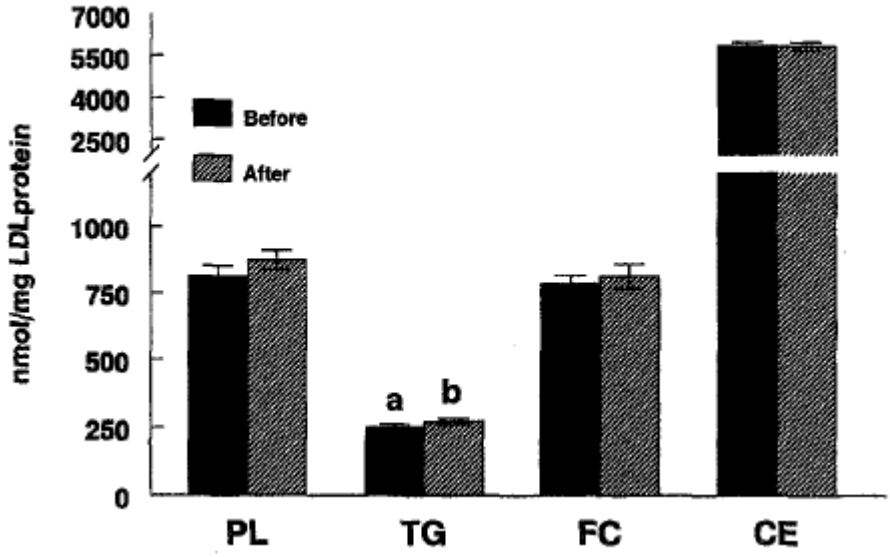

Fig. 1 The composition of the LDL before and after the consumption of 15 g fish oil per day for 5 weeks *(n* = 41). Columns with different letters above them are significantly different at *P* < 0.05. PL = phospholipids; TG = triglycerides; FC = free cholesterol; and CE = cholesteryl ester.

250.9 ± 10.8 nmol/mg LDL protein to 270.9 ± 10.7 nmol/ mg LDL protein *(P* = 0.02). The data were also expressed on a percent basis. The final content of phospholipids was 15.2 ± 0.6%, triglycerides 5.3 ± 0.2%, free cholesterol 7.0 ± 0.4%, cholesterol ester 50.5 ± 1.1% and protein 22.4 ± 0.2%. Expressed in this manner the change in the triglyceride content of LDL was not significant *(P* = 0.09).

The concentration of selected fatty acids before and after the supplementation with fish oil is given in Table 2 in nmol/mg LDL protein. There was an 11% significant increase in the concentration of

polyunsaturated fatty acids, resulting from the increase in the amount of the n-3 fatty acids, EPA, DHA and DPA (docosapentaenoic acid; 20:5 n-3). The concentration of the major fatty acid, linoleic acid, decreased 8.4%, a significant change *(P* = 0.006). There were small (6.7%) but significant *(P* = 0.02) increases in the saturated fatty acid concentration as a result of the increase in the quantity of 16:0 and 18:0. There was a significant decrease *(P* = 0.01) in the mono-unsaturated fatty acid concentration as a result of the decrease in the content of oleic acid. When the data

Table 2 The fatty acid composition of LDL from postmenopausal women before and after supplementation with fish oil (n = 41)[a]

Fatty acid	Before	After	*P*
	nmol/mg LDL protein		
14:0	35 ± 2	35 ± 2	NS[b]
16:0	845 ± 23	899 ± 17	0.05
16:1	94 ± 5	91 ± 4	NS
18:0	239 ± 7	257 ± 6	0.01
18:1(n-9)*t*	35 ± 2	31 ± 2	0.04
18:1(n-9)c	652 ± 19	574 ± 11	0.0001
18:1(n-7)	56 ± 2	63 ± 3	0.006
18:2(n-6)	1503 ± 45	1376 ± 32	0.006
20:0	137 ± 1	17 ± 1	NS
18:3(n-3)	23 ± 3	24 ± 1	NS
20:3(n-6)	58 ± 2	32 ± 1	0.0001
22:0	38 ± 1	42 ± 1	0.01
20:4(n-6)	268 ± 10	255 ± 8	NS
20:5(n-3)	38 ± 2	318 ± 14	0.0001
24:0	32 ± 1	37 ± 1	0.009
22:5(n-3)	14 ± 1	31 ± 1	0.0001
22:6(n-3)	75 ± 3	172 ± 6	0.003
Σ(n-6)	1838 ± 52	1668 ± 36	0.003
Σ(n-3)	150 ± 7	0546 ± 19	0.0001
ΣSFA	1229 ± 32	1311 ± 22	0.02
ΣMUFA	880 ± 24	814 ± 16	0.01
ΣPUFA	1998 ± 55	2213 ± 46	0.001
PI[b]	3061 ± 80	4514 ± 110	0.0001
		Weight percent	
ΣSFA	28.00 ± 0.22	28.10 ± 0.22	NS
ΣMUFA	21.17 ± 0.32	18.42 ± 0.24	0.0001
ΣPUFA	48.20 ± 0.46	50.90 ± 0.38	0.0001
Σ(n-6)	44.23 ± 0.53	37.59 ± 0.45	0.0001
Σ(n-3)	3.96 ± 0.15	13.32 ± 0.33	0.0001

aData are given as means ± SEM; 'PI = Peroxidizability index = (dienoic fatty acids x 1) + (trienoic fatty acids x 2) + (tetraenoic fatty acids x 3) + (pentaenoic fatty acids x 4) + (hexaenoic fatty acids x 5).

were expressed as weight percent of total fatty acids, although the changes in the polyunsaturated and monounsaturated fatty acids prevailed, there were no significant differences in the content of saturated fatty acids.

The effect of the fish oil supplement on the oxidative susceptibility of LDL and its content of α-tocopherol susceptibility of LDL and its content of α-tocopherol are shown in Figure 2. These data have been reported previously[19] and are given here for completeness of the argument. The lag time decreased approximately 18 min. The rate decreased from 1.97 ± 0.08 nmol conjugated dienes/min/mg LDL to 1.61 ± 0.04 nmol conjugated dienes/min/mg LDL, a decrease of approximately 19%. There was no

significant difference in the maximum production of conjugated dienes. The α-tocopherol content increased from 10.1 ± 0.42 nmol/mg LDL protein to 11.5 ± 0.4 nmol/mg LDL protein, an increase of approximately 11 % (P = 0.005).

In an effort to determine which of the fatty acids best predicted the three indices of oxidative susceptibility measured, stepwise multiple regression equations were obtained for each of them before and after supplementation with fish oil using five fatty acids; oleic acid, linoleic acid, arachidonic acid, EPA and DHA as independent variables. Because it is generally argued that the α-tocopherol content of the LDL particles influences its oxidative susceptibility, the concentration of α-tocopherol in LDL susceptibility, the concentration of α-tocopherol in LDL was also used as an independent predictor variable. The equations which resulted and appropriate statistics are given in Table 3. There were no significant interactions for any of the independent terms in any of the equations. As an index of relative prediction error of each of the independent variables on the dependent variable, the standardized regression coefficients were calculated. These terms are given in Table 4.

The equations given in Table 3 range in their predictive value from 21% for the initial maximum production of conjugated dienes to 41% for the initial rate and final production of conjugated dienes. The independent variable that most often contributed to predicting measures of oxidative susceptibility was linoleic acid. It was the major contributor to the variability of the initial rate, final rate and final production of conjugated dienes. It was the second most important predictor for all the other dependent variables. In each instance its appearance in the equation represented an increase in oxidative susceptibility, i.e a decrease in the lag time and an increase in the rate and production of conjugated dienes. The fatty acid that appeared in the equations with second greatest frequency was arachidonic acid. It, too, represented a detrimental effect on the oxidative susceptibility of LDL. Oleic acid contributed to initial lag time and rate and protected LDL from oxidation in that it decreased rate and lengthened lag time. EPA, surprisingly, decreased oxidation rate suggesting a protection from oxidation.

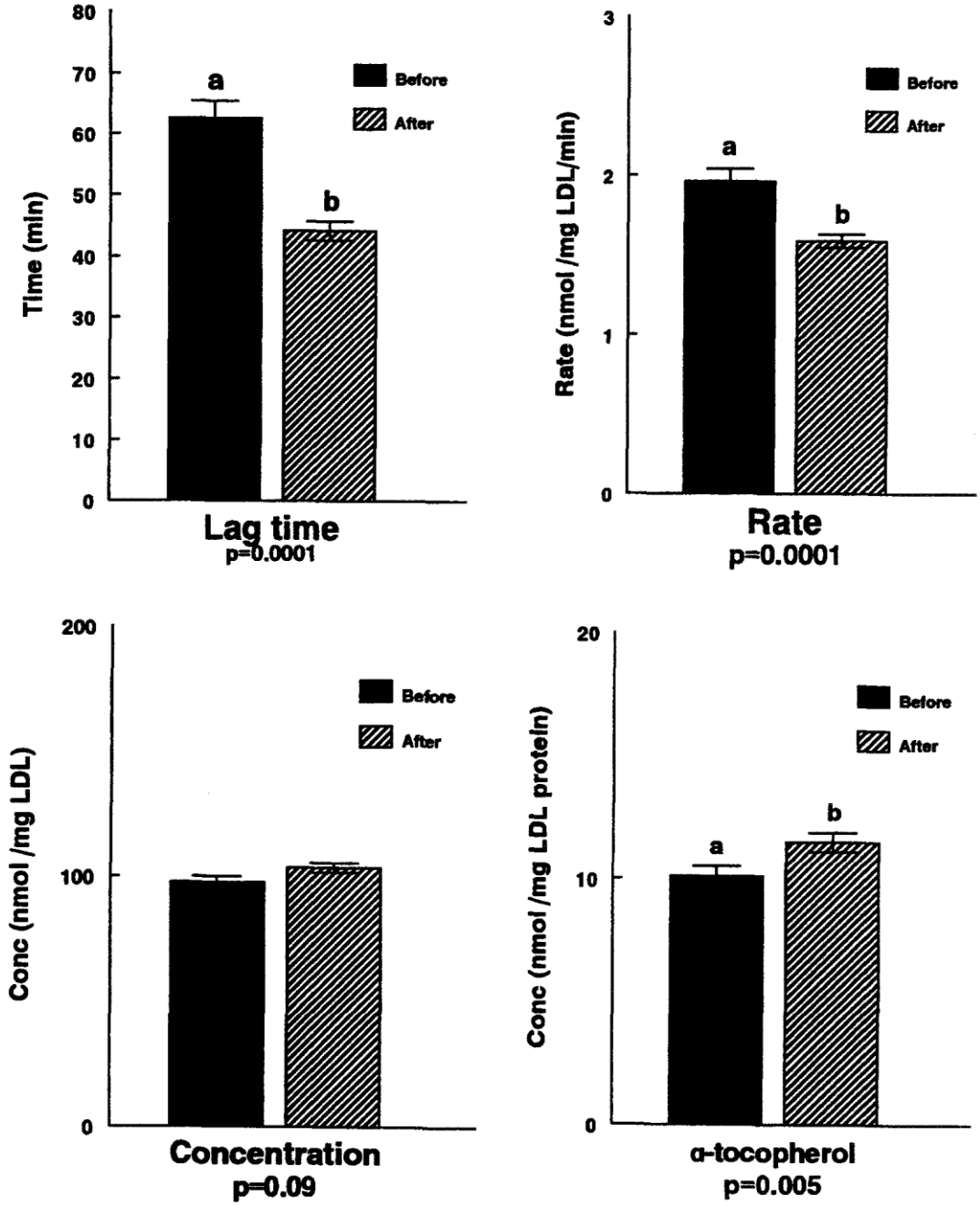

Fig. 2 The oxidizability, lag time (A), rate of formation of conjugated dienes (B), maximum production of conjugated dienes (C) and concentration of α-tocopherol (D) in LDL from postmenopausal women before and after supplementation with fish oil. The bars represent the group means. Bars with different letters above them Fig. 2 The oxidizability, lag time (A), rate of formation of conjugated dienes (B), maximum production of conjugated dienes (C) and concentration of α-tocopherol (D) in LDL from postmenopausal women before and after supplementation with fish oil. The bars represent the group means. Bars with different letters above them are significantly different.

α-Tocopherol appeared only once in any of the equations, and was associated with an increase in the amount of conjugated dienes produced.

Pearson correlation coefficients were calculated for the indices of oxidative susceptibility and the composition of the LDL particle (Table 5). Before the supplement of fish oil, both the rate and maximum production of conjugated dienes were significantly correlated with the cholesteryl ester and cholesterol to protein ratio of LDL, suggesting that the core lipids are mediating the oxidation. After the supplement of fish oil, the most significant correlation was that between phospholipids and the rate of oxidation. The correlation between free cholesterol and rate also became significant. These two correlations and the much

Table 3 Equations determined by stepwise multiple regression which best predict the dependent variables, lag time, rate and maximum production of conjugated dienes using the concentration of oleic acid, linoleic acid, arachidonic acid, EPA, DHA and α-tocopherol in LDL as independent variables

	R	P
Lag Time – before supplementation 44.3 + 93.8 [18:1 n-9]-19.4 [18:2 n-6]-406.5 [20:5 n-3]	0.54	0.005
Lag Time – after supplementation 31.9 + 7.95 [18:2 n-6]	0.26	0.10
Rate – before supplementation 1.10 – 1.97 [18:1 n-9]+1.00 [18:2 n-6]+2.41 [20:4 n-6)	0.64	0.0002
Rate – after supplementation 1.19 + 0.54 [18:2 n-6)-1.01 [20:5 n-3]	0.51	0.003
Diene Production – before supplementation 55.5 + 13.7 [18:2 n-6] + 80.3 [20:4 n-6]	0.46	0.003
Diene Production – after supplementation 34.1 + 1.89 [α-Tocopherol] + 23.2 [18:2 n-6] + 62.8 [20:4 n-6]	0.64	0.0003

Table 4 Standardized regression coefficients for each term in the equations determined by multiple regression to best predict the dependent variables associated with measures of oxidative susceptibility in LDL. The value below the coefficient is the *P* value for that term

	Dependent variables					
	Lag time		Rate		Dienes	
	Before	After	Before	After	Before	After
18:1 n9	0.67 (0.0007)	–	–0.48 (0.01)	–	–	–
18:2 n6	–0.34 (0.06)	–	0.59 (0.003)	0.44 (0.003)	0.26 0.11)	0.37 0.01)
20:4 n6	–	–	0.31 (0.03)	–	0.33 (0.04)	0.24 (0.10)
EPA	–0.32 (0.03)	–	–	–0.35 (0.02)	–	–
DHA	–	–	–	–	–	–
α-tocopherol	–	–	–	–	–	0.35 (0.009)

less significant correlation ($P = 0.06$) between the rate of oxidation and the ratio of cholesterol to protein suggest that after enrichment with EPA and DHA, the surface lipids mediate oxidation.

DISCUSSION

The purpose of this study was to explore the relationship between the oxidative susceptibility of LDL and its concentration of selected unsaturated fatty acids and a- tocopherol and its composition. It is generally thought that oxidation of LDL will be increased as the content of fatty acids containing doubly allylic hydrogen atoms

Table 5 Pearson's correlation coefficients for lipid composition and measures of oxidative susceptibility of LDL. The upper number at each entry is the coefficient while the lower number is its associated *P* value

	Lag time	Rate	Dienes
Before values			
Phospholipids	0.14	0.24	0.10
	(0.39)	(0.13)	(0.53)
Triglycerides	0.23	–0.27	0.13
	(0.16)	(0.09)	(0.42)
Free cholesterol	–0.07	0.26	0.08
	(0.64)	(0.10)	(0.61)
Cholesteryl ester	–0.007	0.47	0.38
	(0.91)	(0.002)	(0.01)
C/P[a]	–0.04	0.54	0.38
	(0.82)	(0.0003)	(0.01)
After values			
Phospholipids	–0.01	0.34	0.20
	(0.93)	(0.03)	(0.21)
Triglycerides	–0.01	–0.07	0.14
	(0.95)	(0.65)	(0.37)
Free cholesterol	0.07	0.33	0.30
	(0.65)	(0.05)	(0.07)
Cholesteryl ester	0.02	–0.07	–0.007
	(0.92)	(0.65)	(0.97)
C/P[a]	–0.09	0.31	0.19
	(0.58)	(0.06)	(0.26)

aThe ratio of cholesterol (free cholesterol plus cholesterol moiety of cholesteryl esters [0.59 x weight of cholesteryl esters] to protein.

increases, as has been observed in chemical systems.' In keeping with this conjecture, LDL obtained from subjects given substantial quantities of mono-unsaturated fatty acids are less prone to oxidize.[11-15] For instance, when oxidative susceptibility was measured in vitro by following the formation of conjugated dienes in LDL obtained from postmenopausal women with adult-onset diabetes on diets high in mono-unsaturated fatty acids, we measured a significantly longer lag time, slower rate of oxidation and lower production of conjugated dienes." This decrease in oxidative susceptibility was accompanied by an increase in the LDL content of oleic acid. The decreased susceptibility of the oleic acid-enriched LDL particle may contribute to the cardioprotective effect of the Mediterranean diet. On the other hand, when the LDL particle is enriched with EPA and DHA, the alteration that occurs to its oxidative susceptibility is less easily understood.

We,[25] as well as others,[24-29] have shown that oxidation of EPA/DHA-enriched LDL is characterized by both a shorter lag time and slower rate of oxidation than those particles that do not contain significant amounts of these fatty acids. The shorter lag time suggests that the presence of these fatty acids increases the oxidative susceptibility of the particle. Paradoxically, the slower rate of formation of conjugated dienes suggests a decrease in oxidative susceptibility.

In this paper using stepwise multiple regression analysis, we have shown that linoleic acid was a significant contributor to all measures of oxidative susceptibility both before and after EPA and DHA

were introduced into the LDL particle. The influence of linoleic acid over-rode that of any other fatty acid. The most obvious explanation for these effects may be the fact that linoleic acid is present in LDL in an amount far greater than any other fatty acid. It provides 36% and 31% of the total fatty acids or 74% and 60% of the PUFA in the EPA/DHAunenriched or enriched-LDL particle, respectively. Viewed on the molecular level, using factors of 22% for the protein content of LDL and 2.5 x 10^6 for its molecular weight,[38] the LDL particle before consumption of fish oil contained approximately 800 molecules of linoleic acid compared to 80 of n-3 fatty acids (linolenic acid, EPA, DPA and DHA). After the consumption of fish oil these values changed to 750 and 300, respectively. Of the n-3 fatty acids, about 175 of the molecules were EPA and 100 DHA.

There are two reasons to suspect that the contradictory effects produced on the oxidative susceptibility of LDL by EPA and DHA cannot be explained solely by its content of linoleic acid. First, while the rate changed by 19^0/o, the concentration changed only 8%. Second, since the ratio of n-3 fatty acid molecules to linoleic acid molecules in an LDL particle changed from 10:1 to 2.4:1, it is reasonable to argue that the n-3 fatty acids, now that they form a more substantive part of the particle, also contribute in a major way to its oxidation rate.

As has been said, the expected response was that LDL enriched with EPA and DHA would be more readily oxidized than LDL that did not contain these fatty acids. This expectation derives from the assumption that the oxidation of PUFA in LDL is explained by classical autoxidation kinetics[39] and proceeds through several steps. The initiation step is the abstraction of a hydrogen atom from a doubly allylic carbon atom to form a carbon-centered radical. This radical then undergoes molecular rearrangement to become a conjugated diene which then reacts with oxygen to give a conjugated diene peroxyl radical. The propagation step is the reaction of the peroxyl radical with another PUFA thus generating another carbon- centered radical. The regeneration of radicals continues until the reaction of the peroxyl radicals to yield non- radical products terminates this process. In classical autoxidation kinetics the oxidizability, a measure of the ease with which a substrate will undergo autoxidation, is determined by a function which contains the ratio of the rate constants for the propagation and termination steps.[16] Over 50 years ago, Holman and Elmer' showed that the rate of oxidation increased as the number of doubly allylic positions increased. Cosgrove et a1[16] refined their work using methyl esters of fatty acids in chloro- benzene and thermally labile azo compounds to control the rate of initiation. They determined that the oxidizability of DHA was five times that of linoleic acid. Recently, using free fatty acids in a more physiologically relevant system and measuring an aldehyde generated by lipid peroxidation using a highly sensitive gas chromatography—mass spectrometry method, Liu et a1[18] also found that the extent of oxidation increased as the number of doubly allylic positions increased.

An explanation of the slower rate of oxidation coupled to the shorter lag time observed in the EPA/DHA-enriched LDL may require deviation from this traditionally accepted model, i.e. in a biological system the rate of oxidation may not increase as do the number of allylic positions. Such conjecture is not without precedent. Cosgrove et al[16] noted that even though classical autoxidation kinetics explained the degradation of methyl esters, it did not explain that of triglycerides of linoleic acid. They found that at low concentrations the monoglycerides of linoleic acid oxidized more readily than the triglycerides. They speculated that this was a result of their aggregation. It has also been shown that phospholipids in homogeneous organic solution do not follow classical autoxidation kinetics [40] Again this was attributed to lipid aggregation. Liu et a1[18] found that the oxidation of EPA was consider-

ably slower than that which would be predicted by the number of doubly allylic positions. Bruna et al[41] reported that in aqueous solution the oxidation rates of EPA and DHA were 14% and 22^{0}/o lower, respectively than those of linoleic acid. Gutteridge[42] measured less MBA in an aqueous dispersion of DHA than arachidonic acid. Hornstra et a1[43] found that whereas human umbilical vein endothelial cells enriched with oleic acid were less susceptible to oxidation and those enriched with linoleic were more susceptible, the oxidative susceptibility of those enriched with EPA and DHA was between these two. Obviously, the matrix in which the oxidative studies are conducted is important to the conclusions that can be drawn. Like the aforementioned investigations, our study provides a precedent for arguing that the highly unsaturated fatty acids are not necessarily oxidized more rapidly than those with fewer double bonds in biological systems. In that event replacing linoleic acid in the LDL particle with EPA and DHA could actually slow the propagation rate, as we observed.

Our data suggest that the rate of oxidation of EPA/ DHA-enriched LDL may be mediated by its surface lipids while that of the unenriched particle is mediated by its core lipids. Mata et a1[44] analyzed the fatty acid content of LDL phospholipids, a surface lipid, and cholesterol esters, a core lipid. They found that DHA preferentially located in the surface phospholipids. Its presence at this site could explain the increased involvement of the surface lipids in the oxidation of LDL after EPA/DHA enrichment.

The functional significance of a slower rate of oxidation of the LDL particle is not known. In the microdomains of the artery wall where extensive oxidative modification of LDL is thought to occur, a slower rate of oxidation might limit the overall pathologic response to the oxidized LDL. Such results would offer another reason to explain the decreased risk of heart disease that occurs with the consumption of fish.[45]

As the conclusions drawn from this study about the role of long-chain, highly unsaturated fatty acids on the oxidative susceptibility of LDL are correlations rather than direct observations, the most serious pitfall in interpreting them is the tendency to over interpret. Collectively, these data suggest that the susceptibility of the LDL particle to oxidize is highly dependent not only on its fatty acid composition but also on the distribution of the fatty acids among the different lipid classes in its surface and core compartments. In particular, linoleic acid, either by its presence or its absence, is the fatty acid that is the most important to the oxidation of LDL. This is true even when the more highly unsaturated fatty acids are introduced into the particle. To fully understand the oxidative susceptibility of the LDL particle requires a thorough knowledge of its fatty acid content. We are currently in the process of conducting more direct observations, such as monitoring oxidation and measuring fatty acid content of LDL phospholipidsd cholesterol esters, to confirm the conclusions drawn from this study.

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