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Review

Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: A systematic review

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Abstract

Greater fish oil consumption has been associated with reduced CVD risk, although the mechanisms are unclear. Plant-source oil omega-3 fatty acids (ALA) have also been studied regarding their cardiovascular effect. We conducted a systematic review of randomized controlled trials that evaluated the effect of consumption of fish oil and ALA on commonly measured serum CVD risk factors, performing meta-analyses when appropriate. Combining 21 trials evaluating lipid outcomes, fish oil consumption resulted in a summary net change in triglycerides of −27 (95% CI −33, −20) mg/dL, in HDL cholesterol of +1.6 (95% CI +0.8, +2.3) mg/dL, and in LDL cholesterol of +6 (95% CI +3, +8) mg/dL. There was no effect of fish oil on total cholesterol. Across studies, higher fish oil dose and higher baseline levels were associated with greater reductions in serum triglycerides. Overall, the 27 fish oil trials evaluating Hgb A_{1c} or FBS found small non-significant net increases compared to control oils. Five studies of ALA were inconsistent in their effects on lipids, Hgb A_{1c} or FBS. Four studies investigating the effects of omega-3 fatty acids on hs-CRP were also inconsistent and non-significant. The evidence supports a dose-dependent beneficial effect of fish oil on serum triglycerides, particularly among people with more elevated levels. Fish oil consumption also modestly improves HDL cholesterol, increases LDL cholesterol levels, but does not appear to adversely affect glucose homeostasis. The evidence regarding the effects of omega-3 fatty acids on hs-CRP is inconclusive, as are data on ALA.

Keywords: Omega-3 fatty acid; Cardiovascular disease; Fish oil; Systematic review; Meta-analysis

Contents

Abbreviations: ALA, alpha linolenic acid (18:3 n-3); CI, confidence interval; CVD, cardiovascular disease; DHA, docosahexaenoic acid (22:6 n-3); EPA, eicosapentaenoic acid (20:5 n-3); FBS, fasting blood sugar; HDL, high density lipoprotein; Hgb A_{1c}, hemoglobin A_{1c}; hs-CRP, highly sensitive C-reactive protein; LDL, low density lipoprotein; VLDL, very low density lipoprotein

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1. Background

The relationship between dietary omega-3 fatty acids and risk of developing CVD began to emerge in the late 1970s [1–3]. Thereafter, a limited number of intervention trials reported lower rates of CVD mortality and sudden death, but not stroke, after supplementation with the long-chain omega-3 fatty acids EPA or DHA; however, the data on the shorter-chain omega-3 fatty acid ALA are far less certain [4]. Potential mechanisms for the cardioprotective effect of omega-3 fatty acids include anti-arrhythmic effects, anti-thrombotic effects, anti-inflammatory effects, lowered blood pressure, improved endothelial function, hypotriglyceridemic effects in hypertriglyceridemic individuals and retarded growth of atherosclerotic plaque [5,6].

EPA and DHA are commonly referred to as very long chain omega-3 fatty acids. The primary sources in the diet of humans are fish, especially dark fleshed fish, and, if consumed, fish oil supplements. ALA is a plant form of omega-3 fatty acid. The major dietary sources in the human diet are soybean and canola oils. In addition, the amounts in flaxseeds and walnuts and their respective oils are high and when consumed can provide relatively high levels of intake. The rate of conversion by humans of ALA to EPA is low, with estimates ranging from 0.2% to 15%, as is the conversion of EPA to DHA [7]. However, high intakes of ALA have been reported to result in significant increases in very long chain omega-3 fatty acids in various body compartments [8,9].

To better understand how both fish oil (and dietary fish) and ALA consumption exert their effects on clinical CVD, we systematically reviewed the literature on various cardiovascular disease risk factors and intermediate markers [10]. We have previously reported on coronary restenosis, intima-media thickness, and exercise tolerance [11]. Here we report on serum markers of CVD risk, including blood lipid and lipoprotein levels (total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride), a measure of inflammation (CRP) and measures of glucose homeostasis (FBS and Hgb A_{1c}).

2. Methods

2.1. Literature search and eligibility criteria

Details of the systematic review and statistical methods have been reported [11]. Briefly, we conducted a systematic review of the English-language literature on omega-3 fatty acids and cardiovascular disease in Medline, Embase, Cochrane Central Register of Controlled Trials, Biological Abstracts, and Commonwealth Agricultural Bureau databases through April 2003. Search terms included the specific omega-3 fatty acids, fish and other marine oils, and omega-3 fatty acid-rich plant oils. We also reviewed additional publications found by domain experts.

We evaluated randomized controlled trials of omega-3 fatty acid interventions, as either supplements or dietary components. The omega-3 fatty acids of particular interest with respect to cardiovascular disease include EPA, DHA, and ALA. To qualify, studies could include only subjects who were either generally healthy; had diabetes, hypertension, or dyslipidemia; or had cardiovascular disease. We excluded studies of omega-3 fatty acid supplementation of >6 g/day or of <4 weeks' duration, and studies that did not quantify fatty acid supplementation or fish amounts.

A large number of studies met the minimum eligibility criteria for the various CVD outcomes we investigated (327 articles), we therefore limited eligibility of studies of lipids, FBS and Hgb A_{1c} to the larger randomized trials, as summarized in Table 1. We determined minimum study size based on a goal of approximately 20 studies per outcome analyzed. We accepted all studies of CRP.

2.2. Quantitative analysis

For analysis, we evaluated the relative change of the outcome compared to placebo – the net difference between the within-treatment effect and the within-placebo effect. For fish oil studies (including dietary fish) of lipids and glucose homeostasis we performed meta-analysis with the DerSimonian and Laird random effects model, which assigns a weight to each study that is based on both the within study

Table 1
Numbers and eligibility criteria for studies of omega-3 fatty acids and cardiovascular risk factors

CVD risk factor	Total studies meeting minimum eligibility criteria	Total randomized studies	Minimum number of subjects consuming omega-3 fatty acids[a]		Analyzed studies
			Randomized controlled trials	Crossover studies	
Lipid profile	182[b]	108	≥60	≥40	25
Hemoglobin A_{1c}	32	22	≥10	≥10	18
Blood sugar, fasting	57	34	≥25	≥15	17
C reactive protein	5	4	All	All	5

[a] Minimum number of subjects refers to all subjects in study consuming omega-3 fatty acids. Specific groups (such as men vs. women, or different doses of fatty acids) may have smaller numbers of subjects.
[b] ≥20 subjects consuming omega-3 fatty acids.

variance and the between-study heterogeneity [12]. When necessary, we estimated the standard error of the net change from reported variance data. We contacted authors for additional data when variance data were missing. For two studies, we made conservative estimates of variance data from other sources, as described in the legend to Fig. 1. Studies of fish oil (or dietary fish) and of ALA were analyzed separately.

We also performed multivariate linear regression analyses (meta-regression) to evaluate the effect of fish oil. In meta-regression, each data point represents the mean effect from each study, instead of data from an individual as in traditional regression. We used the random-effects regression model described by Berkey et al. and Morris [13,14]. We evaluated the effects of fish oil dose, baseline value, study duration, change of outcome value in the control group, and study quality (using dummy variables) on net change levels.

2.3. Quality and applicability assessment

All randomized trials were assessed for both study quality and applicability. Methodological quality refers to the design, conduct, and reporting of the clinical study. Because studies with a variety of design types were evaluated, a three-level classification of study quality and applicability was used as described previously [15]. The quality and applicability classifications, along with the target populations, are described in Table 2.

3. Results

The literature search for all studies of omega-3 fatty acids and cardiovascular disease related conditions yielded 7464 citations. We retrieved and reviewed 807 articles to analyze cardiovascular events, risk factors or intermediate markers. Of these we analyzed 123 articles that reported about 23 different cardiovascular disease risk factors and intermediate markers. Here, we discuss the 52 randomized controlled trials that reported data on the effect of omega-3 fatty acid consumption on the predetermined serum markers of CVD risk.

3.1. Characteristics of all evaluated studies

Fish or other marine oils (EPA and DHA as supplements, dietary fish, or oil spreads) were evaluated by 47 of 52 studies; whereas, six evaluated plant oils (ALA as supplements, vegetable oils, nuts or oil spreads), one of which also evaluated fish oil. EPA + DHA doses ranged from 0.045 to 5.9 g/day, fish diets ranged from 0.9 to 3.8 servings per week, ALA doses ranged from 1.8 g to about 5 g/day. No study examined possible correlations between the effect of omega-3 fatty acid consumption on serum markers and the effect on clinical or cardiovascular disease.

3.2. Lipid profile

We reviewed the 25 largest randomized trials that contained data on omega-3 fatty acid intake and plasma lipids (Table 2) [16–40]. Nineteen studies evaluated fish oil, three fish (or Mediterranean) diets, one various combinations of fish oil and diet, and three plant oils. These studies represented about 8000 subjects. Approximately, two-thirds of the subjects were included in the GISSI study of fish oil supplements [34]. The studies were generally of fair quality, with moderate to broad applicability.

3.2.1. Fish and fish oil

Twenty-one studies included 37 individual study arms of fish oil or fish diet that evaluated any of the lipid profile components (Fig. 1 and Table 2) [16–36]. For all four-lipid profile components, studies were heterogeneous in their results ($P < 0.0001$) with wide ranges of net effects—from 6% to 60% net improvements to 6–14% net worsening. However, in the majority of studies, the net effects on total, LDL, and HDL cholesterol levels were small (<5%). In contrast, most studies of triglycerides found at least a 15% net reduction with fish oil consumption.

Across the studies, random effects model meta-analyses found a significant net improvement of triglycerides with fish oil consumption of −27 (95% CI −33, −20) mg/dL, and of HDL cholesterol of +1.6 (95% CI +0.8, +2.3) mg/dL, and a significant net worsening of LDL cholesterol of +6 (95% CI +3, +8) mg/dL. There was no effect on total cholesterol. The

Table 2
Summary of evaluated evidence for the effect of omega-3 fatty acids on serum markers of CVD risk

Outcome	Omega-3 fatty acid	Dose range (g/day)	No. of randomized studies	No. of subjects[a]	Quality[b]			Applicability[c]			Range of net effects (net % change)	Summary estimate of net effect (95% CI) *P*-value	Explanation for heterogeneity (95% CI)
					A	B	C	I	II	III			
Total cholesterol (mg/dL)	Fish oils	0.045–5.4	19	7853	3	11	5	8	10	1	−19, +21 (−6%, +9%)	0 (–1, +2) NS	Meta-regression: baseline −0.08[d] (−0.15, −0.02)
	ALA	∼1.8 to ∼5	5[e]	1089	1	0	4	1	1	3	−1, +13 (−0.4%, +4%)		None found
LDL (mg/dL)	Fish oils	0.045–5.4	13	6969	3	8	2	4	9	0	−5, +21 (−3%, +14%)	+6 (+3, +8) $P = 0.0006$	None found
	ALA	∼1.8–4.5	3[e]	700	1	0	2	1	1	1	−2, +3 (−1%, +2%)		None found
HDL (mg/dL)	Fish oils	0.045–5.4	17	7353	3	10	4	7	10	0	−3.5, +5.4 (−7%, +12%)	+1.6 (+0.8, +2.3) $P = 0.0003$	Meta-regression: control change −0.38[f] (−0.53, −0.23)
	ALA	∼1.8–4.5	3[e]	700	1	0	2	1	1	1	−1, +1 (−2%, +2%)		None found
Triglycerides (mg/dL)	Fish oils	∼0.1[g]–5.4	17	7803	3	13	1	6	10	1	−80, +6 (−60%, +6%)	−27 (−33, −20) $P < 0.0001$	Meta-regression: baseline −0.16[d] (−0.24, −0.07)[h]; dose −7.8[i] (−10.9, −4.7)[h]
	ALA	∼1.8 to 4.5	3[e]	700	1	0	2	1	1	1	−19, +23 (−10%, +16%)		None found
Fasting blood sugar (mg/dL)	Fish oils	0.6–5.2	17	1427	4	10	3	6	10	1	−29, +25 (−16%, +19%)	+3 (−0.2, +6) $P = 0.09$	Meta-regression: baseline +3.2[d] (+1.5, +4.9); dose +3.1[i] (+0.8, +5.4)
	ALA	4.5–5.9	2	52	1	0	1	1	0	1	−5 (−5%)		None found
Hemoglobin A_{1c} (%)	Fish oils	0.6–4.6	18	578	5	11	2	3	14	1	−0.8, +1.0 (−9%, +13%)	+0.1 (−0.01, +0.2) $P = 0.09$	Homogeneous
	ALA		0										
C-reactive protein (mg/L)	Fish oils	1.6–5.9	3[j]	73	0	2	1	1	1	1	−0.2[k], +1.7 (−14%[k], +35%)		None found
	ALA	2.5% kcal	1	18	0	0	1	1	0	0	+0.1 (+22%)		None found

95% CI = 95% confidence interval.

[a] Receiving omega-3 fatty acids.

[b] A = Least bias; study mostly adheres to the commonly held concepts of good quality, including: formal randomized study; clear description of the population, setting, interventions and comparison groups; appropriate measurement of outcomes; appropriate statistical and analytic methods and reporting; no reporting errors; <20% dropout; clear reporting of dropouts; and no obvious bias. B = Susceptible to some bias; study has some deficiencies but none likely to cause major bias or may be missing information making assessment of the limitations and potential problems difficult. C = Significant bias; study has serious errors in design, analysis, or reporting or may have large amount of missing information or discrepancies in reporting.

[c] I = Sample is representative of the population of interest; sufficiently large to cover both sexes, a wide age range, and other important features of the target population including baseline dietary intake broadly similar to that of the US population. II = Sample is representative of a relevant sub-group of the target population, but not the entire population. III = Sample is representative of a narrow subgroup of subjects only, and not well applicable to other subgroups.

[d] Change in net change (mg/dL) per baseline value (mg/dL). See text for interpretation.

[e] One study of an Indo-Mediterranean diet reported total omega-3 fatty acids.

[f] Change in net change (mg/dL) per change in lipid level (mg/dL) in the control group. See text for interpretation.

[g] 0.9 fish servings per week.

[h] Baseline and dose interact ($P = 0.005$), such that at low baseline values increased dose has smaller effect (e.g., net change from −2 to −14 mg/dL when baseline = 60 mg/dL across the dosage range of 0.21–5.4 g/day) and at high baseline values increased dose has larger effect (e.g., net change from −8 to −108 mg/dL when baseline = 108 mg/dL across the dosage range of 0.21–5.4 g/day).

[i] Change in net change (mg/dL) per omega-3 fatty acid dose (g/day). See text for interpretation.

[j] One study of a Mediterranean diet reported total omega-3 fatty acids.

[k] Based on median values.

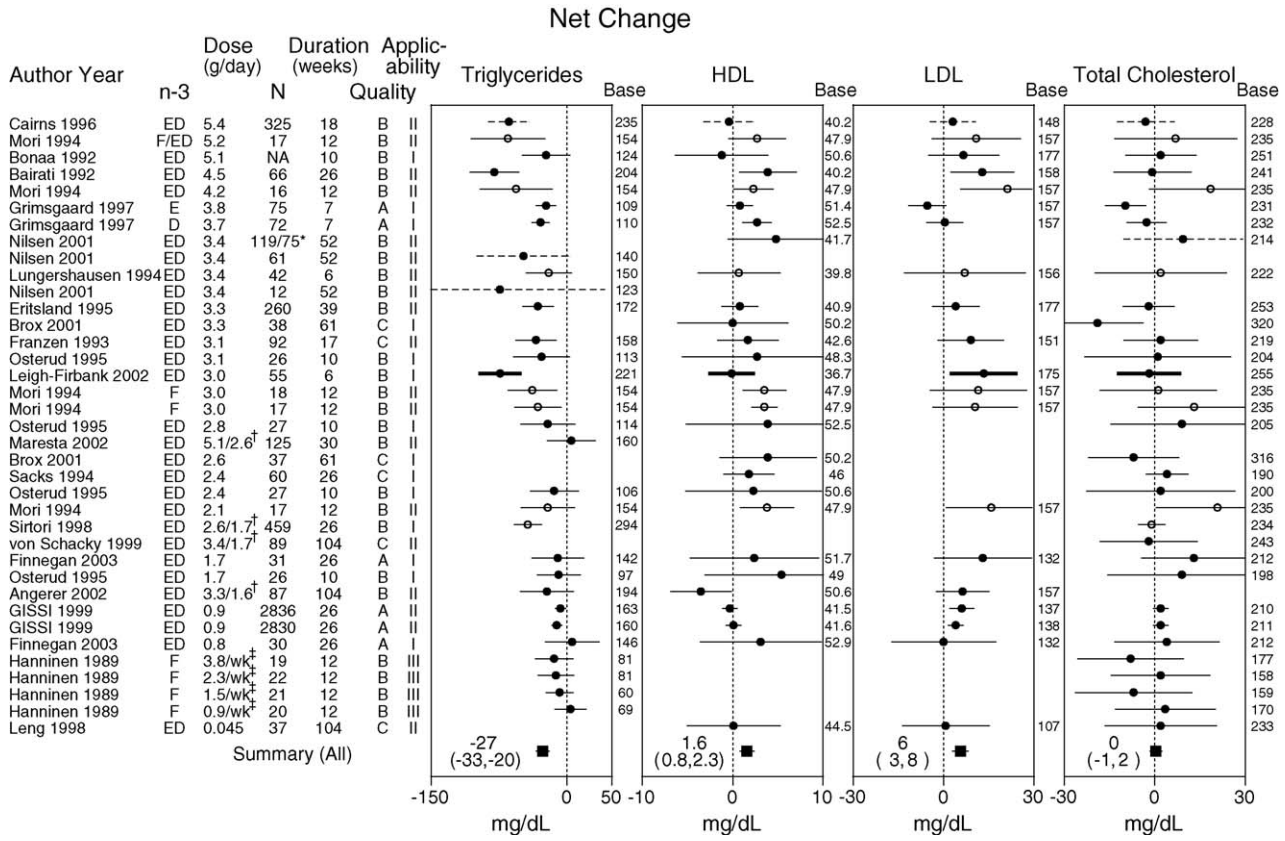

Fig. 1. Meta-analysis of randomized controlled trials of the effect of fish oil on lipid values. The point estimates of the net changes (change in fish oil arm minus change in control arm) and the corresponding 95% CI for individual studies are indicated by circles and bars. The random effects model summary results are indicated by squares and bars near the bottom. Studies are arranged by dose of fish oil. Source of omega-3 fatty acid (n-3) – D = DHA, E = EPA, ED = EPA + DHA, F = Fish, number of subjects consuming fish oil (*N*), duration, study quality and applicability (see Table 2), and baseline values for each lipid are shown. For individual studies, black circles indicate that data came from text or tables; open circles indicate that data were estimated from graphs; thick solid 95% CI indicates that the standard error (S.E.) of the net change was reported; thin solid 95% CI indicates that S.E. of net change estimated from either baseline and final S.E.s or S.E.s of cohort changes; dashed 95% CI indicates that S.E. of net change estimated from other sources. For Cairns [16], no S.E. data were provided, so the standard deviations (S.D.) were assumed to be the same as the largest S.D.s among the other studies. For Nilsen [21], S.E. for triglycerides was estimated based on S.D. for full sample of 120 subjects, and S.E. for total cholesterol was assumed to be the same as the S.E. of the baseline data. (*) $N = 119$ for HDL and 75 for total cholesterol. (†) Initial dose given for 2–3 months, followed by lower dose for remainder of studies. (‡) Servings of fish per week.

effects of fish oil on triglycerides, HDL and LDL cholesterol were all highly significant ($P < 0.001$). Despite the heterogeneity across studies, the meta-analysis results did not substantially (or significantly) change either with removal of the GISSI study – which contributed both the majority of subjects and the majority of weight in the meta-analyses – or with removal of outlier studies.

To further examine the study heterogeneity, meta-regression was performed (Table 2). Across studies, there were significant, independent associations between the effect of fish oil consumption on triglyceride levels and both the dose of fish oil used and the baseline triglyceride levels. Across studies, each increase in fish oil dose of 1 g/day was associated with a decrease in triglycerides of approximately 8 mg/dL. Likewise, each 10 mg/dL increase in the mean baseline triglyceride level was associated with an additional 1.6 mg/dL decrease in triglycerides after fish oil consumption. However, fish oil dose and baseline triglyceride levels interacted with each other, such that in studies with low baseline triglycerides (e.g., 60 mg/dL) higher fish oil dose was predicted to have a small effect (e.g., −2 mg/dL per additional g fish oil), while in studies with high baseline triglycerides (e.g., 294 mg/dL) higher fish oil dose was predicted to have a much larger effect (e.g., −19 mg/dL per additional g fish oil). Study duration was not associated with treatment effect between 4 weeks and 2 years suggesting that once the maximal effect was achieved it was maintained throughout the intervention period. The control rate – the change in triglyceride levels in the control group – and study quality were also not associated with treatment effect.

The four studies that compared different fish oil doses [17,26,33,35] similarly found that the greatest net decreases in triglycerides occurred among subjects consuming the highest doses of fish oil; although no study reported on the statistical significance of this effect. One study likewise found larger effects of fish oil among subjects in successively higher

quartiles of baseline triglyceride levels; although, again no statistical analysis was reported [20]. The effect of duration of fish oil consumptions was inconsistent among four studies that reported outcomes at different time points [21,30,33,35].

Evaluation of the studies that reported HDL cholesterol suggested that interventions in which the subjects in the control arms had a greater (positive) change in their HDL levels from baseline reported a smaller net increase in HDL in the experimental group after fish oil consumption. This possibly implies that fish oil may be most effective at raising (or stabilizing) HDL levels in people whose HDL levels would otherwise decrease with time. Of note, in the GISSI study, the mean HDL level in the control arms rose by 6 mg/dL and the net effect on HDL was smaller than the meta-analysis average across studies (see Fig. 1).

Although across studies fish oil consumption had no significant effect on total cholesterol, baseline total cholesterol was associated with treatment effect, such that each 10 mg/dL increase in the mean baseline total cholesterol was associated with an additional 0.8 mg/dL decrease after fish oil consumption. None of the tested outcomes were associated with the effect of fish oil consumption on LDL cholesterol levels. Individual studies generally confirmed that lack of association of either fish oil dose or duration of fish oil consumption and HDL cholesterol effect [17,21,24,26,29,33,36], LDL effect [17,33,36], or total cholesterol [17,21,24,26,29,30,33,35,36].

Further subgroup analyses and meta-regressions failed to elicit other sources of heterogeneity among the studies. Qualitative review of the studies, which also included specific evaluations of age and sex also failed to uncover an explanation for the large range of net effects seen across studies.

An additional set of meta-analyses were performed to investigate at what point the published randomized trial data were sufficient to yield the same results as were found by the current complete analyses. These cumulative meta-analyses sequentially add studies based on year of publication [41]. Regarding the effect of fish oil on triglycerides, by 1992, after the publication of three studies, meta-analysis revealed a statistically significant improvement ($P < 0.05$). The estimate of the effect size stabilized at −28 mg/dL in 1994 with six studies. Also by 1994, with the publication of six studies, the effect of fish oil on HDL cholesterol was both statistically significant and stable. The effect on LDL cholesterol was statistically significant after publication of the earliest two studies we reviewed in 1992; however, the estimate of the effect size was larger at that time (a net increase of 10 mg/dL) and did not stabilize until the publication of eight studies by 1997. Notably, all cumulative meta-analyses were statistically significant and stable prior to the publication of the GISSI study, with over 5000 subjects consuming fish oil, in 1999.

3.2.2. ALA

Five studies reported on the effect of ALA consumption on lipids (Table 3) [33,37–40]. The studies were mostly of poor quality, but broad applicability. Three studies used plant

Table 3
The effect of ALA on lipids and measures of glucose homeostasis in individual studies

Study	ALA source	Dose (g/day)	No. of subjects[a]	Duration (weeks)	Quality[b]	Applicability[b]	Total cholesterol (mg/dL)			LDL (mg/dL)			HDL (mg/dL)			Triglycerides (mg/dL)			FBS (mg/dL)		
							Base	Net change	*P*-value	Base	Net change	*P*-value	Base	Net change	*P*-value	Base	Net change	*P*-value	Base	Net change	*P*-value
Natvig et al. [37]	Linseed oil	~5	289	26	C	III	246	+1	NS												
Borchgrevink et al. [38]	Linseed oil	~5	100	~37	C	III	289	+13	nd												
Finnegan et al. [33]	Rapeseed / Linseed margarine	4.5	30	26	A	I	217	+2	NS	137	−2	NS	50	+1	NS	147	+23	NS	99	−5	NS
Singh et al. [39]	Indo-Mediterranean diet	1.8	499	104	C[c]	III	221	−20	[d]	141	−19	[d]	45	+2	[d]	163	−22	[d]	108	−5	[d]
de Lorgeril et al. [40]	Mediterranean diet/canola margarine	0.8% kcal	171	104	C	II	240	−1	NS	175	+3	NS	45	−1	NS	190	−19	NS			

95% CI = 95% confidence interval; nd = no data; NS = non-significant.
[a] Receiving omega-3 fatty acids.
[b] See Table 2.
[c] See Refs. [42,43].
[d] $P = 0.0001$.

oil supplements or margarine with 4.5 to about 5 g ALA; two studies used variations on the "Mediterranean diet" with approximately 2 g/day of omega-3 fatty acids. The only study to find significant improvements in all lipids, by Singh et al., was a problematic study [39]. Issues related to this study's reliability have been examined by several bodies [42,43]. The remaining studies generally found small effects (≤2 mg/dL net change) on total cholesterol, LDL, and HDL. An older study reported a 13 mg/dL (4%) net increase in total cholesterol, but no statistical analysis was performed. The remaining two studies that reported triglyceride effects both found non-significant changes of at least 10%, but in opposite directions.

3.3. Glucose homeostasis

We reviewed the 28 largest randomized trials that contained data on omega-3 fatty acid intake and either Hgb A_{1c} or FBS (Table 2) [23,27,30,33,39,44–66]. Of these, 24 evaluated fish oil, three fish (or "Mediterranean") diets, and two plant oils (one evaluated both fish oil and plant oil). These studies represented about 1700 subjects. The studies were generally of fair to good quality, with moderate applicability.

3.3.1. Fish and fish oil

Twenty-seven studies included 35 individual study arms of fish oil or fish diet that evaluated either Hgb A_{1c} or FBS (Fig. 2 and Table 2). Among the studies of FBS there was a wide range of net effects found with fish oil consumption, from 29 mg/dL net reduction to 25 mg/dL net increase. However, in the majority of studies, the net effects were small (<5%). With the exception of two outlier studies [60,65], the net effect of fish oil on Hgb A_{1c} was small (<5%, between −0.4% and 0.4%).

Across the studies, random effects model meta-analyses found a non-significant net increase in FBS with fish oil consumption of +3 (95% CI −0.2, +6) mg/dL and a similarly non-significant net increase in Hgb A_{1c} of +0.1% (95% CI −0.01%, +0.2%). Removal of outlier studies from meta-analysis did not substantially affect the results.

Meta-regression (Table 2) found significant associations across studies between both baseline FBS and fish oil dose and the net effect of fish oil on FBS. Across studies, each increase in fish oil dose of 1 g/day was associated with an increase in FBS of approximately 3 mg/dL. Likewise, each 10 mg/dL increase in the mean baseline FBS level was associated with an additional 3 mg/dL increase in FBS after fish

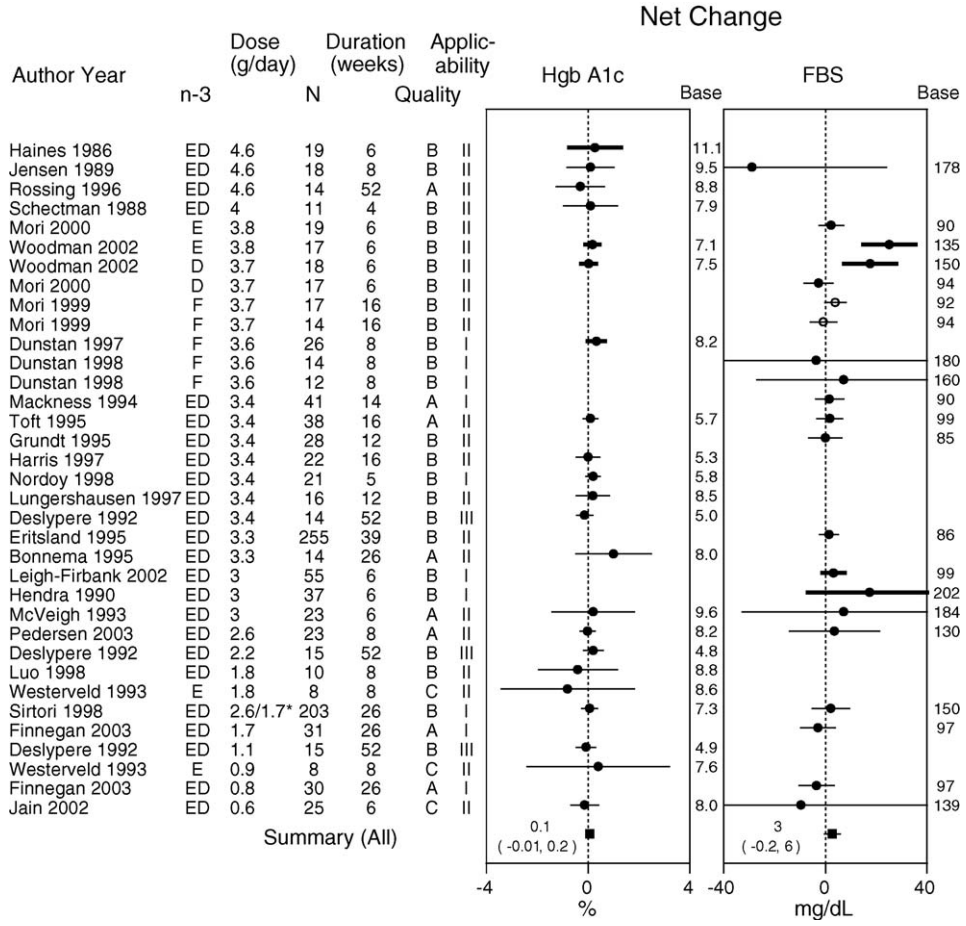

Fig. 2. Meta-analysis of randomized controlled trials of the effect of fish oil on Hgb A_{1c} and FBS (see Fig. 1). (*) Initial dose given for 2 months, followed by lower dose for remainder of study.

Table 4
The effect of omega-3 fatty acids on hs-CRP in individual studies

Study	Omega-3 fatty acid	Dose (g/day)	No. of subjects[a]	Duration (weeks)	Quality[b]	Applicability[b]	Base (mg/L)	Net change (mg/L)	*P*-value
Madsen et al. [67]	Fish oils	5.9 1.7	20 20	12	B	I	[1.07][c] [0.69][c]	[−0.15][d] [+0.02][d]	NS NS
Chan et al. [68]	Fish oils	3.4	12	6	B	II	2.11[e]	+0.05[f]	NS
Mezzano et al. [69]	Total[g]	1.6	21	13	C	III	4.9	+1.7	NS
Junker et al. [70]	ALA	2.5% kcal	18	4	C	I	[0.5][d]	[+0.11][e]	NS

95% CI = 95% confidence interval.
[a] Receiving omega-3 fatty acids.
[b] See Table 2.
[c] Median.
[d] Calculated value from median values.
[e] Geometric mean.
[f] Calculated value from geometric means.
[g] Mediterranean diet. Total omega-3 fatty acids reported.

oil consumption. Dose and baseline levels did not interact with each other; the effects of dose were the same at high and low baseline FBS, and vice versa. The studies of Hgb A_{1c} were statistically homogeneous, implying a lack of variation in findings across studies.

Seven of the 15 studies of FBS, and 14 of 18 Hgb A_{1c} studies included only subjects with diabetes. The remaining studies included subjects with either dyslipidemia or cardiovascular disease (some of whom may also have had diabetes). There were no clear difference in effect of fish oil on either FBS or Hgb A_{1c} based on the eligibility criteria of the studies. Only one FBS study and two Hgb A_{1c} studies compared different doses of fish oil and found no differences in effect [33,59,65]. The studies that measured outcomes at different time points also found no significant differences in net effects [33,44,46,61].

3.3.2. ALA

Two studies reported on the effect of either plant oil margarine or an "Indo-Mediterranean diet" on FBS; no ALA study evaluated Hgb A_{1c}. As with lipids, Singh et al. reported a highly significant improvement in FBS, though the validity of these data are in question [39]. In a good quality study, Finnegan et al. found a similar, though non-significant effect [33].

3.4. hs-CRP

Four prospective studies evaluated the effect of either fish oil or ALA on hs-CRP (Table 4) [67–70]. Two studies evaluated fish oil supplements of various doses, one a "Mediterranean diet", and one an ALA oil diet. Studies were of fair or poor quality with a range of applicability. There was a wide range of mean or median baseline hs-CRP levels across studies and a wide range of calculated net changes in hs-CRP. However, all studies found that there was no significant change in hs-CRP level with omega-3 fatty acid consumption.

4. Discussion

Although the relationship between dietary fish and fish oil and CVD risk has been known for some time, the relationship between omega-3 fatty acids and surrogate circulating markers of CVD risk has been less clear. This area is of interest in light of our previous observations that in randomized studies, compared to placebo, the summary risk ratio of coronary artery restenosis was in favor of fish oil is (0.87 [95% CI 0.73, 1.05]) [11]. However, the data from prospective and cross-sectional studies on carotid IMT were inconsistent. Furthermore, small non-significant improvements in exercise capacity have been reported after fish oil supplementation. There were insufficient data on which to draw conclusions about the effect of ALA on these outcomes. A previous systematic review with meta-regression concluded that increased consumption of fish oil resulted in, on average, a 2 mmHg reduction in both systolic and diastolic blood pressure [5]. Numerous other factors related to CVD, including markers of thrombosis and endothelial function have been evaluated by a small number of studies, but these remain inconclusive [6,10].

The most direct effect of increased intake of omega-3 fatty acids is an increase in the relative proportion of these fatty acids throughout the body, most thoroughly documented in serum, platelet and red blood cell phospholipids, which are the most accessible tissues for testing [10,17,20,32]. However, whether this change in phospholipids is a mediator of CVD is unknown.

The results of this review support the conclusion that the major and most consistent effect of relatively high doses of dietary omega-3 fatty acids on plasma markers of CVD risk was that fish oil consumption decreases triglyceride levels. This outcome was dose dependent and influenced by the baseline plasma triglyceride level of the study subjects. Prior work has suggested that this effect is in part attributable to a decrease in the hepatic production of triglyceride rich particles (VLDL, the lipoprotein responsible for transporting

triglycerides for subsequent delipidation by lipoprotein and hepatic lipases by peripheral tissue and the liver, respectively) and to an increase in fractional clearance rates [71–73]. Additionally, there is some evidence that omega-3 fatty acids increase the conversion rate of VLDL to LDL, similar to fibrate drugs [74].

In both cases, modest decreases in the levels of triglycerides are frequently accompanied by increases in the level of LDL cholesterol. The summary estimate of the change in triglyceride levels with increased fish oil consumption was 27 mg/dL. On an individual basis it is difficult to predict the effect of this change on clinical outcomes. However, triglyceride levels ≥150 mg/dL is one of the components when classifying individuals with metabolic syndrome. Therefore, a change in triglyceride levels as observed with increased fish oil intake could potentially result in a reclassification with regard to metabolic syndrome. Concomitant with lower triglyceride levels, increased fish oils resulted in modestly higher LDL cholesterol (6 mg/dL) and HDL cholesterol levels (1.6 mg/dL), an effect that is consistent with other interventions that reduced triglyceride levels [75]. Since the magnitude of the effect of raising HDL cholesterol levels on CVD is still unclear, and there are no data on the effect of raising both LDL and HDL cholesterol levels, the clinical significance of these changes in lipoprotein levels remains unclear [76]. However, given their modest magnitude, they are unlikely to have a large independent effect on CHD risk.

Concern has been raised that fish oils may worsen glycemic control in diabetic subjects [77,78], although this concern is by no means universal [79,80]. The results of our assessment indicate that within the doses of fish oil provided to the subjects, there was little effect on FBS or Hgb A_{1c} levels. Although there was a certain level of variability among studies there was not an indication of adverse effects of fish oil on glucose homeostasis.

Omega-3 fatty acids have well-established anti-inflammatory effects [81]. Observational data suggests that dietary fish oil and ALA are inversely associated with CRP level, whereas, the intervention data are less consistent [67,82–88]. Intervention studies have also reported an anti-inflammatory effect of fish and fish oil supplements on other inflammatory markers [89,90]. Regardless of vehicle, our analysis indicates that among the few randomized trials there was no significant effect of either ALA, EPA or DHA on hs-CRP levels. The lack of consistency between the observational and some of the intervention studies may be attributable to unidentified factors that co-varied with reported intakes of fish oil that were the responsible agent and difficulties with measuring inflammatory markers other that CRP in large scale observational studies.

The effects of ALA on serum lipids were, for the most part, not consistent with that reported for the very long chain omega-3 fatty acids, EPA and DHA. It is likely that the limited capacity of humans to elongate and desaturate ALA to EPA, even when ALA is fed at high levels, accounts for this inconsistency [8,91,92]. Similarly, it has recently been reported that not only do humans have a limited capacity to convert ALA to EPA, but likewise EPA to DHA [8,9]. However, this latter restriction is unlikely to alter the effect of fish oil or very long chain omega-3 fatty acid supplementation on CVD risk [7].

Overall, while data on the effect of ALA on serum lipids, Hgb A_{1c}, and FBS are sparse, as are data regarding the effect of either fish oil or ALA on CRP, sufficient data have been reported to conclude that fish oil consumption lowers triglyceride levels and has a small beneficial effect on HDL cholesterol, while raising LDL cholesterol levels by a similarly small amount. While questions remain regarding the optimal dose and type of omega-3 fatty acids, the clinical significance of these changes, and possibly which groups of people would best be served by increasing fish oil consumption, it is unlikely that additional studies would change our conclusions regarding overall treatment effect of fish oil. In fact, the publication of the large GISSI study in 1999 did not alter conclusions available in the literature regarding the effect of fish oil on lipid values had meta-analyses been performed at that time. Similarly, future studies would be unlikely to alter the conclusion that fish oil consumption has no substantial effect on glucose homeostasis.

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An ω-3 Polyunsaturated Fatty Acid Concentrate Increases Plasma High-Density Lipoprotein 2 Cholesterol and Paraoxonase Levels in Patients With Familial Combined Hyperlipidemia

Laura Calabresi, Barbara Villa, Monica Canavesi, Cesare R. Sirtori, Richard W. James, Franco Bernini, and Guido Franceschini

A remarkable reduction of plasma concentrations of high-density lipoproteins (HDL), especially of the HDL_2 subfraction, is one of the typical lipoprotein alterations found in patients with familial combined hyperlipidemia (FCHL). Fourteen FCHL patients received 4 capsules daily of Omacor (an ω-3 polyunsaturated fatty acid [ω3 FA] concentrate providing 1.88 g of eicosapentaenoic acid [EPA] and 1.48 g of docosahexaenoic acid [DHA] per day; Pronova Biocare, Oslo, Norway) or placebo for 8 weeks in a randomized, double-blind, crossover study. Plasma triglycerides were 44% lower, and LDL cholesterol and apoliporpotein (apo)B were 25% and 7% higher after Omacor than placebo. HDL cholesterol was higher (+8%) after Omacor than placebo, but this difference did not achieve statistical significance. Omacor caused a selective increase of the more buoyant HDL_2 subfraction; plasma HDL_2 cholesterol and total mass increased by 40% and 26%, respectively, whereas HDL_3 cholesterol and total mass decreased by 4% and 6%. Both HDL_2 and HDL_3 were enriched in cholesteryl esters and depleted of triglycerides after Omacor. No changes were observed in the plasma concentration of major HDL apolipoproteins, LpA-I and LpA-I:A-II particles, lecithin:cholesterol acyltransferase (LCAT), and cholesteryl ester transfer protein (CETP). The plasma concentration of the HDL-bound antioxidant enzyme paraoxonase increased by 10% after Omacor. Omacor may be helpful in correcting multiple lipoprotein abnormalities and reducing cardiovascular risk in FCHL patients.

FAMILIAL combined hyperlipidemia (FCHL) is the most common inherited disorder of lipid metabolism among young survivors of myocardial infarction, with an estimated frequency of 0.3% to 2.0% in the general population.[1] FCHL patients present with elevated plasma cholesterol and/or triglycerides, predominance of small and dense low-density lipoprotein (LDL) particles, and low concentrations of high-density lipoprotein (HDL) cholesterol, particularly HDL_2 cholesterol.[2-4] Each of these lipid/lipoprotein abnormalities may contribute to the high prevalence of coronary heart disease (CHD) in FCHL patients.[5-7]

Because of the increased incidence of CHD in FCHL patients, diet and/or drug treatments aimed at lowering blood lipids and correcting the abnormal lipoprotein phenotype should be initiated early. Some early reports indicated that long-chain ω-3 fatty acids (ω-3 FAs) present in fish oils, mainly eicosapentaenoic and docosahexaenoic acid (EPA and DHA), may lower plasma triglycerides in FCHL patients, with little effects on plasma total, LDL, and HDL cholesterol.[8,9] We recently showed that a concentrate of EPA and DHA ethyl esters, which is used in Europe for the treatment of hypertriglyceridemia,[10] significantly lowers plasma triglycerides, and shifts LDL subclass distribution towards more buoyant particles, without affecting LDL size, in FCHL patients.[11] No changes were observed in plasma HDL cholesterol and apolipoprotein A-I levels.[11]

Despite the minor changes in plasma HDL cholesterol level, ω-3 FA administration to normolipidemic subjects[12,13] or mildly hypercholesterolemic patients with normal plasma HDL levels[14,15] has been shown to shift the distribution of HDL particle towards the more buoyant HDL_2 subfraction. The increase of HDL_2 was secondary to a reduced activity of the lecithin:cholesterol acyltransferase (LCAT) enzyme and of the cholesteryl ester transfer protein (CETP).[12,14] Since a low plasma HDL_2 concentration is a hallmark of FCHL, in the present study we investigated whether a concentrate of EPA and DHA ethyl esters may increase plasma HDL_2 levels in FCHL patients.

MATERIALS AND METHODS

Patients and Experimental Design

Fourteen FCHL patients who had been followed as outpatients for several years at the E. Grossi Paoletti Lipid Clinic were recruited for the study. Patients characteristics and study design have been previously reported.[11] Patients were diagnosed as FCHL when they fulfilled the following criteria[16]: (1) primary hyperlipidemia, defined by a plasma cholesterol and/or triglyceride level exceeding the 90th percentile in the general population, adjusted for age and sex; (2) varying hyperlipidemia phenotype during at least 1 year of follow-up; (3) at least one first-degree relative with a hyperlipidemia phenotype different from the index patient; (4) presence of an LDL phenotype B, defined by a major LDL particle subpopulation with a diameter less than 25.5 nm by nondenaturing polyacrylamide gradient gel electrophoresis (GGE). All patients followed a standard low-fat (30% of calories) diet throughout the study. All participating subjects were fully informed of the

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modalities and end points of the study, which was approved by the Institutional Ethic Committee, and signed an informed consent form.

The study protocol was designed for a randomized, double-blind crossover trial. After a run-in period of 4 weeks, qualifying patients were randomly allocated to receive Omacor (4 g/d) and placebo capsules for 8 weeks in a different sequence. The Omacor capsules (Pronova Biocare, Oslo, Norway) contained 1 g of concentrated ω-3 FAs (92%; 44.4% EPA and 36.2% DHA), and 4 mg of α-tocopherol. The placebo capsules contained corn oil (56.3% linoleic acid), monounsaturated FA (26.8% oleic acid), saturated FA (2.3% stearic acid), and 2.4 mg vitamin E.

Laboratory Procedures

Fasting blood samples were collected at the end of each 8-week treatment phase. Both serum and plasma (Na_2-EDTA, 1 mg/mL) were prepared by low-speed centrifugation at 4°C. Serum aliquots were added immediately with EDTA (1 mg/dL) and NaBr (5.1 mol/L) before storage at 4°C for HDL subfraction separation by rate zonal ultracentrifugation. Plasma aliquots were stored at −80°C for immunoassays and cell cholesterol efflux determination. A serum aliquot was stored at −80°C for determination of paraoxonase (PON) concentration.

Plasma total cholesterol and triglyceride levels were determined with standard enzymatic techniques by using a Roche Diagnostics Cobas autoanalyzer (Indianapolis, IN). Plasma HDL cholesterol levels were routinely measured after precipitation of the apolipoprotein (apo)B-containing lipoproteins by dextran sulfate–$MgCl_2$. Levels of apoA-I and apoA-II were determined by immunoturbidimetry, using commercially available polyclonal antibodies (Boheringer Mannheim, Mannheim, Germany). The plasma concentration of lipoprotein particles containing only apoA-I (LpA-I) and of particles containing both apoA-I and apoA-II (LpA-I:A-II) was determined by electroimmunodiffusion in agarose gel.[17] Plasma concentrations of lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), and paraoxonase were assayed by competitive enzyme-linked immunoassays.[18,19]

HDL subfractions were separated by rate zonal ultracentrifugation in a swinging bucket rotor, as previously described.[20] The lipid contents of the isolated HDL fractions were determined by enzyme techniques; the cholesteryl ester (CE) mass was calculated as (total cholesterol − unesterified cholesterol) × 1.68. Protein contents were measured by the method of Lowry et al[21] using bovine serum albumin as standard.

HDL particle size was analyzed by nondenaturing polyacrylamide gradient gel electrophoresis,[16] using precast 4% to 30% slab gels (Alamo Gels, San Antonio, TX). Aliquots of the $d < 1.21$ g/mL plasma fractions, which had been separated by ultracentrifugation at 100,000 rpm for 5½ hours at 4°C in a Beckman TL 100 ultracentrifuge (Fullerton, CA) equipped with a 100.3 rotor, were applied. Gels were run at 20 V for 15 minutes, then 70 V for 20 minutes, and 125 V for 24 hours at 4°C, stained with Comassie G-250 0.04 % for 24 hours, and scanned with a BioRad Model GS-690 Imaging Densitometer (Hercules, CA). Particle size was calculated with Multi-Analyst/PC Software (BioRad) using thyroglobulin (17.0 m), ferritin (12.2 nm), lactate dehydrogenase (8.15 nm), and bovine serum albumin (6.5 nm) as calibration proteins.

Cell cholesterol efflux to whole plasma was assayed as previously described.[22] Briefly, diluted plasma was incubated with ^{3}H-cholesterol-labeled Fu5AH rat hepatoma cells for 4 hours at 37°C. At the end of this period, the medium was removed, collected into tubes and centrifuged for 5 minutes at 2,000 rpm to remove any floating cells. An aliquot of the medium was then counted for [^{3}H]cholesterol radioactivity (Formula 989, Packard, Groningen, The Netherlands). Cellular lipids were extracted with 2-propanol by overnight incubation at room temperature and radioactivity was measured in an aliquot of the extract (Insta-Fluor, Packard). Cholesterol efflux was calculated as the percentage of total label in each well released to the medium, normalized to the cholesterol efflux obtained with a pool of normolipidemic sera tested in each experiment.

Table 1. Plasma Lipid and Lipoprotein Levels (mg/dL) at Baseline, and During Placebo and Omacor Treatment

	Baseline	Placebo	Omacor
Total cholesterol	270.7 ± 16.1	266.0 ± 10.4	282.5 ± 11.8
Triglycerides	378.1 ± 141.8	375.7 ± 104.5	210.1 ± 29.7*
LDL cholesterol	167.1 ± 12.7	161.5 ± 13.5	202.7 ± 12.2*
HDL cholesterol	39.6 ± 4.0	38.0 ± 3.6	41.2 ± 4.1
HDL_2 cholesterol	6.8 ± 1.9	6.3 ± 1.6	9.5 ± 2.9*
HDL_3 cholesterol	32.9 ± 2.5	32.0 ± 2.3	31.7 ± 2.1
Apolipoprotein A-I	112.9 ± 6.3	113.0 ± 5.8	111.7 ± 7.5
Apolipoprotein A-II	35.6 ± 2.0	35.1 ± 1.7	36.0 ± 1.8
LpA-I	38.4 ± 5.1	37.9 ± 5.0	36.3 ± 4.2
LpA-I:A-II	74.4 ± 4.8	75.1 ± 4.4	75.4 ± 5.2
Apolipoprotein B	135.2 ± 5.6	134.1 ± 7.0	143.8 ± 6.3*

NOTE. Results are expressed as means ± SEM, N = 14.
*Significantly different from baseline and placebo.

Statistical Analyses

The number of subjects needed to detect a difference in HDL_2-C between the 2 treatments of 2.5 mg/dL with a standard deviation of 3.0 mg/dL, a power of 80%, and $\alpha = 0.05$ is 14. Therefore, the study is adequately powered to disprove multiple null hypotheses.

Results are given as means ± SEM, if not otherwise stated. All statistical tests were performed using the SigmaStat computer software (Jandel, San Rafael, CA). Changes caused by treatments were analyzed by using repeated-measures analysis of variance (ANOVA) with post hoc evaluation by the Neuman-Keuls test. A probability value of less than .05 was considered significant.

RESULTS

Omacor was well tolerated; monitoring of drug intake by capsule counting and measurement of FAs profile in plasma phospholipids[11] indicated that compliance to treatment was satisfactory. Plasma total cholesterol did not change after placebo or Omacor treatment; plasma triglycerides were significantly lower after Omacor compared to baseline and placebo (Table 1). Plasma LDL cholesterol and apoB concentrations were 25% and 7% higher after Omacor than placebo.[11]

HDL cholesterol was higher after Omacor (+4% and +8% compared to baseline and placebo), but neither of these differences achieved statistical significance. No changes were observed in the plasma levels of apoA-I and apoA-II, and of LpA-I and LpA-I:A-II particles (Table 1).

Although the HDL cholesterol level did not change, a marked effect was observed on the cholesterol distribution between the major HDL subfractions: HDL_2 cholesterol increased by 40% after Omacor and decreased by 7% after placebo; minor, nonsignificant changes were found in plasma HDL_3 cholesterol (Table 1). Similar changes were observed in total lipoprotein mass; HDL_2 increased from 42.5 ± 14.0 mg/dL (baseline) and 40.1 ± 11.7 mg/dL (placebo) to 53.7 ± 21.3 mg/dL (Omacor), and HDL_3 decreased from 302.7 ± 18.9 mg/dL (baseline) and 302.5 ± 19.0 mg/dL (placebo) to 285.3 ± 15.4 mg/dL (Omacor). Due to these changes, the HDL_2/HDL_3 cholesterol and mass ratios increased from

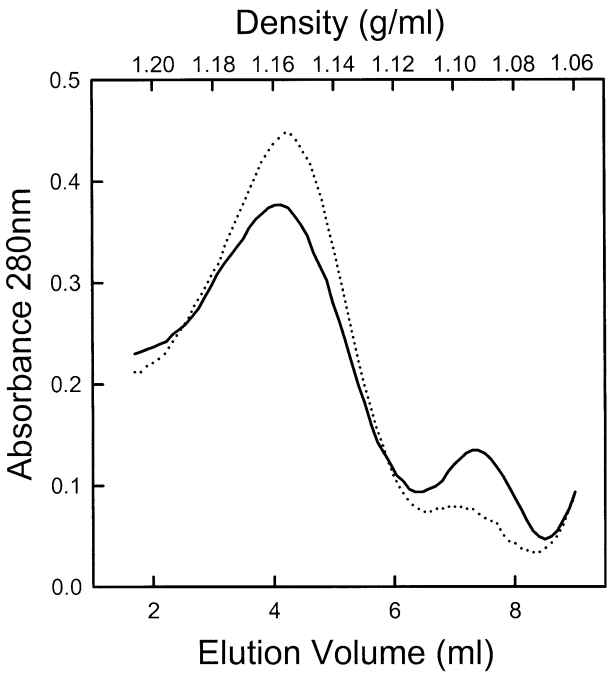

Fig 1. Mean rate zonal ultracentrifugation profiles of HDL subfractions from FCHL patients before (dotted line) and after (continuous line) treatment with Omacor. The left peak represents HDL_3, the right peak represents HDL_2.

0.191 ± 0.042 and 0.141 ± 0.037 (baseline) to 0.283 ± 0.074 and 0.188 ± 0.072 (Omacor), respectively. The structure of HDL subfractions before and after Omacor or placebo was analyzed by evaluating their flotation rate, particle diameter, and chemical composition. The mean elution profiles of HDL after rate zonal ultracentrifugation are reported in Fig 1. After Omacor, the content of slow-floating HDL_3 particles decreased, with a concomitant increase of fast-floating HDL_2. Slight, nonsignificant increases were found in flotation rate of both HDL_3 (elution volume: 4.46 ± 0.12 mL [baseline], 4.53 ± 0.12 mL [Omacor]) and HDL_2 (elution volume: 7.63 ± 0.08 mL [baseline], 7.75 ± 0.07 mL [Omacor]); the mean HDL_3 and HDL_2 particle diameter did not change. Minor variations were also observed in the composition of HDL subfractions (Table 2): both HDL_2 and HDL_3 became CE-enriched and triglycerides-depleted after Omacor, but only the increase in HDL_2 CE content achieved statistical significance.

We also evaluated the plasma concentration of 2 factors critically involved in the determination of the plasma concentration and structure of the HDL, and of their subfractions, ie, LCAT and CETP. No significant changes were observed after either placebo or Omacor (Table 3). By contrast, the plasma concentration of paraoxonase, an antioxidant enzyme that circulates in plasma bound to HDL,[23] increased by 10% after Omacor compared to baseline and placebo (Table 3).

Finally, no significant changes were observed in the capacity of patients' plasma to promote cell cholesterol efflux, expressed as percent cholesterol efflux from Fu5AH cells during a 4-hour incubation with plasma samples collected before (20.0% ± 0.9%) and after placebo (20.3% ± 0.9%) or Omacor (20.3% ± 0.8%).

Table 2. HDL Subfractions Composition at Baseline, and During Placebo and Omacor

	Baseline	Placebo	Omacor
HDL_2			
Free cholesterol	3.43 ± 0.22	3.56 ± 0.24	3.34 ± 0.21
Cholesteryl esters	20.26 ± 1.15	19.99 ± 1.13	22.89 ± 1.28*
Triglycerides	9.59 ± 0.98	8.69 ± 0.77	7.30 ± 1.00
Phospholipids	22.00 ± 1.69	22.04 ± 1.70	22.34 ± 1.40
Proteins	44.73 ± 1.05	45.71 ± 0.8	44.12 ± 0.79
HDL_3			
Free cholesterol	1.99 ± 0.15	1.80 ± 0.08	1.80 ± 0.13
Cholesteryl esters	15.02 ± 0.91	15.08 ± 0.87	15.67 ± 0.64
Triglycerides	8.24 ± 0.84	8.34 ± 0.94	6.74 ± 0.77
Phospholipids	20.84 ± 0.81	20.98 ± 0.79	20.69 ± 0.66
Proteins	53.91 ± 0.78	53.86 ± 0.76	55.11 ± 0.70

NOTE. Results are expressed as percentage of weight, means ± SEM, N = 14.
*Significantly different from baseline and placebo.

DISCUSSION

The dyslipidemia in FCHL patients is characterized by elevations of plasma cholesterol and/or triglycerides, predominance of small and dense LDL, and reduced plasma HDL_2 levels.[2-4] We have previously reported that a concentrate of ω-3 FAs lowers plasma triglycerides, and shifts LDL subclass distribution towards more buoyant particles, without affecting LDL size, in FCHL patients.[11] We show now that the same ω-3 FA concentrate remarkably increases plasma HDL_2 cholesterol and mass levels, without affecting the concentration of the denser HDL_3 subfraction. Moreover, the ω-3 FA concentrate raises the plasma concentration of the HDL-bound, antioxidant enzyme paraoxonase.

Drug treatment of FCHL has essentially focused on the lowering of the total amount of LDL, and the reduction in the atherogenicity of LDL, ie, reduction in the amount of small and dense LDL, with little attention paid to the correction of the defective HDL profile. Statins have little effect[16,24,25] while fibrates increase[24-27] plasma HDL cholesterol in FCHL patients. In the present study, a ω-3 FA concentrate slightly increased plasma HDL cholesterol and did not affect the concentration of major HDL apolipoproteins and lipoprotein particles, but it did enhance the plasma content of the HDL_2 subfraction. The increase of plasma HDL_2 levels may be consequent to the remarkable decrease of plasma triglyceride-rich lipoproteins, leading to a reduced CE transfer out of HDL,[18]

Table 3. Plasma LCAT, CETP, and Paraoxonase Concentrations at Baseline, and During Placebo and Omacor Treatment

	Baseline	Placebo	Omacor
CAT (mg/mL)	4.90 ± 0.44	4.96 ± 0.61	5.03 ± 0.60
CETP (mg/mL)	1.19 ± 0.20	1.20 ± 0.17	1.32 ± 0.27
Paraoxonase (μg/mL)	117.0 ± 7.5	117.2 ± 7.8	129.7 ± 9.3*

NOTE. Results are expressed as means ± SEM, N = 14.
*Significantly different from baseline and placebo.

and accumulation in plasma of CE-enriched and slowly catabolized HDL_2 particles.[28] The observation that ω-3 FAs decrease the net mass transfer of CE from HDL to lower density lipoproteins,[29] without affecting plasma CETP concentration, as shown in the present study, is consistent with this mechanism. However, no significant correlation was found between the reciprocal changes in plasma triglyceride and HDL_2 levels ($r = 0.127$, $P = .67$). Another possibility is that HDL phospholipids enriched with ω-3 FAs are poor substrates for hepatic lipase, as shown for the phospholipase activity of LCAT,[30] preventing hepatic lipase–mediated HDL_2 to HDL_3 conversion, thus again leading to a selective accumulation of HDL_2 in plasma.

Oxidation of LDL is recognized to be a critical early step in atherogenesis, and various investigators have found a correlation between LDL oxidizability and atherosclerosis progression.[31] The small LDL of FCHL patients are more prone to oxidation than normal, large LDL particles,[32] and both LDL size and in vitro oxidizability are correlated with the extent of preclinical atherosclerosis, as measured by B-mode ultrasonography in FCHL patients.[33] Therefore, the accumulation of small, oxidation-prone LDL in the plasma of FCHL patients may contribute to the high CHD risk. The HDL-bound paraoxonase prevents LDL oxidation in vitro[34] and in the arterial wall,[35] a mechanisms that provides a link between HDL, lipoprotein oxidation, and atherosclerosis. Paraoxonase activity and concentration are under genetic and environmental regulation. Environmental factors that alter paraoxonase activity/concentration include cigarette smoking,[36] and alcohol, fat, and vitamin intake.[37-39] There is obvious interest in pharmacological interventions able to increase paraoxonase activity/concentration, especially in high-risk individuals, but very little information has been made available so far. Hormone-replacement therapy was reported to increase paraoxonase activity in postmenopausal women.[40] Among lipid-affecting drugs, simvastatin increased paraoxonase activity in patients with familial hypercholesterolemia,[41] while fibrates had no effect in patients with mixed hyperlipidemia.[42] The present double-blind, placebo-controlled study shows that the administration of an ω-3 FA concentrate to high-risk FCHL patients causes a modest but significant increase of plasma paraoxonase concentration. Some studies have reported significant correlations between paraoxonase activity/concentration and plasma HDL and apoA-I levels[36,43,44]; however, the ω-3 FA concentrate increased plasma paraoxonase concentration without affecting the plasma levels of major HDL apolipoproteins. Paraoxonase has been described as preferentially associated with apoA-I–containing HDL particles,[45] but the increase of paraoxonase induced here by the ω-3 FA concentrate is clearly independent of changes in plasma LpA-I levels. It is noteworthy that a similar rise of paraoxonase activity without changes in plasma HDL cholesterol, apoA-I and LpA-I levels was previously reported in simvastatin-treated hypercholesterolemic patients.[41] Both the ω-3 FA concentrate and simvastatin, however, cause a change in the distribution of HDL subfractions, selectively increasing HDL_2 levels.[46] It thus seems that changes in HDL size/density distribution, rather than variations in total HDL concentrations, are important in mediating the effects of ω-3 FAs and simvastatin on plasma paraoxonase content. Indeed, in vitro studies have shown that large HDL are more efficient than small HDL in promoting the release of paraoxonase from cells and stabilizing the enzyme.[47]

The relevance of the present findings for the objective of reducing the high CHD risk in FCHL patients remains to be defined. FCHL patients present with elevated plasma cholesterol and/or triglycerides, predominance of small and dense LDL particles, and low concentrations of HDL_2 cholesterol,[2-4] and each of these abnormalities may contribute to the high CHD risk.[5-7] The administration of an ω-3 FA concentrate to FCHL patients does not change plasma cholesterol and lowers plasma triglycerides. The potentially harmful increase of LDL cholesterol may be balanced with a shift of LDL distribution towards more buoyant particles.[11] The ω-3 FA concentrate selectively raises the plasma concentrations of the "protective" HDL_2 subfraction,[7,48-51] and of the antioxidant enzyme paraoxonase. All together these changes may imply a beneficial effect of the ω-3 FA concentrate on CHD risk in FCHL patients, which needs to be demonstrated in large prospective studies.

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