Special Article

Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid dose-dependently reduce fasting serum triglycerides

Kathy Musa-Veloso, Malcolm Angus Binns, Alexandra Catherine Kocenas, Theresa Poon, Julie Ann Elliot, Harry Rice, Hilde Oppedal-Olsen, Hilary Lloyd, and Shawna Lemke

The objectives of this review were to determine whether the long-chain omega-3 fatty acids eicosapentaenoic acid and/or docosahexaenoic acid dose-dependently reduce fasting serum triglycerides (TG) and, if so, to create a mathematical model that may be used to predict potential percent reductions in fasting serum TG levels at the recommended intakes of 200–500 mg/day. The assessment included 15 randomized controlled trials that met pre-defined inclusion and exclusion criteria. Across these 15 studies, the dose-response was modeled using a first-order elimination curve. The response variable was defined as percent change from baseline in fasting serum TG, adjusted for the placebo effect. A weighting factor equal to the product of each study's sample size and quality score was used. Using the equation of the dose-response curve, predicted reductions in fasting serum TG levels at the recommended long-chain omega-3 fatty acid intakes of 200–500 mg/day are 3.1 to 7.2%.

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INTRODUCTION

Over the last 10 years, elevated triglyceride (TG) levels have emerged as risk factors for coronary heart disease (CHD), independent of other blood lipid disturbances. In the 8-year Prospective Cardiovascular Münster (PROCAM) study, which included 4,849 middle-aged men, hypertriglyceridemia was found to be an independent risk factor for incident CHD after adjusting for low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, with a fasting TG level ≥200 mg/dL (2.25 mmol/L) associated with a 1.6-fold elevated event risk; moreover, hypertriglyceridemia combined with elevated LDL cholesterol and an elevated LDL : HDL cholesterol ratio (>5) was associated with an approximate 6-fold increase in the risk of incident CHD.[1,2] Likewise, in the 8-year prospective Copenhagen Male Study, the relative risks (RR) of CHD in subjects with TG levels in the second and third tertiles were significantly higher than in subjects with the lowest TG level, even after adjusting for multiple variables, including LDL and HDL cholesterol.[3] In a meta-analysis of 17 population-based prospective studies (including 46,413 men and 10,864 women), the univariate RR of an incident cardiovascular event in subjects with the highest versus the lowest fasting TG level was 1.32 (95% CI, 1.26–1.39) for males and 1.76 (95% CI, 1.50–2.07) for females; after adjusting for multiple variables including HDL cholesterol, risk of an incident cardiovascular event was attenuated, but it was still significantly increased in subjects with the highest versus the lowest fasting TG levels (men: RR, 1.15; 95% CI, 1.05–1.28; women: RR, 1.37; 95% CI, 1.13–1.66).[4] In this same study, it was calculated that for each 1.0 mmol/L (88 mg/dL) increase in fasting TG, the risk of CHD was

Affiliations: *K Musa-Veloso, AC Kocenas, T Poon*, and *J Ann Elliot* are with Cantox Health Sciences International, Mississauga, Ontario, Canada. *MA Binns* is with the University of Toronto, Toronto, Ontario, Canada. *H Rice* is with the Global Organization for EPA and DHA Omega-3s (GOED), Salt Lake City, Utah, USA. *H Oppedal-Olsen* is with Denomega Nutritional Oils AS, Sarpsborg, Norway. *H Lloyd* is with Ocean Nutrition Canada, Dartmouth, Nova Scotia, Canada. *S Lemke* is with Monsanto, St. Louis, Missouri, USA.

Correspondence: *K Musa-Veloso*, Cantox Health Sciences International, Mississauga, Ontario, Canada. E-mail: kmusa-veloso@cantox.com, Phone: +1-905-542-2900, Fax: +1905 5421011.

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increased by approximately 30% in men and 75% in women; after adjusting for HDL cholesterol level, the corresponding rates were lower but still statistically significant (14% in men and 37% in women).

Evidence that elevated TG levels are an independent risk factor for CHD is so compelling that recently, in the Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) of the National Cholesterol and Education Program (NCEP), the fasting TG level considered "normal" was reduced from 200 mg/dL or 2.25 mmol/L (as noted in the 1993 Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults) to 150 mg/dL or 1.69 mmol/L.[5] The European Association for Cardiovascular Prevention and Rehabilitation of the European Society of Cardiology also considers a fasting TG level >150 mg/dL (1.69 mmol/L) to be a risk factor for CHD.[6] In line with these recommendations, the World Health Organization endorses a level of ≤150 mg/dL (≤1.69 mmol/L) as a target for fasting TG.[7] Nutritional interventions that reduce elevated TG levels and/or help maintain TG levels in the normal range (i.e., ≤150 mg/dL or ≤1.69 mmol/L) may be important for reducing CHD risk. One such intervention is the consumption of the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA).[8]

In a review of the effects of the long-chain omega-3 fatty acids on human serum lipids and lipoproteins, it was reported that serum TG levels decrease by approximately 25–30% with long-chain omega-3 fatty acid intakes of approximately 4 g/day, irrespective of the baseline TG level.[9] In another review of randomized controlled trials, the consumption of approximately 2.6 g/day of long-chain omega-3 fatty acids was found to reduce serum TG levels by approximately 27%; moreover, a significant interaction was observed between the baseline TG level and the dose of the long-chain omega-3 fatty acids, such that a larger dose of long-chain omega-3 fatty acids resulted in a greater reduction in TG levels in individuals with a higher baseline TG level than in subjects with a lower baseline TG level.[10] In neither of these reviews was a dose-response relationship assessed between intake of the long-chain omega-3 fatty acids and reduction in TG levels.

Several different scientific and regulatory bodies recommend intakes of the long-chain omega-3 fatty acids in the range of 200–500 mg/day for the generally healthy population.[11–18] It is unclear whether fasting TG levels would improve with consumption of long-chain omega-3 fatty acids in this range. Thus, the objective of the current analysis was to determine if long-chain omega-3 fatty acid-associated reductions in TG levels are dose-dependent, and if so, to develop a mathematical model that could be used to predict the percent reduction from baseline in fasting serum TG at the recommended intakes of the long-chain omega-3 fatty acids (i.e., 200–500 mg/day).

IDENTIFICATION AND ANALYSIS OF STUDIES

Literature search and study selection

The effects of long-chain omega-3 fatty acids on fasting TG levels have been assessed in two comprehensive systematic reviews[9,10]; in the most recent of them, the literature search was conducted through April 2003.[10] To avoid duplication of previously reported findings, the current literature search was limited to studies published in or subsequent to 2002. The preliminary search of the published literature on the long-chain omega-3 fatty acids was very broad and was intended to identify all relevant published data pertaining to supplementation with long-chain omega-3 fatty acids and cardiovascular health. The literature search was conducted in August 2007 using Dialog®, an electronic searching tool, to access a number of databases, including Medline, ToxFile, Agricola, Agris, JICST-EPlus, Biosis Previews, Food Science & Technology Abstracts, Foodline®: Science, NTIS, and Embase. The terms "DHA", "docosahexaenoic acid", "EPA", "eicosapentaenoic acid", "fish", or "fish oil" were searched in conjunction with several cardiovascular disease-related terms, including "triglycerides" and "cholesterol". Abstracts of articles determined to be relevant were reviewed, and pertinent articles were subsequently retrieved and reviewed for inclusion in or exclusion from the current analysis. Reference lists of articles retrieved from the literature search were hand-searched to identify other relevant articles.

Study inclusion and exclusion criteria are specified in Table 1. Any study that did not meet all of the inclusion criteria or that met any one of the exclusion criteria was excluded from this critical review.

Appraisal of study quality

The quality of each included study was assessed by three independent researchers (A.C.K., J.A.E., and T.P.) using the quality appraisal tool outlined in Table 2. The quality appraisal tool was developed using several of the criteria listed in the European Food Safety Authority's guidance document for applicants seeking authorization of a health claim[19]; added to the tool were additional criteria pertinent to the control of potential confounding variables. To permit between-study comparisons in study quality, each study was assigned a "quality score" based on the percentage of items accounted for.

Table 1 **Inclusion and exclusion criteria used for study selection.**

Inclusion criteria	Study was a randomized placebo-controlled trial published in or subsequent to 2002
	Study was published in English as a full-length article in a peer-reviewed journal
	Subjects included in the study had no prior history of cardiovascular disease, though risk factors for cardiovascular disease (e.g., obesity, hypertension, dyslipidemia, type 2 diabetes mellitus, the metabolic syndrome) may have been present
	Effects of long-chain omega-3 fatty acids on fasting triglyceride levels were reported
	The placebo treatment was an inert control known not to affect triglyceride levels
	The amount of long-chain omega-3 fatty acids administered, the length of long-chain omega-3 fatty acid supplementation, and associated tissue levels of long-chain omega-3 fatty acids (to ensure compliance) were quantified
Exclusion criteria	The study was published earlier than 2002
	The study was not a randomized placebo-controlled trial (e.g., systematic review, meta-analysis, opinion letter, position statement, observational study, uncontrolled human intervention study, conference proceeding)
	The study was published in abstract form only
	The study was published in a language other than English
	Subjects included in the study had a history of symptomatic heart disease, angina pectoris, myocardial infarction, or stroke
	The placebo treatment was not inert (i.e., the placebo treatment is known to cause significant modifications in triglyceride levels)
	The amount of long-chain omega-3 fatty acids administered, the length of long-chain omega-3 fatty acid supplementation, and associated tissue levels of long-chain omega-3 fatty acids were not quantified
	The only source of long-chain omega-3 fatty acids was provided as alpha-linolenic acid
	The treatment combined long-chain omega-3 fatty acids with other nutritional or pharmaceutical interventions as treatment
	Effects of long-chain omega-3 fatty acids on fasting triglyceride levels were not reported

Table 2 **Quality appraisal tool.**

Criterion	Criterion established*		
	Yes	No/unknown	Not applicable
Randomization			
Blinding of subjects			
Blinding of outcome assessors			
Power calculations performed for a specific outcome variable			
Subject inclusion and exclusion criteria specified			
Accounted for baseline levels of relevant risk factors/outcome variables			
Accounted for gender			
Accounted for age			
Accounted for smoking			
Accounted for use of lipid-lowering medication			
For women, accounted for menopausal status			
For women, accounted for use of hormone replacement therapy			
Accounted for change in physical activity			
Accounted for change in alcohol intake			
Accounted for change in background diet			
Accounted for change in body weight/BMI			
Compliance of subjects with the intervention reported			
Sufficient detail provided in description of statistical methods			
Analyses included an intent-to-treat analysis			
If crossover study, washout included			
If crossover study, effect of treatment period controlled for			
Subjects completing the study are compared with those who did not			
Subject attrition numerically accounted for			
Reasons for subject attrition provided			

* To permit between-study comparisons in study quality, each study was assigned a "quality score" based on the percentage of criteria accounted for. Criteria scored as "not applicable" were not included in the total score.

Extraction of data from studies

For each of the included studies, the following data were summarized in tabular format: reference; study design; dose of EPA and/or DHA (as free equivalents, per day) and delivery matrix; placebo treatment; duration of intervention; number of subjects completing the study and whether they had normal, borderline-high, or high TG at baseline, as per the American Heart Association, the World Health Organization, and the NCEP/ATP III definitions; baseline TG in the active treatment group; change from baseline TG in the active treatment group; and the net treatment effect (i.e., change from baseline TG in the active treatment group corrected for the change from baseline TG in the placebo group). As an example, in Wu et al.[20] the change from baseline TG following the 6-week administration of 2.14 g/day DHA was −0.25 mmol/L (−17.1%); the corresponding change in the placebo group administered corn oil was 0.04 mmol/L (2.6%). The net treatment effect is therefore calculated as −0.29 mmol/L (−19.7%).

Assessment of dose-response relationship

The response variable was calculated in two ways: 1) as the percent change in TG relative to baseline in the active treatment arm and 2) adjusted for a placebo effect by subtracting the percent change observed in the placebo arm from the percent change in TG in the active treatment arm. All values were expressed as percent change in TG, as it was previously reported that, at a fixed intake of long-chain omega-3 fatty acids, the magnitude of TG lowering is influenced by the baseline TG level.[10] The regressor variable of interest was the intake of EPA and/or DHA (g/day). Parameters describing the relationship between the intake of EPA and/or DHA and reduction in TG level were fit using the nonlinear least squares function (nls) in R (version 2.8.1, The R Foundation for Statistical Computing, Vienna, Austria). A first-order elimination curve was used to model the dose-response curve. An assumption inherent in the first-order elimination curve is that the TG-lowering effect of EPA and/or DHA is saturable; that is, with increased intakes of EPA and/or DHA, the TG-lowering effect reaches a plateau. The first-order elimination curve is described by the following equation:

$$\%\,\text{Change in TG} = a\left(1 - \exp\left[-\ln(2) \times \text{dose}/b\right]\right)$$

Here, *a* represents the maximal percent reduction in TG that can be achieved at high intakes of EPA and/or DHA and *b* represents the dose (in g/day) required to achieve one-half of *a*. The studies were weighted according to the product of each study's sample size and quality score. All studies meeting pre-defined inclusion criteria and none of the exclusion criteria were included in the dose-response assessment, irrespective of the statistical significance of their findings. No study was dropped from the assessment – if it had a small sample size or was of poor quality, it was maintained in the assessment but associated with less weight.

OVERVIEW OF FINDINGS

Characteristics of included studies

A total of 15 randomized controlled trials meeting predefined inclusion criteria and none of the exclusion criteria were identified (Table 3). Six of the studies were conducted in Europe[22,24,27,30,33,34]; three in North America[20,23,28]; one in South America[32]; three in Australia[21,26,31]; and two in Asia.[20,25] Twelve of the studies had a parallel group design while three had a crossover design with a random sequence assignment (Table 3). The effects of long-chain omega-3 fatty acids on TG levels were reported to be similar in studies with either a crossover design or a parallel design[9]; thus, the dose-response assessment included all studies. The quality scores of the 15 studies ranged from 54.5% to 89.5%, with the median score being 72.7%. Limitations identified in each of the studies are summarized in Table 4.

Of the 15 randomized controlled trials included in the current assessment, 12 conducted statistical comparisons between groups.[20,21,23–25,27–32,34] Of these 12 studies, all seven studies with a quality score of ≥72.7%[21,23–25,27–29] and one of five studies with a quality score of <72.7%[34] reported significant decreases in TG levels compared to the placebo group. The mean dose of long-chain omega-3 fatty acids administered in studies with a quality score ≥72.7% was 2.1 g/day, which is similar to the 2.5 g/day mean dose administered in studies with a quality score <72.7%. The mean sample size amongst the studies with a quality score ≥72.7% was 76, while for the studies with a quality score <72.7%, the mean sample size was 52. This difference indicates that the lower-quality studies may have had insufficient power to detect statistically significant reductions in fasting serum TG. In addition, the lower-quality studies did not control for many potential confounding variables; thus, the inability to detect statistically significant effects on fasting serum TG may be due to poorer methodological robustness (Table 4).

The amounts of long-chain omega-3 fatty acids (EPA and/or DHA) administered (as free fatty acid equivalents) ranged from 209 mg/day to 5.6 g/day, with an average intake across the 15 studies of 2.3 g/day. In eight of the studies, the long-chain omega-3 fatty acids were administered as dietary supplements (in capsules), while in the remaining seven studies, the long-chain omega-3 fatty

Table 3 **Summary of studies assessing effects of long-chain omega-3 fatty acids on serum fasting triglyceride levels.**

Reference	Study design	Quality score*	DHA and/or EPA (g/day) as free equivalents (source)	Placebo	Matrix	Duration (weeks)	Subjects		Fasting serum triglyceride level (mmol/L)		
							Total number completing study	TG classification at study entry†	Baseline	Change from baseline (%)	Change from baseline corrected for placebo effect (%)‡
Chan et al. (2002)[21]	R, DB, PC, P	89.5%	3.2 (fish oil)	Corn oil	Capsules	6 (3-week run-in)	26 obese M	BH, H	2.1§	–0.5 (–25.0%)	–0.4 (–21.2%)
Leigh-Firbank et al. (2002)[22]	R, DB, PC, X	82.4%	3.01 (fish oil)	Olive oil	Capsules	6 (12-week washout)	55 M	N, BH, H	N/A	N/A	–0.83 (–33.3%)
Maki et al. (2005)[23]	R, DB, PC, P	77.3%	1.60 (DHA-rich algal oil)	Olive oil	Capsules	6	57 M and F with below-average levels of HDL-CH	BH	2.02	–0.48 (–23.8%)	–0.32 (–15.3%)
Dyerberg et al. (2004)[24]	R, DB, PC, P	73.7%	3.01 (fish oil)	Regular fat	Foods	8 (12-week follow-up)	51 M	N	1.38§	–0.35 (–26.1%)	–0.45 (–35.7%)
Hamazaki et al. (2003)[25]	R, DB, PC, P	73.3%	0.860 (fish oil)	Linoleic and oleic acid	Soy milk	12	41 M and F	N, BH	1.83	–0.32 (–17.5%)	–0.37 (–20.6%)
Hill et al. (2007)[26]	R, DB, PC, P	72.7%	1.92 (tuna fish oil)	Sunflower oil	Capsules	12	35 overweight M and F with HTN	BH	1.71§	–0.23 (–13.9%)	–0.21 (–12.7%)
Lovegrove et al. (2004)[27]¶	R, DB, PC, P	72.7%	2.37 (fish oil)	Olive oil	Capsules	12 (2-week run-in)	84 M and F	N, BH, H	1.44§	–0.30 (–21.4%)	–0.4 (–28.1%)
Maki et al. (2003)[28]**	R, DB, PC, P	72.7%	0.209 (DHA-rich algal oil)	Regular eggs	Eggs	6	150 M and F	N, BH, H	2.57	–0.26 (–10.1%)	–0.17 (–6.3%)
Stark and Holub (2004)[29]	R, DB, PC, X	72.7%	2.8 (DHA-rich algal oil)	Corn and soy oil	Capsules	4 (washout ≥6 weeks)	26 postmenopausal F receiving and not receiving HRT	N, BH	1.61	–0.32 (–19.9%)	–0.27 (–16.8%)
Goyens and Mensink (2006)[30]	R, DB, PC, P	68.2%	1.6 (fish oil)	Oleic acid	Foods	6 (3-week run-in)	24 elderly	N, BH	1.10	–0.18 (–16.4%)	–0.04 (–4.0%)
Murphy et al. (2007)[31]	R, DB, PC, P	68.2%	1.0 (cod fish oil)	Regular foods	Foods	24	74 overweight M and F	BH	1.85§	0 (0%)	–0.1 (–5.0%)
Castro et al. (2007)[32]	R, DB, PC, P	59.1%	0.460 (fish oil)	Soy oil	Milk	6	36 M and F	N	1.16	0.6 (51.7%)	–0.09 (–0.3%)
Wu et al. (2006)[20]	R, SB, PC, P	57.1%	2.14 (DHA-rich algal oil)	Corn oil	Eating/ cooking oil	6 (2-week run-in)	25 amenorrheic premenopausal F on vegetarian diets	N	1.44§	–0.25 (–17.1%)	–0.29 (–19.7%)
Browning et al. (2007)[33]	R, PC, X	54.5%	4.2 (fish oil)	Oleic acid linoleic acid	Capsules	12 (4-week washout)	18 overweight premenopausal F	N	0.89	–0.15 (–16.9%)	–0.17 (–19.1%)
Buckley et al. (2004)[34]††	R, DB, PC, P	54.5%	5.6 (fish oil)	Oleic acid	Capsules	4	42 M and F	N	1.21§	–0.35 (–29.9%)	–0.26 (–22.8%)

* See Methods section and Table 2 for details on the quality appraisal tool used to assess the quality of the studies.

† TG levels were classified as normal (≤150 mg/dL or 1.69 mM); borderline-high (150–199 mg/dL or 1.69–2.25 mM); high (200–499 mg/dL or 2.26–5.63 mM); or very high (≥500 mg/dL or ≥5.64 mM), according to the American Heart Association, World Health Organization (2005), and National Cholesterol and Educaiton Program/Adult Treatment Panel III quartiles.

‡ Calculated by subtracting the change (percent change) from baseline in the placebo group from the change (percent change) from baseline in the treatment group.

§ TG levels were measured in plasma,w so values were increased by 3% to account for the 3% higher TG level in serum.

¶ Study had four groups in total, two groups of Europeans that consumed the placebo or fish oil, and two groups of British Indo-Asians that consumed the placebo or fish oil. There was no significant effect of ethnicity on outcome, so the results for the placebo groups and the fish oil groups were averaged.

** Statistical analyses were conducted within each group based on BMI ≥ 30 kg/m^2 or BMI < 30 kg/m^2. To calculate a crude estimate of the change in TG in each group (irrespective of BMI), the levels reported for the two BMI categories were averaged.

†† Study had three groups in total; one control group and two treatment groups (a high EPA group and a high DHA group). Because the total amount of long-chain omega-3 fatty acids administered in the treatment groups was the same, as were the baseline characteristics of the subjects, results are expressed as averages for the two treatment groups.

Abbreviations: BH, borderline-high; DB, double-blind; F, females; H, high; HDL-CH, high-density lipoprotein cholesterol; HRT, hormone replacement therapy; HTN, hypertension; M, males; N, normal; P, parallel; PC, placebo-controlled; R, randomized; SB, single-blind; X, crossover.

Table 4 **Quality scores and study limitations.**

Reference	Proportion of quality appraisal tool items accounted for		Study limitations
	Fraction	Quality score (%)	
Chan et al. (2002)[21]	17/19	89.5	Power calculations not performed Intent-to-treat analysis not included
Leigh-Firbank et al. (2002)[22]	14/17	82.4	Power calculations not performed Compliance of subjects with the intervention not reported Study did not control for the effect of the treatment period
Maki et al. (2005)[23]	17/22	77.3	Study did not account for smoking, menopausal status, or the use of hormone replacement therapy Intent-to-treat analysis not included Subjects completing the study were not compared with those who did not
Dyerberg et al. (2004)[24]	14/19	73.7	Study did not account for the use of lipid-lowering medication Study did not account for changes in physical activity Compliance of subjects with the intervention not reported Intent-to-treat analysis not included Subjects completing the study were not compared with those who did not
Hamazaki et al. (2003)[25]	14/19	73.7	Power calculations not performed Study did not account for menopausal status Study did not account for changes in alcohol intake or background diet Compliance of subjects with the intervention not reported
Hill et al. (2007)[26]	16/22	72.7	Study did not account for smoking, menopausal status, or use of hormone replacement therapy Study did not account for changes in alcohol intake Intent-to-treat analysis not included Subjects completing the study were not compared with those who did not
Lovegrove et al. (2004)[27]	16/22	72.7	Power calculations not performed Study did not account for smoking or menopausal status Study did not account for changes in body weight/BMI Reasons for subject attrition not provided Subjects completing the study were not compared with those who did not
Maki et al. (2003)[28]	16/22	72.7	Power calculations not performed Study did not account for smoking or menopausal status Study did not account for changes in body weight/BMI Reasons for subject attrition not provided Subjects completing the study were not compared to those who did not
Stark and Holub (2004)[29]	16/22	72.7	Power calculations not performed Study did not account for smoking or use of lipid-lowering medication Study did not account for changes in physical activity Intent-to-treat analysis not included Subjects completing the study were not compared with those who did not
Goyens and Mensink (2006)[30]	15/22	68.2	Study did not account for smoking, menopausal status, or use of hormone replacement therapy Study did not account for changes in physical activity or alcohol intake Intent-to-treat analysis not included Subjects completing the study were not compared with those who did not
Murphy et al. (2007)[31]	15/22	68.2	Study did not account for smoking, menopausal status, or the use of hormone replacement therapy Study did not account for changes in physical activity or alcohol intake Intent-to-treat analysis not included Subjects completing the study were not compared with those who did not
Castro et al. (2007)[32]	13/22	59.1	Power calculations not performed Study did not account for gender, smoking, menopausal status, or use of hormone replacement therapy Study did not account for changes in physical activity or alcohol intake Intent-to-treat analysis not included Subjects completing the study were not compared to those who did not

Table 4 **Continued**

Reference	Proportion of quality appraisal tool items accounted for		Study limitations
	Fraction	Quality score (%)	
Wu et al. (2006)[20]	12/21	57.1	Outcome assessors were not blinded Power calculations not performed Study did not account for smoking or use of lipid-lowering medication Study did not account for changes in physical activity, alcohol intake, or background diet Intent-to-treat analysis not included Subjects completing the study were not compared with those who did not
Browning et al. (2007)[33]	12/22	54.5	Subjects and outcome assessors not blinded Study did not account for use of hormone replacement therapy Study did not account for changes in physical activity, alcohol intake, background diet, or body weight/BMI Intent-to-treat analysis not included Study did not control for effect of treatment period Subjects completing the study were not compared to those who did not
Buckley et al. (2004)[34]	12/22	54.5	Power calculations were not performed Study did not account for smoking, menopausal status, or the use of hormone replacement therapy Study did not account for changes in physical activity, alcohol intake, or background diet Intent-to-treat analysis not included Reasons for subject attrition not provided Subjects completing the study were not compared to those who did not

acids were administered as components of enriched foods ($n = 6$ studies) or as eating/cooking oils ($n = 1$ study). Study durations ranged from 4 to 24 weeks, with the majority of studies having a duration of 6 weeks ($n = 7$ studies) or 12 weeks ($n = 4$ studies). The number of subjects studied (per group) ranged from 13 to 77; collectively, 747 subjects were studied in the 15 trials. Fasting TG levels were reported in serum in seven of the studies[23,25,28–30,32,33] and in plasma in eight of the studies.[20–22,24,26,27,31,34] According to the Laboratory Methods Committee of the Lipid Research Clinics Program,[35] values for fasting TG average 3% higher for serum than for plasma; thus, for studies reporting fasting plasma TG levels, values were expressed as fasting serum TG levels by applying a 3% correction factor. Five studies included subjects with normal TG levels, three included subjects with borderline-high TG levels, and the remaining seven studies included combinations of subjects with either normal, borderline-high, or high TG levels, as defined by the American Heart Association, the World Health Organization, and the NCEP/ATP III.

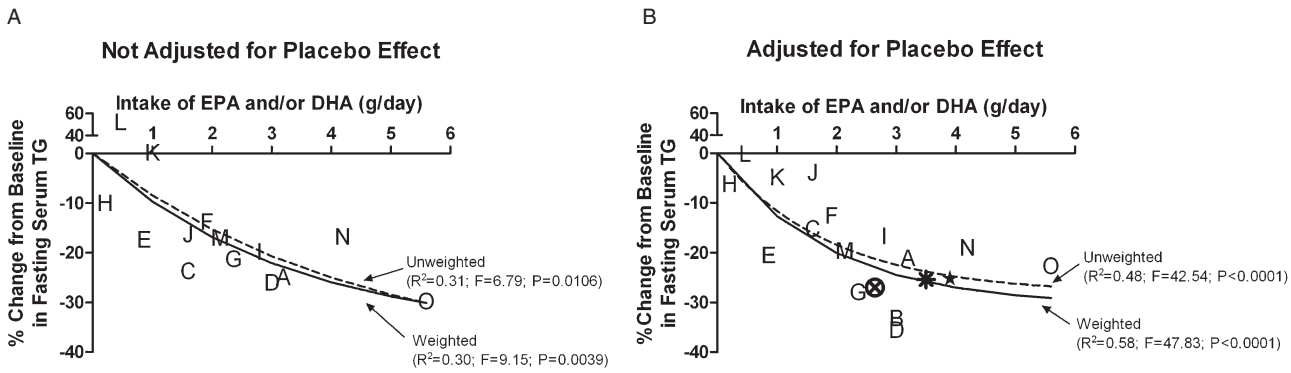
Dose-response relationship

In Figure 1, the percent change from baseline in fasting serum TG level is expressed as a function of intake of the long-chain omega-3 fatty acids EPA and/or DHA. The square of the multiple correlation coefficient ($R^2 = 1 - \text{Residual SS/Total}_C \text{ SS}$) is improved by correcting the percent change from baseline in TG level for the effect observed in the placebo group; it is further improved by weighting the data points by the product of each study's sample size and quality score (Table 5). Using the equation of this line $\{y = a(1 - \exp[-\ln(2) \times \text{dose/b}])$, where $a = -30.7069$ and $b = 1.3042\}$, predicted changes in fasting serum TG levels at the recommended intakes of EPA and/or DHA of 200–500 mg/day are −3.1 (95% CI, −5.9 to −0.31%) to −7.2% (95% CI, −11.6 to −2.5%).

DISCUSSION

Effects of applying weights to studies

It has been known for approximately 25 years that high-dose fish oil reduces TG levels.[9,10,36] The objectives of the current assessment were to determine whether the long-chain omega-3 fatty acid-induced reductions in fasting serum TG levels are dose-dependent and, if so, to generate a mathematical model that can be used to predict percent change from baseline in fasting serum TG at the recommended long-chain omega-3 fatty acid intakes of 200–500 mg/day.



Letter	Reference	Quality score (%)	Total sample size	Weight
H	Maki et al. (2003)[28]	72.7	150	10,905.0
B	Leigh-Firbank et al. (2002)[22]	82.4	110	9,064.0
G	Lovegrove et al. (2004)[27]	72.7	84	6,106.8
K	Murphy et al. (2007)[31]	68.2	74	5,046.8
C	Maki et al. (2005)[23]	77.3	57	4,406.1
I	Stark and Holub (2004)[29]	72.7	52	3,780.4
D	Dyerberg et al. (2004)[24]	73.7	51	3,758.7
E	Hamazaki et al. (2003)[25]	73.7	41	3,021.7
F	Hill et al. (2007)[26]	72.7	35	2,544.5
A	Chan et al. (2002)[21]	89.5	26	2,327.0
O	Buckley et al. (2004)[34]	54.5	42	2,289.0
L	Castro et al. (2007)[32]	59.1	36	2,127.6
N	Browning et al. (2007)[33]	54.5	36	1,962.0
J	Goyens and Mensink (2006)[30]	68.2	24	1,636.8
M	Wu et al. (2006)[20]	57.1	25	1,427.5
✳	Harris (1997)[9] (16 parallel design studies)	N/A	N/A	N/A
★	Harris (1997)[9] (16 crossover design studies)	N/A	N/A	N/A
⊗	Balk et al. (2006)[10] (37 data sets)	N/A	N/A	N/A

Figure 1 **Percent change from baseline in fasting serum triglycerides is a function of intake of EPA and/or DHA.** (**A**) Percent change from baseline in TG was not adjusted for the change observed in the placebo group. Data from Leigh-Firbank et al.[22] were not included, as change from baseline was not reported and could not be calculated from the data provided. (**B**) Percent change from baseline in TG was adjusted for the change observed in the placebo group by subtracting the effect in the placebo group from the effect in the active treatment group. To conduct the weighted assessments, the product of each study's sample size and quality score was used. The dose-response was modeled using a first-order elimination curve % Change in TG = a (1 – exp[-ln(2) × dose/b]), where *a* represents the maximal percent reduction in TG and *b* represents the dose (in g/day) required to achieve one-half of *a*. See Table 5 for estimates of the *a* and *b* parameters for the various analyses.

Table 5 **Results of nonlinear regression analyses.**

Analysis	a (95% CI)	b (95% CI)	R^2	*F*-value	*P*-value
Not corrected for placebo effect					
Unweighted	–42.182 (–187.0, 102.6)	3.087 (–12.4545, 18.62847)	0.31	6.79	0.0106
Weighted	–36.738 (–123.5, 49.994)	2.261 (–6.232, 10.754)	0.30	9.15	0.0039
Corrected for placebo effect					
Unweighted	–28.2681 (–46.7166, –9.8196)	1.3142 (–0.5543, 3.1827)	0.48	42.54	<0.0001
Weighted	–30.7069 (–51.2222, –10.1916)	1.3042 (–0.5338, 3.1422)	0.58	47.83	<0.0001

In the current assessment, the relationship between intake of EPA and/or DHA and percent reduction in fasting serum TG level was modeled using a first-order elimination curve. As can be seen in Figure 1, the square of the correlation coefficient is highest after adjusting for the placebo effect and after using weighting factors equal to the product of each study's sample size and quality score. The importance of applying weights to studies and of adjusting the effect in the active treatment group for the effect in the placebo group is exemplified in one study,[32] which had a low study weight relative to the other studies (see legend of Figure 1 for study weights, which are presented in descending order of study quality index). In the study of Castro et al.[32], which included subjects with normal baseline serum TG levels, an approximate 50% increase in fasting serum TG was reported in both the active treatment and the placebo groups. Since this effect occurred in both groups, the increase in fasting serum TG was likely unrelated to treatment allocation but to some other factor associated with the study. Inclusion of the study by Castro et al.[32] in the analysis of percent change from baseline, unadjusted for the placebo effect (Panel A of Figure 1), results in an R-squared value of 0.31. Once the approximate 50% increase in fasting TG in the active treatment group is adjusted to account for the approximate 50% increase in fasting TG in the placebo group, the study becomes less of an outlier (hashed line, Panel B of Figure 1), and the R-squared value increases to 0.48. When the study weights are then applied to the placebo-adjusted values (solid line, Panel B of Figure 1), the study by Castro et al.[32] has less impact on the nonlinear regression model, resulting in an R-squared value of 0.58; this means that 58% of the variability in the response variable (percent change from baseline in serum TG, adjusted for the placebo effect) was explained by the EPA and/or DHA doses.

Congruency of predicted reductions in fasting serum TG with results of other reviews

Harris[9] and Balk et al.[10] conducted comprehensive systematic reviews of the effects of the long-chain omega-3 fatty acids on serum lipoproteins. In the study by Harris,[9] the mean percent reduction in TG level from baseline amongst individuals with baseline TG levels <200 mg/dL (<2.25 mmol/L) was 25.4% (across 16 parallel design studies with a mean long-chain omega-3 fatty acid intake of 3.5 g/day) and 25.2% (across 16 crossover design studies with a mean long-chain omega-3 fatty acid intake of 3.9 g/day). In the study by Balk et al.,[10] the mean percent reduction in TG level from baseline amongst individuals with a mean baseline TG level of 144.7 mg/dL (1.63 mmol/L) was 27% (mean intake of the long-chain omega-3 fatty acids was 2.64 g/day across the 37 data sets included). The analyses conducted by Harris[9] and Balk et al.[10] are similar to our assessment with respect to the subjects' baseline TG levels, the correction of treatment effects for effects observed in the placebo group, and the weighting of studies according to sample size; thus, the summary data points reported by Harris[9] and by Balk et al.[10] have been superimposed onto Panel B of Figure 1. As can be seen, the data points fall on or close to the weighted line of best fit, indicating that, for subjects with normal, borderline-high, or high TG levels, the response variable is adequately described by our nonlinear regression model. It should be noted that, as in our assessment, Harris[9] did not include studies in which the source of the long-chain omega-3 fatty acids was fish; in contrast, Balk et al.[10] did include studies in which the source of the long-chain omega-3 fatty acids was fish. Despite this difference in methodology, the effects of the long-chain omega-3 fatty acids on TG levels appear to be similar, whether consumed as part of a fish meal or in other matrices.

The TG-lowering efficacy of prescription omega-3 fatty acid concentrates in subjects with moderate hypertriglyceridemia (150–500 mg/dL) was recently assessed by Skulas-Ray et al.[37] In that study, the extent of TG lowering was found to be a function of baseline TG level, a finding that has been reported previously [10] and that reinforces the importance of expressing effects on serum TG as percent changes from baseline. From the 19 randomized controlled trials reviewed by Skulas-Ray et al.,[37] it was concluded that the percent change in TG from baseline with consumption of 4 g/day prescription omega-3 fatty acid concentrates (equivalent to approximately 3.4 g/day of the long-chain omega-3 fatty acids) is approximately 30%. This reduction is very similar to the 26% reduction from baseline in fasting serum TG predicted by our mathematical model presented here. Although the majority of studies reviewed by Skulas-Ray et al.[37] were conducted in subjects with familial hyperlipidemia or with a previous history of cardiovascular disease, all of the subjects had a baseline fasting serum TG level of <500 mg/dL; this is similar to the baseline fasting serum TG levels that characterized subjects in the 15 randomized controlled trials included in our assessment. The similarity in the reductions in TG levels predicted in our review and suggested by Skulas-Ray et al.,[37] at a long-chain omega-3 fatty acid intake of 3.4 g/day, reinforces the robustness of our mathematical model in predicting reductions in fasting serum TG in subjects with normal, borderline-high, or high fasting serum TG levels. In contrast, in studies of prescription omega-3 fatty acid concentrates involving subjects with very high levels of fasting serum TG (i.e., in excess of 500 mg/dL), reductions of as much as 45% from baseline and 60% (after correcting for the placebo effect) have been reported.[38] As the maximum TG lowering achievable in

our mathematical model is approximately 31%, it is likely that effects in subjects with very high TG levels are underestimated by our model – a fact that is not surprising given that none of the 15 randomized controlled trials in our assessment included subjects with very high TG levels.

Predicted reductions in fasting serum TG at recommended long-chain omega-3 fatty acid intakes

Using the equation of the first-order elimination curve, the predicted reductions in fasting serum TG levels at the recommended long-chain omega-3 fatty acid intakes of 200–500 mg/day are 3.1% to 7.2%. In the majority of the 15 randomized controlled trials included in our assessment, the long-chain omega-3 fatty acids were administered in supplemental form, and subjects were either instructed not to consume fish or were already low-level fish consumers. Even on a low-fish diet, the background dietary intake of the long-chain omega-3 fatty acids could be 100–200 mg/day. The effects on TG levels of background dietary intakes of the long-chain omega-3 fatty acids would have been accounted for, given that the change from baseline in fasting serum TG in the placebo group was subtracted from that in the long-chain omega-3 fatty acid group. Thus, the reduction in fasting TG observed in the active treatment group is attributed to the intake of supplemental long-chain omega-3 fatty acids.

The number of studies in which the dose of EPA and/or DHA was between 200 and 500 mg/day was limited to two. In one study, the consumption of 209 mg/day DHA from DHA-enriched eggs for 6 weeks resulted in a 6.3% reduction in fasting TG (after correcting for the placebo effect) in subjects with normal, borderline-high, or high fasting TG at baseline[28]; in the second study, the consumption of 460 mg/day of long-chain omega-3 fatty acids for 6 weeks resulted in a 0.3% reduction in fasting TG (after correcting for the placebo effect) in subjects with normal fasting TG at baseline.[32] Despite this lack of effect on TG levels, this study[32] is flawed, given that in the active and the placebo groups, fasting TG levels increased by approximately 50% for no apparent reason. Hamazaki et al.[25] reported a 20.6% reduction in fasting TG following the 12-week consumption of 860 mg/day EPA+DHA in subjects with normal or borderline-high fasting TG at baseline. Although the dose administered in the study of Hamazaki et al.[25] was greater than the 200–500 mg/day dose range of interest, these results suggest that low intakes of EPA+DHA are beneficial for improving fasting TG.

Given that a limited number of low-dose studies was identified when the literature search was restricted to studies published in or subsequent to 2002, several review articles were reviewed to identify additional clinical studies in which the administered dose of EPA and/or DHA was low (i.e., between 200 and 500 mg/day).[9,10,39–46] It was determined that in three studies, the dose of EPA+DHA administered was between 200 and 500 mg/day.[47–49] In a parallel, single-blinded, randomized study, the consumption of approximately 300 mg/day of EPA+DHA (as part of a fish oil-enriched bread) for 4 weeks resulted in a 16.7% reduction in TG levels in nine healthy subjects with normal baseline TG levels[48]; the reduction was not corrected for the placebo effect as the placebo group was administered margarine, which increased TG levels substantially and was thus presumed to be hydrogenated. In another study by the same group, the administration of a bread containing 500 mg/day EPA+DHA and oat fiber for 4 weeks resulted in a significant reduction in TG levels compared to the administration of a bread containing oat fiber only in subjects with high baseline TG levels.[49] The change from baseline in TG levels (after correcting for the placebo effect) was −30.8%.[49] In a third study conducted by a different research group, the administration of a fish oil in capsule form (285 mg/day for 12 weeks) to pre- and postmenopausal women with normal baseline TG levels resulted in an 18.9% reduction in TG levels (after adjusting for the change in the control group, which was not administered a placebo).[47] Although the TG reductions observed in these three additional low-dose studies are greater than those predicted by the first-order elimination equation, it should be noted that none of these studies would have met the inclusion criteria defined in Table 1. Specifically, the study by Baker and Najadah[47] was not placebo-controlled and the placebo treatments administered in the studies by Saldeen et al.[48] and Liu et al.[49] were not inert with respect to the effects on TG levels. Nonetheless, these studies cumulatively suggest that long-chain omega-3 fatty acid intakes of 200–500 mg/day may be beneficial for reducing TG levels.

Further evidence that long-chain omega-3 fatty acid doses of 200–500 mg/day reduce fasting TG comes from studies in which the fatty acids were consumed via fish. These studies were excluded from the current dose-response assessment for several reasons. The estimation of omega-3 fatty acid intake from fish is subject to measurement error; moreover, substituting fish for other protein sources, such as meat, introduces changes in a multiplicity of variables, making it difficult to discern the proportion of TG lowering that is attributable strictly to the long-chain omega-3 fatty acids. Furthermore, subject blinding and inclusion of a placebo group would be difficult to achieve in clinical studies in which omega-3 fatty acids are consumed via fish. Although fish studies were not included in the current assessment, it is noteworthy that dose-dependent reductions in TG levels have been demonstrated in studies in which low intakes of EPA and

DHA were from fish. For example, in a randomized parallel-design dose-response study involving 100 male adult subjects (mean age, 23.4 years) with normal fasting TG at baseline, the consumption of 0.9 servings of fish per day (equivalent to approximately 100 mg/day of the long-chain omega-3 fatty acids) was not associated with reductions in fasting TG; however, consumption of 1.5, 2.3, and 3.8 servings of fish per week (equivalent to approximately 170, 260, and 420 mg/day of the long-chain omega-3 fatty acids)[50] resulted in reductions in fasting TG of 13.4, 15.0, and 17.4%, respectively (corrected for the change in the control group).

Influence of baseline fasting serum TG level

The effects of long-chain omega-3 fatty acids on serum TG levels in subjects with normal serum TG levels are not well characterized in the published literature. Of the 15 randomized controlled trials included in the current assessment, five were conducted in subjects with normal baseline TG levels (≤150 mg/dL or ≤1.7 mmol/L). In one study,[32] no improvement in TG levels following the 6-week consumption of 460 mg/day DHA+EPA was reported. The results of this study[32] are subject to speculation for reasons already discussed. In the other four studies conducted in subjects with normal baseline fasting TG levels, fasting TG levels were reduced by 19.1 to 35.7% (after correcting for the placebo effect) following the 4- to 12-week consumption of 2.1–5.6 g/day of long-chain omega-3 fatty acids.[20,24,33,34] These results suggest that even subjects with normal fasting TG levels may benefit from the consumption of long-chain omega-3 fatty acids.

It has previously been reported that, at a fixed intake of long-chain omega-3 fatty acids, the magnitude of TG lowering is influenced by the baseline TG level[10]; thus, in the current assessment, predicted reductions in TG levels were calculated relative to baseline TG levels. Consequently, at a fixed intake of long-chain omega-3 fatty acids, a person with a higher baseline TG level will experience a greater absolute reduction in fasting serum TG than a person with a lower baseline TG level. For example, at the recommended long-chain omega-3 fatty acid intake of 200–500 mg/day, predicted reductions in fasting serum TG levels are 3.1 to 7.2%. In an individual with a TG level of 150 mg/dL (considered normal), the absolute reduction in fasting serum TG is predicted to be 4.65 to 10.8 mg/dL. In an individual with a TG level of 350 mg/dL (considered high), the absolute reduction in fasting serum TG is predicted to be 10.9 to 25.2 mg/dL. Improvements in TG levels are expected in all individuals, though those with higher baseline TG levels are expected to experience larger absolute reductions in TG levels.

Strengths of scientific assessment

There are several strengths associated with the current analysis. First, to be included in the assessment, each study had to report the effects of long-chain omega-3 fatty acids not only on fasting TG levels, but also on circulating levels of long-chain omega-3 fatty acids. Since it is well known that consumption of long-chain omega-3 fatty acids results in increased circulating levels, the reporting of such measures can be considered an objective measure of compliance in both the active treatment and the placebo groups. Second, to isolate the effects of long-chain omega-3 fatty acids on fasting TG from other experimental variables that may have influenced TG levels, net effects were calculated by subtracting the change from baseline in TG in the placebo group from the corresponding change in the active treatment group. Third, because studies were weighted according to their sample size and quality score (which was largely dependent on the methodological robustness of the study and whether potential confounding variables were adequately controlled for), studies with larger sample sizes and higher quality scores had the greatest influence in the nonlinear regression model.

CONCLUSION

Recommendations for long-chain omega-3 fatty acid intakes of 200–500 mg/day stem from consistent scientific evidence, suggesting a lowering of CHD risk with these intakes. While there are several biological mechanisms for the cardioprotective effects of the long-chain omega-3 fatty acids, their role in decreasing fasting TG levels has emerged as yet another plausible/additional mechanism. It is well established that intakes of large doses of long-chain omega-3 fatty acids reduce TG levels in subjects with very high baseline TG levels.[9,10] The current assessment demonstrates that TG levels are dose-dependently reduced by long-chain omega-3 fatty acids, and a mathematical model has been generated to estimate the percent change from baseline in fasting serum TG as a function of intake of long-chain omega-3 fatty acids. Using this mathematical model, at the recommended intakes of long-chain omega-3 fatty acids of 200–500 mg/day, predicted changes in fasting serum TG levels are −3.1 (95% CI, −5.9 to −0.31%) to −7.2% (95% CI, −11.6 to −2.5%). Since there is large day-to-day variability in fasting TG measures,[51] the clinical relevance of such reductions in fasting TG is difficult to interpret; nevertheless, significant evidence has accumulated indicating that fasting TG levels are an independent risk factor for cardiovascular disease.[1–4] Thus, efforts to reduce fasting TG levels, including the daily consumption of

200–500 mg EPA and/or DHA, may have significant public health benefits.

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