

Systematic Review with Meta-analysis

Impact of low v. moderate intakes of long-chain *n*-3 fatty acids on risk of coronary heart disease

Kathy Musa-Veloso^{1*}, Malcolm A. Binns², Alexandra Kocenas¹, Catherine Chung¹, Harry Rice³, Hilde Oppedal-Olsen⁴, Hilary Lloyd⁵ and Shawna Lemke⁶

¹Cantox Health Sciences International, An Intertek Company, 2233 Argentia Road, Suite 308, Mississauga, Ontario, Canada, L5N 2X7

²University of Toronto, Toronto, Ontario, Canada

³Global Organization for EPA and DHA Omega-3s (GOED), Salt Lake City, UT, USA

⁴Denomega Nutritional Oils AS, Sarpsborg, Norway

⁵Ocean Nutrition Canada, Dartmouth, Nova Scotia, Canada

⁶Monsanto, St Louis, MO, USA

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Abstract

The objective of the present study was to determine whether the consumption of ≥ 250 v. < 250 mg of the long-chain *n*-3 fatty acids (*n*-3 LCFA) per d is associated with a reduction in the risk of fatal and non-fatal CHD in individuals with no prior history of CHD. A comprehensive and systematic review of the published scientific literature resulted in the identification of eight prospective studies (seven cohorts and one nested case-control study) that met predefined inclusion criteria. Relative to the consumption of < 250 mg *n*-3 LCFA per d, the consumption of ≥ 250 mg/d was associated with a significant 35.1% reduction in the risk of sudden cardiac death and a near-significant 16.6% reduction in the risk of total fatal coronary events, while the risk of non-fatal myocardial infarction was not significantly reduced. In several meta-analyses, which were based on US studies, risk of CHD death was found to be dose-dependently reduced by the *n*-3 LCFA, with further risk reductions observed with intakes in excess of 250 mg/d. Prospective observational and intervention data from Japan, where intake of fish is very high, suggest that *n*-3 LCFA intakes of 900 to 1000 mg/d and greater may confer protection against non-fatal myocardial infarction. Thus, the intake of 250 mg *n*-3 LCFA per d may, indeed, be a minimum target to be achieved by the general population for the promotion of cardiovascular health.

Key words: EPA: DHA: *n*-3 Fatty acids: Heart disease: Coronary heart disease

CHD is the leading cause of illness and death in the UK, the USA and globally^(1–3). Presently, CHD causes 7.2 million deaths per year, accounting for approximately 35% of all CVD deaths and 10.5% of all deaths worldwide⁽¹⁾. Each year, approximately 137 500 individuals in the UK and 607 000 individuals in the USA die from CHD-related events^(1–3). The majority of identified CHD risk factors are modifiable, making CHD largely preventable⁽⁴⁾.

In 1978, Dyerberg *et al.*⁽⁵⁾ reported that Greenland Inuit have high circulating levels of the long-chain *n*-3 fatty acid (*n*-3 LCFA) EPA and a very low prevalence of atherosclerotic disease. Since the publication of this report, EPA, and its longer-chain counterpart, DHA, have been studied for their

potential roles in attenuating the risk of CHD. EPA and DHA can be synthesised from their essential 18-carbon precursor, α -linolenic acid via a series of desaturation and elongation steps; however, *in vivo* human studies have demonstrated that less than 5.0% of α -linolenic acid is converted to EPA, and less than 0.5% of α -linolenic acid is converted to DHA^(6,7). Consumption of dietary sources of pre-formed EPA and DHA may therefore be important for reducing the risk of CHD.

In subjects with no prior history of CHD, prospective observational studies are key in understanding the intake of the *n*-3 LCFA that is most likely to be cardioprotective. In prospective observational studies, intakes of the *n*-3 LCFA are assessed via

Abbreviations: HR, hazard ratio; *n*-3 LCFA, long-chain *n*-3 fatty acid; RR, relative risk.

* **Corresponding author:** Dr K. Musa-Veloso, fax +1 905 542 1011, email kmusa-veloso@cantox.com

some validated tool (typically a FFQ), and subjects are stratified according to their estimated intakes of the *n*-3 LCFA. The incidence of coronary events in subjects in the highest stratification is then compared relative to subjects in the lowest stratification. The interpretation of findings from different observational studies requires that intakes of the *n*-3 LCFA in the reference group be comparable. Thus, the primary objective of the present assessment was to determine whether the consumption of ≥ 250 mg of the *n*-3 LCFA per d is associated with a reduced risk of fatal or non-fatal coronary events relative to the consumption of < 250 mg/d in subjects with no prior history of CHD. The cut-off of 250 mg/d was chosen because this intake was recently determined to be cardioprotective by the European Food Safety Authority⁽⁸⁾ and by the North American branch of the International Life Sciences Institute (ILSI North America)⁽⁹⁾; the latter has endorsed an intake of 250–500 mg/d.

Methods

Identification and selection of studies

The original literature search was conducted in November 2008 using Dialog, an electronic searching tool, to access a number of databases including Medline[®], Agricola, Allied and Complementary Medicine Database[™], Manual, Alternative, and Natural Therapy[™] (MANTIS[™]), CAB Abstracts, Foodline[®]: Science, BIOSIS[®] previews, Federal Register, Elsevier Biobase, Foodline[®]: Market, Embase[®], DIOGENES[®] FDA (Food and Drug Administration) Regulatory Updates, FDAnews, Adis Clinical Trials Insight, and BCC Market Research. The exposure terms 'DHA', 'docosahexaenoic acid', 'EPA', 'eicosapentaenoic acid', 'fish oil' and 'omega 3' were searched in conjunction with the outcome terms 'heart disease', 'cardiovascular disease', 'coronary', 'sudden cardiac death', 'myocardial infarct', 'infarct', 'CVD', 'CHD', 'ischemia', 'ischemic', 'angina', 'arrhythmia', 'arrhythmias', 'arrhythmic', 'primary prevention', 'secondary prevention', 'stenosis', 'restenosis', 'atherosclerosis', 'atherosclerotic', 'plaque' and 'artery'. The literature search was restricted to human studies. Exposure and outcome terms were searched for in all fields available within each database. An updated literature search was conducted in August 2010 to identify any potentially pertinent studies published since the original literature search. The same exposure and outcome terms were used in the updated literature search; however, the search was restricted to human studies published in or subsequent to 2008.

Literature identified from the literature searches was filtered in three stages; at each stage of literature filtration, the study inclusion and exclusion criteria listed in Table 1^(10,11) were applied to determine the relevance of the study.

Stage 1. Titles of articles were reviewed, and abstracts of titles determined to be potentially relevant were retrieved.

Stage 2. Abstracts were reviewed, and full-length articles of abstracts determined to be potentially relevant were retrieved.

Stage 3. Full-length articles were reviewed, and those determined to meet all of the inclusion criteria and to not

meet any one of the exclusion criteria specified in Table 1 were included.

The original literature search, as well as the updated literature search, was conducted by A. K.

Extraction of data from studies

The data collected from the identified studies included the reference (first author's name and publication year), the name of the cohort (if applicable), the number of participants,

Table 1. Inclusion and exclusion criteria used to filter pertinent identified literature

Inclusion criteria
A study was included in the present assessment if it met all of the following inclusion criteria:
(a) It was a randomised controlled trial or prospective observational study (cohort or nested case–control);
(b) It was published in English as a full-length article in a peer-reviewed journal;
(c) Subjects included in the study were free of known CHD at baseline, though risk factors for CHD (obesity, hypertension, dyslipidaemia, type 2 diabetes mellitus, metabolic syndrome, etc) may have been present*;
(d) Effects or associations between long-chain <i>n</i> -3 fatty acids and either fatal and/or non-fatal coronary events were reported separately‡;
(e) For randomised controlled trials:
(i) The amount of long-chain <i>n</i> -3 fatty acids administered, the length of long-chain <i>n</i> -3 fatty acid supplementation, and associated tissue levels of long-chain <i>n</i> -3 fatty acids were quantified;
(ii) If a co-intervention was administered, the effects of EPA and DHA on CHD risk could be isolated from the effect of the co-intervention;
(iii) The placebo group consumed < 250 mg long-chain <i>n</i> -3 fatty acids/d, while the active treatment groups received ≥ 250 mg long-chain <i>n</i> -3 fatty acids/d
(f) For prospective observational studies:
(i) Food intake was assessed using a validated tool and intake of the long-chain <i>n</i> -3 fatty acids was quantified‡;
(ii) The reference group consumed < 250 mg long-chain <i>n</i> -3 fatty acids/d, while the comparator group(s) received ≥ 250 mg long-chain <i>n</i> -3 fatty acids/d
A study was excluded from the present analysis if:
(1) It was not primary research (for example, opinion letter, position statement, systematic review§, meta-analysis§);
(2) It was published in abstract form only;
(3) It was published in a language other than English;
(4) It was an animal or <i>in vitro</i> study;
(5) It was an uncontrolled human intervention study or a retrospective observational study;
(6) Outcomes were unrelated to either fatal or non-fatal coronary events ;
(7) The only source of <i>n</i> -3 fatty acids was provided as α -linolenic acid;
(8) The study was a kin publication without a unique dataset

* Studies conducted in subjects free of known heart disease at baseline were considered to be more applicable to the general population than studies conducted in subjects with established heart disease at baseline.

‡ Because the aetiology of fatal and non-fatal coronary events may be different, and because others have reported differing effects of the long-chain *n*-3 fatty acids on fatal v. non-fatal coronary events (He *et al.*⁽¹⁰⁾; Oh⁽¹¹⁾), a study was included in the present assessment only if it reported on each of these outcomes separately.

‡ To be included in the present assessment, measures of long-chain *n*-3 fatty acid intake had to be provided. Studies reporting intakes of fish or frequency of fish consumption were not included in the present assessment.

§ Although reviews and meta-analyses were not included in the present assessment, reference lists of these articles were scanned to ensure that all relevant publications were identified.

|| While CHD and cerebrovascular disease are both subsets of CVD, the present assessment was intended to assess the effects of the long-chain *n*-3 fatty acids EPA and DHA on the risk of CHD only.

Table 2. Characteristics of included studies

Reference	Country	Cohort	Subjects (n)	% Men	Age at baseline (years)	Duration of follow-up (years)	Exposure assessment			
							Frequency	Tool and validation	Database used to analyse intake data	Variables accounted for in the multivariate model
Albert <i>et al.</i> ⁽¹⁵⁾	USA	The US Physicians' Health Study	20 551	100	40–84	≤ 11	1; administered at 1 year	Validated self-administered semi-quantitative FFQ used to assess the frequency of fish and shellfish consumption*	Marine n-3 LCFA intakes were estimated using USDA and similar food composition tables†	Age; aspirin and β-carotene assignment; evidence of CVD before 12-month questionnaire; BMI; smoking status; history of diabetes, hypertension, and hypercholesterolaemia; alcohol consumption; vigorous exercise; use of vitamins C and E, and multivitamins
Ascherio <i>et al.</i> ⁽¹⁶⁾	USA	The Health Professionals' Follow-up Study	44 895	100	40–75	6	4; administered at study entry (1986) and follow-up (1988, 1990, 1992)	Validated self-administered semi-quantitative FFQ used to assess the frequency of fish and shellfish consumption*	Marine n-3 LCFA intakes were estimated using USDA and similar food composition tables†	Age; BMI; smoking; alcohol consumption; history of hypertension, diabetes, and hypercholesterolaemia; family history of MI before 60 years of age; profession; quintile group for intake of n-3 fatty acids
Dolecek & Grandits ⁽¹⁷⁾	USA	The Multiple Risk Factor Intervention Trial	6285‡	100	35–57	6–8	5; administered at study entry and follow-up (study years 1, 2, 3, 6)	24 h dietary recall for assessment of fish intake administered by interviewer	n-3 LCFA intakes were estimated using the University of Minnesota Nutrition Coordinating Center Food Table, version 11	Age; race; smoking; diastolic blood pressure; blood HDL- and LDL-cholesterol concentrations
Hu <i>et al.</i> ⁽¹⁸⁾	USA	Nurses' Health Study	84 688	0	34–59	16	5; administered at study entry (1980) and follow-up (1984, 1986, 1990, 1994)	Validated self-administered semi-quantitative FFQ used to assess the frequency of fish and shellfish consumption*	Marine n-3 LCFA intakes were estimated using USDA food composition tables and 1984 US landing data§	Age; time periods; smoking status; BMI; alcohol intake; menopausal status; postmenopausal hormone use; vigorous to moderate activity; aspirin use; multi vitamin use; vitamin E supplementation; history of hypertension, hypercholesterolaemia, or diabetes; intake of trans-fat, dietary fibre, and PUFA:SFA
Morris <i>et al.</i> ⁽¹⁹⁾	USA	The US Physicians' Health Study	21 185	100	40–84	4	1; administered at 1 year	Validated self-administered semi-quantitative FFQ used to assess the frequency of fish and shellfish consumption*	Marine n-3 LCFA intakes were estimated using USDA and similar food composition tables	Level of fish consumption; age; aspirin and β-carotene assignment; smoking; alcohol consumption; obesity; diabetes mellitus; vigorous exercise; parental history of MI before age 60 years; history of hypertension and hypercholesterolaemia; vitamin use; SFA intake

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Table 2. Continued

Reference	Country	Cohort	Subjects (n)	% Men	Age at baseline (years)	Duration of follow-up (years)	Exposure assessment			
							Frequency	Tool and validation	Database used to analyse intake data	Variables accounted for in the multivariate model
Mozaffarian <i>et al.</i> ⁽²⁰⁾	USA	The Cardiovascular Health Study	3910	39.1	72.5	9.3	1; administered at study entry	Validated self-administered semi-quantitative FFQ used to assess the frequency of fish and shellfish consumption*	n-3 LCFA intakes were estimated using the USDA and Harvard University food composition databases	Age; sex; education; diabetes; smoking; pack-years of smoking; tuna/other fish and fried fish/fish sandwich consumption; BMI; systolic blood pressure; blood HDL- and LDL-cholesterol concentrations; C-reactive protein; intake of SFA, alcohol, beef/pork, and fruits and vegetables
Mozaffarian <i>et al.</i> ⁽²¹⁾	USA	Health Professionals' Follow-up Study	10 982	100	40–75	14	4; administered at study entry (1986) and follow-up (1990, 1994, 1998)	Validated self-administered, picture-sort, semi-quantitative version of the NCI FFQ for assessment of usual intake of fish; non-picture sort for assessment of summary dietary measures†	Marine n-3 LCFA intakes were estimated using USDA food composition tables and 1984 US landing data§	Age; BMI; smoking; physical activity; history of diabetes, hypertension, and hypercholesterolaemia; aspirin use; alcohol use; intake of protein, SFA, dietary fibre, MUFA, trans-fatty acids, total energy, and ALA
Pietinen <i>et al.</i> ⁽²²⁾	Finland	The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study	21 930	100	50–69	5–8 (median 6.1)	1; administered at study entry	Validated self-administered, semi-quantitative, picture-sort FFQ for assessment of usual intakes of n-3 LCFA from fish**	n-3 LCFA intakes calculated using University of Helsinki food composition tables††	Age; smoking; BMI; blood pressure; intakes of energy, alcohol, and fibre; education; physical activity

LCFA, long-chain fatty acids; USDA, US Department of Agriculture; MI, myocardial infarction; NCI, National Cancer Institute; ALA, α -linolenic acid.

* Validated by comparing FFQ (administered twice with 1 year between administrations) with two 1-week dietary records (taken 6 months apart) and percentage of n-3 LCFA in adipose tissue in a random sample of 127 men aged 45 to 70 years and living in the Boston area.

† Four fish items were included: dark meat fish (such as bluefish), 1.37 g n-3 LCFA per portion; canned tuna, 0.69 g n-3 LCFA per portion; other fish, 0.17 g n-3 LCFA per portion; seafood (shrimp, lobster or scallops), 0.46 g n-3 LCFA per portion.

‡ Includes subjects who were in the control group of the intervention.

§ Four fish items were included: dark meat fish (mackerel, salmon, sardines, bluefish, or swordfish), 1.51 g n-3 LCFA per 84 to 140 g serving; canned tuna, 0.42 g n-3 LCFA per 84 to 112 g serving; other fish, 0.48 g n-3 LCFA per 84 to 140 g serving; seafood (shrimp, scallops, lobster), 0.32 g n-3 LCFA per 98 g serving. n-3 LCFA compositions were calculated by weighting the mean values of n-3 LCFA for the most common types of fish according to US landing data in 1984 (US Department of Commerce).

|| Four fish items were included (serving sizes, but not n-3 LCFA content per serving were included in the publication): dark meat fish (mackerel, salmon, sardines, bluefish, swordfish), 113 to 170 g/serving; canned tuna, serving size not specified; other fish, 113 to 170 g/serving; seafood (shrimp, lobster, or scallops, as main dish), serving size not specified.

¶ The semi-quantitative picture-sort FFQ version was validated against 24 h dietary recalls and against plasma phospholipid EPA and DHA in fifty-six participants.

** Validated in a pilot study in which baseline and end-of-treatment FFQ results were compared with 24 daily food records (spread out over 6 months) in a similar sample population of men.

†† The food composition table contained data for thirty-three types of fresh and processed fish, fish liver, and roe commodities from the Helsinki area.

the proportion of men, the mean age or age range of the participants, the duration of follow-up, the number of times exposure (i.e. intake of EPA and DHA) was assessed, the methods used to assess the exposure and the outcome (i.e. incidence of fatal or non-fatal coronary events), the count of events, the number of person years, and the different covariates for which relative risk (RR) or hazard ratio (HR) rates were adjusted. The studies were summarised in alphabetical order, according to the last name of the first author. Relevant data were extracted independently by two individuals (A. K. and K. M.-V.).

Statistical analyses

For strata corresponding to an intake of less than 250 mg/d and for strata corresponding to an intake of 250 mg/d or more, the cardiac event counts were pooled, as were the number of person years. To take into account adjustments made to the RR or HR in the multivariate models, rather than pooling the raw event counts, the adjusted RR or HR were used to calculate a 'pseudo number of events' in each stratum⁽¹²⁾; pseudo event counts were then used to arrive at a pooled count of pseudo events for the n-3 LCFA intakes of interest (i.e. < 250 mg/d and ≥ 250 mg/d) using the following rationale. The simple RR for the *i*th level of an exposure variable is defined as:

$$RR_i = \frac{\text{events}_i / \text{person years}_i}{\text{events}_0 / \text{person years}_0},$$

and solving for the event count in the *i*th level yields:

$$\text{events}_i = \frac{\text{events}_0}{\text{person years}_0} \times RR_i \times \text{person years}_i.$$

Given fixed person years for both target and reference strata and a fixed event count in the reference stratum, a pseudo event rate was calculated that would be expected to produce the reported adjusted RR (*aRR*_{*i*}) as follows:

$$\text{pseudo events}_i = \frac{\text{events}_0}{\text{person years}_0} \times aRR_i \times \text{person years}_i.$$

RR or HR for each study were calculated from the pooled pseudo event count and person years or persons, if data on person years were not reported. RR of sudden cardiac death, fatal coronary events, and non-fatal myocardial infarction in subjects consuming 250 mg/d or more *v.* < 250 mg/d of the n-3 LCFA were calculated using Comprehensive Meta Analysis Software (version 2.2.046; Biostat Inc., Englewood, NJ, USA). Because the studies identified were conducted in populations with ethnic, cultural, and socio-economic diversities, the random-effects model (which assumes that the populations studied differed from each other in ways that could make an impact on the risk of fatal coronary events)⁽¹³⁾ was chosen.

Results

The literature search resulted in the identification of 4828 unique titles, of which 672 were determined to be potentially

relevant. Abstracts of articles determined to be relevant were reviewed, and potentially pertinent articles (*n* 20) were subsequently retrieved and reviewed for inclusion in or exclusion from the present analysis. Of the twenty full publications retrieved, nine met the inclusion criteria specified in Table 1; however, one of these studies⁽¹⁴⁾ could not be included in the present meta-analysis, given that neither the number of persons nor the number of person years in each stratification was reported. No attempt was made to obtain these data from the authors. The additional literature search conducted in August 2010 resulted in the identification of 753 unique titles. Of these identified titles, forty-five were determined to be potentially relevant and their abstracts were reviewed (A. K.). Based on their abstracts, fourteen potentially pertinent full publications were retrieved and screened using the inclusion and exclusion criteria specified in Table 1; none met all of the inclusion criteria. Thus, the present meta-analysis included only studies identified in the literature search that was conducted in November 2008.

All eight of the publications identified were prospective cohort studies (Table 2)^(15–22). Of these, seven studies were conducted in the USA and one was conducted in Finland. The cohort studies varied in length of follow-up from 4 to 16 years (Table 2). Altogether, the studies evaluated the experience of 214 426 individuals aged 34 to 84 years at baseline (Table 2). Of the studies, one was based on a cohort made up exclusively of women (the Nurses' Health Study⁽¹⁸⁾); six publications were based on cohorts made up exclusively of men (the Health Professionals' Follow-up Study^(16,21), the Physicians' Health Study^(15,19), the Multiple Risk Factor Intervention Trial⁽¹⁷⁾ and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study⁽²²⁾); one publication was based on a nested case-control study made up of men and women (the Cardiovascular Health Study⁽²⁰⁾). All of the eight publications used validated methods to assess n-3 LCFA intakes (Table 2).

The CHD outcomes assessed in each of the eight observational studies are summarised in Table 3^(15–22). The ranges of n-3 LCFA intakes in each of the eight observational studies, as well as the pooled pseudo event counts and person years are summarised in Tables 4^(15,20,21), 5^(16–18,20,22,23) and 6^(16,18–21,23) for sudden cardiac death, fatal coronary events and non-fatal myocardial infarction, respectively.

The association between n-3 LCFA intake and risk of sudden cardiac death was assessed in three studies. In these three studies, sudden cardiac death was defined as death within 1 h of symptom onset^(15,21) or death within 5 min of symptom onset⁽²⁰⁾. As can be seen in Fig. 1, across the three studies, the RR of death from a sudden cardiac event was significantly lower in subjects who consumed ≥ 250 mg n-3 LCFA/d relative to subjects who consumed < 250 mg n-3 LCFA/d (RR 0.649; 95% CI 0.535, 0.786; *P* < 0.0001).

The association between n-3 LCFA intake and the risk of fatal coronary events was assessed in five studies. In four of these studies, the definition of fatal coronary events included (but was not limited to) sudden cardiac death^(16–18,22); in the fifth study, the definition of fatal coronary events did not

Table 3. CHD outcomes assessed in each study

Reference	Outcomes measured	Outcome classification		
		Fatal coronary events	Sudden cardiac death	Non-fatal coronary events
Albert <i>et al.</i> ⁽¹⁵⁾	Sudden death (all deaths occurring within 1 h of symptom onset and/or witnessed cardiac arrest)		✓	
Ascherio <i>et al.</i> ⁽¹⁶⁾	Fatal CHD (death due to CHD; includes sudden deaths (deaths within 1 h of symptom onset))	✓		
Dolecek & Grandits ⁽¹⁷⁾	Non-fatal MI (WHO criteria*)			✓
Hu <i>et al.</i> ⁽¹⁸⁾	Fatal CHD (unspecified ICD-9 codes)	✓		
Morris <i>et al.</i> ⁽¹⁹⁾	Fatal CHD (fatal MI or when CHD was the plausible/presumed cause of death)	✓		
Mozaffarian <i>et al.</i> ⁽²⁰⁾	Non-fatal MI (definite and probable MI (WHO criteria*)†)			✓
	Non-fatal MI (WHO criteria*)			✓
	Total IHD death (fatal MI or CHD death)	✓		
	Non-fatal MI (WHO criteria*)			✓
	Arrhythmic IHD death (fatal MI or CHD death with arrhythmia preceding or occurring within 5 min of symptoms)		✓	
Mozaffarian <i>et al.</i> ⁽²¹⁾	Non-fatal MI (WHO criteria*, plus blood troponin measurements)			✓
	Incident sudden death (diagnosed when CHD death occurred within 1 h of symptom onset, with no other plausible cause of death)		✓	
Pietinen <i>et al.</i> ⁽²²⁾	Fatal CHD (ICD-9 410–414)	✓		

MI, myocardial infarction; ICD, International Classification of Diseases; ECG, electrocardiogram.

* WHO criteria for MI: chest pain, the presence of cardiac enzymes in blood samples, and ECG changes.

† Definite non-fatal MI, diagnosed using records of self-reported MI confirmed by using the WHO criteria; probable non-fatal MI, MI with hospital admission but no medical records for verification.

include sudden cardiac death⁽²⁰⁾. As can be seen in Fig. 2, across the five studies, the RR of death from a fatal coronary event was nearly significantly lower in subjects who consumed ≥ 250 mg *n*-3 LCFA/d relative to subjects who consumed < 250 mg *n*-3 LCFA/d (RR 0.834; 95% CI 0.679, 1.025; $P=0.085$).

The association between *n*-3 LCFA intake and the risk of non-fatal coronary events was assessed in five studies. In all five studies, the outcome assessed was non-fatal myocardial infarction^(16,18–21). As can be seen in Fig. 3, across the five studies, there was no significant difference in the risk of a non-fatal myocardial infarction between subjects who consumed ≥ 250

mg *n*-3 LCFA/d relative to subjects who consumed < 250 mg *n*-3 LCFA/d (RR 0.934; 95% CI 0.824, 1.060; $P=0.290$).

Discussion

Strengths and limitations of the present meta-analysis

The present meta-analysis is associated with several strengths. Studies that reported the intake of fish only, rather than actual intakes of the *n*-3 LCFA, were excluded from the assessment, given that the *n*-3 LCFA composition of fish can vary substantially, particularly between fatty fish and

Table 4. Sudden cardiac death – pooled pseudo events and person years at intakes of < 250 v. ≥ 250 mg/d

Reference	Long-chain <i>n</i> -3 fatty acid intake (mg/d)	Adjusted RR	95% CI	<i>P</i> (trend)	Pooled results		
					Intake (mg/d)	Pseudo event count	Person years
Albert <i>et al.</i> ⁽¹⁵⁾	<10.0 †	1	Reference	0.21	<250	119	190957
	10.0– <90.0 †	0.58	0.28, 1.21				
	90.0– <163.3 †	0.34*	0.15, 0.75				
	163.3– <246.7 †	0.60	0.29, 1.27				
	≥ 246.7 †	0.43*	0.20, 0.93				
Mozaffarian <i>et al.</i> ⁽²⁰⁾	0	1	Reference	0.001*	≥ 250	32	62820
	128	0.86	0.45, 1.63				
	267	0.81	0.40, 1.66				
	547	0.50	0.23, 1.07				
	919	0.32*	0.15, 0.70				
Mozaffarian <i>et al.</i> ⁽²¹⁾	<11.2 g <i>n</i> -6/d, <250 mg EPA + DHA/d	1	Reference	NA	<250	114	23111‡
	≥ 11.2 g <i>n</i> -6/d, <250 mg EPA + DHA/d	0.76	0.52, 1.11				
	<11.2 g <i>n</i> -6/d, ≥ 250 mg EPA + DHA/d	0.52*	0.34, 0.79				
	≥ 11.2 g <i>n</i> -6/d, ≥ 250 mg EPA + DHA/d	0.60*	0.39, 0.93				

RR, relative risk; NA, not applicable.

* $P < 0.05$.

† Daily intake was calculated by dividing the given monthly intake (0.3–7.4 g marine *n*-3 fatty acids/month) by 30 and multiplying by 1000.

‡ Persons were reported, not person years.

Table 5. Fatal coronary events – pooled pseudo events and person years at intakes of < 250 v. ≥ 250 mg/d

Reference	Long-chain n-3 fatty acid intake (mg/d)	Adjusted RR	95% CI	P (trend)	Pooled results		
					Intake (mg/d)	Pseudo event count	Person years
Ascherio <i>et al.</i> ⁽¹⁶⁾	70	1	Reference	1.0	<250	152	148 964
	150	1.14	0.78, 1.66				
	240	0.96	0.65, 1.42				
	340	1.04	0.71, 1.53				
	580	1.06	0.72, 1.55				
Dolecek & Grandits ⁽¹⁷⁾	0.0	1	Reference	0.0150*	<250	155	5004†
	9.0	1.01	0.66, 1.56‡				
	46	0.87	0.56, 1.35‡				
	153	0.87	0.56, 1.35‡				
	664	0.59*	0.36, 0.98‡				
Hu <i>et al.</i> ⁽¹⁸⁾	77§	1	Reference	0.002*	<250	263	1 048 917
	118§	0.93	0.70, 1.24				
	171§	0.69*	0.50, 0.94				
	221§	0.54*	0.38, 0.75				
	481§	0.62*	0.44, 0.88				
Mozaffarian <i>et al.</i> ⁽²⁰⁾	0	1	Reference	0.002*	<250	114	11 480
	128	0.78	0.47, 1.28				
	267	0.77	0.45, 1.32				
	547	0.53*	0.30, 0.96				
	919	0.47*	0.27, 0.82				
Pietinen <i>et al.</i> ⁽²²⁾	200	1	Reference	0.118	<250	126	26 032
	300	0.93	0.72, 1.20				
	400	0.98	0.76, 1.27				
	500	1.07	0.83, 1.37				
	800	1.24	0.97, 1.58				

RR, relative risk.

* P < 0.05.

† Persons were reported, not person years.

‡ CI values were not provided and so were calculated from the number of events and person years provided in the report.

§ Actual long-chain n-3 fatty acid intakes were reported in the Iso *et al.*⁽²³⁾ study; in the Hu *et al.*⁽¹⁸⁾ study, long-chain n-3 fatty acid intakes were expressed only as median intake, percentage of energy.

lean white fish. Moreover, as the minimum effective intake of the n-3 LCFA for reducing the risk of CHD may vary according to history of CHD and according to whether the event is fatal or non-fatal, studies were included only if subjects recruited were free of known CVD at baseline and if associations were reported separately for fatal and non-fatal coronary events. Furthermore, for a study to be included, the reference or comparator group had to have an n-3 LCFA intake of < 250 mg/d. This latter criterion is important, given that in populations with already high background intakes of the n-3 LCFA (i.e. ≥ 250 mg/d), further reductions in the risk of CHD may not be discernable. In the present analysis, eight prospective studies were found to meet these and additional inclusion criteria and to contain information sufficient for the pooling of pseudo event counts and person years across the different intake strata (i.e. < 250 v. ≥ 250 mg n-3 LCFA/d).

A notable limitation of the present analysis is that, within a study, the pooling and defining of n-3 LCFA intakes as '< 250 mg/d' and '≥ 250 mg/d' were based on mean or median n-3 LCFA intakes, typically reported as quintiles. For example, in the study by Morris *et al.*⁽¹⁹⁾, n-3 LCFA intakes were reported as ranges across five quintiles: < 71.4 mg/d; 71.4 to < 142.9 mg/d; 142.9 to < 242.9 mg/d; 242.9 to < 328.6 mg/d; and ≥ 328.6 mg/d. Consequently, the RR of non-fatal coronary events with consumption of ≥ 250 v. < 250 mg/d was calculated as the pooled risk in the

fourth and fifth quintiles relative to the pooled risk in the first three quintiles. While subjects in the first three quintiles clearly had an n-3 LCFA intake of < 250 mg/d, some subjects in the fourth quintile also may have had an n-3 LCFA intake of < 250 mg/d. Thus, across the studies, the categorisation of intakes as '< 250 mg/d' and '≥ 250 mg/d' represents approximations.

All eight studies included in the present assessment were prospective, observational studies in which consumption of the n-3 LCFA was estimated from self-administered semi-quantitative FFQ^(15,16,18–22) or dietary recall⁽¹⁷⁾. As in all observational studies, there is always the potential for measurement error with respect to the exposure of interest. One of the criteria for study inclusion was that food intake had to be assessed using a validated tool and intake of the n-3 LCFA had to be quantified. Several of the studies were found to use the same tool to assess n-3 LCFA intakes^(15,16,18–20). Only marine sources of the n-3 LCFA were considered in the majority of the studies; other sources of pre-formed n-3 LCFA, such as eggs, were not considered in the majority of the analyses. Likewise, in none of the analyses was the conversion of α-linolenic acid to the n-3 LCFA considered. Despite these limitations, estimated intakes of the n-3 LCFA correlated well with the percentage of n-3 LCFA in adipose tissue^(15,16,18–20) and in plasma phospholipids⁽²¹⁾, indicating that the estimated intakes were likely to be reasonable and representative of actual intakes.

Table 6. Non-fatal myocardial infarction – pooled pseudo events and person years at intakes of < 250 v. ≥ 250 mg/d

Reference	Long-chain <i>n</i> -3 fatty acid intake (mg/d)	Adjusted RR	95% CI	<i>P</i> (trend)	Pooled results		
					Intake (mg/d)	Pseudo event count	Person years
Ascherio <i>et al.</i> ⁽¹⁶⁾	70	1	Reference	0.44	<250	322	148 964
	150	0.93	0.72, 1.21				
	240	0.89	0.68, 1.16				
	340	0.78	0.59, 1.03				
	580	1.09	0.85, 1.41				
Hu <i>et al.</i> ⁽¹⁸⁾	77†	1	Reference	0.003*	<250	656	1 048 917
	118†	0.93	0.76, 1.15				
	171†	0.84	0.68, 1.05				
	221†	0.78*	0.62, 0.98				
	481†	0.73*	0.57, 0.93				
Morris <i>et al.</i> ⁽¹⁹⁾	<71.4‡	1	Reference	0.99	<250	153	65 254
	71.4– < 142.9‡	1.5	1.0, 2.3				
	142.9– < 242.9‡	1.3	0.9, 2.0				
	242.9– < 328.6‡	1.2	0.8, 1.9				
	≥ 328.6‡	1.1	0.7, 1.8				
Mozaffarian <i>et al.</i> ⁽²⁰⁾	0	1	Reference	0.10	<250	126	10 964
	128	0.81	0.51, 1.26				
	267	0.71	0.44, 1.15				
	547	0.75	0.46, 1.21				
	919	0.67	0.42, 1.07				
Mozaffarian <i>et al.</i> ⁽²¹⁾	< 11.2 g <i>n</i> -6/d, < 250 mg EPA + DHA/d	1	Reference	NA	<250	769	23 111§
	≥ 11.2 g <i>n</i> -6/d, < 250 mg EPA + DHA/d	1.09	0.93, 1.28				
	< 11.2 g <i>n</i> -6/d, ≥ 250 mg EPA + DHA/d	1.16	0.99, 1.36				
	≥ 11.2 g <i>n</i> -6/d, ≥ 250 mg EPA + DHA/d	1.09	0.91, 1.29				

RR, relative risk; NA, not applicable.

* *P* < 0.05.† Actual long-chain *n*-3 fatty acid intakes were reported in the Iso *et al.*⁽²³⁾ study; in the Hu *et al.*⁽¹⁸⁾ study, long-chain *n*-3 fatty acid intakes were expressed only as median intake, percentage of energy.‡ Daily intake calculated by dividing given weekly intake (0.5–2.3 g *n*-3 fatty acids/week) by 7 and multiplying by 1000.

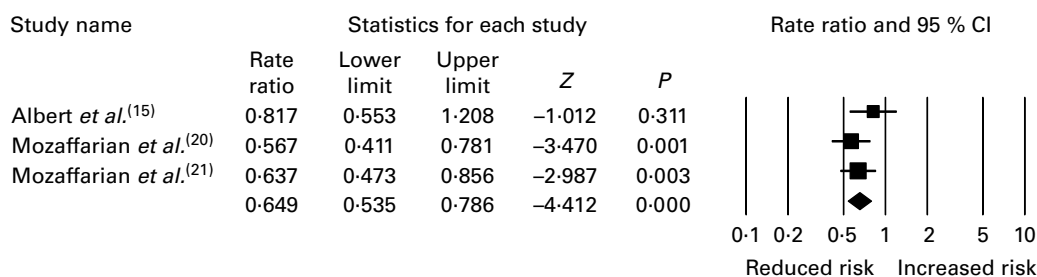
§ Persons were reported, not person years.

Effects on fatal coronary events

It has been determined that relative to the consumption of < 250 mg of the *n*-3 LCFA/d, the consumption of ≥ 250 mg of the *n*-3 LCFA/d was associated with a significant 35.1% (*P* < 0.0001) reduction in the risk of sudden cardiac death and a near-significant 16.7% (*P* = 0.085) reduction in the risk of total fatal coronary events. Females represented 6.7% of the subjects on which the assessment of sudden cardiac death risk was based and 53.8% of the subjects on which the assessment of fatal CHD risk was based. Of the five studies included in the assessment of fatal CHD risk, all but one included sudden cardiac death in their definition of fatal CHD; thus, despite the low representation of women in the sudden cardiac death risk assessment, it is likely that the finding of a significant 35.1% reduction in the risk of sudden cardiac

death is applicable to both males and females. Therefore, relative to the consumption of < 250 mg of the *n*-3 LCFA/d, consumption of ≥ 250 mg of the *n*-3 LCFA/d is associated with a significant reduction in the risk of sudden cardiac death and a near-significant reduction in the risk of total fatal coronary events.

There is evidence that further reductions in the risks of sudden cardiac death or fatal coronary events are possible at intakes of *n*-3 LCFA above 250 mg/d. In three of the five observational studies included in the present fatal coronary events meta-analysis, statistically significant inverse trends were noted between *n*-3 LCFA intakes and risk of fatal coronary events above an intake of 250 mg EPA and DHA/d^(17,18,20). Moreover, in a recent meta-analysis of six US epidemiological studies⁽²⁴⁾, a significant inverse dose–response between intake of the *n*-3 LCFA beyond 250 mg/d and risk of CHD

**Fig. 1.** Risk of sudden cardiac death at a long-chain *n*-3 fatty acid intake of ≥ 250 v. < 250 mg/d.

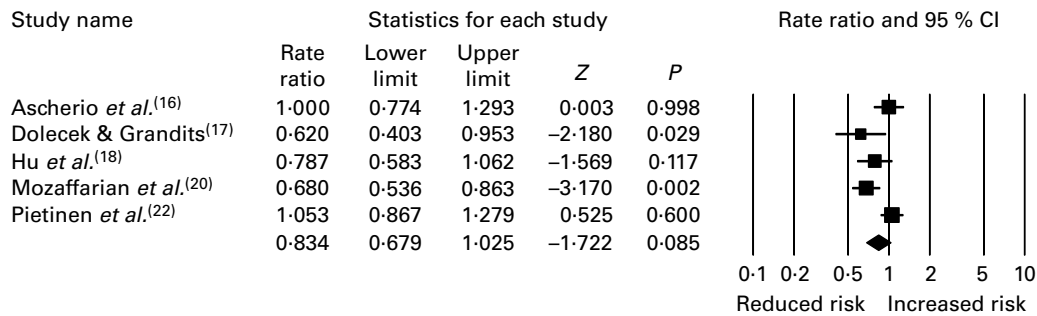


Fig. 2. Risk of fatal coronary events at a long-chain n-3 fatty acid intake of ≥ 250 v. < 250 mg/d.

death in subjects previously free of CHD was noted. Likewise, in three meta-analyses^(10,25,26), incremental decreases in risk of CHD death have been reported with fish intakes greater than one portion of 170 g per week (an amount cited by Mozaffarian & Rimm⁽²⁷⁾ to be approximately equivalent to an intake of 250 mg n-3 LCFA/d).

Several population-based studies conducted in Japan, a country with very high background dietary intakes of the n-3 LCFA, suggest that n-3 LCFA intakes are not associated with reductions in risk of fatal coronary events; however, the results of these studies are difficult to interpret as n-3 LCFA intakes were very high, even in the reference groups. In the Japan Public Health Center-Based Study⁽²⁸⁾, in which 41 578 Japanese men and women aged 40 to 59 years at baseline were followed, for 11 years, no significant association was observed between n-3 LCFA intake and risk of sudden cardiac death or risk of fatal coronary events. The median intake of EPA + DHA in the lowest (reference) intake group was 300 mg/d. According to the results of the present assessment, everyone would have been protected from sudden cardiac death and fatal coronary events – a fact supported by the finding of only thirty-seven cases of sudden cardiac death and sixty-two cases of fatal coronary events in the entire cohort of 41 578 subjects. Likewise, in the Japan Collaborative Cohort Study for Evaluation of Cancer Risk, in which 57 972 Japanese men and women were followed for 12.7 years, no significant reduction in risk of death from cardiac arrest, IHD or myocardial infarction was noted in subjects consuming increasing amounts of the n-3 LCFA; however, in the lowest intake group (the reference quintile), intakes of the n-3 LCFA were already at approximately 1000 mg/d⁽²⁹⁾.

Likewise in the Takayama Study, in which 29 079 Japanese men and women were followed for 7 years, a high intake of the n-3 LCFA in the first/reference quintile (i.e. 410 mg/d for males and 332 mg/d for females) probably precluded the observation of an effect of the fatty acids in attenuating the risk of CVD mortality⁽³⁰⁾. In all of these prospective cohort studies, the minimum effective dose required for protection from CHD death, which is proposed to be 250 mg/d, was already being consumed by the vast majority of the population.

In the Japan EPA Lipid Intervention Study, which was a randomised, open-label, blinded study conducted in subjects with elevated serum total and LDL-cholesterol concentrations (≥ 6.5 and 4.4 mmol/l, respectively), subjects were randomised to receive either statins alone or in combination with 1800 mg EPA/d⁽³¹⁾. In a subset of the subjects with no known history of coronary artery disease (n 14 981), no significant reductions in either sudden cardiac death or fatal myocardial infarction were observed in the EPA group relative to the control group⁽³¹⁾. Based on the reported intakes of the n-3 LCFA in Japanese prospective cohort studies^(28–30), it is assumed that subjects in the control group would have already been consuming > 250 mg n-3 LCFA/d from their background diets, and so would have already been protected against sudden cardiac death and fatal coronary events, as predicted by the results of the present analysis.

Effects on non-fatal coronary events

In the present assessment, n-3 LCFA intakes of 250 mg/d or more did not reduce the risk of non-fatal myocardial infarction relative to intakes of < 250 mg/d. All of the studies in which

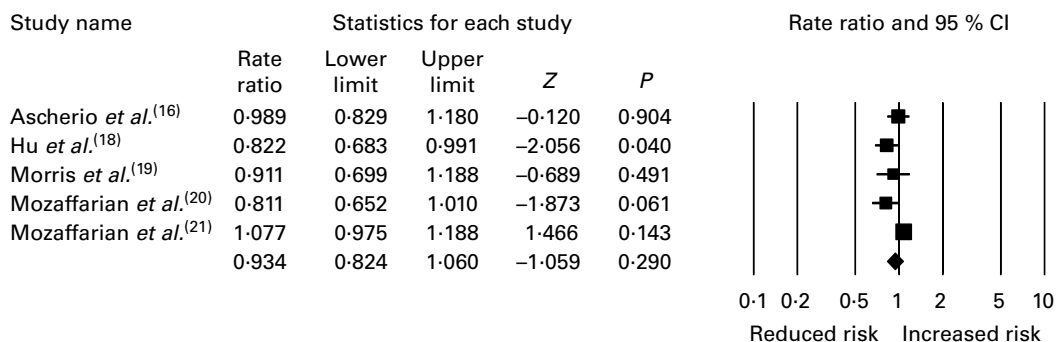


Fig. 3. Risk of non-fatal myocardial infarction at a long-chain n-3 fatty acid intake of ≥ 250 v. < 250 mg/d.

the risk of non-fatal coronary events was assessed were conducted in the USA. The highest *n*-3 LCFA intake reported was 919 mg/d by Mozaffarian *et al.*⁽²⁰⁾. It is indicated from data from other studies that for a reduction in the risk of non-fatal coronary events, *n*-3 LCFA intakes of approximately 900 to 1000 mg/d may be required. In the Japan Public Health Center-Based Study⁽²⁸⁾, the risk of non-fatal myocardial infarction was significantly reduced with intakes of EPA + DHA > 600 mg/d, with a significant dose–response noted. At an intake of 900 mg of the *n*-3 LCFA/d, risk of non-fatal myocardial infarction was reduced by 39%, while at intakes of 1300 and 2100 mg/d, risk was reduced by 43 and 67%, respectively. In the meta-analysis conducted by He *et al.*⁽¹⁰⁾, the risk of non-fatal myocardial infarction was found to be significantly reduced (by 21%) only in the highest intake group (fish intake \geq 5 times per week) compared with those consuming fish less than once per month. In the Japan EPA Lipid Intervention Study, amongst subjects with no known history of coronary artery disease (*n* 14 981), the risk of non-fatal coronary events was nearly significantly reduced in patients in the 1800 mg EPA/d group relative to patients in the control group (HR 0.80; 95% CI 0.61, 1.05; *P*=0.102)⁽³¹⁾. In a sub-analysis of these subjects with high TAG levels and low HDL-cholesterol levels (\geq 1500 and < 400 mg/l, respectively), the risk of total coronary events was significantly reduced by 53% in the EPA group relative to the control group (HR 0.47; 95% CI 0.23, 0.98; *P*=0.043)⁽³²⁾. Included in the definition of ‘total’ coronary events were fatal and non-fatal coronary events, and risk according to the type of coronary event was not reported⁽³²⁾. Based on incidence data reported by Yokoyama *et al.*⁽³¹⁾ for the entire cohort of subjects free of heart disease at baseline, there were 214 non-fatal coronary events and twenty-one fatal coronary events. Therefore, it is likely that the significant risk reduction noted in the subset of subjects with high TAG levels and low HDL-cholesterol levels administered EPA was due to a reduction in non-fatal coronary events. Additional studies will be helpful in understanding whether the risk of non-fatal myocardial infarction can be reduced with greater intakes of the *n*-3 LCFA in subjects with no known history of CHD. This conclusion is in agreement with other recent critical reviews^(25,27).

Implications of findings

The Technical Committee on Dietary Lipids of ILSI North America recently recommended that the dietary reference intake for the *n*-3 LCFA should be 250–500 mg/d⁽⁹⁾. The European Food Safety Authority has proposed 250 mg of the *n*-3 LCFA/d as the labelling reference intake value, based on their conclusion that this intake would be important for the maintenance of cardiovascular health and that little additional benefit has been observed at higher intakes⁽⁸⁾. From a public health perspective, it would be useful to understand whether an *n*-3 LCFA intake of 250 mg/d should be considered an absolute target intake or a minimum target intake.

The present meta-analysis indicates that relative to the consumption of < 250 mg of the *n*-3 LCFA/d, consumption of \geq 250 mg of the *n*-3 LCFA/d is associated with a significant

reduction in the risk of sudden cardiac death. Insufficient data precluded a robust assessment of whether intakes of the *n*-3 LCFA in excess of 250 mg/d would be associated with further reductions in the risk of sudden cardiac death or other fatal coronary events compared with an intake of 250 mg/d. Data from Japanese prospective cohort and observational studies suggest that increased intakes of the *n*-3 LCFA are not associated with further reductions in the risk of fatal coronary events; however, *n*-3 LCFA intakes in the reference groups were very high, thereby limiting the interpretation of the study findings^(28–31). Several American prospective observational studies and meta-analyses indicate that the risk of death from CHD is further reduced with intakes of the *n*-3 LCFA in excess of 250 mg/d^(17,18,20,24). Taking into account these observations as well as emerging data that the risk of non-fatal coronary events may be reduced with *n*-3 LCFA intakes of 900 to 1000 mg/d^(10,28,31,32), it appears that 250 mg/d could be considered a minimum target intake, rather than an absolute target.

Mozaffarian & Rimm⁽²⁷⁾ concluded that above an intake of 250 mg of the *n*-3 LCFA/d, risk of CHD death was not further attenuated. This conclusion was considered by the European Food Safety Authority and probably had an impact on the selection of 250 mg/d as the absolute labelling reference intake value for the *n*-3 LCFA, as well as their conclusion that *n*-3 LCFA intakes in excess of 250 mg/d are probably inconsequential with regards to further reducing the risk of CHD⁽⁹⁾. The dose–response assessment conducted by Mozaffarian & Rimm⁽²⁷⁾ is impressive in that reductions in the risk of CHD death were apparent, despite combining results from primary and secondary prospective cohort and randomised controlled trials conducted throughout the world. However, given the multitude of assumptions on which the dose–response assessment was based, it is not possible to consider the *n*-3 LCFA intake of 250 mg/d as an absolute efficacious dose above which there are no additional benefits. The limitations of the dose–response assessment include the following:

- (1) In several of the studies included by Mozaffarian & Rimm⁽²⁷⁾, only the intake or frequency of intake of fish was reported. In some cases, the level of detail collected with respect to the type of fish consumed was so minimal that the derivation of *n*-3 LCFA intakes would be associated with considerable measurement error. For example, in the prospective cohort study by Osler *et al.*⁽³³⁾, only one question was used to assess the frequency of fish consumption, and the authors themselves commented that ‘it was not possible to separate intake of fish into white and fatty types’. Thus, it is unclear how *n*-3 LCFA intakes were estimated by Mozaffarian & Rimm⁽²⁷⁾ in their dose–response assessment. Of the twenty studies included in their dose–response assessment, in six studies, insufficient data precluded a robust estimation of *n*-3 LCFA intake^(33–38). Thus, in these studies, the quantification of *n*-3 LCFA intakes may be associated with considerable measurement error and misclassification of exposure.

- (2) As already described, the protective effects of the *n*-3 LCFA against CHD death are not discernable in studies in which even the lowest intake/reference group is already consuming excessive amounts of the *n*-3 LCFA^(28–31). Inclusion of such studies in a dose–response assessment may cause the cardioprotective benefits of the *n*-3 LCFA at higher doses to be indiscernible. Although Mozaffarian & Rimm⁽²⁷⁾ applied a scaling factor of 0.7 to studies where reference group *n*-3 LCFA intakes were between 150 and 500 mg/d and of 0.6 to studies where reference group *n*-3 LCFA intakes were > 500 mg/d, the rationale for these cut-offs is unclear, particularly as a significant reduction in the risk of CHD would be expected at both reference group intakes. The exclusion of studies in which the reference group *n*-3 LCFA intake was in excess of 150 mg/d reportedly had no bearing on the dose–response assessment⁽²⁷⁾; however, it is unclear why an intake of 150 mg/d was chosen as the cut-off.
- (3) In the initial dose–response, which included subjects with and without a known history of coronary artery disease, there was a clear attenuation in risk of CHD death up to an *n*-3 LCFA intake of 250 mg/d, with no further risk reductions with higher intakes⁽³¹⁾ (Fig. 4(a)). In a subsequent dose–response assessment, which was

restricted to studies conducted in subjects with no known history of CHD⁽⁹⁾, there was a continuous reduction in the risk of CHD death up to and beyond an *n*-3 LCFA intake of 500 mg/d, despite the maintenance, in the dose–response assessment, of the Japanese studies in which *n*-3 LCFA intakes in the reference group were well in excess of the intake presumed to be efficacious (Fig. 4(b)). The results of the second dose–response assessment suggest that for the population at large, 250 mg of the *n*-3 LCFA/d should be the minimum target intake, and not the absolute target intake. For the purposes of establishing recommended intakes for the general population, the most representative and relevant studies are those in which subjects were free of known CHD upon study entry⁽²⁴⁾.

Concluding remarks

Data from the present assessment support a significant 35.1% reduction in the risk of sudden cardiac death and a near-significant 16.6% reduction in the risk of non-fatal coronary events with the consumption of ≥ 250 mg of the *n*-3 LCFA/d relative to the consumption of < 250 mg/d, in subjects previously free of known CHD. Ideally, support for these effects should come from randomised controlled trials; however, it is difficult to conduct such studies wherein the primary endpoint of interest is CHD in subjects free of known CHD at baseline. Observational studies allow for prolonged follow-up in a more representative sample of the population, under conditions more typical than those found in a controlled clinical trial. Moreover, restricting the assessment to prospective studies ensured that biases common to retrospective studies, such as recall bias and selection bias, were limited.

While there were insufficient data in the present analysis to determine whether *n*-3 LCFA intakes in excess of 250 mg/d elicit further reductions in the risk of either fatal or non-fatal coronary events, several American studies and meta-analyses suggest that, indeed, with intakes in excess of 250 mg/d, additional reductions in the risk of death from CHD are achieved^(17,18,20,24). Moreover, data from prospective observational and intervention studies in Japan indicate that *n*-3 LCFA intakes of approximately 900 to 1000 mg/d may protect against the risk of non-fatal coronary events in subjects free of known CHD at baseline^(10,28,32). The present evidence suggests that 250 mg/d of the *n*-3 LCFA should be considered a minimum target intake and not an absolute target intake.

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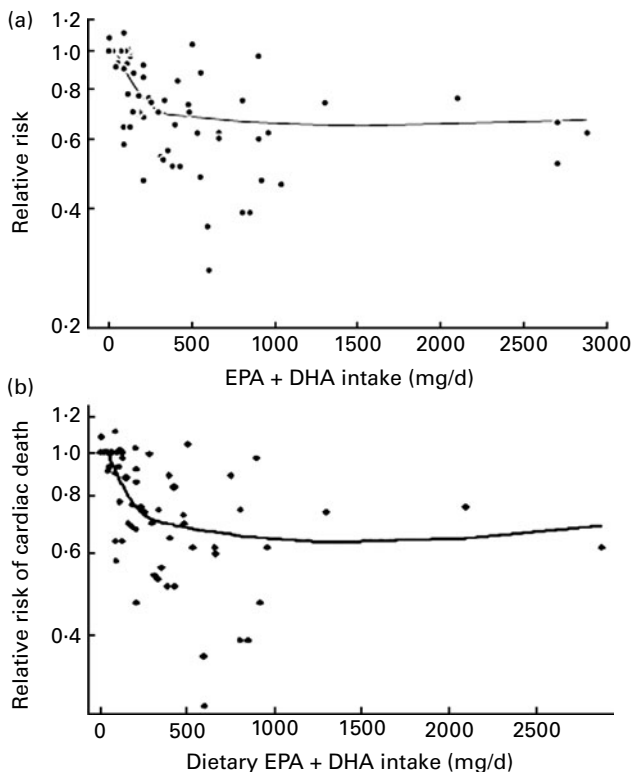


Fig. 4. (a) Relationship between intake of fish or fish oil and relative risks of CHD death in prospective cohort studies and randomised clinical trials. Reprinted with permission from the publication by Mozaffarian & Rimm (2006)⁽²⁷⁾. Copyright ©2006 American Medical Association. All rights reserved. (b) Meta-analysis of estimated dietary EPA + DHA consumption from sea-food and risk of cardiac death in generally healthy populations of individuals without known heart disease. Reprinted with permission from the publication by Harris *et al.*⁽⁹⁾ (American Society for Nutrition).

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