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Marine *n*-3 fatty acids and CVD: new insights from recent follow-up studies and clinical supplementation trials

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Marine *n*-3 PUFA exert beneficial effects that might inhibit atherosclerosis and reduce vascular disease. Previous studies have, however, reported conflicting results and here we have summarised the early history and the most recent findings from follow-up studies and randomised clinical trials investigating marine *n*-3 PUFA in relation to the risk of atherosclerotic CVD. Most follow-up studies have suggested that the intake of marine *n*-3 PUFA may be associated with a lower risk of CVD. Recent studies have also shown that it is important to focus on substitution issues and dietary patterns. Further, the use of gold standard biomarkers of fatty acid exposure such as adipose tissue should be encouraged. Findings from clinical supplemental trials have shown conflicting results and findings from previous meta-analyses and guidelines have generally not supported the use of fish oil supplements for the prevention of CVD. However, a recent meta-analysis including three recent large clinical trials with fish oil supplements reported a moderate beneficial effect on cardiovascular endpoints. Interestingly, results from a large clinical trial (REDUCE-IT) have suggested that supplementation with a high dose of purified EPA ethyl ester for 4·9 years significantly and markedly reduced the risk of cardiovascular events in patients with CVD and mild hypertriglyceridaemia; findings that need to be confirmed. While it seems appropriate to recommend consumption of fish, particular fatty fish for prevention of CVD, an effect of fish oil supplements is probably at best marginal perhaps apart from patients with hypertriglyceridaemia.

Marine *n*-3 PUFA: EPA: DHA: Atherosclerotic CVD

Marine *n*-3 PUFA are organic acids that naturally contain two or more double bonds in their carbon chain, which can be found in seafood and especially in oily fish such as mackerel, salmon, herring, anchovies, tuna and sardines[1,2]. Marine *n*-3 PUFA may after ingestion become incorporated into cell membranes and storage pools, utilised in energy production and converted into lipid signalling molecules that may influence a myriad of molecular pathways. Marine *n*-3 PUFA have attracted great interest in cardiovascular research since Danish scientists from Aalborg in Denmark, Bang and Dyerberg reported that traditionally living Greenland Eskimos had a low prevalence of myocardial infarction, a markedly reduced platelet reactivity and an antiatherogenic lipid profile compared with Danes and suggested this could be due to the Inuit diet that was extremely rich in marine *n*-3 PUFA in particular derived from seal and whale[3–5]. Based on several expeditions and investigations, Dyerberg and colleagues in 1978 put forward their hypothesis that marine *n*-3 PUFA in particular EPA might protect against atherosclerosis and thrombosis[4]. This made investigators from other

Abbreviations: DCH, Diet, Cancer and Health; HR, hazard ratio; DPA, docosapentaenoic acid; PAD, peripheral artery disease.
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parts of the world report their findings relating fish consumption to a reduced risk of CVD from populations in Alaska, several populations from the United States, Japan and the Netherlands[6,7].

Beneficial effects of marine *n*-3 PUFA important to vascular disease risk may include anti-inflammatory effects, a lowering of plasma TAG, a reduction of platelet reactivity and heart rate, and improvement of endothelial dysfunction among others[8]. Also, marine *n*-3 PUFA have been suggested to prevent fatal arrhythmias and sudden cardiac death, and to stabilise atherosclerotic plaques[1]. Marine *n*-3 PUFA are composed of EPA (20 : 5 *n*-3), docosapentaenoic acid (DPA; 22 : 5 *n*-3) and DHA (22 : 6 *n*-3). Interestingly, the biological effects of these marine *n*-3 PUFA may differ. Thus, EPA might be more important in atherothrombosis, while DHA probably may be important for anti-arrhythmic effects of marine *n*-3 PUFA although these findings have not been consistent[9]. Also, EPA and DHA may have different effects on inflammation[10]. Less is known of the biological effects of DPA.

The objective of the present paper was to summarise recent findings from previous observational follow-up studies and randomised clinical trials investigating marine *n*-3 PUFA in relation to the risk of atherosclerotic CVD including not only CHD, but also ischaemic stroke and peripheral artery disease (PAD).

Follow-up studies on marine *n*-3 PUFA and atherosclerotic CVD

Marine n-*3 PUFA and CHD*

Several follow-up studies have investigated the intake of marine *n*-3 PUFA in relation to the risk of CHD and a previous systematic review and meta-analysis of observational follow-up studies including 422 786 participants and a total of 9089 CHD cases concluded that the intake of total marine *n*-3 PUFA might be associated with a lower risk of CHD[11]. Further, a pooled analysis of previous observational biomarker studies has supported that marine *n*-3 PUFA may be associated with a lower risk of fatal CHD[12]. However, few studies have examined associations between the complementary measures of exposure of both total and individual *n*-3 PUFA and risk of CHD.

The Danish Diet, Cancer and Health (DCH) cohort represents a valuable source to study associations between the fatty acids and development of health outcomes because detailed information on diet and lifestyle was collected at baseline including an adipose tissue biopsy from the buttock[13]. The DCH cohort was established between 1993 and 1997 and included a total of 57 053 men and women[13]. The adipose tissue content of marine *n*-3 PUFA is considered the gold standard biomarker of intake and metabolism of these fatty acids[14]. Early findings from the DCH cohort suggested indications of inverse associations between the intake of total marine *n*-3 PUFA, EPA, DPA and DHA and the risk of acute coronary syndrome in men, while no consistent pattern of associations were found in women[15]. Also, indications of a lower risk of acute coronary syndrome were observed in analyses of adipose tissue content of total marine *n*-3 PUFA, EPA, DHA and DPA in men, whereas no consistent pattern of association was observed amongst women[16]. However, a later study from the same cohort with a longer follow-up including more cases found indications of lower rates of myocardial infarction among subjects in the highest quintile of intake of total marine *n*-3 PUFA, EPA, DPA and DHA in both men and women[17]. Further, a study investigating adipose tissue content of marine *n*-3 PUFA also found that EPA and DHA might be inversely related to the risk of myocardial infarction[18]. In contrast, adipose tissue content of DPA was associated with a higher risk of myocardial infarction[18]. A possible explanation for this finding may be that DPA apart from fish can also be found in meat.

Marine n-*3 PUFA and ischaemic stroke*

We have recently reviewed follow-up studies reporting on the association between the intake of marine *n*-3 PUFA and the risk of ischaemic stroke[19]. In brief, findings from the Nurses' Health Study[20], the Health Professionals Follow-up Study[21] and the Swedish Mammography Cohort[22] have indicated that the intake of EPA + DHA may be associated with a lower risk of total ischaemic stroke. Also, indications of lower rates of total ischaemic stroke were observed among both men and women in the MORGEN Study[23], but the confidence intervals were wide and the point estimates were not statistically significant. In contrast, five other follow-up studies found no consistent pattern of associations between the intake of EPA + DHA or total marine *n*-3 PUFA and the risk of total ischaemic stroke[24–29]. Also, previous biomarker studies investigating the association between the content of EPA + DHA or total *n*-3 PUFA in blood compartments and the risk of total ischaemic stroke have not given consistent results[30–34]. However, most studies have not discriminated between intake of individual marine *n*-3 PUFA, which may be of importance due to possible differences in biological effects. Further, recent findings suggest that the association between marine *n*-3 PUFA and risk of ischaemic stroke may differ according to the aetiological origin as ischaemic stroke is a heterogeneous condition. The TOAST classification system has been widely used for categorisation of ischaemic stroke into subtypes including large artery atherosclerosis, small-vessel occlusion, cardio-embolism, other aetiology (rare causes) or undetermined aetiology[35]. Ischaemic strokes due to large artery atherosclerosis is believed to be mainly caused by atherosclerosis, whereas ischaemic strokes due to small-vessel occlusion may occur as a result of atherosclerosis or lipohyalinosis affecting the smaller penetrating arteries[35,36]. Ischaemic strokes due to cardio-embolism are mainly due to atrial fibrillation or flutter, while ischaemic strokes of other aetiology include rare causes of stroke such as vasculopathies, arterial dissections and hypercoagulable states[35]. Few studies have evaluated associations between *n*-3 PUFA and the risk of

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ischaemic stroke subtypes, and findings have been conflicting[19]. Thus, a study based on pooled analyses from three large American cohorts found inverse associations between the content of DHA in plasma phospholipids and the risk of atherothrombotic stroke (large artery atherosclerosis and small-vessel occlusions), while no associations were found for EPA and DPA[34]. However, the content of DPA in plasma phospholipids was inversely associated with the risk of cardio-embolic stroke, whereas no associations were observed between EPA and DHA and the risk of cardio-embolic stroke[34]. In contrast, we reported in a large follow-up study of more than 1800 total ischaemic stroke cases using data from the DCH cohort that a high intake of EPA and DHA was associated with a lower risk of ischaemic stroke due to large artery atherosclerosis and surprisingly also a higher risk of ischaemic stroke due to cardio-embolism[29]. No consistent pattern of associations was observed between the intake of EPA and DHA and the risk of ischaemic stroke due to small-vessel occlusions, but indications of a higher risk of ischaemic stroke due to cardio-embolism and small-vessel occlusion were observed when comparing the highest quartile of DPA with the lowest although not statistically significant[29]. A major advantage of this study was the use of adipose tissue content of marine *n*-3 PUFA, which showed that the content of EPA in adipose tissue was associated with a lower risk of ischaemic stroke due to large artery atherosclerosis and small-vessel occlusion (Fig. 1), whereas the content of DPA in adipose tissue was associated with a higher risk of ischaemic stroke due to cardio-embolism[29].

Marine n-*3 PUFA and peripheral artery disease*

Limited knowledge exists regarding the role of marine *n*-3 PUFA and development of PAD. However, in a recent case-cohort study including 863 cases of validated PAD based on data from the DCH cohort we found that a high content of EPA + DHA in adipose tissue was inversely associated with the risk of PAD after a median 13·5 years of follow-up[37]. Analyses of the individual *n*-3 PUFA showed that in particular a high content of EPA in adipose tissue was associated with a lower risk of PAD, while the association between a high content of DHA in adipose tissue and the risk of PAD was weaker and statistically non-significant (Fig. 1)[37]. In contrast, indications of a positive association were observed between the DPA content in adipose tissue and the risk of PAD[37].

Randomised clinical trials with marine *n*-3 PUFA

Several clinical trials have been conducted to study the effect of marine long-chain *n*-3 PUFA on the risk of CVD; however, the results have been conflicting.

In the seminal DART study published in 1989, a total of 2033 male survivors of myocardial infarction were randomly assigned to receive or not receive one of three dietary recommendations and one of those included advice to increase the intake of oily fish[38]. Subjects advised to increase their intake of fatty fish had a markedly lower risk of all-cause mortality after 2 years follow-up compared with those not advised to increase their intake of fatty fish (Hazard ratio (HR) 0·71, 95 % CI 0·54, 0·93), which was ascribed to a beneficial effect on cardiovascular deaths[38]. This led to suggestions of an anti-arrhythmic effect of marine *n*-3 PUFA in line with previous *in vitro* studies[39] and animal studies[40]. The DART study was novel in showing dietary advices on increased fish consumption might reduce mortality, but the dietary intervention was complex, and an open-label design was used. Another landmark study, the GISSI-Prevenzione trial, was the first major clinical trial to evaluate the clinical effect of fish oil supplementation in patients with a myocardial infarction[41]. In this study, 11 324 subjects with a recent myocardial infarction were randomly allocated to four treatment groups using an open-label design including supplements of EPA + DHA (1 g daily), vitamin E (300 mg daily), both or neither (control group)[41]. After 3·5 years follow-up, subjects who received EPA + DHA had a lower risk of a primary composite endpoint of death non-fatal myocardial infarction and non-fatal stroke compared with controls (HR 0·90, 95 % CI 0·82, 0·99) and a substantial lower risk of sudden death (HR 0·74, 95 % CI 0·58, 0·93)[41]. This was the first study suggesting that fish oil supplements might reduce CVD risk. Further, 18 645 subjects with hypercholesterolaemia enrolled in the JELIS trial from Japan randomly assigned using an open-label design to receive 1·8 g purified EPA and statins daily had a lower risk of coronary events after 4·6 years follow-up compared with subjects receiving statins only (HR 0·81, 95 % CI 0·69, 0·95)[42]. In contrast, supplementation of 0·4 g EPA + DHA daily was not associated with a lower risk of major cardiovascular events compared with controls in 4837 subjects with a prior myocardial infarction enrolled in the double-blinded Alpha Omega trial (HR 1·01, 95 % CI 0·87, 1·17)[43]. Also, several other previous clinical supplemental trials have not supported that *n*-3 PUFA lower the risk of CVD[44]. A meta-analysis from 2018 including 77 917 individuals from ten clinical supplemental trials concluded that supplementation with *n*-3 PUFA had no significant association with fatal or non-fatal CHD or any vascular event[45].

Latest evidence from randomised clinical supplementation trials

Recently, the results of three large trials using fish oil supplements have been published. The ASCEND study investigated whether supplementation with 1 g EPA + DHA daily compared with olive oil was associated with the risk of a composite endpoint of non-fatal myocardial infarction, ischaemic stroke, transient ischaemic attack or vascular death in 15 480 subjects with diabetes mellitus and without evidence of CVD[46]. After 7·4 years of follow-up, no significant difference in the risk of the composite endpoint was observed between those assigned to receive EPA + DHA and those assigned to receive

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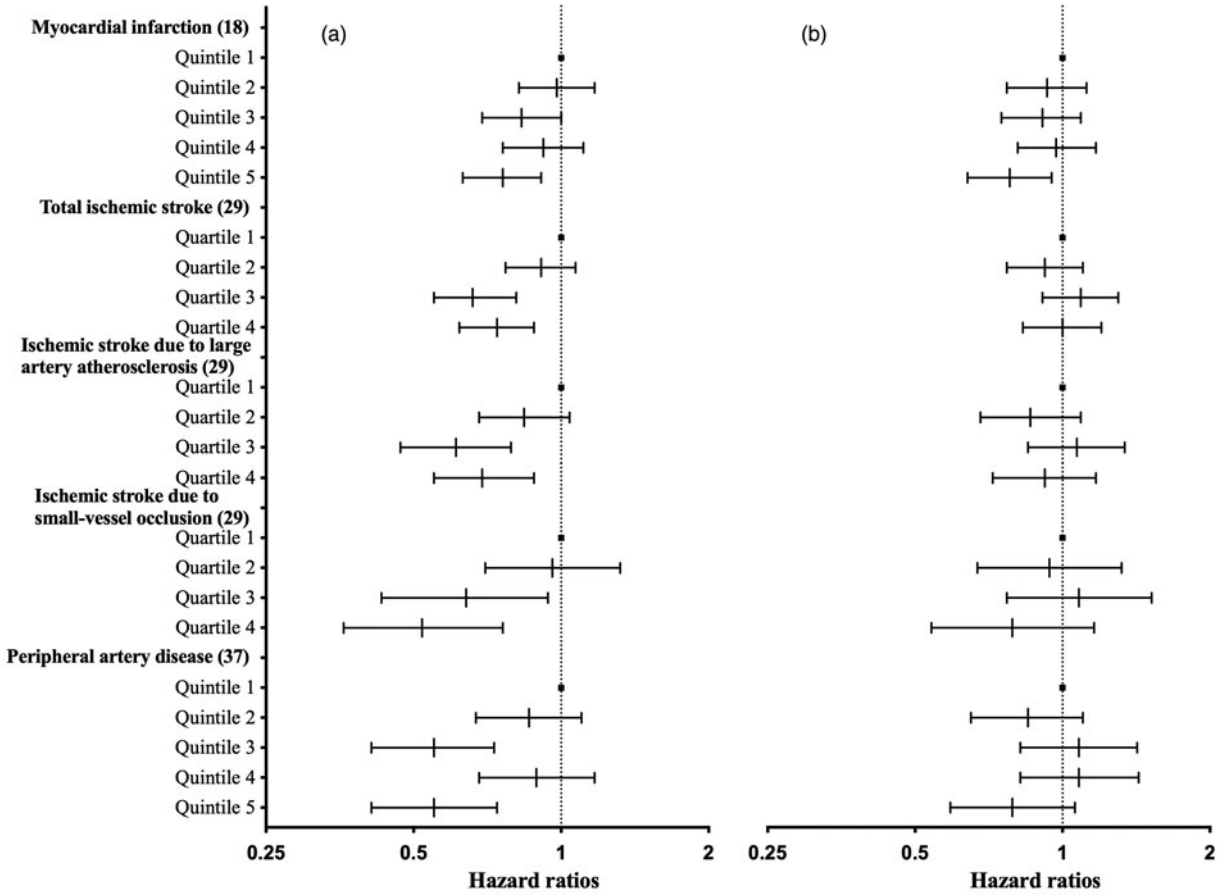

Fig. 1. Associations between content of EPA (a) and DHA (b) in adipose tissue and development of vascular disease including myocardial infarction, ischaemic stroke and peripheral artery disease in studies originating from the Danish Diet, Cancer and Health cohort.

placebo (HR 0·97, 95 % CI 0·87, 1·08). Also, supplementation with 1 g EPA + DHA daily was not statistically significantly associated with the risk of major cardiovascular events defined as a composite of myocardial infarction, stroke or death from cardiovascular cause among 25 871 participants followed-up for 5·3 years in the VITAL study[47]. However, secondary analyses of the ASCEND study[46] indicated that EPA + DHA might lower the risk of vascular death, whereas secondary analyses from the VITAL study[47] indicated that EPA + DHA was associated with a lower risk of myocardial infarction compared with placebo.

Interestingly, the REDUCE-IT study investigated the effect of supplementation of a total dose of 4 g purified EPA ethyl ester against placebo on the risk of cardiovascular events among 8179 subjects with established CVD or with diabetes and at least one additional risk factor and mildly elevated plasma TAG[48]. After a median 4·9 years follow-up, markedly lower rates of a composite endpoint of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, coronary revascularisation or unstable angina pectoris was observed in those assigned to EPA ethyl ester supplementation compared with placebo (HR 0·75, 95 % CI 0·68, 0·83)[48].

Finally, a recent meta-analysis including thirteen clinical trials including the three studies mentioned earlier concluded that marine *n*-3 PUFA supplementation lowered the risk of myocardial infarction, total CHD and total CVD[49]. However, the observed effect estimates were modest.

Strengths and limitations of present evidence

Randomised controlled trials with hard endpoints are considered the gold standard of scientific evidence. However, in the context of nutritional research carefully conducted prospective epidemiological studies represent a valuable approach to study the role of marine *n*-3 PUFA in relation to development of CVD. Previous clinical trials investigating marine *n*-3 PUFA supplementation on CVD risk have been conducted in subjects with preexisting CHD or at high risk of CHD and the interventions have been relatively short in the context of a disease with a long aetiological period as atherosclerosis that may begin in early life and develop over a lifetime. In contrast, clinical supplemental trials have offered the possibility to investigate high doses of marine *n*-3

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PUFA on CVD risk that may be difficult to achieve through the diet.

Marine *n*-3 PUFA in present guidelines

The present British National Institute for Health Care Guidelines recommend subjects at high risk or with established CVD to eat at least two portions of fish weekly including a portion of oily fish[50]. Similar recommendations regarding fish intake are encouraged in the European Guidelines on CVD prevention in clinical practice[51]. American Guidelines from 2018 give advice that one or two seafood meals should be included to reduce the risk of CVD especially when seafood replaces the intake of less healthy foods[2]. Also, supplementation with *n*-3 PUFA has been considered reasonable for patients with prevalent CHD in the most recent guidelines from the American Heart Association[44]. Further, the 2019 ESC/EAS Guidelines introduced new recommendations suggesting that the use of *n*-3 PUFA (icosapent ethyl 2 × 2 g daily) in subjects in high risk of CVD or above with elevated plasma TAG should be considered in combination with statins[52].

Conversely, epidemiological studies have offered the possibility to study associations between marine *n*-3 PUFA and development of CVD over 15–20 years or more follow-up. However, random measurement error is an inevitable limitation of epidemiological studies relying on self-reported intakes. Therefore, the use of objective biomarkers of marine *n*-3 PUFA exposure in addition to estimated intakes represents an important approach to study the role of marine *n*-3 PUFA in relation to the risk of CVD although it should be acknowledged that both estimated intakes and biomarkers of exposure are indicators of an underlying dietary pattern. Further, epidemiological studies offer the possibility to study modelling of isoenergetic substitutions with other foods and several studies, which is of interest as several studies from the DCH cohort have suggested that the association between the intake of fish and risk of myocardial infarction, ischaemic stroke and PAD depends on the foods that is being replaced[53–55].

Conclusion

Several observational follow-up studies have indicated that marine *n*-3 PUFA may be associated with a lower risk of myocardial infarction and ischaemic stroke caused by atherosclerosis and PAD. Further, the use of adipose tissue as a gold standard long-term objective biomarkers have generally shown stronger associations for EPA than DHA in adipose tissue on myocardial infarction and ischaemic stroke caused by atherosclerosis and PAD (Fig. 1). Early clinical supplementation trials supported a beneficial role of marine *n*-3 PUFA in lowering the risk of CVD although more recent studies have questioned these findings. Differences in study designs, composition of interventions, trial duration and other methodological variations might explain some of the inconsistencies observed. One of the most consistent biochemical findings after increased the intake of *n*-3 PUFA has been a reduction in plasma TAG. It is therefore of outmost interest that supplementation with 4 g purified EPA ethyl esters in the REDUCE-IT study showed a significant and pronounced effect on major cardiovascular events in subjects with mildly elevated plasma TAG. However, these results need to be confirmed by other studies.

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Conflict of Interest

None.

Authorship

All authors contributed to planning the outline of the present paper. C. S. B. wrote the first draft of the manuscript. E. B. S., L. T. M. and K. H. critically revised the manuscript and contributed with intellectual content to the paper. All authors read and approved the final manuscript.

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