Proceedings of the Nutrition Society (2020), **79**, 22–29 doi:10.1017/S0029665119000582
 First published online 15 April 2019

The Nutrition Society Winter Meeting was held at the Royal Society of Medicine, London on 4–5 December 2018

Conference on 'Optimal diet and lifestyle strategies for the management of cardio-metabolic risk'
Symposium 1: Dietary fatty acids

Marine and plant-based *n*-3 PUFA and atherosclerotic cardiovascular disease

Christian S. Bork[1]*, Stine K. Venø[1], Anne N. Lasota[2], Søren Lundbye-Christensen[3,4] and Erik B. Schmidt[1,3]

[1]*Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark*
[2]*Department of Vascular Surgery, Aalborg University Hospital, Aalborg, Denmark*
[3]*Department of Clinical Medicine, Aalborg University, Aalborg, Denmark*
[4]*Unit of Clinical Biostatistics, Aalborg University Hospital, Aalborg, Denmark*

n-3 PUFA may exert favourable effects on several processes that may inhibit the atherosclerotic process. However, the role of *n*-3 PUFA in lowering the risk of atherosclerotic CVD (ASCVD) has been fiercely debated. In the present paper, we summarise the main findings from previous follow-up studies of intake and studies using adipose tissue as an objective biomarker to investigate exposure to *n*-3 PUFA in relation to ASCVD risk and discuss some perspectives for further research. The majority of previous studies investigating intake of marine- and plant-based *n*-3 PUFA have focused on CHD while other ASCVD such as ischaemic stroke and peripheral artery disease have been less studied. However, recent data from Danish Diet, Cancer and Health cohort suggest that marine *n*-3 PUFA may be inversely associated with risk of myocardial infarction, ischaemic stroke and peripheral arterial disease caused by atherosclerosis. The effect of the plant-derived *n*-3 PUFA α-linolenic acid on ASCVD is less clear and several gaps in the literature remain to be explored.

α-linolenic acid: EPA: DHA: Atherosclerotic cardiovascular disease

Atherosclerosis is the underlying disease process that may result in atherosclerotic CVD (ASCVD) including myocardial infarction, ischaemic stroke and peripheral arterial disease (PAD).

n-3 PUFA are organic acids that contain more than one double bond in the aliphatic chain[1]. *n*-3 PUFA can be divided into the short-chain *n*-3 PUFA, α-linolenic acid (ALA, 18:3*n*-3) and the longer-chain (LC) *n*-3 PUFA EPA (20:5*n*-3), docosapentaenoic acid (DPA, 22:5*n*-3) and DHA (22:6*n*-3)[2].

ALA is found in high concentrations in walnuts and plant oils based on rapeseed, soyabean and flaxseed, but can also be acquired from other sources such as green leafy vegetables, whole-grain cereals, margarines, dairy products and meat[3–5]. The primary source of EPA and DHA is seafood especially oily fish such as salmon, anchovies, herring, mackerel, tuna and sardines[6]. ALA is the most abundant *n*-3 PUFA in the diets of subjects who do not consume very high amounts of oily fish or concentrated supplements of EPA and/or DHA[7]. The typical intake of *n*-3 PUFA in adults varies substantially by country, but most Western populations consume approximately 0·5 to 2·3 g/d ALA[7] and 0·1 to 0·6 g/d EPA + DHA. ALA can be converted into EPA and DHA by a series of desaturation, elongation and β-oxidation reactions[8]. The conversion efficiency of

Abbreviations: ALA, α-linolenic acid; ASCVD, atherosclerotic CVD; DCH, Diet, Cancer and Health; HR, hazard ratio; LC, longer-chain; PAD, peripheral arterial disease.

***Corresponding author:** Christian Bork, email c.bork@rn.dk

ALA into EPA and DHA appears to be limited in human subjects with reported conversion percentages of ALA to EPA <10 % and to DHA <1 %[7,9]. However, the conversion efficiency appears to be better in women and especially in those of reproductive age[10]. Also, interplay between fatty acids and genetics are believed to influence the metabolism of ALA[11].

ALA has been ascribed anti-inflammatory, anti-atherosclerotic and anti-thrombotic effects[12], but controversy remains whether these suggested mechanisms of action can be attributed to the effects of ALA *per se* or its role as a precursor for LC *n*-3 PUFA and beyond. Accumulating evidence suggests that EPA and DHA may exert favourable effects on several biological processes that may influence atherosclerosis development including beneficial effects on inflammation, blood pressure, TAG, platelet reactivity, endothelial function and stabilisation of atherosclerotic plaques[1,13].

Dietary studies assessing consumption of *n*-3 PUFA using self-reported questionnaires are prone to measurement error, which may result in an attenuation of observed associations and loss of statistical power. In contrast, the content of *n*-3 PUFA in human tissue may reflect an objective measure of intake and metabolism and the endogenous exposure of *n*-3 PUFA[11,14], which may be more important from a biological point of view. Shorter-term biomarkers may include the content of *n*-3 PUFA in serum, plasma or erythrocytes reflecting the exposure from days to months, whereas the content in adipose tissue is believed to represent the long-term exposure during the previous 1–2 years[11,14]. Thus, adipose tissue content of *n*-3 PUFA is considered the gold standard biomarker to investigate the role of *n*-3 PUFA in relation to chronic diseases such as ASCVD[11].

Here, we will summarise the main findings from previous follow-up studies of intake and studies using adipose tissue as an objective biomarker to investigate exposure to *n*-3 PUFA in relation to ASCVD risk and discuss some perspectives for further research.

α-Linolenic acid and atherosclerotic CVD

ALA has been proposed to be an important component of the Mediterranean diet[15], which has been suggested to lower the risk of major cardiovascular events[16]. Most previous studies investigating the association between intake of ALA and ASCVD have focused on CHD and to some extent also on ischaemic stroke.

α-Linolenic acid and CHD

Several follow-up studies have investigated the association between ALA intake and the rate of total or fatal CHD[4,5,17–27]. Early findings from the mid-1980s based on data from the Usual Care group of the Multiple Risk Factor Intervention Trial (*n* 6250) suggested that intake of ALA expressed as percentage of total kilocalories was inversely associated with mortality from CHD in analyses including 175 cases identified during 10·5 years of follow-up amongst US men[20]. Also, early findings from the US Health Professionals Follow-up Study (*n* 43 757) indicated an inverse association between ALA expressed as a percentage of total energy intake and the rate of myocardial infarction including 734 events amongst men followed for 6 years[21]. However, the association was only statistically significant in analyses including adjustment for traditional risk factors and dietary risk factors and no statistically significant association was found between an ALA intake ranging between 0·9 g/d in the first quintile to 1·5 g/d in the fifth quintile in analyses of 229 cases of fatal myocardial infarction[21]. A Finnish study conducted amongst 21 930 male smokers followed for a median of 6·1 years found no consistent association between energy-adjusted ALA intake and the rate of total CHD (1399 cases) or fatal CHD (635 cases)[22]. However, indications of a lower rate of fatal CHD were observed in analyses including additional adjustment for *trans* fatty acids, MUFA and SFA when comparing an ALA intake of median 2·5 g/d in the fifth quintile with an intake of 0·9 g/d in the first quintile (hazard ratio (HR) 0·75, 95 % CI 0·52, 1·10)[22]. Also, an inverse association between energy-adjusted ALA intake and the rate of fatal CHD (2697 cases) was observed amongst men and women followed for nearly 15 years in the Singapore Chinese Health Study (*n* 60 298) when comparing an average intake of 0·86 g/d in the fourth quartile with an intake of 0·36 g/d in the first quartile (HR 0·82, 95 % CI 0·71, 0·93)[23]. In an updated report from the Health Professionals Follow-up Study including 14 years of follow-up, Mozaffarian *et al.*[24] suggested that intake of energy-adjusted ALA intake was associated with a lower rate of CHD in subjects with intake of LC *n*-3 PUFA below 100 mg/d, whereas no consistent association was observed amongst subjects with a higher intake of LC *n*-3 PUFA. Initial analyses from the Nurses Health Study (*n* 76 283) with 10 years of follow-up suggested an inverse association between the energy-adjusted intake of ALA and the rate of fatal CHD (232 cases) in analyses including adjustment for traditional CHD risk factors and dietary risk factors, while no consistent pattern of association was observed between ALA intake and the rate of non-fatal myocardial infarction (597 cases)[4]. The lowest rates of fatal CHD were observed when comparing an ALA intake of 1·36 g/d in the fifth quintile with an intake of 0·71 g/d in the first quintile (HR 0·55, 95 % CI 0·32, 0·94)[4]. However, in an updated report from the Nurses' Health Study of 18 years of follow-up, no consistent pattern of association was observed between energy-adjusted ALA intake and the rate of fatal CHD (641 cases) or non-fatal myocardial infarction (1604 cases)[25]. Also, several other follow-up studies have not confirmed an inverse association between ALA intake and the rate of CHD or fatal CHD[17–19,26,27]. A pooled analysis of eight American and European cohorts investigated the association between energy-adjusted ALA intake and the rate of CHD and fatal CHD amongst 148 675 women and 80 368 men[19]. During 4–10 years

https://doi.org/10.1017/S0029665119000582 Published online by Cambridge University Press

of follow-up, 3337 total CHD and 1191 fatal CHD events occurred amongst men, while 1156 total CHD and 560 fatal CHD events occurred amongst women[19]. In analyses including adjustment for traditional risk factors for CHD, energy-adjusted intake of ALA was not associated with the rate of total or fatal CHD in men or women[19]. However, in analyses including additional adjustment for dietary factors, indications of lower rates of total CHD (HR 0·85, 95 % CI 0·72, 1·01) for each additional 1 g ALA consumed) and fatal CHD (HR 0·77, 95 % CI 0·58, 1·01) were observed in men, whereas no consistent associations were observed in women[19]. The median energy-adjusted ALA intake was 1·01 g/d in women (80 % central range: 0·58; 1·64 g/d) and 1·17 g/d in men (80 % central range: 0·64; 1·62 g/d)[19]. Data from the Diet, Cancer and Health (DCH) cohort (*n* 53 901) suggested that energy-adjusted ALA intake was not appreciably associated with the rate of myocardial infarction in men (2124 cases) or women (854 cases) during 17 years of follow-up in analyses adjusted for established CHD risk factors[5]. However, an indication of a lower rate of myocardial infarction was observed amongst men in analyses including additional adjustment for dietary risk factors when comparing the highest quintile of intake (≥2·54 g/d) with the lowest (<1·67 g/d; HR 0·90, 95% CI 0·76, 1·07)[5]. A recent systematic review and meta-analysis suggested a modest non-linear inverse association between ALA intake and CHD with the lowest rates of CHD up to a daily intake of ALA of 1·4 g/d and a dose-dependent inverse association between ALA intake and the rate of fatal CHD[28]. In this meta-analysis, the authors generally included effect estimates derived from statistical models including adjustment for CHD risk factors and dietary factors when available[28].

Few studies have investigated adipose tissue content of ALA in relation to risk of CHD[5,29–33]. A case–control study including both men and women from Costa Rica reported a statistically significant inverse association between adipose tissue content of ALA and the odds of non-fatal myocardial infarction (1819 cases)[29]. The lowest odds for non-fatal myocardial infarction were observed when comparing the adipose tissue content of ALA in the ninth decile (median 0·88 %) with the lowest decile (0·36 %) in analyses including CHD risk factors and SFA, linoleic acid and *trans* fatty acids (OR: 0·37, 95 % CI 0·23, 0·59)[29]. However, no significant difference in the odds of non-fatal myocardial infarction was observed between the seventh decile and tenth decile of adipose tissue content of ALA (0·70 % to 1·04 %)[29]. Few other smaller case–control studies have explored the associations between adipose tissue content of ALA and myocardial infarction, but results have been inconsistent[30–33]. In analyses including adjustment for established CHD risk factors, a positive association between adipose tissue content of ALA and myocardial infarction was observed in men (1994 cases) and a weak U-shaped association was observed in women (770 cases) amongst subjects enrolled in the DCH cohort, but none of these associations were statistically significant[5]. In women, the lowest rates of myocardial infarction was observed around the median adipose tissue content of ALA (0·82 %)[5].

α-Linolenic acid and ischaemic stroke

Few studies have investigated associations between intake of ALA and the risk of ischaemic stroke[18,27,34–36]. In the MORGEN Study, lower rates of total ischaemic stroke with energy-adjusted intake of ALA were reported amongst men and women (*n* 19 896) including 144 cases identified during a median of 10·5 years[27]. When comparing with the lowest quintile, the HR in the second to fifth quintile of ALA intake were 0·63 (95 % CI 0·39, 1·02); 0·45 (95 % CI 0·26, 0·77), 0·55 (95 % CI 0·33, 0·92) and 0·70 (95 % CI 0·43, 1·12), respectively[27]. The energy-adjusted ALA intake ranged between a median of 1·0 g/d in the first quintile to 1·9 g/d in the fifth quintile[27]. In the Cardiovascular Health Study (*n* 2583) a cohort of US men and women, a median ALA intake of 2·44 % of total fatty acids in the fifth quintile was associated with a non-significant lower rate of total ischaemic stroke (HR 0·70, 95 % CI 0·47, 1·04) compared with an intake of 1·33 % in the first quintile, but no consistent pattern of association was observed across quintiles in analyses of 278 cases identified during 12 years of follow-up[18]. In addition, no consistent association was found between intake of ALA expressed as percentage of total energy intake and the rate of total ischaemic stroke in 807 cases identified during 22 years of follow-up in the Women's Health Study (*n* 38 392)[35]. Further, an energy-adjusted ALA intake ranging between a mean of 0·9 g/d in the first quintile to 1·5 g/d in the fifth quintile was not associated with the rate of total ischaemic stroke amongst women in the Swedish Mammography Cohort (*n* 34 670), which included 1310 cases identified during a median of 10·4 years of follow-up[34]. Neutral findings were also reported between the energy-adjusted intake of ALA and the rate of total ischaemic stroke amongst men and women enrolled in the DCH cohort (*n* 55 018) including 1859 cases identified during 13·5 years of follow-up[36]. Also, no consistent pattern of association was observed between ALA intake and the rate of ischaemic stroke subtypes including ischaemic stroke due to large artery atherosclerosis, small-vessel occlusions or cardioembolisms[36]. The energy-adjusted ALA intake in the first quintile was 1·10 g/d and 2·60 g/d in the fifth quintile.

There is limited knowledge on the association between adipose tissue content of ALA and the rate of ischaemic stroke. However, a recent case–cohort study based on data from the DCH cohort reported a U-shaped pattern of association between adipose tissue content of ALA and the rate of total ischaemic stroke, but the association was not statistically significant ($P = 0·172$) in analyses including 1755 cases identified during 13·4 years of follow-up[37]. In analyses of ischaemic stroke subtypes, a statistically significant U-shaped association was observed between adipose tissue content of ALA and the rate of ischaemic stroke due to large artery atherosclerosis ($P = 0·017$) with the lowest rates observed around the median adipose tissue content of ALA

https://doi.org/10.1017/S0029665119000582 Published online by Cambridge University Press

(0·84 %)[37]. When comparing with the lowest quintile, the HR in the second to fifth quintile of adipose tissue content of ALA and the rates of ischaemic stroke due to large artery atherosclerosis were 0·72 (95 % CI 0·48, 1·08); 0·63 (95 % CI 0·41, 0·96); 0·83 (95 % CI 0·56, 1·22) and 0·95 (95 % CI 0·65, 1·40) respectively[37]. In contrast, no consistent association was found between adipose tissue content of ALA and the rate of ischaemic stroke due to small-vessel occlusion and a positive statistically non-significant association was observed between ALA and the rate of ischaemic stroke due to cardioembolism[37].

α-Linolenic acid and peripheral arterial disease

To our knowledge, no previous follow-up studies have explored the associations between intake and adipose tissue content of ALA in relation to development of PAD.

Longer-chain *n*-3 fatty acids and atherosclerotic CVD

A large number of epidemiological studies has investigated dietary intake of LC *n*-3 PUFA in relation to risk of CVD with the vast majority of studies focusing on CHD while ischaemic stroke and PAD have been less studied.

Longer-chain n-*3 PUFA and CHD*

Since the late 1970s where Dyerberg and colleagues in a seminal paper hypothesised that LC *n*-3 PUFA might protect against atherosclerosis and thrombosis[38] this has been the subject of multiple investigations. The protective effect of seafood against CHD reported in Greenland Inuit was confirmed in several epidemiological studies of other populations, which previously has been reviewed in detail[6,39]. In the following, we will focus on studies using data from the DCH cohort, which have investigated both dietary intake and adipose tissue content of LC-*n*-3 PUFA in relation to risk of CHD[40–43]. The reported median intake of total LC *n*-3 PUFA in this cohort was approximately 700 mg/d with EPA and DHA constituting 180 mg/d and 430 mg/d, respectively[42]. Early findings from the DCH cohort (*n* 53 802) suggested indications of inverse associations between intake of total LC *n*-3 PUFA, EPA, DPA and DHA and the rate of acute coronary syndrome in men, whereas no consistent pattern of associations were found in women after adjustment for established risk factors and potential dietary factors[40]. Acute coronary syndrome was defined as unstable angina pectoris and non-fatal and fatal myocardial infarction and during 7·6 years of follow-up 852 male and 272 female cases were identified[40]. Also, indications of lower rates of acute coronary syndrome were observed in analyses of adipose tissue content of total LC *n*-3 PUFA, EPA, DHA and DPA amongst men (779 cases) although only statistically significant when comparing the fifth quintile of total *n*-3 PUFA and DHA with the first quintile, respectively[41]. No consistent pattern of association was observed amongst women (233 cases)[41]. However, a recent study based on the DCH cohort (*n* 54 904) including 17 years of follow-up of investigating intake of LC *n*-3 PUFA on myocardial infarction found indications of lower rates of myocardial infarction amongst subjects in the highest quintile of intake of total *n*-3 PUFA, EPA, DPA and DHA compared with the lowest quintile in both men (2136 cases) and women (892 cases) although only modestly amongst men[42]. In analyses of adipose tissue content of LC *n*-3 PUFA, indications of inverse associations were observed between EPA and myocardial infarction and between DHA and myocardial infarction (2814 cases)[43]. In contrast, a high content of DPA in adipose tissue was associated with a higher rate of myocardial infarction[43]. In analyses including adjustment for traditional risk factors, HR of 0·76 (95 % CI 0·63, 0·91) and 0·78 (95 % CI 0·64, 0·95) were found for EPA and DHA when comparing the highest and lowest quintile, respectively[43].

Longer-chain n-*3 PUFA and ischaemic stroke*

Some studies have investigated intake of LC *n*-3 PUFA in relation to risk of total ischaemic stroke[34,35,44–51]. Early findings from women enrolled in the Nurses' Health Study (*n* 79 839) suggested indications of an inverse association between energy-adjusted intake of EPA + DHA and the rate of total ischaemic stroke in 303 cases identified during 14 years of follow-up[44]. The HR in the second to fifth quintile of EPA + DHA intake were 0·83 (95 % CI 0·59, 1·18), 0·67 (95 % CI 0·47, 0·98), 0·82 (95 % CI: 0·57, 1·18) and 0·71 (95 % CI 0·46, 1·10), respectively in analyses adjusted for cardiovascular and dietary risk factors when compared with the lowest quintile[44]. The energy-adjusted intake of EPA + DHA was 77 mg/d in the first quintile and 481 mg/d in the fifth quintile[44]. Also, an inverse association between energy-adjusted EPA + DHA intake and the rate of total ischaemic stroke was observed amongst men followed during 12 years in the Health Professional Follow-up Study (*n* 43 671) although not statistically significantly comparing the highest quintile of intake with the lowest[45]. The HR in the second to fifth quintile of EPA + DHA intake were 0·56 (95 % CI 0·35, 0·88); 0·63 (95 % CI 0·40, 0·98); 0·54 (95 % CI 0·32, 0·91) and 0·73 (95 % CI 0·43, 1·25) in 377 cases adjusted for cardiovascular and dietary risk factors when compared with the lowest quintile, respectively[45]. The energy-adjusted intake of EPA + DHA was below 50 mg/d in the first quintile and above 600 mg/d in the fifth quintile[45].

Lower rates of total ischaemic stroke were also found across quintiles of energy-adjusted EPA + DHA intake in women followed for 10·4 years in the Swedish Mammography Cohort (*n* 34 670)[34]. The HR in the second to fifth quintile of EPA + DHA intake were 0·88 (95 % CI 0·74, 1·04); 0·84 (95 % CI 0·70, 1·01); 0·83 (95 % CI 0·69, 0·99) and 0·83 (95 % CI 0·69, 0·99) in analyses of 1310 cases adjusted for cardiovascular risk factors and dietary risk factors when compared with the lowest quintile, respectively[34]. The intake of EPA + DHA ranged between a median of 131 mg in

https://doi.org/10.1017/S0029665119000582 Published online by Cambridge University Press

the first quintile and 559 mg in the fifth quintile[34]. In the MORGEN study, lower rates of total ischaemic stroke with intake of energy-adjusted EPA + DHA in analyses including adjustment for cardiovascular and dietary risk factors were observed in both men (80 cases) and women (64 cases), but the CI were wide and not statistically significant[47]. The median intake of EPA + DHA in this Dutch population ranged between 36 mg in the first quintile to 225 mg in the fifth quintile in women and between 44 mg and 241 mg in the fifth quintile in men[47]. In contrast, five other follow-up studies did not find consistent associations between intake of EPA + DHA and the rate of total ischaemic stroke[35,46,48,50,51].

A recent study based on data from the DCH cohort investigated the association between dietary intake and adipose tissue content of LC *n*-3 PUFA and the risk of total ischaemic stroke and ischaemic stroke subtypes during 13·5 years of follow-up[49]. In analyses of dietary intake, no consistent associations were found between intake of total *n*-3 PUFA, EPA or DHA and the rate of total ischaemic stroke while DPA was associated with a higher rate of total ischaemic stroke in 1879 cases when comparing the highest quartile of intake with the lowest[49]. However, in analyses of ischaemic stroke subtypes lower rates of ischaemic stroke due to large artery atherosclerosis were associated with a high intake of total LC *n*-3 PUFA (HR 0·69, 95 % CI 0·50, 0·95), EPA (HR: 0·66, 95 % CI: 0·48, 0·91) and DHA (HR 0·72, 95 % CI 0·53, 0·99) in analyses including 319 cases[49]. In contrast, indications of higher rates of ischaemic stroke due to cardioembolisms were observed with intake of LC *n*-3 PUFA, while no consistent associations were observed between LC *n*-3 PUFA and ischaemic stroke due to small-vessel occlusions[49]. No association was observed between the content of total LC *n*-3 PUFA in adipose tissue and the rate of total ischaemic stroke in 1755 cases[49]. However, the content of EPA in adipose tissue was inversely associated with the rate of total ischaemic stroke when comparing the highest with the lowest quartile (HR 0·74 (95 % CI 0·62, 0·88)[49]. In contrast, the content of DPA in adipose tissue seemed to be associated with a higher rate of total ischaemic stroke, while no association was found for DHA[49]. Thus, the associations between LC *n*-3 PUFA seemed to differ amongst subtypes of ischaemic stroke. Interestingly, the content of EPA in adipose tissue was inversely associated with the rate of ischaemic stroke due to large artery atherosclerosis in analyses of 300 cases with a HR of 0·52 (95 % CI 0·36, 0·76) when comparing the highest with the lowest quartile of EPA in adipose tissue[49]. Thus, the content of EPA in adipose tissue was inversely associated with a lower rate of ischaemic stroke due to small-vessel occlusion in analyses in 781 cases with a HR of 0·69 (95 % CI 0·55, 0·88) while a higher rate of ischaemic stroke due to cardioembolism was observed in subjects with a high content of EPA in adipose tissue[49]. The content of DPA and DHA in adipose tissue was associated with higher rates of ischaemic strokes due to cardioembolism while no consistent association was found for ischaemic strokes due to large artery atherosclerosis or small-vessel occlusions[49]. The observation that both intake and adipose tissue content of EPA in particular may be associated with a lower rate of ischaemic stroke due to large artery atherosclerosis is of interest because this subtype of ischaemic stroke is believed to be caused mainly by atherosclerosis.

Longer-chain n-*3 PUFA and peripheral arterial disease*

To our knowledge, no previous large follow-up studies have investigated the association between the intake of LC *n*-3 PUFA and the risk of PAD. However, a recent case–cohort study based on data from the DCH cohort including 870 incident PAD cases suggested that a high content of EPA + DHA and in particular of EPA in adipose tissue was associated with a lower rate of PAD during 13·5 years of follow-up[52]. The content of EPA in adipose tissue was associated with a dose-dependent lower rate of PAD with a HR of 0·55 (95 % CI 0·41, 0·74) when comparing the highest quintile of EPA with the lowest[52]. The association between DHA indicated a lower rate of PAD above the median content in adipose tissue with a HR of 0·79 (95 % CI 0·59, 1·06) when comparing the highest with the lowest quintile[52]. In contrast, the content of DPA in adipose tissue seemed to be associated with a higher rate of PAD[52].

Conclusions

Follow-up studies indicate that intake of LC *n*-3 PUFA derived mainly from seafood may be inversely associated with the risk of myocardial infarction, ischaemic stroke caused by atherosclerosis and PAD, whereas the role of the mainly plant-derived *n*-3 PUFA ALA in ASCVD is less clear with conflicting results reported. The use of adipose tissue as a gold standard long-term objective biomarker to reflect intake and metabolism has supported the view that EPA, but maybe also DHA, may be associated with a lower risk of atherosclerotic vascular disease. Recent results from the DCH cohort have generally shown stronger associations for EPA than DHA in adipose tissue on myocardial infarction, ischaemic stroke caused by atherosclerosis and PAD. However, whether the observed difference is due to different biological effects related to atherosclerosis remains unclear and further studies investigating the independent biological effects of EPA and DHA are warranted[53]. The use of complementary measures of exposure including estimated intake and the content in adipose tissue or other biomarkers of LC *n*-3 PUFA may each contribute with valuable information despite their different limitations. Estimation of dietary intake of *n*-3 PUFA are prone to measurement error, which is inevitable due to self-reported intakes of foods and calculation of nutrient intakes based on food composition tables that may be imprecise. In contrast, the content of *n*-3 PUFA in adipose tissue may represent a more objective marker of intake. The use of biomarkers may be of particular importance to reflect exposure to ALA because important sources such as plant oils and margarines may be used in food preparation or included in convenience

https://doi.org/10.1017/S0029665119000582 Published online by Cambridge University Press

foods and may therefore be difficult to recognise and quantify in questionnaires[12]. However, the content of PUFA in adipose tissue may also be influenced by metabolism[7]. Endogenous conversion of ALA may contribute to tissue levels of LC *n*-3 PUFA, predominantly EPA, although the significance of this is not clear because high intakes of linoleic acid relative to ALA in most Western populations may favour conversion of linoleic acid over ALA due to metabolism by shared enzymes[7]. Also, intake of LC *n*-3 PUFA may inhibit the metabolism of ALA[7]. Interestingly, most studies reporting lower risk of atherosclerotic vascular disease with intake of ALA has been conducted in populations with a relatively low intake of LC *n*-3 PUFA and the previous findings by Mozaffarian *et al.*[24] support the view that the association between ALA intake and ASCVD may depend on intake of LC *n*-3 PUFA. Thus, the neutral findings of the associations between ALA intake and myocardial infarction, ischaemic stroke and PAD in the DCH cohort might be due to the high intake of LC *n*-3 PUFA in this population[5,36,37]. Also, competitive mechanisms between ALA and the linoleic acid may affect the association between ALA and ASCVD.

Several studies investigating intake of *n*-3 PUFA in relation to ASCVD included adjustment for both cardiovascular risk factors and potential dietary risk factors in the same statistical model. Analyses including adjustment for dietary factors may contribute with insight in the aetiology to exposure to *n*-3 PUFA by limiting potential residual confounding from diet. Previous studies that have investigated associations between *n*-3 PUFA and ASCVD in analyses including adjustment for both cardiovascular risk factors and diet have used a variety of different nutrients such as other PUFA, MUFA, SFA, *trans* fatty acids, vitamins, fibre, β-carotene, electrolytes or proteins while some did include adjustment for foods such as fruit, vegetables or meat as well. However, the interpretation of the observed measures of association may not be straightforward if major restrictions are introduced to the underlying dietary pattern by adding of dietary covariates and the public health relevance may be reduced. Therefore, analyses including traditional risk factors of the outcome of interest and analyses including dietary risk factors in addition to traditional risk factors using separate statistical models represent two different approaches which each may contribute with a more detailed interpretation of the associations of interest.

Total ischaemic stroke is a heterogeneous condition and the different associations observed between LC *n*-3 PUFA and ischaemic stroke subtypes in the DCH cohort may suggest that in studies of aetiology separate analyses of ischaemic stroke subtypes may contribute to a deeper understanding of the underlying biology of exposure to LC *n*-3 PUFA.

In summary, prospective studies using estimated intakes or adipose tissue content of marine and the plant-derived *n*-3 PUFA have contributed with important knowledge on the role of these PUFA in relation to development of ASCVD, but several gaps in the literature remains to be elucidated.

Financial Support

C. S. B. has received a grant from The Danish Heart Foundation (grant number 17-R115-A7415-22060), Helene and Georg Jensens and Ethel Merethe and Christian Pontoppidan's Fund. The funding agencies had no role in writing of the manuscript or decision to publish.

Conflict of Interest

None.

Authorship

C. S. B. and E. B. S. planned the study. C. S. B. wrote the first draft of the manuscript. All authors contributed to planning the outline of this paper. S. K. V., A. N. L., S. L. C. and E. B. S. critically revised the manuscript and contributed with intellectual content to the paper. All authors read and approved the final manuscript.

References

1. De Caterina R (2011) n-3 Fatty Acids in Cardiovascular Disease. *N Engl J Med* **364**, 2439–2450.
2. Calder PC (2015) Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochim Biophys Acta* **1851**, 469–484.
3. Gebauer SK, Psota TL, Harris WS *et al.* (2006) n-3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *Am J Clin Nutr* **83**, 1526–1535.
4. Hu FB, Stampfer MJ, Manson JE *et al.* (1999) Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. *Am J Clin Nutr* **69**, 890–897.
5. Bork CS, Jakobsen MU, Lundbye-Christensen S *et al.* (2016). Dietary intake and adipose tissue content of alpha-linolenic acid and risk of myocardial infarction: a Danish cohort study. *Am J Clin Nutr* **104**, 41–48.
6. Rimm EB, Appel LJ, Chiuve SE *et al.* (2018) Seafood long-chain n-3 polyunsaturated fatty acids and cardiovascular disease: a science advisory from the American Heart Association. *Circulation* **138**, e35–e47.
7. Baker EJ, Miles EA, Burdge G *et al.* (2016) Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. *Prog Lipid Res* **64**, 30–56.
8. Burdge GC & Calder PC (2005) Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev* **45**, 581–597.
9. Burdge GC (2018) Is essential fatty acid interconversion an important source of polyunsaturated fatty acids in humans? *Br J Nutr* **27**, 1–28.
10. Burdge GC & Wootton SA (2002) Conversion of α-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr* **88**, 411–420.
11. Hodson L, Skeaff CM & Fielding BA (2008) Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res* **47**, 348–380.
12. Rajaram S (2014) Health benefits of plant-derived alpha-linolenic acid. *Am J Clin Nutr* **100**, 443–448.

https://doi.org/10.1017/S0029665119000582 Published online by Cambridge University Press

13. Mozaffarian D & Wu JH (2011) Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol* **58**, 2047–2067.
14. Arab L & Akbar J (2002) Biomarkers and the measurement of fatty acids. *Public Health Nutr* **5**, 865–871.
15. de Lorgeril M & Salen P (2007) Mediterranean diet and n-3 fatty acids in the prevention and treatment of cardiovascular disease. *J Cardiovasc Med* **8** (Suppl. 1), 38–41.
16. Estruch R, Ros E, Salas-Salvadó J *et al.* (2018) Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. *N Engl J Med* **378**, e34.
17. Oomen CM, Ocké MC, Feskens EJ *et al.* (2001) Alpha-linolenic acid intake is not beneficially associated with 10-y risk of coronary artery disease incidence: the Zutphen Elderly Study. *Am J Clin Nutr* **74**, 457–463.
18. Fretts AM, Mozaffarian D, Siscovick DS *et al.* (2014) Plasma phospholipid and dietary α-linolenic acid, mortality, CHD and stroke: the Cardiovascular Health Study. *Br J Nutr* **112**, 1206–2013.
19. Vedtofte MS, Jakobsen MU, Lauritzen L *et al.* (2014) Association between the intake of alpha-linolenic acid and the risk of CHD. *Br J Nutr* **112**, 735–743.
20. Dolecek T (1992) Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial. *Proc Soc Exp Biol Med* **200**, 177–182.
21. Ascherio A, Rimm EB, Giovannucci EL *et al.* (1996) Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. *Br Med J* **313**, 84–90.
22. Pietinen P, Ascherio A, Korhonen P *et al.* (1997) Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am J Epidemiol* **145**, 876–887.
23. Koh AS, Pan A, Wang R *et al.* (2015) The association between dietary omega-3 fatty acids and cardiovascular death: the Singapore Chinese Health Study. *Eur J Prev Cardiol* **22**, 364–372.
24. Mozaffarian D, Ascherio A, Hu FB *et al.* (2005) Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. *Circulation* **111**, 157–164.
25. Albert CM, Oh K, Whang W *et al.* (2005) Dietary alpha-linolenic acid intake and risk of sudden cardiac death and coronary heart disease. *Circulation* **112**, 3232–3238.
26. Vedtofte MS, Jakobsen MU, Lauritzen L *et al.* (2011) Dietary a-linolenic acid, linoleic acid, and n-3 long-chain PUFA and risk of ischemic heart disease. *Am J Clin Nutr* **94**, 1097–1103.
27. de Goede J, Verschuren WM, Boer JM *et al.* (2011) Alpha-linolenic acid intake and 10-year incidence of coronary heart disease and stroke in 20,000 middle-aged men and women in the Netherlands. *PLoS One* **6**, e17967.
28. Wei J, Hou R, Xi Y *et al.* (2018) The association and dose-response relationship between dietary intake of α-linolenic acid and risk of CHD: a systematic review and meta-analysis of cohort studies. *Br J Nutr* **119**, 83–89.
29. Campos H, Baylin A & Willett WC (2008) α-Linolenic acid and risk of nonfatal acute myocardial infarction. *Circulation* **118**, 339–345.
30. Guallar E, Aro A, Jiménez FJ *et al.* (1999) Omega-3 fatty acids in adipose tissue and risk of myocardial infarction: the EURAMIC study. *Arterioscler Thromb Vasc Biol* **19**, 1111–1118.
31. Pedersen JI, Ringstad J, Almendingen K *et al.* (2000) Adipose tissue fatty acids and risk of myocardial infarction – a case-control study. *Eur J Clin Nutr* **54**, 618–625.
32. Kark JD, Kaufmann NA, Binka F *et al.* (2003) Adipose tissue n-6 fatty acids and acute myocardial infarction in a population consuming a diet high in polyunsaturated fatty acids. *Am J Clin Nutr* **77**, 796–802.
33. Lopes C, Aro A, Azevedo A *et al.* (2007) Intake and adipose tissue composition of fatty acids and risk of myocardial infarction in a male Portuguese community sample. *J Am Diet Assoc* **107**, 276–286.
34. Larsson SC, Virtamo J & Wolk A (2012) Dietary fats and dietary cholesterol and risk of stroke in women. *Atherosclerosis* **221**, 282–286.
35. Rhee J, Kim E, Buring J *et al.* (2017) Fish consumption, Omega-3 fatty acids, and risk of cardiovascular disease. *Am J Prev Med* **52**, 10–19.
36. Bork CS, Venø SK, Lundbye-Christensen S *et al.* (2018) Dietary intake of α-linolenic acid is not appreciably associated with risk of Ischemic stroke among middle-aged Danish men and women. *J Nutr* **148**, 952–958.
37. Bork CS, Venø SK, Lundbye-christensen S *et al.* (2018) Adipose tissue content of alpha-linolenic acid and the risk of ischemic stroke and ischemic stroke subtypes: a Danish case-cohort study. *PLoS One* **13**, e0198927.
38. Dyerberg J, Bang HO, Stoffersen E *et al.* (1978) Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* **2**, 117–119.
39. Alexander DD, Miller PE, Van Elswyk ME *et al.* (2017) A meta-analysis of randomized controlled trials and prospective cohort studies of eicosapentaenoic and docosahexaenoic long-chain Omega-3 fatty acids and Coronary Heart Disease Risk. *Mayo Clin Proc* **92**, 15–29.
40. Joensen AM, Schmidt EB, Dethlefsen C *et al.* (2010) Dietary intake of total marine n-3 polyunsaturated fatty acids, eicosapentaenoic acid, docosahexaenoic acid and docosapentaenoic acid and the risk of acute coronary syndrome – a cohort study. *Br J Nutr* **103**, 602–607.
41. Joensen AM, Overvad K, Dethlefsen C *et al.* (2011) Marine n-3 polyunsaturated fatty acids in adipose tissue and the risk of acute coronary syndrome. *Circulation* **124**, 1232–1238.
42. Gammelmark A, Nielsen MS, Bork CS *et al.* (2016) Association of fish consumption and dietary intake of marine n-3 PUFA with myocardial infarction in a prospective Danish cohort study. *Br J Nutr* **116**, 167–177.
43. Gammelmark A, Nielsen MS, Bork CS *et al.* (2016) Adipose tissue content of marine n-3 polyunsaturated fatty acids is inversely associated with myocardial infarction. *J Am Coll Cardiol* **67**, 1008–1009.
44. Iso H, Rexrode KM, Stampfer MJ *et al.* (2001) Intake of fish and omega-3 fatty acids and risk of stroke in women. *JAMA* **285**, 304–312.
45. He K, Rimm EB, Merchant A *et al.* (2002) Fish consumption and risk of stroke in men. *JAMA* **288**, 3130–3136.
46. Montonen J, Järvinen R, Reunanen A *et al.* (2009) Fish consumption and the incidence of cerebrovascular disease. *Br J Nutr* **102**, 750–756.
47. de Goede J, Verschuren WM, Boer JM *et al.* (2012) Gender-specific associations of marine n-3 fatty acids and fish consumption with 10-year incidence of stroke. *PLoS One* **7**, e33866.
48. Kippler M, Larsson SC, Berglund M *et al.* (2016) Associations of dietary polychlorinated biphenyls and long-chain omega-3 fatty acids with stroke risk. *Environ Int* **94**, 706–711.

https://doi.org/10.1017/S0029665119000582 Published online by Cambridge University Press

49. Venø SK, Bork CS, Jakobsen MU *et al.* (2019) Marine n-3 polyunsaturated fatty acids and the risk of ischemic stroke. *Stroke* **50**, 274–282.
50. Wallström P, Sonestedt E, Hlebowicz J *et al.* (2012) Dietary fiber and saturated fat intake associations with cardiovascular disease differ by sex in the Malmö Diet and Cancer Cohort: a prospective study. *PLoS One* **7**, e31637.
51. Yamagishi K, Iso H, Date C *et al.* (2008) Fish, omega-3 polyunsaturated fatty acids, and mortality from cardiovascular diseases in a nationwide community-based cohort of Japanese men and women the JACC (Japan Collaborative Cohort Study for Evaluation of Cancer Risk) Study. *J Am Coll Cardiol* **52**, 988–996.
52. Lasota A, Grønholdt M, Bork CS *et al.* (2018) Marine n-3 fatty acids and the risk of peripheral arterial disease. *J Am Coll Cardiol* **72**, 1576–1584.
53. Innes JK & Calder PC (2018) The differential effects of eicosapentaenoic acid and docosahexaenoic acid on cardiometabolic risk factors: a systematic review. *Int J Mol Sci* **19**, E532.

https://doi.org/10.1017/S0029665119000582 Published online by Cambridge University Press