Adipose tissue n-3/n-6 fatty acids ratios versus n-3 fatty acids fractions as predictors of myocardial infarction

Chiusolo, Simona; Bork, Christian Sørensen; Gentile, Francesco; Lundbye-Christensen, Søren; Harris, William S.; Schmidt, Erik Berg; De Caterina, Raffaele

Published in:
American Heart Journal

DOI (link to publication from Publisher):
10.1016/j.ahj.2023.03.019

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

Adipose tissue n-3/n-6 fatty acids ratios versus n-3 fatty acids fractions as predictors of myocardial infarction

Simona Chiusolo, MD [a,*], Christian Sørensen Bork, MD, PhD [b,*], Francesco Gentile, MD [f], Søren Lundbye-Christensen, PhD [c,d,c], William S. Harris, PhD [e], Erik Berg Schmidt, MD, DMSc [b,c], and Raffaele De Caterina, MD, PhD [f] *Modena, Italy; Aalborg, Denmark; Aalborg, Denmark; Aalborg, Denmark; Sioux Falls, SD; Pisa, Italy*

ABSTRACT

Background Tissue levels of n-3 polyunsaturated fatty acids (PUFAs) have been inversely related with risk of myocardial infarction (MI). Whether ratios of n-3 to n-6 PUFAs, reflecting both dietary intake of n-3 PUFAs and competing n-6 PUFAs, are better predictors of future MI than n-3 PUFA fractions is unclear. We aimed at investigating whether such ratios in adipose tissue better predict MI than n-3 PUFA fractions.

Methods Subcutaneous adipose tissue biopsies were obtained in a random sample (n = 3,500) of the Diet, Cancer and Health cohort (n = 57,053). Adipose tissue content of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), alpha-linolenic acid (ALA), arachidonic acid (AA) and linoleic acid was determined using gas chromatography. Fractions of selected n-3 PUFAs and n-3/n-6 PUFA ratios were correlated to the 15-year occurrence of MI in a case-cohort design.

Results A total of 2,406 participants experienced an MI during follow-up. Adipose tissue total marine n-3 PUFAs, EPA+DHA, EPA, EPA/AA, DHA/AA and (EPA + DPA + DHA)/AA were all inversely associated with risk of incident MI. Evaluating the predictive power (Harrel's C-index) of the selected metrics, fractions of marine n-3 PUFAs and ratios of EPA/AA, DHA/AA, (EPA + DHA)/AA and (EPA + DPA + DHA)/AA all refined risk prediction over age and sex alone. At multivariable analyses, however, the above ratios were the only metrics providing additional risk prediction. Differences in ratios were related to differences in food intake.

Conclusions Both adipose tissue n-3 PUFAs fractions and ratios of n-3 PUFAs/AA were associated with a lower occurrence of MI, but ratios provided superior risk prediction. Dietary strategies affecting n-3/n-6 PUFA ratios should be further investigated for prediction of MI with dietary interventions at the population level and in intervention studies. (Am Heart J 2023;262:38–48.)

Background

Clinical manifestations of cardiovascular disease (CVD) may be prevented through population-level strategies, including dietary approaches.[1] Among these, dietary consumption of n-3 polyunsaturated fatty acids (PUFAs) has been associated with reduced risk of both fatal and non-fatal cardiovascular (CV) events, including myocardial infarction (MI).[2] PUFAs in membrane phospholipids influence physical and biologic properties of cell membranes.[2] Linoleic acid (LA) (18:2 n-6) and alpha-linolenic acid (ALA) (18:3 n-3) are essential nutrients. The former can be converted to arachidonic acid (AA) (20:4 n-6); the latter to eicosapentaenoic acid (EPA) (20:5 n-3), Docosapentaenoic acid (DPA, 22:5 n-3), and docosahexaenoic acid (DHA) (22:6 n-3). N-3 and n-6 PUFAs compete for

From the [a]Cardiology Division, University of Modena and Reggio Emilia, Modena Hospital, Modena, Italy, [b]Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark, [c]Department of Clinical Medicine, Aalborg University, Aalborg, Denmark, [d]Unit of Clinical Biostatistics, Aalborg University Hospital, Aalborg, Denmark, [e]Fatty Acid Research Institute and the Department of Internal Medicine, Sanford School of Medicine, University of South Dakota, Sioux Falls, SD, [f]Cardiology Division, Pisa University Hospital and University of Pisa, Pisa, Italy

**SC and CSB equally contributed to this work.*

Abbreviations: PUFA, polynsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; LA, linoleic acid; ALA, alfa-linolenic acid; DPA, docosapentaenoic acid.

Submitted September 18, 2022; accepted March 31, 2023

Reprint requests: Raffaele De Caterina, MD, PhD, Chair of Cardiology, University of Pisa, and Cardiovascular Division, Pisa University Hospital, Via Paradisa 2, 56124 Pisa, Italy.
E-mail address: raffaele.decaterina@unipi.it.
0002-8703

https://doi.org/10.1016/j.ahj.2023.03.019

the sn-2 positions in membrane phospholipids and for enzymes that convert members of both families into a wide array of bioactive oxylipins.[3,4] Current Western diets are low in n-3 but rich in n-6 PUFAs[5] as opposed to estimates of the ratios of these FA families throughout most of human evolution.[6,7] There is evidence from epidemiologic studies for an inverse relationship between n-3 PUFAs and risk of CVD, while studies on intakes[8-10] and biomarker-based data[11,12] have reported modest associations of n-6 PUFAs with CVD risk.[13] CV benefits of n-3 PUFAs appear to be larger—if anything—than those of n-6 PUFAs. As these 2 families of FA compete for incorporation in membrane phospholipids, it is plausible that their biological effects are in competition.

Fractions of PUFAs in tissues in general,[14,15] and in the adipose tissue in particular,[16] provide an objective measure of long-term PUFA intake, and are considered more reliable than largely used dietary questionnaires based on self-reported estimates of intake.[17,18] Thus, the halftime of adipose tissue is 1 to 2 years, which makes adipose tissue the gold standard biomarker of PUFAs.[16] The fraction of n-3 PUFAs in adipose tissue has previously been shown to be inversely associated with the risk of MI in the Diet, Cancer and Health cohort,[19,20] but whether fractions of marine n-3 PUFAs or ratios of n-3 to n-6 PUFAs in adipose tissue provide better prediction on development of future MIs remains unknown.

We here aimed at investigating comparative associations and predictive power of marine n-3 PUFAs fractions and selected ratios of n-3 to n-6 PUFAs in adipose tissue with the occurrence of MI over a long period of follow-up, and put these metrics in relation with food intakes obtained through dietary questionnaires. We speculate that such information may inform dietary advice.

Methods

The diet, cancer and health cohort

The present study was based on data accrued from the Danish Diet, Cancer and Health cohort, which has previously been described in detail.[21] Briefly, from 1993 to 1997, 57,053 men and women from the general population, aged 50 to 65 years, born in Denmark, living in and around Aarhus and Copenhagen and with no previous diagnosis of cancer, were enrolled. During enrollment, all participants also completed a detailed questionnaire on health status, social factors, physical activity, education, smoking habits and alcohol consumption. In addition, information on body weight, height, and blood pressure were collected, and adipose tissue samples were obtained (see below). Furthermore, all participants completed a semi-quantitative 192-item food frequency questionnaire. This food frequency questionnaire was developed during the planning of the Diet, Cancer and Health cohort[22] and has been validated against 2 times 7-day diet records.[23]

Subjects with a prior diagnosis of MI as well as participants with missing information on exposure and/or covariates were excluded from the analysis (Figure 1). Adipose tissue samples were collected at baseline, but PUFA composition was determined only in subjects experiencing an MI during follow-up (N = 2,406) and in a subcohort of randomly selected subjects (n = 3,500) and analyzed using a case-cohort design. The study was conducted according to the Helsinki Declaration, and approved by the ethics review boards of the participating institutions and the Danish Data Protection Agency. All participants gave written informed consent to the use of their data for scientific purposes.

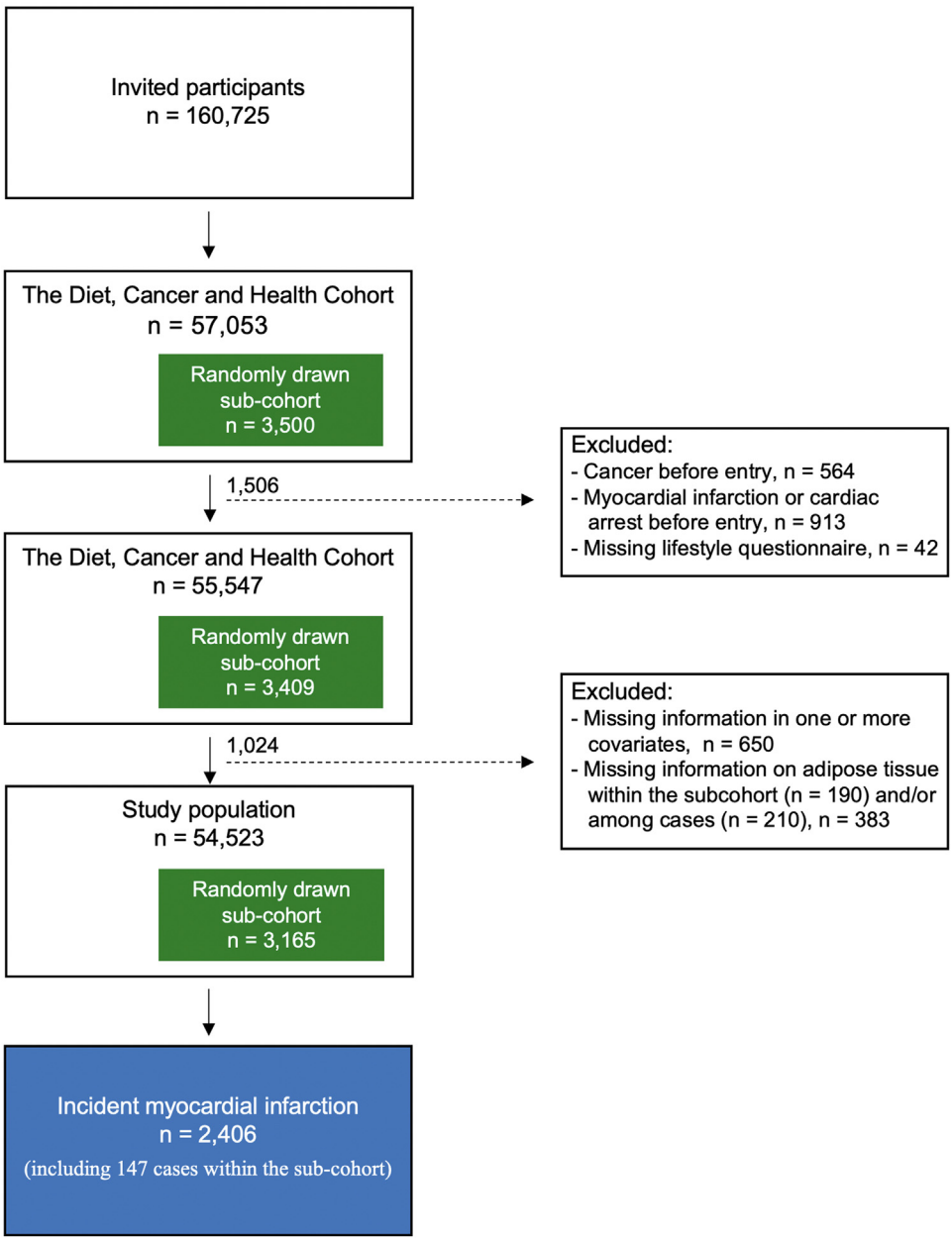
Adipose tissue biopsy and analysis

Biopsy specimens of subcutaneous adipose tissue were obtained from the buttocks of all participants using a Luer-lock syringe (Terumo, Terumo Corp, Tokyo, Japan).[24] Biopsy tissue were flushed with nitrogen and stored at −150 °C. Before analysis, biopsies were thawed and 1 to 2 mg of adipose tissue prewarmed at 50 °C for 10 minutes. In the next step, the fat tissue was dissolved in heptane at 50 °C, and FAs were trans-esterified by 2 mol/L KOH in methanol at 50 °C for 2 minutes, according to the International Union of Pure and Applied Chemistry Standard Methods for the Analysis of Oils, Fats, and Derivatives. FA composition was determined through gas chromatography by using a Varian 3900 gas chromatograph with a cyanopropyl phase (CP)-8400 autosampler (Varian, Middleburg, the Netherlands) equipped with a flame ionization detector. Split injection mode, a CP-sil 88 60-m length × 0.25-mm internal diameter capillary column, temperature programming from 90 °C to 210 °C, and constant flow were used. Helium was the carrier gas, and commercially available standards (Nu-Chek-Prep Inc, Elysian, MN) were used to identify the individual FAs. Results for individual FAs were given as weight percentage (fraction) of total FAs. The between-assay coefficients of variation were: for EPA 6.4%; for DPA 3.5%; for DHA 4.1%; for ALA 1.9%; for AA 3.9%; and for LA 0.8%.

Outcome assessment

Incident cases of MI were identified through nationwide Danish registries (International Classification of Disease [ICD]-8 codes 410.00–410.99; ICD-10 codes: I21.0–I21.9) including the Danish National Patient Register[25] and the Danish Causes of Death Register.[26] A previous validation study including MI cases up to 2003 found a positive predictive value >92% for MI based on review of medical records when diagnoses were obtained from a hospital ward.[19,27] All validated cases of MI from the validation study were immediately accepted as cases for the present study and from January 2004 through July 2013, all participants with an incident MI diagnosed from a hospital ward were accepted as cases without further

Figure 1



Flowchart of cases and subcohort participants.

validation. All other potential cases were validated by reviewing a complete list of diagnoses and interventional procedures recorded in the Danish National Patient Register. Additionally, cases of cardiac arrest (ICD-8: 427.27 and ICD-10: I46.0-I46.9) were also included if the arrest was considered to be related to MI after assessment of each individual case. Participants were followed until incident MI, death, emigration from Denmark or end of the 15 years follow-up from baseline evaluation.

Statistical analyses

We used hazard rate ratios (HRs) to investigate the associations between adipose tissue fraction of n-3 PUFAs or ratios of n-3 to n-6 PUFAs with the occurrence of incident MI. We interpreted the obtained HRs as measures of relative risk. PUFAs in adipose tissue were categorized into quintiles. HRs with 95% confidence intervals (CI) were estimated using weighted Cox proportional hazard regression with time-in-study as the underlying time

scale. All cases were assigned a weight equal to one, whereas all noncases in the subcohort were assigned a weight calculated as the ratio between the number of noncases in the cohort divided by the number of noncases in the subcohort.[28] This weighting approach has previously been shown to perform well in a validation study compared with most other commonly used case-cohort estimators.[29] Each noncase in the subcohort was assigned a weight of 17.3 (52,117/3,018). Standard errors were estimated using a robust variance estimator.

The predictive performance of the exposure metrics of interest was quantified by the Harrell's C-index.[30] The C-index (sometimes called C-statistic) is considered a rank-correlation measure, and corresponds to the area under the receiver operating characteristics in a logistic regression.[31] A perfect predictive performance has a C-index = 1, while the absence of any predictive performance has a C-index = 0.5. The predictive performance of the exposures was reported *as the difference in C-index* when adding the exposure to the model.

We examined the associations and predictive performance for MI of the fraction of marine n-3 PUFAs (EPA + DPA + DHA, EPA + DHA or EPA) and the following ratios (R) of n-3 and n-6 PUFAs:

- R1, ratio between the principal 20-carbon atom key products of the n-3 and n-6 pathways (EPA/AA);
- R2, ratio between the n-3 pathway final product and the key product of the n-6 pathway (DHA/AA);
- R3, ratio between the major biological active marine n-3 PUFAs and the key product of the n-6 pathway [(EPA + DHA)/AA];
- R4, ratio between the total fraction of long-chain n-3 PUFAs and the key product of the n-6 pathway [(EPA + DPA + DHA)/AA];
- R5, ratio between the total fraction of n-3 PUFAs and the sum of the key product and main precursor within n-6 PUFAs [(EPA + DPA + DHA + ALA)/(AA + LA)];
- R6, ratio between total fraction of n-3 PUFAs excluding DPA and the total fraction of n-6 PUFAs ([EPA +DHA + ALA]/[AA + LA]).

We conducted age- and sex-adjusted analyses (Model 1) as well as multivariable analyses (Model 2) including additional adjustment for duration of schooling (≤7, 8-10, or >10 years), smoking (never, former or current smoker of <15, 15-25, >25/day), physical activity (h/week), waist circumference (cm), body mass index (kg/m^2), alcohol intake (g/day) and history of diabetes mellitus, hypertension and hypercholesterolemia. "Hypercholesterolemia," in particular, was defined as self-reported hypercholesterolemia and/or use of lipid-lowering treatment. "Hypertension" was defined as self-reported hypertension and/or use of anti-hypertensive medications." Exposures of interest and continuous adjustment covariates were modeled using restricted cubic splines with 3 knots.

In order to allow a practical translation of differences here investigated in fractions vs ratios into differences in dietary intakes, we finally created radar plots relating the underlying dietary pattern with adipose tissue content of EPA + DHA (fraction) and EPA/AA (ratio) within the subcohort. To do this, with data available on 18 selected foods and beverages through dietary questionnaires submitted to participants in the cohort at enrollment, we calculated the median residual energy-adjusted intakes of these dietary components as occurring in the highest and lowest quintile of adipose tissue EPA + DHA fraction and in the highest and lowest quintile of EPA/AA ratio, and expressed such intakes as percent of the overall median intakes in the cohort. Thus, interquintile differences in the selected FA metrics were related to food intakes as derived from the questionnaire. We then plotted these interquintile differences in the indicated adipose tissue FA fraction and ratio for each of the 18 food categories assessed in the questionnaire. This visual representation, already previously adopted by us,[32] allows to pinpoint which differences in dietary intake correlates (and likely—by reasonable inference—causes) relevant differences in FA incorporation indices here investigated, as well as, eventually, differences in the occurrence of MI.

Probability (*P*) values <.05 were considered statistically significant. We used the STATA, version 16 (StataCorp LP, Lakeway Drive College Station, TX) software for statistical analyses.

Results

Study population

From the original cohort and after exclusions we identified 2,406 participants with an incident diagnosis of MI (Figure 1). Baseline characteristics of cases and the subcohort (N = 3,165) are provided in Table 1. Established risk factors were more prevalent among participants who later became cases compared with participants in the subcohort. Cases were characterized by a larger proportion of men, current smokers and individuals with a shorter duration of schooling, higher age, alcohol intake, body mass index, and waist circumference, as well as with lower levels of physical activity.

Associations between fractions of n-3 PUFAs and ratios of n-3 to n-6 PUFAs with MI

In analyses adjusted for age and sex (Model 1), we found that both the fraction of EPA + DHA, EPA and the ratios EPA/AA, DHA/AA, (EPA + DHA)/AA and (EPA + DPA + DHA)/AA were inversely associated, in a dose-dependent manner, with the risk of MI, while no appreciable associations were found for EPA + DHA + DPA, (EPA + DPA + DHA + ALA)/(LA + AA) and (EPA + DHA + ALA)/(LA + AA) (Table 2). Similar patterns of association were observed in analyses adjusted for established risk factors (Model 2), but the observed

Table 1. Baseline characteristics of the study population

	Subcohort (n = 3,165)	Myocardial infarction (n = 2,406)
Sex (%)		
Males	53.5	72.2
Females	46.5	27.8
Age at enrolment (y)	56.3	58.3
Length of schooling (%)		
≤7 y	32.8	43.2
8-10 y	45.0	40.3
>10 y	22.2	16.5
Smoking (%)		
Never	34.9	20.7
Former	29.1	26.7
Current <15 g/d	13.4	15.6
Current 15-25 g/d	15.8	23.7
Current >25 g/d	6.8	13.2
Physical activity (hours/week)	2.5 (0.0; 8.0)	2.0 (0.0; 7.5)
Waist circumference (cm)[1]	90 (73; 106)	95 (79; 111)
Body mass index (kg/m^2)[1]	25.7 (21.8; 31.2)	26.8 (22.6; 32.7)
Alcohol intake (g/d)[1]	13.7 (1.7; 48.1)	13.9 (1.2; 56.9)
Hypercholesterolemia	7.4	13.0
Hypertension	15.8	25.6
Diabetes mellitus	2.1	4.8
Fatty acids in adipose tissue		
EPA + DPA + DHA (%)	0.63 (0.40; 0.99)	0.62 (0.39; 0.94)
EPA + DHA (%)	0.36 (0.21; 0.61)	0.35 (0.20; 0.57)
EPA (%)	0.10 (0.05; 0.16)	0.09 (0.05; 0.16)
EPA/AA (ratio %)	0.26 (0.16; 0.46)	0.25 (0.16: 0.44)
DHA/AA (ratio %)	0.72 (0.40; 1.30)	0.67 (0.38: 1.21)
EPA + DHA/AA (ratio %)	1.72 (1.11. 2.79)	1.65 (1.08; 2.61)
EPA + DPA + DHA/AA (ratio %)	0.14 (0.11; 0.17)	0.14 (0.11; 0.17)
EPA + DPA + DHA + ALA/AA + LA (ratio %)	0.11 (0.09; 0.14)	0.11 (0.09; 0.14)
EPA + DHA + ALA/AA + LA (ratio %)	0.98 (0.57; 1.74)	0.92 (0.55; 1.62)

Continuous covariates are presented by the median (10th; 90th percentile) and categorical variables are given in percentage.

associations were attenuated compared to analyses adjusted for age and sex alone (Table 2).

Predictive performance of fractions of n-3 PUFAs and ratios of n-3 to n-6 PUFAs on future MI

In analyses adjusted for age and sex, we found that the fraction of total marine n-3 PUFAs, EPA + DHA, EPA, as well as ratios of EPA/AA, DHA/AA, (EPA + DHA)/AA and (EPA + DPA + DHA)/AA refined risk prediction over age and sex alone (Table 3). However, the EPA/AA ratio, the (EPA + DHA)/AA ratio, the DHA/AA ratio and the (EPA + DPA + DHA)/AA ratio were the only metrics that provided additional statistically significant risk predictions in multivariable analyses, with the lowest *P*-value achieved with the EPA/AA ratio (Table 3).

Dietary patterns underlying marine n-3 PUFAs and the EPA/AA ratio

By correlating adipose tissue FA analyses with results of the dietary questionnaires, the radar plot created visually describes the underlying average dietary pattern in subjects in the highest and lowest quintile of EPA + DHA and in those in the highest and lowest quintile of EPA/AA, respectively. Interquintile differences (highest quintile minus lowest quintile in EPA + DHA vs highest minus lowest quintile of EPA/AA) were overall not prominent, and practically absent for medium and fatty fish, but mostly apparent in categories depicting the intake of vegetable oils and mayonnaises, margarines, butter and animal fat, processed meat and fruit and vegetables, which are food categories that reflect the intake of n-6 PUFAs (Figure 2).

Discussion

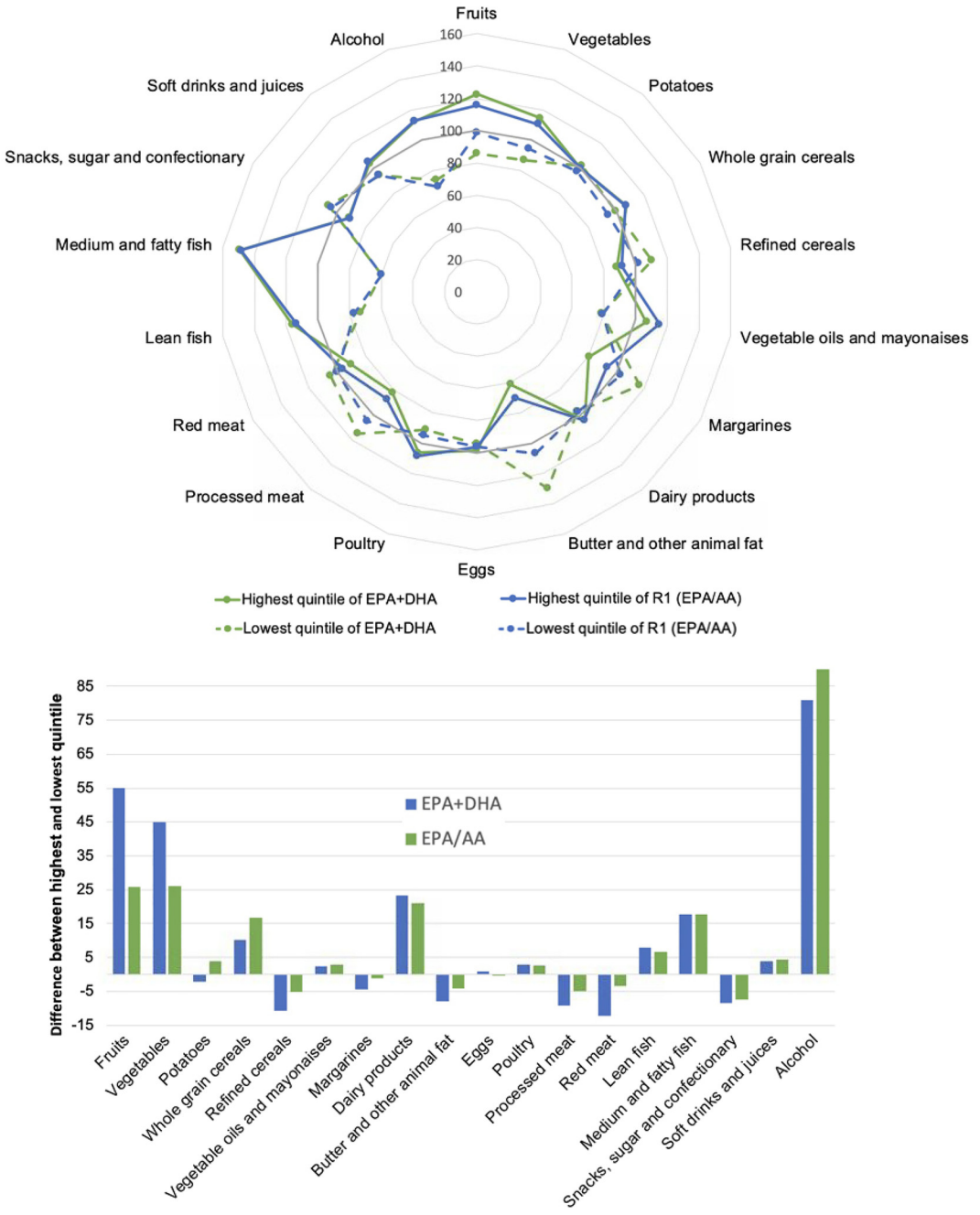
In this large case-cohort study, we found that the fraction in adipose tissue of EPA + DHA, EPA and ratios of EPA/AA, DHA/AA, (EPA + DHA)/AA and (EPA + DPA + DHA)/AA were all inversely associated, in a dose-dependent manner, with the risk of MI during 15 years of follow-up. In analyses exploring the predictive performance measured by Harrel's C-index, we found that the fraction of marine n-3 PUFAs as well as ratios of EPA/AA, DHA/AA and (EPA + DPA + DHA)/AA in adipose tissue refined risk prediction over age and sex alone. However, in multivariable analyses the above ratios were the only metrics that provided additional statistically significant risk predictions. The incremental value

Table 2. Associations between adipose tissue fraction of n-3 PUFAs and ratios of n-3/n-6 PUFAs and risk of myocardial infarction

	Model 1 Hazard ratio (95% CI)	Model 2 Hazard ratio (95% CI)
EPA+DPA+DHA		
Q1 (0.15-0.48)	1 (reference)	1 (reference)
Q2 (0.48-0.58)	1.06 (0.89; 1.26)	1.09 (0.90; 1.31)
Q3 (0.58-0.69)	0.94 (0.79; 1.12)	0.98 (0.81; 1.19)
Q4 (0.69-0.84)	0.98 (0.82; 1.17)	1.10 (0.90; 1.34)
Q5 (0.84-2.36)	0.79 (0.66; 0.94)	0.80 (0.65; 0.99)
EPA+DHA		
Q1 (0.06-0.26)	1 (reference)	1 (reference)
Q2 (0.26-0.32)	0.86 (0.72; 1.03)	0.82 (0.68; 0.99)
Q3 (0.32-0.40)	0.87 (0.73; 1.03)	0.91 (0.75; 1.10)
Q4 (0.40-0.51)	0.83 (0.70; 1.00)	0.91 (0.75; 1.11)
Q5 (0.51-1.76)	0.63 (0.52; 0.76)	0.65 (0.53; 0.80)
EPA		
Q1 (0.02-0.07)	1 (reference)	1 (reference)
Q2 (0.07-0.09)	0.99 (0.84; 1.17)	1.01 (0.84; 1.21)
Q3 (0.09-0.11)	0.84 (0.70; 0.99)	0.85 (0.70; 1.04)
Q4 (0.11-0.14)	0.87 (0.73: 1.04)	0.88 (0.73; 1.07)
Q5 (0.14-0.50)	0.71 (0.60; 0.85)	0.75 (0.61; 0.92)
R1 (EPA/AA)		
Q1 (0.06-0.18)	1 (reference)	1 (reference)
Q2 (0.18-0.23)	0.96 (0.80; 1.14)	1.05 (0.87; 1.28)
Q3 (0.23-0.29)	0.82 (0.68; 0.98)	0.90 (0.74; 1.10)
Q4 (0.29-0.38)	0.66 (0.55; 0.79)	0.82 (0.67; 1.00)
Q5 (0.38-2.00)	0.57 (0.47; 0.69)	0.77 (0.62; 0.95)
R2 (DHA/AA)		
Q1 (0.10-0.50)	1 (reference)	1 (reference)
Q2 (0.50-0.64)	0.86 (0.72; 1.02)	0.85 (0.70; 1.03)
Q3 (0.64-0.80)	0.86 (0.72; 1.02)	0.96 (0.79; 1.15)
Q4 (0.80-1.05)	0.65 (0.55; 0.78)	0.79 (0.66; 0.96)
Q5 (1.05-6.00)	0.56 (0.47; 0.67)	0.74 (0.61; 0.91)
R3 (EPA + DHA)/AA		
Q1 (0.59-1.28)	1 (reference)	1 (reference)
Q2 (1.28-1.58)	0.88 (0.74; 1.04)	0.91 (0.75; 1.10)
Q3 (1.58-1.89)	0.78 (0.65; 0.92)	0.89 (0.73; 1.08)
Q4 (1.89-2.33)	0.65 (0.55; 0.78)	0.80 (0.66; 0.97)
Q5 (2.33-10.73)	0.55 (0.46; 0.66)	0.75 (0.61; 0.92)
R4 (EPA + DPA + DHA)/AA		
Q1 (0.04-0.12)	1 (reference)	1 (reference)
Q2 (0.12-0.13)	0.85 (0.71; 1.01)	0.90 (0.75; 1.09)
Q3 (0.13-0.14)	0.78 (0.66; 0.93)	0.90 (0.74; 1.09)
Q4 (0.14-0.16)	0.69 (0.58; 0.82)	0.84 (0.69; 1.03)
Q5 (0.16-0.30)	0.57 (0.48; 0.69)	0.79 (0.64; 0.97)
R5 (EPA + DPA + DHA + ALA)/(LA + AA)		
Q1 (0.03-0.10)	1 (reference)	1 (reference)
Q2 (0.10-0.11)	1.12 (0.93; 1.34)	1.00 (0.82; 1.23)
Q3 (0.11-0.12)	1.21 (1.01; 1.45)	1.09 (0.89; 1.33)
Q4 (0.12-0.13)	1.18 (0.99; 1.42)	1.09 (0.89; 1.33)
Q5 (0.13-0.24)	1.06 (0.89; 1.28)	0.91 (0.74; 1.12)
R6 (EPA + DHA + ALA)/(LA + AA)		
Q1 (0.25-0.70)	1 (reference)	1 (reference)
Q2 (0.70-0.88)	1.11 (0.92; 1.33)	1.08 (0.88; 1.32)
Q3 (0.88-1.10)	1.20 (1.00; 1.44)	1.12 (0.92; 1.37)
Q4 (1.10-1.41)	1.12 (0.93; 1.35)	1.10 (0.90; 1.34
Q5 (1.41-8.00)	1.07 (0.89; 1.28)	0.97 (0.79; 1.18)

AA, arachidonic acid; *ALA*, alpha-linolenic acid; *BMI*, body mass index; *CI*, confidence interval; *DHA*; docosahexaenoic acid; *DPA*, docosapentaenoic acid; *EPA*, eicosapentaenoic acid; *HR*, Hazard ratio; *LA*, linoleic acid; *PUFA*, polyunsaturated fatty acids; *Q*, quintile (with ranges of values).
Model 1 included baseline age and sex; Model 2 included the variables of model A and duration of schooling, smoking, physical activity, waist circumference, body mass index, alcohol intake, hypercholesterolemia, hypertension and diabetes mellitus.

Figure 2



Upper panel: Radar plot of the median intake of selected foods and beverages in the highest and lowest quintile of adipose tissue fraction of EPA + DHA and EPA/AA within the subcohort, relating differences in fatty acid tissue fraction to differences in food intake. The dots represent the percent interquintile differences in relation to the median intake of the specific food in the cohort. Lower panel: Differences between the highest and lowest quintiles in either the EPA + DHA fraction or in the EPA to AA ratio in the various classes of food intakes investigated by the dietary questionnaire.

Table 3. Additive predictive power, measured by Harrel's C-index, of marine n-3 PUFAs fractions and ratios of n-3/n-6 PUFAs in relation to development of myocardial infarction

Reference models	Model 1		Model 2	
	0.658 (0.644; 0.672)	*P*-value	0.725 (0.712; 0.738)	*P*-value
ΔC-index (95% CI)				
EPA + DPA + DHA	0.003 (0.000; 0.006)	.026	0.001 (-0.001; 0.002)	.376
EPA + DHA	0.005 (0.002; 0.009)	.005	0.001 (-0.001; 0.003)	.222
EPA	0.006 (0.002: 0.009)	.003	0.001 (-0.001; 0.003)	.176
R1 (EPA/AA)	0.010 (0.005; 0.015)	<.001	0.002 (0.000; 0.004)	.026
R2 (DHA/AA)	0.012 (0.006; 0.017)	<.001	0.002 (0.000; 0.004)	.043
R3 (EPA + DHA/AA)	0.012 (0.006; 0.017)	<.001	0.002 (0.000; 0.004)	.030
R4 (EPA + DPA + DHA)/AA	0.010 (0.005; 0.015)	<.001	0.002 (0.000; 0.003)	.048
R5 (EPA + DPA + DHA + ALA)/(LA + AA)	0.001 (-0.002; 0.003)	.571	0.000 (-0.001; 0.002)	.559
R6 (EPA + DHA + ALA)/(AA + LA)	0.001 (-0.001; 0.003)	.493	0.000 (-0.001; 0.002)	.544

AA, arachidonic acid; *ALA*, alpha-linolenic acid; *BMI*, body mass index; *CI*, confidence interval; *DHA*; docosahexaenoic acid; *DPA*, docosapentaenoic acid; *EPA*, eicosapentaenoic acid; *HR*, Hazard ratio; *LA*, linoleic acid; *PUFA*, polyunsaturated fatty acids.
Model 1 included baseline age and sex.
Model 2 included the variables of model A and duration of schooling, smoking, physical activity, waist circumference, body mass index, alcohol intake, hypercholesterolemia, hypertension and diabetes mellitus.

of the ratios over the fractions could be related to the dietary intake of foods rich in n-6 FA, thus strongly suggesting that dietary competition between food categories rich in n-3 and n-6 FA occurs and this influences the future risk of MI.

To our knowledge, this is the first study investigating and reporting associations of ratios of marine n-3/n-6 PUFAs in adipose tissue with the long-term risk of incident MI, and to explore the predictive performance of these ratios in comparison with n-3 PUFA fractions.

A previous cross-sectional study suggested correlations between imbalance in the ratio of serum n-3 to n-6 PUFAs—mainly EPA/AA—and the occurrence of an acute coronary syndrome.[33] Retrospective studies have reported associations of a reduced ratio of (EPA + DHA) to AA with early-onset acute coronary syndrome[34] or of the EPA/AA ratio with the complexity of coronary artery lesions.[35] Largely because of the uncertain relation of n-6 PUFAs with CV risk—found for LA to be also inverse and directionally similar to n-3 PUFAs,[12,36,37] for uncertainties in describing metabolites of n-6 PUFAs as tout-court proinflammatory, and for uncertainties in expressing n-3 to n-6 PUFAs ratios for FAs differing in chain length and differing number of unsaturations (reviewed in[38]), the entire concept of the n-3 to n-6 PUFAs ratio has been criticized.[38] Our study now, however, advances this research area in precisely defining several types of ratios, comparing these metrics with fractions of n-3 PUFAs, and relating such metrics with the long-term occurrence of MI in an extended follow-up. Additional important strengths of our study are the use of measurements of n-3 and n-6 PUFAs in adipose tissue aspirates—proven to be stable over years[39] and considered a gold standard biomarker of their long-term dietary intake[40,41]—and with only a few subjects lost to follow-up. Outcome data derive from the nationwide Danish national patient register and were validated through a review of medical records to ensure a correct diagnosis of MI according to current criteria. In addition, study participants were extensively characterized at baseline, and we could perform an extensive adjustment for potential confounders. A final strength is the correlation of the interquintile differences of the adipose tissue fraction of EPA + DHA and of the EPA/AA ratio with differences in food intake, providing a translational message for possible dietary advice.

We also recognize limitations. Disadvantaged population groups tend to have a less healthy diet, and are often underrepresented in cohort studies requiring active participation, as in the Diet, Cancer and Health study.[21] Furthermore, we excluded all participants with MI before the study period, but not participants with other CVD who may have been at increased risk of MI. We did not have access to plasma or cells (eg, red blood cells), so we were unable to compare the fraction of n-3 PUFAs in adipose tissue with that in plasma or cells. The content of PUFAs in adipose tissue is considered the best long-term biomarker of intake of PUFAs reflecting the intake over 1 to 2 years, whereas content in plasma or erythrocytes may reflect intake over days to few months.[16] Limited data exist on the relationship between the content of PUFAs in plasma or cells and adipose tissue, but previous findings in adults (n = 75) suggested a high correlation between content of EPA (r = 0.57) and modest correlations between AA (r = 0.32) and LA (r = 0.27) in plasma phospholipids and adipose tissue, while no appreciable correlation was found for DHA.[42] Also, another study in adults (n = 27) reported high correlations between content of PUFAs in plasma nonesterified fatty acids and adipose tissue.[43] However, it should be emphasized that the markedly lower time frame that circulating biomarkers

in plasma and cells reflect PUFA consumption compared with adipose tissue implies that correlations between PUFAs in these lipid fractions will not be very high.

The fraction of n-3 PUFAs in adipose tissue depends not only on dietary intake but also on conversion from other FAs and on the metabolism and distribution of FAs into different tissues in the body. However, humans can convert ALA, a shorter essential n-3 PUFA mainly derived from plants, into EPA and DPA to only a very limited degree, and further conversion to DHA is even smaller[44]; to this regard, previous data have documented correlations between EPA, DHA and AA dietary intake and adipose tissue fractions.[16] Also, the conversion of LA to AA only occurs to a limited extent in humans, and tissue levels are likely mainly determined by food sources rich in AA, such as meat and eggs.[45] Another limitation is that changes in diet (and lifestyle habits) during follow-up may not be captured by analyses on a single biopsy specimen taken at the beginning of the follow-up, but this limitation is most important when examining dietary exposures than FA tissue incorporation, which objectively reflects the average dietary intake on an extended time period (1-2 years),[16] We also recognize that the "fraction" of n-3 FAs, here analyzed in contrast with several ratios of n-3 and n-6 FAs as to its relation with the incident risk of MI, is itself a "ratio," being a percentage of the n-3 FA analyzed over the total FAs. Still, for practical purposes, the "n-3 to n-6 FA ratio," when expressed in specific terms, appears to be a useful concept to describe relationships with outcomes and dietary patterns. Finally, albeit the predictive performance of ratios here investigated proved to be statistically superior to that of fractions alone, differences in predictive performance were small when considering established risk factors for MI, probably reflecting the predominant negative association of n-3 PUFAs over the effect of n-6 PUFAs and the possible intercorrelations of risk factors themselves with dietary habits.

Another limitation was that we did not have information on lipid profile and were therefore not able to explore mechanistic insight into associations between n-3 PUFAs or ratios of n-3/n-6 PUFAs and plasma lipoproteins. LA has been associated with lowering of low-density lipoprotein cholesterol (LDL-C), while marine n-3 PUFAs obtained through dietary sources may lower triglyceride levels, but not have no appreciable effect on plasma LDL-C. Few studies have suggested that marine n-3 PUFAs may increase and improve the functionality of high-density cholesterol (HDL-C).[46] However, the clinical implications of potential HDL-C raising effects of marine n-3 PUFAs on CVD risk are uncertain as HDL-C raising drugs have not proven to reduce CVD and recent Mendelian randomization studies have suggested that HDL-C is not causally associated with CVD risk.[47]

The radar plot depicting dietary intakes in extreme quintiles of the FA metrics investigated, offered a descriptive insight into the underlying dietary pattern of the individuals analyzed. As expected, between-quintile differences for EPA + DHA fraction and the EPA/AA ratio were minor. Both interquintile differences are characterized by differences in consumption of fatty fish. The 2 metrics, however, differ in that the EPA/AA ratio appears more related than the EPA + DHA fraction to intakes of vegetable oils and mayonnaises, margarines, butter and animal fat, processed meat and fruit and vegetables, which are food categories that reflect the intake of n-6 PUFAs as well as intake of saturated fat. These differences are not large but suggest that not only a high consumption of fatty fish, but also a low consumption of some n-6 FA-containing foods affect the risk of developing future MIs.

Conclusions

Subjects from a general population with higher ratios of EPA/AA, DHA/AA, (EPA + DHA)/AA, total marine n-3 FA/AA or with high EPA and EPA + DHA fractions in adipose tissue had a lower future occurrence of MI in this large case-cohort study, providing an answer to the original research question. The selected ratios showed improved prediction of future MI, also after multiple adjustment for covariates, over metrics of n-3 FA fractions in adipose tissue alone. The interplay between n-3 and n-6 PUFAs in the diet and in membrane incorporation should therefore be considered in future studies for better risk stratification, and also in intervention studies with n-3 PUFAs, such as the recent REDUCE-IT trial,[48] to predict subjects with a higher response to treatment in reducing risk of future MI and—possibly—of other atherothrombotic events.

Acknowledgments

We are indebted to the many investigators participating in the Danish Diet, Cancer and Health study providing data entered in the database eventually used for correlations with fatty acid incorporation.

Funding

The Danish Cancer Society funded the Diet, Cancer and Health cohort. No extramural funding was used to support this work. The authors are solely responsible for the design and conduct of this study, all study analyses and drafting and editing of this paper and its final contents.

Author Contributions

SC, CSB, SLC, EBS, and RDC contributed to the conceptualization and planning of the formal statistical analyses. SC and CSB conducted the statistical analyses supervised by SLC. SC, CSB and RDC prepared the tables and figures, and wrote the first draft of the manuscript. All authors contributed to a critical interpretation of the results and

to the manuscript content. All authors read and approved the final manuscript.

Conflicts of Interest

None reported.

References

1. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937–52.
2. De Caterina R. n-3 fatty acids in cardiovascular disease. N Engl J Med 2011;364:2439–50.
3. Beermann C, Möbius M, Winterling N, et al. sn-position determination of phospholipid-linked fatty acids derived from erythrocytes by liquid chromatography electrospray ionization ion-trap mass spectrometry. Lipids 2005;40:211–18.
4. Bork CS, Baker EJ, Lundbye-Christensen S, et al. Lowering the linoleic acid to alpha-linoleic acid ratio decreases the production of inflammatory mediators by cultured human endothelial cells. Prostaglandins Leukot Essent Fatty Acids 2019;141:1–8.
5. Centers for disease control and prevention. national health and nutrition examination survey. Questionnaires, datasets, and related documentation. https://wwwn.cdc.gov/nchs/nhanes [accessed 26 February 2022].
6. Eaton SB, Eaton 3rd SB, Sinclair AJ, et al. Dietary intake of long-chain polyunsaturated fatty acids during the paleolithic. World Rev Nutr Diet 1998;83:12–23.
7. Simopoulos AP, Leaf A, Salem Jr N. Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. Ann Nutr Metab 1999;43:127–30.
8. Hu FB, Bronner L, Willett WC, et al. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. JAMA 2002;287:1815–21.
9. He K, Song Y, Daviglus ML, et al. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. Circulation 2004;109:2705–11.
10. Zheng J, Huang T, Yu Y, et al. Fish consumption and CHD mortality: an updated meta-analysis of seventeen cohort studies. Public Health Nutr 2012;15:725–37.
11. Kark JD, Kaufmann NA, Binka F, et al. 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 2003;77:796–802.
12. Marklund M, Wu JHY, Imamura F, et al. Biomarkers of dietary omega-6 fatty acids and incident cardiovascular disease and mortality. Circulation 2019;139:2422–36.
13. Sacks FM, Lichtenstein AH, Wu JHY, et al. Dietary fats and cardiovascular disease: a presidential advisory from the American Heart Association. Circulation 2017;136:e1–e23.
14. Harris WS, Luo J, Pottala JV, et al. Red blood cell polyunsaturated fatty acids and mortality in the Women's Health Initiative Memory Study. J Clin Lipidol 2017;11:250–9 e5.
15. Harris WS, Tintle NL, Etherton MR, Vasan RS. Erythrocyte long-chain omega-3 fatty acid levels are inversely associated with mortality and with incident cardiovascular disease: the Framingham Heart Study. J Clin Lipidol 2018;12:718–27 e6.
16. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res 2008;47:348–80.
17. Guasch-Ferre M, Babio N, Martinez-Gonzalez MA, et al. Dietary fat intake and risk of cardiovascular disease and all-cause mortality in a population at high risk of cardiovascular disease. Am J Clin Nutr 2015;102:1563–73.
18. Gammelmark A, Nielsen MS, Bork CS, et al. Association of fish consumption and dietary intake of marine n-3 PUFA with myocardial infarction in a prospective Danish cohort study. Br J Nutr 2016;116:167–77.
19. Joensen AM, Overvad K, Dethlefsen C, et al. Marine n-3 polyunsaturated fatty acids in adipose tissue and the risk of acute coronary syndrome. Circulation 2011;124:1232–8.
20. Gammelmark A, Nielsen MS, Bork CS, et al. Adipose tissue content of marine N-3 polyunsaturated fatty acids is inversely associated with myocardial infarction. J Am Coll Cardiol 2016;67:1008–9.
21. Tjønneland A, Olsen A, Boll K, et al. Study design, exposure variables, and socioeconomic determinants of participation in Diet, Cancer and Health: a population-based prospective cohort study of 57,053 men and women in Denmark. Scand J Public Health 2007;35:432–41.
22. Overvad K, Tjønneland A, Haraldsdóttir J, et al. Development of a semiquantitative food frequency questionnaire to assess food, energy and nutrient intake in Denmark. Int J Epidemiol 1991;20:900–5.
23. Tjønneland A, Overvad K, Haraldsdóttir J, et al. Validation of a semiquantitative food frequency questionnaire developed in Denmark. Int J Epidemiol 1991;20:906–12.
24. Beynen AC, Katan MB. Rapid sampling and long-term storage of subcutaneous adipose-tissue biopsies for determination of fatty acid composition. Am J Clin Nutr 1985;42:317–22.
25. Schmidt M, Schmidt SA, Sandegaard JL, et al. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol 2015;7:449–90.
26. Juel K, Helweg-Larsen K. The Danish registers of causes of death. Dan Med Bull 1999;46:354–7.
27. Joensen AM, Jensen MK, Overvad K, et al. Predictive values of acute coronary syndrome discharge diagnoses differed in the Danish National Patient Registry. J Clin Epidemiol 2009;62:188–94.
28. Kalbfleisch JD, Lawless JF. Likelihood analysis of multi-state models for disease incidence and mortality. Stat Med 1988;7:149–60.
29. Petersen L, Sørensen TI, Andersen PK. Comparison of case-cohort estimators based on data on premature death of adult adoptees. Stat Med 2003;22:3795–803.
30. Sanderson J, Thompson SG, White IR, et al. Derivation and assessment of risk prediction models using case-cohort data. BMC Med Res Methodol 2013;13:113.
31. Harrell FE, Jr, Lee KL, Califf RM, et al. Regression modelling strategies for improved prognostic prediction. Stat Med 1984;3:143–52.
32. Lasota AN, Grønholdt MM, Bork CS, et al. Marine n-3 fatty acids and the risk of peripheral arterial disease. J Am Coll Cardiol 2018;72:1576–84.
33. Nishizaki Y, Shimada K, Tani S, et al. Significance of imbalance in the ratio of serum n-3 to n-6 polyunsaturated fatty acids in patients with acute coronary syndrome. Am J Cardiol 2014;113:441–5.

34. Yagi S, Aihara K, Fukuda D, et al. Reduced ratio of eicosapentaenoic acid and docosahexaenoic acid to arachidonic acid is associated with early onset of acute coronary syndrome. Nutr J 2015;14:111.
35. Hayakawa S, Yoshikawa D, Ishii H, et al. Association of plasma ω-3 to ω-6 polyunsaturated fatty acid ratio with complexity of coronary artery lesion. Intern Med 2012;51:1009–14.
36. Harris WS, Mozaffarian D, Rimm E, et al. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. Circulation 2009;119:902–7.
37. Farvid MS, Ding M, Pan A, et al. Dietary linoleic acid and risk of coronary heart disease: a systematic review and meta-analysis of prospective cohort studies. Circulation 2014;130:1568–78.
38. Harris WS. The Omega-6:Omega-3 ratio: a critical appraisal and possible successor. Prostaglandins Leukot Essent Fatty Acids 2018;132:34–40.
39. Katan MB, Harryvan JL, van de Bovenkamp P. n-3 fatty acids in human fat tissue aspirates are stable for up to 6 y. Eur J Clin Nutr 2003;57:816–18.
40. Tjønneland A, Overvad K, Thorling E, Ewertz M. Adipose tissue fatty acids as biomarkers of dietary exposure in Danish men and women. Am J Clin Nutr 1993;57:629–33.
41. Huang X, Sjögren P, Cederholm T, et al. Serum and adipose tissue fatty acid composition as biomarkers of habitual dietary fat intake in elderly men with chronic kidney disease. Nephrol Dial Transplant 2014;29:128–36.
42. Ogura T, Takada H, Okuno M, et al. Fatty acid composition of plasma, erythrocytes and adipose: their correlations and effects of age and sex. Lipids 2010;45:137–44.
43. Hellmuth C, Demmelmair H, Schmitt I, et al. Association between plasma nonesterified fatty acids species and adipose tissue fatty acid composition. PLoS One 2013;8:e74927.
44. Baker EJ, Miles EA, Burdge GC, et al. Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. Prog Lipid Res 2016;64:30–56.
45. Rett BS, Whelan J. Increasing dietary linoleic acid does not increase tissue arachidonic acid content in adults consuming Western-type diets: a systematic review. Nutr Metab (Lond) 2011;8:36.
46. Siri-Tarino PW. Effects of diet on high-density lipoprotein cholesterol. Curr Atheroscler Rep 2011;13:453–60.
47. Mach F, Baigent C, Catapano AL, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J 2020;41:111–88.
48. Bhatt DL, Steg PG, Miller M, et al. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. N Engl J Med 2019;380:11–22.