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Dietary intake of marine n-3 polyunsaturated fatty acids and future risk of venous thromboembolism

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Abstract

Background: Studies on the association between long-chained n-3 polyunsaturated fatty acids (n-3 PUFAs) and risk of venous thromboembolism (VTE) are conflicting, potentially due to challenges related to assessment of n-3 PUFA intake and changes in diet during follow-up.

Objectives: To investigate whether dietary intake of marine n-3 PUFAs was associated with risk of incident VTE in a population-based cohort with repeated assessments of n-3 PUFA intake.

Methods: We recruited 21 970 participants (after excluding 7570 with incomplete data) from the fourth (1994-1995) and sixth (2007-2008) surveys of the Tromsø Study, and recorded incident VTEs up to 2016. Intake of n-3 PUFAs was computed from self-reported consumption of fat and lean fish, fish spread, and supplements. Cox proportional hazards regression models with n-3 PUFA intake as a time-varying variable were used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) for VTE across quartiles (Q) of n-3 PUFA intake.

Results: There were 541 incident VTEs during follow-up. Compared to Q1, subjects in Q2-4 had 22%-26% lower risk of VTE (HR Q2 0.74, 95% CI 0.57-0.96; HR Q3 0.77, 95% CI 0.59-0.99; HR Q4 0.78, 95% CI 0.61-1.00). The association was most pronounced for provoked VTE, particularly provoked pulmonary embolism (PE), with risk estimates of 0.42 (95% CI 0.25-0.72), 0.40 (95% CI 0.23-0.68), and 0.61 (95% CI 0.38-0.96) for Q2-4, respectively.

Conclusions: Dietary intake of marine n-3 PUFAs was associated with a lower risk of VTE, particularly provoked PE. The association displayed a threshold pattern and suggested a protective effect of an n-3 PUFA intake ≥4.7 g/week.

KEYWORDS
deep vein thrombosis, diet, omega-3 fatty acids, pulmonary embolism, risk factors, venous thromboembolism

Essentials

- It is uncertain if intake of marine fatty acids (n-3 PUFAs) influences the risk of venous thromboembolism (VTE).
- The association was explored accounting for variation in dietary intake over time.
- Intake of n-3 PUFAs exceeding 4.7 g/week was associated with 22%-26% lower VTE risk.
- The association was most pronounced for provoked pulmonary embolism (PE), with 39%-60% lower risk.

1 | INTRODUCTION

Venous thromboembolism (VTE) is a common cardiovascular disease (CVD) with an annual incidence of 1-2 per 1000 in adult western populations.[1-3] VTE constitutes a significant public health burden due to debilitating long-term complications and a potentially fatal outcome.[4,5] Contemporary data have shown that the incidence of VTE has remained stable or increased slightly over the past decades,[6,7] which contrasts with the declining rate observed in arterial CVD.[8,9] Thus, there is a need to identify new strategies to reduce the burden of VTE.

Food habits may have a significant influence on health. Dietary intake of fish and marine food products is associated with several health benefits including lower risk of fatal and non-fatal arterial CVD,[10-12] and is now implemented in dietary guidelines worldwide.[12-14] The beneficial effects are largely attributed to the essential long-chained n-3 polyunsaturated fatty acids (n-3 PUFAs, ie, eicosapentaenoic acid [EPA], docosapentaenoic acid [DPA] and docosahexaenoic acid [DHA]).[10-12] n-3 PUFAs have also been associated with key pathways in the VTE pathogenesis, including downregulation of inflammation,[15] tissue-factor expression,[16,17] platelet function,[18] and platelet–endothelium interactions.[19,20] Despite this, epidemiological data on the association between fish or n-3 PUFA intake and the risk of incident VTE is conflicting. Two prospective cohorts, the Tromsø Study[21] and the Atherosclerosis Risk in Communities (ARIC) Study,[22] reported a beneficial association. In contrast, results from the Iowa Women's Health Study suggested a small adverse association between fish intake and the risk of VTE.[23] Finally, the Diet Cancer and Health (DCH) Study[24] and a cohort of US nurses and physicians[25] did not find any association between intake of fish or n-3 PUFAs and VTE risk.

The inconsistent findings regarding the association between fish or n-3 PUFA consumption and VTE risk in longitudinal studies may partly be attributed to methodological aspects, including exposure assessment and data handling. First, the content of n-3 PUFAs in fatty fish can be up to seven- to eightfold higher than in lean fish, and dietary supplements comprise even higher n-3 PUFA concentrations.[26,27] It is therefore possible to have a high intake of (lean) fish and simultaneously a relatively low intake of n-3 PUFAs. This has been accounted for in various ways depending on the available data in the published studies. The exposure assessment has varied from frequency of fish intake regardless of n-3 PUFA content,[23] via frequency of fatty and lean fish intake with or without fish oil supplements,[21,24] to estimated total intake of n-3 PUFAs based on answers in food frequency questionnaires.[22,25] Of note, only one of the published studies has validated the information of intake obtained from self-reported questionnaires against objective measurement of n-3 PUFA concentrations in serum.[21] Moreover, the majority of studies have assessed fish or n-3 PUFA intake at baseline only.[21,23,24] As dietary habits may change during a long follow-up, these risk estimates may be subjected to regression dilution bias.[28]

In the present study, we computed a comprehensive variable of total intake of marine n-3 PUFAs based on contributions from lean fish, fat fish, and supplements. We also included fish as spread, which contains a higher amount of n-3 PUFAs per serving unit than lean fish does for dinner (Table S1). Further, the intake of n-3 PUFAs was modeled as a time-varying variable to account for dietary changes during follow-up. Finally, as a potential effect of n-3 PUFAs could be explained merely by substitution of otherwise unhealthy foods, we also investigated the effect of total fish intake on the risk of VTE, regardless of n-3 PUFA content.

2 | METHODS

2.1 | Study population

Participants (n = 29 648) were recruited from the fourth and sixth survey of the Tromsø Study, conducted in 1994 to 1995 and 2007 to 2008, respectively. The Tromsø Study is a prospective, single-center population-based study with repeated health surveys of the inhabitants of Tromsø, Norway. Detailed methodology of the Tromsø Study has been published elsewhere.[29] Briefly, the entire (Tromsø 4) or parts (Tromsø 6) of the adult population were invited to participate, and the attendance rates ranged from 66% in Tromsø 6%-77% in Tromsø 4. Participants were aged 25-97 years and 30-87 years at study entry in Tromsø 4 and 6, respectively. Individuals not officially registered as inhabitants of the municipality of Tromsø at baseline (n = 23), participants with a known history of VTE (n = 85), and those with incomplete data on fish intake and use of fish oil supplement (n = 7570), were excluded from the study. Accordingly, the study population comprised 21 970 unique individuals. Of these, 11 832 participated in Tromsø 4 only, 4246 in Tromsø 6 only, and 5892 in both surveys. Those attending both surveys had their exposure data updated and contributed with two observation periods, yielding 27 862 observation periods in total. The study was approved by the Regional Committee for Medical and Health Research Ethics, and all participants provided written informed consent prior to inclusion.

2.2 | Measurements

Exposure information was obtained via physical examinations, non-fasting blood samples and self-administered questionnaires. Height

TABLE 1 Characteristics according to observational periods across quartiles of marine n-3 PUFA intake in the Tromsø Study[a]

	Q1 (n = 6970)	Q2 (n = 7012)	Q3 (n = 6967)	Q4 (n = 6913)
Quartile range, g/wk	<4.7	4.7-13.4	>13.4-29.1	>29.1
Age, y	41 (12)	47 (14)	49 (14)	56 (14)
Sex, male	47.1 (3282)	50.6 (3546)	40.8 (3539)	46.4 (3210)
BMI, kg/m^2	25.1 (4.0)	25.6 (3.9)	25.8 (4.0)	26.1 (4.0)
Serum total cholesterol, mmol/L	5.71 (1.21)	5.82 (1.24)	5.82 (1.21)	5.88 (1.22)
Serum HDL cholesterol, mmol/L	1.45 (0.38)	1.48 (0.40)	1.50 (0.42)	1.56 (0.44)
Serum triglycerides, mmol/L	1.54 (1.01)	1.55 (1.00)	1.52 (1.00)	1.46 (0.89)
Systolic blood pressure, mmHg	130 (17)	133 (20)	133 (20)	138 (23)
Diastolic blood pressure, mmHg	76 (11)	77 (11)	77 (11)	78 (11)
Current smoking	38.0 (2640)	31.6 (2210)	27.6 (1916)	22.4 (1541)
Diabetes	1.6 (108)	2.3 (158)	2.6 (181)	3.5 (241)
History of CVD	3.2 (221)	5.7 (398)	6.8 (471)	10.3 (700)
History of cancer	1.7 (116)	2.7 (192)	3.5 (243)	5.9 (410)
Higher education[b]	34.4 (2391)	36.0 (2511)	40.0 (2775)	38.0 (2615)

Values are mean (±SD) or percentage (count).

BMI, body mass index; CVD, cardiovascular disease (angina pectoris, stroke, myocardial infarction); HDL, high-density lipoprotein; PUFAs, polyunsaturated fatty acid; Q, quartile; SD, standard deviation.

[a]Based on 27 862 observational periods from 21 970 individuals in the period between 1994 and 2016.

[b]≥15 y of education (corresponding to 3 y in university or academy).

(in cm) and weight (in kg) were measured with participants dressed in light clothes with no shoes, and BMI was calculated as weight divided by the square of height (in m, kg/m^2). Blood pressure and serum lipid concentrations were assessed according to procedures described previously.[30] Information on fish intake, use of dietary supplements, education, current smoking, diabetes, and prior CVD (comprising myocardial infarction, angina pectoris, and stroke) were based on self-administered questionnaires. Information on cancer history was obtained from the Norwegian Cancer Registry.

2.3 | Assessment and validation of marine n-3 PUFA and fish intake

Information on dietary intake was obtained by self-administered questionnaires.[31] In Tromsø 4, participants aged <70 years were asked to report how frequently they consumed lean and fat fish for dinner and how often they used fish as spread on bread (never, <1, 1/week, 2-3/week, 4-5/week, or daily). Participants aged ≥70 years received a similar questionnaire, but with fewer options (never, <1/week, 1/week or ≥2/week). These assessments were repeated in Tromsø 6, however, the frequency options differed slightly from those in the Tromsø 4 questionnaire (0-1/month, 2-3/month, 1-3/week, 4-6/

week, or 1-2/day. In both surveys, the participants were also asked to report whether they used fish oil or any supplements containing n-3 PUFAs (never, sometimes, or daily). In order to estimate the total intake of marine n-3 PUFAs, we first calculated the average content of n-3 PUFAs in different food items and supplements based on information obtained from official web resources (Table S1).[26,27] Standard serving sizes were defined according to recommendations from the Norwegian Directorate of Health.[13] One serving unit of fish for dinner was defined as 200 g, and one serving unit of fish as bread spread was 25 g (Table S1). Total weekly intake of fish was calculated as the sum of frequency (of lean and fat fish) multiplied by serving size. Total weekly intake of marine n-3 PUFAs was calculated as the sum of frequency multiplied by amount of n-3 PUFAs per serving derived from intake of fat and lean fish, fish as bread spread and fish oil supplements. Frequencies reported as ranges in the questionnaire (eg, 2-3/week) were recoded to the mean value (eg, 2.5/week). For participants aged ≥70 years in Tromsø 4, the highest frequency option (≥2/week) was recoded to 2.5/week. In the questionnaires, it was possible to report fish for dinner daily. As this option was considered unrealistic and appeared to be relatively uncommon (<2%), it was recoded to 2.5/week.

In order to validate the self-reported intake of marine n-3 PUFAs, the serum concentration of marine derived n-3 PUFAs (EPA, DPA
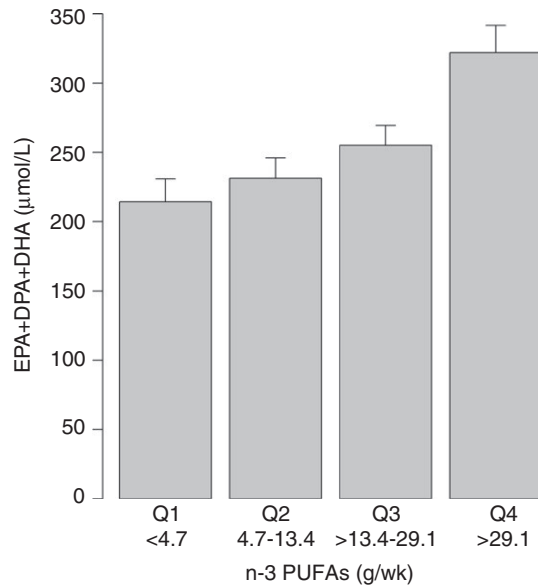
FIGURE 1 Serum n-3 PUFA concentration across quartiles of self-reported weekly intake of marine n-3 PUFAs in the Tromsø Study (1994). Values are means with 95% CI. CI, confidence interval; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; PUFAs, polyunsaturated fatty acids; Q, quartiles

and DHA) in the cholesterol-ester fraction was measured in a sub-group of participants (n = 1167) in Tromsø 4, according to procedures described previously.[21]

2.4 | Outcome assessment

Incident VTE during follow-up was identified by searching the hospital discharge registry, the radiology procedure registry, and the autopsy registry at the University Hospital of North Norway (UNN). UNN exclusively provides diagnostic work-up and treatment of VTE in the study region, and the discharge registry comprises both outpatient contacts and hospitalizations. Trained personnel reviewed the medical records for all potential VTEs. An event was recorded if all of the following criteria were present: signs and symptoms consistent with VTE, a diagnosis of deep vein thrombosis (DVT) or pulmonary embolism (PE) was made by a physician on basis of objective diagnostic procedures (compression ultrasonography, venography, spiral computed tomography, perfusion-ventilation scan or pulmonary angiography), and anticoagulant treatment was initiated (unless contraindicated). Cases identified in the autopsy registry were included if VTE was documented as the cause of death or reported as a significant contributor to death.

All events were categorized as provoked or unprovoked based on the presence of provoking factors at the time of diagnosis. Provoked VTE was defined as recent surgery or trauma (within 8 weeks prior to the event), an acute medical condition (acute myocardial infarction, acute ischemic stroke, major infectious disease), active cancer, immobilization (bed rest ≥3 days, confinement to wheelchair, long distance travel ≥4 hours within the last 14 days), or other provoking

TABLE 2 Characteristics of VTE events in the Tromsø Study[a]

Characteristics	
Age at incident VTE, y	67 (13)
Male	51.6 (279)
Clinical presentation	
DVT	56.6 (306)
PE	43.4 (235)
Provoked VTE	57.8 (313)
Clinical risk factors	
Pregnancy/puerperium	0.6 (3)
Heredity[b]	4.1 (22)
Other medical conditions[c]	19.3 (82)
Provoking factors[d]	
Surgery	16.1 (87)
Trauma	10.4 (56)
Acute medical conditions	11.3 (61)
Cancer[e]	24.4 (132)
Immobilization[f]	16.8 (91)
Other[g]	4.6 (25)

DVT, deep vein thrombosis; PE, pulmonary embolism; VTE, venous thromboembolism.

[a]n events = 541 in the period 1994-2016. Values are mean (±SD) or percentage (count).

[b]VTE reported in a first-degree relative before the age of 60 y.

[c]Myocardial infarction, ischemic stroke, heart failure or chronic obstructive lung disease within the previous year.

[d]One patient may have multiple provoking factors.

[e]Cancer disease present at the time of VTE diagnosis.

[f]Bed rest ≥3 d, wheelchair user, plaster cast, air travel ≥4 h or long automobile travel <14 d prior to VTE.

[g]Other factors specified as provoking in the medical record (eg, intravascular catheters).

factors specified in the medical record (eg, intravascular catheter). The remaining cases were categorized unprovoked. The events were also classified as DVT or PE based on localization, and coexisting DVT and PE was classified as PE.

2.5 | Statistical analysis

For each participant, person-years of follow-up were accrued from the date of enrollment in Tromsø 4 (1994-1995) or Tromsø 6 (2007-2008) until the date of incident VTE, death, migration or the end of the study period (December 31, 2016), whichever came first. For participants attending both surveys, the first observation period ended on the date of Tromsø 6, and exposure information was updated for the second observation period. Participants who experienced a VTE in one period were excluded from the subsequent observation period. Participants who died (n = 2355) or moved from the municipality of Tromsø (n = 3525) during follow-up were censored at these respective time points.

Statistical analyses were performed with STATA version 14 (Stata Corp, College Station, TX) and R version 3.3.3

TABLE 3 IRs and HRs with 95% CIs for VTE, PE, and DVT, overall and stratified by the presence of provoking factors, across quartiles of weekly intake of marine n-3 PUFAs in the Tromsø Study[a]

n-3 PUFA intake (g/wk)	Person-years	VTE events	Crude IR (95% CI)[b]	HR model 1 (95% CI)[c]	P value	HR model 2 (95% CI)[d]	P value
Total VTE							
Q1 < 4.7	96 809	120	1.24 (1.04-1.48)	1.00	0.12	1.00	0.13
Q2 4.7-13.4	88 864	120	1.35 (1.13-1.61)	0.75 (0.58-0.96)		0.74 (0.57-0.96)	
Q3 > 13.4-29.1	81 104	129	1.59 (1.34-1.89)	0.77 (0.6-1)		0.77 (0.59-0.99)	
Q4 > 29.1	71 711	172	2.40 (2.07-2.79)	0.78 (0.61-1)		0.78 (0.61-1)	
Unprovoked VTE							
Q1 < 4.7	96 809	46	0.48 (0.36-0.63)	1.00	0.81	1.00	0.82
Q2 4.7-13.4	88 864	49	0.55 (0.42-0.73)	0.81 (0.54-1.21)		0.81 (0.54-1.21)	
Q3 > 13.4-29.1	81 104	59	0.73 (0.56-0.94)	0.94 (0.64-1.4)		0.94 (0.63-1.40)	
Q4 > 29.1	71 711	73	1.02 (0.81-1.28)	0.89 (0.60-1.31)		0.89 (0.6-1.31)	
Provoked VTE							
Q1 < 4.7	96 809	74	0.76 (0.6-0.96)	1.00	0.07	1.00	0.07
Q2 4.7-13.4	88 864	71	0.89 (0.63-1.01)	0.71 (0.51-0.99)		0.70 (0.51-0.98)	
Q3 > 13.4-29.1	81 104	69	0.85 (0.67-1.08)	0.66 (0.47-0.93)		0.65 (0.47-0.91)	
Q4 > 29.1	71 711	99	1.38 (1.13-1.68)	0.72 (0.52-0.98)		0.72 (0.52-0.98)	
Total DVT							
Q1 < 4.7	96 809	60	0.62 (0.48-0.8)	1.00	0.51	1.00	0.50
Q2 4.7-13.4	88 864	74	0.83 (0.66-1.05)	0.95 (0.68-1.35)		0.95 (0.67-1.34)	
Q3 > 13.4-29.1	81 104	81	1.01 (0.81-1.26)	1.02 (0.72-1.43)		1.01 (0.72-1.42)	
Q4 > 29.1	71 711	90	1.26 (1.26-1.54)	0.88 (0.62-1.24)		0.87 (0.62-1.23)	
Unprovoked DVT							
Q1 < 4.7	96 809	23	0.24 (0.16-0.36)	1.00	0.87	1.00	0.87
Q2 4.7-13.4	88 864	26	0.29 (0.20-0.43)	0.88 (0.5-1.55)		0.88 (0.50-1.54)	
Q3 > 13.4-29.1	81 104	35	0.43 (0.31-0.6)	1.15 (0.67-1.97)		1.14 (0.67-1.96)	
Q4 > 29.1	71 711	38	0.53 (0.39-0.73)	0.96 (0.56-1.66)		0.96 (0.56-1.66)	
Provoked DVT							
Q1 < 4.7	96 809	37	0.38 (0.28-0.53)	1.00	0.32	1.00	0.31
Q2 4.7-13.4	88 864	48	0.54 (0.41-0.72)	1.00 (0.65-1.54)		1.00 (0.65-1.54)	
Q3 > 13.4-29.1	81 104	46	0.57 (0.42-0.76)	0.93 (0.6-1.45)		0.93 (0.60-1.44)	
Q4 > 29.1	71 711	52	0.73 (0.55-0.95)	0.82 (0.53-1.28)		0.82 (0.53-1.28)	
Total PE							
Q1 < 4.7	96 809	60	0.62 (0.48-0.8)	1.00	0.11	1.00	0.13
Q2 4.7-13.4	88 864	46	0.52 (0.39-0.69)	0.55 (0.37-0.81)		0.54 (0.37-0.8)	
Q3 > 13.4-29.1	81 104	47	0.58 (0.44-0.77)	0.53 (0.36-0.79)		0.53 (0.36-0.78)	
Q4 > 29.1	71 711	82	1.14 (0.92-1.42)	0.68 (0.47-0.96)		0.69 (0.48-0.98)	
Unprovoked PE							
Q1 < 4.7	96 809	23	0.24 (0.16-0.36)	1.00	0.59	1.00	0.6
Q2 4.7-13.4	88 864	23	0.26 (0.17-0.39)	0.74 (0.41-1.33)		0.74 (0.41-1.33)	
Q3 > 13.4-29.1	81 104	24	0.30 (0.20.0.44)	0.75 (0.42-1.34)		0.74 (0.42-1.33)	
Q4 > 29.1	71 711	35	0.49 (0.35-0.68)	0.81 (0.46-1.41)		0.81 (0.47-1.42)	

(Continues)

TABLE 3 (Continued)

n-3 PUFA intake (g/wk)	Person-years	VTE events	Crude IR (95% CI)[b]	HR model 1 (95% CI)[c]	P value	HR model 2 (95% CI)[d]	P value
Provoked PE							
Q1 < 4.7	96 809	37	0.38 (0.28-0.53)	1.00	0.10	1.00	0.11
Q2 4.7-13.4	88 864	23	0.26 (0.17-0.39)	0.43 (0.25-0.73)		0.42 (0.25-0.72)	
Q3 > 13.4-29.1	81 104	23	0.28 (0.19-0.43)	0.41 (0.24-0.69)		0.40 (0.23-0.68)	
Q4 > 29.1	71 711	47	0.66 (0.49-0.87)	0.60 (0.38-0.94)		0.61 (0.38-0.96)	

CI, confidence interval; DVT, deep vein thrombosis; HR, hazard ratio; IR, incidence rate; PE, pulmonary embolism; PUFAs, polyunsaturated fatty acids; VTE, venous thromboembolism.
[a]Based on data from the Tromsø Study in the period 1994-2016, analyzed by Cox proportional hazards regression models.
[b]Per 1000 person-years.
[c]Adjusted for age (as time scale).
[d]Model 1 + sex and BMI.

(The R Foundation for Statistical Computing, Vienna, Austria). Demographics and clinical characteristics across quartiles of n-3 PUFA and fish intake were reported as means with SD. Crude incidence rates (IRs) for VTE were calculated across quartiles and expressed as number of events per 1000 person-years. Cox proportional hazards regression models with age as time scale were used to estimate hazard ratios (HRs) with 95% CIs for VTE with the lowest quartile as reference. P-values for linear trends across quartiles were also calculated. HRs were estimated for total VTE, for PE and DVT, and for provoked and unprovoked VTE. The analyses were performed in two adjustment models. Model 1 included age (as time scale), and model 2 additionally included sex and BMI. In addition to analyzing across quartiles of n-PUFA intake, Cox analyses with a restricted cubic spline with four knots fitted to the n-PUFA intake values were performed and plotted.

The proportional hazards assumption was evaluated and confirmed based on Schoenfeld residuals using a global test. Statistical interactions between sex and n-3 PUFA intake (sex*n-3 PUFA intake) and sex and fish intake (sex*fish intake) were tested by including the cross-product terms separately in the fully adjusted models, and no interactions were found.

To evaluate the validity of the self-reported data, mean serum concentrations of n-3 PUFAs across quartiles of self-reported intake of n-3 PUFAs were displayed in a histogram for a subgroup of participants (n = 1167). Linear regression analysis was used to test the association between quartiles of self-reported intake and serum concentration of n-3 PUFAs. Finally, because dietary habits may change over time, we performed sensitivity analyses with follow-up restricted to maximum 5 years in order to limit potential bias due to regression dilution.

3 | RESULTS

The mean age at enrollment was 46 ± 14 years and 48.6% of the of the study population were men. The median intake of n-3 PUFAs and fish per week was 11.1 g (IQR: 3.6-27.8) and 406 g (IQR: 250-700) at baseline, respectively. Characteristics, assessed at the start of each observational period, across quartiles of total n-3 PUFA intake are reported in Table 1. Mean age, serum high-density lipoprotein (HDL) cholesterol, blood pressure, and the prevalence of diabetes, cancer and CVD increased with higher intake of n-3 PUFAs, while mean serum triglyceride concentration was lowest in those with the highest intake and the proportion of smokers was highest in those with the lowest intake. A similar pattern was observed across quartiles of total fish intake (Table S2). Serum concentration of marine derived n-3 PUFAs across quartiles of self-reported intake in the subgroup of participants (n = 1167) are shown in Figure 1. There was a significant dose-dependent increase in serum concentration with higher intake of n-3 PUFAs (P < 0.001).

There were 541 incident VTE events during 338 488 person-years of follow-up, and the crude IR was 1.6 per 1000 person-years (95% CI 1.5-1.7). Median duration per observation period was 11.6 years (range: 7 days to 22 years). The mean age at incident VTE was 67 ± 13 years and 52% of the VTE events occurred in men. Further, 57% of the events presented as DVTs and 58% were provoked, with cancer as the most common provoking factor (Table 2). VTE characteristics according to quartiles of n-3 PUFA intake are shown in Table S3. There was a striking trend showing that surgery was a less prevalent provoking factor with increasing n-3 PUFA intake.

The HRs of VTE across quartiles of weekly n-3 PUFA intake are presented in Table 3. Overall, individuals with an n-3 PUFA intake above the reference (≥4.7 g/week) had a significant 22%-26% lower risk of VTE. The largest risk difference was observed between the two lowest quartiles and there was no evidence for additional protection with increased weekly intake (Model 1, P = 0.12). Sub-analyses revealed that the association was largely driven by an effect on provoked VTE (HR model 1 Q2: 0.71, 95% CI 0.51-0.99, P = 0.07) and particularly provoked PE (HR model 1 Q2: 0.43, 95% CI 0.25-0.73, P = 0.10). The risk estimates were essentially unchanged after further adjustment for sex and BMI. Figures 2 and 3 visualize the relationship between n-3 PUFA intake modeled as a restricted cubic spline and the risk of total VTE and provoked PE, respectively. There was a steep decline in risk
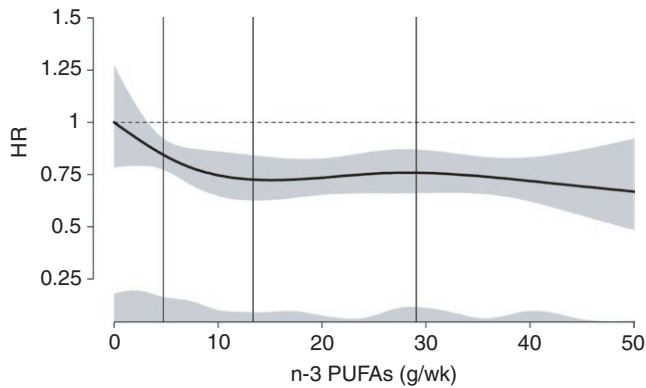
FIGURE 2 Dose-response relationship between weekly intake of marine n-3 PUFAs (modelled as a restricted cubic spline with four knots) and the risk of VTE. The regression model is adjusted for age (as time scale), sex, and BMI. The solid line represents the HR and the shaded area shows the 95% CI. The density plots shows the distribution of n-PUFAs intake, and the vertical lines represent the 25th, 50th, and 75th percentiles. BMI, body mass index; CI, confidence interval; HR, hazard ratio; PUFAs, polyunsaturated fatty acids; VTE, venous thromboembolism
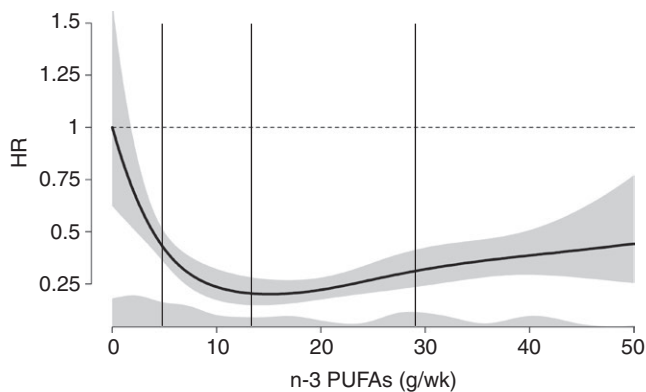

FIGURE 3 Dose-response relationship between weekly intake of marine n-3 PUFAs (modelled as a restricted cubic spline with four knots) and the risk of provoked PE. The regression model is adjusted for age (as time scale), sex and BMI. The solid line represents the HR and the shaded area shows the 95% CI. The density plots shows the distribution of n-PUFAs intake, and the vertical lines represent the 25th, 50th, and 75th percentiles. BMI, body mass index; CI, confidence interval; HR, hazard ratio; PE, pulmonary embolism; PUFAs, polyunsaturated fatty acids

with an n-3 PUFA intake above the first quartile (ie, ≥4.7 g/week), and no evidence for further beneficial effects with increasing intake.

Due to the lack of a dose-dependent effect of n-3 PUFA intake on the risk of VTE, the three upper quartiles (Q2-4) were merged for further analyses (Table 4). Overall, the risk of VTE was 24% (HR 0.76, 95% CI 0.62-0.94) lower in those with an n-3 PUFA intake ≥4.7 g/week. Again, the largest effect sizes were observed in relation to provoked VTE (HR 0.69, 95% CI 0.53-0.91) and provoked PE (HR 0.48, 95% CI 0.32-0.72).

Results from the sensitivity analyses with follow-up restricted to 5 years are shown in Table S4. Overall, the trends were similar as in the main analyses. However, the point estimates indicated a slightly stronger association between intake of n-3 PUFAs and the risk of VTE, although the confidence intervals were wider. There were only 189 VTE events during the initial 5 years of follow-up, resulting in low statistical power for these analyses. When the risk of VTE was explored across quartiles of weekly fish intake, regardless of n-3 PUFA content, no significant associations were observed (Table S5).

Baseline characteristics of included participants and those excluded due to incomplete data on fish intake and use of fish oil supplements are shown in Table S6. Included participants were younger, healthier and were more likely to have higher education compared to those who were excluded.

4 | DISCUSSION

In the present study, we investigated the association between self-reported intake of marine n-3 PUFAs validated against measurements of serum concentration, and the risk of incident VTE in a large population-based cohort taking changes in food habits into consideration. We observed an inverse association between the intake of n-3 PUFAs and VTE risk that displayed a threshold pattern occurring at a moderate weekly consumption (>first quartile, ≥4.7 g/week). The association was mainly driven by an effect on provoked events, and particularly PE. On the other hand, total weekly intake of fish was not associated with the risk of VTE, which supports the hypothesis that n-3 PUFAs exert the protective effect.

Previous reports on the association between n-3 PUFAs or fish intake and the risk of VTE have been conflicting, and a recent systematic review concluded that a risk modifying effect of fish consumption has meager support in the literature.[32] However, the present results are in support of two previous studies suggesting that a moderate intake of fish or n-3 PUFAs is associated with a lower risk of VTE.[21,22] In our former report from the Tromsø Study, we investigated the association between the weekly frequency of fish for dinner and the risk of VTE.[21] A total of 23 621 men and women were followed for a median of 15.8 years. We found that fish intake ≥3 times per week was associated with a non-significant 22% lower risk of VTE compared to intake 1-2 times per week, while an intake ≥3 times per week in combination with fish oil supplements was associated with a significant 48% lower risk compared to intake one to two times weekly without supplements. This suggests that the beneficial effect may be mediated by n-3 PUFAs. Further, in the ARIC study, a cohort of 14 962 middle-aged adults, a 30%-45% lower VTE risk was reported for an n-3 PUFA intake exceeding 0.7 g/week, and a similar effect was observed for fish intake exceeding one serving per week.[22] Information on dietary intake was based on self-report at baseline with reassessment after 6 years, and cumulative averages were calculated for participants with two measurements during the 12.5-year follow-up. However, data from two other cohort studies suggest no or non-significant associations. In the DCH Study, an intake of fatty fish at baseline exceeding 35 g/week in women and 49 g/week in men was associated with a non-significant 20%-40%

TABLE 4 IRs and HRs with 95% CIs for VTE, PE, and DVT, overall and stratified by the presence of provoking factors, by weekly intake of marine n-3 PUFAs[a]

n-3 PUFA intake	Person-years	VTE events	Crude IR (95% CI)[b]	HR (95% CI)[c]
Total VTE				
Q1[d]	96 809	120	1.24 (1.04-1.48)	1
Q2-Q4[e]	241 678	421	1.74 (1.58-1.92)	0.76 (0.62-0.94)
Unprovoked VTE				
Q1[d]	96 809	46	0.48 (0.36-0.63)	1
Q2-Q4[e]	241 678	181	0.75 (0.65-0.87)	0.88 (0.63-1.23)
Provoked VTE				
Q1[d]	96 809	74	0.76 (0.61-0.96)	1
Q2-4[e]	241 678	239	0.99 (0.87-1.12)	0.69 (0.53-0.91)
Total DVT				
Q1[d]	96 809	60	0.62 (0.48-0.8)	1
Q2-Q4[e]	241 678	245	1.02 (0.90-1.15)	0.95 (0.71-1.27)
Unprovoked DVT				
Q1[d]	96 809	23	0.24 (0.16-0.36)	1
Q2-Q4[e]	241 678	99	0.41 (0.34-0.5)	0.99 (0.62-1.59)
Provoked DVT				
Q1[d]	96 809	37	0.38 (0.28-0.53)	1
Q2-Q4[e]	241 678	146	0.60 (0.51-0.71)	0.92 (0.63-1.33)
Total PE				
Q1[d]	96 809	60	0.62 (0.48-0.8)	1
Q2-Q4[e]	241 678	175	0.72 (0.62-0.84)	0.59 (0.43-0.80)
Unprovoked PE				
Q1[d]	96 809	23	0.24 (0.16-0.36)	1
Q2-Q4[e]	241 678	82	0.34 (0.27-0.42)	0.77 (0.47-1.24)
Provoked PE				
Q1[d]	96 809	37	0.38 (0.28-0.53)	1
Q2-Q4[e]	241 678	93	0.38 (0.31-0.47)	0.48 (0.32-0.72)

CI, confidence interval; DVT, deep vein thrombosis; HR, hazard ratio; IR, incidence rate; PE, pulmonary embolism; PUFAs, polyunsaturated fatty acid; Q, quartile; VTE, venous thromboembolism.
[a]Based on data from the Tromsø Study in the period 1994-2016, analyzed by Cox proportional hazards regression models.
[b]Per 1000 person-years.
[c]Adjusted for age (as time scale), sex and BMI.
[d]<4.7 g/wk.
[e]≥4.7 g/wk.

lower risk of unprovoked VTE,[24] while a large US study of 129 430 adults did not find any association between the intake of n-PUFAs or fish and VTE risk.[25] Finally, in the Iowa Women's Health Study, ≥2.5 servings of fish per week was associated with a 22% higher risk of VTE compared to <0.5 serving per week.[23]

Most studies reporting on the association between n-3 PUFAs or fish intake and the risk of VTE have assessed intake at baseline only.[21,23,24] However, as dietary behavior is likely to fluctuate over time, non-differential misclassification of participants may occur during a long follow-up, typically leading to regression dilution and underestimation of the true association.[33,34] In the present study, we addressed this issue by including n-3 PUFA intake as a time-varying variable in the regression analyses where the exposure information was updated for those participating in both surveys. This resulted

in a shorter time interval between assessment of exposure and outcome for a part of the study population (27%). Still, sensitivity analyses with observation time further restricted to maximum 5 years yielded stronger risk estimates, suggesting that our main analyses are still subject to regression dilution and that the true association between n-3 PUFA intake and VTE risk is likely to be stronger.

Theoretically, a high intake of fish may substitute otherwise unhealthy foods and evoke health effects irrespective of the contents in fish (eg, n-3 PUFAs). In the present study, we found that the beneficial association was restricted to the intake of marine n-3 PUFAs, and no relationship was observed between total fish intake and the risk of VTE. Similarly, studies reporting separate analyses for intake of lean and fatty fish (ie, low and high n-3 PUFA content) have generally found largest effect sizes in relation to fatty fish and fish

oil supplements.[21,24] This suggests that a high-fish diet may not be sufficient, and that n-PUFAs are key components to modulate the risk of VTE. We further observed that the nature of the association between the intake of n-3 PUFAs and VTE risk displayed a threshold pattern, where the largest risk difference occurred between the two lowest quartiles. A similar pattern was also observed in the ARIC Study[22] and in the DCH Study,[24] which suggests that a low intake of n-3 PUFAs may be a risk factor for VTE. However, due to the study location, the average intake of n-3 PUFAs was relatively high in our study, and the exact threshold for an effect remains to be identified.

We found that the beneficial association between intake of n-3 PUFAs and VTE was strongest in relation to provoked events. We further observed that the prevalence of surgery as a provoking factor decreased with increasing intake of n-PUFAs. Interestingly, this is in accordance with ecological data from Norway showing that the incidence of postoperative VTE dropped markedly during World War II (1940-1945),[35] a period in which the diet was characterized by a high intake of fish and a low intake of saturated fat.[36] Interpreted in light of the thrombosis potential model,[37] this may suggest that an individual with an adequate n-3 PUFA intake has a lower baseline risk of VTE or a lower incidence of VTE-related triggers and comorbidities, compared to an individual with inadequate intake. Given that other characteristics are equal, it follows that a given provoking factor more readily exceeds the threshold for thrombus formation under inadequate intake of n-3 PUFAs. Potential pathways for an protective effect of n-3 PUFAs on VTE risk include downregulation inflammation,[15] tissue-factor expression,[16,17] platelet function,[18] and platelet–endothelium interactions.[19,20] Further, as PE traditionally has been considered as a complication to DVT, it may also be speculated that n-PUFAs influence the clot structure, providing more stable clots that are less prone to embolization. However, PE may also occur due to whole-clot embolization, de novo formation in the lungs or have a cardiac origin.[38] Consequently, the protective effect of n-3 PUFAs on provoked VTE, particularly PE, could be explained by a lower prevalence of VTE-related disease (eg, atrial fibrillation).[39,40]

The major strengths of our study include a large cohort with high participation rates, a comprehensive and validated exposure variable, and thoroughly validated outcomes. We also accounted for changes in dietary habits during follow-up with repeated assessments and including n-3 PUFA intake as a time-varying variable in our analyses. However, some limitations of the study merit consideration. There were substantial exclusions due to incomplete questionnaires, and the included participants were younger and healthier compared with those who were excluded. This influences the generalizability of the study population, but the study still addresses the principal association between intake of n-3 PUFAs and the risk of VTE. However, several factors may influence the precision of our risk estimates. The reproducibility of self-reported fish intake in the Tromsø study has been reported to be moderate (Spearman correlation coefficient = 0.41-0.56),[41] and in general, dietary questionnaires may only reflect 20% of n-3 PUFAs levels measured in erythrocytes.[42] However, these are misclassifications unrelated to the outcome, which would lead to

underestimated risk estimates. Additionally, although we reassessed exposure during follow-up, the median duration of the observation periods was still relatively long in our study (12 years). As confirmed by the sensitivity analyses with maximum 5 years of follow-up, the main results are probably subject to regression dilution and the true association likely underestimated. Moreover, residual confounding cannot be excluded, although the risk estimates were approximately similar when more extensively adjusted models were tested. Finally, there were slight differences in the questionnaires used the fourth and the sixth survey of the Tromsø Study. However, serum triglyceride concentrations predicatively decreased with increasing n-3 PUFA intake,[43] and there was a significant dose-dependent increase in serum concentration of marine n-3 PUFAs with higher reported intake, both observations supporting the validity of our variable.

In conclusion, we found lower risk of VTE with a weekly intake of ≥4.7 g of marine n-3 PUFAs, with no evidence for increased protection with higher intake. The association appeared to be driven by an effect on provoked events, and particularly provoked PE. The findings should be replicated in future studies with objectively assessed n-3 PUFA concentrations.

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RELATIONSHIP DISCLOSURE

All authors state that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

The authors' responsibilities were as follows: T. Isaksen conducted research, analyzed the data, interpreted the results, and wrote the paper; L. H. Evensen interpreted the results and wrote the paper; S. H. Johnsen and B. K. Jacobsen interpreted the results; K. Hindberg analyzed the data and interpreted the results; S. K. Brækkan and J.-B. Hansen designed the study, conducted research, and interpreted the results. All authors critically revised the manuscript for important intellectual content and read and approved the final version of the manuscript.

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REFERENCES

1. Huang W, Goldberg RJ, Anderson FA, Kiefe CI, Spencer FA. Secular trends in occurrence of acute venous thromboembolism: the Worcester VTE study (1985-2009). Am J Med. 2014;127:829–839.e5.

2. Næss IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrøm J. Incidence and mortality of venous thrombosis: a population-based study. J Thromb Haemost. 2007;5:692–9.

3. Cushman M, Tsai AW, White RH, et al. Deep vein thrombosis and pulmonary embolism in two cohorts: the longitudinal investigation of thromboembolism etiology. Am J Med. 2004;117:19–25.

4. Heit JA. Venous thromboembolism: disease burden, outcomes and risk factors. J Thromb Haemost. 2005;3:1611–7.

5. Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ 3rd. Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. Arch Intern Med. 1998;158:585–93.

6. Arshad N, Isaksen T, Hansen JB, Braekkan SK. Time trends in incidence rates of venous thromboembolism in a large cohort recruited from the general population. Eur J Epidemiol. 2017;32:299–305.

7. Heit JA, Ashrani A, Crusan DJ, McBane RD, Petterson TM, Bailey KR. Reasons for the persistent incidence of venous thromboembolism. Thromb Haemost. 2017;117:390–400.

8. Mannsverk J, Wilsgaard T, Mathiesen EB, et al. Trends in modifiable risk factors are associated with declining incidence of hospitalized and nonhospitalized acute coronary heart disease in a population. Circulation. 2016;133:74–81.

9. Vangen-Lønne AM, Wilsgaard T, Johnsen SH, Løchen ML, Njølstad I, Mathiesen EB. Declining incidence of ischemic stroke: what is the impact of changing risk factors? The Tromso Study 1995 to 2012. Stroke. 2017;48:544–50.

10. Wang C, Harris WS, Chung M, et al. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. Am J Clin Nutr. 2006;84:5–17.

11. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation. 2002;106:2747–57.

12. Rimm EB, Appel LJ, Chiuve SE, et al. Seafood long-chain n-3 polyunsaturated fatty acids and cardiovascular disease: a science advisory from the American Heart Association. Circulation. 2018;138:e35–47.

13. The Norwegian Directorate of Health. Kostråd om fisk og sjømat (Dietary advice on fish and seafood). [Accessed 2018 June 22] Available from https://helsenorge.no/kosthold-og-ernaring/kostrad/spis-fisk-oftere

14. U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015–2020 Dietary Guidelines for Americans. 8th edition. [Accessed 2018 June 22] Available from https://health.gov/dietaryguidelines/2015/guidelines/

15. Surette ME. The science behind dietary omega-3 fatty acids. CMAJ. 2008;178:177–80.

16. Moertl D, Berger R, Hammer A, et al. Dose-dependent decrease of platelet activation and tissue factor by omega-3 polyunsaturated fatty acids in patients with advanced chronic heart failure. Thromb Haemost. 2011;106:457–65.

17. Hansen JB, Olsen JO, Wilsgård L, Østerud B. Effects of dietary supplementation with cod liver oil on monocyte thromboplastin synthesis, coagulation and fibrinolysis. J Intern Med Suppl. 1989;731:133–9.

18. Hansen JB, Olsen JO, Wilsgard L, Lyngmo V, Svensson B. Comparative effects of prolonged intake of highly purified fish oils as ethyl ester or triglyceride on lipids, haemostasis and platelet function in normolipaemic men. Eur J Clin Nutr. 1993;47:497–507.

19. Hansen JB, Svensson B, Wilsgard L, Osterud B. Serum enriched with n-3 polyunsaturated fatty acids inhibits procoagulant activity in endothelial cells. Blood Coagul Fibrinolysis. 1991;2:515–9.

20. De Caterina R, Cybulsky MA, Clinton SK, Gimbrone MA Jr, Libby P. Omega-3 fatty acids and endothelial leukocyte adhesion molecules. Prostaglandins Leukot Essent Fatty Acids. 1995;52:191–5.

21. Hansen-Krone IJ, Enga KF, Sudduth-Klinger JM, et al. High fish plus fish oil intake is associated with slightly reduced risk of venous thromboembolism: the Tromso Study. J Nutr. 2014;144:861–7.

22. Steffen LM, Folsom AR, Cushman M, Jacobs DR Jr, Rosamond WD. Greater fish, fruit, and vegetable intakes are related to lower incidence of venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology. Circulation. 2007;115:188–95.

23. Lutsey PL, Steffen LM, Virnig BA, Folsom AR. Diet and incident venous thromboembolism: the Iowa Women's Health Study. Am Heart J. 2009;157:1081–7.

24. Severinsen MT, Overvad K, Andersen VL, Tjønneland A, Schmidt EB, Kristensen SR. Fish intake and venous thromboembolism: a Danish follow-up study. Thromb Res. 2014;133:352–6.

25. Varraso R, Kabrhel C, Goldhaber SZ, Rimm EB, Camargo CA Jr. Prospective study of diet and venous thromboembolism in US women and men. Am J Epidemiol. 2012;175:114–26.

26. Institute of Marine Research. Seafood data. [Accessed 2018 August 17] Available from https://sjomatdata.nifes.no/#search/

27. Norwegian Food Safety Authority. Matvaretabellen 2018 (Table of food composition 2018). [Accessed 2018 August 17] Available from http://www.matvaretabellen.no/

28. Hutcheon JA, Chiolero A, Hanley JA. Random measurement error and regression dilution bias. BMJ. 2010;340:c2289.

29. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njølstad I. Cohort profile: the Tromsø Study. Int J Epidemiol. 2012;41:961–7.

30. Braekkan SK, Mathiesen EB, Njølstad I, Wilsgaard T, Størmer J, Hansen JB. Family history of myocardial infarction is an independent risk factor for venous thromboembolism: the Tromso study. J Thromb Haemost. 2008;6:1851–7.

31. Jacobsen BK, Nilsen H. High education is associated with low fat and high fibre, beta-carotene and vitamin C. Computation of nutrient intake based on a short food frequency questionnaire in 17,256 men and women in the Tromsø Study. Nor Epidemiol. 2000;10:57–62.

32. Mattiuzzi C, Cervellin G, Franchini M, Lippi G. Fish intake and venous thromboembolism: a systematic literature review. Clin Appl Thromb Hemost. 2016;22:309–13.

33. Mishra GD, McNaughton SA, Bramwell GD, Wadsworth ME. Longitudinal changes in dietary patterns during adult life. Br J Nutr. 2006;96:735–44.

34. Clarke R, Shipley M, Lewington S, et al. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. Am J Epidemiol. 1999;150:341–53.

35. Jensen RA. Postoperative thrombosis-emboli; their frequency in the period 1940-1948 at the III department of Ulleval Sykehus and the surgical department of Akers Sykehus. Acta Chir Scand. 1952;103:263–75.

36. Strom A. Examination into the diet of Norwegian families during the war-years 1942-1945. Acta Med Scand. 1948;131 (suppl 214):47.

37. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet. 1999;353:1167–73.

38. van Langevelde K, Sramek A, Vincken PW, van Rooden JK, Rosendaal FR, Cannegieter SC. Finding the origin of pulmonary emboli with a total-body magnetic resonance direct thrombus imaging technique. Haematologica. 2013;98:309–15.

39. Enga KF, Rye-Holmboe I, Hald EM, et al. Atrial fibrillation and future risk of venous thromboembolism:the Tromso study. J Thromb Haemost. 2015;13:10–6.

40. Massaro M, Scoditti E, Carluccio MA, De Caterina R. Basic mechanisms behind the effects of n-3 fatty acids on cardiovascular disease. Prostaglandins Leukot Essent Fatty Acids. 2008;79:109–15.

41. Jacobsen BK, Bønaa KH. The reproducibility of dietary data from a self-administered questionnaire. The Tromso Study. Int J Epidemiol. 1990;19:349–53.

42. von Schacky C. Omega-3 fatty acids in cardiovascular disease—an uphill battle. Prostaglandins Leukot Essent Fatty Acids. 2015;92:41–7.

43. GISSI-Prevenzione Investigators (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico). Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet. 1999;354:447–55.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.