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Dietary intake of marine polyunsaturated fatty acids and incidence, recurrence and mortality related to venous thromboembolism

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Let me tell a secret. In all these years, I have adopted a little nickname for TREC for private humorous use, the Ivory Tower. In true ivory towers, people are happily disconnected from the rest of the world in favor of their own favorite pursuits, perhaps indulging themselves in extravagancies. The nickname was inspired by noticeable differences from my previous work as a nurse at the hospital. For instance, I did not anticipate invitations to that many conferences, including Nice, Amsterdam, Longyearbyen, Toronto, Marseille, Berlin and Glasgow, and I hold travels to be among the richest of experiences. However, good mockery takes truthfulness lightly, and the scientific tower was high, exposed and merciless. I would have fallen off long ago without all of you, whom I now want to thank.

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SUMMARY

Venous thromboembolism (VTE) is a common and serious cardiovascular disease, though advancing knowledge about risk factors has not yet led to decreased incidence. Marine n-3 PUFAs have several beneficial effects related to hemostasis, and our goal was to investigate the association between dietary intake of marine n-3 PUFAs and the risk of VTE in the general population. Only few studies have investigated this topic previously with conflicting results. However, there are several methodological challenges in epidemiological studies that could obscure the effect of marine n-3 PUFAs on VTE risk. Our main efforts to improve methods include a comprehensive and validated measure of marine n-3 PUFAs in the diet and the use of updated measurements through follow-up.

Paper I is a large prospective cohort study representing the general population in the municipality of Tromsø. There were 21 970 participants in Tromsø 4 and 6 that provided information of fish consumption and other n-3 PUFAs sources. The participants were followed in the period 1994-2016. Dietary n-3 PUFAs intake exceeding 4.7 grams per week was associated with a 22-26% lower risk of VTE. Higher intake did not add to the effect, and this apparent threshold effect was larger for provoked VTE (30-35% reduced risk) and PE (31-47% reduced risk).

Paper II is a prospective cohort study of 595 VTE patients recruited from the Tromsø Study. In this study, we investigated the overall association between dietary intake of marine n-3 PUFAs and risk or recurrent VTE and overall mortality. In analysis contrasting the higher and the lower tertile of n-3 PUFAs intake, we found a weak association between intake of n-3 PUFAs and risk of recurrent VTE overall. However, we found a 55% reduced risk of recurrence in patients with unprovoked VTE, a 49% reduced risk in cancer-free patients, and a 51% reduced risk in deep vein thrombosis (DVT) patients, in subjects with high compared to low intake of n-3 PUFAs. The inverse associations were more evident when follow-up was

restricted to the period after discontinuation of anticoagulant therapy. We found no association between intake of n-3 PUFAs and mortality after incident VTE.

Major surgeries are trigger events that cause a large proportion of all VTEs in the population. In Paper III, we conducted a case-crossover study and investigated whether the trigger effect of major surgery was modified with n-3 PUFAs intake. We recruited 445 patients with VTE that occurred within the period between 1994 and December 31, 2012. We categorized the VTE patients according to tertiles of n-3 PUFAs. We found a significant lower odds ratio of having a major surgery as a trigging event in the VTE patients with a high n-3 PUFAs intake compared to those with low intake (ORs: 4.1 vs. 11.8).

NORSK SAMMENDRAG – NORWEGIAN SUMMARY

Venøs tromboembolisme (VTE) er en vanlig og alvorlig karsykdom, men økende kunnskap om risikofaktorer har ennå ikke ført til redusert forekomst av VTE. Marine omega-3 fettsyrer (n-3 PUFAs) har flere gunstige effekter relatert til hemostase, og vårt mål var å undersøke sammenhengen mellom inntak av marine n-3 PUFAs i kosten og risikoen for VTE i befolkningen. Det har tidligere blitt gjennomført få studier om emnet og disse har vist til dels motstridende resultater. Dette kan delvis forklares av at tidligere studier har brukt ulike mål på fiskeinntak (total fiskeinntak, mager og fet fisk, og estimert inntak av n-3 PUFAs) og i ulik grad tatt hensyn til at fiskeinntaket varierer over tid. I våre studier har vi estimert inntaket av marine n-3 PUFAs i kostholdet og brukt repeterte målinger av inntaket av n-3 PUFAs gjennom oppfølgingstiden.

Artikkel I er en stor prospektiv kohortstudie som representerer befolkningen generelt i Tromsø kommune. Det var 21 970 deltakere som hadde oppgitt komplette data om inntak av fisk til middag og andre kilder til n-3 PUFAs i dietten i Tromsø 4 og 6. Disse ble fulgt i perioden 1994-2016. Vi fant at n-3 PUFAs inntak på mer enn 4,7 gram per uke var assosiert med en 22-26% lavere risiko for VTE. Høyere inntak ga ikke økt effekt. Terskeleffekten var størst for provosert VTE (30-35% redusert risiko) og PE (31-47% redusert risiko).

Artikkel II er en prospektiv kohortstudie av 595 VTE-pasienter rekruttert fra Tromsø-studien. I denne studien undersøkte vi sammenhengen mellom inntaket av marine n-3 PUFAs i kostholdet og risiko for tilbakevendende VTE og generell dødelighet. I analyser som sammenliknet høyt og lavt inntak av n-3 PUFAs, fant vi en beskjeden, men gunstig effekt på sammenhengen mellom inntak av n-3 PUFAs og risikoen for tilbakevendende VTE totalt. I tilleggsanalyser fant vi imidlertid en 55% redusert risiko for tilbakefall hos pasienter med uprovosert VTE, en 49% redusert risiko hos kreftfrie pasienter, og en 51% redusert risiko etter dyp venetrombose (DVT) pasienter hos personer med høyt inntak av n-3 PUFAs. De

gunstige assosiasjonene ble forsterket når oppfølgingen ble begrenset til perioden etter seponering av antikoagulasjonsbehandling. Vi fant ingen sammenheng mellom inntak av n-3 PUFAs og dødelighet etter VTE.

Store kirurgiske inngrep er en utløsende faktorer for 15-22% av VTE tilfellene i befolkningen. I artikkel III gjennomførte vi en case-crossover-studie og undersøkte om triggereffekten av større kirurgi varierte ut fra n-3 PUFAs inntak i dietten. Vi kategoriserte VTE-pasienter i henhold til tertiler av n-3 PUFAs. Vi fant en betydelig lavere odds-ratio for å ha gjennomgått større kirurgiske inngrep som en utløsende faktor for VTE hos pasienter med høyt inntak av PUFAs n-3 sammenlignet med lavt inntak (OR: 4,1 mot 11,8).

LIST OF PAPERS

The Thesis is based on the following papers:

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

Trond Isaksen, Line H. Evensen, Stein Harald Johnsen, Bjarne K. Jacobsen, Kristian Hindberg, Sigrid K. Brækkan, John-Bjarne Hansen

Res Pract Thromb Haemost. 2019; 3:59–69

II **Dietary Intake of Marine Polyunsaturated n-3 Fatty Acids and Risk of Recurrent Venous Thromboembolism**

Trond Isaksen, Line H. Evensen, Sigrid K. Brækkan, John-Bjarne Hansen

Thromb Haemost. 2019; 12:2053-2063

III **Impact of dietary marine n-3 polyunsaturated fatty acids on surgery as a trigger for venous thromboembolism – results from a case-crossover study**

Trond Isaksen, Line H. Evensen, Kristian Hindberg, Sigrid K. Brækkan, John-Bjarne Hansen

Manuscript

ABBREVIATIONS

ACCP	American College of Chest Physicians
ADP	Adenosine diphosphate
ALA	Alpha linolenic acid
ARA	Arachidonic acid
ARIC	The Atherosclerosis Risk in Communities
ATP	Adenosine triphosphate
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CD39	Entonucleoside triphosphate diphosphohydrolase-1
COX1	Cyclooxygenase-1
CRP	C-reactive protein
CTEPH	Chronic thromboembolic pulmonary hypertension
CTPA	Mulitidetector row compuded tomographic pulmonary angiography
CVD	Cardiovascular disease
DCH	The Danish Diet, Cancer and Health Study
DHA	Docosahexaenoic acid
DOAC	Direct oral anticoagulants
DPA	Docosapentaenoic acid
DVT	Deep vein thrombosis
EPA	Eicosapentaenoic acid
EV	Extracellular vesicles
F	Factor
FVL	Factor V Leiden
GP	Glycoprotein
IR	Incidence rate
IWHS	Iowa Women's Health Study
LA	Linoleic acid
LMWH	Low molecular weight heparin
MI	Myocardial infarction
NETs	Neutrophil extracellular traps
NHS	The Nurses' Health Study and Health Professionals Follow-up Study
NO	Nitric oxide
P2	ATP or ADP receptor
PAI-1	Plasminogen activator inhibitor-1
PAR	Protease-activated receptor
PE	Pulmonary embolism
PGI_2	Prostacyclin I2
PS	Phosphatidylserine
PUFAs	Polyunsaturated fatty acids
PY	Person year
TF	Tissue factor
TP	Thromboxane receptor

TxA_2	Thromboxane A2
VCAM1	Vascular cell adhesion protein1
VKA	Vitamin K antagonist
VTE	Venous thromboembolism
vWF	Von Willebrand factor

1. INTRODUCTION

1.1 Epidemiology of venous thromboembolism

Venous thromboembolism (VTE) is a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE). Approximately two thirds of VTE events present as DVTs in the lower extremities and one-third as PEs (1, 2). Less frequently, VTE occur in upper extremity veins (1-4%) or in other large veins in the abdomen like mesenteric veins and vena porta, or even cranially in dural venous sinuses (0.05-0.5%) (3-7). Altogether, VTE is the third most common cardiovascular disease after myocardial infarction (MI) and stroke, and afflicts 1-2 per 1000 annually (8-10). Incidence rates (IRs) increase exponentially with age (11). VTE is rare in childhood with incidence rates less than 0.06 per 1000 annually (12). At the age 20-30, the rate is around 0.5, increasing to one per 1000 roughly around the age of 60. This rate doubles approximately around the age of 70, and at advanced age beyond 80 the rate may exceed 10 per 1000 annually (9, 13). In the Tromsø Survey, the mean age at first life-time VTE was 69 years (14). Large epidemiological studies on VTE are predominantly from Western societies that share characteristics in age composition, prevalence of lifestyle-related diseases and development of health care (1, 13, 15-19). These studies show annual incidence rates of VTE in the range between 0.63 and 1.83 per 1000 individuals in the adult population. Differences between estimates in different studies could arise from data selection, study designs, case definitions and availability of data. However, VTE incidence also vary by ethnicity. People with African ancestry have somewhat higher incidence of VTE than those with Western European ancestry, followed by Southern European origin, whereas the lowest rate is observed in people with East-Asian-Pacific ancestry. The latter group may have a 70-80% lower incidence rate of VTE than Caucasians (1, 20, 21).

Studies on VTE trends indicate stable or increasing incidence rates over the recent decades, with somewhat different figures for DVTs and PEs considered separately (8, 9, 13,

14, 22-27). Rates of PE seem to have increased after year 2000. In the Tromsø Study, the overall incidence rate of VTE increased from 1.6 per 1000 in 1996/97 to 2.0 in 2010/11. The increase in PE was also observed in the Tromsø Study, rising from 0.45 per 1000 in 1996/97 to 1.1 in 2011/12, whereas incidence of DVT slightly decreased from 1.1 in 1996/97 to 0.9 in 2011/12 (14). A proportion of the increase in PE is attributed to the introduction of highly sensitive multidetector row computed tomographic pulmonary angiography (CTPA). The use of CTPA in PE-diagnostics increased rapidly after year 2000. In a study addressing overdiagnosis (finding clinically unimportant emboli) by CTPA in the U.S., an 81% increase in the incidence of PE was observed comparing the period 1993-1998 (before CTPA) to 1998-2006 (after the introduction of CTPA), while the corresponding rate of PE related deaths actually decreased by 8% (27). Although more frequent use of CTPA probably explain much of the increasing incidence of PE, the overall epidemiological trends seem paradoxical compared with the trends of MI and stroke. In these fields, the use of highly sensitive diagnostic methods have increased considerably too, while incidence- and mortality rates have decreased by 30-50% in many Western countries over the same time-span (28-30). An investigation from the Tromsø Study showed that the incidence rates (age- and sexadjusted) of total coronary heart diseases (primarily sudden deaths and ST-segment MI) decreased by an average of 3% each year between 1994 and 2010 (31). Interestingly, changes in modifiable coronary risk factors, such as smoking and hypercholesterolemia, accounted for 66% of the observed decrease in this study.

Classical symptoms and signs of DVT include swelling, redness and pain in the afflicted limb, whereas symptoms of PE include shortness of breath, chest pain, hemoptysis and syncope. These symptoms and signs are somewhat unspecific and could overlap with other, less serious conditions. This, together with a generally lower awareness of VTE disease

in the population compared to stroke and heart attack, makes it challenging for patients to suspect VTE as the cause of the symptoms in an initial phase (32).

Long-term complications after VTE constitute a major health burden in the society (33). The most common long-term complication after DVT is termed post-thrombotic syndrome (PTS), and involves degrees of chronic symptoms and signs in the affected limb, including pain, edema, discoloration, varicose veins, sensation of heaviness, cramps and ulcers. PTS afflicts 20-50% of DVT patients, and is associated with reduced quality of life and up to a 50% increased risk of work-related disability (34, 35). The risk of PTS is highest after an ileo-femoral DVT (36, 37), and a 2-3 fold increased risk has been reported in patients with subtherapeutic anticoagulant treatment (38, 39). Catheter-directed thrombolysis vs. standard non-invasive treatment reduced the absolute risk of PTS with 14% after a 2 year follow-up in an open-label randomized trial (40). Other risk factors for PTS include ipsilateral recurrent DVT and obesity (41), while there are inconsistent findings for age and sex as risk factors (42).

Long-term complications of PE include persisting shortness of breath, and in one study, 19% of PE patients had incomplete reperfusion after 2 years follow-up (43). The most severe long-term complication of PE involves fibrotic transformation of residual thrombi in the pulmonary arteries, which causes degrees of persistent obstruction of pulmonary arteries and elevated circulatory pressure in the right heart chambers, i.e., chronic thromboembolic pulmonary hypertension (CTEPH). Symptoms of CTEPH include hypoxemia and right-sided heart failure, and the condition is associated with elevated mortality risk (44). CTEPH afflicts 1-4% of all PE cases (45, 46). The prevalence of CTEPH is somewhat difficult to determine as it is not subjected to routine diagnostics after PE, and develops gradually up to two years after an incident PE. Moreover, 25-42% of patients with chronic pulmonary hypertension

have no evidence of a previous PE (45, 47). Risk factors for CTEPH include previous PE, younger age, unprovoked PE and severe perfusion defects (46).

Up to 30% of all VTE patients experience recurrence within 10 years (48). The risk of recurrence is highest during the first year and decreases over time (49). In the Tromsø Study, IRs (95% CIs) of recurrence per 100 person-years after 0-6 months, 6 months-1 year, 5-10 years, and after 10 years were 9.2, 6.3, 3.5, 2.3 and 2.4 respectively (50). A recurrent PE is more likely to occur when the first event was a PE and a recurrent DVT is more likely to occur after a first DVT (51, 52). In the Tromsø Study this tendency applied to approximately 70% of all recurring VTE events (50). Moreover, the risk of recurrence is 1.5 to 2.0-fold higher after a DVT compared with after a PE (51, 53, 54). In addition, men have a more than doubled risk of recurrence compared to women (55). Other risk factors for recurrent VTE include hereditary thrombophilia, active cancer, occult cancer, neurologic paresis and neurosurgery, high BMI and higher age (48, 56-59). Risk factors for incident VTE may not constitute equivalent risk factors for a recurrent event. This phenomenon is referred to as the paradox of recurrence (60). For instance, there is little difference between men and women in risk of a first VTE, and while age is the most important predictor for the first VTE, it is only a weak predictor for recurrence. The phenomenon is partly explained by the fact that a second VTE always arises from the highly selected group of those who have had a VTE in the first place, a group that differs from the general population (60). The recurrence paradox will be revisited in chapter 1.5.

The risk of recurrence is also related to provoking factors for the first VTE. Therefore, the convention to categorize VTEs as either provoked or unprovoked, and whether or not these provoking factors were transient or persistent, has prognostic and treatment implications (61). Important transient provoking factors include acute medical conditions, surgery, trauma and plaster cast immobilization, whereas examples of persisting provoking factors are

malignant diseases and paresis. For instance, major surgery is a typical transient provoking factor, because the risk of VTE is high immediately after the procedure, and then drops rapidly within a few days or weeks (62). If no recognized provoking factor is documented, a VTE is considered as unprovoked. Guidelines on anticoagulant treatment aim to balance the benefits of terminating thrombi growth and prevent recurrence, to the risk of bleeding complications. The long-term risk of recurrence after a VTE provoked by a transient risk factor such as a surgery is low, and consequently, short-term treatment of 3-6 months is recommended (63). The highest risk of recurrence is observed when provoking factors are persistent. In such cases, long-term treatment until contraindicated or throughout life is recommended. Unprovoked VTEs have an intermediate risk of recurrence, which indicates that unknown risk factors exist and are likely to persist (60). In unprovoked VTE, American College of Chest Physicians (ACCP) guidelines recommend short-term treatment except for patients with unprovoked proximal DVT (the category with highest recurrence risk), given low or medium bleeding risk (63).

VTE is associated with an increased risk of mortality. The one month cumulative mortality risk vary between 6-11%, increasing to 17-36% after one year (1, 9, 13, 36, 64), and 30-52% after eight years (1, 13). Mortality rates (95% CI) after VTE were estimated in the Tromsø Study in the period 1994-2012, and were as follows (per 100 PY), 0-6 months: 9.2 (6.3-13.9), 6 months-1 year: 6.3 (3.8-10.3), 1-5 years: 3.5 (2.6-4.6), 5-10 years: 2.3 (1.5-3.7), and finally, after 10 years: 2.4 (1.0-5.3) (50). The observation that 25% of PE cases present with sudden death (16) underlines the severity of PE. The one-month mortality rate for PE patients is 8-16% (first PE event) (1, 13). In general, the risk of sudden death after PE is 18-fold higher than after a DVT event (16), and PE is considered as one of the most preventable lethal hospital acquired complications (65, 66). Most VTE-related all-cause mortality confine within the first year after the event, partly driven by concurrent malignancy, but even after 30

years of follow-up, a 1.5-fold increased risk of mortality after DVT, and a 2.7- fold increased risk after PE has been reported (67).

VTE is treated with anticoagulant drugs (63). Acute treatment with low molecular weight heparin (LMWH) the first few days followed by continued treatment with vitamin K antagonist (VKA) monotherapy became widespread during the 1940's, and was standard medication for several decades (65). In cancer patients, LMWH is favored over VKA for continued therapy due to less bleeding complications (68). Exceptional and comparatively rare treatments in the acute phase of severe or life-threatening VTE include use of systemic unfractionated heparin or alteplase, catheter-directed thrombolysis and thrombectomy. Nowadays, direct oral anticoagulants (DOACs) increasingly replace VKA therapy (61), mainly due to lower bleeding risk, and ease of administration that does not require regular monitoring, as is the case with VKA therapy (69).

1.2 Pathophysiology of venous thromboembolism

Until the 17th century, only anecdotal documentation of conditions that we now recognize as VTE are known, and the first descriptions of DVT as a clotting disorder began with pregnancy-related DVT (65). The modern understanding of the pathophysiology of VTE began in the mid-19th century with the German pathologist and physicist Rudolph Virchow. Virchow correctly inferred that thrombi developing in the deep veins of the legs (DVTs) could dislodge and cause the even more severe condition of PE. Moreover, Virchow postulated three broad risk categories involved in the pathogenesis of VTE; i) stasis of the blood flow, ii) endothelial injury or dysfunction, and iii) hypercoagulability (65, 70). Virchow's triad (Figure 1) illustrates that one or more of the three categories must be present, or interplay to cause VTE, and captures the multifactorial nature of the disease. Known major

risk factors for VTE like paresis, surgery and inheritable thrombophilia represent well each of these three risk categories.

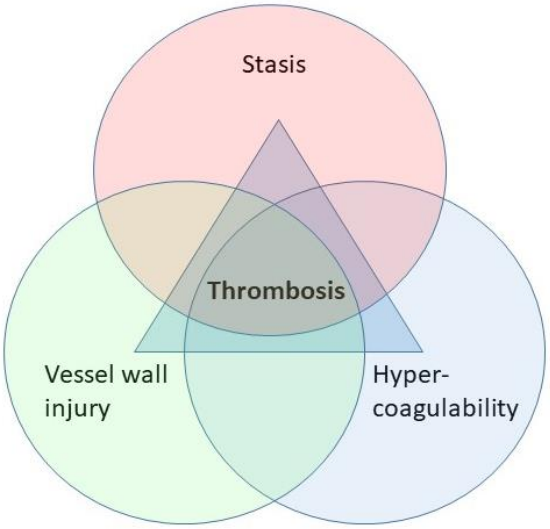

Figure 1. Virchow's triad consist of three main risk categories for venous thromboembolism, stasis, vessel wall injury and hypercoagulability.

1.2.1 Hemostasis

Hemostasis refer to prosesses that prevent and stop bleeding, and a healthy circulation is maintained by the regulations of procoagulant and anticoagulant systems (71). At the site of an injury, bleeding is stopped by the formation of a clot made up of circulating constituents. Excess clot formation is simultaneously restricted by the fibrinolytic system, that eventually dissolve the clot altogether and restore the vessel integrity. VTE and other thrombotic diseases can therefore be viewed as an inappropriate form of hemostasis at the wrong place (71).

1.2.2 The mechanistic hypothesis for venous thrombosis

Autopsy studies suggest that VTEs typically originate in the valvular sinuses in the lower extremities (72-74), and a positive correlation between number of valves (e.g. varying with

height) and risk of VTE has been reported (75). The valves' function is to direct the blood flow, although they also create circulatory deadlocks in the valve pockets (Figure 2). Even though muscle work facilitates circulatory turnover (76), these microenvironments frequently become oxygen-deprived (77).

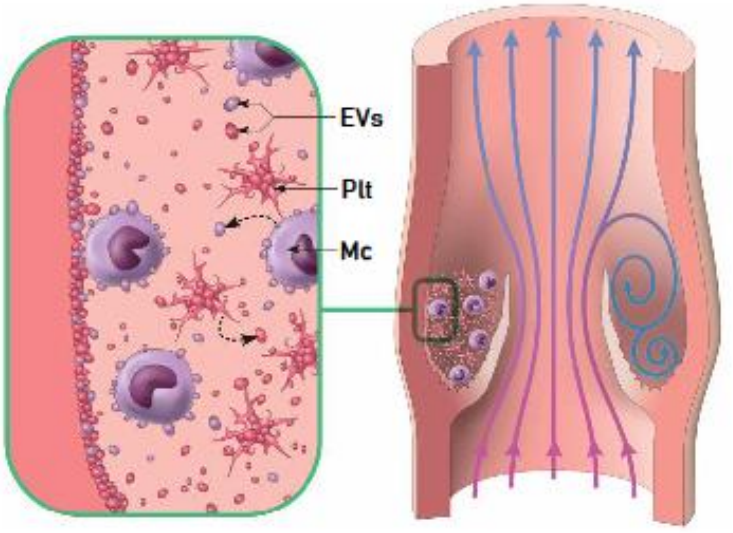

Figure 2. The valve sinuses are vulnerable to low circulatory turnover and hypoxia. The endothelial layer and trapped cells such as monocytes, platelets and EVs in such an environment, could shift toward prothrombotic expressions. VTE is often initiated at these sites.

Endothelial cells in the venous valves actually constitute phenotypes that tolerate more hypoxia than the endothelial cells elsewhere (78). However, under prolonged hypoxic stress, endothelial cells express P-selectin, E-selectin and vascular cell adhesion protein1 (VCAM1). These adhere to platelets and other blood elements like leukocytes, neutrophils, and leucocyte-derived extracellular vesicles (EVs) that express tissue factor (TF). The aggregated elements have the potential to initiate or positively feedback clot formation (79). Expression of plasminogen activator inhibitor-1 (PAI-1) and activated neutrophils that release extracellular traps (NETs) may also contribute to the hypercoagulable state in the valvular sinuses (80, 81). The proposed mechanism of these events is illustrated in Figure 3.

Remarkably, Virchow's triad of endothelial dysfunction, stasis and hypercoagulability seems to apply at the cellular level in the modern understanding of VTE pathogenesis.

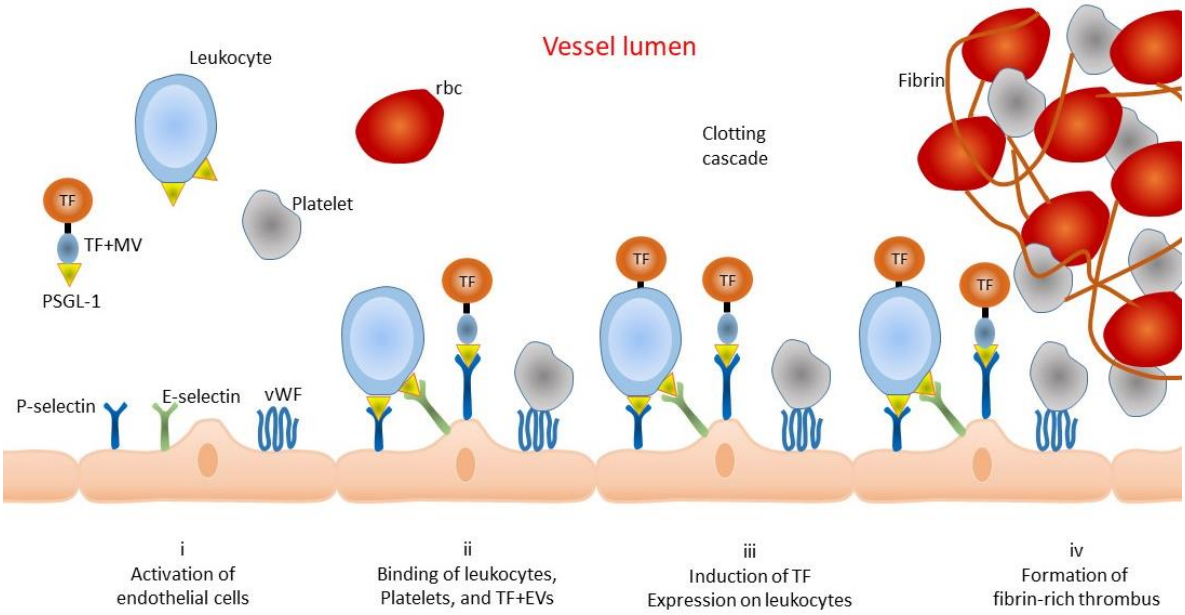

Figure 3. A proposed mechanism for venous thrombosis. Venous thrombosis may be trigged through the intrinsic pathway by i), hypoxic stress causing endothelial cells to express adhesive proteins (P-selectin, E-selectin and von Willebrand Factor (vWF)), ii) circulation leukocytes, platelets, TF and extracellular vesicles (EVs) binding to the activated endothelium, and iii), activation of bound leukocytes that express more TF. VTE occurs if the local activation of the coagulation cascade overwhelms the surrounding anticoagulant expression in the adjacent healthy vessel lumen. Adapted from Mackman 2012 (73).

1.3 The role of hemostatic factors in hemostasis

1.3.1 The role of platelets in hemostasis

Primary hemostasis is the first response to vessel damage. Primary hemostasis include vasoconstriction and the formation of a platelet plug that seal a vessel damage (82). Platelets are discoid cells 2.0-3.0 μm wide, which originate from the cytoplasm of polyploid megakaryocytes in the bone marrow. They have a typical life span of 5-9 days (83, 84). The platelets enter the bloodstream anucleated, though with granules and lysosomes ready to release at activation, causing reaction cascades (82, 84, 85). Platelet activation is mainly driven by increased intracellular Ca^{2+} level, that is regulated by cyclic adenosine

monophosphate (cAMP) in platelets (86). Signals transmitted through glycoproteins in the platelet membrane and certain contents of the granules are stimuli that induce increased Ca^{2+} levels and platelet activation (83). Activated platelets transform from discoid and circulating, to amoeboid-shaped and aggregating, a process that occurs in distinct steps (87, 88). The steps overlap and represent a simplification of platelet activation. The first step is **adhesion and initiation**, next **excretion and propagation**, and finally **stabilization** through cross-linked fibrin bonds (87). Figure 4 shows several of the involved mechanisms in platelet activation.

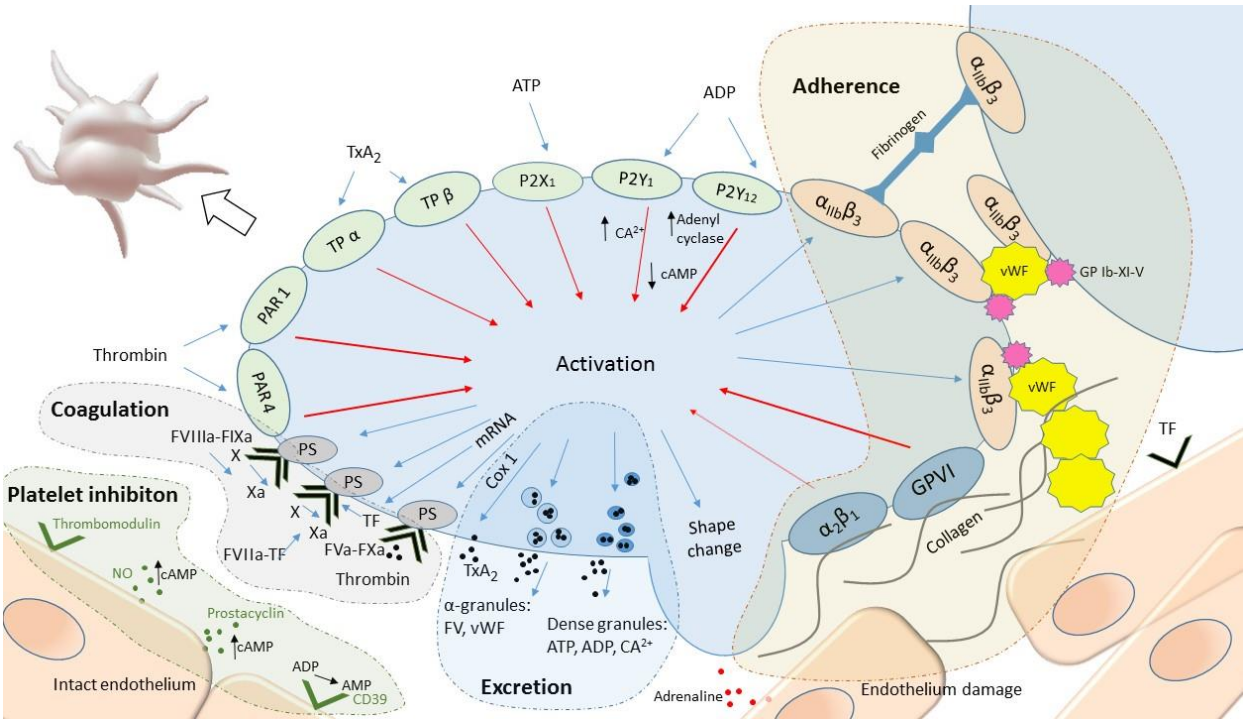

Figure 4. Platelet activation mechanisms. Platelet activation can initiate when glycoprotein VI (GPVI) and integrin $\alpha_2\beta_1$ adhere to collagen. The binding cause granule **excretion** of signalling molecules that bind to G-protein-coupled receptors (PAR, TP and P2), in an inside-out signalling pathway. **Adherence** to other platelets and additional bonding to collagen are mediated by the activation of integrin $\alpha_{IIb}\beta_3$, expression of GPIb-IX-V, von Willebrand factor (vWF) and fibrinogen bonds. The activated platelet facilitates **coagulation** by excreting factor V and factor XI, and by expressing phosphatidylserines (PS) that are binding sites for coagulation factor complexes. To the lower left corner, main endothelial expression of **platelet inhibitors** are shown (Modifyed after Storey 2008 (89), Ruggeri 2002 (87), Adams 2009 (90). Activated platelet image: colourbox.com.

1.3.1.1 Activation by adhesion to collagen

At a vessel injury, collagen is exposed to the bloodstream. Circulating platelets bind to collagen in glycoprotein VI (GPVI) that cause platelet activation and the release of dense- and α-granules, and activation of phospholipase A2 (Figure 4). Phospholipase A2 releases the omega-6 fatty acid arachidonic acid (ARA) from the platelet cell membrane (91). The released constituents take part in further platelet activation and propagation. When activated, the platelet express integrin $\alpha_{IIb}\beta_3$. Integrin $\alpha_{IIb}\beta_3$ is an abundant and unique platelet adhesive receptor (92) that form bridging bonds with the glycoprotein (GP) von Willebrand Factor (vWF) (93). vWF exists circulating, and in large polymers in endothelial cells, and has several binding sites. vWF and integrin $\alpha_{IIb}\beta_3$ form bridging bonds to collagen that resist high circulatory stress (83) (Figure 5).

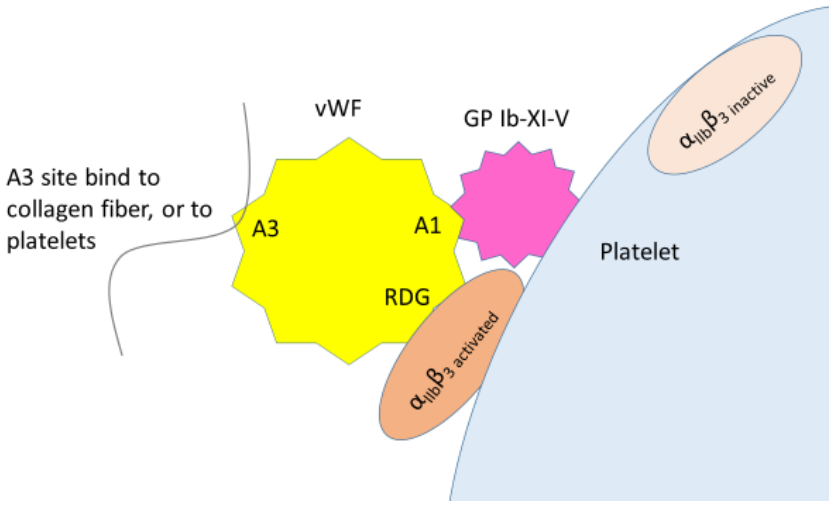

Figure 5. Bridging bonds with von Willebrand factor (vWF) in platelet adhesion. vWF has several binding sites that can bind between vWF and collagen, and between vWF and platelets (83).

1.3.1.2 Excretion and propagation

In platelets, cyclooxygenase 1 (COX1) cyclize free ARA to endoperoxides, which are catalyzed to thromboxane A_2 (TxA_2) by thromboxane syntetase (94). TxA_2 diffuses through

the platelet cell membrane and binds to the TxA_2-receptors (TPα and TPβ), causing activation of platelets and vasoconstriction in vessels (91). At activation, ADP, ATP and Ca^{2+} released from dense granules, enhance platelet activation through the $P2X_1$, $P2Y_1$ and $P2Y_{12}$ receptors (84). This stimulates downregulation of cAMP that reduces Ca^{2+} efflux. At the same time, more Ca^{2+} ions are available outside the platelet due to the degranulation. An effect of these inside-out signaling pathways is the recruitment of nearby platelets.

Platelet activation becomes irreversible at the point where integrin $\alpha_{IIb}\beta_3$ is activated. In addition to the role of integrin $\alpha_{IIb}\beta_3$ in attaching the platelet to collagen as described, it is involved in the formation of fibrinogen crosslink and aggregation with other platelets (87, 93), through bonds with fibrinogen, or through bonds with vWF and GP-1b-XI-V (Figure 4 and 5).

Platelets also interact with, and adhere to, stimulated endothelial cells and monocytes (95). Activated platelets transmit inflammatory mediators, altering functions of endothelial cells to facilitate chemotaxis, adhesion and transmigration of monocytes to the site of the vessel damage (85). Activated platelets thus stimulate the endothelium in the vessel wall to shift from antithrombotic to pro-thrombotic phenotypes (96). Activated platelets can also recruit monocytes by releasing chemokines (97). Notably, under pathological stimuli, monocytes express TF, a primary initiator of the coagulation cascade (98).

Activated platelets strongly stimulate coagulation activation (as described in the next paragraph) by the release of α- and dense granules that contain coagulation factor V, factor XI, and negatively charged phospholipids in the bloodstream. Activated platelets express phosphatidylserines (PS) on the outer cell membrane that facilitate the assembly and activation of coagulation factor complexes, i.e. the prothombinase complex, the FVIIa-TF complex and the FVIIIa-FIXa complex (99-101).

1.3.2 The role of coagulation in hemostasis

The coagulation cascade is a catalytic pathway that culminates with fibrin deposition, which crosslink and stabilizes a developing blood clot. The cascade has an amplifying effect downstream the chain of reactions, in which coagulation factors (F), i.e. zymogens or serine proteases together with co-factors, are activated (a) and catalyze the next step. The coagulation factors' roman numerics follow the sequence of their discovery and not the sequence of events. The coagulation cascade has two entry points, the intrinsic and extrinsic pathways. The extrinsic and intrinsic pathways coalescence in a common pathway at the point where FX is activated (FXa). Figure 6 shows a simplification of the coagulation cascade.

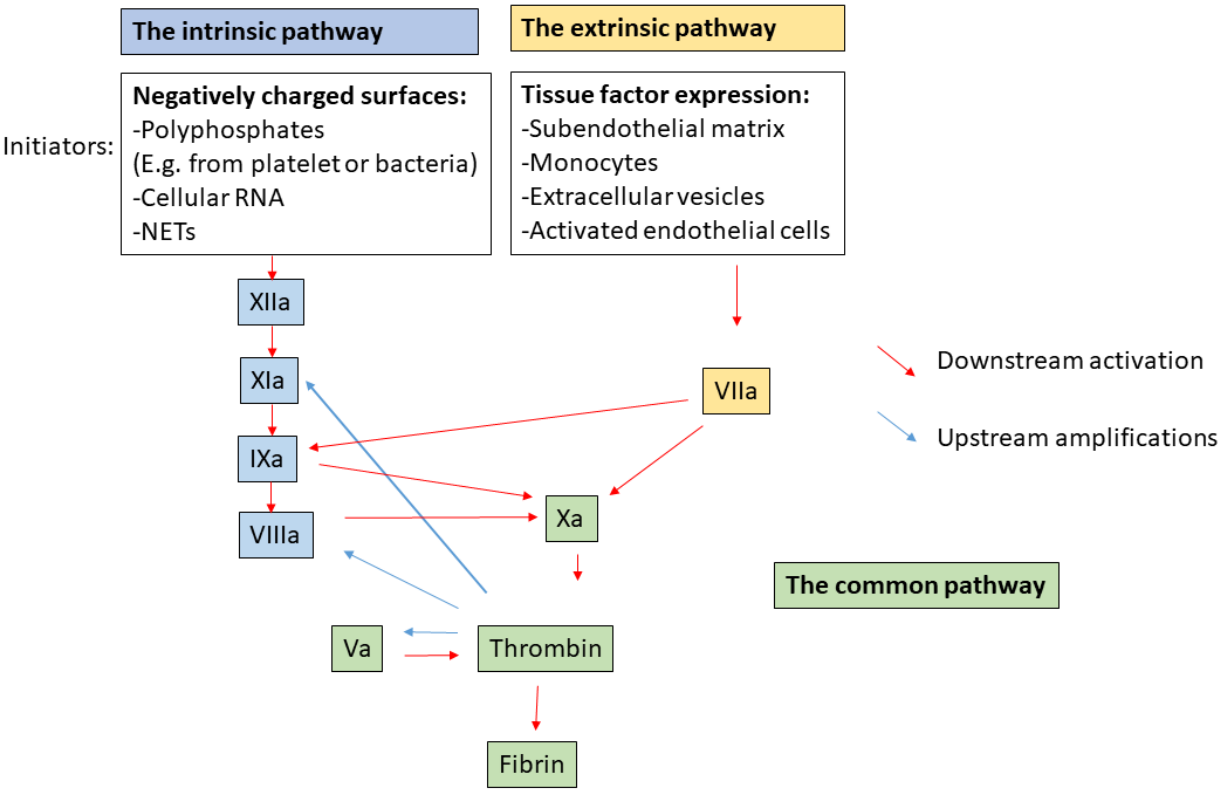

Figure 6. An overview of the chain of reaction in the coagulation cascade. The extrinsic pathway is initiated by TF. The intrinsic pathway initiates through negatively charged surfaces. Both pathways coalescence in the common pathway and the formation of thrombin that cleaves fibrinogen into fibrin. Thrombin also causes upstream positive feedback in the cascade. The outburst of fibrin that stabilizes a growing blood clot is the functional endpoint of the cascade.

The extrinsic pathway initiates with vessel damage and exposure of sub-endothelial cells that express tissue factor (TF). Monocytes and endothelial cells can also express TF under pathological conditions (102, 103). TF acts as a cofactor for FVII and the TF-FVIIa complex activates factors IX and X, entering the common pathway (90, 104).

The intrinsic pathway is activated through negatively charged contact, including cellular RNA, polyphosphate from activated platelets or bacteria, and collagen (73). The intrinsic pathway initiates by the activation of FXII, causing a chain of coagulation factor activation following the sequence of FXIIa, FXIa, and FIXa. FXIa forms a complex with thrombin-activated FVIIIa as a co-factor. Next, the FVIIIa-FIXa complex activates FX that enters the common pathway (90).

In **the common pathway**, FXa forms a complex with FV (90). The FXa-FVa prothombinase complex binds to negatively charged phospholipids on cell membranes, including PS on activated platelets (83, 101). The FXa-FVa complex (prothombinase) catalyzes prothrombin to thrombin that catalyzes fibrinogen to fibrin, which is the functional endpoint of the coagulation cascade. Fibrin eventually cross-links platelets and other blood cells that stabilize the growing platelet plug. Within the coagulation cascade, there are several amplifying interactions. For instance, FVIIa stimulates the activation of FIX (104). Thrombin facilitates the activation of FXI, VIII and FV upstream. Thrombin also activates FXIII that stabilizes the fibrin matrix with covalent bonds (90). In addition, thrombin act as a platelet activator through PAR1 and PAR4 receptors (101).

It can be assumed that the intrinsic contact pathway is an important contributor to VTEs, e.g. under the pro-thrombotic microenvironments within deep veins (105). This is in contrast to a myocardial infarction, where endothelial rupture and release of accumulated atherosclerotic plaque with TF causes a rapid coagulation response through the extrinsic pathway (106, 107).

1.3.4 The role of the endothelium in hemostasis

A healthy endothelial layer in vessels constitutes an antithrombotic surface (105). Endothelial cells repress platelet activation by the expression of prostacyclin I_2 (PGI_2), nitric oxide (NO) and the ligand entonucleoside triphosphate diphosphohydrolase-1 (CD39). ARA released in endothelial cells enters a slightly different enzymatic pathway compared to platelets. In endothelial cells, ARA-derived endpoeroxides are synthesized to PGI_2 by prostacyclin syntetase. PGI_2 act as a vasodilator, and upregulates cAMP levels in platelets that inhibit activation (108). NO upregulates platelets cyclic guanosine monophosphate (cGMP), which together with cAMP stimulate Ca^{2+} efflux in platelets (109). CD39 hydrolyses ATP and ADP and reduces the levels of these platelet activators (110).

Healthy endothelial cell expressions also downregulate coagulation. Endothelial protein C receptors promote the activation of protein C in the presence of thrombomodulin, and activated protein C (APC) inhibits FVIIIa and FVa (111). Endothelial tissue factor pathway inhibitor (TFPI) reversibly inhibits FXa and the TFPI-FXa complex inhibits the FVIIa-TF complex (96). Endothelial expression of heparan sulfate enhances the binding of antihrombin to thrombin that inhibits factor Xa, and tissue plasminogen activator (TPA) catalyzes plasminogen to plasmin that breaks down fibrin (112).

Interestingly, ARA, thrombin, FVa and VIIIa promote platelet activation and thrombosis formation at the site of endothelial damage, whereas they are elements in anti-thrombotic expressions in a healthy endothelium. This situation dependent function assists to limit blood clotting to the damaged site.

1.4 Risk factors of venous thromboembolism

VTE is a multicausal disease with inherited or acquired risk factors, and often several factors need to occur simultaneously to cause thrombosis (113). A risk factor is associated with an increased probability of an adverse outcome, and is potentially, but not necessarily a causal factor (114). The thrombosis potential model is a theory that describe the risk of VTE as a function of inherited and acquired risk factors over time (Figure 7) (113). VTE develops when the thrombosis potential, due to the combination of inherited and acquired risk factors, exceeds a critical thrombosis threshold. In the thrombosis potential model, age is a universal risk factor that increases the risk over time. Transient risk factors such as surgery would add to the underlying risk temporarily, and may drive the potential risk over the thrombosis threshold. It follows that a transient risk factor for VTE tolerated at young age could cause VTE at older age. Inherited and persistent risk factors such as the Factor V Leiden Mutation (FVL) would add to the impact of age through life, and it follows that VTE could develop at younger age in carriers than in non-carriers.

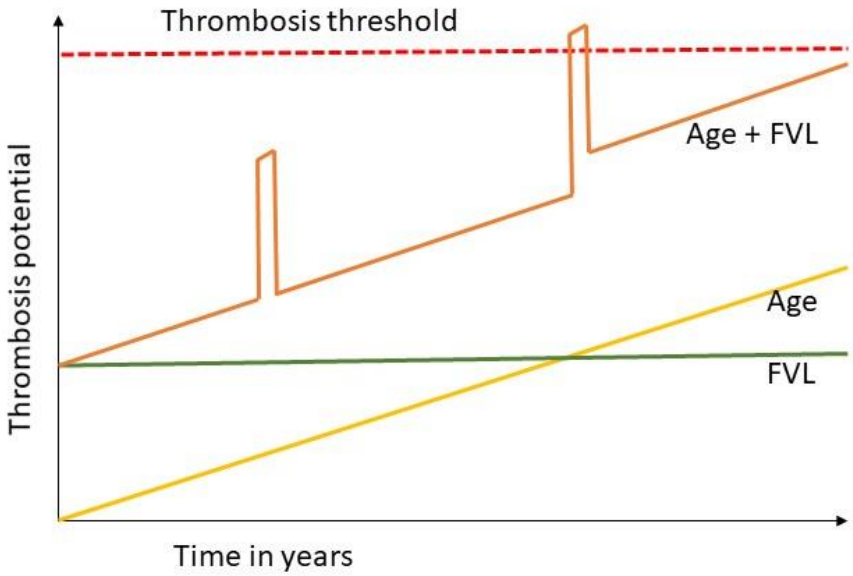

Figure 7. The thrombosis potential model describes VTE risk as a dynamic and accumulative process. The thrombosis potential inevitably increases with age, and the outcome of any transient provoking factors during life such as surgery, depend on the combined impact of all risk factors at that time. An inheritable and persistent risk factor such as Factor V Leiden would add to the impact of age throughout life. Adapted from Rosendaal, Lancet. 1999 (113).

1.4.1 Acquired risk factors

Acquired risk factors for VTE include age, obesity, cancer, immobilization, major surgery, congestive heart failure, varicose veins, fractures, use of estrogen, trauma, pregnancy, birth and puerperium, myocardial infarction, stroke and infections (115). Age and obesity are examples of risk factors with high prevalence in the general population, whereas other risk factors such as surgery and cancer are less prevalent, but impose high relative risk.

Age is a well established risk factor for VTE (116). Almost 90% of VTE patients have passed 40 years at the time of their first event (115). A report from the Tromsø Study showed an 11-fold higher risk of VTE for those above 70 compared to those below 50 years (117). Advanced age is associated with several characteristics that could influence the risk of VTE. Elderly have increased levels of fibrinogen, FVIII, FIX, and increased platelet activity (118). Degenerative changes in the vein walls and venous valves also come with age (119), as well elevated markers of C-reactive protein (CRP) and interleukin-6 that indicate an ongoing low-

grade inflammation (118). Other risk factors, such as general immobilization and many comorbidities are also more common with advancing age. However, in a study of people ≥70 years in the Tromsø population, there was no evidence that the higher risk of VTE in elderly could be attributed to malignancies (120).

Obesity, defined as a body mass index (BMI) ≥30kg/m^2 is associated with a 2-3-fold increased risk of VTE (121, 122). Results from the Tromsø Study have further shown that waist circumference identify more people at risk and display higher effect sizes for VTE than BMI (123), and that weight change per se, in particular weight gain in those already obese, increases the risk (124). Several mechanisms could be involved in this association. Abdominal obesity causes increased intra-abdominal pressure with subsequent iliofemoral venous pressure and stasis (125). Obesity is also associated with increased hepatic synthesis of fibrinogen, FVII, FVIII and TF, increased inflammation and reduced fibrinolysis (126-128). Results from several recent Mendelian Randomization studies have suggested a causal relationship between obesity and risk of VTE (129-131).

Height is a known risk factor for VTE, particularly in men (132). In men, a 34% increased risk per 10 cm increase in height was reported from the Tromsø Study (133). Height increases the vessel area at risk, hydrostatic pressure and sheer number of venous valves, which possibly explains stature as a risk factor for VTE (76).

Cancer is a major risk factor for VTE and is associated with a 5-7 fold-increased risk (134-137). Among all VTEs, 18-22% are associated with active malignancy (9, 117). However, the risk varies with the cancer site, stage, histological type and time since cancer diagnosis (136, 138). VTE is reported to occur more frequently in pancreatic cancer, mesothelioma, in cancers with unknown primary site, lung cancer, brain cancer and cancer in the gastrointestinal tract, as reported from a large UK cohort (136). The relative risk may be highest with pancreatic cancer and brain cancer (137). Metastatic cancer and patient-related

comorbidities further increase the risk. There are several suggested mechanisms in cancer-related VTE. Tumor growth can cause physical obstruction and stasis in vessels. Active cancer is associated with chronic pro-thrombotic changes including increased TF expression, activated platelets, release of EVs and an increased inflammatory state (139, 140). Chemotherapy has toxic and pro-thrombotic effects on vessel wall endothelial cells (141). Importantly, cancer types with high mortality introduce a bias that tend to overestimate the risk of VTE. Analysis taking competing risk of death into account specifies the risk of VTE more appropriately in cancers with high mortality (142).

Major surgery in general anesthesia for more than 30 minutes is one of the most important hospital-related risk factors for VTE. Around 20% of VTEs occurring in the general population are trigged by major surgery, despite the routine use of thromboprophylaxis (9, 22). About 8 out of 1000 patients undergoing surgery develop a post-operative VTE, and recent surgery in general confers a 4-22 fold increased risk of VTE (143). The risk depends on the type of surgery. The highest incidences are reported after total hip arthroplasty, major vascular surgery, and invasive neurosurgery (144). However, minor surgeries involving the larger vessels such as venous catheters and pacemaker insertion are associated with up to 10-fold increased risk of VTE (135, 145).

Trauma, depending on the extent, is a major risk factor for VTE. In a study of 349 patients with multiple trauma 201 (58%) had an asymptomatic DVT, whereas 3 (<1%) had clinical symptoms and 3 (<1%) died from PE (146).

VTE occurs in 1-2 per 1000 **pregnancies**, an incidence rate corresponding to a 4-5 fold increased risk compared to non-pregnant women of reproductive age (147, 148). Delivery involves a significant risk of bleeding, and the organism has apparently adapted to the challenge by upregulating FI, FII, FV, FIII, FIX and FXII, and downregulating protein C (149). During the 6 first weeks after delivery, the **puerperium**, the risk of VTE even

increases up to 4-5 fold as compared with the risk during pregnancy, which might be caused by endothelial damage in pelvic vessels during delivery (148).

Another risk factor for VTE restricted to women is the use of **estrogen** taken as contraceptives or medication to relieve menopausal symptoms. Estrogens cause increased levels of coagulation factors, and a 2-7 fold increased risk of VTE, dependent on estrogen type and indication (150, 151). The risk is transient and resolves after approximately a year of use. The risk of VTE by use of estrogen as contraceptives is defensible partly because the baseline risk of VTE in young women is low, and the pregnancy-related risk of VTE is higher than the risk related to estrogen use.

A diversity of reasons for **immobilization** including lower extremity plaster-cast immobilization, neurological paresis, confinement to bed or wheelchair, bed-rest for more than three days and long-haul travels are associated with an approximately doubled risk of VTE (152, 153). The increased VTE risk with immobilization is likely due to venous stasis.

Hospitalization per se is also a risk factor for VTE. Hospitalization is likely to involve several of the already described risk factors such as cancer, surgery and immobilization. Hence, hospitalization is associated with a 40-100 fold increased risk for VTE as compared to community residents (15, 154).

Acute medical conditions include ischemic stroke, myocardial infarction and congestive heart failure, severe respiratory disease, severe infections and rheumatologic disorders. The risk of VTE associated with acute illness is typically transient. The VTE risk after ischemic stroke, myocardial infarction (MI) and acute infections was investigated in the Tromsø Study (155, 156). After ischemic stroke, IRs (per 1000 PY) after one, 3 and >3 months were 82, 47 and 8, respectively (155). After MI, incidence rates were 18 within 6 months and 7 within 6 months and 1 year. In a case-crossover study investigating acute

infection as trigger for VTE, an odds ratio (ORs) of 20 for acute infection as a trigger was reported (156). Moreover, a synergistic effect (OR: 141) of infection and immobilization was demonstrated. In the Tromsø Study 14% of VTE patients had an acute medical condition within 8 weeks before the event (14).

1.4.2 Inherited risk factors

Family and twin studies have shown that genetic factors probably account for 50-60% of the variation in VTE risk (157, 158), and a family history of VTE is an independent risk factor for VTE (157, 159-161). Genetic variants that inhibit or downregulate endogenous anticoagulants are referred to as loss-of-function mutations (162). Numerous mutations could cause loss of function in the same pathway, and more than 250 different gene variants are known to cause **antithrombin deficiency**, a condition that is present in 0.2% of the population (163). Antithrombin is a serine protease inhibitor produced in the liver that binds to and inhibits the function of thrombin and FXa. Glycosaminoglycans like heparin bind to and enhance the function of antithrombin, and heparins are widely used in medical anticoagulation (164). Antithrombin deficiency is associated with a 10-50 fold increased risk of VTE (165). Other loss-of-function mutations include **protein C and protein S deficiency**, which are present in 0.03-0.2% of the population, and are associated with an approximately 10-fold increased risk of VTE in heterozygous carriers (166-168).

Conversely, gain-of-function mutations upregulate normal proteins involved in hemostasis. Gain-of-function mutations include FV Leiden (FVL), Prothrombin gene mutation (PT20210A), variants increasing the levels of fibrinogen, FVIII, FIX, FX and FXI, and non-O blood groups (162). The prevalence of FVL mutation is high in Caucasians (5%) (169, 170), and is associated with a 2-5 fold increased risk of VTE in heterozygous carriers and a 10-80 fold increased risk in homozygous carriers (171). FVL affects the FV gene and

causes degrees of APC resistance. Prothrombin mutation G20210A, prevalent in 2% of the population, is associated with an approximate 3-fold increased risk of VTE. The mutation is associated with increased levels of prothrombin (172, 173). Non-O blood type is highly prevalent (60% of the world's population) and is thereby a significant contributor to the overall incidence of VTE despite a modest 1.5-2.0 fold increased relative risk (172, 174). Levels of vWF and FVIII are higher in non-O blood groups (175).

Modern genomics have allowed for hypothesis-free search for genetic variants associated with VTE. However, few of the recently discovered variants imposes >30% increased risk of VTE, and common variants may only account for 5-20% of VTE heritability (170, 176). This observation has implications for the prospects in this field. Genetic variants associated with a modestly increased risk of VTE are unlikely to be indicators for thromboprophylaxis due to the balancing of side effects. On the other hand, there is a potential to discover combinations of risk factors yielding a high risk that could exceed the threshold for prophylactic intervention.

1.4.3 VTE triggers

A trigger is a broad term that simply refers to an event that initiates another. In VTE etiology, triggers are considered as risk factors for VTE that are transient and discrete events limited to particular dates or periods (61). Typical triggers for VTE include several of the discussed risk factors including surgery, hospitalization, infections, temporal immobilization and acute medical conditions.

1.5 Marine derived polyunsaturated fatty acids

In 1956 H. M. Sinclear linked dietary fatty acid composition with “diseases of civilization”, a term that included thrombotic diseases. He noted as follows in the Lancet: “Eskimos have high dietary fat and little atherosclerotic plaque”, and “Norwegians and also Eskimos uncontaminated by so-called civilization fare well by taking marine foods” (177). Later, in 1976, Bang and Dyerberg compared the diet of Danes with the Greenland Inuits and observed that even though the total intake of fat was similar, Inuits had relatively higher intakes of marine long-chained omega-3 polyunsaturated fatty acids (n-3 PUFAs) (178). Bang and Dyerberg reported that the relative high total fat intake appeared contradictory to a lower level of serum cholesterol and triglycerides measured in the Inuits, and suggested that marine long-chained n-3 PUFAs have special metabolic effects. In a second study, Bang and Dyerberg found that plasma n-3 PUFAs levels were significantly higher and that bleeding-time was longer in Greenland Inuits than in Danes (179). In 1980, these associations were also reported from Japan, another population with low incidence of thrombotic disease, in a study comparing inhabitants in a fishing village to those in a farming village (180). Since then, marine fatty acids have been subjected to extensive research related to numerous diseases, and marine derived n-3 PUFAs are widely recommended as a component in a healthy diet (181-183).

1.5.2 Structure and properties

Fatty acids are a diverse group of molecules characterized by repeating series of hydrophobic methylene groups (a carbon chain with hydrogen bonds) and an acyl compound that facilitate the composition of complex lipids (184, 185). The human diet contains a mixture of >20 different types of fatty acids, mainly with a carbon chain length of 12-22 (186). Functions of fatty acids include energy supply, storage, insulation, and cell membrane structure, though

fatty acids also provide substances to physiological processes. Life would not sustain without them. The number of carbons, double bonds and their position are the main basis of nomenclature, and naturally occurring fatty acids generally share the characteristics of a cis-isomer configuration and an even number of carbons (187).

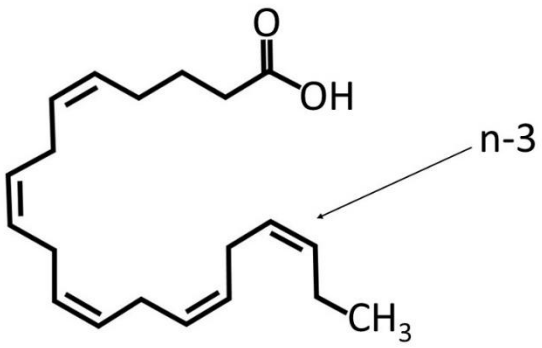

Figure 7. Structure of eicosapentaenoic acid (EPA). The omega-3 fatty acid family is named after the last double bound in the chain, which occur three carbons away from the terminal methyl group (n-3). The label C20:5n-3 specify the number of carbons and number of double bonds as well (Adapted from Wikimedia).

Marine derived polyunsaturated fatty acids (n-3 PUFAs) have a chain length of 18, 20 or 22 carbon atoms with 3-6 double bounds (n) (184). The last double bound in the carbon chain occurs three carbons away from the terminal methyl group, giving them the popularized name omega-3 (187, 188). The marine derived long-chained n-3 PUFAs include eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). These fatty acids mainly origin from marine algae that enter the human food chain through the marine fauna. In contrast to the long-chained n-3 PUFAs, the shorter chained alpha linolenic acid (ALA, C18:3n-3) is also abundant in terrestrially derived plant oils. In 1929, it was discovered that mammals do not possess enzymes able to synthesize double bonds at the n-3 and n-6 position of the carbon chain. Therefore, n-3 PUFAs in humans depend on dietary sources, and are referred to as essential fatty acids (189, 190). Although ALA is 15-25 fold more abundant than EPA and DHA in the

typical Western diet, it only represents about 0.5% of total fatty acids concentration in plasma cells and tissue phospholipids, whereas concentrations of EPA and DHA usually are higher. (191). This indicates a significant affinity to take up the long chained n-3 PUFAs. However, in mammals ALA and linoleic acid (LA, C18:2n-6) can be extended to EPA and the omega-6 arachidonic acid (ARA, C20:4n-6) respectively through elongation and desaturation (190). In a study where participants were given high doses of radioisotope labeled ALA and a background diet high in saturated fat, a 6% conversion from ALA to EPA was observed, a rate that was considerably slower under alternative background diets (192). Increased rates of ALA to EPA conversion has been reported in non-fish consumers, and the rate is generally higher in women in childbearing ages (193). Intake of LA also modifies the conversion rate because it is a competitive inhibitor of ALA for the Δ^5 and Δ^6 desaturase enzymes (194). Elongation from EPA to DHA may be <0.5% (193), or does not occur in humans (195), whereas reconversion of DHA to EPA readily occurs (192, 195). In all, the conversion of ALA to long-chained n-3 PUFAs is inefficient and unpredictable, while long-chained n-3 PUFAs are readily taken up through dietary marine food or supplements (192, 196).

1.5.3 Physiological effects of n-3 PUFAs on hemostasis and thrombosis

Dietary n-3 PUFAs are incorporated into cellular membranes of all tissues, and the extent of incorporation depends on the intake (186). Increased intake of n-3 PUFAs is measurable in cellular membranes within days (197). n-3 PUFAs increases cell membrane fluidity which is considered to enhance trans-membrane functionality (186).

Cell membrane polyunsaturated phospholipids constitute a pool of fatty acids used as components in the diverse eicosanoid lipid family of signaling molecules (198). The effect and type of eicosanoid that are produced differ between cell types (187). Eicosanoids have short half-lives and exert a local effect. Among the eicosanoids, prostaglandins and

prostacyclins have hemostatic regulatory functions in platelets and in endothelial cells (198). The prostaglandin thromboxane A2 (TxA_2) is of particular interest because it causes platelet activation and vasoconstriction (83). Thromboxanes and other eicosanoids are formed in response to cell perturbation, and in the first step phospholipase releases ARA and EPA into the cytoplasm. In platelets, and also in monocytes, neutrophils and eosinophils, the COX-1 enzyme cyclize ARA and EPA released from the cell membranes to endoperoxides, which next are metabolized to thromboxane by thromboxane syntethase. In platelets, ARA is a precursor of the 2-series of thromboxane (TxA_2). However, EPA is a competitive inhibitor of ARA for the COX enzyme, and EPA is a precursor to the 3-series of thromboxanes (TxA_3) (Figure 8). TxA_3 ability to activate platelets may only be 1/10 of TxA_2 (199, 200). In platelets, prostaglandin H_3 (PGH_3) is the immediate parent compound of TxA_3, and a proportion of PGH_3 rapidly degrades to PGD_3 that increases platelet cAMP and Ca^{2+} efflux that counteracts platelet activation (200).

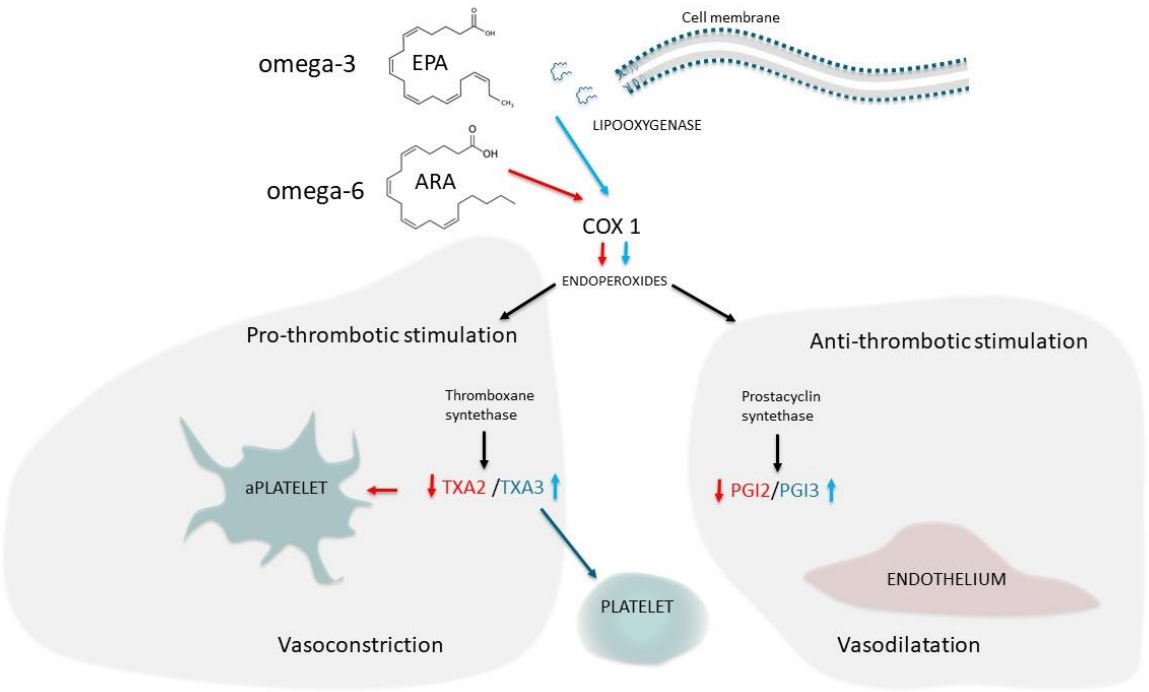

Figure 8. EPA is a competitive inhibitor of ARA for the COX enzyme, and EPA is a precursor of the considerably less potent 3-series of prostaglandins.

Several studies have shown that increased intake of EPA through fish oil increases the levels of the 3-series of eicosanoids at the expense of the ARA derived 2-series of eicosanoids (201-205). A meta-analysis involving 52 publications with a majority of randomized controlled trials, concluded that fish oil supplements reduce platelet aggregation in healthy subjects (206). In medicine, inhibition of the COX-enzyme and thereby the metabolism of TxA2, is an efficient and well-established way to suppress platelet aggregation. This is achieved with acetylsalicylic acid (aspirin) that binds to, and irreversibly inhibits the COX enzyme. Although the role of platelets in VTE is not fully understood, accumulating evidence indicates that platelets contribute to thrombosis in animal models and in humans (207, 208). Two clinical trials (WARFSA and ASPIRE) indicated that a low dose of aspirin reduced recurrent VTE after the completion of anticoagulant treatment (209), and a meta-analysis estimated a 42% risk reduction of recurrent VTE with aspirin therapy (210).

The antithrombotic effects of n-3 PUFAs are not restricted to platelet reactivity. In The Atherosclerosis Risk in Communities (ARIC) Study, a cross sectional study of more than 15 000 individuals, a high fish intake (>1 servings per day) was associated with lower levels of the coagulation proteins fibrinogen (-2.9mg/dL), FVIII (-3.3%), vWF (-2.7%), and a slightly increased level of protein C (211). High doses of n-3 PUFAs possibly attenuate procoagulant activity in endothelial cells. In an experiment with umbilical vein endothelial cell cultures, 43% lower TF activity was reported when exposed to serum from volunteers who had a daily intake of 25 ml cod liver oil for 8 weeks, as compared with the same persons before the high dose fish-oil intervention (212). The expression of TF in monocytes may also decrease with intake of n-3 PUFAs. In a controlled trial where 40 healthy volunteers had 25 ml cod liver oil for 8 weeks, TF expression in monocytes decreased with 40% (213). Bleeding time is considered to reflect platelet-endothelial interactions, and the bleeding time is shortened in response to exercise (214). A randomized controlled trial in patients with

hypercholesterolemia showed that intake of fish oil completely inhibited the reduction in bleeding time after a standardized exercise, indicating an inhibition of the platelet-endothelial interaction (215).

1.5.3 Dietary marine polyunsaturated fatty acids and risk of venous thromboembolism

A possible link between marine food intake and incidence of surgery related VTE can be inferred from historic data in Norway during World War II. An observational study from 1940-1948 in two hospitals in Oslo, showed a temporal decline in incidence of VTE after surgery (216). Compared with the stable or slightly increasing trends of incident VTEs as described in several studies over the recent decades, the observed U-shaped curve in surgery related VTE during the war years appeared exceptional. The study adequately accounted for important confounders like type of surgery and immobilization that varied in the period. The reason for the transient decline is not known, but it occurred in parallel with dietary changes during the war period, including higher intake of fish and cod-liver oil, and lower intake of red meat and dairy products (217).

The effects of n-3 PUFAs intake on key pathways involved in hemostasis have motivated five large prospective cohort studies to investigate the association between fish intake and risk of VTE. In the ARIC study (2007), a prospective study following 14 962 participants over 12 years, the risk of VTE was estimated over quintiles (Qnt) of fish servings (218). The lower Qnt reference corresponded to less than one fish serving per week and Qnt2-5 corresponded to having more than one and up to more than three servings per week. A 30-45% lower risk of VTE was reported in Qnt2-5, suggesting a threshold effect. In analysis over quintiles of total n-3 PUFAs intake in the diet, a similarly reduced risk of 30-46% was reported. Conflicting result were reported in the Iowa Women's Health Study (IWHS, 2009) where 37 393 women aged 55-69 years at inclusion were followed for 19 years. This study

found that ≥2.5 as compared to <0.5 servings of fish per week was associated with a 22% increased risk of VTE (219). The study also reported no association between total intake of omega-3 fatty acids and the risk of VTE. The Nurses' Health Study and Health Professionals Follow-up Study (NHS, 2012) included 129 430 individuals who were followed from 1984 to 2008 (220). Food frequency questionnaires were answered every 2-4 years and the most recent one was used as basis for analysis. The study reported negligible effects when contrasting high versus low intake of fish or n-3 PUFAs. A non-significant 5% reduced risk for women and a 4% reduced risk for men were found. In analysis of total n-3 PUFAs intake, a corresponding and still non-significant risk reduction of 6 and 8% were reported. In The Danish Diet, Cancer and Health Study (DCH, 2014), 57 054 participants aged 50-64 were followed between 1993 and 2006, and fat fish and total fish intake was assessed at baseline (221). In analysis of quintiles of total fish intake, and using the lower Qnt as reference, no association with VTE risk was found. However, fat fish intake in Qnt2-5 compared to Qnt1 was associated with a non-significant 18-38% risk reduction in men, and 29-44% reduced risk in women for unprovoked VTE. The models were adjusted for use or no use of fish oil. Our group has previously conducted a cohort study on this topic. Using a single baseline measurement in Tromsø 4, 23 621 participants aged 25-97 were followed from 1994/95 to 2012. The weekly frequency of lean and fat fish for dinner combined with use or no use of fish oil was collected. A 48% lower risk for VTE was observed for those who reported fish for dinner ≥3 times per week and additionally used fish oil supplements, compared with 1-2 times per week with no use of fish oil (222). The cohort studies are summed up in Table 2.

Table 2. An overview of existing prospective cohort studies investigating fish intake and risk of VTE. Fish intake and n-3 PUFAs intake have different definitions between studies.

First author (Study)	Study population (Age/group)	n	Main finding (High vs. low intake) Focus of fish intake as exposure	Fat fish/ All omega 3 (High vs. low intake) Focus on omega-3 intake as exposure
Steffen et al. 2007 (ARIC)	45-64 years	14,962	30% reduced risk	30% reduced risk
Lutsey et al. 2009 (IWHS)	Women 55-69	37,393	22% increased risk	Na
Varraso et al. 2012 (NHS)	30-75 years	129,430	No association	No association
Severinsen et al. 2014 (DCH)	50-64 years	57,054	No association	20-40% reduced risk
Hansen-Krone 2014 (TROMSØ)	25-97 years	23,621	22% reduced risk	48% reduced risk

Two small Japanese studies have investigated serum EPA/ARA ratios and the risk of VTE. The first study was a cross sectional study of 144 out-patients of which 12 had PE. The PE patients had a lower EPA/ARA ratio than the non-PE patients (223). The second study was a case-control study that included 45 patients with acute VTE who were compared to age-, gender-, and BMI matched healthy individuals. Serum levels of ARA where higher whereas levels of EPA was lower among the VTE patients, a tendency that was stronger among the younger half of the participants (<58 years old) (224).

To our knowledge, only the SWITCO65+ study of elderly patients have previously investigated the association between n-3 PUFAs and the risk of recurrent VTE, and VTE related mortality (225). Levels of n-3 PUFAs in erythrocytes were measured at the time of the first VTE. Low levels were compared to medium and high levels of n-3 PUFAs based on the 25^{th} and 75^{th} percentile. After 6 months, medium and high levels were associated with a 61-83% reduced risk of recurrent VTE. No association was observed after 3 years of follow-up. All-cause mortality risk was 66-71% reduced after 6 months and 33-45% reduced after 3 years. A methodological advantage in this study was the use of an objective measurement of

the n-3 PUFAs status. However, the study did not separate marine derived n-3 PUFAs from terrestrial plant derived n-3 PUFA (ALA) that has different properties than the marine long chained n-3 PUFAs. For instance, ALA do not inhibit platelet activation.

A beneficial effect of marine long-chained n-3 PUFAs on pathways involved in VTE formation is supported theoretically, and in several experimental and observational studies. However, the results are conflicting in epidemiological prospective cohort studies that aim to assess dietary marine food intake at a population level and the risk of VTE. This could have several explanations as the investigated populations differ in age composition and in average fish intake. For instance, a 6-fold difference between the lowest and the highest intakes was shown from a study comparing fish intake in 10 European countries (226). Importantly, the exposure variable definition also varies across the studies. The content of n-3 PUFAs varies substantially between lean fish, fat fish, fish as bread spread and in fish oil supplements. Therefore, the n-3 PUFAs intake could vary considerably with the same dietary intake of fish. In the available prospective cohort studies, the exposure variable was mostly expressed as fish servings for dinner per week, and was rarely validated against circulating levels of n-3 PUFAs.

2. AIMS OF THE THESIS

The aims of the thesis were:

1) To investigate the association between intake of marine n-3 PUFAs and the risk of incident VTE in a cohort recruited from the general population using repeated measurements (Paper I).

2) To investigate the association between intake of marine n-3 PUFAs and the risk of VTE recurrence and mortality in patients with incident VTE (Paper II).

3) To investigate whether dietary intake of marine n-3 PUFAs intake modifies the trigger effect of major surgery on VTE risk in a case-crossover study (Paper III).

3. STUDY POPULATION AND METHODS

3.1 The Tromsø Study

The first survey of the Tromsø Study was conducted in 1974 to investigate the causes of the high rate of cardiovascular diseases among men in the north of Norway, and thereby to find strategies to prevent heart attack and stroke in the population (227). Both sexes were included in the second survey (1979/80), and to date, seven surveys have been conducted representing the broader population, and survey goals and measurements have diversified along the way. Today, the Tromsø Study represents a single-center, population based cohort with prospective data relevant for a wide range of disease outcomes. We used data from the 4th and 6th surveys. The 4th survey, conducted in 1994/95, was the broadest initiative to date, inviting all inhabitants in the municipality of Tromsø above the age of 25, and 27 158 (77%) participated. In the 6th survey, conducted in 2007/08, a selection of the population aged between 30 and 87 years were invited and 12 984 attended (66%).

3.1.1 Study design

In Paper I, we conducted a prospective cohort study with repeated measurements. We followed participants from the date of enrollment in Tromsø 4 and in Tromsø 6 respectively, until the date of a VTE, migration, death, or until end of follow-up at December 31, 2016 (whichever occurred first). Out of 29 540 unique individuals, 21 970 had complete information on n-3 PUFAs intake and were included. Among these, 5 892 participated in both surveys. The participants who took part in two surveys were given two observational periods with updated exposure data for the second period, unless they had VTE or were censored in the first period between Tromsø 4 and 6. During follow-up, 541 of the included had a first lifetime VTE. We used Cox proportional hazard regression models with age as time scale and calculated hazard ratios (HRs) with 95% confidence intervals (CIs) for VTE across quartiles (Qrt) of total fish and n-3 PUFAs intake.

In Paper II, we recruited patients with a first lifetime VTE from the 4th and the 6th surveys of the Tromsø Study in order to investigate the association between intake of marine n-3 PUFAs and risk of recurrent VTE and mortality. Out of 896 who had a first lifetime VTE, 595 had complete information on n-3 PUFAs intake and were included. During follow-up, 98 had recurrent VTE and 227 died. We used Cox regression models and calculated HRs for VTE and mortality across tertiles (T) of n-3 PUFAs intake. End of follow up was 31 December, 2016.

In Paper III, we conducted a case-crossover study involving cases with incident VTE (n=445), all recruited from Tromsø 4 and followed until December 31, 2012. The aim of this study was to assess the trigger effect of major surgery across categories of n-3 PUFAs intake. The patients' medical record was searched for major surgery and hospitalization in a 90 days hazard period before the VTE event, and in four control periods. The four 90-days periods within the 6-18 months period before the VTE were defined as control-periods (Figure 9). A 90-days washout period was included to avoid carry-over effects. We used conditional logistic regression to calculate regression coefficients and corresponding odds ratios (ORs) for the presence of major surgery in the 90-days hazard period according to tertiles of n-3 PUFAs intake.

Figure 9. An overview of the case-crossover design. Triggers for VTE are registered within 3 months preceding the event, i.e. the hazard period. For comparison, occurrence of risk factors are also registered in four preceding 3-month control periods. Between the hazard period and the control-periods, a 3-month wash-out period was included to avoid carry-over effects.

3.2 Baseline measurements

At the survey visits, data were collected by physical examination, blood sampling and self-administered questionnaires. Blood pressure measurements were taken three times with 2-minutes intervals with a Dinamap Vital Signs Monitor, and the mean of the last two measurements was recorded. Height and weight were measured with light clothing and without shoes, and BMI was calculated as weight divided by the square in height (kg/m^2). Non-fasting blood samples were drawn from an antecubital vein, and serum was prepared by centrifugation after one hour respite at room temperature. Analyzes were undertaken at the Department of Clinical Chemistry, University Hospital of North Norway (UNN). GPO-PAP kits (Boering Mannheim) were used to analyze serum total triglycerides. In Tromsø 4, serum concentration of EPA, DPA and DHA in the cholesterol ester fraction was analyzed in a subset of 1167 participants. In this analysis, 200 µl serum was extracted according to the method of Folch et al. (228). The separation of cholesterol-esters was achieved with a solvent system consisting of petroleum ether/diethyl ether/acetic acid. Next, individual phospholipid classes were separated by liquid chromatography (Agilent Technologies model 1100 series), and then quantified by capillary gas chromatography (Agilent Technologies model 6890). Lipomics Technologies, Inc. (West Sacramento, CA) performed the latter analysis according to the method of Watkins et al. (229). Information on diet, smoking habits, education, history of CVD, stroke and diabetes was obtained from self-administered questionnaires.

3.2.1 Assessment of n-3 PUFAs intake

In Tromsø 4 and 6, participants were asked how frequently they had fat or lean fish for dinner, fish as spread on bread and how many months a year they used fish oil supplements. In Tromsø 4, the questionnaire for those ≥70 years was simplified and had fewer items that included the predefined maximum option range. For participants aged ≥70 years in Tromsø 4,

the highest frequency option was ≥2/week which was recoded to 2.5/week. In the Tromsø 4 questionnaire, it was possible for those aged < 70 to report fish for dinner daily. This option was rarely chosen (<2%) and possibly unrealistic, and it was therefore recoded to 2.5/week, corresponding to the maximum option for those aged > 70. In addition, the answer options were slightly different in Tromsø 4 and in Tromsø 6 (Tromsø 4, < 70 years: never, < 1/month, 1/week, 2-3/week, 4-5/week or daily. Tromsø 4, >70 years old: never, < 1/month, 1/week or ≥2/week. Tromsø 6: 0-1/month, 1-3/week or 4-6/week). We recoded the option 4-6/week in Tromsø 6 to 2.5/week to adapt to the maximum option with the Tromsø 4 questionnaires. Frequencies reported as ranges (e.g, 1-3/week) were recoded to the mean value (e.g, 1.5/week).

We calculated an average n-3 PUFAs content in the different items as given in Institute of Marine Research, Seafood data (NIFES) and in the Norwegian Food Safety Authority (Matportalen) (230, 231). Accordingly, 100 grams of fat and lean fish contains 3.1 and 0.4 grams of n-3 PUFAs, respectively, fish as spread contains 0.9 grams per 25 grams, whereas 15 ml of fish oil supplements contains 3.6 grams of n-3 PUFAs. The total weekly intake of n-3 PUFAs was calculated as the sum of frequency multiplied by serving size. According to the Norwegian Directorate of Health, a standard serving unit of fish for dinner was defined as 200 grams, spread on bread as 25 grams and a spoon of fish oil as 15 ml. An overview of marine n-3 PUFAs in different dietary items is shown in Table 3.

Table 3 Dietary marine n-3 PUFAs in different food items

	n-3 PUFAs/100 g	Serving unit	n-3 PUFAs/serving unit
Lean fish for dinner	0.4	200 g	0.8
Fat fish for dinner	3.0	200 g	6.1
Fat fish as spread	3.7	25 g	0.9
Fish-oil supplement	0.24/ml	15 ml	3.6

Based on information from https://www.nifes.no and http://matportalen.no/

3.2.2 Assessment of major surgery as a VTE trigger in the case-crossover study

Trained personnel thoroughly reviewed the patients' medical records, and information on VTE triggers such as major surgery in the hazard and control periods was extracted using a standardized form. Major surgery was defined as procedures involving organs within the chest, abdomen, pelvic cavity, the cranium, and for knee and hip surgeries, or surgery with general anesthesia >30 minutes (232).

3.3 Outcome assessment

3.3.1 Venous thromboembolism

VTE events within the Tromsø Study cohort during follow-up were identified and recorded. The University Hospital in North Norway (UNN) is the only hospital serving the Tromsø municipality, and exclusively provides diagnostics, hospitalization and outpatient consultation for VTE patients. The following discharge codes were used to identify the VTE cases: For the period 1994-1998 relevant International Classification of Diseases (ICD) 9th codes for VTE diagnosis were 325, 415.1, 451, 452, 453, 671.3, 671.4, 671.9, and for the period 1999-2016, the ICD 10th revision codes I26, I80, I82, I67.6, O22.3, O22.5, O878.1 and O87.3. The computerized registry of autopsy diagnosis was also searched, and cases were registered if VTE was the cause of death (part one of the death certificate) or a significant condition (part two of the death certificate). In addition, the radiology procedure registry was searched to identify any cases accidently not given the relevant medical diagnosis code, but yet had an objectively confirmed VTE.

The medical record of all potential VTE cases was reviewed and validated by trained personnel with no knowledge of the baseline variables. The following four criteria were mandatory to register a VTE: i) A VTE had to be objectively confirmed by radiology procedures, i.e. compression ultrasonography, venography spiral computed tomography,

perfusion-ventilation scan or autopsy. ii) A diagnosis of VTE was indicated by a physician in the medical record. iii) Signs and symptoms consistent with the VTE diagnosis were present. iv) The VTE diagnosis had to require anticoagulant treatment (heparin, warfarin, thrombolysis) or vascular surgery, and if no treatment was given, contraindications had to be specified in the patient's medical record. For cases indicated by autopsy diagnosis, the VTE had to have caused, or significantly contributed to the death of the patient. All VTEs were classified as either PEs or DVTs. Only a minority of cases with PE are examined for a simultaneous DVT, because the additional diagnostics have none or minimal treatment consequences. Hence, isolated PEs could not be categorized separately. VTE cases were further classified as provoked or unprovoked based on the presence of provoking factors within 8 weeks ahead of the event. Provoking factors encompassed major surgery or trauma, acute medical conditions (acute MI, ischemic stroke or major infectious disease), active cancer and a high grade of immobilization (bed-rest for more than 3 days, wheelchair use or long distance travel over 4 hours within two weeks ahead of the VTE). Recurrent VTEs were identified and validated according to the same criteria in cases who had a previous VTE.

3.3.3 Date of cancer and death

Date of cancer diagnosis were obtained from the Cancer Registry of Norway, whereas date of death was collected from the Norwegian Population Registry.

4. MAIN RESULTS

4.1 Paper 1

DIETARY INTAKE OF MARINE n-3 POLYUNSATURATED FATTY ACIDS AND FUTURE RISK OF VENOUS THROMBOEMBOLISM.

Polyunsaturated fatty acids (n-3 PUFAs) have beneficial effects on key pathways in the pathogenesis of thrombosis. Previous studies on the relationship between dietary intake of fish- or n-3 PUFAs and the risk of venous thromboembolism (VTE) have shown inconsistent results, potentially due to challenges in assessing the total n-3 PUFAs intake and taking into account changes in the diet during follow-up. Our aim was to investigate the association between intake of n-3 PUFAs of marine origin and the risk of incident VTE in a population-based cohort. We included 21 970 individuals aged 25-97 years from the fourth (1994-95) and sixth (2007-08) surveys of the Tromsø study, and incident VTE events were recorded to December 31, 2016. Weekly intake of n-3 PUFAs was calculated from self-reported fish for dinner, fish oil supplements and from fat fish as bread spread. For participants attending both surveys, data were updated and they contributed with two observation periods. Total weekly intake of n-3 PUFAs in grams was divided in quartiles (Qrt1:<4.7, Qrt2:4.7-13.4, Qrt3:13.4-29.1, Qrt4:>29.1).

There were 451 incident VTE events during a median follow-up time of 11.6 years. Compared to Qrt1, subjects in Qrt2-4 had 22-26% lower risk of VTE after adjustment for age, sex and BMI (HR Qrt2 0.74, 95% CI 0.57-0.96; HR Qrt3 0.77, 95% CI 0.59-0.99; HR Qrt4 0.78, 95% CI 0.61-1.00). The association was more pronounced for provoked VTE, and provoked pulmonary embolism (PE), and the HRs for provoked PE were 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 Qrt2-4, respectively.

In conclusion, dietary intake of marine n-3 PUFAs exceeding 4.7 g/week was associated with a lower risk of VTE, particularly of provoked PE. The results suggest a threshold protective effect of n-3 PUFAs intake.

4.2 Paper 2

DIETARY INTAKE OF MARINE POLYUNSATURATED N-3 FATTY ACIDS AND RISK OF RECURRENT VENOUS THROMBOEMBOLISM

Venous thromboembolism (VTE) is associated with a significant risk of recurrence, particularly following an unprovoked or a cancer-related index event. However, the impact of marine n-3 PUFAs intake on the risk of recurrent VTE remains virtually unexplored. Our aim was to investigate the association between dietary intake of n-3 PUFAs and the risk of recurrence and all-cause mortality in VTE-patients derived from the general population. A total of 595 patients with incident VTE and complete data on dietary intake of n-3 PUFAs were derived from the 4th (1994-95) and the 6th (2007-08) surveys of the Tromsø Study, and categorized according to tertiles (T) of weekly intake in grams (T1: <8.2, T2: 8.2-29.1, T3: >29.1). Participants entered the study on the date of the incident event and were followed until recurrence, death, migration or the end of the study period (December 31, 2016).

During a median follow-up of 3.6 years, there were 98 recurrent VTEs. We found no overall association between intake of n-3 PUFAs and risk of recurrent VTE. However, high intake was associated with inverse associations after unprovoked VTEs (HR 0.45, 95% CI 0.20-1.01), and after DVT (HR 0.49, 95% CI: 0.24-0.97). The risk reduction was more pronounced in cancer free patients (HR 0.51, 95% CI: 0.27-0.95). There were 227 deaths during a median follow-up of 4.5 years. No association was observed between intake of n-3 PUFAs and the risk mortality after incident VTE.

In conclusion, we found that a high dietary intake of n-3 PUFAs was associated with lower risk of recurrent VTE following an unprovoked first event, DVT and in cancer-free patients. We found no association between intake of n-3 PUFAs and mortality after incident VTE

4.3 Paper 3

IMPACT OF DIETARY MARINE n-3 POLYUNSATURATED FATTY ACIDS ON SURGERY AS A TRIGGER FOR VENOUS THROMBOEMBOLISM – RESULTS FROM A CASE-CROSSOVER STUDY

Surgery is an important trigger of venous thromboembolism (VTE) and previous observational studies suggest that dietary intake of n-3 polyunsaturated fatty acids (n-3 PUFAs) lower the risk of incident VTE. However, it is unknown whether n-3 PUFAs intake influences the effect of surgery as a trigger for VTE, and the aim of the study was to investigate whether intake of n-3 PUFAs modifies the trigger effect of surgery.

We conducted a case-crossover study with 445 VTE patients recruited from the Tromsø Study, a population-based cohort. Self-reported intake of n-3 PUFAs was collected at baseline, and information on surgery and hospitalization was registered during the 90-day period prior to the VTE event (hazard period) and in four preceding 90-day control periods. Conditional logistic regression was used to estimate odd ratios (ORs) with 95% confidence intervals (CIs) of surgery as a VTE trigger, and these were compared across tertiles of n-3 PUFAs intake.

The overall OR of surgery was 7.6 (95% CI 5.2-11.1), and a higher intake of n-3 PUFAs mitigated the association. The ORs across tertiles of n-3 PUFAs intake were 11.8 (95% CI 6.1-22.6), 8.1 (95% CI 4.2-15.7) and 4.1 (95% CI 2.1-8.2). The effect was strongest for pulmonary embolism (PE), with ORs of 14.7 (95% CI 5.5-39.6), 7.0 (95% CI 2.1-23.5) and 2.8 (95% CI 1.1-7.4), respectively. In conclusion, a high intake of n-3 PUFAs strongly attenuated the effect of surgery as a trigger for VTE, and particularly for PE.

5. GENERAL DISCUSSION

5.1 Methodological consideration

5.1.1 Study design

Paper I and II have a prospective cohort study design, whereas Paper III is a case-crossover study, and all papers are based on data from the Tromsø Study, a large population-based cohort. Our results should be interpreted in the light of strengths and limitations of the study designs and the risk of bias.

In a cohort study, a population of interest is first defined. In Paper I, the study population represents the general population of Tromsø, Norway, whereas the study population in Paper II consists of VTE cases. A cohort study usually starts with a baseline visit where study variables are measured. Participants are then followed until a defined outcome occurs, or up to a defined end date of the study. Follow-up is censored, i.e. ends, when continuation becomes impractical or impossible, as with migration, withdrawal from the study or death. In general, the cohort design is well suited to estimate the incidence rate or absolute risk of a disease in the population, and relative risks (e.g., HRs) according to exposure status can readily be estimated (233). Cohort studies are considered well suited to investigate common diseases, such as VTE. Cohort studies are expensive to conduct because they typically enroll a large number of participants who are followed over a long time (234). A methodological strength of cohort studies is that the exposure is measured before the outcome of interest occurs, and a clear temporal sequence is a criterion for causal inference. Causal relationships has previously been established in cohort studies, and merits include the first recognitions of now well-known cardiovascular risk factors such as diet, exercise and hypertension (235) and the verification of a causal link between cigarette smoking and lung-cancer (236). Yet, causal inference is one of the main challenges in cohort studies, as further explained in section 5.4.1.

A case-crossover study design have similarities with a case-control study. A comparison between these two approaches is useful to explain the case-crossover design used in Paper III. In a case-control study, the level of a risk factor in cases is compared with that of healthy controls, and the relative risk of the outcome is usually expressed as an odds ratio (OR). A case-control design is cost-efficient and suited to study rare outcomes, because cases could be scarce even in large cohort studies leading to low statistical power. Even though controls typically are age- and sex -matched, a disadvantage in case-control studies is that cases and controls could still differ systematically and cause a bias (e.g. selection bias, as explained in the next section). Whereas the case-control design basically address the question "why me?", the case-crossover design rather addresses the question "why now?". Triggers such as hospitalization and surgery are transient exposures and the effect is confined to limited periods (hazard periods) (61). Such time windows can be used as control periods in the same individual, and this is utilized in the case-crossover study design. Since cases serve as their own controls, all persisting characteristics such as age, sex, genetic predispositions and socioeconomic status, are in theory controlled for through the design. The case-crossover design is well suited to study the effect of transient triggers (237). In the case-crossover study in Paper III, we recruited VTE cases derived from the Tromsø study, and investigated the effect of n-3 PUFAs intake on major surgery as a trigger for VTE. It is challenging to define the proper duration of the hazard period. Increased risk of VTE after major surgery tend to resolve within 3 months [61]. However, 3 months is an approximation of the hazard period and the effect of certain surgeries tend to resolve within 2 months. Our definition of a 90 days hazard period was probably long enough to account for most of the effect of major surgery. An inappropriate lengthy hazard period would tend to include triggers that had no true effect at the time of the VTE, which would cause underestimated risk estimates. A too short hazard period would fail to take into account all relevant trigger events. We included a 90 days

washout period, which presumably would prevent carry-over effects of trigger events from the control-periods to the hazard period. A drawback of the case-crossover design is that it does not yield absolute risks or incidence rates.

5.1.2 Errors and bias

Errors are inevitable in studies. Random errors, or sampling errors, could affect study results in any direction. Improved measurement accuracy and increasing sample sizes will minimize the effect of random errors and increase the precision of estimates (114). In contrast, **bias** refers to systematic errors causing study results to be different from true results in a specific direction (238). **Selection bias** refers to an unequal chance of being recruited into the study based on either the exposure or the outcome of interest. As a consequence, the study results may not by representative for the target population. In cohort studies, selection bias on the basis of outcomes is unlikely as participants are recruited to the study before the outcome occurs. However, cohort studies are vulnerable to **non-response bias**, referring to the fact that non-responders to study invitations tend to differ in some respects from those enrolled. In the Tromsø study, relatively more non-responders were males, or belonged to the oldest or youngest group. These characteristics of non-responders are reported from other large cohort studies as well, together with lower socio-economic status (239). It is likely to assume that those with the highest burdens of disease also are less likely to participate than those who are healthy or suffer from milder burdens. It follows that non-responders could differ in levels of exposure and risk of outcomes. Within our study, we measured different intakes of n-3 PUFAs across age groups, and since younger and elder had lower participation rates, a degree of non-response bias must be expected. Non-responder bias reduces how well a study represent the general population, and our study may be less representative for the youngest and oldest segments of the general population.

Information bias denotes the tendency to place study participants in an incorrect exposure or outcome category, which will lead to misclassification. **Differential misclassification** refers to an erroneous categorization of study participants that relates to the outcome. One example is **recall bias**, referring to differences in cases and controls due to a strong motivation in cases to search their mind thoroughly for any exposures that could have caused their diagnosis, a motivation not present in healthy controls (240). Another example is **reversed causation**, which implies that the outcome is a causal factor of the exposure, and not vice-versa. Differential misclassification causes biased study results in one or the other direction (238). Prospective cohort studies are principally less vulnerable to differential misclassification as the exposure is measured before the outcome occurs. For instance, in our studies it would be unlikely that those who got VTE during follow-up were more prone to over- or underestimate their marine food intakes at baseline. Cohort studies can influence outcome assessment if a baseline measurement causes increased surveillance for the outcome. The marine food intake as measured in the Tromsø surveys were unlikely to influence the likelihood of VTE diagnostics and cause surveillance bias, and in addition, those who registered the outcome were blinded to the exposure. **Non-differential misclassification** describes the situation where the probability to be erroneously categorized is unrelated to the outcome status. Non-differential misclassification generally leads to an underestimation of the association under investigation (238). Self-administered questionnaires can have an inherent degree of non-differential misclassification. In dietary questionnaires in general and in the Tromsø surveys, participants are often asked to approximate their typical food intakes per week during the last year within fixed ranges (e.g. 1-3 times per week, etc), because it cannot be expected that people are aware of their accurate intake of marine food over long time-spans. Moreover, there were slight differences in the questionnaires used the fourth and the sixth survey of the Tromsø Study, possibly causing non-differential misclassification. Still,

our assessment of marine food intake was comprehensive as fish oil supplements and fat fish as bread spread was included in both questionnaires. Self-reported intake of marine food is the strongest predictor of n-3 PUFAs levels (241-243), and in a subgroup of 1167 individuals, we demonstrated a significant dose-dependent increase of marine long chained n-3 PUFAs in the serum cholesterol ester fraction with higher self-reported intake of n-3 PUFAs ($p < 0.001$) (244). In addition, baseline triglyceride levels decreased with increasing self-reported n-3 PUFAs intake. It is therefore likely to assume that the calculated quartiles of n-3 PUFAs intake fairly well classified four distinct levels of exposures of marine n-3 PUFAs. However, the variance in EPA+DHA levels explained by self-reported intake is generally low, and in a Spanish cross-sectional study of 198 subjects at high CVD risk, 12% of the variance in EPA+DHA content in erythrocytes was explained by self-reported intake (241). Other contributing biological factors on EPA+DHA levels include BMI, smoking, heritability, and an inverse relationship with high intake of saturated fatty acids is also demonstrated (241, 245).

In Paper III we focused on surgery as a trigger for VTE. Triggers in general could also be subjected to degrees of non-differential misclassifications. Certain hospital-related triggers such as immobilization and major infections can build up gradually and are challenging to classify consistently in time and extent. In addition, we had no access on information about infections and immobilization outside the hospital setting. In contrast, hospitalizations and surgeries are well-defined in time with presumably few unregistered instances. In this respect, surgery was well suited to use in the case-crossover study design. Hospitalization is a major trigger for VTE that increases the risk of VTE up to a 100-fold (15, 154, 246). However, hospitalizations are heterogeneous and can involve serious disease with multiple interventions associated with high risk of VTE, or little intervention and risk. The effect of n-3 PUFAs would probably vary with different trigger situations. A low effect of n-3 PUFAs on a given

hospital related trigger would tend to weaken an association between n-3 PUFAs intake and hospitalization as a trigger, whereas a higher effect of n-3 PUFAs would tend to strengthen the association. Although our focus on major surgery represent a narrowly defined trigger that consistently causes damaged vessel walls, stasis and hypercoagulability, different surgeries are still associated with varying risk of postoperative VTEs. For instance, VTE occurs three times as often after orthopedic surgeries (1.2%) as compared to gastrointestinal surgeries (0.4%) (247).

Competing risk refer to another type of selection bias. An assumption of the Cox regression model is that censoring events and loss of follow-up are non-informative to the outcome, i.e., individuals who remain in the study should have the same risk of developing the outcome as those who are no longer under follow-up. Censoring due to high mortality of a disease is a competing event that could alter the probability that the other event occur. For instance, recurrent VTE is more common in cancer patients than in VTE patients without cancer. High mortality rates could cause substantial censoring and loss of follow-up time in cancer patients. A general effect of competing risk of death is a tendency to overestimate the risk of the disease of interest, e.g. as confirmed in VTE risk in patients with pancreatic cancer (142). In Paper II, we calculated relative risk of recurrent VTE taking competing risk of death into account, as there could be a different n-3 PUFAs intake among the censored. However, we observed no effect of competing risk by death on our results.

Analyzing recurring events (Paper II), introduces a form of selection bias called **index event bias**. Since patients are selected based on the occurrence of an index event (i.e. first VTE), multiple risk factors for the disease tend to accrue within this narrow study population. For instance, in studies of first VTE the estimated increased risk in a person with a prothrombotic genotype is compared with the general population without the genotype, and where the majority have a low burden of other VTE risk factors. This balance changes when

risk factors are compared within individuals who have suffered from a first VTE. When the risk of recurrence for the individual with a prothrombotic genotype is compared with other patients with a first VTE, this selected population carry a higher burden of VTE risk factors than the general population and the impact of the genotype is less apparent (248). The accruement of individuals with risk factors causes a conflict with the Cox regression model assumption that individuals and events are independent. Recurrence studies are in general at risk of introducing dependency between risk factors, causing recognized risk factor for the first event to appear neutral or even reversed for the second event. Index event bias is a likely explanation of the observation that FVL mutation imposes up to a 5-fold increased risk for a first VTE, and only a 1.2-fold increased risk for recurrence (60). In Paper II, we found a moderate negative association between high n-3 PUFAs intake and risk of recurrence after unprovoked VTE and in cancer free patients, and no association for all VTEs. However, HRs could be underestimated due to index event bias.

5.1.3 Modifiable risk factors and regression dilution bias

Modifiable risk factors represent another source of non-differential misclassification in cohort studies. This may introduce **regression dilution bias** with underestimation of the true association between the exposure and the outcome (249, 250). Intake of n-3 PUFAs could change over time and this was only partly taken into account in two of the previous cohort studies that applied an accumulated average of repeated measurements (218, 220). Possible regression dilution bias is a limitation of all cohort studies with single baseline measurements of modifiable exposures (250). For instance, it has been shown that the use of single baseline measurements of risk factors for cardiovascular disease significantly underestimated the true association (251). In Paper I, we used updated measurements rather than an accumulated

average of n-3 PUFAs intake in the analysis in an effort to minimize regression dilution bias. However, only 5892 out of the study population of 21970 participated in two surveys, and our efforts to minimize the effect of dietary changes were therefore suboptimal. Therefore, we also performed a sensitivity analysis where we restricted follow-up time to 5 years after baseline. The results of the sensitivity analysis showed stronger associations between intake of n-3 PUFAs and risk of VTE, indicating an underestimated effect of n-3 PUFAs in the main analysis due to regression dilution. In relevance to Paper II, the experience of having a first VTE event could have a modifying effect on n-3 PUFAs intake after baseline, because fish intake and n-3 PUFAs intake have been widely recommended for vascular health (182). This would add to the potential regression dilution bias in the study of n-3 PUFAs intake and VTE recurrence in Paper II, and our risk estimates may be underestimated.

5.1.4 Confounding

A weakness of cohort studies is that **confounders** cannot be ruled out through the study design. Confounders are variables that influence both the outcome and the exposure of interest, but are not in the causal path between the exposure and the outcome (233). Basically, confounding mixes up causal and non-causal relationships. Confounders result in either strengthening or weakening of an association. There are several methods to address confounders. In the Cox model, confounders can be adjusted for (i.e., included as covariates) in order to subtract their contribution to the association under investigation. Such multivariable adjustment models have the advantage of conserving statistical power. Confounders can also be controlled for by stratification, for instance by conducting separate analysis for the sexes or across age groups. However, stratification always imply loss of statistical power and multivariate models are therefore often preferred. Stratification is still an appropriate strategy to account for **statistical interaction**, for instance in models where a

covariate has a multiplicative effect on a second covariate (238). The consequence of interaction is that the risk estimate will vary across strata of this particular covariate. In such cases, stratified analysis according to the effect modifier would be appropriate.

In our main analysis in Paper I, the risk estimates were adjusted for age, sex and BMI. Age was probably the strongest confounder in the analysis because elderly eat more fish, and the risk of VTE is strongly dependent with age. We used age as time-scale to control for age as a confounder (252). The effect of age-adjustment was large, flipping n-3 PUFAs intake from seemingly to be a risk factor to being a protective factor (This was observed by running an analysis with calendar time and no age adjustment). Sex and BMI were also possible confounders in our analyses. Males may have relatively higher risk of VTE than women at higher age (117), and we observed slight differences between the sexes in n-3 PUFAs intake. Risk of VTE and intake of n-3 PUFAs also vary with BMI, but additional adjustment for sex and BMI had negligible effects. Age is not a strong predictor for recurrence, and in Paper II we included age as a covariate in the Cox model with time on study as the time-scale. In the analysis of mortality after VTE, we included systolic blood pressure and smoking as covariates by using a stepwise backward elimination method (Starting with all candidate variables and eliminate statistically non-significant variables stepwise each time the model is run). In the analysis of VTE recurrence and mortality, the effect of age and other possible confounders in the multivariate models were modest. Although multivariate Cox models can strengthen causal inference by adjusting for confounders, unknown or unaccounted confounding variables could still cause the observed association. Such **residual confounding** represent a crucial weakness in the inference of causality in cohort studies. If possible, supporting evidence should be provided to strengthen assumptions of causality in cohort studies. Bradford Hills 9 criteria for establishing epidemiologic evidence of causality is a practical tool for this purpose (253). Briefly, Hill postulated that the more a study can

demonstrate strength (effect-size), consistency (reproducibility), specificity, temporality (the cause must precede the effect), a dose-response relationship, plausibility (a plausible theory of the causation should be present), coherence (agreement between the cohort study and experiments), experimental validations and analogy to other circumstances, the more likely there is a true causal link.

Randomized controlled trials (RCT) and Mendelian randomization studies represent superior study designs to infer causality. In RCTs, individuals are randomly assigned to the exposure of interest, which thereby brake any underlying links to confounders (Section 5.1.4), even if they are unknown. However, RCTs have some practical limitations. Inclusion criteria in RCTs are often narrow and tend to result in a selection of relatively healthy individuals (233). The results in RCTs may therefore not represent the general population. Moreover, some interventions could cause unintended harmful effects, and ethical regulations are therefore strict compared to cohort studies (233, 254). Theoretically, it would be possible to conduct a RCT on n-3 PUFAs intake and risk of VTE. However, such a study would be practically demanding because a large number of individuals would need to be enrolled and to be followed for a long time to achieve sufficient statistical power.

Mendelian randomization studies are novel approaches that can control for confounders and thereby reveal causal pathways. Mendelian randomization studies exploit allele allocation in meiosis as a natural randomization procedure (238). The design is possible if a gene variant inflicts the exposure of interest, for instance increasing the likelihood for obesity (129). A possibility in the field of n-3 PUFAs could be genes that control the transformation of fatty acids, for instance the conversion of ALA to EPA (255). Meiotic allocation to an exposure is unbiased similar to the randomization in an RCT, though there are known exceptions to consider. Mendelian Randomization is not appropriate for low risk variants and in ethnic heterogeneous populations where the effect of the risk allele could vary

between groups. In meiosis, some alleles are inherited in linkage with other genetic variants, which could introduce a confounding effect (256).

5.1.5 Generalizability

The **generalizability**, or external validity of the study, refers to what degree results from a study applies to other populations (257). Internal validity refers to which extent the study measured the effect it intended to measure (258). The generalizability in cohort studies relies on the definition of inclusion and exclusion criteria, the participation rate and loss to follow-up. The participation rate in the Tromsø Study has been high, and was 77% and 66% in the 4th and 6th surveys respectively. Men and women aged 25-97 years old were enrolled, and the distribution of risk factors in the Tromsø Study is comparable with other western populations (9, 10). This indicates that the Tromsø cohort represents the general population well. However, participation rates were lower among the younger (<40 years) and the older (>80 years), and in both surveys, the participation rate was lower among men than women (227). These traits are typical for cohort studies and reduces the generalizability in the groups with lower attendance (239). An advantage of cohort studies is that participants are selected before the outcome occurs. Therefore, errors would affect individuals with high or low intake of n-3 PUFAs equally and probably have a low influence on the generalizability of the study results.

5.1.6 Missing data

Missing data are expected in large epidemiological studies (259). Missing data can be particularly common in self-administered questionnaires as unassisted respondents frequently leave some items unanswered (260). This was evident in our studies as well. In Paper I, 34% had one or more missing item in the questionnaires on marine food, and the total intake of n-3 PUFAs could not be calculated. In contrast, a baseline visit examination such as systolic

blood pressure in Tromsø 4 and 6 was missing in <0.003% of the participants. Reasons for high rates of missing data in self-administered questionnaires could be diverse. For instance, unclear language and difficult questionnaire designs, or lack of motivation to answer the full questionnaire could contribute. Missing data causes loss of statistical power, and can cause biased estimates similar to non-response bias. There are several types of missing data. Data missed completely at random (MCAR) are unrelated to the study variables. In such cases, analyses based on complete cases would be unbiased. Data missing at random (MAR) signifies that the missing data mechanism is unrelated to the missing variables but may be related to other observed values. Data are missed not at random (MNAR) when the missing data mechanism is related to the missing values (260). The most common and simplest method to deal with missing data is to do a complete case analysis, in which all individuals with missing data are excluded. Computational techniques such as multiple imputation can be applied to assign probable values to replace missing data, and have been shown to represent actual data well, which thus improves statistical power and risk estimate precision. However, use of imputation depends on the missing mechanism and are not guaranteed to adjust for the presumed bias (259). Imputation can be considered in MAR and MCAR situations. In our study population, the participants with missing n-3 PUFAs data were older (mean age was 53 years vs. 46 years), more likely to be current smokers (37.3% vs. 32.9%) having a history of CVD (11.6% vs. 5.45%) and cancer (5% vs. 2.7%). More people had higher education in the group with complete data (36.4% vs. 19.9%). In all, the group with missing n-3 PUFAs data were somewhat older and had more comorbidities, which most likely explains a higher incidence rate of VTE (2.9 vs. 1.6 per 1000 per year). The missing data mechanism was not completely unrelated to the study variables, though in complex relationship to other variables. In addition, our data on total n-3 PUFAs intake represented the sum of several variables with widely different contributions of n-3 PUFAs from lean fish, fat fish, fat fish as spread and fish

oil supplement. Imputation based on an assumption of MAR in our study would be ambiguous, and we chose to conduct complete-case analysis. Consequences are similar as in non-response bias, which can influence the generalizability of the study results. Our study population was probably more homogenous than the source population due to missing data. Still, the study's internal validity addresses the principal association between intake of n-3 PUFAs and the risk of VTE. Actually, homogeneity in the study population is often preferred in RCTs and in animal experiments to reduce random sampling errors and confounders, ultimately to isolate the causal effect from noise (261).

5.2 Discussion of the main results

5.2.1 Dietary intake of n-3 PUFAs and risk of incident VTE

In Paper I, we found an inverse association between self-reported intake of marine n-3 PUFAs and the risk of incident VTE in a large population-based cohort using repeated measurement and a time varying Cox model. Our results suggested that n-3 PUFAs intake exceeding the lowest quartile (4.7 grams per week) was associated with 22-26% lower risk of VTE, and there was no further reduced risk with a higher intake. The association was mainly driven by provoked events, and particularly PEs. We also investigated the effect of fish intake irrespective of n-3 PUFAs content, and found no association with overall risk of VTE.

Results from previous studies have shown conflicting results (218-222, 262, 263). In the ARIC study, a 30-45% lower VTE risk was observed for n-3 PUFAs intake exceeding 0.7 g/week, and a similar effect was observed for fish intake exceeding one serving per week (218). In the DCH Study, 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% lower risk of unprovoked VTE (221). The NHS reported no association between the intake of fish or n-3 PUFAs and VTE risk (220). Finally, the IWHS study reported that ≥2.5 servings of fish per week at baseline was associated with a 22% increased risk of VTE compared to <0.5 serving per week (219). Factors that could explain some of the variations in the reported results include different definitions of the exposure variable and handling of potential changes in diet during follow-up. Intake of n-3 PUFAs or fish intake was assessed at baseline only (219, 221, 222), or the cumulative average of two or more measurements were used (218, 220). Dietary behavior could change during the course of follow-up causing non-differential misclassification, and the reported relative risks could therefore be underestimated due to regression dilution (264, 265). In Paper I, we addressed changes in dietary habits by updating the self-reported intake for those participating in both surveys. The time-varying model was

somewhat suboptimal as only 27% participated in two surveys. However, we also conducted analyzes with follow-up time restricted to maximum 5 years, which reduced the average time between n-3 PUFAs measurement and VTE event. These analysis yielded stronger effect sizes and suggests that the HRs in the main analysis were underestimated due to regression dilution bias.

An intuitive alternative explanation of our results is that high intake of fish merely substitutes otherwise unhealthy meals, which in turn could cause the observed reduced risk of VTE according to n-3 PUFAs intake. In our study population, consumption of lean fish was more common than fat fish (>70% reported to have lean fish for dinner once or more per week), and lean fish have low concentrations of n-3 PUFAs compared to fat fish (230, 231, 266). The n-3 PUFAs intake could therefore vary substantially with the same amount of dietary intake of fish. Our analysis of total fish intake irrespective of n-3 PUFAs concentration (fish oil supplements were not included in this analysis) therefore largely represented lean fish intake. Accordingly, we found no association between total fish intake and risk of VTE. This observation is in accordance with the previous cohort studies that reported separate analyses for intake of lean and fatty fish (i.e. low and high n- 3 PUFAs content). Generally, they found largest effect sizes between intake of fatty fish and fish oil supplements and risk of VTE, and lower effect sizes in analyses on lean fish intake (221, 222). This suggests that the total content of n-3 PUFAs in the diet, rather than fish intake were key components that modulated the risk of VTE.

The inverse association between intake of n-3 PUFAs and VTE was stronger in relation to provoked events and PEs. This propensity corresponds with the observation of a temporal decline in postsurgical VTEs during World War II, a period characterized with high intake of marine food and low intake of saturated fat (216, 217). More recently, a Japanese study reported an inverse association between fresh fish consumption or n-3 PUFAs intake

and risk of fatal PE (DVTs were not studied) in a cohort of 90 791 individuals with a median follow-up time of 19.2 years (267). For fresh fish intake exceeding >1 time per month up to daily, a 64-83% reduced risk of fatal PE was reported. Moreover, total n-3 PUFAs intake (including plant derived ALA) exceeding the lower 10^{th} percentile (<0.8 grams per day) was associated with 64-74% reduced risk. As in our study, no association was observed when a variable of fish low in n-3 PUFAs content was used as exposure variable.

The larger effect of n-3 PUFAs intake on PEs was not shown before we conducted our study. It could be speculated that n-3 PUFAs have an effect on the clot structure that reduces the risk of embolization of clots in the deep veins. Recently, platelet expression of cell adhesion molecules (E-cadherins) was shown to strongly influence platelet aggregation, clot stability and clot retraction in a murine model (268). As n-3 PUFAs have a downregulating effect on platelet activation, an effect on clot stability may also be possible. Most PEs probably originate in the lower extremities, however, de novo formation in the lungs or embolus of cardiac origins may possibly account for a substantial proportion of cases (269). It is therefore another possibility that the effect of n-3 PUFAs on provoked VTE, particularly PE, could be explained by a lower prevalence of VTE-related disease (e.g. atrial fibrillation) in those with a high intake (270, 271).

Our observation of a modest protective effect of dietary intake of marine n-3 PUFAs on the risk of incident VTE is consistent with findings in other cohort studies (218, 221, 222). The measurement of dietary n-3 PUFAs intake was likely subjected to some degree of non-differential misclassification at baseline and through dietary changes over the course of follow-up, which are likely to cause underestimated risk estimates. The observation that the risk was confined to provoked events and PEs was also in accordance with the scarce literature on the topic (216, 217, 267). The results should be interpreted with caution as residual confounders cannot be ruled out in a prospective cohort study design (114). In

conflict with our findings, a recent large Mendelian randomization study reported a 13% increased risk of VTE for genetically increased EPA levels. The study investigated single nucleotide polymorphisms (SNPs) in the gene that encodes Δ5-desaturase, an enzyme involved in the conversion of ALA to EPA (255). However, methodological limitations in this study included pleiotropic effects of the investigated SNPs, and that Δ5-desaturase functionality is also associated with the levels of other fatty acids. For instance, Δ5-desaturase also controls the conversion of LA to the pro-inflammatory arachidonic acid (ARA), and in a small Japanese case-control study, VTE patients were found to have higher ARA levels and lower EPA/ARA ratios than controls without VTE (224).

We found that a low intake of dietary marine n-3 PUFAs was associated with increased risk of future provoked VTEs or PEs, which suggest a beneficial effect with a relatively modest increased intake. Future studies should focus on whether the observed association is causal, as there is an inherent risk of residual confounders in cohort studies.

5.2.2 Dietary intake of n-3 PUFAs and risk of VTE recurrence

In Paper II, we found that a high intake of n-3 PUFAs (upper tertile, >29.1 g/week) was associated with a lower risk of recurrent VTE compared with a low intake (lower tertile, <8.2 g/week) in patients with unprovoked incident VTE events and in those with DVT. There was no association between n-3 PUFAs intake and risk of mortality after incident VTE. Prior to our report, there was limited data on the association between marine n-3 PUFAs intake and the risk of recurrence and mortality in patients with incident VTE. In a study of elderly (SWITCO65+), high levels of n-3 PUFAs in erythrocyte membranes was associated with a 61 to 83% lower risk of recurrence and a 66 to 71% lower risk of mortality after 6 months of follow-up (225). After 3 years, a 45% lower risk of mortality was reported. The observed reduced risk of recurrence appeared to be transient, as no association was reported after 3

years. This contrast our findings where we observed the effect on recurrence only after 2 years and no association with mortality. However, there were differences in the study populations and study design that could partly explain the differences in the study results.

The n-3 PUFAs intake in our study population was high and was calculated only from marine foods, whereas the n-3 PUFAs erythrocyte level variable in the SWITCO65+ study was not restricted to marine origin. Thus, the influence of plant derived ALA (α-linolenic acid) in the SWITCO65+ study may have led to different findings in the two studies (272-274). The patients included in SWITCO65+ were also older than those in our study (75 vs. 67 years), and whereas 29% of the patients had a history of previous VTE in the SWITCO65+ study, our study population was limited to those with a first lifetime VTE. In our study, we only observed a beneficial association of n-3 PUFAs on recurrence risk in those with a high intake, which indicates only a moderate beneficial effect of n-3 PUFAs on the recurrence risk. A moderate effect is in accordance with the observation that the association was mainly confined to those with unprovoked index events. The risk for a recurrent VTE after an unprovoked event is moderate. In comparison, the risk of recurrence after a VTE provoked by a transient risk factor is low, whereas the risk of recurrence is high in patients with persistent risk factors (61, 113). Hence, a moderate effect of n-3 PUFAs could be difficult to detect in those at low risk, and conversely, a moderate effect could be overwhelmed in those at high risk. In a sensitivity analysis, we excluded patients with active cancer, who are at high risk of recurrence. In cancer free patients we observed a beneficial effect of high n-3 PUFAs intake, supporting the notion that the effects of n-3 PUFAs may have been overwhelmed in individuals with high recurrence risk.

Apparently conflicting, we observed that high intake of n-3 PUFAs was associated with an increased risk of recurrence after a PE index event. PE typically recurs as a new PE which is a more serious condition than DVT (50). However, the current guidelines for

antithrombotic therapy recommend the same duration of anticoagulant treatment for PE and DVT under most circumstances (63). In our study, most patients with PE were under anticoagulant treatment for a longer time period than those with DVT. Specifically, in our data, representing treatment of VTE patients from 1994 to 2016, we found that 45% of patients with PE were scheduled with anticoagulant treatment for 1 year, which applied to only 13% of patients with DVT. This could partly explain the apparent lack of effect of n-3 PUFAs on recurrence after PEs in our study. The risk of recurrent VTE is highest within the first year (48, 50), and in our study 53% of the recurrences occurred during the first year. It is therefore possible that patients with an index PE and under anticoagulant treatment the first year achieved no additional beneficial protective effect of n-3 PUFAs intake on top of the anticoagulant treatment. Conversely, DVT patients with shorter duration of the anticoagulant treatment seemingly had an effect of a high intake of n-3 PUFAs. In a separate analysis, we therefore restricted the follow-up time to the period after completion of anticoagulant treatment. The results consistently showed inverse associations between high n-3 PUFAs intake and recurrence after overall VTE and subtypes of VTE (unprovoked, provoked, DVT, and PE), indicating that anticoagulant treatment overwhelm any effect of n-3 PUFAs.

In contrast to the results of the SWITCO 65+ study (225), we did not observe an association between n-3 PUFAs and risk of mortality in patients with VTE. Observational studies on the effects of fish- or n-3 PUFAs intake on mortality are conflicting (275-278). Possibly, the long chained marine n-3 PUFAs are associated with a lower risk of CVD related death, while ALA is associated with a lower risk of death from other causes (273, 279). Of relevance, the most frequently reported causes of death in VTE patients are cancer, PE, infections, and other cardiovascular or respiratory diseases (9, 280, 281). Consequently, a high proportion of deaths due to non-CVD causes in VTE patients could explain the lack of a beneficial association between marine n-3 PUFAs and all-cause mortality in our study.

Unfortunately, we did not have the possibility to assess distinct causes of death. However, the results did not change after exclusion of patients with active cancer.

We observed a reduced risk of recurrent VTE with high intake of n-3 PUFAs mainly in cancer-free patients and in patients after discontinuation of anticoagulant treatment. As the risk of recurrence is relatively high, a protective effect of dietary marine n-3 PUFAs would be valuable knowledge for VTE patients, though future studies are necessary to confirm whether this relationship is causal.

5.2.3 Modification of the effect of surgery as a trigger for VTE by intake of n-3 PUFAs

In Paper III, we conducted a case-crossover study and the results indicated that a high intake of n-3 PUFAs strongly attenuated the effect of surgery as a trigger of VTE. The ORs for surgery across tertiles of higher n-3 PUFAs intake displayed an inverse relationship, where the ORs were 11.8 (95% CI 6.1-22.6) for tertile 1, 8.1 (95% CI 4.2-15.7) for tertile 2 and 4.1 (95% CI 2.1-8.2) for tertile 3. Subgroup analyses further showed that this effect across n-3 PUFAs intake was largest in PE, rather than DVT. For PE, the ORs according to surgery were 14.7 (95% CI 5.5-39.6) for tertile 1, 7.0 (95% CI 2.1-23.5) for tertile 2 and 2.8 (95% CI 1.1-7.4) for tertile 3.

Previous investigations on surgery as a trigger for VTE and the relationship with n-3 PUFAs intake are scarce. During World War II, data from two hospitals in Norway showed that the incidence rates of VTEs after surgery decreased from approximately 40 per 1000 before the war, to <10 per 1000 during the war (216). Three years after the war, the incidence rate had risen to 20-30 per 1000. This U-shaped temporal decline correlated with a temporal increase in fish and fish oil intake during the war (217), and could indicate a beneficial effect

of marine n-3 PUFAs. Moreover, in Paper I, we observed an apparent stronger protective effect of n-3 PUFAs on the risk of provoked VTEs compared to unprovoked VTEs.

Prothrombotic trigging effects of major surgery include vessel wall injury, initiation of coagulation and altered hemodynamics, which basically affect all three categories in Vichow's triad (endothelial injury, stasis of blood flow, hypercoagulability) (282). The risk of VTE after major surgery is transient and peaks within three weeks after the procedure (62). Measured pro-thrombotic responses after major surgery include increased inflammatory mediators (144), coagulation factors (FVII, FV, FVIII and fibrinogen) and monocyte activation (283), platelet activation (284) and NETs formation, which are all associated with an increased risk of VTE (80). Crucially, several of the factors that trigger VTE after surgery are both transient and modifiable, and a lowered VTE rate is therefore anticipated with lowered pro-thrombotic responses. According to the thrombosis potential model, VTE only develops if the thrombosis potential, due to combined impact from risk factors, exceeds the threshold for VTE (113). Interestingly, a beneficial effect of marine n-3 PUFAs on pro-thrombotic factors that are associated with surgery, have been reported in ex vivo experiments. Specifically, TF expression in endothelial cells is downregulated with n-3 PUFAs in vitro (212), and the potential to activate monocytes and platelets was reduced experimentally ex vivo (186, 213). Moreover, a 40% reduction of the synthesis of TF and a 10-20% reduction in serum arachidonic acid levels was shown in a trial of participants who were given 25 ml cod liver oil per day for 8 weeks. In this study, the authors concluded that activation of extrinsic pathways of coagulation was suppressed by dietary enrichment of n-3 PUFAs (213). No experimental studies have investigated the effect of n-3 PUFAs on the risk of surgery-related VTE. However, a placebo-controlled trial of 567 patients with newly created arteriovenous fistulas (AVF) investigated whether the AVF patency could improve with fish oil intake. Those randomized to fish oil (4 grams/d in three months) had reduced

intervention rates for acute thrombosis (285), whereas there were no beneficial effect on other endpoints such as AVF usability, or time to AVF patency loss (285, 286). Comparable results were reported in a RCT that involved synthetic arteriovenous grafts (287). However, these interventions may be questionable analogues for major surgeries in the general population, and the studies were also naturally limited to patients with severe renal failure.

The risk reduction of surgery-related VTE by n-3 PUFAs was strongest for surgery-related PE. In general PEs are considered to be a complication of DVT, and it may be speculated that a high n-3 PUFAs intake alters the clot structure with more stable clots that are less likely to embolize. Alternatively, many PEs could also have a cardiac origin or develop de novo in the lungs (269). Thus, a lower rate of PE-related post-surgical complications (e.g., atrial fibrillation) could be observed in those with a high n-3 PUFAs intake (270, 288).

The case-crossover design is robust against confounding from persisting patient characteristics since cases serve as their own controls. However, the average age varied across the n-3 PUFAs tertiles, and the impact of surgery as a trigger potentially varies across age groups. Yet, the beneficial impact of high n-3 PUFAs intake on surgery as a VTE trigger remained present in sensitivity analyses where we used two different approaches to control for age as a potential confounder. Finally, confounding by factors that change over time within individuals remains possible. Although the n-3 PUFAs intake was assessed prospectively, the triggers for VTE were documented with a prior knowledge that a subsequent VTE would occur. This is a retrospective approach that could influence the decision to document certain triggers and introduce a bias. For instance, minor surgeries with negligible trigger effects could be included erroneously. Future interventional studies should investigate whether there is a true causal relationship between n-3 PUFAs intake and risk of post-surgical VTE, as suggested in our study.

6. CONCLUSIONS

1) We found that weekly intake of marine n-3 PUFAs above 4.7 grams per week (a threshold effect) was associated with a 22-26% lower risk of VTE. We observed a stronger association for PE than DVT, and particularly for provoked PE (39-60% reduced risk).

2) Overall, we observed a moderate effect of dietary intake of n-3 PUFAs on the risk of recurrent VTE and no association with all-cause mortality. However, sub analysis showed a 55 % lower risk of recurrent VTE after unprovoked index events and a 49% reduced risk in cancer-free patients. These findings suggest that VTE patients under intermediate risk of recurrence may benefit from a high n-3 PUFAs intake.

3) We observed a beneficial effect of n-3 PUFAs intake on major surgery as a trigger for VTE. Odds ratio for surgery decreased with high intake of n-3 PUFAs in the hazard compared to the control periods (OR 11.8 with low intake, 8.1 with medium intake, and 4.1 with high intake).

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PAPER I

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ORIGINAL ARTICLE

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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

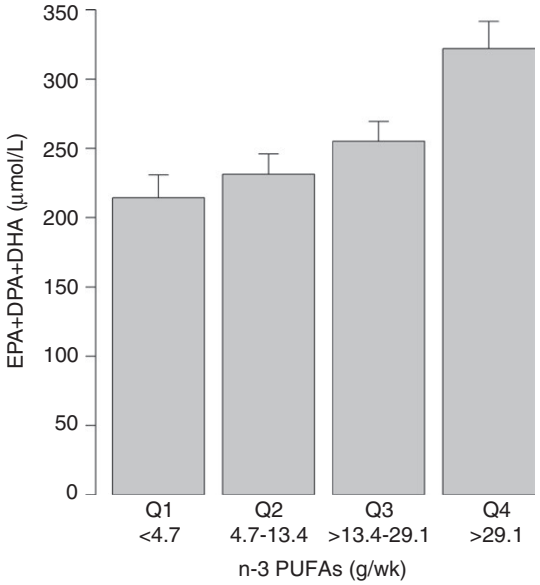

FIGURE 1 Serum n-3 PUFA concentration across quartiles of self-reported weekly intake of marine n-3 PUFAs in the Tromsø Study (1994). Values are means with 95% CI. CI, confidence interval; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; PUFAs, polyunsaturated fatty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

and DHA) in the cholesterol-ester fraction was measured in a subgroup 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 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.

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).

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

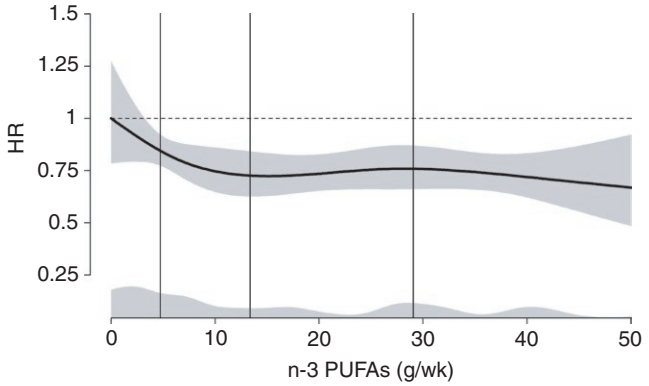

FIGURE 2 Dose-response relationship between weekly intake of marine n-3 PUFAs (modelled as a restricted cubic spline with four knots) and the risk of VTE. The regression model is adjusted for age (as time scale), sex, and BMI. The solid line represents the HR and the shaded area shows the 95% CI. The 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

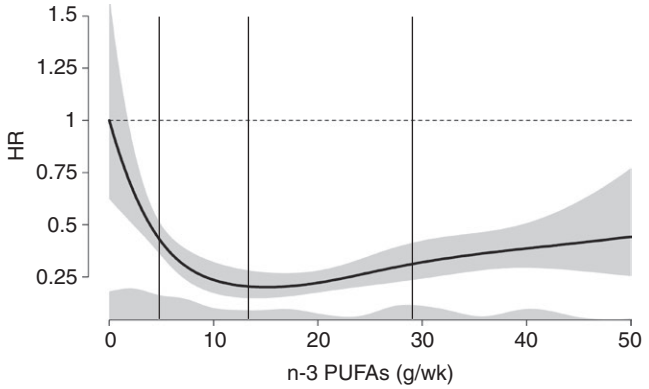

FIGURE 3 Dose-response relationship between weekly intake of marine n-3 PUFAs (modelled as a restricted cubic spline with four knots) and the risk of provoked PE. The regression model is adjusted for age (as time scale), sex and BMI. The solid line represents the HR and the shaded area shows the 95% 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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SUPPORTING INFORMATION

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

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Estimated weight and content of n-3 PUFAs in normal serving units of marine food items obtained from official web resources (1-3)

Food item	**n-3 PUFAs**	**Serving unit**	**n-3 PUFAs per serving unit**
Lean fish	0.43 g/100 g	200 g	0.8 g
Fat fish	3.03 g/100 g	200 g	6.1 g
Fat fish spread	3.70 g/100 g	25 g	0.9 g
Cod liver oil/ supplements	0.24 g/ml	15 ml	3.6 g

PUFA polyunsaturated fatty acid

Supplemental Table 2. Characteristics according to observational periods across quartiles of fish intake in the Tromsø Study*

	Q1 (n=7473)	**Q2 (n=6609)**	**Q3 (n=7352)**	**Q4 (n=6428)**
Quartile range, g/week	≤250	251-503	504-706	≥707
Age, years	41 (12)	46 (14)	51 (14)	56 (13)
Sex, male	45.6 (3405)	47.4 (3133)	50.6 (3717)	51.7 (3322)
BMI, kg/m^2	25.2 (4.0)	25.3 (3.8)	25.8 (4.0)	26.5 (4.1)
Serum total cholesterol, mmol/L	5.60 (1.16)	5.80 (1.20)	5.97 (1.27)	5.86 (1.21)
Serum HDL cholesterol, mmol/L	1.46 (0.39)	1.50 (0.41)	1.52 (0.42)	1.52 (0.43)
Serum triglycerides, mmol/L	1.52 (1.00)	1.50 (0.98)	1.52 (0.99)	1.53 (0.95)
Systolic blood pressure, mmHg	129 (17)	132 (19)	136 (21)	137 (23)
Diastolic blood pressure, mmHg	75 (11)	77 (11)	78 (12)	79 (12)
Current smoking	34.3 (2554)	31.5 (2075)	28.2 (2065)	25.3 (1613)
Diabetes	1.6 (119)	2.0 (134)	2.6 (190)	3.9 (245)
History of CVD	3.1 (232)	5.1 (334)	7.8 (568)	10.3 (656)
History of cancer	1.9 (142)	2.8 (186)	3.9 (290)	5.3 (343)
Higher education†	39.5 (2948)	37.7 (2486)	33.2 (2424)	38.1 (2434)

CVD cardiovascular disease (angina pectoris, stroke, myocardial infarction), *Q* quartile.

*Based on 27862 observational periods from 21970 individuals in the period between 1994 and 2016. Values are mean (SD) or percentage (count).

†≥15 years of education (corresponding to 3 years in university or academy).

Supplemental Table 3. Characteristics of VTE events in the Tromsø Study across quartiles of weekly intake of marine n-3 PUFAs*

	VTEs in Q1 (n=120)	**VTEs in Q2 (n=120)**	**VTEs in Q3 (n=129)**	**VTEs in Q4 (n=172)**
Characteristics				
Age at incident VTE, years	60 (13)	67 (14)	68 (13)	71 (11)
Male	54.2 (65)	52.7 (62)	49.6 (64)	51.2 (88)
Clinical presentation				
DVT	50.0 (60)	61.7 (74)	63.6 (82)	52.3 (90)
PE	50.0 (60)	38.3 (46)	36.4 (47)	47.7 (82)
Provoked VTE	61.7 (74)	59.2 (71)	53.9 (69)	57.6 (99)
Clinical risk factors				
Pregnancy/puerperium	0.8 (1)	0.8 (1)	-	0.6 (1)
Heredity†	4.2 (5)	4.2 (5)	3.1 (4)	4.7 (8)
Other medical conditions‡	15.5 (15)	17.4 (19)	24.5 (23)	20.0 (25)
Provoking factors§				
Surgery	22.5 (27)	19.2 (23)	13.3 (17)	11.6 (20)
Trauma	13.3 (16)	8.3 (10)	8.6 (11)	11.1 (19)
Acute medical conditions	10.8 (13)	15.8 (19)	7.8 (10)	11.1 (19)
Cancer¶	23.3 (28)	21.7 (26)	21.1 (27)	29.7 (51)
Immobilization**	20.8 (25)	20.0 (/24)	12.4 (16)	15.1 (26)
Other††	2.5 (3)	5.0 (6)	5.5 (7)	5.2 (9)

DVT deep vein thrombosis, *PE* pulmonary embolism, Q quartile, *VTE* venous thromboembolism

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

†VTE reported in a first-degree relative before the age of 60 years.

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

§One patient may have multiple provoking factors.

¶Cancer disease present at the time of VTE diagnosis.

**Bed rest ≥3 days, wheelchair user, plaster cast, air travel ≥4 h or long automobile travel < 14 days prior to VTE.

††Other factors specified as provoking in the medical record (e.g., intravascular catheters).

Supplemental 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, with follow-up limited to maximum **five** years*

n-3 PUFA intake	Person-years	VTE events	Crude IR (95% CI) †	HR (95% CI) ‡
Total VTE				
Q1§	38176	35	0.92 (0.66-1.28)	1.00
Q2-4¶	144441	154	1.07 (0.91-1.25)	0.50 (0.34-0.73)
Unprovoked VTE				
Q1§	38176	10	0.26 (0.14-0.49)	1.00
Q2-4¶	144441	59	0.41 (0.32-0.53)	0.74 (0.36-1.50)
Provoked VTE				
Q1§	38176	25	0.65 (0.44-0.97)	1.00
Q2-4¶	144441	95	0.66 (0.54-0.80)	0.41 (0.26-0.65)
Total DVT				
Q1§	38176	20	0.52 (0.34-0.81)	1.00
Q2-4¶	144441	84	0.58 (0.47-0.72)	0.50 (0.30-0.83)
Unprovoked DVT				
Q1§	38176	4	0.10 (0.04-0.28)	1.00
Q2-4¶	144441	26	0.18 (0.12-0.26)	1.00 (0.33-3.06)
Provoked DVT				
Q1§	38176	16	0.42 (0.26-0.68)	1.00
Q2-4¶	144441	58	0.40 (0.31-0.52)	0.39 (0.22-0.70)
Total PE				
Q1§	38176	15	0.39 (0.24-0.65)	1.00
Q2-4¶	144441	70	0.48 (0.38-0.61)	0.50 (0.28-0.89)
Unprovoked PE				
Q1§	38176	6	0.16 (0.07-0.35)	1.00
Q2-4¶	144441	33	0.23 (0.16-0.32)	0.59 (0.24-1.45)
Provoked PE				
Q1§	38176	9	0.24 (0.12-0.45)	1.00
Q2-4¶	144441	37	0.26 (0.19-0.35)	0.44 (0.21-0.94)

DVT deep vein thrombosis, *IR* incidence rate, *PE* pulmonary embolism, *VTE* venous thromboembolism

*Based on data from the Tromsø Study in the period 1994-2016, analyzed by Cox proportional hazards regression models.

†Per 1000 person-years.

‡Adjusted for age (as timescale), sex and BMI.

§<4.7 g/week.

¶≥4.7 g/week.

Supplemental Table 5. IRs and HRs with 95% CIs for VTE, PE and DVT, overall and stratified by the presence of provoking factors, across quartiles of total weekly fish intake in the Tromsø Study*

Fish intake (g/week)	Person years	VTE events	Crude IR (95% CI) †	HR model 1 (95% CI) ‡	p for trend	HR model 2 (95% CI) §	p for trend
Total VTE							
Q 1 ≤250	97504	96	0.98 (0.81-1.20)	1.00	0.63	1.00	0.92
Q 2 251-503	86255	111	1.29 (1.07-1.55)	0.93 (0.71-1.23)		0.93 (0.71-1.23)	
Q 3 >503-706	90127	173	1.92 (1.65-2.23)	1.00 (0.77-1.29)		0.98 (0.75-1.27)	
Q 4 >706	64602	161	2.49 (2.14-2.91)	1.03 (0.79-1.35)		0.99 (0.76-1.29)	
Unprovoked VTE							
Q 1 ≤250	97504	41	0.42 (0.31-0.57)	1.00	0.76	1.00	0.60
Q 2 251-503	86255	50	0.58 (0.44-0.76)	0.99 (0.65-1.50)		0.99 (0.65-1.51)	
Q 3 >503-706	90127	74	0.82 (0.65-1.03)	1.00 (0.67-1.49)		0.99 (0.66-1.47)	
Q 4 >706	64602	62	0.96 (0.75-1.23)	0.94 (0.62-1.42)		0.90 (0.59-1.36)	
Provoked VTE							
Q 1 ≤250	97504	55	0.56 (0.43-0.73)	1.00	0.40	1.00	0.60
Q 2 251-503	86255	61	0.71 (0.55-0.91)	0.89 (0.62-1.29)		0.89 (0.62-1.29)	
Q 3 >503-706	90127	99	1.10 (0.90-1.34)	1.00 (0.71-1.40)		0.97 (0.69-1.37)	
Q 4 >706	64602	98	1.52 (1.24-1.85)	1.10 (0.77-1.56)		1.05 (0.74-1.48)	
Total DVT							
Q 1 ≤250	97504	54	0.55 (0.42-0.72)	1.00	0.48	1.00	0.60
Q 2 251-503	86255	60	0.70 (0.54-0.90)	0.92 (0.64-1.34)		0.93 (0.64-1.34)	
Q 3 >503-706	90127	105	1.17 (0.96-1.41)	1.14 (0.81-1.60)		1.12 (0.80-1.58)	
Q 4 >706	64602	87	1.35 (1.09-1.66)	1.06 (0.74-1.52)		1.04 (0.72-1.48)	
Unprovoked DVT							
Q 1 ≤250	97504	25	0.26 (0.17-0.38)	1.00	0.96	1.00	0.82
Q 2 251-503	86255	20	0.23 (0.15-0.36)	0.66 (0.36-1.19)		0.66 (0.36-1.19)	
Q 3 >503-706	90127	44	0.49 (0.36-0.66)	1.00 (0.60-1.67)		0.98 (0.59-1.64)	
Q 4 >706	64602	33	0.51 (0.36-0.72)	0.84 (0.49-1.46)		0.81 (0.47-1.40)	
Provoked DVT							
Q 1 ≤250	97504	29	0.30 (0.21-0.43)	1.00	0.38	1.00	0.42
Q 2 251-503	86255	40	0.46 (0.34-0.63)	1.16 (0.71-1.87)		1.16 (0.72-1.88)	
Q 3 >503-706	90127	61	0.68 (0.53-0.87)	1.25 (0.79-1.98)		1.25 (0.79-1.97)	
Q 4 >706	64602	53	0.82 (0.63-1.07)	1.24 (0.77-1.99)		1.22 (0.76-1.96)	
Total PE							
Q 1 ≤250	97504	42	0.43 (0.32-0.58)	1.00	0.95	1.00	0.65
Q 2 251-503	86255	51	0.59 (0.45-0.78)	0.94 (0.62-1.42)		0.94 (0.62-1.42)	
Q 3 >503-706	90127	68	0.75 (0.59-0.96)	0.83 (0.56-1.24)		0.80 (0.54-1.20)	
Q 4 >706	64602	74	1.15 (0.91-1.44)	0.99 (0.67-1.48)		0.93 (0.62-1.38)	
Unprovoked PE							
Q 1 ≤250	97504	16	0.16 (0.10-0.27)	1.00	0.70	1.00	0.60
Q 2 251-503	86255	30	0.35 (0.24-0.50)	1.49 (0.81-2.76)		1.50 (0.81-2.78)	
Q 3 >503-706	90127	30	0.33 (0.23-0.48)	1.01 (0.54-1.90)		1.00 (0.53-1.87)	
Q 4 >706	64602	29	0.45 (0.31-0.65)	1.08 (0.57-2.05)		1.04 (0.55-1.98)	
Provoked PE							
Q 1 ≤250	97504	26	0.27 (0.18-0.39)	1.00	0.80	1.00	0.89
Q 2 251-503	86255	21	0.24 (0.16-0.37)	0.61 (0.34-1.09)		0.61 (0.34-1.08)	
Q 3 >503-706	90127	38	0.42 (0.31-0.58)	0.72(0.43-1.21)		0.69 (0.41-1.15)	
Q 4 >706	64602	45	0.70 (0.52-0.93)	0.93 (0.56-1.55)		0.85 (0.51-1.41)	

DVT deep vein thrombosis, *IR* incidence rate, *PE* pulmonary embolism, *VTE* venous thromboembolism.

*Based on data from the Tromsø Study in the period 1994-2016, analyzed by Cox proportional hazards regression models.

†Per 1000 person-years.

‡Adjusted for age (as timescale).

§Model 1 + sex and BMI.

Supplemental Table 6. Baseline characteristics of included participants and those excluded due to incomplete data on fish intake and use of fish oil supplements*

Baseline characteristics	**Included (n=21970)**	**Excluded (n=7570)**
Incidence rate of VTE (n events) †	1.6 (541)	2.9 (324)
Age, years	46 (14)	53 (17)
Sex, male	48.6 (10865)	44.4 (3361)
BMI, kg/m^2	25.4 (3.9)	25.6 (4.2)
Serum cholesterol, mmol/L	5.86 (1.25)	6.25 (1.37)
Serum HDL, mmol/L	1.49 (0.41)	1.50 (0.42)
Serum triglycerides, mmol/L	1.52 (1.00)	1.64 (1.12)
Systolic blood pressure, mmHg	133 (20)	140 (24)
Diastolic blood pressure, mmHg	77 (12)	80 (14)
Current smoking	32.9 (7210)	37.3 (2810)
Diabetes	2.04 (447)	3.24 (244)
History of CVD	5.45 (1191)	11.6 (871)
History of cancer	2.74 (601)	5.0 (381)
Higher education‡	36.4 (7977)	19.9 (1490)

BMI body mass index, *CVD* cardiovascular disease (angina pectoris, stroke, myocardial infarction), *HDL* high-density lipoprotein, *VTE* venous thromboembolism

*Based on data from the Tromsø Study in the period 1994-2016. Values are mean (±SD) or percentage (count)

†per 1000 person-years

‡≥15 years of education (corresponding to 3 years in university or academy).

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PAPER II

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

Running head: Omega-3 fatty acids and recurrent venous thromboembolism

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ABSTRACT

Background: Limited knowledge exists on the association between intake of long-chained n-3 polyunsaturated fatty acids (n-3 PUFAs) and risk of recurrence and all-cause mortality in patients with venous thromboembolism (VTE).

Objectives: To investigate whether intake of marine n-3 PUFAs was associated with risk of recurrence and mortality in patients with incident VTE.

Methods: A total of 595 patients with incident VTE and available data on n-3 PUFA intake were derived from the Tromsø Study surveys 4 (1994-95) and 6 (2007-08). Weekly intake of n-3 PUFAs was categorized as low, medium and high based on tertiles. Recurrent VTEs and all-cause mortality were registered up to December 31, 2016. Hazard ratios (HRs) were calculated using Cox-regression models with the low intake-category as reference.

Results: There were 98 recurrent VTEs and 227 deaths during follow-up. Overall, we found no association between intake of n-3 PUFAs and risk of recurrent VTE. However, inverse associations were found for high intakes in patients with unprovoked VTE (HR 0.45, 95% CI 0.20-1.01), cancer-free patients (HR 0.51, 95% CI: 0.27-0.95), and DVT patients (HR 0.49, 95% CI: 0.24-0.97). The inverse associations were more evident when follow-up was restricted to the time after discontinuation of anticoagulant therapy. No association was observed between intake of n-3 PUFAs and mortality after incident VTE.

Conclusions: A high dietary intake of marine n-3 PUFAs was associated with lower risk of recurrent VTE after unprovoked index events, DVT and in cancer-free patients.

Keywords: Diet – Omega-3 fatty acids – Recurrence – Risk factors – Venous thromboembolism

INTRODUCTION

Venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common and burdensome disease associated with serious consequences (1, 2). The one- and eight-year mortality after VTE is 22-36% and 30-52%, respectively, with the highest rates observed after acute PE (3-6). Notably, patients with VTE have an elevated risk of mortality for up to three decades after the incident event when compared to the general population (7). Recurrent events are common, especially during the first year after the incident event, and the ten-year cumulative incidence of recurrence is 30-40% (4-6). The risk of recurrence is dependent on the etiology of the index event. The lowest risk is observed in patients with VTE provoked by a major transient risk factor (e.g., major surgery), an intermediate risk is observed in patients where a provoking factor cannot be identified, and the highest risk is recognized in presence of a major persistent risk factor (e.g., cancer) (8). Although anticoagulant therapy efficiently prevents VTE recurrence, such treatment is most often time-limited as the benefit must be balanced against the risk of bleeding (9, 10). In order to improve strategies for secondary prevention, there is a need to identify life-style factors that are inversely associated with adverse outcomes after VTE.

Fish and marine food products are advocated as important determinants of a healthy diet, and the long-chained n-3 polyunsaturated fatty acids (n-3 PUFAs; eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA)) are identified as key bioactive compounds (11-13). n-3 PUFAs are reported to be associated with downregulation of inflammation (14), platelet function (15), platelet endothelium-interactions (16, 17) and tissue factor expression (18), which are key pathways in the VTE pathogenesis. We and others have previously suggested a favorable association between dietary intake of fish and marine food products, particularly those high in n-3 PUFAs, and the risk of incident VTE (19-22). Specifically, in our recent report based on the Tromsø Study, we found that moderate and

high intake of marine n-3 PUFA was associated with 22-26% lower risk of VTE (22). However, the role of n-3 PUFAs in relation to complications following VTE, such as recurrence and mortality, is largely unknown.

To our knowledge, only one study has investigated the association between n-3 PUFAs and risk of recurrence and mortality after VTE. In the Swiss Cohort of Elderly Patients with Venous Thromboembolism (SWITCO65+), n-3 PUFA (marine- and plant-based) levels in the erythrocyte membrane were measured in VTE patients aged ≥65 years, and the six-month and three-year risks of recurrence and mortality were investigated (23). They found that medium or high content of n-3 PUFAs in the erythrocyte membrane was associated with a lower six-month recurrence risk, and a lower risk of mortality at both time points. However, although, the fatty acid composition in the erythrocyte membrane reflects the fatty acid composition in the diet (24, 25), the association between dietary intake of n-3 PUFAs and risk of recurrent VTE has not been studied. Further, whether the reported associations apply to VTE patients with a wider age-range and in a longer time-perspective, is not known. Finally, the plant-based and the marine-derived n-3 PUFAs may not be equally important in this context. Therefore, the aims of the present study was to investigate the association between dietary intake of marine n-3 PUFAs and the risk of recurrence and all-cause mortality in VTE patients recruited from a population-based cohort with long follow-up.

METHODS

Study population

The Tromsø Study is a population-based single-center cohort study with repeated health surveys of inhabitants of the Tromsø municipality in Norway (26). The study was approved by the Regional Committee for Medical and Health Research Ethics, and all participants provided written informed consent prior to inclusion. Patients with incident VTE were recruited among participants in the fourth (1994-95) and sixth (2007-08) surveys of the Tromsø study. The source population comprised of 29,538 unique individuals, of which 10,304 took part in both surveys. The participants were followed from study inclusion to the end of the study period (December 31, 2016). All incident VTE events were identified and adjudicated by trained personnel searching the hospital discharge registry, the radiology procedure registry and the autopsy registry at the University Hospital of North Norway (UNN), as previously described in detail (27). UNN is the only hospital and the exclusive provider of relevant diagnostic radiology and hospital care in the study region, and manages essentially all in- and outpatients in Tromsø with a VTE diagnosis. Adjudication criteria for VTE were (i) signs and symptoms DVT or PE, (ii) objective confirmation by radiological procedures (compression ultrasonography, venography, spiral computed tomography, perfusion-ventilation scan or pulmonary angiography) or autopsy (only including cases where VTE was a significant contributor, or cause of death), (iii) a diagnosis of DVT or PE in the patient journal, and (iv) treatment initiation unless contraindications were specified.

There were of 896 incident VTE events during follow-up, and these formed the basis of our study population. Participants (n=301) with incomplete information on n-3 PUFA intake were excluded, and 595 patients with incident VTE were included in the present study. A comparison of included VTE patients and those excluded due to incomplete data on fish intake and use of fish oil supplements is shown in Table S1.

All VTE events were classified as provoked or unprovoked based on the presence of provoking factors at the time of diagnosis. Provoking factors were major surgery or trauma (within 8 weeks prior to the event), acute medical condition (acute myocardial infarction, acute ischemic stroke, major infectious disease), cancer, immobilization (bed rest ≥3 days or confinement to wheelchair, long distance travel ≥4 h. within the last 14 days) or a factor specifically described as provoking in the medical record, e.g. intravascular catheter. A VTE was classified as unprovoked if no provoking factor was reported.

Characteristics

Information on anthropometry, dietary habits, physical activity and medical history was obtained from physical examinations and self-administered questionnaires at inclusion in Tromsø 4 or 6. Height, weight and blood pressure were measured by standardized procedures, as described elsewhere (27). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). History of cardiovascular disease (CVD), diabetes, alcohol intake, physical activity, education level and smoking habits were reported via questionnaires. Information on cancer was obtained from the Cancer Registry of Norway. Active cancer was recorded when a cancer diagnosis was made in the time period from two years before to one year after the incident VTE event. Information on preplanned duration of anticoagulant treatment was obtained from the patients' medical records.

Assessment of marine n-3 PUFA intake

The assessment of marine n-3 PUFA intake in the Tromsø Study has been described in detail previously (22, 28). In brief, all participants in Tromsø 4 and 6 were asked to complete food

frequency questionnaires (FFQs), which included questions about how frequently they had fat fish and lean fish for dinner, how frequently they had fish as bread spread, and how often they used fish-oil supplements. In order to estimate the total intake of 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 (11, 29, 30), as previously described in detail (22). One serving unit of fish for dinner was defined as 200 g, and one serving unit of fish as bread spread was defined as 25 g. Total weekly intake of n-3 PUFAs was calculated by summarizing the amount of n-3 PUFAs derived from a person's weekly intake of fat and lean fish, fish as bread spread and fish oil supplements. For participants with data on n-3 PUFAs intake in both Tromsø 4 and 6, the data with shortest proximity to the incident VTE event (before or after) was used, as it was assumed to be the most relevant intake. For the remaining, the available data, either from Tromsø 4 or 6, was used. Eventually, there were 342 and 253 participants with data form Tromsø 4 and 6, respectively.

Outcome assessment

All recurrent VTE events during follow-up were identified and adjudicated using the same procedure as described for the incident events. Data on mortality was obtained from the Norwegian Population Registry.

Statistical analysis

Person-years of follow were accrued from the date of the incident VTE to the date of recurrent VTE, death, migration or the end of the study period (December 31, 2016), whichever occurred first. Recurrent VTE was not included as a censoring event in the analyses on mortality. The study population was categorized into tertiles according to the

estimated intake of n-3 PUFAs. The ranges were <8.19, 8.19-29.1 and >29.1 g/week for low, medium and high intake, respectively.

Crude incidence rates of VTE recurrence (IRs) and mortality rates (MRs) according to n-3 PUFA intake were calculated and expressed as number of events per 100 person-years with 95% confidence intervals (CIs). Cox proportional hazards regression models were used to estimate hazard ratios (HRs) with 95% CIs for recurrence and mortality with the lowest tertile of n-3 PUFA intake as the reference. Calendar time was used as time scale for the recurrence analyses, and attained age was used as time scale in the mortality analyses. The analyses were performed in two models for VTE recurrence and two models for mortality. For recurrence, model 1 was unadjusted and model 2 was adjusted for age, sex and BMI. In addition, to account for death as a competing event to VTE recurrence, sub-distribution hazard ratios (SHR) were calculated according to the method by Fine and Gray (31). For mortality, model 1 included age (as time scale), while model 2 additionally included sex, BMI, systolic blood pressure and smoking. For both outcomes, sensitivity analyses excluding participants with active cancer at the time of incident VTE, were performed. Moreover, we performed sensitivity analysis where follow-up started at the time of discontinuation of anticoagulant treatment. IRs/MRs and HRs for recurrence and mortality according to n-3 PUFA intake were calculated overall, and in subgroups stratified by characteristics of the incident event (unprovoked and provoked, DVT and PE). The proportional hazards assumption was evaluated and verified on the basis of Schoenfeld residuals. The cumulative incidence of VTE recurrence according to n-3 PUFA intake was visualized in 1-Kaplan-Meier plots. Statistical analyses were performed with STATA version 15.1 (Stata Corp, College Station, TX, USA) and R version 3.3.3 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Characteristics of first and recurrent VTE according to categories of n-3 PUFAs intake are summarized in Table 1. Overall, the mean age at incident VTE was 67 (±13) years and 51% were men. Further, 38% of the incident VTE events were unprovoked and 58% presented as a DVT. The age at incident VTE increased with increasing intake of n-3 PUFAs. Likewise, the prevalence of CVD increased across tertiles, while the prevalence of cancer was lowest in the middle tertile. The mean age at recurrent VTE was 69 (±12) years, and 61% were men.

Recurrence

Among the 595 patients with incident VTE, there were 98 recurrences during a median follow-up of 3.6 years. HRs of recurrence according to n-3 PUFA intake are shown in Table 2. Overall, there was no significant association between n-3 PUFA intake and risk of recurrence. Compared to the lowest tertile, the HR for tertile 2 was 1.09 (95% CI: 0.69-1.74) and the HR for tertile 3 was 0.77 (95% CI: 0.45-1.30) in the multivariable model (model 2). However, analyses stratified by characteristics of the incident event showed that a high n-3 PUFA intake (i.e. tertile 3) was associated with a 55% lower recurrence risk after unprovoked VTE (HR model 2: 0.45, 95% CI 0.20-1.01), and a 51% lower risk after DVT (HR model 2: 0.49, 95% CI 0.24-0.97). Further adjustment for alcohol consumption and physical activity did not affect our results (data not shown).

The ten-year cumulative incidence of recurrence after VTE according to tertiles of n-3 PUFA intake is shown in Fig. 1 and 2. The beneficial effect of a high n-3 PUFA intake on recurrence risk in patients with unprovoked VTE and DVT appeared to occur approximately two years after the incident event.

Sensitivity analyses excluding participants with active cancer (n=100) at the time of incident VTE are shown in Table 3. The results were comparable to the main analyses, but the inverse associations between n-3 PUFA intake and recurrence risk were stronger. Specifically, a high intake of n-3 PUFAs (tertile 3) was associated with a significant 49% lower risk of recurrence (HR model 2: 0.51, 95% CI: 0.27-0.95), which was most pronounced in those with unprovoked VTE (HR model 2: 0.41, 95% CI: 0.17-1.03) and DVT (HR model 2: 0.31, 95% CI: 0.13-0.71). However, in contrast to the main analyses, a high n-3 PUFA intake was also associated with a non-significant lower risk of recurrence after provoked VTE (HR: 0.66, 95% CI: 0.27-1.57). We observed stronger inverse associations when start of follow-up was restricted to the period after discontinuation of anticoagulant treatment (Table S2). For high n-3 PUFA intake we observed significantly reduced risk of recurrence after overall VTE (HR model 2: 0.42, 95% CI: 0.21-0.82), unprovoked VTE (HR model 2: 0.31, 95% CI: 0.12-0.83), and after DVT (HR model 2: 0.30, 95% CI: 0.13-0.73). Additionally, we observed a non-significant reduced recurrence risk after provoked VTE and after PE in this analysis (HR model 2: 0.61, 95% CI: 0.24-1.55, and HR: 0.70, 95% CI: 0.24-2.04, respectively). In analyses taking competing risk by death into account, the SHRs were generally comparable to the HRs obtained from the Cox regression models (Tables 2 and 3).

All-cause mortality

There were 227 deaths during a median follow-up of 4.5 years. HRs of all-cause mortality according to n-3 PUFA intake are shown in Table 4. Overall, there was no association between intake of n-3 PUFAs and the risk of mortality after incident VTE in the multivariable model (HR tertile 2: 0.96, 95% CI: 0.69-1.35; HR tertile 3: 1.02, 95% CI: 0.73-1.43). Similarly, no association was observed between n-3 PUFA intake and mortality risk in analyses stratified by characteristics of the incident event. The results from the sensitivity

analyses restricted to participants without active cancer were generally comparable to the results from the main analyses (Table 5). However, a non-significant lower mortality risk was observed after provoked VTE in those with a high n-3 PUFA intake (HR model 2: 0.68, 95% CI: 0.39-1.20).

DISCUSSION

In the present study, we investigated the association between dietary intake of marine n-3 PUFAs and the risk of VTE recurrence and all-cause mortality in patients with incident VTE recruited from a general population. We found that a high intake of n-3 PUFAs (upper tertile, >29.1 g/week) was associated with a lower recurrence risk compared to a low intake (lower tertile, <8.2 g/week) in patients with unprovoked incident events and in those with DVT. In contrast, there was no association between n-3 PUFAs intake and risk of mortality after incident VTE. The confidence intervals were wide, particularly in some subgroups, and our findings should therefore be interpreted with caution.

We and others have previously reported on the association between fish or n-3 PUFA intake and the risk of incident VTE. Although findings have been inconsistent, the available data suggests that intake of n-3 PUFAs may lower the risk of incident VTE (19-21). In our previous report, we found an inverse association between intake of marine n-3 PUFAs and risk of VTE, while there was no association between total fish intake and VTE risk (22). Marine n-3 PUFAs have an impact on several molecular pathways and risk factors of relevance in the VTE pathogenesis. These include downregulation of inflammation (14), tissue-factor expression (18, 32), platelet function (15), platelet endothelium-interactions (16, 17), and possibly hepatic excretion of coagulation factors (33). Moreover, n-3 PUFAs alternate cell membrane permeability and functionality, and have antiarrhythmic properties (14).

To our knowledge, our study is among the first to explore the association between marine n-3 PUFAs and the risk of recurrence and mortality in patients with incident VTE. Reiner and colleagues investigated the association between levels n-3 PUFAs in the erythrocyte membrane, and the risk of recurrence and mortality in elderly VTE patients (23). Although there were few recurrent events (n=22), they found that a moderate or high level of n-3 PUFAs was associated with a 61-83% lower risk of recurrence and 66-71% lower risk of mortality at six months of follow-up. After three years, a moderate level was still associated with a 45% lower risk of mortality, while there was no association between n-3 PUFA levels and recurrence risk. As the fatty acid composition in the erythrocyte membrane reflects the diet (24, 25), these findings suggest that a medium or high dietary intake of n-3 PUFAs may be associated with a better prognosis after VTE, although the effect on recurrence risk appeared to be transient (23). Apparently conflicting, we found that a high dietary intake of n-3 PUFAs was associated with 55% and 51% lower recurrence risk after unprovoked VTE and DVT, respectively, and that the effect occurred almost two years after the incident event. Although the different exposure assessments hamper a direct comparison of the studies, there are some plausible explanations for the diverging findings. First, the n-3 PUFA intake our study was high, and the upper tertile cut-off equals more than four grams per day, which is higher than the doses used in most clinical trials on prevention (34). Moreover, Reiner et al. (23) included PUFAs derived from both marine sources (i.e. EPA, DPA and DHA) and plants (i.e. alpha-linolenic acid, ALA) in their exposure variable. The impact of ALA in relation to health outcomes is less investigated and an effect is probably modest compared to marine-derived n-3 PUFAs (35-37). Therefore, the association between n-3 PUFAs and recurrence risk may have been underestimated due to the inclusion of ALA in the study by Reiner et al. (23). Further, the patients included in SWITCO65+ were almost ten years older than those in

the present study (75 years vs. 67 years), and 29% of the patients had a history of previous VTE, while we exclusively included incident cases.

The beneficial association of n-3 PUFAs on recurrence risk in our study was restricted to those with a high weekly intake, suggesting a mild protective effect of n-3 PUFA intake. Stratified analyses further revealed that the association was mainly driven by an effect in patients with unprovoked incident events. The risk of recurrence largely depends on the etiology of the incident event, which also reflects the underlying predisposition (baseline risk) for thrombosis (8, 38). Assuming a mild protective effect of n-3 PUFAs on VTE recurrence, it is plausible that the effect is most prominent in those with an intermediate recurrence risk, such as patients with unprovoked VTE. Conversely, a small effect may be difficult to observe in those with a low risk (e.g. VTE provoked by major surgery), and may be overwhelmed in those with a high risk (e.g. VTE provoked by active or progressive cancer). This is supported by our sensitivity analyses showing lowered risk estimates, even for provoked events, when patients with active cancer were excluded.

We further observed a difference in the influence of n-3 PUFA intake on the risk of recurrence after DVT and PE. Apparently, a high intake was associated with a lower recurrence risk after DVT, and a higher risk of recurrence after PE. Although PE typically recur as a new PE and is regarded as a more severe form of VTE (39), the current guidelines for antithrombotic therapy do not distinguish between the two disease entities (9). However, in our data, which reflects clinical practice from 1994 to 2016, we found that 45% of patients with PE were scheduled anticoagulant treatment for ≥1 year, while this applied to only 13% of patients with DVT. The risk of recurrence is highest during the first year after incident VTE (4, 5), and 53% of the recurrences in the present study occurred during this period. Potentially, DVT patients receiving anticoagulant treatment for a shorter time-period may have benefitted more from a high intake of n-3 PUFAs, while an effect in patients with PE

may have been masked by long-term treatment. We addressed this point by restricting the follow-up time to the period after completion of anticoagulant treatment. Interestingly, this approach consistently showed inverse associations between high n-3 PUFA intake and recurrence after overall VTE and subtypes of VTE (unprovoked, provoked, DVT and PE).

In contrast to the findings by Reiner and colleagues (23), we did not observe any association between n-3 PUFAs and the risk of mortality in patients with VTE. Although observational studies on the effect of n-3 PUFAs on cause-specific mortality in individuals without known CVD have shown somewhat diverging results (40, 41), it may be suggested that marine-derived n-3 PUFAs are primarily associated with a lower risk of CVD-related death, while ALA is associated with a lower risk of death from non-CVD causes (36, 42). The most frequently reported causes of death in patients with VTE are cancer, PE, infections, and other cardiovascular or respiratory diseases (3, 43, 44). Potentially, a relatively higher proportion of deaths due to non-CVD causes in VTE-patients could explain the lack of a beneficial association between marine n-3 PUFAs and all-cause mortality in our study. Nevertheless, the results did not change after exclusion of patients with active cancer.

The main strengths of our study include unselected VTE patients with a wide age distribution, a validated exposure variable, long follow-up and thoroughly validated outcomes. Limitations of the study include an observational design, which does not allow for random allocation to exposure groups, and residual confounding may be present. Further, a substantial proportion of participants were excluded due to incomplete information on n-3 PUFA intake, and the included participants were younger and healthier compared to those excluded. This may influence the generalizability of our findings. Moreover, although we previously reported a significant trend between self-reported intake of marine n-3 PUFAs and serum concentrations (22), the correlation coefficients are generally reported to be low (45). Due to limited study power, the confidence intervals were wide, and our findings should

therefore be interpreted with caution. Finally, a long follow-up may introduce regression dilution as dietary habits may change over time. Such misclassification may influence the precision of our estimates, but is unlikely to be related the outcomes of the study, and would typically lead to an underestimation of the true association.

In conclusion, a high intake of marine n-3 PUFAs was associated with a lower risk of VTE recurrence, which was most pronounced after unprovoked VTE and DVT, and in cancer-free patients. In contrast, there was no association between n-3 PUFAs and the risk of all-cause mortality in patients with incident VTE.

CONFLICT OF INTEREST

None declared.

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TABLES AND FIGURES

Table 1 Characteristics of patients with incident VTE (n=595) and recurrent events (n=98) according to tertiles of weekly intake of n-3 PUFAs. The Tromsø Study (1994-2016)

n-3 PUFAs (g/week)	All	T1 (<8.19)	T2 (8.19-29.1)	T3 (>29.1)
First VTE				
Number of patients	595	200	201	194
DVT	57.5 (342)	53.0 (106)	63.2 (127)	56.2 (109)
PE	42.5 (253)	47.0 (94)	36.8 (74)	43.8 (85)
Unprovoked	38.0 (226)	42.0 (84)	47.8 (96)	42.3 (82)
Duration of anticoagulant treatment				
< 3 months	20.0 (119)	31.9 (38)	37.0 (44)	31.1 (37)
3-6 months	42.5 (253)	30.4 (77)	35.2 (89)	34.4 (87)
6-12 months	20.5 (122)	45.1 (55)	29.5 (36)	25.4 (31)
> 12 months	17.0 (101)	29.7 (30)	31.7 (32)	38.6 (39)
Male sex	51.1 (304)	54.5 (109)	47.8 (96)	51.0 (99)
Age at incident VTE	67 ± 13	63 ± 14	67 ± 13	71 ± 12
BMI, kg/m^2	27.4 ± 4.5	27.1 ± 4.3	27.4 ± 4.3	27.7 ± 4.4
Diabetes	4.9 (29)	3.0 (6)	6.0 (12)	5.7 (11)
History of CVD	13.1 (78)	7.5 (15)	14.4 (29)	17.5 (34)
Cancer	16.8 (100)	18.0 (36)	13.9 (28)	18.6 (36)
Current smoking	28.2 (168)	40.0 (80)	26.9 (54)	17.5 (34)
Systolic BP, mmHg	142 ± 24	138 ± 23	142 ± 24	147 ± 25
Second VTE (by index event):				
Number of patients	98	35	38	25
DVT	72.4 (71)	77.1 (27)	81.6 (31)	52.0 (13)
PE	27.6 (27)	22.9 (8)	18.4 (7)	48.0 (12)
Unprovoked	52.0 (51)	51.4 (18)	63.2 (24)	36.0 (9)
Male sex	61.2 (60)	77.1 (27)	55.3 (21)	48.0 (12)
Age at second VTE	69 ± 12	67 ± 13	69 ± 12	71 ± 12
BMI, kg/m^2	27.0 ± 4.5	27.1 ± 6.0	27.0 ± 3.3	26.8 ± 3.9
Cancer	18.4 (18)	11.4 (4)	13.2 (5)	36.0 (9)
Current smoking	20.4 (20)	31.4 (11)	23.7 (9)	20.0 (5)
Systolic BP, mmHg	139 ± 20	135 ± 20	143 ± 20	139 ± 21

BMI body mass index, *BP* blood pressure, *CVD* cardiovascular disease (angina pectoris, stroke or myocardial infarction), *DVT* deep vein thrombosis, *PE* pulmonary embolism, *PUFAs* polyunsaturated fatty acids, *T* tertile, *VTE* venous thromboembolism.

Values are mean (standard deviation) or percentage (count)

Table 2 Incidence rates (IRs) and hazard ratios (HRs) with 95% confidence intervals (CI) for recurrent venous thromboembolism (VTE) by tertiles of weekly intake of marine n-3 polyunsaturated fatty acids (PUFAs). The Tromsø Study 1994-2016.

n-3 PUFA intake (g/week)	Person-years	VTE-events	Crude IR (95% CI)*	Model 1 HR (95% CI)†	Model 2 HR (95% CI)‡	SHR (95% CI)§
VTE						
T1 <8.19	1111	35	3.1 (2.3-4.4)	Ref.	Ref.	Ref.
T2 8.19-29.1	1080	38	3.5 (2.6-4.8)	1.10 (0.69-1.74)	1.09 (0.69-1.74)	1.19 (0.76-1.87)
T3 >29.1	916	25	2.7 (3.7-4.0)	0.86 (0.51-1.43)	0.77 (0.45-1.30)	0.83 (0.49-1.42)
Unprovoked VTE						
T1 <8.19	537	18	3.4 (2.1-5.3)	Ref.	Ref.	Ref.
T2 8.19-29.1	591	24	4.1 (2.7-6.1)	1.20 (0.65-2.21)	1.13 (0.61-2.10)	1.12 (0.61-2.08)
T3 >29.1	448	9	2.0 (1.0-3.9)	0.59 (0.26-1.31)	0.45 (0.20-1.01)	0.42 (0.18-1.00)
Provoked VTE						
T1 <8.19	575	17	3.0 (1.8-4.8)	Ref.	Ref.	Ref.
T2 8.19-29.1	487	14	2.9 (1.7-4.9)	0.96 (0.47-1.96)	1.00 (0.49-2.04)	1.10 (0.56-2.19)
T3 >29.1	468	16	3.4 (2.1-5.6)	1.21 (0.60-2.43)	1.25 (0.61-2.56)	1.35 (0.70-2.61)
PE						
T1 <8.19	540	8	1.5 (0.7-3.0)	Ref.	Ref.	Ref.
T2 8.19-29.1	378	7	1.9 (0.9-3.9)	1.18 (0.43-3.27)	0.97 (0.35-2.71)	1.10 (0.41-2.95)
T3 >29.1	405	12	3.0 (1.7-5.2)	1.93 (0.78-4.75)	1.55 (0.62-3.87)	1.64 (0.65-4.12)
DVT						
T1 <8.19	571	27	4.7 (3.2-6.9)	Ref.	Ref.	Ref.
T2 8.19-29.1	703	31	4.4 (3.1-6.3)	0.94 (0.56-1.57)	1.00 (0.59-1.69)	1.12 (0.67-1.86)
T3 >29.1	511	13	2.5 (1.5-4.4)	0.54 (0.28-1.06)	0.49 (0.24-0.97)	0.54 (0.27-1.08)

CI confidence interval, *DVT* deep vein thrombosis, *PE* pulmonary embolism, *SHR* subdistribution hazard ratio, *T* tertile.

*****Per 100 person-years

†Unadjusted

‡Adjusted for age, sex and body mass index

§Competing risk model, adjusted for age, sex and body mass index

Table 3 Incidence rates (IRs) and hazard ratios (HRs) for recurrent venous thromboembolism (VTE) by tertiles of weekly intake of marine n-3 polyunsaturated fatty acids (n-3 PUFAs) after excluding individuals with active cancer. The Tromsø Study 1994-2016

n-3 PUFA intake (g/week)	Person-years	VTE-events	Crude IR (95% CI)*	Model 1 HR (95% CI)†	Model 2 HR (95% CI)‡	SHR (95% CI)§
VTE						
T1 <8.19	1026	31	3.0 (2.1-4.3)	Ref.	Ref.	Ref.
T2 8.19-29.1	1005	33	3.3 (2.3-4.6)	1.07 (0.65-1.74)	1.01 (0.61-1.66)	1.09 (0.67-1.77)
T3 >29.1	826	16	1.9 (1.2-3.2)	0.64 (0.35-1.17)	0.51 (0.27-0.95)	0.56 (0.30-1.03)
Unprovoked VTE						
T1 <8.19	524	16	3.1 (1.9-5.0)	Ref.	Ref.	Ref.
T2 8.19-29.1	570	21	3.7 (2.4-5.7)	1.20 (0.62-2.29)	1.13 (0.59-2.18)	1.15 (0.60-2.22)
T3 >29.1	407	7	1.7 (0.8-3.6)	0.55 (0.23-1.34)	0.41 (0.17-1.03)	0.38 (0.15-1.00)
Provoked VTE						
T1 <8.19	502	15	3.0 (1.8-5.0)	Ref.	Ref.	Ref.
T2 8.19-29.1	433	12	2.8 (1.6-4.9)	0.92 (0.43-1.96)	0.88 (0.40-1.90)	0.97 (0.47-2.04)
T3 >29.1	419	9	2.1 (1.1-4.1)	0.76 (0.33-1.77)	0.66 (0.27-1.57)	0.79 (0.36-1.73)
PE						
T1 <8.19	500	8	1.6 (0.8-3.2)	Ref.	Ref.	Ref.
T2 8.19-29.1	346	6	1.7 (0.8-3.9)	1.02 (0.35-2.96)	0.75 (0.26-2.18)	0.91 (0.33-2.52)
T3 >29.1	354	8	2.3 (1.3-4.5)	1.35 (0.50-3.62)	0.99 (0.36-2.71)	1.07 (0.41-2.81)
DVT						
T1 <8.19	526	23	4.4 (2.9-6-6)	Ref.	Ref.	Ref.
T2 8.19-29.1	660	27	4.1 (2.8-6.0)	0.93 (0.53-1.63)	0.98 (0.55-1.73)	1.04 (0.60-1.81)
T3 >29.1	472	8	1.7 (0.8-3.4)	0.39 (0.17-0.88)	0.31 (0.13-0.71)	0.35 (0.15-0.81)

CI confidence interval, *DVT,* deep vein thrombosis, *PE* pulmonary embolism, *SHR* subdistribution hazard ratio, *T* tertile.

*****Per 100 person-years

†Unadjusted

‡Adjusted for age, sex and body mass index

§Competing risk model, adjusted for age, sex and body mass index

Table 4 Mortality rates (MRs) and hazard ratios (HRs) for all-cause mortality across tertiles of weekly intake of marine n-3 polyunsaturated fatty acids (n-3 PUFAs). The Tromsø Study 1994-2016.

n-3 PUFA intake (g/week)	Person-years	Deaths	Crude MR (95% CI)*	Model 1 HR (95% CI)†	Model 2 HR (95% CI)‡
VTE					
T1 <8.19	1302	72	5.5 (4.4-7.0)	Ref.	Ref.
T2 8.19-29.1	1220	76	6.2 (5.0-7.8)	0.97 (0.70-1.34)	0.96 (0.69-1.35)
T3 >29.1	1033	79	7.6 (6.1-9.5)	1.01 (0.72-1.40)	1.02 (0.73-1.43)
Unprovoked VTE					
T1 <8.19	658	17	2.6 (1.6-4.2)	Ref.	Ref.
T2 8.19-29.1	682	24	3.5 (2.4-5.3)	1.11 (0.59-2.08)	1.08 (0.56-2.10)
T3 >29.1	510	26	5.1 (3.5-7.5)	1.26 (0.68-2.35)	1.26 (0.67-2.36)
Provoked VTE					
T1 <8.19	644	55	8.5 (6.6-11.1)	Ref.	Ref.
T2 8.19-29.1	537	51	9.5 (7.2-12.5)	0.98 (0.66-1.44)	1.02 (0.69-1.52)
T3 >29.1	523	53	10.1 (7.7-13.3)	0.91 (0.61-1.35)	0.94 (0.62-1.42)
PE					
T1 <8.19	586	29	4.9 (3.4-7.1)	Ref.	Ref.
T2 8.19-29.1	403	23	5.7 (3.8-8.6)	0.95 (0.54-1.68)	0.95 (0.53-1.68)
T3 >29.1	457	35	7.7 (5.5-10.7)	1.21 (0.73-2.02)	1.16 (0.69-1.96)
DVT					
T1 <8.19	716	43	6.0 (4.5-8.1)	Ref.	Ref.
T2 8.19-29.1	818	53	6.5 (5.0-8.5)	0.95 (0.63-1.44)	0.98 (0.64-1.49)
T3 >29.1	575	44	7.6 (5.7-10.3)	0.87 (0.56-1.35)	0.94 (0.60-1.48)

CI confidence interval, *DVT* deep vein thrombosis, *PE* pulmonary embolism, *T* tertile.

*Per 100 person-years

†Adjusted for age (as time-scale)

‡Model 1 + sex, body mass index, systolic blood pressure and smoking

Table 5 Mortality rates (MRs) and hazard ratios (HRs) for all-cause mortality by tertiles of weekly intake of marine n-3 polyunsaturated fatty acids (n-3 PUFAs) excluding individuals with active cancer. The Tromsø Study 1994-2016.

n-3 PUFA intake (g/week)	Person-years	Deaths	Crude MR (95% CI)*	Model 1 HR (95% CI)†	Model 2 HR (95% CI)‡
VTE					
T1 <8.19	1203	43	3.6 (2.7-4.8)	Ref.	Ref.
T2 8.19-29.1	1138	53	4.7 (3.6-6.1)	1.03 (0.68-1.55)	1.00 (0.66-1.51)
T3 >29.1	921	51	5.5 (4.2-7.3)	0.96 (0.63-1.45)	0.91 (0.60-1.40)
Unprovoked VTE					
T1 <8.19	631	14	2.2 (1.3-3.7)	Ref.	Ref.
T2 8.19-29.1	653	17	2.6 (1.6-4.2)	0.95 (0.46-1.95)	0.92 (0.43-1.97)
T3 >29.1	456	22	4.8 (3.2-7.3)	1.34 (0.68-2.64)	1.28 (0.64-2.57)
Provoked VTE					
T1 <8.19	572	29	5.1 (3.5-7.3)	Ref.	Ref.
T2 8.19-29.1	483	35	7.3 (5.2-10.1)	1.09 (0.66-1.80)	1.04 (0.62-1.74)
T3 >29.1	465	29	6.2 (4.3-9.0)	0.75 (0.43-1.29)	0.68 (0.39-1.20)
PE					
T1 <8.19	546	18	3.3 (2.1-5.2)	Ref.	Ref.
T2 8.19-29.1	368	14	3.8 (2.3-6.4)	0.88 (0.42-1.83)	0.86 (0.41-1.80)
T3 >29.1	401	23	5.7 (3.8-8.6)	1.33 (0.71-2.51)	1.21 (0.63-2.30)
DVT					
T1 <8.19	657	25	3.8 (2.6-5.6)	Ref.	Ref.
T2 8.19-29.1	770	39	5.1 (3.7-6.9)	1.05 (0.63-1.76)	1.11 (0.66-1.88)
T3 >29.1	520	28	5.4 (3.7-7.8)	0.75 (0.43-1.31)	0.80 (0.45-1.41)

CI confidence interval, *DVT* deep vein thrombosis, *PE* pulmonary embolism, *T* tertile.

*****Per 100 person-years

†Adjusted for age (as time-scale)

‡Model 1 + sex, body mass index, systolic blood pressure and smoking

Figure 1 Ten-year cumulative incidence of recurrent venous thromboembolism (VTE) by tertiles of weekly intake of n-3 PUFAs for the total follow-up (Panel A) and follow-up restricted to the period after discontinuation of anticoagulant treatment (Panel B). Low intake: <8.19 g/week, medium intake: 8.19-29.1 g/week, and high intake: >29.1 g/week. *PUFAs* polyunsaturated fatty acids.

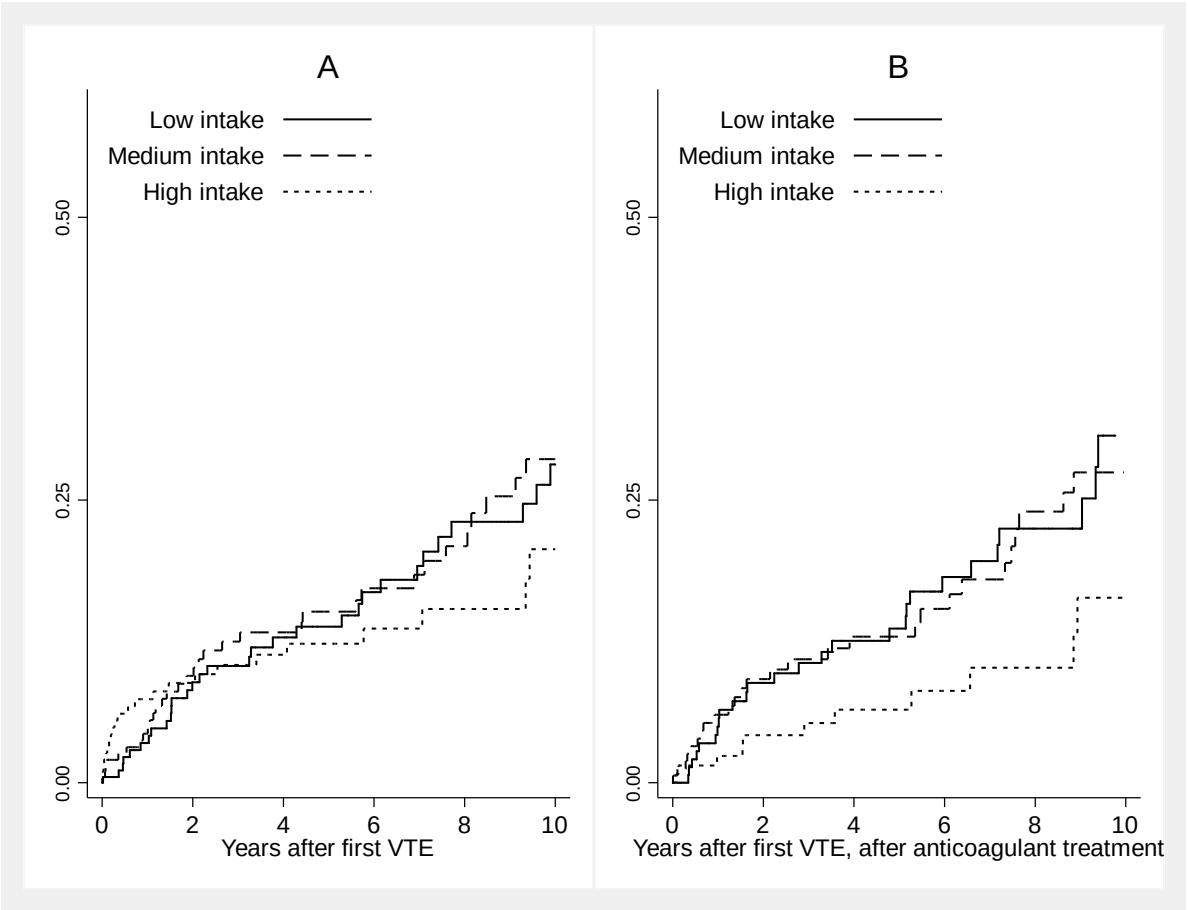
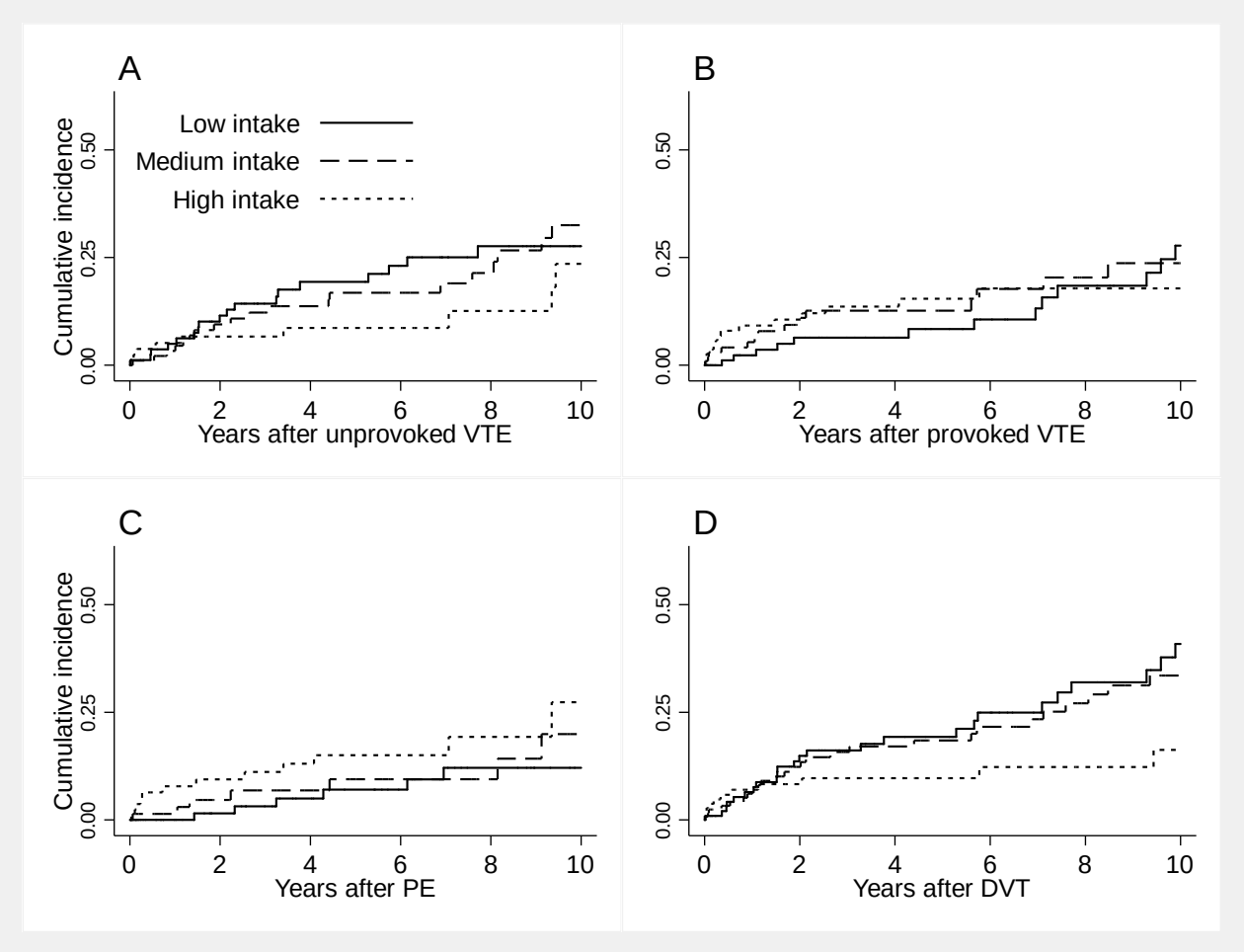


Figure 2 panel A-D. Cumulative incidence of recurrent venous thromboembolism (VTE) by tertiles of weekly intake of n-3 PUFAs according to the index event (A: unprovoked VTE, B: provoked VTE, C: Pulmonary embolism (PE), D: Deep vein thrombosis (DVT)). Low intake: <8.19 g/week, medium intake: 8.19-29.1 g/week and high intake: >29.1 g/week.



SUPPLEMENTARY MATERIAL

Table S1 Comparison of included and excluded VTE-patients

	Included (n=595)	**Excluded (n=304)**
Incident VTE		
DVT	57 (342)	62 (187)
PE	43 (253)	38 (117)
Unprovoked	44 (262)	41 (124)
Male sex	51 (304)	42 (129)
Age at incident VTE	67 ± 13	71 ± 13
Body mass index, kg/m^2	27.4 ± 4.5	27.1 ± 4.5
Diabetes	5 (29)	3 (10)
History of CVD	13 (78)	18 (55)
Cancer	17 (100)	16 (48)

CVD cardiovascular disease (angina pectoris, stroke or myocardial infarction), *DVT* deep vein thrombosis, *PE* pulmonary embolism, *VTE* venous thromboembolism.

Values are mean (standard deviation) or percentage (count)

Table S2

Incidence rates (IRs) and hazard ratios (HRs) with 95% confidence intervals (CI) for recurrent venous thromboembolism (VTE) by tertiles of weekly intake of marine n-3 polyunsaturated fatty acids (PUFAs) in sensitivity analyses with follow-up restricted to the period after discontinuation of anticoagulant therapy. The Tromsø Study 1994-2016.

n-3 PUFA intake (g/week)	Person-years	VTE-events	Crude IR (95% CI)*	Model 1 HR (95% CI)†	Model 2 HR (95% CI)‡	SHR (95% CI)§
VTE						
T 1	935	31	3.3 (2.3-4.7)	Ref.	Ref.	Ref.
T 2	927	32	3.5 (2.4-4.9)	1.03 (0.63-1.69)	0.99 (0.60-1.63)	1.03 (0.62-1.68)
T 3	747	13	1.7 (1.0-3.0)	0.53 (0.28-1.02)	0.42 (0.21-0.82)	0.48 (0.25-0.94)
Unprovoked VTE						
T 1	425	15	3.5 (2.1-5.9)	Ref.	Ref.	Ref.
T 2	502	22	4.4 (2.9-6.7)	1.24 (0.64-2.40)	1.14 (0.58-2.23)	1.18 (0.61-2.30)
T 3	361	6	1.7 (0.7-3.7)	0.48 (0.19-1.25)	0.31 (0.12-0.83)	0.33 (0.12-0.89)
Provoked VTE						
T 1	511	16	3.1 (1.9-5.1)	Ref.	Ref.	Ref.
T 2	424	10	2.4 (1.3-4.4)	0.76 (0.34-1.68)	0.79 (0.35-1.77)	0.77 (0.35-1.69)
T 3	386	7	1.8 (0.9-3.8)	0.63 (0.26-1.56)	0.61 (0.24-1.55)	0.69 (0.29-1.64)
PE						
T 1	415	8	1.9 (1.0-3.9)	Ref.	Ref.	Ref.
T 2	294	6	2.0 (0.9-4.5)	1.02 (0.35-2.95)	0.67 (0.23-1.95)	0.74 (0.26-2.15)
T 3	321	6	1.9 (0.8-4.2)	0.99 (0.34-2.87)	0.70 (0.24-2.04)	0.82 (0.29-2.32)
DVT						
T 1	521	23	4.4 (2.9-6.6)	Ref.	Ref.	Ref.
T 2	634	26	4.1 (2.8-6.0)	0.92 (0.53-1.62)	0.99 (0.56-1.76)	1.11 (0.63-1.95)
T 3	426	7	1.6 (0.8-3.4)	0.38 (0.16-0.88)	0.30 (0.13-0.73)	0.36 (0.15-0.86)

CI confidence interval, *DVT* deep vein thrombosis, *PE* pulmonary embolism, *SHR* subdistribution hazard ratio, *T* tertile.

*****Per 100 person-years

†Unadjusted

‡Adjusted for age, sex and body mass index

§Competing risk model, adjusted for age, sex and body mass index

PAPER III

Impact of dietary marine n-3 polyunsaturated fatty acids on surgery as a trigger for venous thromboembolism – results from a case-crossover study

Running head: n-3 PUFAs and surgery as a trigger for venous thromboembolism

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Abstract

Background: Surgery is an important trigger of venous thromboembolism (VTE) and previous observational studies suggest that dietary intake of n-3 polyunsaturated fatty acids (n-3 PUFAs) lowers the risk of incident VTE. However, it is unknown whether n-3 PUFAs intake influences the effect of surgery as a trigger for VTE.

Objective: To investigate whether intake of n-3 PUFAs modifies the effect of surgery as a trigger of VTE.

Methods: We conducted a case-crossover study with 445 VTE patients recruited from the population-based Tromsø Study. Self-reported intake of n-3 PUFAs was collected at study inclusion, and information on surgery was registered during the 90-day period prior to the VTE event (hazard period) and in four preceding 90-day control periods. Patients were stratified on tertiles (T) of n-3 PUFAs intake, and conditional logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) of surgery as a VTE trigger.

Results: The overall OR of surgery was 7.6 (95% CI 5.2-11.1). Higher intake of n-3 PUFAs mitigated the association, as the ORs of surgery were 11.8 (95% CI 6.1-22.6) in T1, 8.1 (95% CI 4.2-15.7) in T2 and 4.1 (95% CI 2.1-8.2) in T3 of n-3 PUFAs intake. The effect was strongest for pulmonary embolism (PE), with ORs of 14.7 (95% CI 5.5-39.6), 7.0 (95% CI 2.1-23.5) and 2.8 (95% CI 1.1-7.4) in T1-3, respectively.

Conclusions: A high intake of n-3 PUFAs strongly attenuated the effect of surgery as a trigger for VTE, and particularly for PE.

Keywords: Diet – Omega-3 fatty acids – Venous thromboembolism – Surgery – Case-crossover

Introduction

Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common and potentially life-threatening condition. The incidence of VTE has remained stable or slightly increased over the past decades, and it is recognized as a significant contributor to the global burden of disease (1-3). Surgery is an important risk factor, and surgery-related VTE is estimated to account for 15-22% of all incident events in the population (1, 4, 5). Despite awareness of the great preventive potential to improve patient safety in hospitals, the proportion of VTEs in the population that can be attributed to surgery has increased slightly during the last decades (4). Whether lifestyle factors, such as diet, may modify the effect of VTE triggers, such as surgery, remains elusive. However, ecological data from Norway demonstrated a temporal decrease in the incidence of postoperative VTE during World War II, which coincided with substantially increased intake of fish in the population (6, 7).

n-3 polyunsaturated long-chained fatty acids (n-3 PUFAs, i.e. eicosapentaenoic, docosapentaenoic and docosahexaenoic acid) are essential nutrients mainly found in foods of marine origin (8). The content of n-3 PUFAs varies and may be up to eightfold higher in fat fish than in lean fish, while dietary supplements have the highest concentrations (9, 10). n-3 PUFAs have been found to influence key pathways in the VTE pathogenesis, including downregulation of inflammation (11), tissue-factor expression (12, 13), platelet function and platelet-endothelium interactions (14, 15). Observational studies on the association between fish intake and the risk of incident VTE have shown conflicting results (16). However, sub-analyses according to the total content of n-3 PUFAs in the diet indicated that a higher n-3 PUFAs intake was associated with a lower VTE risk (17-19), and particularly provoked VTE (20).

The objective of the present study was to investigate whether intake of n-3 PUFAs modified the effect of surgery as a trigger for VTE. We used a case-crossover design to test the hypothesis that a higher n-3 PUFAs intake reduced the risk of surgery-related VTE. This design is well-suited for studying transient exposures and theoretically eliminates confounding by fixed covariates due to self-matching (i.e., each case serves as his/her own control) (21).

Methods

Study population

The source population included participants from the fourth survey (1994-95) of the Tromsø Study, a single center population-based cohort with repeated health surveys. All inhabitants living in the Tromsø municipality above 24 years were invited and 27 158 participated (77% of the eligible). Detailed methodology and description of the Tromsø Study can be found elsewhere (22). Incident VTE events during follow-up (1994-2012) were identified by searching the hospital discharge registry, the radiology procedure registry and the autopsy registry at the University Hospital of North Norway (UNN). The UNN exclusively provides VTE diagnostics and treatment in the study region. All potential cases were adjudicated according to the following four criteria: (i) signs and symptoms of PE or DVT, (ii) presence of a thrombus confirmed by radiology, (iii) a diagnosis of PE or DVT in the medical records, and (iv) initiation of treatment (unless contraindications were specified) (23). The study was approved by the Regional Committee for Medical and Health Research Ethics, and all participants provided written informed consent.

Study design

There were 707 incident VTE events during follow-up, and these formed the basis of the case-crossover study. We defined five exposure periods; the hazard period was the 90-days preceding the VTE and four 90-day control periods. A 90-day washout period was included between the hazard and control periods in order to avoid carryover effects (Figure 1). The duration of the hazard and control periods were defined based on the timeframe that major transient risk factors are generally considered to influence VTE risk (24).

Assessment of n-3 PUFAs intake

Total dietary intake of n-3 PUFAs in grams per week was estimated based on reported intake in either Tromsø 4 (1994-95) or Tromsø 6 (2007-08), depending on which surveys that was closest to the VTE event in time. Only those with dietary information on these items were included in the study (n=445). Briefly, participants were asked how frequently they had lean and fat fish for dinner, used fish as bread spread and took fish oil supplements. Information on the average n-3 PUFAs content in different marine food products was provided by official web resources (9, 10). On average, fat fish for dinner, lean fish for dinner and fish as spread was estimated to contain 3.0 grams, 0.4 grams and 3.7 grams of n-3 PUFAs per 100 grams, respectively. One serving size of fish oil supplements contained 3.6 grams of n-3 PUFAs. A dinner serving was defined as 200 grams and fish spread as 25 grams, according to recommendations by the Norwegian Directorate of Health (25). Further details on the assessment of n-3 PUFAs intake are described elsewhere (20).

Definition of major surgery

Trained personnel reviewed the medical records for each VTE case and systematically collected information on surgical procedures and other relevant information occurring during the exposure periods. This included risk factors for VTE, diagnostic procedures and laboratory test, surgical and medical treatment, and diagnoses given during admissions, day care and outpatient visits. We did not have any information from healthcare visits outside the hospital setting. Surgery was recorded for procedures involving organs within the chest, abdomen, pelvic cavity, the cranium, knee and hip surgeries, or surgery with general anesthesia >30 minutes (26). Surgery was dichotomized according to presence or absence during the hazard and control periods.

Statistical analysis

Baseline characteristics are shown as means ± standard deviation (SD) or percentage (count), overall and by tertiles of n-3 PUFAs intake. Conditional logistic regression was used to estimate regression coefficients with standard error (SE) and corresponding odds ratios (ORs) with 95% confidence intervals (CIs) for the presence of surgery in hazard versus control periods. To evaluate the differences of the impact of surgery between tertiles of n-3 PUFAs intake, we obtained *p*-values based on the ratio (Z-score) of the regression coefficients and their SEs (27). The threshold for significance was set to 0.05. The n-3 PUFAs intake increased substantially with age. Since the effect of surgery as a VTE trigger also could vary with age, we performed two sensitivity analyses to control for age as a potential confounder for the observed differences in ORs between tertiles. In the first approach, we defined tertiles based on the residuals from a linear regression of n-3 PUFAs on age. This approach removes the age-dependency, and the exposure variable can be interpreted as the deviation from the

expected n-3 PUFAs intake in relation to a given persons age. In the second approach, we performed a matched analysis, in which individuals across the three tertiles were matched on age (±2 years), which ensures an approximately identical age distribution in all three tertiles. As the matching process produces slightly different matched sets in each draw, we ran the matching and logistic regressions 1000 times and presented the mean OR from these 1000 iterations as the risk estimate of each tertile. Analysis were performed in STATA version 15.0 (Stata Corporation, College Station, TX, USA) and R version 4.0.1 (The R Foundation for Statistical Computing 2018).

Results

Characteristics of the 445 patients included at the time of VTE are shown in Table 1. The overall mean age was 67 ± 14 years and 50.8% were women. There were 57.7% presenting with DVT and 42.2% with PE ± DVT. The mean age increased with increasing intake of n-3 PUFAs, whereas the sex distribution was similar across tertiles.

The frequencies of surgery in the hazard and control periods according to n-3 PUFAs intake are shown in Table 2. Overall, surgery occurred more frequently in the hazard period (19%) than in the control periods (3%), and the OR for surgery in the hazard versus the control periods was 7.6 (95% CI 5.2-11.1) (Table 3). The ORs for surgery as a VTE trigger decreased across tertiles of higher n-3 PUFAs intake, and were 11.8 (95% CI 6.1-22.6) for tertile 1, 8.1 (95% CI 4.2-15.7) for tertile 2 and 4.1 (95% CI 2.1-8.2) for tertile 3, respectively (Table 3 and Fig. 2; p=0.03 for the comparison of tertile 1 and tertile 3). Subgroup analyses indicated that the OR gradient across n-3 PUFAs intake was driven by PE, rather than DVT. For PE, the ORs according to surgery were 14.7 (95% CI 5.5-39.6) for tertile 1, 7.0 (95% CI

2.1-23.5) for tertile 2 and 2.8 (95% CI 1.1-7.4) for tertile 3 (p=0.02 for the comparison of tertile 1 and tertile 3).

The mean age varied across the n-3 PUFAs categories, and the impact of surgery as a trigger could potentially vary across age groups. Therefore, we performed two sensitivity analysis to adjust for age as a potential confounder. In the first sensitivity analysis, the residual approach yielded tertiles with essentially similar age-distribution (supplementary Table 1). The ORs of surgery as a VTE trigger were 8.7 (95% CI 4.9-15.4) in tertile 1, 11.9 (95% CI 5.4-26.3) in tertile 2, and 4.4 (95% CI 2.2-8.6) in tertile 3. In the second sensitivity analysis we used a matching approach, and the mean ORs for surgery as a VTE trigger were 9.6 (95% CI 4.6-20.0) in tertile 1, 11.0 (95% CI 4.8-26.6) in tertile 2 and 4.1 (95% CI 1.8-10.0) in tertile 3. The frequencies of surgery in the hazard and control periods according to n-3 PUFAs intake in the residual approach are shown in supplementary Table 2.

Discussion

In this population-based case-crossover study of VTE patients, we found that the impact of surgery as a trigger for VTE was lower in those with a high intake of n-3 PUFAs. Our findings suggest that a high intake of n-3 PUFAs may attenuate the effect of surgery as a trigger for VTE, and indicates the need for future studies on the role of n-3 PUFAs in prevention of surgery-related VTE.

Several observational studies have investigated the association between intake of n-3 PUFAs (or fat fish as a proxy for n-3 PUFAs intake) and the risk of incident VTE. In the Atherosclerosis Risk in Communities (ARIC) Study, a cohort of 14 962 adults, an n-3 PUFAs intake exceeding 0.7 grams/week was associated with 30-46% lower VTE risk (17). Further, among 57 054 adults in the Diet, Cancer and Health (DCH) Study, an intake of fat fish

exceeding 35 grams/week in women and 49 grams/week in men was associated with 20-40% lower VTE risk (18). In the Tromsø Study, an early report including 23 621 individuals found that fish for dinner ≥3 times/week in combination with fish oil supplements was associated with 48% lower risk of VTE (19). Recently, we found that a weekly n-3 PUFAs intake exceeding 4.7 grams was associated with 24% lower risk of VTE in the Tromsø Study, with strongest risk reduction for provoked events (31%) and PE (41%) (20).

Historical data displayed a clear and transient reduction in surgery-related VTE occurring in parallel with dietary changes, including a high dietary intake of n-3 PUFAs, in Norway during the second World War (6, 7). These findings suggested that n-3 PUFAs could have a protective effect against surgery-related VTE. This notion is further supported by the present study demonstrating that a higher intake of n-3 PUFAs modifies the impact of surgery as a trigger of VTE. A large proportion (15-22%) of the VTE burden worldwide can be attributed to surgery (1, 4, 5). Despite awareness and improved preventive strategies (28, 29), this proportion has slightly increased during the last decades (4). In the present study, surgery was confirmed to be a strong trigger associated with an 8-fold higher risk of VTE, and the OR for VTE by surgery decreased in a dose-dependent manner across tertiles of increasing n-3 PUFAs intake.

As proposed by Virchow, thrombus formation occurs as a result from changes in the vessel wall, blood flow (stasis) and blood coagulation (30). Surgery is a discrete event accompanied by several prothrombotic processes. Within hours or the first few days after surgery, higher levels of inflammatory markers (31) and coagulation factors (FVII, FV, FVIII and fibrinogen) as well as activation of monocytes (32) and platelets (33) can be measured. Furthermore, immobilization with subsequent stasis is a strong trigger for VTE (34, 35), which frequently accompanies surgical procedures. According to the thrombosis potential model, VTE occurs when the accumulation of risk factors overwhelms the anticoagulant

mechanisms and exceeds the thrombosis threshold (36). Data from experimental studies suggest several pathways through which n-3 PUFAs may diminish the trigger-effect of surgery. Specifically, tissue factor expression in endothelial cells have been downregulated experimentally in vitro by n-3 PUFAs (14), and the potential to activate monocytes and platelets has been reduced experimentally in vivo (11, 13). Additionally, a 40% reduction of the synthesis of tissue factor and a 10-20% reduction in serum arachidonic acid levels were shown in a trial where participants were given 25 ml cod liver oil daily for 8 weeks (13). In the latter study, the authors concluded that activation of the extrinsic pathway of coagulation may be suppressed by dietary enrichment of n-3 PUFAs. Thus far, no experimental study has investigated the effect of n-3 PUFAs intake on the risk of surgery-related VTE.

In the present study, the dose-dependent risk reduction of surgery-related VTE by n-3 PUFAs was strongest for surgery-related PE. As PE frequently develops as a complication of DVT, it may be speculated that a high n-3 PUFAs intake facilitates a more stable clot structure that is less likely to embolize. However, PE may also have a cardiac origin or develop *de novo* in the lungs (37). Although confounding by established comorbidities are controlled for by the study design in the present study, an alternative explanation may be a lower rate of PE-related post-surgical complications (e.g., atrial fibrillation) in those with a high n-3 PUFAs intake (38, 39).

Strengths of the present study include a large number of VTE cases recruited from a population-based cohort with high attendance rate and thorough adjudication of VTE events. Furthermore, the case-crossover design is well suited to study transient risk factors while controlling for time-invariant confounders, as participants act as their own controls. Some limitations also merit consideration. The information on triggers was collected retrospectively using medical records, and rely on thorough documentation by the treating health professionals. However, surgery is a procedure that is normally well-documented in medical

records and the likelihood of misclassification of surgery in hazard and control periods is low. Further, the case-crossover design is susceptible to confounding by factors that change over time within individuals. Assessment of n-3 PUFAs intake is challenging. The reliability of self-reported food intake is uncertain, and in the Tromsø Study the reproducibility of self-reported intake of fish is reported to be moderate (Spearman correlation coefficient = 0.41-0.56) (40). In the present study, we accounted for differential contributions from fat and lean marine foods as well as from supplements, and only those who with complete dietary information on these items were included. The n-3 PUFAs variable used in the present study corresponded well with serum levels of n-3 PUFAs, as shown in our previous report (20). However, the included participants were somewhat healthier and younger compared with the excluded (20), which limits the generalizability or our results. There was a varying time-lag between the assessment of n-3 PUFAs intake and the VTE event that add to the uncertainty of the n-3 PUFAs intake at the time of the event. However, this uncertainty presumably constitutes non-differential misclassification, which might dilute OR differences across n-3 PUFAs categories. Finally, even though confounding is controlled for within the case-crossover design, our comparison of ORs across the n-3 PUFAs categories could potentially be confounded by age differences in n-3 PUFAs intake. Yet, the beneficial impact of high n-3 PUFAs intake on surgery as a VTE trigger was still present in sensitivity analyses where we used two different approaches to control for age as a potential confounder.

In conclusion, a high intake of n-3 PUFAs appeared to mitigate the effect of major surgery as a trigger for VTE, and PE in particular. Whether n-3 PUFAs may exert a preventive effect in patients undergoing surgery is an intriguing question that may be properly addressed in a randomized controlled trial.

Conflict of Interest

None declared.

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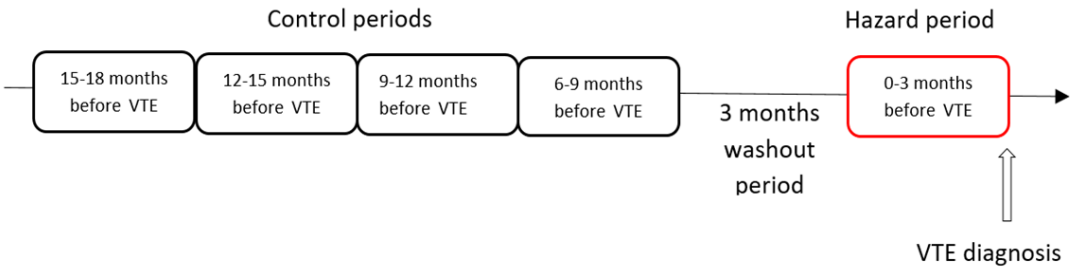

Figure 1 The case-crossover design. For each case of venous thromboembolism, surgery was recorded in the 90-day hazard period prior to the event and in four preceding 90-day control periods, separated by a 90-day washout period.

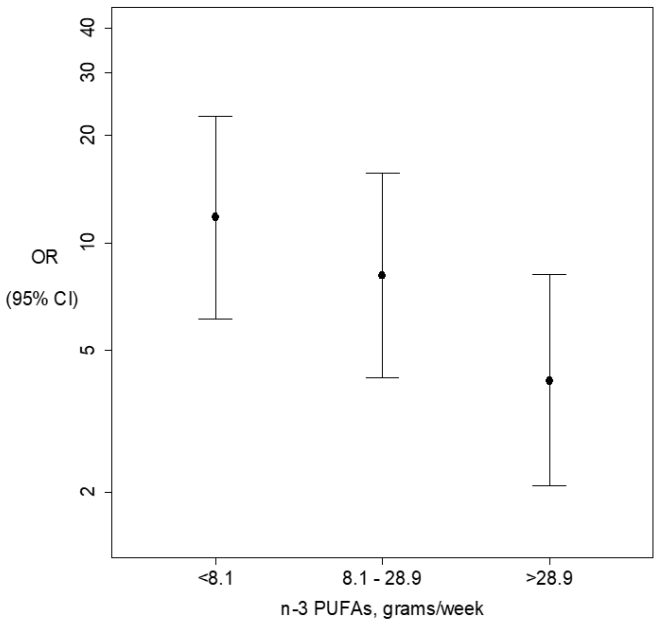

Figure 2 Odds ratios (OR) with 95% confidence intervals (CI) for surgery as a VTE trigger according to tertiles of weekly intake of n-3 polyunsaturated long-chained fatty acids (n-3 PUFAs).

Tables and Figures

Table 1 Characteristics of participants at the time of venous thromboembolism (VTE) diagnosis according to tertiles of n-3 polyunsaturated long-chained fatty acids (n-3 PUFA) intake (n=445).

		n-3 PUFA intake		
	All (n=445)	**Tertile 1 (n=148)**	**Tertile 2 (n=155)**	**Tertile 3 (n=142)**
Tertile range, grams/week	-	<8.1	8.1-28.9	>28.9
Age, years (± SD)	66.8 (±13.5)	62.6 (±14.3)	66.4 (±13.6)	72.0 (±10.9)
Male sex, n (%)	219 (49.2)	70 (47.3)	79 (51.0)	70 (49.3)
DVT, n (%)	257 (57.7)	80 (54.1)	98 (66.2)	79 (55.6)
PE, n (%)	188 (42.2)	68 (45.9)	57 (36.8)	63 (44.4)
Surgery-related VTE				
During hospitalization (n, %)	22 (26)	10 (12)	10 (12)	2 (2)
After hospitalization (n, %)	62 (74)	27 (32)	20 (24)	15 (18)

DVT, deep vein thrombosis; n-3 PUFAs, polyunsaturated long-chained fatty acids; PE, pulmonary embolism; VTE, venous thromboembolism.

Table 2 Distribution of surgery in the hazard and control periods according to tertiles of n-3 polyunsaturated long-chained fatty acids (n-3 PUFA) intake.

	Tertile 1 (n=148)		**Tertile 2 (n=155)**		**Tertile 3 (n=142)**	
	Hazard	**Control**	**Hazard**	**Control**	**Hazard**	**Control**
All VTE, n (%*)	37 (25)	15 (3)	30 (19)	21 (3)	17 (12)	17 (3)
PE, n (%)	19 (28)	6 (2)	8 (14)	6 (3)	7 (11)	10 (4)
DVT, n (%)	18 (23)	9 (3)	22 (22)	15 (4)	10 (13)	7 (2)

DVT, Deep vein thrombosis; n-3 PUFAs, polyunsaturated long-chained fatty acids; PE, Pulmonary embolism; VTE, venous thromboembolism.

*Percentage of all VTE events within each tertile category.

Table 3 Regression coefficients (β) with standard error (SE) and corresponding odds ratios (ORs) with 95% confidence intervals (CIs) for surgery as a trigger of incident venous thromboembolism (VTE) according to intake of n-3 polyunsaturated long-chained fatty acids (n-3 PUFAs).

			OR (95% CI)					
	All (n=445)	**Tertile 1 (n=148)**		**Tertile 2 (n=155)**		**Tertile 3 (n=142)**		
All VTE	7.6 (5.2-11.1)	11.8 (6.1-22.6)		8.1 (4.2-15.7)		4.1 (2.1-8.2)		
PE	6.8 (3.9-12.0)	14.7 (5.5-39.6)		7.0 (2.1-23.5)		2.8 (1.1-7.4)		
DVT	8.3 (5.0-13.8)	9.7 (4.0-23.2)		8.6 (3.9-18.9)		6.3 (2.3-17.5)		
			β (SE)					
All VTE	2.0 (0.19)	2.5 (0.33)		2.1 (0.34)		1.4 (0.35)		
PE	1.9 (0.29)	2.7 (0.50)		1.9 (0.62)		1.0 (0.49)		
DVT	2.1 (0.26)	2.3 (0.45)		2.2 (0.40)		1.8 (0.52)		
		Tertile 1 vs. Tertile 2			**Tertile 1 vs. Tertile 3**		**Tertile 2 vs. Tertile 3**	
		Δβ	**Z-score**	***p*-value**	**Δβ**	**Z-score**	***p*-value**	**Δβ**	**Z-score**	***p*-value**
All VTE	n/a	0.37	0.79	0.43	1.05	2.17	**0.03**	0.67	1.39	0.16
PE	n/a	0.75	0.94	0.35	1.66	2.36	**0.02**	0.91	1.15	0.25
DVT	n/a	0.12	0.19	0.85	0.42	0.62	0.53	0.31	0.47	0.64

DVT, deep vein thrombosis; CI, confidence interval; n-3 PUFAs, polyunsaturated long-chained fatty acids; OR, odds ratio; PE, pulmonary embolism; SE, standard error; VTE, venous thromboembolism

Supplementary Table 1 Characteristics of participants at the time of venous thromboembolism (VTE) diagnosis, and ORs for surgery in the hazard period according to tertiles based on residuals from a linear regression of n-3 PUFAs on age.

		Residual tertiles		
	All (n=445)	**Tertile 1 (n=149)**	**Tertile 2 (n=148)**	**Tertile 3 (n=148)**
Tertile range, residuals	-	< -9.8	-9.8-8.4	>8.4
Age, years (± SD)	66.8 (±13.5)	67.8 (±13.3)	63.9 (±14.9)	68.6 (±11.8)
Male sex, n (%)	219 (49.2)	71 (47.6)	74 (50.0)	74 (50.0)
DVT, n (%)	257 (57.7)	77 (51.7)	98 (66.2)	82 (55.4)
PE, n (%)	188 (42.2)	72 (48.3)	50 (33.8)	66 (44.6)
		ORs for surgery in the hazard period:		
All VTE	7.6 (5.2-11.1)	8.7 (4.9-15.4)	11.9 (5.4-26.3)	4.4 (2.2-8.6)
PE	6.8 (3.9-12.0)	11.9 (5.1-28.0)	3.6 (0.9-14.5)	4.0 (1.5-10.7)
DVT	8.3 (5.0-13.8)	6.4 (2.9-14.0)	20.2 (6.9-58.9)	4.7 (1.9-12.1)

DVT, deep vein thrombosis; n-3 PUFAs, polyunsaturated long-chained fatty acids; PE, pulmonary embolism; VTE, venous thromboembolism.

Supplementary Table 2 Distribution of surgery in the hazard and control periods according to residuals of n-3 polyunsaturated long-chained fatty acids (n-3 PUFA) intake.

	Tertile 1 (n=149)		Tertile 2 (n=148)		Tertile 3 (n=148)	
	Hazard	**Control**	**Hazard**	**Control**	**Hazard**	**Control**
All VTE, n (%*)	39 (26)	21 (4)	27 (18)	15 (3)	18 (12)	17 (3)
PE, n (%)	22 (31)	9 (3)	4 (8)	5 (3)	8 (16)	8 (3)
DVT, n (%)	17 (21)	12 (4)	23 (24)	10 (3)	10 (13)	9 (3)

DVT, Deep vein thrombosis; n-3 PUFAs, polyunsaturated long-chained fatty acids; PE, Pulmonary embolism; VTE, venous thromboembolism.

*Percentage of all VTE events within each tertile category.

APPENDIX

Helseundersøkelsen i Tromsø

Hovedformålet med Tromsøundersøkelsene er å skaffe ny kunnskap om hjerte-karsykdommer for å kunne forebygge dem. I tillegg skal undersøkelsen øke kunnskapen om kreftsykdommer og andre alminnelige plager som f.eks. allergier, smerter i muskulatur og nervøse lidelser. Vi ber deg derfor svare på noen spørsmål om forhold som kan ha betydning for risikoen for disse og andre sykdommer.

Skjemaet er en del av Helseundersøkelsen som er godkjent av Datatilsynet og av Regional komite for medisinsk forskningsetikk. Svarene brukes bare til forskning og behandles strengt fortrolig. Opplysningene kan senere bli sammenholdt med informasjon fra andre offentlige helseregistre etter de regler som Datatilsynet og Regional komite for medisinsk forskningsetikk gir.

Hvis du er i tvil om hva du skal svare, sett kryss i den ruten som du synes passer best.

Det utfylte skjema sendes i vedlagte svarkonvolutt. Portoen er betalt.

På forhånd takk for hjelpen!

Med vennlig hilsen

Fagområdet medisin
Universitetet i Tromsø **Statens helseundersøkelser**

Hvis du ikke ønsker å besvare spørreskjemaet, sett kryss i ruten under og returner skjemaet. Da slipper du purring.

Jeg ønsker ikke å besvare spørreskjemaet ..... 17 ❑

Dag Mnd År

Dato for utfylling av skjema: ..... 18 ...../...../.....

OPPVEKST

I hvilken kommune bodde du da du fylte 1 år?

..... 24 - 28

Hvis du ikke bodde i Norge, oppgi land i stedet for kommune.

Hvordan var de økonomiske forhold i familien under din oppvekst?

- Meget gode ..... 29 ❑
- Gode ..... ❑
- Vanskelige ..... ❑
- Meget vanskelige ..... ❑

Hvor mange av de første 3 årene av ditt liv
- bodde du i by? ..... 30 _____ år
- hadde dere katt eller hund i hjemmet? ..... 31 _____ år

Hvor mange av de første 15 årene av ditt liv
- bodde du i by? ..... 32 _____ år
- hadde dere katt eller hund i hjemmet? ..... 34 _____ år

BOLIG

Hvem bor du sammen med?

Sett ett kryss for hvert spørsmål og angi antall.		Ja	Nei	Antall
Ektefelle/samboer	36	❑	❑	
Andre personer over 18 år	37	❑	❑	_____
Personer under 18 år	40	❑	❑	_____

Hvor mange av barna har plass i barnehage? ..... 43 _____

Hvilken type bolig bor du i?
- Enebolig/villa ..... 45 ❑ 1
- Gårdsbruk ..... ❑ 2
- Blokk/terrasseleilighet ..... ❑ 3
- Rekkehus/2-4 mannsbolig ..... ❑ 4
- Annen bolig ..... ❑ 5

Hvor stor er din boenhet? ..... 46 _____ m^2

I omtrent hvilket år ble boligen bygget? ..... 49 _____

		Ja	Nei
Er boligen isolert etter 1970?	53	❑	❑
Bor du i underetasje/kjeller?	54	❑	❑
Hvis "Ja", er gulvbelegget lagt på betong?	55	❑	❑

Hvordan er boligen hovedsakelig oppvarmet?
- Elektrisk oppvarming ..... 56 ❑
- Vedfyring ..... ❑
- Sentralvarmeanlegg oppvarmet med:
  - Parafin ..... ❑
  - Elektrisitet ..... ❑

		Ja	Nei
Er det heldekkende tepper i stua?	60	❑	❑
Er det katt i boligen?	61	❑	❑
Er det hund i boligen?	62	❑	❑

ARBEID

Hvis du er i lønnet eller ulønnet arbeid, hvordan vil du beskrive ditt arbeid?
- For det meste stillesittende arbeid? ..... 63 ❑ 1
  (f.eks. skrivebordsarbeid, montering)
- Arbeid som krever at du går mye? ..... ❑ 2
  (f.eks. ekspeditørarb., lett industriarb., undervisning)
- Arbeid hvor du går og løfter mye? ..... ❑ 3
  (f.eks. postbud, pleier, bygningsarbeid)
- Tungt kroppsarbeid? ..... ❑ 4
  (f.eks. skogsarb., tungt jordbruksarb., tungt bygn.arb.)

Kan du selv bestemme hvordan arbeidet ditt skal legges opp?
- Nei, ikke i det hele tatt ..... 64 ❑ 1
- I liten grad ..... ❑ 2
- Ja, i stor grad ..... ❑ 3
- Ja, det bestemmer jeg selv ..... ❑ 4

		Ja	Nei
Har du skiftarbeid, nattarbeid eller går vakter?	65	❑	❑

Har du noen av følgende yrker (heltid eller deltid)?

Sett ett kryss for hvert spørsmål.		Ja	Nei
Sjåfør	66	❑	❑
Bonde/gårdbruker		❑	❑
Fisker		❑	❑

EGNE SYKDOMMER

Har du noen gang hatt:
Sett ett kryss for hvert spørsmål. Oppgi alderen ved hendelsen.
*Hvis det har skjedd flere ganger, hvor gammel var du **siste** gang?*

	Ja	Nei	Alder
Lårhalsbrudd 69	❑	❑	_____
Brudd ved håndledd/underarm 72	❑	❑	_____
Nakkesleng (whiplash) 75	❑	❑	_____
Skade som førte til sykehusinnleggelse 78	❑	❑	_____
Sår på magesekken 81	❑	❑	_____
Sår på tolvfingertarmen 84	❑	❑	_____
Magesår-operasjon 87	❑	❑	_____
Operasjon på halsen 90	❑	❑	_____

Har du eller har du hatt:
Sett ett kryss for hvert spørsmål.

	Ja	Nei
Kreftsykdom 93	❑	❑
Epilepsi (fallesyke)	❑	❑
Migrene	❑	❑
Kronisk bronkitt	❑	❑
Psoriasis	❑	❑
Benskjørhet (osteoporose) 98	❑	❑
Fibromyalgi/fibrositt/kronisk smertesyndrom	❑	❑
Psykiske plager som du har søkt hjelp for	❑	❑
Stoffskiftesykdom (skjoldbruskkjertel)	❑	❑
Sykdom i leveren	❑	❑
Nyrestein 103	❑	❑
Blindtarmsoperasjon	❑	❑
Allergi og overfølsomhet		
Atopisk eksem (f.eks. barneeksem)	❑	❑
Håndeksem	❑	❑
Høysnue	❑	❑
Matvareallergi 108	❑	❑
Annen overfølsomhet (ikke allergi)	❑	❑

Hvor mange ganger har du hatt forkjølelse, influensa, "ræksjuka" og lignende siste halvår? ..110 ____ ganger

	Ja	Nei
Har du hatt dette siste 14 dager? 112	❑	❑

SYKDOM I FAMILIEN

Kryss av for de slektningene som har eller har hatt noen av sykdommene:
Kryss av for "Ingen" hvis ingen av slektningene har hatt sykdommen.

	Mor	Far	Bror	Søster	Barn	Ingen
Hjerneslag eller hjerneblødning 113	❑	❑	❑	❑	❑	❑
Hjerteinfarkt før 60 års alder 119	❑	❑	❑	❑	❑	❑
Kreftsykdom 125	❑	❑	❑	❑	❑	❑
Astma 131	❑	❑	❑	❑	❑	❑
Mage/tolvfingertarm-sår 137	❑	❑	❑	❑	❑	❑
Benskjørhet (osteoporose) 143	❑	❑	❑	❑	❑	❑
Psykiske plager 149	❑	❑	❑	❑	❑	❑
Allergi 155	❑	❑	❑	❑	❑	❑
Diabetes (sukkersyke) 161	❑	❑	❑	❑	❑	❑
– alder da de fikk diabetes 167	____	____	____	____	____	____

SYMPTOMER

	Ja	Nei
Hoster du omtrent daglig i perioder av året? 177	❑	❑
Hvis "Ja":		
Er hosten vanligvis ledsaget av oppspytt? 178	❑	❑
Har du hatt slik hoste så lenge som i en 3 måneders periode i begge de to siste år? 179	❑	❑
Har du hatt episoder med piping i brystet? 180	❑	❑
Hvis "Ja", har dette oppstått:		
Sett ett kryss for hvert spørsmål.		
Om natten 181	❑	❑
Ved luftveisinfeksjoner	❑	❑
Ved fysiske anstrengelser	❑	❑
Ved sterk kulde	❑	❑
Har du merket anfall med plutselig endring i pulsen eller hjerterytmen siste år? 185	❑	❑

Hvor ofte er du plaget av søvnløshet?

Aldri, eller noen få ganger i året 186	❑ 1
1-2 ganger i måneden	❑ 2
Omtrent en gang i uken	❑ 3
Mer enn en gang i uken	❑ 4

Hvis du er plaget av søvnløshet i perioder, når på året er du mest plaget?

Ingen spesiell tid 187	❑ 1
Særlig i mørketiden	❑ 2
Særlig i midnattsoltiden	❑ 3
Særlig vår og høst	❑ 4

	Ja	Nei
Har du det siste året vært plaget av søvnløshet slik at det har gått ut over arbeidsevnen? 188	❑	❑

Hvor ofte er du plaget av hodepine?

Sjelden eller aldri 189	❑ 1
En eller flere ganger i måneden	❑ 2
En eller flere ganger i uken	❑ 3
Daglig	❑ 4

Hender det at tanken på å få alvorlig sykdom bekymrer deg?

Ikke i det hele tatt 190	❑ 1
Bare i liten grad	❑ 2
En del	❑ 3
Ganske mye	❑ 4

BRUK AV HELSEVESENET

Hvor mange ganger har du siste året, på grunn av egen helse eller sykdom, vært:
*Sett **0** hvis du **ikke** har hatt slik kontakt.*

	Antall ganger siste år
Hos vanlig lege/legevakt 191	_____
Hos psykolog eller psykiater	_____
Hos annen legespesialist utenfor sykehus	_____
På poliklinikk 197	_____
Innlagt i sykehus	_____
Hos bedriftslege	_____
Hos fysioterapeut 203	_____
Hos kiropraktor	_____
Hos akupunktør	_____
Hos tannlege 209	_____
Hos naturmedisiner (homøopat, soneterapeut o.l.)	_____
Hos håndspålegger, synsk eller "leser"	_____

LEGEMIDLER OG KOSTTILSKUDD

Har du det siste året periodevis brukt noen av de følgende midler daglig eller nesten daglig? Angi hvor mange måneder du brukte dem.
*Sett **0** hvis du **ikke** har brukt midlene.*

Legemidler
- Smertestillende ....215 _____ mnd.
- Sovemedisin .... _____ mnd.
- Beroligende midler .... _____ mnd.
- Medisin mot depresjon ....221 _____ mnd.
- Allergimedisin .... _____ mnd.
- Astmamedisin .... _____ mnd.

Kosttilskudd
- Jerntabletter ....227 _____ mnd.
- Kalktabletter eller benmel .... _____ mnd.
- Vitamin D-tilskudd .... _____ mnd.
- Andre vitamintilskudd ....233 _____ mnd.
- Tran eller fiskeoljekapsler .... _____ mnd.

Har du de siste 14 dager brukt følgende legemidler eller kosttilskudd?
*Sett **ett** kryss for **hvert** spørsmål.*

	Ja	Nei
Legemidler		
Smertestillende medisin 237	❑	❑
Febersenkende medisin	❑	❑
Migrenemedisin	❑	❑
Eksemsalve	❑	❑
Hjertemedisin (ikke blodtrykksmedisin)	❑	❑
Kolesterolsenkende medisin 242	❑	❑
Sovemedisin	❑	❑
Beroligende medisin	❑	❑
Medisin mot depresjon	❑	❑
Annen nervemedisin	❑	❑
Syrenøytraliserende midler 247	❑	❑
Magesårsmedisin	❑	❑
Insulin	❑	❑
Tabletter mot diabetes (sukkersyke)	❑	❑
Tabletter mot lavt stoffskifte (thyroxin)	❑	❑
Kortisontabletter 252	❑	❑
Annen medisin	❑	❑
Kosttilskudd		
Jerntabletter	❑	❑
Kalktabletter eller benmel	❑	❑
Vitamin D-tilskudd	❑	❑
Andre vitamintilskudd 257	❑	❑
Tran eller fiskeoljekapsler	❑	❑

VENNER

Hvor mange gode venner har du som du kan snakke fortrolig med og gi deg hjelp når du trenger det? ....259 ____ gode venner
Tell ikke med de du bor sammen med, men ta med andre slektninger!

Hvor mange av disse gode vennene har du kontakt med minst en gang i måneden? ....261 ____

	Ja	Nei
Føler du at du har nok gode venner? 263	❑	❑

Hvor ofte tar du vanligvis del i foreningsvirksomhet som f.eks. syklubb, idrettslag, politiske lag, religiøse eller andre foreninger?
- Aldri, eller noen få ganger i året ....264 ❑ 1
- 1-2 ganger i måneden .... ❑ 2
- Omtrent en gang i uken .... ❑ 3
- Mer enn en gang i uken .... ❑ 4

KOSTVANER

Hvis du bruker smør eller margarin på brødet, hvor mange skiver rekker en liten porsjonspakning vanligvis til? Vi tenker på slik porsjonspakning som du får på fly, på kafé o.l. (10-12 gram).

Den rekker til omtrent ....265 _____ skiver

Hva slags fett blir vanligvis brukt til **matlaging** (ikke på brødet) i din husholdning?
- Meierismør ....266 ❑
- Hard margarin .... ❑
- Bløt (Soft) margarin .... ❑
- Smør/margarin blanding .... ❑
- Oljer ....270 ❑

Hva slags type brød (kjøpt eller hjemmebakt) spiser du vanligvis?
Sett ett eller to kryss!

	Loff	Fint brød	Kneip-brød	Grov-brød	Knekke-brød
Brødtypen ligner mest på:	❑ 271	❑	❑	❑	❑ 275

Hvor mye (i **antall** glass, kopper, poteter eller brødskiver) spiser eller drikker du vanligvis **daglig** av følgende matvarer?
*Kryss av for **alle** matvarene.*

	0	Færre enn 1	1-2	3-4	5-6	Mer enn 6
Helmelk (søt eller sur) (glass) 276	❑	❑	❑	❑	❑	❑
Lettmelk (søt eller sur) (glass)	❑	❑	❑	❑	❑	❑
Skummet melk (søt eller sur) (glass)	❑	❑	❑	❑	❑	❑
Te (kopper)	❑	❑	❑	❑	❑	❑
Appelsinjuice (glass)	❑	❑	❑	❑	❑	❑
Poteter 281	❑	❑	❑	❑	❑	❑
Brødskiver totalt (inkl. knekkebrød)	❑	❑	❑	❑	❑	❑
Brødskiver med						
– fiskepålegg (f.eks. makrell i tomat)	❑	❑	❑	❑	❑	❑
– magert kjøttpålegg (f.eks. skinke)	❑	❑	❑	❑	❑	❑
– fetere kjøttpålegg (f.eks. salami)	❑	❑	❑	❑	❑	❑
– gulost 286	❑	❑	❑	❑	❑	❑
– brunost	❑	❑	❑	❑	❑	❑
– kaviar	❑	❑	❑	❑	❑	❑
– syltetøy og annet søtt pålegg	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	❑ 6

Hvor mange **ganger i uka** spiser du vanligvis følgende matvarer?
*Kryss av for **alle** matvarene.*

	Aldri	Færre enn 1	1	2-3	4-5	Omtrent daglig
Yoghurt 290	❑	❑	❑	❑	❑	❑
Kokt eller stekt egg	❑	❑	❑	❑	❑	❑
Frokostblanding/havregryn o.l.	❑	❑	❑	❑	❑	❑
Middag med						
– rent kjøtt	❑	❑	❑	❑	❑	❑
– pølser/kjøttpudding/-kaker	❑	❑	❑	❑	❑	❑
– feit fisk (f.eks. laks/uer) 295	❑	❑	❑	❑	❑	❑
– mager fisk (f.eks. torsk)	❑	❑	❑	❑	❑	❑
– fiskeboller/-pudding/-kaker	❑	❑	❑	❑	❑	❑
– grønnsaker	❑	❑	❑	❑	❑	❑
Majones, remulade o.l.	❑	❑	❑	❑	❑	❑
Gulrøtter 300	❑	❑	❑	❑	❑	❑
Blomkål/kål/brokkoli	❑	❑	❑	❑	❑	❑
Epler/pærer	❑	❑	❑	❑	❑	❑
Appelsiner, mandariner o.l.	❑	❑	❑	❑	❑	❑
Sukkerholdige leskedrikker	❑	❑	❑	❑	❑	❑
Sukkerfrie («Light») leskedrikker	❑	❑	❑	❑	❑	❑
Sjokolade	❑	❑	❑	❑	❑	❑
Vafler, kaker o.l. 307	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	❑ 6

ALKOHOL

Hvor ofte pleier du å drikke	øl?	vin?	brennevin?
Aldri, eller noen få ganger i året	❑ 308	❑	❑ 1
1-2 ganger i måneden	❑	❑	❑ 2
Omtrent 1 gang i uken	❑	❑	❑ 3
2-3 ganger i uken	❑	❑	❑ 4
Omtrent hver dag	❑	❑	❑ 5 310

Omtrent hvor ofte har du i løpet av siste år drukket alkohol tilsvarende minst 5 halvflasker øl, en helflaske vin eller 1/4 flaske brennevin?

- Ikke siste år ..... 311 ❑ 1
- Noen få ganger ..... ❑ 2
- 1 - 2 ganger per måned ..... ❑ 3
- 1 - 2 ganger i uken ..... ❑ 4
- 3 eller flere ganger i uken ..... ❑ 5

I omtrent hvor mange år har ditt alkoholforbruk vært slik du har svart i spørsmålene over? ..... 312 _____ år

SLANKING

Omtrent hvor mange ganger har du bevisst prøvd å slanke deg? Sett **0** hvis ingen forsøk.

- – før 20 år ..... 314 _____ ganger
- – senere ..... 316 _____ ganger

Hvis du har slanket deg, omtrent hvor mange kilo har du på det meste gått ned i vekt?

- – før 20 år ..... 318 _____ kg
- – senere ..... 320 _____ kg

Hvilken vekt ville du være tilfreds med (din "trivselsvekt")? ..... 322 _____ kg

UFRIVILLIG URINLEKKASJE

Hvor ofte har du ufrivillig urinlekkasje?

- Aldri ..... 325 ❑ 1
- Ikke mer enn en gang i måneden ..... ❑ 2
- To eller flere ganger i måneden ..... ❑ 3
- Ukentlig eller oftere ..... ❑ 4

Dine kommentarer:

BESVARES BARE AV KVINNER

MENSTRUASJON

Hvor gammel var du da du fikk menstruasjon første gang? ..... 326 _____ år

Hvis du ikke lenger har menstruasjon, hvor gammel var du da den sluttet? ..... 328 _____ år

Når du ser bort fra svangerskap og barselsperiode, har du noen gang vært blødningsfri i minst 6 måneder? 330	Ja ❑	Nei ❑

Hvis "Ja", hvor mange ganger? ..... 331 _____ ganger

Hvis du fremdeles har menstruasjon eller er gravid:

Hvilken dato startet din siste menstruasjon? ..... 333 dag/ mnd/ år ___/___/___

Bruker du vanligvis smertestillende legemidler for å dempe menstruasjonsplager? 339	Ja ❑	Nei ❑

SVANGERSKAP

Hvor mange barn har du født? ..... 340 _____ barn

Er du gravid nå? 342	Ja ❑	Nei ❑	Usikker ❑

Har du i forbindelse med svangerskap hatt for høyt blodtrykk og/eller eggehvite (protein) i urinen? 343	Ja ❑	Nei ❑

Hvis "Ja", i hvilket svangerskap?

	Svangerskap Første	Senere
For høyt blodtrykk 344	❑	❑
Eggehvite i urinen 346	❑	❑

Hvis du har født, fyll ut for hvert barn barnets fødselsår og omtrent antall måneder du ammet barnet.

Barn:	Fødselsår:	Antall måneder med amming:
1	348 _____	_____
2	_____	_____
3	356 _____	_____
4	_____	_____
5	364 _____	_____
6	_____	_____

PREVENSJON OG ØSTROGEN

Bruker du, eller har du brukt:	Nå	Før	Aldri
P-pille (også minipille) 372	❑	❑	❑
Hormonspiral	❑	❑	❑
Østrogen (tabletter eller plaster) 374	❑	❑	❑
Østrogen (krem eller stikkpiller)	❑ 1	❑ 2	❑ 3

Hvis du bruker p-pille, hormonspiral eller østrogen; hvilket merke bruker du nå?

376 .....

Hvis du bruker eller har brukt p-pille:

Alder da du begynte med P-piller? ..... 380 _____ år

Hvor mange år har du tilsammen brukt P-piller? ..... 382 _____ år

Dersom du har født, hvor mange år brukte du P-piller før første fødsel? ..... 384 _____ år

Hvis du har sluttet å bruke P-piller:

Alder da du sluttet? ..... 386 _____ år

Takk for hjelpen! Husk å postlegge skjemaet idag!

Helseundersøkelsen i Tromsø

Helseundersøkelsen i Tromsø

for dem som er 70 år og eldre.

Hovedformålet med Tromsøundersøkelsene er å skaffe ny kunnskap om hjerte-karsykdommer for å kunne forebygge dem. De skal også øke kunnskapen om kreftsykdommer og alminnelige plager som f.eks. allergier, smerter i muskulatur og nervøse lidelser. Endelig skal de gi kunnskap om hvorledes den eldste delen av befolkningen har det. Vi ber deg derfor svare på spørsmålene nedenfor.

Skjemaet er en del av Helseundersøkelsen som er godkjent av Datatilsynet og av Regional komite for medisinsk forskningsetikk. Svarene brukes bare til forskning og behandles strengt fortrolig. Opplysningene kan senere bli sammenholdt med informasjon fra andre offentlige helseregistre etter de regler som Datatilsynet og Regional komite for medisinsk forskningsetikk gir.

Hvis du er i tvil om hva du skal svare, sett kryss i den ruten som du synes passer best.

Det utfylte skjema sendes i vedlagte svarkonvolutt. Portoen er betalt.

På forhånd takk for hjelpen!

Med vennlig hilsen

Fagområdet medisin
Universitetet i Tromsø

Statens helseundersøkelser

Hvis du ikke ønsker å besvare spørreskjemaet, sett kryss i ruten under og returner skjemaet. Da slipper du purring.

Jeg ønsker ikke å besvare spørreskjemaet ..... 17 ❑

Dato for utfylling av skjema: ..... 18 Dag ...../ Mnd ...../ År .....

OPPVEKST

I hvilken kommune bodde du da du fylte 1 år?

..... 24 -28

Hvis du ikke bodde i Norge, oppgi land i stedet for kommune.

Hvordan var de økonomiske forhold i familien under din oppvekst?

- Meget gode ..... 29 ❑ 1
- Gode ..... ❑ 2
- Vanskelige ..... ❑ 3
- Meget vanskelige ..... ❑ 4

Hvor gamle ble dine foreldre?

- Mor ble ..... 30 _____ år
- Far ble ..... 32 _____ år

BOLIG

Hvem bor du sammen med?

Sett ett kryss for hvert spørsmål og angi antall.	Ja	Nei	Antall
Ektefelle/samboer 34	❑	❑	
Andre personer over 18 år 35	❑	❑	_____
Personer under 18 år 38	❑	❑	_____

Hvilken type bolig bor du i?

- Enebolig/villa ..... 41 ❑ 1
- Gårdsbruk ..... ❑ 2
- Blokk/terrasseleilighet ..... ❑ 3
- Rekkehus/2-4 mannsbolig ..... ❑ 4
- Annen bolig ..... ❑ 5

Hvor lenge har du bodd i boligen du bor i nå? ..... 42 _____ år

	Ja	Nei
Er boligen tilpasset til dine behov? 44	❑	❑
Hvis "Nei", er det problemer med:		
Plassen i boligen 45	❑	❑
Ujevn, for høy eller for lav temperatur 46	❑	❑
Trapper 47	❑	❑
Toalett 48	❑	❑
Bad/dusj 49	❑	❑
Vedlikehold 50	❑	❑
Annet (spesifiser) 51	❑	❑
Ønsker du å flytte til en eldrebolig? 52	❑	❑

TIDLIGERE ARBEID OG ØKONOMI

Hvordan vil du beskrive det arbeidet du hadde de siste 5-10 årene før du ble pensjonist?

- For det meste stillesittende arbeid? ..... 53 ❑ 1
  (f.eks. skrivebordsarbeid, montering)
- Arbeid som krever at du går mye? ..... ❑ 2
  (f.eks. ekspeditørarbeid, husmor, undervisning)
- Arbeid hvor du går og løfter mye? ..... ❑ 3
  (f.eks. postbud, pleier, bygningsarbeid)
- Tungt kroppsarbeid? ..... ❑ 4
  (f.eks. skogsarb., tungt jordbruksarb., tungt bygn.arb.)

Har du hatt noen av følgende yrker (heltid eller deltid)?

Sett ett kryss for hvert spørsmål.	Ja	Nei
Sjåfør 54	❑	❑
Bonde/gårdbruker 55	❑	❑
Fisker 56	❑	❑

Hvor gammel var du da du ble pensjonert? ..... 57 _____ år

Hva slags pensjon har du?

- Minstepensjon ..... 59 ❑
- Tilleggspensjon ..... 60 ❑

Hvordan er din økonomi nå?

- Meget god ..... 61 ❑ 1
- God ..... ❑ 2
- Vanskelig ..... ❑ 3
- Meget vanskelig ..... ❑ 4

HELSE OG SYKDOM

Er helsen din blitt forandret det siste året?

- Ja, dårligere ..... 62 ❑ 1
- Nei, uforandret ..... ❑ 2
- Ja, bedre ..... ❑ 3

Hvordan synes du at helsen din er nå i forhold til andre på samme alder?

- Mye dårligere ..... 63 ❑ 1
- Litt dårligere ..... ❑ 2
- Omtrent lik ..... ❑ 3
- Litt bedre ..... ❑ 4
- Mye bedre ..... ❑ 5

EGNE SYKDOMMER

Har du noen gang hatt:

Sett ett kryss for hvert spørsmål. Oppgi alderen ved hendelsen. Hvis det har skjedd flere ganger, hvor gammel var du siste gang?

	Ja	Nei	Alder
Lårhalsbrudd 64	❑	❑	_____
Brudd ved håndledd/underarm 67	❑	❑	_____
Nakkesleng (whiplash) 70	❑	❑	_____
Skade som førte til sykehusinnleggelse 73	❑	❑	_____
Sår på magesekken 76	❑	❑	_____
Sår på tolvfingertarmen 79	❑	❑	_____
Magesår-operasjon 82	❑	❑	_____
Operasjon på halsen 85	❑	❑	_____

Har du eller har du hatt:

Sett ett kryss for hvert spørsmål.	Ja	Nei
Kreftsykdom 88	❑	❑
Epilepsi (fallesyke)	❑	❑
Migrene	❑	❑
Parkinsons sykdom	❑	❑
Kronisk bronkitt	❑	❑
Psoriasis 93	❑	❑
Benskjørhet (osteoporose)	❑	❑
Fibromyalgi/fibrositt/kronisk smertesyndrom	❑	❑
Psykiske plager som du har søkt hjelp for	❑	❑
Stoffskiftesykdom (skjoldbruskkjertel)	❑	❑
Sykdom i leveren 98	❑	❑
Gjentatt, ufrivillig urinlekkasje	❑	❑
Grønn stær	❑	❑
Grå stær	❑	❑
Slitasjegikt (artrose)	❑	❑
Leddgikt 103	❑	❑
Nyrestein	❑	❑
Blindtarmsoperasjon	❑	❑
Allergi og overfølsomhet		
Atopisk eksem (f.eks. barneeksem)	❑	❑
Håndeksem	❑	❑
Høysnue 108	❑	❑
Matvareallergi	❑	❑
Annen overfølsomhet (ikke allergi)	❑	❑

Hvor mange ganger har du hatt forkjølelse, influensa, "ræksjuka" og lignende siste halvår? 111 ____ ganger

	Ja	Nei
Har du hatt dette de siste 14 dager? 113	❑	❑

SYKDOM I FAMILIEN

Kryss av for de slektningene som har eller har hatt noen av sykdommene:

Kryss av for "Ingen" hvis ingen av slektningene har hatt sykdommen.

	Mor	Far	Bror	Søster	Barn	Ingen
Hjerneslag eller hjerneblødning 114	❑	❑	❑	❑	❑	❑
Hjerteinfarkt før 60 års alder 120	❑	❑	❑	❑	❑	❑
Kreftsykdom 126	❑	❑	❑	❑	❑	❑
Høyt blodtrykk 132	❑	❑	❑	❑	❑	❑
Astma 138	❑	❑	❑	❑	❑	❑
Benskjørhet (osteoporose) 144	❑	❑	❑	❑	❑	❑
Slitasjegikt (artrose) 150	❑	❑	❑	❑	❑	❑
Psykiske plager 156	❑	❑	❑	❑	❑	❑
Alderdomssløvhet 162	❑	❑	❑	❑	❑	❑
Diabetes (sukkersyke) 168	❑	❑	❑	❑	❑	❑
– alder da de fikk diabetes 174	__	__	__	__	__	

SYMPTOMER

	Ja	Nei
Hoster du omtrent daglig i perioder av året? 184	❑	❑
Hvis "Ja":		
Er hosten vanligvis ledsaget av oppspytt? 185	❑	❑
Har du hatt slik hoste så lenge som i en 3 måneders periode i begge de to siste år? 186	❑	❑
Har du hatt episoder med piping i brystet? 187	❑	❑
Hvis "Ja", har dette oppstått:		
Sett ett kryss for hvert spørsmål.		
Om natten 188	❑	❑
Ved luftveisinfeksjoner	❑	❑
Ved fysiske anstrengelser	❑	❑
Ved sterk kulde 191	❑	❑
Har du merket anfall med plutselig endring i pulsen eller hjerterytmen siste år? 192	❑	❑
Har du gått ned i vekt siste året? 193	❑	❑
Hvis "Ja":		
Hvor mange kilo? 194	_____ kg	

Hvor ofte er du plaget av søvnløshet?

- Aldri, eller noen få ganger i året ..... 196 ❑ 1
- 1-2 ganger i måneden ..... ❑ 2
- Omtrent en gang i uken ..... ❑ 3
- Mer enn en gang i uken ..... ❑ 4

Hvis du er plaget av søvnløshet i perioder, når på året er du mest plaget?

- Ingen spesiell tid ..... 197 ❑ 1
- Særlig i mørketiden ..... ❑ 2
- Særlig i midnattsoltiden ..... ❑ 3
- Særlig vår og høst ..... ❑ 4

	Ja	Nei
Pleier du å ta en lur på dagen? 198	❑	❑
Føler du at du vanligvis får nok søvn?	❑	❑

Er du plaget av:	Nei	Litt	I stor grad
Svimmelhet 200	❑	❑	❑
Dårlig hukommelse	❑	❑	❑
Kraftløshet	❑	❑	❑
Forstoppelse 203	❑	❑	❑

Hender det at tanken på å få alvorlig sykdom bekymrer deg?

- Ikke i det hele tatt ..... 204 ❑
- Bare i liten grad ..... ❑
- En del ..... ❑
- Ganske mye ..... ❑

LEGEMLIGE FUNKSJONER

Klarer du selv disse gjøremålene i det daglige uten hjelp fra andre?	Ja	Med noe hjelp	Nei
Gå innendørs i samme etasje 205	❑	❑	❑
Gå i trapper	❑	❑	❑
Gå utendørs	❑	❑	❑
Gå ca. 500 meter	❑	❑	❑
Gå på toalettet	❑	❑	❑
Vaske deg på kroppen 210	❑	❑	❑
Bade eller dusje	❑	❑	❑
Kle på og av deg	❑	❑	❑
Legge deg og stå opp	❑	❑	❑
Spise selv	❑	❑	❑
Lage varm mat 215	❑	❑	❑
Gjøre lett husarbeid (f.eks. oppvask)	❑	❑	❑
Gjøre tyngre husarbeid (f.eks. gulvvask)	❑	❑	❑
Gjøre innkjøp	❑	❑	❑
Ta bussen	❑	❑	❑

	Ja	Vanskelig	Nei
Kan du høre vanlig tale (evt. med høreapparat)? 220	❑	❑	❑
Kan du lese (evt. med briller)? 221	❑	❑	❑

Er du avhengig av noen av disse hjelpemidlene?

	Ja	Nei
Stokk 222	❑	❑
Krykke	❑	❑
Gåstol (rullator)	❑	❑
Rullestol	❑	❑
Høreapparat	❑	❑
Trygghetsalarm 227	❑	❑

BRUK AV HELSEVESENET

Hvor mange ganger har du siste året, på grunn av egen helse eller sykdom, vært: *Sett 0 hvis du ikke har hatt slik kontakt.*	Antall ganger siste år
Hos vanlig lege/legevakt 228	_____
Hos psykolog eller psykiater	_____
Hos annen legespesialist utenfor sykehus	_____
På poliklinikk 234	_____
Innlagt i sykehus	_____
Hos fysioterapeut	_____
Hos kiropraktor 240	_____
Hos akupunktør	_____
Hos tannlege	_____
Hos fotterapeut 246	_____
Hos naturmedisiner (homøopat, soneterapeut o.l.)	_____
Hos håndspålegger, synsk eller "leser"	_____

Har du hjemmehjelp?	Ja	Nei
Privat 252	❑	❑
Kommunal	❑	❑

Har du hjemmesykepleie? ..... ❑ ❑

Er du fornøyd med helse- og hjemmetjenesten i kommunen?	Ja	Nei	Vet ikke
Prinsippet med fast lege 255	❑	❑	❑
Hjemmesykepleien	❑	❑	❑
Hjemmehjelpen	❑	❑	❑

Er du trygg på at du kan få hjelp av helse- og hjemmetjenesten hvis du trenger det?

- Trygg ..... 258 ❑ 1
- Ikke trygg ..... ❑ 2
- Svært utrygg ..... ❑ 3
- Vet ikke ..... ❑ 4

LEGEMIDLER OG KOSTTILSKUDD

Har du det siste året periodevis brukt noen av de følgende midler daglig eller nesten daglig? Angi hvor mange måneder du brukte dem.
Sett 0 hvis du ikke har brukt midlene.

Legemidler

- Smertestillende ..... 259 _____ mnd.
- Sovemedisin ..... _____ mnd.
- Beroligende midler ..... _____ mnd.
- Medisin mot depresjon ..... 265 _____ mnd.
- Allergimedisin ..... _____ mnd.
- Astmamedisin ..... _____ mnd.
- Hjertemedisin (ikke blodtrykksmedisin) ..... 271 _____ mnd.
- Insulin ..... _____ mnd.
- Tabletter mot diabetes (sukkersyke) ..... _____ mnd.
- Tabletter mot lavt stoffskifte (thyroxin) ..... 277 _____ mnd.
- Kortisontabletter ..... _____ mnd.
- Midler mot forstoppelse ..... _____ mnd.

Kosttilskudd

- Jerntabletter ..... 283 _____ mnd.
- Vitamin D-tilskudd ..... _____ mnd.
- Andre vitamintilskudd ..... _____ mnd.
- Kalktabletter eller benmel ..... 289 _____ mnd.
- Tran eller fiskeoljekapsler ..... _____ mnd.

FAMILIE OG VENNER

Har du nær familie som kan gi deg hjelp og støtte når du trenger det? 293	Ja	Nei
	❑	❑

Hvis "Ja": Hvem kan gi deg hjelp?

- Ektefelle/samboer ..... 294 ❑
- Barn ..... ❑
- Andre ..... ❑

Hvor mange gode venner har du som du kan snakke fortrolig med og gi deg hjelp når du trenger det? ..297 ____ **gode venner**
Tell ikke med dem du bor sammen med, men ta med andre slektninger!

	Ja	Nei
Føler du at du har nok gode venner? 299	❑	❑

Føler du at du hører med i et fellesskap (gruppe av mennesker) som stoler på hverandre og føler forpliktelse overfor hverandre (f.eks. i politisk parti, religiøs gruppe, slekt, naboskap, arbeidsplass eller organisasjon)?

- Sterk tilhørighet ..... 300 ❑ 1
- Noe tilhørighet ..... ❑ 2
- Usikkert ..... ❑ 3
- Liten eller ingen tilhørighet ..... ❑ 4

Hvor ofte tar du vanligvis del i foreningsvirksomhet som f.eks. syklubb, idrettslag, politiske lag, religiøse eller andre foreninger?

- Aldri, eller noen få ganger i året ..... 301 ❑ 1
- 1-2 ganger i måneden ..... ❑ 2
- Omtrent en gang i uken ..... ❑ 3
- Mer enn en gang i uken ..... ❑ 4

KOSTVANER

Hvor mange måltider spiser du vanligvis daglig (middag og brødmåltid)? ..... 302 _____ Antall

Hvor mange ganger i uken spiser du varm middag? ..... 304 _____

Hva slags type brød (kjøpt eller hjemmebakt) spiser du vanligvis?
Sett ett eller to kryss.

	Loff	Fint brød	Kneip-brød	Grov-brød	Knekke-brød
Brødtypen ligner mest på:	❑ 306	❑	❑	❑	❑ 310

Hva slags fett blir til vanligvis brukt til *matlaging* (ikke på brødet) i din husholdning?

- Meierismør ..... 311 ❑
- Hard margarin ..... ❑
- Bløt (Soft) margarin ..... ❑
- Smør/margarin blanding ..... ❑
- Oljer ..... 315 ❑

Hvor mye (i antall glass, poteter eller brødskiver) spiser/drikker du vanligvis daglig av følgende matvarer?
Kryss av for alle matvarene.

	Ingen	Mindre enn 1	1-2	3 og mer
Melk alle sorter (glass) 316	❑	❑	❑	❑
Appelsinjuice (glass)	❑	❑	❑	❑
Poteter	❑	❑	❑	❑
Brødskiver totalt (inkl. knekkebrød) ...	❑	❑	❑	❑
Brødskiver med				
– fiskepålegg (f.eks. makrell i tomat)	❑	❑	❑	❑
– gulost	❑	❑	❑	❑
– kaviar 322	❑ 1	❑ 2	❑ 3	❑ 4

Hvor mange ganger i uka spiser du vanligvis følgende matvarer?
Kryss av for alle matvarene.

	Aldri	Sjeldnere enn 1	1	2 og mer
Yoghurt 323	❑	❑	❑	❑
Kokt eller stekt egg	❑	❑	❑	❑
Frokostblanding/havregryn o.l.	❑	❑	❑	❑
Middag med				
– rent kjøtt	❑	❑	❑	❑
– feit fisk (f.eks. laks/uer)	❑	❑	❑	❑
– mager fisk (f.eks. torsk) 328	❑	❑	❑	❑
– grønnsaker (rå eller kokte)	❑	❑	❑	❑
Gulrøtter (rå eller kokte)	❑	❑	❑	❑
Blomkål/kål/brokkoli	❑	❑	❑	❑
Epler/pærer	❑	❑	❑	❑
Appelsiner, mandariner o.l. 333	❑ 1	❑ 2	❑ 3	❑ 4

TRIVSEL

Hvordan trives du med å bli gammel - alt i alt?

- Godt ..... 334 ❑ 1
- Ganske bra ..... ❑ 2
- Opp og ned ..... ❑ 3
- Dårlig ..... ❑ 4

Hvordan ser du på livet fremover?

- Lyst ..... 335 ❑ 1
- Ikke så verst ..... ❑ 2
- Nokså bekymret ..... ❑ 3
- Mørkt ..... ❑ 4

BESVARES BARE AV KVINNER

MENSTRUASJON

Hvor gammel var du da du fikk menstruasjon første gang? ..... 336 _____ år

Hvor gammel var du da menstruasjonen sluttet? ..... 338 _____ år

SVANGERSKAP

Hvor mange barn har du født? ..... 340 _____ barn

Hvis du har født, fyll ut for hvert barn barnets fødselsår og omtrent antall måneder du ammet barnet.
Hvis du har født mer enn 6 barn, noter fødselsår og antall måneder med amming for dem nederst på siden.

Barn:	Fødselsår:	Antall måneder med amming:
1	342 _____	_____
2	346 _____	_____
3	_____	_____
4	_____	_____
5	358 _____	_____
6	_____	_____

Har du i forbindelse med svangerskap hatt for høyt blodtrykk og/eller eggehvite (protein) i urinen? ..... 366 Ja ❑ Nei ❑

Hvis "Ja", i hvilket svangerskap?

	Svangerskap Første	Senere
For høyt blodtrykk 367	❑	❑
Eggehvite i urinen 369	❑	❑

ØSTROGEN-MEDISIN

Bruker du, eller har du brukt, østrogen-medisin?

	Nå	Før	Aldri
Tabletter eller plaster 371	❑	❑	❑
Krem eller stikkpiller 372	❑	❑	❑

Hvis du bruker østrogen, hvilket merke bruker du nå?

..... 373

Dine kommentarer:

Takk for hjelpen! Husk å postlegge skjemaet idag!
Helseundersøkelsen i Tromsø

Tromsø-undersøkelsen

Skjemaet skal leses optisk. Vennligst bruk blå eller sort penn. Du kan ikke bruke komma, bruk blokkbokstaver.

2007 – 2008 KONFIDENSIELT

HELSE OG SYKDOMMER

1 **Hvordan vurderer du din egen helse sånn i alminnelighet?**

- ☐ Meget god
- ☐ God
- ☐ Verken god eller dårlig
- ☐ Dårlig
- ☐ Meget dårlig

2 **Hvordan synes du at helsen din er sammenlignet med andre på din alder?**

- ☐ Mye bedre
- ☐ Litt bedre
- ☐ Omtrent lik
- ☐ Litt dårligere
- ☐ Mye dårligere

3 **Har du eller har du hatt?**

	Ja	Nei	Alder første gang
Hjerteinfarkt	☐	☐	
Angina pectoris *(hjertekrampe)*	☐	☐	
Hjerneslag/hjerneblødning	☐	☐	
Hjerteflimmer *(atrieflimmer)*	☐	☐	
Høyt blodtrykk	☐	☐	
Beinskjørhet *(osteoporose)*	☐	☐	
Astma	☐	☐	
Kronisk bronkitt/emfysem/KOLS	☐	☐	
Diabetes	☐	☐	
Psykiske plager *(som du har søkt hjelp for)*	☐	☐	
Lavt stoffskifte	☐	☐	
Nyresykdom, unntatt urinveisinfeksjon	☐	☐	
Migrene	☐	☐	

4 **Har du langvarige eller stadig tilbakevendende smerter som har vart i 3 måneder eller mer?**

☐ Ja ☐ Nei

5 **Hvor ofte har du vært plaget av søvnløshet de siste 12 måneder?**

- ☐ Aldri, eller noen få ganger
- ☐ 1-3 ganger i måneden
- ☐ Omtrent 1 gang i uken
- ☐ Mer enn 1 gang i uken

6 **Under finner du en liste over ulike problemer. Har du opplevd noe av dette den siste uken (til og med i dag)?** (Sett ett kryss for hver plage)

	Ikke plaget	Litt plaget	Ganske mye	Veldig mye
Plutselig frykt uten grunn	☐	☐	☐	☐
Føler deg redd eller engstelig	☐	☐	☐	☐
Matthet eller svimmelhet	☐	☐	☐	☐
Føler deg anspent eller oppjaget	☐	☐	☐	☐
Lett for å klandre deg selv	☐	☐	☐	☐
Søvnproblemer	☐	☐	☐	☐
Nedtrykt, tungsindig	☐	☐	☐	☐
Følelse av å være unyttig, lite verd	☐	☐	☐	☐
Følelse av at alt er et slit	☐	☐	☐	☐
Følelse av håpløshet mht. framtida	☐	☐	☐	☐

BRUK AV HELSETJENESTER

7 **Har du i løpet av de siste 12 måneder vært hos:** Hvis JA; Hvor mange ganger?

	Ja	Nei	Ant ggr
Fastlege/allmennlege	☐	☐	
Psykiater/psykolog	☐	☐	
Legespesialist utenfor sykehus *(utenom fastlege/allmennlege/psykiater)*	☐	☐	
Fysioterapeut	☐	☐	
Kiropraktor	☐	☐	
Annen behandler *(homøopat, akupunktør, fotsoneterapeut, naturmedisiner, håndspålegger, healer, synsk el.l)*	☐	☐	
Tannlege/tannpleier	☐	☐	

8 **Har du i løpet av de siste 12 måneder vært på sykehus?**

	Ja	Nei	Ant ggr
Innlagt på sykehus	☐	☐	
Konsultasjon ved sykehus uten innleggelse;			
Ved psykiatrisk poliklinikk	☐	☐	
Ved annen sykehuspoliklinikk	☐	☐	

9 **Har du gjennomgått noen form for operasjon i løpet av de siste 3 årene?**

☐ Ja ☐ Nei

BRUK AV MEDISINER

10 **Bruker du, eller har du brukt, noen av følgende medisiner?** (Sett ett kryss for hver linje)

	Aldri brukt	Nå	Før	Alder første gang
Medisin mot høyt blodtrykk	☐	☐	☐	
Kolesterolsenkende medisin	☐	☐	☐	
Medisin mot hjertesykdom	☐	☐	☐	
Vanndrivende medisin	☐	☐	☐	
Medisin mot beinskjørhet *(osteoporose)*	☐	☐	☐	
Insulin	☐	☐	☐	
Diabetesmedisin *(tabletter)*	☐	☐	☐	
Stoffskiftemedisinene Thyroxin/levaxin	☐	☐	☐	

11 **Hvor ofte har du i løpet av de siste 4 ukene brukt følgende medisiner?** (Sett ett kryss pr linje)

	Ikke brukt siste 4 uker	Sjeldnere enn hver uke	Hver uke, men ikke daglig	Daglig
Smertestillende på resept	☐	☐	☐	☐
Smertestillende reseptfrie	☐	☐	☐	☐
Sovemidler	☐	☐	☐	☐
Beroligende medisiner	☐	☐	☐	☐
Medisin mot depresjon	☐	☐	☐	☐

12 **Skriv ned alle medisiner – både de med og uten resept – som du har brukt regelmessig i siste 4 ukers periode.** (Ikke regn med vitaminer, mineraler, urter, naturmedisin, andre kosttilskudd etc.)

Får du ikke plass til alle medisiner, bruk eget ark.

VED FRAMMØTE vil du bli spurt om du har brukt antibiotika eller smertestillende medisiner de siste 24 timene. Om du har det, vil vi be om at du oppgir preparat, styrke, dose og tidspunkt

FAMILIE OG VENNER

13 **Hvem bor du sammen med?** (Sett kryss for hvert spørsmål og angi antall)

	Ja	Nei	Antall
Ektefelle/samboer	☐	☐	
Andre personer over 18 år	☐	☐	
Personer under 18 år	☐	☐	

14 **Kryss av for de slektninger som har eller har hatt**

	Foreldre	Barn	Søsken
Hjerteinfarkt	☐	☐	☐
Hjerteinfarkt før fylte 60 år	☐	☐	☐
Angina pectoris *(hjertekrampe)*	☐	☐	☐
Hjerneslag/hjerneblødning	☐	☐	☐
Beinskjørhet *(osteoporose)*	☐	☐	☐
Magesår/tolvfingertarmsår	☐	☐	☐
Astma	☐	☐	☐
Diabetes	☐	☐	☐
Demens	☐	☐	☐
Psykiske plager	☐	☐	☐
Rusproblemer	☐	☐	☐

15 **Har du nok venner som kan gi deg hjelp når du trenger det?**

☐ Ja ☐ Nei

16 **Har du nok venner som du kan snakke fortrolig med?**

☐ Ja ☐ Nei

17 **Hvor ofte tar du vanligvis del i foreningsvirksomhet som for eksempel syklubb, idrettslag, politiske lag, religiøse eller andre foreninger?**

☐ Aldri, eller noen få ganger i året
☐ 1-2 ganger i måneden
☐ Omtrent 1 gang i uken
☐ Mer enn en gang i uken

ARBEID, TRYGD OG INNTEKT

18 **Hva er din høyeste fullførte utdanning?** (Sett ett kryss)

☐ Grunnskole, framhaldsskole eller folkehøyskole
☐ Yrkesfaglig videregående, yrkesskole eller realskole
☐ Allmennfaglig videregående skole eller gymnas
☐ Høyskole eller universitet, mindre enn 4 år
☐ Høyskole eller universitet, 4 år eller mer

19 **Hva er din hovedaktivitet?** (Sett ett kryss)

☐ Yrkesaktiv heltid
☐ Yrkesaktiv deltid
☐ Arbeidsledig
☐ Hjemmeværende
☐ Pensjonist/trygdet
☐ Student/militærtjeneste

20 **Mottar du noen av følgende ytelser?**

- ☐ Alderstrygd, førtidspensjon (AFP) eller etterlattepensjon
- ☐ Sykepenger (er sykemeldt)
- ☐ Rehabiliterings-/attføringspenger
- ☐ Uføreytelse/pensjon, hel
- ☐ Uføreytelse/pensjon, delvis
- ☐ Dagpenger under arbeidsledighet
- ☐ Overgangstønad
- ☐ Sosialhjelp/-stønad

21 **Hvor høy var husholdningens samlede bruttoinntekt siste år?** Ta med alle inntekter fra arbeid, trygder, sosialhjelp og lignende.

- ☐ Under 125 000 kr
- ☐ 125 000-200 000 kr
- ☐ 201 000-300 000 kr
- ☐ 301 000-400 000 kr
- ☐ 401 000-550 000 kr
- ☐ 551 000-700 000 kr
- ☐ 701 000 -850 000 kr
- ☐ Over 850 000 kr

22 **Arbeider du utendørs minst 25 % av tiden, eller i lokaler med lav temperatur, som for eksempel lager-/industrihaller?**

- ☐ Ja
- ☐ Nei

FYSISK AKTIVITET

23 **Hvis du er i lønnet eller ulønnet arbeid, hvordan vil du beskrive arbeidet ditt?**

- ☐ For det meste stillesittende arbeid *(f.eks. skrivebordsarbeid, montering)*
- ☐ Arbeid som krever at du går mye *(f.eks ekspeditørarbeid, lett industriarbeid, undervisning)*
- ☐ Arbeid der du går og løfter mye *(f.eks postbud, pleier, bygningsarbeider)*
- ☐ Tungt kroppsarbeid

24 **Angi bevegelse og kroppslig anstrengelse i din fritid. Hvis aktiviteten varierer meget f eks mellom sommer og vinter, så ta et gjennomsnitt. Spørsmålet gjelder bare det siste året.** (Sett kryss i den ruta som passer best)

- ☐ Leser, ser på fjernsyn eller annen stillesittende beskjeftigelse
- ☐ Spaserer, sykler eller beveger deg på annen måte minst 4 timer i uken *(her skal du også regne med gang eller sykling til arbeidsstedet, søndagsturer med mer)*
- ☐ Driver mosjonsidrett, tyngre hagearbeid, snømåking e.l. *(merk at aktiviteten skal vare minst 4 timer i uka)*
- ☐ Trener hardt eller driver konkurranseidrett regelmessig og flere ganger i uka

25 **Hvor ofte driver du mosjon?** (Med mosjon mener vi at du f.eks går en tur, går på ski, svømmer eller driver trening/idrett)

- ☐ Aldri
- ☐ Sjeldnere enn en gang i uken
- ☐ En gang i uken
- ☐ 2-3 ganger i uken
- ☐ omtrent hver dag

26 **Hvor hardt mosjonerer du da i gjennomsnitt?**

- ☐ Tar det rolig uten å bli andpusten eller svett.
- ☐ Tar det så hardt at jeg blir andpusten og svett
- ☐ Tar meg nesten helt ut

27 **Hvor lenge holder du på hver gang i gjennomsnitt ?**

- ☐ Mindre enn 15 minutter
- ☐ 15-29 minutter
- ☐ 30 minutter – 1 time
- ☐ Mer enn 1 time

ALKOHOL OG TOBAKK

28 **Hvor ofte drikker du alkohol?**

- ☐ Aldri
- ☐ Månedlig eller sjeldnere
- ☐ 2-4 ganger hver måned
- ☐ 2-3 ganger pr. uke
- ☐ 4 eller flere ganger pr.uke

29 **Hvor mange enheter alkohol** (en øl, et glass vin, eller en drink) **tar du vanligvis når du drikker?**

- ☐ 1-2
- ☐ 3-4
- ☐ 5-6
- ☐ 7-9
- ☐ 10 eller flere

30 **Hvor ofte drikker du 6 eller flere enheter alkohol ved en anledning?**

- ☐ aldri
- ☐ sjeldnere enn månedlig
- ☐ månedlig
- ☐ ukentlig
- ☐ daglig eller nesten daglig

31 **Røyker du av og til, men ikke daglig?**

- ☐ Ja
- ☐ Nei

32 **Har du røykt/røyker du daglig?**

- ☐ Ja, nå
- ☐ Ja, tidligere
- ☐ Aldri

33 **Hvis du har røykt daglig tidligere, hvor lenge er det siden du sluttet?**

Antall år ☐☐

34 **Hvis du røyker daglig nå eller har røykt tidligere: Hvor mange sigaretter røyker eller røykte du vanligvis daglig?**

Antall sigaretter ☐☐

35 **Hvor gammel var du da du begynte å røyke daglig?**

Antall år ☐☐

36 **Hvor mange år til sammen har du røykt daglig?**

Antall år ☐☐

37 **Bruker du, eller har du brukt, snus eller skrå?**

- ☐ Nei, aldri
- ☐ Ja, men jeg har sluttet
- ☐ Ja, av og til
- ☐ Ja, daglig

KOSTHOLD

38 **Spiser du vanligvis frokost hver dag?**

☐ Ja ☐ Nei

39 **Hvor mange enheter frukt og grønnsaker spiser du i gjennomsnitt per dag?** (Med enhet menes f.eks. en frukt, glass juice, potet, porsjon grønnsaker)

Antall enheter ☐☐

40 **Hvor mange ganger i uken spiser du varm middag?**

Antall ☐☐

41 **Hvor ofte spiser du vanligvis disse matvarene?**
(Sett ett kryss pr linje)

	0-1 g pr. mnd	2-3 g pr.mnd	1-3 g pr.uke	4-6 g pr.uke	1-2 g pr. dag
Poteter	☐	☐	☐	☐	☐
Pasta/ris	☐	☐	☐	☐	☐
Kjøtt *(ikke kvernet)*	☐	☐	☐	☐	☐
Kvernet kjøtt *(pølser, hamburger o.l)*	☐	☐	☐	☐	☐
Grønnsaker, frukt, bær	☐	☐	☐	☐	☐
Mager fisk	☐	☐	☐	☐	☐
Feit fisk *(f.eks.laks, ørret, makrell, sild, kveite,uer)*	☐	☐	☐	☐	☐

42 **Hvor mye drikker du vanligvis av følgende?**
(Sett ett kryss pr. linje)

	Sjelden/ aldri	1-6 glass pr. uke	1 glass pr. dag	2-3 glass pr. dag	4 glass el. mer pr. dag
Melk, kefir, yoghurt	☐	☐	☐	☐	☐
Fruktjuice	☐	☐	☐	☐	☐
Brus/leskedrikker med sukker	☐	☐	☐	☐	☐

43 **Hvor mange kopper kaffe og te drikker du daglig?**
(sett 0 for de typene du ikke drikker daglig)

	Antall kopper
Filterkaffe	☐☐
Kokekaffe/presskanne	☐☐
Annen kaffe	☐☐
Te	☐☐

44 **Hvor ofte spiser du vanligvis fiskelever?**
(For eksempel i mølje)

☐ Sjelden/aldri ☐ 1-3 g i året ☐ 4-6 g i året
☐ 7-12 g i året ☐ Oftere

45 **Bruker du følgende kosttilskudd?**

	Daglig	Iblant	Nei
Tran, trankapsler	☐	☐	☐
Omega 3 kapsler *(fiskeolje,selolje)*	☐	☐	☐
Kalktabletter	☐	☐	☐

SPØRSMÅL TIL KVINNER

46 **Er du gravid nå?**

☐ Ja ☐ Nei ☐ Usikker

47 **Hvor mange barn har du født?**

Antall ☐☐

48 **Hvis du har født, fyll ut for hvert barn: fødselsår og vekt samt hvor mange måneder du ammet.**
(Angi så godt som du kan)

Barn	Fødselsår	Fødselsvekt i gram	Ammet ant.mnd
1	☐☐☐☐	☐☐☐☐	☐☐
2	☐☐☐☐	☐☐☐☐	☐☐
3	☐☐☐☐	☐☐☐☐	☐☐
4	☐☐☐☐	☐☐☐☐	☐☐
5	☐☐☐☐	☐☐☐☐	☐☐
6	☐☐☐☐	☐☐☐☐	☐☐

49 **Har du i forbindelse med svangerskap hatt for høyt blodtrykk?**

☐ Ja ☐ Nei

50 **Hvis Ja, i hvilket svangerskap?**

☐ Første ☐ Senere

51 **Har du i forbindelse med svangerskap hatt protein (eggehvite) i urinen?**

☐ Ja ☐ Nei

52 **Hvis Ja, i hvilket svangerskap?**

☐ Første ☐ Senere

53 **Ble noen av disse barna født mer enn en måned for tidlig (før termin) pga. svangerskapsforgiftning?**

☐ Ja ☐ Nei

54 **Hvis Ja, hvilke(t) barn**

Barn 1	Barn 2	Barn 3	Barn 4	Barn 5	Barn 6
☐	☐	☐	☐	☐	☐

55 **Hvor gammel var du da du fikk menstruasjon første gang?**

Antall år ☐☐

56 **Bruker du for tiden reseptpliktige legemidler som påvirker menstruasjonen?**

P-pille, hormonspiral eller lignende ☐ Ja ☐ Nei
Hormonpreparat for overgangs-alderen ☐ Ja ☐ Nei

VED FRAMMØTE vil du få utfyllende spørsmål om menstruasjon og eventuell bruk av hormoner. Skriv gjerne ned på et papir navn på hormonpreparater du har brukt, og ta det med deg. Du vil også bli spurt om din menstruasjon har opphørt og eventuelt når og hvorfor.

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