

Received Date : 06-May-2016

Revised Date : 30-Sep-2016

Accepted Date : 17-Oct-2016

Article type : Original Article - Clinical Haemostasis and Thrombosis

Omega-3 Fatty Acids Predict Recurrent Venous Thromboembolism or Total Mortality in Elderly Patients with Acute Venous Thromboembolism

Martin F. Reiner, MD,*† Simona Stivala, PhD,*† Andreas Limacher, PhD,‡ Nicole R. Bonetti,
MD,*† Marie Méan, MD,§ Michael Egloff, MD,† Nicolas Rodondi, MD,¶** Drahomir Aujesky,
MD,¶ Clemens von Schacky, MD,†† Thomas F. Lüscher, MD,‡‡§§ Giovanni G. Camici,
PhD,§§ Jürg H. Beer, MD,*†

*Center for Molecular Cardiology, Laboratory for Platelet Research, University of Zurich, Schlieren, Switzerland; †Department of Internal Medicine, Cantonal Hospital of Baden, Baden, Switzerland; ‡Clinical Trials Unit Bern, Department of Clinical Research, and Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland; §Division of General Internal Medicine, Lausanne University Hospital, Lausanne, Switzerland; ¶Department of General Internal Medicine, Bern University Hospital, University of Bern, Bern, Switzerland; **Institute of Primary Health Care (BIHAM), University of Bern, Bern, Switzerland ††Department of Preventive Cardiology, Ludwig-Maximilian-University Munich, Munich, Germany; ‡‡Department of Cardiology, University Heart Center, University Hospital Zurich, Zurich, Switzerland; §§Center for Molecular Cardiology, University of Zurich, Schlieren, Switzerland

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jth.13553

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Running Head: Omega-3 and Recurrent Venous Thromboembolism

Prof. Dr. med. Jürg Hans Beer, M.D., F.A.C.P.

Head of the Department of Medicine

Cantonal Hospital Baden

Im Ergel 1, CH-5404 Baden, Switzerland and

Laboratory for Platelet Research, Center for Molecular Cardiology

University of Zurich, Switzerland

Phone: +41 56 486 25 02; Fax: +41 56 486 25 09; E-mail: hansjuerg.beer@ksb.ch

Essentials

- The role of omega-3 fatty acids (n-3 FAs) in recurrent venous thromboembolism (VTE) is unknown.
- Association of n-3 FAs with recurrent VTE or total mortality was investigated in 826 patients.
- Whole blood n-3 FAs were inversely correlated with recurrent VTE or total mortality.
- Major and non-major bleeding was not increased in patients with higher levels of n-3 FAs.

Summary

Background: The role of omega-3 fatty acids (n-3 FAs) in recurrent venous thromboembolism (VTE) remains unknown.

Objectives: To investigate the association of n-3 FAs with recurrent VTE or total mortality at six months and three years.

Methods: N-3 FAs were assessed in 826 patients aged ≥ 65 years, categorized into low, medium, and high based on the 25th and 75th percentile. Mean follow up was 29 months.

Results: At six months, subjects with medium (adjusted hazard ratio [HR], 0.37; 95% confidence interval [CI], 0.22 to 0.62) and high n-3 FA levels (adjusted HR, 0.36; 95% CI, 0.20 to 0.67) were less likely to develop recurrent VTE or total mortality, compared with low n-3 FAs. At three years, medium levels (adjusted HR, 0.67; 95% CI, 0.47 to 0.96) were associated with lower risk of recurrent VTE or total mortality. As compared with low n-3 FAs, the adjusted subhazard ratio [SHR] of recurrent VTE was 0.39 (95% CI, 0.15 to 0.99) in patients with medium and 0.17 (95% CI, 0.03 to 0.82) in patients with high n-3 FAs. The cumulative incidence of recurrent VTE was lower in the medium and high n-3 FA groups as compared with low n-3 FAs, but seems to have worn off after 3 years. Incidence of major and non-major bleeding was not greater in the high n-3 FA groups.

Conclusion: Higher levels of n-3 FA were associated with a lower risk of recurrent VTE or total mortality in elderly patients with VTE, but not with greater bleeding risk.

Key words Bleeding, deep vein thrombosis, omega-3 fatty acids, pulmonary embolism, recurrent venous thromboembolism

Introduction

Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE), remains a major cause of cardiovascular mortality [1]. The annual incidence is 108 per 100,000 person-years in the US [1], and incidence rates increase exponentially with age for both DVT and PE [1, 2]. More patients present with DVT than PE, but the latter accounts for the majority of VTE-related deaths [3]. VTE is considered to be a chronic, recurrent disease, and despite anticoagulant and thrombolytic therapy [4, 5], roughly one third of patients experience recurrent VTE within ten years [1], peaking at six months after the index VTE [2]. Importantly, the mortality risk remains increased up to 30 years after index VTE [6]. Therefore, recurrent VTE is responsible for substantial comorbidities and healthcare costs [7], and identifying strategies to reduce the burden of recurrent VTE is crucial.

Omega-3 fatty acids (n-3 FAs), including long-chain, marine-derived eicosapentaenoic acid (EPA, 20:5 n-3), docosahexaenoic acid (DHA, 22:6 n-3), docosapentaenoic acid (DPA), and the short-chain, plant-derived alpha-linolenic acid (ALA, 18:3 n-3), have been shown to prevent thrombotic events, cardiovascular disease and mortality in experimental [8-10] and clinical studies [11-14]. We found that treatment of mice with ALA reduces atherosclerosis [10] and arterial thrombosis [9] via anti-inflammatory [10], antiplatelet [8] and anticoagulant [9] mechanisms; similar properties have been described for long-chain marine-derived n-3

FAs [15]. Inflammation, coagulation, and platelet activation are the hallmarks of VTE and VTE-related death. Indeed, clinical studies have found an inverse association of fish or n-3 FA intake with incident VTE [16, 17]. However, the association of n-3 FAs with VTE recurrence and mortality remains unclear.

Elderly patients with prior VTE are at high risk for recurrent VTE due to their advanced age and frequent pro-inflammatory and pro-thrombotic comorbidities, such as cancer, cerebrovascular disease, immobility, hypertension, and heart failure [18, 19]. Experimental studies have suggested an important involvement of tissue factor-bearing microparticles [20, 21] and inflammatory mediators such as neutrophil extracellular traps [22-24] and P-selectin [25, 26] in the pathogenesis of venous thrombosis. Correspondingly, these mediators have been linked to VTE in clinical studies [24, 27, 28]. Furthermore, platelets play a fundamental role in venous thrombus formation [26]. N-3 FAs have been shown to exert antiplatelet and anticoagulant properties by reducing platelet activation [8], vascular tissue factor activity [9], levels of plasma tissue factor [29], and P-selectin [29], indicating a potential implication of n-3 FA in preventing venous thrombosis.

In the present study, we investigate the association of whole blood n-3 FAs with recurrent VTE or total mortality and major and non-major bleeding in a prospective multicenter cohort study of elderly patients with acute VTE.

Materials and Methods

Study population

The study was conducted as part of the Swiss Cohort of Elderly Patients with Venous Thromboembolism (SWITCO65+; ClinicalTrials.gov Identifier: NCT00973596), a prospective multicenter cohort study designed to assess short- and long-term medical outcomes in elderly patients with acute VTE [18]. Patients were screened at all five university and four

high-volume non-university hospitals in Switzerland from September 2009 to March 2012 (n=1863). Patients aged ≥ 65 years with objectively confirmed acute, symptomatic VTE (proximal and/or distal DVT and/or PE) were included in the study [18]. Symptomatic DVT was defined as an acute onset of leg pain or swelling and incomplete compressibility of a venous segment on ultrasonography or an intraluminal filling defect on contrast venography [18]. Additional diagnostic criteria for iliac and caval DVT also included abnormal duplex flow patterns compatible with thrombosis or an intraluminal filling defect on spiral computed tomography or magnetic resonance imaging venography [18]. Symptomatic PE was defined as a positive spiral computed tomography or pulmonary angiography, a high-probability ventilation-perfusion scan, or proximal DVT documented by compression ultrasonography or contrast venography in patients with acute chest pain, new or worsening dyspnea, hemoptysis, or syncope [18]. Exclusion criteria were inability to provide informed consent, conditions incompatible with follow-up, language barriers, thrombosis at a different site than lower limb, catheter-related thrombosis, or previous enrolment in the cohort [18].

After exclusion of 860 patients (398, no consent; 285, inability to provide consent, 192, follow up not possible; 21, other site than lower limb; 7, catheter-related thrombosis; 51, language barriers; multiple reasons for exclusion may apply), 1003 patients were enrolled and 177 patients were excluded from the current study (8, patients denying use of data; 4, early withdrawals; 165, no bio samples available); finally, n-3 FAs were analyzed in 826 patients. The ethics committee of every participating center approved the study, and eligible patients provided informed consent.

Baseline data collection and follow-up

At baseline, study nurses prospectively collected demographic information (age and gender), comorbid conditions, laboratory findings, VTE-related treatment before and after the event, and concomitant antiplatelet therapy using standardized data collection forms.

Follow-up contact included one telephone interview and two face-to-face evaluations during the first year followed by semi-annual contacts and periodic reviews of the patient's hospital chart. During each contact, study nurses interviewed patients to obtain information about the date and type of clinical events (recurrent VTE, mortality). In case of an event, this information was complemented by reviewing medical charts and interviewing patients' primary care physicians and family members. Patients were followed up at a mean of 29 months.

Blood sampling and whole blood fatty acid composition

At the baseline visit EDTA-anticoagulated blood was drawn after minimal venostasis; aliquots of samples were cryovialled in 3 ml polypropylene tubes and temporarily stored at -80°C at each participating centre before they were transported to and stored at the SWITCO65+ biobank at the Central Laboratory of Hematology of Lausanne University hospital, Switzerland. For whole-blood fatty acid (EPA + DHA + DPA + ALA) determination, samples were shipped on dry ice to Omegametrix GmbH, Planegg, Germany (no prior freeze-thaw cycles). Whole-blood composition was analysed according to the HS-Omega-3 Index® methodology as previously described [30]. Fatty acid methyl esters were generated from whole blood by acid transesterification and analyzed by gas chromatography using a GC2010 Gas Chromatograph (Shimadzu, Duisburg, Germany) equipped with a SP2560, 100-m column (Supelco, Bellefonte, PA) hydrogen as carrier gas. Fatty acids were identified by comparison with a standard mixture of fatty acids characteristic of erythrocytes. Results are given as fatty acids expressed as a percentage of total identified fatty acids after response factor correction. The coefficient of variation for fatty acid levels was 5%. Analyses were quality controlled according to DIN ISO 15189. Samples were labelled with individual 8-digit European article number barcodes and sent to Omegametrix for n-3 FA

determination. Subsequently, bar codes with corresponding fatty acid contents were provided to the statistician. All researchers involved in fatty acid determination were blinded.

End points

Our primary endpoint was recurrent VTE or total mortality. Recurrent VTE during follow-up was defined as new fatal or new non-fatal PE, or new DVT (proximal and/or distal) [18]. Diagnosis of recurrent VTE during follow-up was established with the following criteria: for DVT, abnormal results on ultrasonography; for PE, CT, or angiography, showing new intraluminal defects or ventilation-perfusion lung scan showing a high-probability pattern with new perfusion defects. A new proximal DVT, associated with new PE symptom(s) (shortness of breath, chest pain, syncope) was also considered as recurrent PE [18].

Our secondary endpoints included recurrent VTE, total mortality, and major or non-major bleeding. Major bleeding was defined as a fatal bleeding, symptomatic bleeding in a critical organ (intracranial, intraspinal, intraocular, retroperitoneal, intraarticular, pericardial, or intramuscular with compartment syndrome), bleeding with a reduction of haemoglobin ≥ 20 g/L, or bleeding leading to the transfusion ≥ 2 units of packed red blood cells [18]. Non-major bleeding was defined as bleeds that did not fulfill the criteria for major bleeding but required medical attention [18].

Statistical analysis

We categorized n-3 FA into low, medium, and high levels based on pre-specified cut-offs, the 25th and 75th percentile (low, 2.7 – 4.9%; medium, 4.9 – 6.6%; high, 6.6 – 11.6%). Cumulative incidences of recurrent VTE or total mortality and bleeding were estimated by the Kaplan-Meier method, and survivor functions between groups were compared using the logrank test. Associations between n-3 FA levels and the time to a first VTE recurrence, or a first major or non-major bleeding were assessed by competing risk regression accounting for

death as a competing event, according to Fine and Gray's method [31]. The method yields subhazard ratios (SHR) with corresponding 95% confidence intervals (CI) and p-values for the event of primary interest. For total mortality and the composite of VTE recurrence or total mortality, an ordinary Cox regression was calculated with robust standard errors. We adjusted the models for risk factors that had previously been shown to be associated with VTE recurrence, total mortality, and bleeding. Recurrent VTE or total mortality was adjusted for age, gender, overt PE, cancer, heart failure, chronic lung disease, body mass index, provoked VTE, prior VTE, and periods of anticoagulation as a time-varying covariate. Bleeding was adjusted for age, cancer, history of major bleeding, overt PE, antiplatelet therapy, and periods of anticoagulation as a time-varying covariate. VTE recurrence was adjusted for age, gender, body mass index, cancer, provoked VTE, prior VTE, and periods of anticoagulation as a time-varying covariate. Total mortality was adjusted for age, gender, overt PE, cancer, immobilization, heart failure, chronic lung disease, and periods of anticoagulation as a time-varying covariate. Missing values in covariates used for adjustment were rare (<1%) and thus assumed to be absent. In addition, we performed a sensitivity analysis excluding cancer patients (n=678) as cancer is associated with a higher incidence of VTE [1], and there is evidence that higher intake of n3 FAs is associated with a lower incidence and progression of cancer [32]. All analyses were performed using Stata 13 (Stata Corporation, College Station, Texas). Reported STROBE guidelines have been the basis for reporting our results [33].

Results

Study population

Patients had a mean age of 75 years, and 46 % were female. Patients' baseline characteristics were comparable across n-3 FA categories. Exceptions were a lower prevalence of arterial hypertension in the high n-3 FA group, a higher prevalence of cancer in the low n-3 FA group, and differences in the type of initial anticoagulation (Table 1).

Recurrent VTE or total mortality after six months

After 6 months, the cumulative incidence of the primary endpoint, recurrent VTE or total mortality, was inversely associated with levels of n-3 FA (low, 18.1%; medium, 7.7%; high, 7.3%) (Fig. 1A). After adjustment, patients with high (HR, 0.36; 95% CI, 0.20 to 0.67) and medium (HR, 0.37; 95% CI, 0.22 to 0.62) n-3 FA levels were less likely to develop recurrent VTE or total mortality than patients with low levels (Table 2). Similarly, the individual secondary end points, recurrent VTE alone (low, 5.8%; medium, 2.3%; high, 1.0%) and total mortality alone (low, 16.7%; medium, 5.9%; high, 6.3%) occurred less frequently in patients with medium and high n-3 FA levels (Fig. 1B). After adjustment, medium (SHR, 0.39; 95% CI, 0.15 to 0.99) and high levels (SHR, 0.17; 95% CI, 0.03 to 0.82) were associated with a lower incidence of recurrent VTE, compared to low levels (Table 2). Medium (SHR, 0.29; 95% CI, 0.16 to 0.51) and high n-3 FA levels (SHR, 0.34; 95% CI, 0.17 to 0.68) were also associated with a lower risk of total mortality (Table 2). At 6 months, a total of 71 deaths were observed: 31 deaths (43.7%) were attributable to cancer, 17 (23.9%) to PE (4 [5.6%] PE-related, 13 [18.3%] possibly PE-related), 4 (5.6%) to bleeding, 4 (5.6%) to sepsis, 4 (5.6%) to pulmonary causes other than PE, 3 (4.2%) to infection, 1 (1.4%) to acute coronary syndrome, 1 (1.4%) to left ventricular failure, 2 (2.8%) to others, and 4 (5.6%) to unknown causes.

Recurrent VTE or total mortality after three years

At 3 years, the cumulative incidence of the primary endpoint differed between n-3 FA levels (low, 35.7%; medium, 27.1%; high, 32.9%). Medium levels (HR 0.67; 95% CI, 0.47 to 0.96) were associated with a lower risk of recurrent VTE or total mortality than low n-3 FA levels (Table 2 and Fig. 3A). The occurrence rate of the secondary endpoint, recurrent VTE, was comparable between the groups after three years (low, 15.1%; medium, 13.7%; high, 16.7%) (Table 2 and Fig. 3B). However, incidence of total mortality differed among the

groups (low, 28.5%; medium, 18.9%; high, 20.8%) (Table 2). Medium (HR, 0.55; 95% CI, 0.37 to 0.82) and high n-3 FAs (HR, 0.67; 95% CI, 0.42 to 1.06) were associated with a decreased risk of total mortality at three years, although the latter did not reach statistical significance (Table 2). At 3 years, a total of 165 deaths were observed: 59 deaths (35.8%) were attributable to cancer, 30 (18.1%) to PE (7 [4.2%] PE-related, 23 [13.9%] possibly PE-related), 13 (7.9%) to sepsis, 12 (7.3%) to infection, 11 (6.7%) to bleeding, 9 (5.5%) to left ventricular failure, 6 (3.6%) to pulmonary causes other than PE, 3 (1.8%) to acute coronary syndrome, 2 (1.2%) to stroke, 2 (1.2%) to suicide, 5 (3.0%) to others, and 13 (7.9%) to unknown causes.

Bleeding after six months and three years

The cumulative incidence of major or non-major bleeding was different across n-3 FA categories at six months (low, 20.4%; medium, 12.5%; high, 16.0%) (Fig. 2, Table 2), interestingly with the highest bleeding rates in patients with low n-3 FA levels; however, the differences did not reach statistical significance. After adjustment, patients with medium levels (SHR 0.64; 95% CI, 0.41 to 0.98) but not patients with high n-3 FA levels (SHR 0.82; 95% CI, 0.51 to 1.32) had a reduced risk of bleeding compared to patients with low levels (Table 2 and Fig. 2), suggesting a potential benefit of medium n-3 FA levels in terms of short-term bleeding risk. At three years, the cumulative incidence of bleeding did not differ between n-3 FA categories (low, 32.3%; medium, 34.5%; high, 32.9%) (Table 2 and Fig. 4).

Kaplan-Meier curves suggest a non-linear rather than a linear relationship between n-3 FAs and outcomes. Nevertheless, we performed analyses using n-3 FAs as a continuous measure, and found a consistent pattern of results for all endpoints similar to categorical analyses (Supplementary Table 1).

Sensitivity analysis of patients without cancer

The prevalence of active cancer differed significantly across n-3 FA groups and was higher in patients with low n-3 FAs. Furthermore, cancer was a major cause of total mortality both at six months and three years. To investigate the association of n-3 FAs with recurrent VTE or total mortality and bleeding independently of cancer, we performed a sensitivity analysis excluding active cancer patients. Baseline characteristics were comparable, with the exceptions of arterial hypertension and type of initial anticoagulation (Supplementary Table 2). Similar to the primary analysis, recurrent VTE occurred less frequently in patients with high (SHR 0.17; 95% CI, 0.03 to 0.94) and medium (SHR 0.33; 95% CI, 0.12 to 0.95), compared with low n-3 FA levels after 6 months. Moreover, higher n-3 FAs were not associated with higher bleeding rates (Supplementary Table 3). Patients with medium and high n-3 FA levels were less likely to develop the primary endpoint recurrent VTE or total mortality as well as the secondary endpoint total mortality; however, this associations did not reach statistical significance (Supplementary Table 3). At 3 years, n-3 FAs in patients without active cancer were not associated with recurrent VTE, total mortality, or bleeding (Supplementary Table 3).

Discussion

This prospective multicenter cohort study investigated the association of total whole blood n-3 FAs with recurrent VTE or total mortality and with major and non-major bleeding in elderly patients with acute VTE. In this study, n-3 FAs were inversely associated with the cumulative incidence of recurrent VTE or total mortality. At six months, patients with higher n-3 FA levels had a risk reduction of more than 60% for developing recurrent VTE or total mortality. Importantly, the individual secondary endpoints, recurrent VTE, and total mortality, occurred significantly less frequently in patients with medium and high compared to low n-3 FA levels. At three years, only medium n-3 FAs were associated with a significant risk reduction of 33%

for developing recurrent VTE or total mortality. Importantly and consistently, medium and high levels of n-3 FAs were not associated with greater bleeding rates at either six months or three years. Since a reduced incidence of cancer has been reported in patients with higher intake of n-3 FAs and cancer contributed substantially to total mortality in the current study, we undertook a sensitivity analysis excluding patients with active cancer. This still revealed a substantial and clinically relevant risk reduction of 67% and 83% for developing recurrent VTE after six months in patients with medium and high n-3 FAs, respectively at six months.

The association of n-3 FAs or fish intake with VTE has previously been examined in epidemiological studies and provided inconsistent results. Some studies have reported no association of fish or n-3 FAs with incident VTE [34-36] whereas others have found a weak correlation of fish consumption with the reduction of provoked VTE [17]. Steffen et al. reported a lower incidence of VTE associated with higher quintiles of fish consumption and n-3 FAs and suggested a threshold pattern effect rather than a linear trend [16]. These studies have assessed fish and n-3 FA intake by food frequency questionnaires, which have recently been shown to be hampered by up to 50% of participants providing implausible data [37]. In addition, n-3 FA uptake is not only affected by intake but also by absorption, food interaction, genetics, and catabolism, among others [38]. Thus, determining whole blood n-3 FA content by gas chromatography, an analytical procedure with low variability [38], reflects patients' n-3 FA status more reliably and appropriately.

We and others have found plausible mechanisms in experimental studies by which n-3 FAs may prevent venous thrombosis including anti-inflammatory, antiplatelet and anticoagulant properties [8, 9, 39]. Recently, we have reported that ALA did not prevent acute venous thrombosis in mice in a model of vena cava stenosis or in a model of photochemical-induced venous endothelial injury [40]. Consistent with these results, long-chain n-3 FAs did not prevent venous thrombosis in a model of stasis and hypercoagulability [41], whereas others have demonstrated beneficial effects of the long-chain n-3 FA EPA [39]. These inconsistent results in rodents may be difficult to translate to the human situation, especially to elderly

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patients with multiple risk factors, a setting which cannot be modeled in animals. On the other hand, differences in pathophysiology may explain the apparent discrepancy. The animal models consist of an acute and short-term injury with a relatively short exposure to n-3 FAs, whereas low-dose and long-term protective mechanisms of n-3 FAs may apply in elderly patients with proinflammatory and prothrombotic backgrounds. Age is a main risk factor for VTE [1] and is also associated with other risk factors for VTE such as hypertension, heart failure, cancer, and stroke [18]. Studies have demonstrated that n-3 FAs reduce blood pressure [11] and heart failure [42], and n-3 FA were associated with a reduction of some cancer types [32]. This suggests that the substantial reduction of recurrent VTE or total mortality observed in this study may in part be due to additional non-coagulation-related protective effects of n-3 FAs.

Bleeding occurred significantly less frequently in the medium group and tended to be so in the high n-3 FA group (Table 2) after six months. In line with this observation, a recent study reported no association of high n-3 FA levels with bleeding rates in patients with acute myocardial infarction and antithrombotic therapy [43]. Also, randomized clinical trials did not report increased bleeding rates in patients treated with n-3 FAs [44, 45], and a recent meta-analysis found no association between n-3 FAs and hemorrhagic strokes [46]. The findings that high n-3 FA levels are associated with a lower incidence of recurrent VTE, but not with higher bleeding rates, appear attractive for potential future applications. In addition, given that patients with low n-3 FAs are not at risk for major bleeding, they may be good candidates for receiving prolonged anticoagulant therapy.

Patients in the current prospective cohort study were recruited from September 2009 to March 2012, and were followed for a mean of 29 months. Thus, our data reflect recent clinical practice. The overall proportion of the primary endpoint was 10.0% and 28.0% at six months and three years, respectively. The 6-month incidence rate of VTE recurrence was 5.8 (95% CI, 3.8 to 8.7) per 100 patient-years. Incidence rates were similar to previous

studies reporting 7% within 6 months [47], 7.7% within the first year [48] and 11.1 (95% CI, 10.5–11.8) per 100 patient years within 6 months [2].

Limitations of the current study include the absence of novel oral anticoagulants, which were not approved for clinical use during the study period; ~~which~~ this could have affected the primary endpoint and interfered with the beneficial association of medium and high n-3 FA levels with VTE or total mortality. Further, the number of events of some endpoints, particularly of recurrent VTE after 6 months with 22 events is low, which may decrease power. Nevertheless, significant associations, in line with our hypothesis were observed. Likewise, only few event rates and broad confidence intervals were found in the sensitivity analysis resulting in low power. However, the data is supportive of our findings, considering the significantly lower event rates of recurrent VTE in patients with higher n-3 FA levels and furthermore, the coherent trend of lower recurrent VTE and/or mortality in patients with higher n-3 FA levels. The current observational study found associations of n-3 FAs with recurrent VTE and mortality; however, a causal relationship may be investigated in intervention trials. The current study was performed in patients above 65 years of age; therefore, the findings cannot be extrapolated to the general population. In line with previous reports [11, 32], confounding variables were observed, including cancer and hypertension. Despite extensive adjustment, we cannot exclude the possibility that the association between n-3 FA levels and outcomes may be due to residual confounding.

In conclusion, in elderly patients with acute VTE, medium and high levels of total whole blood n-3 FAs were associated with a lower risk of recurrent VTE or total mortality, but not with higher bleeding rates at six months. After three years, patients with medium n-3 FAs levels were less likely to develop recurrent VTE or total mortality. Although the current observational study suggests potential protective effects of n-3 FAs on recurrent VTE and total mortality, the efficacy of preventing recurrent VTE or total mortality by n-3 FA supplementation remains to be investigated in interventional trials.

Addendum

M. F. Reiner designed the study, analyzed and interpreted the data, wrote the manuscript and approved the final version. A. Limacher designed the study, analyzed and interpreted the data, wrote the manuscript and approved the final version. S. Stivala interpreted the data, contributed to the discussion and approved the final version. N. R. Bonetti interpreted the data, contributed to the discussion and approved the final version. M. Méan designed the study, interpreted the data, wrote the manuscript and approved the final version. M. Egloff interpreted the data, contributed to the discussion and approved the final version. N. Rodondi designed the study, interpreted the data, contributed to the discussion and approved the final version. D. Aujesky designed the study, interpreted the data, wrote the manuscript and approved the final version. C. von Schacky measured whole blood n-3 FA content, interpreted the data, wrote the manuscript and approved the final version. T. F. Lüscher interpreted the data, wrote the manuscript and approved the final version. G. G. Camici interpreted the data, wrote the manuscript and approved the final version. J. H. Beer designed the study, analyzed and interpreted the data, wrote the manuscript and approved the final version.

Acknowledgements

We would like to thank our technician Sara Gobbato for help with the sample preparation. The Swiss National Science Foundation supported the SWITCO65+ study (grant no. 33CSCO-122659/139470), the current sub-study (grant no. 310030_144152/1 to J. H. Beer, 310030_166576 to TFL and 310030_147017 to G. G. Camici), and the Foundation Kardio, Baden, Switzerland. We thank all participating centers and collaborators of SWITCO65+.

Disclosure of Conflict of Interests

T. F. Lüscher and J. H. Beer have received research and educational grants and honoraria from Bayer HealthCare, Boehringer Ingelheim and Daiichi Sankyo outside this study. C. von Schacky has founded Omegamatrix, a laboratory performing fatty acid analysis. The other authors state that they have no conflict of interest.

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Figure Titles and Legends

Figure 1. Kaplan–Meier Estimates of Time to Recurrent VTE or Total Mortality by n-3 FA levels up to 6 Months.

A) At 6 months, the cumulative incidence of recurrent VTE or total mortality differed among levels of n-3 FA (low, 18.1%; medium, 7.7%; high, 7.3%). After adjustment, patients with medium (hazard ratio [HR], 0.37; 95% confidence interval [CI], 0.22 to 0.62) and high n-3 FA levels (HR, 0.36; 95% CI, 0.20 to 0.67) had a lower risk of recurrent VTE or total mortality.

B) Also, cumulative incidence of recurrent VTE differed among n-3 FA levels (low, 5.8 %; medium, 2.3%; high, 1.0%). After adjustment, patients with medium (subhazard ratio [SHR], 0.39; 95% CI, 0.15 to 0.99) and high n-3 FA levels (SHR, 0.17; 95% CI, 0.03 to 0.82) had a lower risk of recurrent VTE.

Figure 2. Kaplan–Meier Estimates of Time to Major or Non-Major Bleeding by n-3 FA levels up to 6 Months.

After 6 months, major and non-major bleeding occurred less often in patients with medium and high levels of total n-3 FA, however, differences were not significant (low, 20.4%; medium, 12.5%; high, 16.0%). After adjustment, patients with medium (subhazard ratio [SHR], 0.64; 95% confidence interval [CI], 0.41 to 0.98), but not high levels (SHR, 0.82; 95% CI 0.51 to 1.32) had fewer bleeding events.

Figure 3. Kaplan–Meier Estimates of Time to Recurrent VTE or Total Mortality by n-3 FA levels up to 3 Years.

A) At 3 years, the cumulative incidence of recurrent VTE or total mortality differed among levels of n-3 FAs (low, 35.7%; medium, 27.1%; high, 32.9%). After adjustment, patients with medium levels (hazard ratio [HR], 0.67; 95% confidence interval [CI], 0.47 to 0.96), but not patients with high levels of n-3 FAs (HR, 0.88; 95% CI, 0.60 to 1.30) had a lower risk for

recurrent VTE or total mortality. **B)** At 3 years, cumulative incidence of recurrent VTE differed among n-3 FA levels (low, 15.1%; medium, 13.7%; high, 16.7%). After adjustment, patients with medium (subhazard ratio [SHR], 0.90; 95% CI, 0.54 to 1.52) and high n-3 FA levels (SHR, 1.13; 95% CI, 0.63 to 2.01) had a lower risk of recurrent VTE.

Figure 4. Kaplan–Meier Estimates of Time to Major or Non-Major Bleeding by n-3 FA levels up to 3 Years.

Cumulative incidences of major and non-major bleeding did not differ significantly between n-3 FA groups (low, 32.3%; medium, 34.5%; high, 32.9%).

Tables

Table 1. Baseline characteristics by n-3 FA levels.

	All	Low n-3 FA	Medium n-3 FA	High n-3 FA
	n (%) or median (IQ-range)	n (%) or median (IQ-range)	n (%) or median (IQ-range)	n (%) or median (IQ-range)
Total N	N = 826	N = 207	N = 412	N = 207
Age	75.0 (69.0; 81.0)	75.0 (69.0; 79.0)	75.0 (69.0; 82.0)	75.0 (70.0; 82.0)
Gender (female)	376 (46%)	96 (46%)	188 (46%)	92 (44%)
Body mass index	26.4 (24.0; 29.8)	26.3 (23.5; 29.8)	26.8 (24.2; 30.0)	25.8 (23.8; 29.4)

Overt PE	579 (70%)	145 (70%)	284 (69%)	150 (72%)
Prior VTE	240 (29%)	71 (34%)	111 (27%)	58 (28%)
Major surgery during the last 3 months	120 (15%)	33 (16%)	64 (16%)	23 (11%)
Current oestrogen therapy during the last 3 months	27 (3%)	5 (2%)	15 (4%)	7 (3%)
Immobilization during the last 3 months	180 (22%)	42 (20%)	92 (22%)	46 (22%)
Provoked index VTE	241 (29%)	59 (29%)	126 (31%)	56 (27%)
Active cancer	148 (18%)	50 (24%)	68 (17%)	30 (14%)
History of major bleeding	81 (10%)	15 (7%)	38 (9%)	28 (14%)
Arterial hypertension	531 (64%)	136 (66%)	279 (68%)	116 (56%)
Chronic or acute heart failure	100 (12%)	24 (12%)	55 (13%)	21 (10%)
Treated atrial fibrillation	11 (1%)	5 (2%)	5 (1%)	1 (0%)
Diabetes mellitus	127 (15%)	39 (19%)	64 (16%)	24 (12%)
Cerebrovascular disease (stroke, TIA)	80 (10%)	20 (10%)	37 (9%)	23 (11%)
Chronic lung disease	117 (14%)	29 (14%)	55 (13%)	33 (16%)
Chronic renal disease	149 (18%)	46 (22%)	73 (18%)	30 (14%)
Anemia	315 (38%)	92 (44%)	149 (36%)	74 (36%)

Platelet count <150 G/l	122 (15%)	32 (15%)	59 (14%)	31 (15%)
Creatinine >107 umol/l	182 (22%)	55 (27%)	84 (20%)	43 (21%)
Aspirin	228 (28%)	59 (29%)	119 (29%)	50 (24%)
Concomitant antiplatelet therapy	257 (31%)	66 (32%)	136 (33%)	55 (27%)
Polypharmacy	426 (52%)	116 (56%)	212 (51%)	98 (47%)
Anticoagulation prior to index VTE	42 (5%)	16 (8%)	18 (4%)	8 (4%)
Type of initial parenteral anticoagulation				
Low molecular weight heparin	411 (50%)	107 (52%)	213 (52%)	91 (44%)
Unfractionated heparin	244 (30%)	72 (35%)	111 (27%)	61 (29%)
Fondaparinux	142 (17%)	26 (13%)	69 (17%)	47 (23%)
Danaparoid	1 (0%)	0 (0%)	1 (0%)	0 (0%)
None	28 (3%)	2 (1%)	18 (4%)	8 (4%)
Initial vitamin K antagonist therapy	714 (86%)	166 (80%)	363 (88%)	185 (89%)

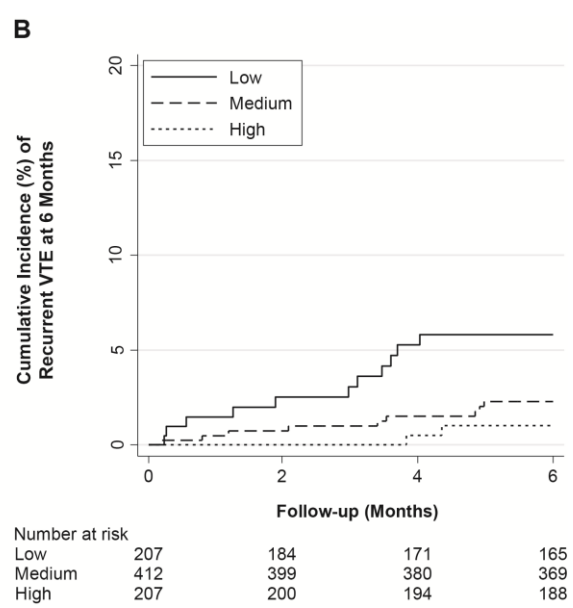
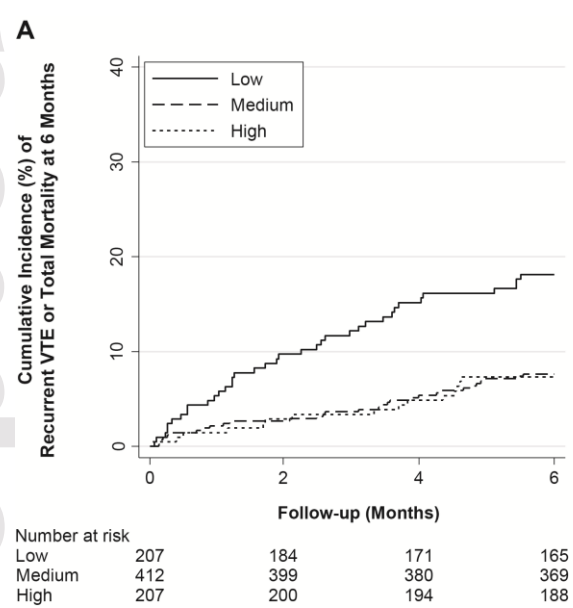
Values were missing in body mass index (1%), anaemia (6%), platelet count (6%), and creatinine (7%). Provoked VTE was defined as presence of ≥ 1 of the following factors: major surgery, oestrogen therapy, or immobilization during the last 3 months; polypharmacy was defined as prescription of more than four different drugs. IQ = interquartile, n-3 FA = omega-3 fatty acids; PE = pulmonary embolism, TIA = transient ischemic attack, VTE = venous thromboembolism.

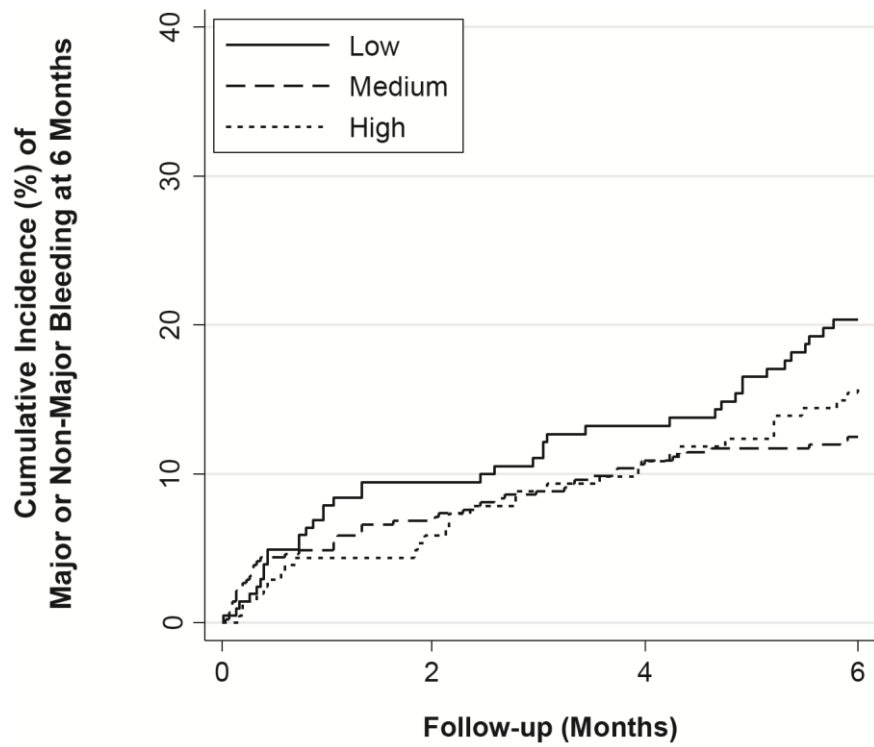
Table 2. Association of n-3 FA levels with clinical endpoints at 6 months and 3 years.

	Level	N° events/ patient-years	IR per 100 patient- years (95% CI)	Crude SHR or HR (95% CI)	Adjusted SHR or HR (95%-CI)
Clinical Endpoints at 6 Months					
VTE recurrence or death	Low	37 / 90.0	41.1 (29.8 to 56.8)	1 (reference)	1 (reference)
	Medium	31 / 194.2	16.0 (11.2 to 22.7)	0.39 (0.24 to 0.63)	0.37 (0.22 to 0.62)
	High	15 / 98.3	15.3 (9.2 to 25.3)	0.37 (0.21 to 0.68)	0.36 (0.20 to 0.67)
VTE recurrence	Low	11 / 90.0	12.2 (6.8 to 22.1)	1 (reference)	1 (reference)
	Medium	9 / 194.2	4.6 (2.4 to 8.9)	0.40 (0.17 to 0.97)	0.39 (0.15 to 0.99)
	High	2 / 98.3	2.0 (0.5 to 8.1)	0.18 (0.04 to 0.79)	0.17 (0.03 to 0.82)
Overall mortality	Low	34 / 90.8	37.4 (26.8 to 52.4)	1 (reference)	1 (reference)
	Medium	24 / 195.6	12.3 (8.2 to 18.3)	0.33 (0.20 to 0.56)	0.29 (0.16 to 0.51)
	High	13 / 98.7	13.2 (7.7 to 22.7)	0.36 (0.19 to 0.67)	0.34 (0.17 to 0.68)
Any bleeding	Low	39 / 83.5	46.7 (34.1 to 63.9)	1 (reference)	1 (reference)
	Medium	50 / 180.0	27.8 (21.1 to 36.6)	0.63 (0.42 to 0.96)	0.64 (0.41 to 0.98)
	High	32 / 91.7	34.9 (24.7 to 49.3)	0.80 (0.50 to 1.26)	0.82 (0.51 to 1.32)
Clinical Endpoints at 3 Years					
VTE recurrence or death	Low	68 / 390.7	17.4 (13.7 to 22.1)	1 (reference)	1 (reference)
	Medium	100 / 908.8	11.0 (9.0 to 13.4)	0.65 (0.47 to 0.88)	0.67 (0.47 to 0.96)
	High	63 / 439.7	14.3 (11.2 to 18.3)	0.83 (0.59 to 1.18)	0.88 (0.60 to 1.30)
VTE recurrence	Low	24 / 390.7	6.1 (4.1 to 9.2)	1 (reference)	1 (reference)
	Medium	45 / 908.8	5.0 (3.7 to 6.6)	0.90 (0.55 to 1.48)	0.90 (0.54 to 1.52)
	High	28 / 439.7	6.4 (4.4 to 9.2)	1.11 (0.64 to 1.93)	1.13 (0.63 to 2.01)
Overall mortality	Low	55 / 411.3	13.4 (10.3 to 17.4)	1 (reference)	1 (reference)
	Medium	70 / 952.4	7.3 (5.8 to 9.3)	0.57 (0.40 to 0.81)	0.55 (0.37 to 0.82)
	High	40 / 473.2	8.5 (6.2 to 11.5)	0.65 (0.43 to 0.98)	0.67 (0.42 to 1.06)

Any bleeding	Low	57 / 345.0	16.5 (12.7 to 21.4)	1 (reference)	1 (reference)
	Medium	120 / 784.2	15.3 (12.8 to 18.3)	1.01 (0.74 to 1.39)	1.00 (0.72 to 1.38)
	High	60 / 399.8	15.0 (11.7 to 19.3)	1.00 (0.69 to 1.44)	1.00 (0.69 to 1.44)

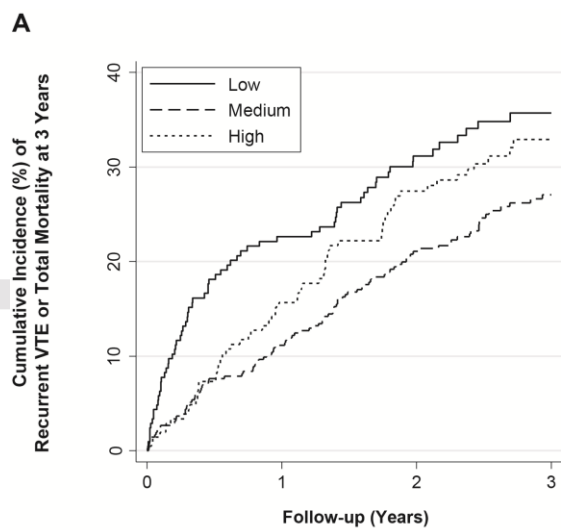
Recurrent VTE or total mortality was adjusted for age, gender, overt PE, cancer, heart failure, chronic lung disease, BMI, provoked VTE, prior VTE, and periods of anticoagulation as a time-varying covariate. Bleeding was adjusted for age, cancer, history of major bleeding, overt PE, antiplatelet therapy, and periods of anticoagulation as a time-varying covariate. VTE recurrence was adjusted for age, gender, BMI, cancer, provoked VTE, prior VTE, and periods of anticoagulation as a time-varying covariate. Total mortality was adjusted for age, gender, overt PE, cancer, immobilization, heart failure, chronic lung disease, and periods of anticoagulation as a time-varying covariate. HR = hazard ratio, IR = incidence rate, n-3 FA = omega-3 fatty acids, SHR = subhazard ratio, VTE = venous thromboembolism.





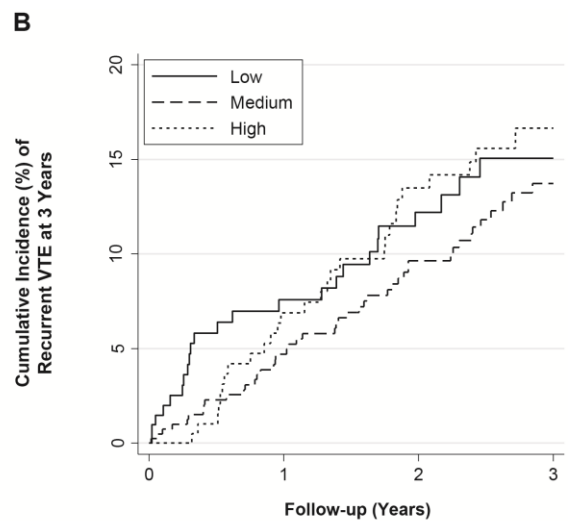
Number at risk

	0	1	2	3	4	5	6
Low	207	171	159	145			
Medium	412	376	344	330			
High	207	191	177	164			



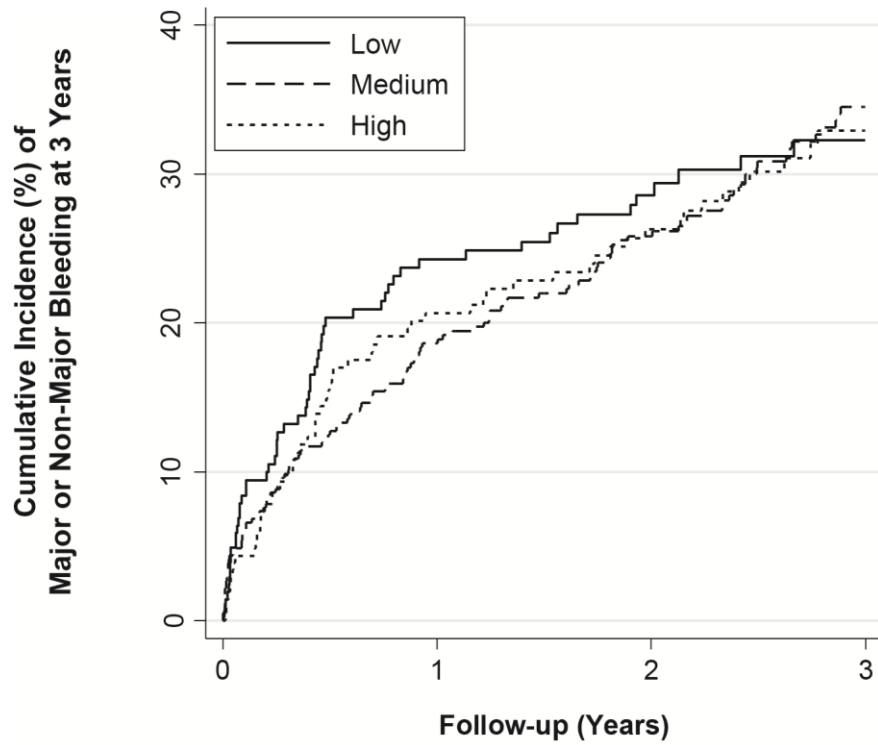
Number at risk

	0	1	2	3
Low	207	152	102	54
Medium	412	350	265	124
High	207	171	129	47



Number at risk

	0	1	2	3
Low	207	152	102	54
Medium	412	350	265	124
High	207	171	129	47



Number at risk		Follow-up (Years)		
Low	207	132	90	46
Medium	412	297	227	98
High	207	150	121	45