Dietary omega-3 alpha-linolenic acid does not prevent venous thrombosis in mice

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Summary
Venous thromboembolism (VTE) is a leading cause of cardiovascular death. Omega-3 fatty acids (n-3 FA) exhibit protective effects against cardiovascular disease. Others and our group have reported that the plant-derived n-3 FA alpha-linolenic acid (ALA) displays antiinflammatory, anticoagulant and antiplatelet effects, thereby reducing atherosclerosis and arterial thrombosis in mice fed a high ALA diet. Since procoagulant factors such as tissue factor and fibrin as well as platelets and leukocytes are crucially involved in the development of VTE, we investigated possible protective effects of dietary ALA on venous thrombus formation in a mouse model of stenosis- and furthermore, in a mouse model of endothelial injury-induced venous thrombosis. Four week old C57BL/6 mice underwent four weeks of high (7.3g%) or low ALA (0.03g%) treatment before being exposed to inferior vena cava (IVC) stenosis for 48 hours or laser injury of the endothelium of the internal jugular vein (IJV). Thrombus generation frequency, thrombus size and composition (IVC stenosis group) and time to thrombus formation (endothelial injury group) were assessed. In addition, plasma glycocalcin, a marker of platelet activation, platelet P-selectin and activated integrin expression as well as plasma thrombin generation was determined, but did not reveal any significant differences between the groups. Despite its protective properties against arterial thrombus formation, dietary ALA did not protect against venous thrombosis neither in the IVC stenosis nor the endothelial injury model, further indicating that the biological processes involved in arterial and venous thrombosis are different.

Keywords
Alpha-linolenic acid, cardiovascular disease, deep vein thrombosis, omega-3 fatty acids, venous thromboembolism

Introduction
Deep-vein thrombosis (DVT) and pulmonary embolism (PE), collectively known as venous thromboembolism (VTE), are major causes of cardiovascular morbidity and mortality. The incidence of VTE increases exponentially with age in both men and women and reaches over 250,000 events annually in the US population (1). However, its diagnosis remains challenging, since many of such events occur without symptoms, as silent PEs or as incidental findings. Further, in some instances they manifest as abrupt PE with fatal outcome, a major complication of DVT and thus, adequate treatment may not always be applicable (2). In line with this, the prevention of DVT may be of increasing importance to reduce the burden of VTE.

Different triggers have been typically linked to the development of DVT such as stasis, activation or injury of the endothelium or changes in the composition of coagulation factors and deficiencies of naturally occurring anticoagulants. Neutrophils, monocytes, platelets and red blood cells (RBC) as well as coagulation factors including tissue factor (TF) and fibrin are involved in the pathogenesis of DVT induced by flow restriction (3), a frequent trigger that occurs during immobilisation, pregnancy and aging (4).

Numerous clinical (5–8) and experimental studies (9–11) have reported beneficial effects of omega-3 fatty acids (n-3 FA) on the cardiovascular system. In particular, plant-derived n-3 FA alpha-linolenic acid (ALA) reduces atherosclerosis (12), arterial thrombosis (13) and decreases platelet activation (14) in mice fed an ALA-enriched diet. However, whether dietary alpha-linolenic acid has an effect on the development of venous thrombosis remains unknown.

Unlike arterial thrombosis, which is initiated by endothelial damage, venous thrombosis may be triggered by stasis, endothelial
injury and/or the local release of procoagulant mediators without vessel injury. Furthermore, venous thrombi are rich in red blood cells and fibrin and are therefore referred to as red clot compared to the white, platelet-rich arterial thrombi (15, 16). Tissue factor, the main initiator of the extrinsic coagulation cascade, seems to play a crucial role in venous thrombus formation, since TF-deficient mice do not develop venous thrombosis in a mouse model of vena cava stenosis (3). Furthermore, inflammation has been strongly linked to DVT (17) and von Brühl and others could demonstrate an early recruitment of leukocytes, predominately neutrophils and monocytes, through the endothelial adhesion molecule P-selectin in response to venous blood flow restriction (3, 18). Also, platelets interact with endothelial-released von Willebrand factor (VWF) via glycoprotein (GP)Iib and further with leukocytes and are therefore involved in the progression of DVT (3) and DNA extracellular traps, which are released from activated neutrophils, have been shown to bind platelets as well as red blood cells (19–21).

In previous studies employing murine models, we could show that ALA decreases atherosclerosis and displays anti-inflammatory effects by reducing the expression of the adhesion molecule vascular cell adhesion molecule 1 (VCAM-1) and by negatively affecting T-cell migration into atherosclerotic plaques (12). Furthermore, dietary ALA prevented arterial thrombus formation by reducing vascular TF activity after two weeks of high ALA treatment in mice. In addition, thrombin- as well as collagen induced platelet aggregation was diminished in murine whole blood ex vivo (13). ALA also reduced platelet GPIb-VWF interaction under high shear flow conditions (14).

These findings point towards a potential protective effect of the plant-derived n-3 FA ALA on venous thrombus formation. In the present study we investigate the effects of dietary ALA on the prevention of DVT in a mouse model of inferior vena cava (IVC) stenosis and a mouse model of endothelial injury-induced venous thrombosis of the internal jugular vein (IJV).

Materials and methods
Animals and diet
C57BL/6 wild-type mice were kept under specific-pathogen free condition in a 12-hour (h) light-dark cycle. Four-week-old rodents were fed either a high ALA (7.3 g%; Research Diets, New Brunswick, NJ, USA; n = 11) or low ALA (0.03 g%; Research Diets; n = 11) diet for four weeks. Alpha-linolenic acid was given as flaxseed oil and substituted with cacao butter in the control group as published previously (14). After four weeks of treatment animals underwent IVC stenosis and were sacrificed 48 h after the surgical procedure to investigate venous thrombus formation. In a second approach, the IJV of rodents was exposed to laser-induced injury of the endothelium and time to thrombus formation was assessed.

Experimental procedures involving mice were reviewed and approved by the Institutional Animal Care and Use Committee of Boston Children’s Hospital (protocol number 11–03–1919) or the institutional animal care committee (licence number TVA 174/2011; Kommission für Tierversuche des Kantons Zürich, Switzerland).

Inferior vena cava stenosis model
IVC stenosis was performed as described previously (21, 22). Briefly, mice were anaesthetised using 3.5 % isoflurane in 100 % oxygen and placed in supine position, maintaining anaesthesia with 2 % isoflurane. A midline laparotomy was performed, intestines were moved aside and the IVC was separated from the surrounding connective tissue. Side branches between the renal and iliac veins were ligated. Finally, the IVC was ligated distal to the renal veins over a 30-gauge needle using a 7–0 polypropylene suture. The needle was removed subsequently, which led to a 90 % stenosis of the IVC and thereby to a marked blood flow restriction. The needle was removed subsequently, which led to a 90 % stenosis of the IVC and thereby to a marked blood flow restriction. During the whole surgical procedure, 0.9 % sterile saline was applied to prevent drying. After IVC stenosis, the peritoneum was closed using a 6–0 monofilament absorbable vicryl suture, skin

Figure 1: Venous thrombosis after 48 h stenosis of the IVC.
A) Venous thrombus formation in mice treated with high or low ALA for four weeks did not differ between the two groups (high ALA 6 out of 11, 55%; low ALA 7 out of 11, 64%, p = ns). B) Furthermore, lengths of thrombi were comparable in both treated and untreated mice (high ALA 2.41 ± 0.98 mm; low ALA 2.27 ± 0.86 mm; n = 11 per group; p = ns).
was closed using a 6–0 nylon suture, and venous thrombus formation was observed 48 h after surgery. The entire procedure was performed using aseptic technique. Mice received buprenorphine (0.1 mg/kg s.c.) as an analgesic 20 minutes (min) before surgery and every 8–12 h subsequently.

**Laser-induced thrombosis of the internal jugular vein**

Laser-induced thrombosis of the internal jugular vein was performed in accordance to laser-induced arterial thrombosis of the carotid artery (13). Mice were anaesthetised by sodium pentobarbital (87 mg/kg) intraperitoneal (i.p.) administration. To induce endothelial damage, rose bengal (20.8 mg/kg) diluted in phosphate-buffered saline was injected into the tail vein and after midline neck incision, the right internal jugular vein was exposed under a binocular operating microscope. Thereafter, a green laser light beam (1.5 mW green laser light 540nm, Mellesgriot Inc., Rochester, NY, USA) was pointed at the vessel and kept at a fixed distance of 12 cm. Thrombus formation was observed visually every 3 min for a maximum of 30 min and time to thrombus formation was measured.

**Plasma glycocalicin and glycocalicin index**

An ELISA 96-well plate was pre-incubated with the antibody 7A9 (5 µg/ml; against the extracellular part of GPIb; a kind gift from Bernard Nieswandt, Würzburg, Germany) overnight. After washing, plates were blocked with BSA 5 %-milk 5 %-PBS for 2 h at 37°C and incubated with the standard or samples at 37°C for 1 h. Plates were washed three times, and the HRP-conjugated detection antibody 15E3 was added. After another washing step, TMB substrate solution (BD Bioscience, Heidelberg, Germany) was applied for 5 min, and the reaction was terminated using sulphuric acid 0.5 M. Absorbance was detected at 450 nm (Spectramax, Molecular Probes, Eugene, OR, USA) and the plasma concentration (µg/ml) was calculated according to the standard. The glycocalicin index (GI) is expressed as plasma glycocalicin concentration (GC) per 250,000 platelets (µg/ml/250,000 platelets).

**Platelet P-selectin and αIIbβIII expression**

Sodium citrate (3.8%) anticoagulated whole blood (1:9 v/v) was obtained by cardiac puncture after euthanasia of mice. After centrifugation (125 g, 8 min, room temperature) platelet-rich plasma (PRP) was washed twice with wash buffer (140 mM NaCl, 5 mM KCl, 12 mM sodium citrate, 10 mM glucose, 12.5 mM sucrose, pH 6.0) and platelets were resuspended in platelet buffer (10 mM HEPES, 140 mM NaCl, 3 mM KCl, 0.5 mM MgCl₂ hexahydrate, 0.5 mM NaHCO₃, 10 mM glucose, pH=7.4). Platelets were activated using thrombin (0.1 U/ml) and incubated with anti-P-selectin and anti-activated integrin αIIbβIII antibodies (Emfret analytics, Eibelstadt, Germany). After 15 min the reaction was stopped by adding 200 µl phosphate-buffered saline (PBS). Mean fluorescence intensity (MFI) for P-selectin and αIIbβIII as well as the fold increase of thrombin-stimulated compared to resting platelets was analysed using a FacsCanto (BD Bioscience, Heidelberg, Germany).

**Plasma thrombin generation**

Plasma thrombin generation was assessed by calibrated automated thrombogram (23). Platelet-poor plasma (PPP) was obtained by centrifugation (10,000 RPM, 10 min, 4°C) and mixed with either PPP-Reagent (Thrombinoscope BV, Maastricht, The Netherlands) containing TF (f.c. 6 pM) and phospholipids (f.c. 4.8 µM), or thrombin calibrator (Thrombinoscope BV). Afterwards a fluorogenic thrombin substrate (Z-Gly-Gly-Arg-AMC, Thrombinoscope BV) together with Fluo-Buffer (Hepes buffer, pH 7.35, 20 mM Hepes, with BSA 60 mg/ml, Thrombinoscope BV) and CaCl₂ (246 mM) was added and thrombin generation was measured by Fluoroskan™ Ascent reader (Thermo Labsystems, Helsinki, Finland).
land) and calculated by Thrombinoscope software (Thrombinoscope BV). Thrombin lag time (min), time to thrombin peak (min), thrombin peak (nmol) and endogenous thrombin potential (ETP) (area under the thrombin generation curve, nmol thrombin x min) were determined.

**Histological analysis of thrombi**

Snap frozen blocks of thrombi were sectioned 6 µm on a LEICA cryostat, air-dried and fixed in acetone for 10 min at room temperature. Haematoxylin and eosin (H/E) staining was performed with dyes from Waldeck GmbH (Division Chroma, Münster, Germany). Images were analysed using an Olympus BX51 microscope with a BP73 camera with the Olympus cellSens software (Olympus, Münster, Germany).

**Immunofluorescence analysis**

Thrombi were embedded in Optimal Cutting Temperature medium, snap frozen and cryosectioned at 10 µm thickness. After an overnight incubation in zinc fixative (100 mM Tris-HCl, 37 mM zinc chloride, 23 mM zinc acetate, 3.2 mM calcium acetate), slides were washed thoroughly in PBS. Tissue sections were permeabilised using 0.1 % Triton X-100, 0.1 % sodium citrate for 10 min at 4°C and blocked with 3 % bovine serum albumin (BSA) for 1 h at 37°C. The primary antibodies anti-von Willebrand factor (Dako A0082, 2 µg/ml; Dako, Glostrup, Denmark) and anti-CD41 (BD Bioscience 553847, 1 µg/ml) were diluted in 0.3 % BSA in PBS containing 0.05 % Tween-20 and were incubated overnight at 4°C. After washing, AlexaFluor 488 and AlexaFluor 555-conjugated secondary antibodies (1.5 µg/ml) were incubated for 2 h at room temperature. DNA was counterstained using Hoechst 33342 (Invitrogen, Karlsruhe, Germany; 1 µg/ml). Images were acquired using a Zeiss Axiovert inverted widefield fluorescence microscope with Axiovision software. Composite images were generated using the MosaicJ plug-in for ImageJ software (24).

**Statistical analysis**

Data is expressed as mean ± SEM. Thrombus frequencies were compared using a Chi-square test on a contingency table. For all other analyses, unpaired two-tailed Student’s t-tests were performed to test statistical significance and a probability value below 0.05 was considered as significant.

**Results**

**High ALA-containing diet did not prevent venous thrombus formation nor affect thrombus size in the IVC stenosis model**

Mice were fed a high or low ALA diet for four weeks. At eight weeks of age an IVC stenosis, which results in endothelial activation without vessel injury, was placed. Venous thrombus

![Figure 3: Plasma thrombin generation after IVC stenosis for 48 hours. A) Endogenous thrombin potential, the area under thrombin generation curve, was comparable in plasma obtained from mice treated with either high (643.1 ± 26.2 nmol x min, n = 5) or low ALA (576.5 ± 75.8 nmol x min, n = 3) diet after 48 h of IVC stenosis. B) Thrombin peak did not differ significantly (high ALA 95.44 ± 6.6 nmol; low ALA 81.40 ± 8.0 nmol; n = 5/3; p = ns). Thrombin lag time and time to peak were similar in both groups (data not shown).](https://www.thrombosis-online.com/article-image.png)
formation was observed in six out of 11 (55%) mice treated with a high ALA diet and occurred in seven out of 11 animals in the control group (64%) after 48 h of IVC stenosis (▶ Figure 1A). Further, thrombus length, measured after 48 h of blood flow restriction, was comparable in mice fed a high and low ALA diet (high ALA 2.41 ± 0.98 mm; low ALA 2.27 ± 0.86 mm; n = 11 per group; p = ns) (▶ Figure 1B).

**Plasma glycocalicin and glycocalicin index were not affected by high ALA treatment after IVC stenosis**

Plasma glycocalicin, the extracellular part of the platelet receptor GPIb, a marker of platelet activation (25), was measured in murine plasma of high and low ALA-treated mice after 48 h of IVC stenosis and did not show a significant difference (high ALA 32.43 ± 2.64 µg/ml; low ALA 29.91 ± 1.98 µg/ml; n = 7/6; p = ns) (▶ Figure 2A). Also when normalised to the platelet count (glycocalicin index) the values were comparable (high ALA 15.04 ± 2.91 µg/ml/250 000 platelets; low ALA 11.13 ± 1.83 µg/ml/250 000 platelets; n = 7/6; p = ns) (▶ Figure 2B).

**Plasma thrombin generation did not differ between low and high ALA treated mice before and after IVC stenosis**

Endogenous thrombin potential (high ALA 643.1 ± 26.2 nmol x min; low ALA 576.5 ± 75.8 nmol x min; n = 5/3; p = ns) (▶ Figure 3A), thrombin peak (high ALA 95.44 ± 6.6 nmol; low ALA 81.40 ± 8.0 nmol; n = 5/3; p = ns) (▶ Figure 3B), thrombin lag time and time to peak (data not shown) as measured by calibrated automated thrombography in plasma of mice after 48 h of IVC stenosis were comparable in both groups. Measurements were also comparable in plasma obtained from mice before surgical interventions (data not shown).

**Leukocyte, platelet and RBC content as well as fibrin and VWF fraction were comparable in thrombi from high and low ALA-treated mice**

To analyse the thrombus structure, venous thrombi were harvested and stained for neutrophils (Ly-6G), monocytes (CD68), platelets (CD41), fibrin (anti-fibrinogen) and VWF (anti-VWF), whereas the RBC content was assessed in H/E stained sections. In line with previous publications (3, 21), neutrophils and monocytes appeared to be more abundant in venous thrombi compared to lymphocytes. No differences in the cellular composition as well as in the fibrin and VWF content of thrombi was observed between the intervention and the untreated group (▶ Figure 4, representative pictures).

**Time to laser-induced venous thrombosis was similar in low and high ALA treated rodents**

To further evaluate the effects of high ALA treatment in a second mouse model of venous thrombosis, injury of the venous endothelium of the IJV was induced; time to venous thrombosis in the IJV was measured by visual observation of thrombus formation in regular intervals of 3 min. Mean duration of time to venous thrombosis in high ALA (10.29 ± 1.3 min, n = 7) and in low ALA treated mice (10.71 ± 2.3 min, n = 7) did not differ significantly (▶ Figure 5).

**Platelet P-selectin and αIIbβIII expression was equal in mice treated with high or low ALA diet after laser injury**

Platelet activation and thrombin-induced activatability was measured by P-selectin and activated integrin αIIbβIII expression
on the surface of platelets obtained after laser-injury-induced venous thrombosis. Both groups showed comparable baseline levels of P-selectin (high ALA 33.29 ± 7.8 MFI; low ALA 34.43 ± 8.8 MFI; n = 7 per group; p = ns) as well as αIIbβIII (high ALA 16.0 ± 5.9 MFI; low ALA 9.29 ± 1.1 MFI; n = 7 per group; p = ns) expression. After stimulation with thrombin (0.1 U/ml) both groups were still comparable (P-selectin: high ALA 237.1 ± 46.0 MFI; low ALA 238.3 ± 36.9 MFI; n = 7 per group; p = ns; αIIbβIII: high ALA 35.43 ± 5.5 MFI; low ALA 37.0 ± 4.7 MFI; n = 7 per group; p = ns) (▶Figure 6). In line with this, also the fold increase of stimulated vs. unstimulated platelets did not differ (data not shown). Likewise, we did not detect a difference in platelet activation as well as thrombin-induced activatability in platelets from mice prior surgery (data not shown).

**Figure 6: Platelet activation and activatability after laser-injury.** A) Platelet P-selectin expression after endothelial-injury in mice was comparable in high ALA (33.29 ± 7.8 MFI; n = 7) and low ALA 34.43 ± 8.8 MFI; n = 7) treated rodents at baseline and after stimulation with thrombin (0.1 U/ml) (high ALA 237.1 ± 46.0 MFI; low ALA 238.3 ± 36.9 MFI; n = 7 per group; p = ns). B) Further, activated integrin αIIbβIII was not affected by high ALA diet in unstimulated (high ALA 16.0 ± 5.9 MFI; low ALA 9.29 ± 1.1 MFI; n = 7 per group; p = ns) and stimulated conditions (high ALA 35.43 ± 5.5 MFI; low ALA 37.0 ± 4.7 MFI; n = 7 per group; p = ns).

**Plasma thrombin generation was not affected after laser-induced thrombosis by high ALA treatment**

Murine coagulation was assessed by thrombin generation in plasma of mice after laser injury; endogenous thrombin potential (high ALA 488.5 ± 35.3 nmol x min; low ALA 444.3 ± 31.4 nmol x min; n = 7 per group; p = ns) (▶Figure 7A), thrombin peak (high ALA 81.81 ± 2.2 nmol; low ALA 86.08 ± 1.7 nmol; n = 7 per group; p = ns) (▶Figure 7B) as well as thrombin lag time and time to peak (data not shown) were comparable.

**Discussion**

The plant-derived n-3 FA ALA exerts protective effects against cardiovascular disease (CVD), in particular against arterial thrombus formation (13) and platelet activation (14). In this study we tested the hypothesis whether dietary ALA also protects from DVT in a mouse model of IVC stenosis for 48 h and additionally, in a mouse model of endothelial injury-induced venous thrombosis of the IJV.

In mice after a treatment period of 4 weeks with ALA, no protective effects on the formation of venous thrombosis after IVC stenosis were observed. DVT occurred with a similar frequency in both the treated and untreated group and also the size of thrombi was comparable. Furthermore, analysis of the individual thrombus composition in terms of cellular and non-cellular content did not reveal differences between the groups. In line with previous reports approximately 60% of mice developed DVT and neutrophils and monocytes among the leukocytes were the most prevalent cells in venous thrombi (3). Neutrophil, monocyte, lymphocyte, RBC and platelet content as well as fibrin and VWF levels were...
comparable in thrombi of both groups. Further, time to venous thrombus formation after endothelial damage did not differ significantly between high and low ALA treated mice. In line with this observation, platelet activation as well as plasma thrombin generation were similar between the experimental groups in both mouse models.

The reported findings do not support our hypothesis of the potential protective effects of dietary ALA on venous thrombus formation in mice. The neutral results of this study may in part be due to the acute onset and the short-term trigger (48 h) for DVT in this mouse model of IVC stenosis and therefore, nutritionally increased ALA may not be sufficient to compensate for the acute increase in inflammation after IVC stenosis. Indeed, in this model the marked stasis induced by the ligation, may be so dominant that potential protective effects of ALA on procoagulant factors may have been overruled. However, only approximately 60% of mice generate DVT due to IVC stenosis and thus, a reduction of the high degree of stenosis may not be sufficient to adequately provoke venous thrombosis and whether high ALA treatment may be protective in a mouse model using a weaker but therefore prolonged trigger remains unknown.

The adhesion molecule P-selectin has been suggested to be crucially involved in the early leukocyte recruitment in stenosis-induced DVT (3); however, the role of VCAM-1 has not been specifically determined in this model and may be of minor significance. Furthermore, it has been suggested that leukocytes, in particular neutrophils may be sufficient to propagate thrombus formation by binding platelets and red blood cells through their release of nuclear chromatin (neutrophil extracellular traps) (20), which is important for the generation of venous thrombosis (19–21). An additional explanation could include that platelets may play a less paramount role in the venous stenosis- and endothelial injury-induced thrombosis model compared to arterial thrombosis (4, 26). The duration of high ALA diet may be another issue, although nutritionally achievable concentrations of ALA inhibit platelet activation and aggregation ex vivo after an exposure time of only 1 h and also arterial thrombosis has been prevented in mice treated with an ALA-enriched diet after two weeks (13). Platelet activation as measured by plasma glyocalcin as well as platelet P-selectin and αIIbβIII expression did not differ in the current study, although it has been reported previously that platelet aggregation in response to collagen and thrombin was reduced after a high ALA diet for two weeks (13). The different observa-

**What is known about this topic?**

- Venous thrombosis may be triggered by endothelial injury or a reduction in blood flow and subsequent endothelial activation leading to an early recruitment of leukocytes and platelets via the adhesion molecules P-selectin and VWF, respectively.
- Monocyte-derived tissue factor is crucially involved in blood flow restriction-induced venous thrombosis, as TF-deficient mice do not develop DVT.
- Omega-3 fatty acids have been shown to exert protective cardiovascular effects; in particular, the plant-derived alpha-linolenic acid has been reported to prevent arterial thrombosis, atherosclerosis and platelet activation by decreasing vascular tissue factor expression and activity, endothelial VCAM-1 expression and by reducing platelet aggregation in response to thrombin and collagen as well as platelet adhesion to VWF.

**What does this paper add?**

- Despite its protective effects in arterial thrombosis, dietary alpha-linolenic acid did not prevent stenosis- or laser injury-induced venous thrombosis in mice, underscoring the distinct pathophysiological mechanisms promoting arterial and venous thrombosis.
- The described effects of dietary alpha-linolenic acid on platelet aggregation and vascular tissue factor expression in a mouse model of arterial thrombosis did not translate into similar beneficial effects in stasis- or endothelial injury-induced venous thrombosis.
Nomenclature and abbreviations

ALA, alpha-linolenic acid; CVD, cardiovascular disease; DVT, deep-vein thrombosis; ETP, endogenous thrombin potential; GC, glyocalcin; Gl, glyocalcin index; IV, internal jugular vein; IVC, inferior vena cava; MCV, mean corpuscular volume; MFI, mean fluorescence intensity; MPO, myeloperoxidase; n-3 FA, omega-3 fatty acid(s); PPP, platelet-poor plasma; PRP, platelet-rich plasma; RBC, red blood cell(s); TF, tissue factor; PE, pulmonary embolism; SMC, smooth muscle cell(s); VCAM-1, vascular cell adhesion molecule 1; VTE, venous thromboembolism; VWF, von Willebrand factor; WBC, white blood cell(s).

...tions are likely to be due to the diverse triggers of platelet activation and/or the experimental readouts.

The degree of TF inhibition, which is crucial in the prevention of arterial thrombosis, is another point of discussion. We have shown that ALA reduces expression of TF in endothelial cells and smooth muscle cells (13). However, TF is not only expressed in vascular cells but is also released from blood monocytes as the major source of circulating TF (27) and indeed, monocyte-derived TF has been implicated in the coagulation process during stenosis-induced DVT (3). Thus it is unlikely that ALA strongly inhibited the monocyte-derived TF.

Due to the aging population and the associated increase in the prevalence of DVT (1), preventive strategies, particularly if nutritionally achievable are of major interest. Omega-3 FA acids have been found to have beneficial effects on the cardiovascular system in clinical (5–7, 28) and experimental studies (12–14). However, the present study does not support the concept of protective effects of dietary ALA on the development of venous thrombosis in mice.

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Conflicts of interest

None declared.

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