Clinical Nutrition 37 (2018) 948–957

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Fish oil LC-PUFAs do not affect blood coagulation parameters and bleeding manifestations: Analysis of 8 clinical studies with selected patient groups on omega-3-enriched medical nutrition

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ARTICLE INFO

Article history:
Received 29 September 2016
Accepted 22 March 2017

Keywords:
LC-PUFA
EPA
DHA
Omega-3
Coagulation
Bleeding

SUMMARY

Background & aims: The increased consumption of fish oil enriched-products exposes a wide diversity of people, including elderly and those with impaired health to relatively high amounts of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs). There is an ongoing debate around the possible adverse effects of n-3 LC-PUFAs on bleeding risk, particularly relevant in people with a medical history of cardiovascular events or using antithrombotic drugs.

Methods: This analysis of 8 clinical intervention studies conducted with enteral medical nutrition products containing fish oil as a source of n-3 LC-PUFAs addresses the occurrence of bleeding-related adverse events and effects on key coagulation parameters (Prothrombin Time [PT], (activated) and Partial Thromboplastin Time [(a)PTT]).

Results: In all the patients considered (over 600 subjects treated with the active product in total), with moderate to severe disease, with or without concomitant use of antithrombotic agents, at home or in an Intensive Care Unit (ICU), no evidence of increased risk of bleeding with use of n-3 LC-PUFAs was observed. Furthermore there were no statistically significant changes from baseline in measured coagulation parameters.

Conclusion: These findings further support the safe consumption of n-3 LC-PUFAs, even at short-term doses up to 10 g/day of eicosapentaenoic acid + docosahexaenoic acid (EPA + DHA) or consumed for up to 52 weeks above 1.5 g/day, in selected vulnerable and sensitive populations such as subjects with gastrointestinal cancer or patients in an ICU. We found no evidence to support any concern raised with regards to the application of n-3 LC-PUFAs and the potentially increased risk for the occurrence of adverse bleeding manifestations in these selected patient populations consuming fish oil enriched medical nutrition.

1. Introduction

Polyunsaturated fatty acids (PUFAs) are fatty acids which contain more than one double bond in their structure. The two main classes of PUFAs are the omega-6 (n-6) and omega-3 (n-3). N-

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http://dx.doi.org/10.1016/j.clnu.2017.03.027

3 PUFAs include α-linolenic acid (ALA; 18:3Δ9c,12c,15c or C18:3n-3), and 3 long-chain PUFAs (LC-PUFAs): eicosapentaenoic acid (EPA; 20:5Δ5c,8c,11c,14c,17c or C20:5n-3), docosapentaenoic acid (DPA; 22:5Δ7c,10c,13c,16c,19c or C22:5n-3) and docosahexaenoic acid (DHA; 22:6Δ4c,7c,10c,13c,16c,19c or C22:6n-3). The main dietary sources of EPA, DPA and DHA are fatty fish and fish oils produced either from fatty fish or from livers of lean fish. Other sources include human milk and oils from marine mammals, krill or marine algae. N-3 LC-PUFA enriched-foods such as milk, cheeses or spreads and food supplements are also available on the market. The body can convert ALA to EPA, DPA and DHA but this conversion rate, especially to DHA, is generally limited and inadequate to provide LC-PUFAs in sufficient amount to reach the recommended levels.

N-3 LC-PUFAs are involved in a variety of physiological processes, and their intake is associated with positive effects on cardiovascular health, brain function, immunity and inflammation [1,2].

These potential health benefits have led several expert committees to define recommended intakes in healthy populations. The EFSA panel on Dietetic Products, Nutrition and Allergies has proposed an Adequate Intake of 250 mg/day for EPA + DHA for healthy adults for the primary prevention of cardiovascular disease [3]. For people with cardiovascular disease or a medical history of cardiovascular disease, recommended n-3 LC-PUFA intakes are higher. For instance, the American Heart Association advises subjects with history of coronary heart disease to consume 1 g EPA + DHA per day from fish or supplements [4].

A consequence of an increased use of fish oil supplements is that a wide diversity of people, including elderly and those with impaired health will be exposed to n-3 LC-PUFAs in relatively high amounts. From the early observations in the Greenland Inuit population of a significantly longer bleeding-time associated with their very high n-3 LC-PUFA intakes [5], an ongoing debate exists around the possible adverse effects of n-3 LC-PUFAs on bleeding risk. This might be particularly relevant considering the specific recommendations to people with a medical history of cardiovascular events or patients in preparation for upper gastrointestinal surgery, who frequently use antithrombotic drugs (anticoagulant (AC) or platelet aggregation inhibitors (PAI) drugs) [6].

EPA and DHA are incorporated into cell membranes, where they shift the n-3/n-6 ratio of LC-PUFAs, partly replacing the n-6 LC-PUFA arachidonic acid (AA) which is the precursor for the synthesis of many eicosanoids, including prostaglandins, thromboxanes, and leukotrienes. At higher n-3 LC-PUFA concentrations, the competition with AA for cyclooxygenase enzymes leads to a reduction in synthesis of thromboxane A2, a potent promoter of platelet aggregation, and an increase in the formation of thromboxane A3 from EPA, which is a weak platelet aggregation factor [7]. N-3 LC-PUFAs are also suspected to have an impact on levels of some blood coagulation factors, but the results reported from different studies are not consistent. There are some reports that increased intake of n-3 LC-PUFAs leads to a decrease in blood levels of prothrombin, von Willebrand factor and factor V, and in an increase in protein C level in plasma [8,9]. These effects provide plausible mechanisms for reduced blood coagulation and could account for the observations in Inuits. These effects have also raised a concern about the potential effects of high n-3 LC-PUFA intake on blood coagulation in various patient groups.

In order to evaluate the safety of n-3 LC-PUFAs, we looked at the effect of n-3 LC-PUFA-enriched enteral medical nutrition products in patients included in Nutricia sponsored human intervention studies conducted after 2007. Blood coagulation parameters and bleeding-related adverse events were specifically assessed. Eight clinical studies with enteral products enriched in n-3 LC-PUFAs at or above 1.5 g EPA + DHA/day in persons with a variety of different diseases were evaluated.

2. Materials and methods

Six published and one unpublished randomized (registration number NTR1966), double-blind, controlled clinical studies involving n-3 LC-PUFA enriched enteral medical nutrition products performed by Nutricia Research between March 2007 and February 2013 were reviewed specifically for effects on coagulation parameters and (bleeding-related) adverse effects [10–15]. An open label extension (OLE) study in which all subjects received a n-3 LC-PUFA-enriched product was also reviewed [16]. The majority of studies took place in Europe (Netherlands, Germany, Spain, France, Belgium, Spain, Italy and UK), one study was solely conducted in the US, and one study also had recruitment centres in Argentina, Australia, Brazil and Thailand. All protocols had originally been reviewed and approved by the local ethical committees and the studies fully conformed with the principles of the "Declaration of Helsinki" (52nd WMA General Assembly, Edinburgh, Scotland, October 2000), Good Clinical Practice guidelines and with local legislation of the country in which the research was conducted.

2.1. Study populations

Three studies were conducted in oncology patients. One study was carried out in subjects with newly diagnosed oesophageal cancer [11], and the other two included patients with a variety of tumour types and locations, not under treatment during the study period, with the majority including lower gastrointestinal or breast cancers [10,11]. The remaining studies included one performed in human immunodeficiency virus-1 (HIV-1) infected patients not on antiretroviral therapy [12], one in mechanically ventilated patients in intensive care units (ICUs) [15], and three in patients with Alzheimer's disease (AD). Two of the latter trials were in drug naïve, mild AD subjects [14,16], and the other one included subjects with more advanced disease on AD medication [13].

Except in NUSPEC and BITE studies, the consumption of any other food supplements containing vitamins, minerals and/or omega-3 fatty acids or fish oil was not allowed by the subjects. In the 2 other studies, it was not forbidden but was not recommended either and strictly monitored.

All subjects included in the 8 clinical trials were adults and gave their informed written consent, or this was obtained from their representative in the case of the patients in an ICU. Detailed inclusion or exclusion criteria can be found in the scientific publications of the studies.

2.2. Study products

Three studies investigated the effects of an energy dense protein-rich nutritionally complete oral nutritional supplement designed for patients with cancer [10,11]. Three other studies were performed with Souvenaid® (Nutricia NV, Zoetermeer, The Netherlands), an oral product for the dietary management of early AD, currently available on the market [13,14,16], and one with a nutritional concept consisting of a special blend of fibres, proteins and fats [12]. In the study from van Zanten et al. [15], performed in critically ill ICU patients, a high-protein tube feed, enriched with immune-modulating nutrients was used.

Levels of n-3 LC-PUFAs administered orally ranged from 1.5 to 3.6 g/day. In the tube feeding study, target energy intake was 25 kcal/kg body weight/day. For a 70-kg adult, this corresponded to 6.8 g/day EPA + DHA. Feeding was introduced gradually towards this target. The minimal mean intake of 1.5 g EPA + DHA (+/−1.2 g) was reported at day 1 whereas the highest mean intake of 5.6 g/day (+/−2.8 g) was reported at day 9. In practice for some subjects, the maximum EPA + DHA intake was 10.2 g/day. In all studies where

oral products were used, the intervention was a supplement to the subject's diet, whereas in the tube feeding study, the product was the sole source of nutrition. Intervention periods varied from 8 days up to 52 weeks.

In the context of this paper, all n-3 LC-PUFA-enriched products are referred to as "Active" products, and the medical nutrition products not enriched with n-3 LC-PUFAs as "Control".

The product details are summarised in Table 1.

2.3. EPA/DHA incorporation

Blood samples were collected at baseline and at different time points in order to investigate the EPA + DHA incorporation either in erythrocyte membranes, in white blood cells, in plasma or in blood [10,11,13,15–17]. Only the BITE study did not investigate these parameters.

2.4. Coagulation parameters

During the studies, parameters for coagulation function were investigated. Prothrombin time (PT) was measured in all studies except in the EIIC-RT study [10], whereas the activated Partial Thromboplastin Time (aPTT) was investigated in 4 out of 8 studies [13–16]. In the EIIC-RT study, the PT time was replaced by the Partial Thromboplastin Time (PTT). Platelet count was also investigated in the EIIC, EIIC-RT, NUSPEC and BITE studies [10–12]. Each analysis was conducted according to the local protocol of the study site.

2.5. (Serious) adverse events

Details of any (S)AE reported spontaneously by the subjects or observed by the Investigator or medical staff were recorded. The nature of the event (diagnosis or major symptoms/signs), start and end dates, severity, product-relatedness, action(s) taken regarding the (S)AE, action taken regarding the study product, and participant outcome were recorded during all clinical studies as part of the safety evaluation.

2.6. Concomitant medications

At each visit, the investigator obtained information about intake of (any) medication and nutritional supplements, either physician prescribed or not. The product name of the consumed medication or nutritional supplement, daily dosage taken and period of use were reported. Other fish-oil enriched products (food or supplements) were not authorized during the study periods.

2.7. Statistical analysis

In all studies, analyses were performed on the All-Subjects-Treated (AST) population (all subjects who received at least one dose of study product). All data were reprocessed and statistical analyses were performed using SAS version 9.4. For the coagulation parameters, figures presented in this paper are the treatment difference for change from baseline at the end of the product consumption period with 95% confidence interval (CI) (error bars). It was considered not appropriate to conduct a meta-analysis since the populations, doses, exposure durations and time points of measurements were not homogeneous within the selected clinical studies.

For adverse events and concomitant antithrombotic medications, descriptive analyses only are presented. Data on concomitant medications were not available for the METAPLUS study.

3. Results

A total of 1561 subjects were randomized. Seven hundred and eighty subjects received at least one intake of n-3 LC-PUFA enriched product (active) and 777 received the control product (not enriched with EPA + DHA) in the AST population. A total of 1245 participants completed the studies of which 617 received products enriched with EPA + DHA.

The incorporation of EPA, DHA and/or total n-3 PUFAs in white blood cell membranes, erythrocyte membranes or plasma was analysed in almost all studies. Detailed results are available in previous publications for the EIIC-RT, NUSPEC, S-CONNECT, SOUVENIR II, METAPLUS and SOUVENIR II OLE studies [10,11,13–16]. Analyses have also been conducted in the EIIC study but have not been published. In this study, fish oil supplementation resulted in plasma and red blood cells n-3 PUFA levels similar those reached in the EIIC-RT study. Only in the BITE study these parameters were not investigated. All the results showed a statistically significant increase of all these parameters in all matrices analysed, except for the percentage of DHA in white blood cells in the EIIC study. These results indicate a high adherence to intervention during the clinical studies (data not shown).

Blood coagulation parameters were not analysed in all the subjects. In the Souvenaid® trials, only selected study centres performed these analyses [13,14,16]. In total, at least one blood coagulation parameter was analysed in 822 subjects of which 408 received a n-3 LC-PUFA enriched product and 339 received a control product.

In SOUVENIR II OLE study [16], all subjects included had also previously been included in SOUVENIR II study [14]. Indeed 77.9% of subjects from this last study agreed to participate in the open label extension phase, and all subjects received Souvenaid®, the product containing n-3 LC-PUFAs. As a consequence, some subjects took the product for 48 weeks (group named A–A in the tables), whereas others took it for 24 weeks only, during the extension phase (group named C–A in the tables).

3.1. Safety and tolerance

All (S)AE and blood parameters analyses were calculated on the AST population.

Table 2 summarizes the number and proportion of patients experiencing one or more serious adverse events (SAEs) in each study.

Among the SAEs, only one (diarrhoea) in METAPLUS study was judged as possibly related to the Active product. Four SAEs were considered as possibly related to the control product.

Table 3 shows the number of subjects with at least one adverse event (AE), the number of AEs occurring during the clinical trials and the number of AEs with a potential relationship with study product consumption.

A specific review of bleeding-related (S)AE(s) was also performed on the 8 clinical studies. The results are described in Table 4.

A total of 14 bleeding-related SAEs were reported in 14 subjects in 3 clinical studies: NUSPEC, S-CONNECT and METAPLUS studies. Nine bleeding-related SAEs occurred in the active groups and 5 in the control groups. Cerebral/intracranial haemorrhage and gastrointestinal haemorrhage were the most frequent reported bleeding-related SAEs.

A total of 45 bleeding-related (S)AEs occurred in 7 studies (no bleeding-related AE was reported in EIIC-RT study): Twenty five (S)AEs were reported in 20 subjects in the active groups compared to 20 (S)AEs in 20 subjects in the control groups. Half of the bleeding-related AEs were reported in METAPLUS study, conducted in ICU

Table 1
Overview of clinical studies reviewed and n-3 LC-PUFA containing products used.

Study name, first author, year of publication [reference]	Product	Population	Number of subjects in AST population (active/control)	Fatty acid profile of product used							Duration
				Daily dose of EPA + DHA (g/day)	Source	EPA (g/day)	DHA (g/day)	ALA (g/day)	DPA (g/day)	Ratio n-6/n-3	
Randomized clinical trials (RCTs)											
EIIC-RT Faber, 2013 [10]	Energy dense protein-rich nutritionally complete supplement	Mixed population of cancer patients on radiotherapy	38 (20/18)	3.6	Oil from various species (incl. anchovy, mackerel, sardine, tuna)	2.4	1.2	0.37	0.27	1.15	8 days
NUSPEC Faber, 2015 [11]		Oesophageal cancer patients	65 (31/34)								28 d up to 49 days
EIIC		Mixed population of cancer patients	31 (16/15)								8 days
Unpublished											
BITE Cahn, 2013 [12]	NR100157: nutritional concept consisting of a special blend of fibres, proteins and fats	HIV+ patients not receiving anti-retroviral therapy	340 (168/172)	1.8	Sardine and anchovy oils	1.2	0.6	0.04	0.2	0.46	52 weeks
S-CONNECT Shah, 2013 [13]	Souvenaid®	Mild to moderate AD patients	524 (264/260)	1.5	Tuna oil	0.3	1.2	0.03	0.05	0.18	24 weeks
SOUVENIR II Scheltens, 2012 [14]			258 (129/129)								24 weeks
METAPLUS Van Zanten, 2014 [15]	High-protein enteral nutrition formula with immune-modulating nutrients	Mechanically ventilated critically ill patients	301 (152/149)	Mean intakes: 1.5–5.6 g (Day 1–Day 9) Max intake: 10.2 g	Sardine and/or anchovy and/or mackerel oil	0.23 g/ 100 kcal	0.16 g/ 100 kcal	0.098 g/ 100 kcal	0.004 g/ 100 kcal	1.2	Median duration: 12 days (8–21 [Q1–Q3])
Open label extension (OLE)											
SOUVENIR II OLE[a] Olde Rikkert, 2015 [16]	Souvenaid®	Mild to moderate AD patients	201 from Souvenir II (97 A–A/104 C–A)[b]	1.5	Tuna oil	0.3	1.2	0.03	0.05	0.18	24 weeks

Only Souvenaid® is a commercially available medical enteral nutrition product.
[a] Souvenir II OLE study was an extension of the Souvenir II study, so the subjects participating in the OLE study were also participants of the Souvenir II study.
[b] A–A: Active–Active; C–A: Control–Active, the first term corresponding to the belonging group in the Souvenir II study, but all subjects in the OLE study received the fish-oil enriched product.

Table 2
Overview of serious adverse events (SAEs) reported in each study.

Study name [reference]	Total number of subjects with at least one SAE (% total group subjects)		Total number of SAEs (% of total SAEs)	
	Active	Control	Active	Control
EIIC	0 (0.0)	0 (0.0)	0	0
EIIC-RT [10]	0 (0.0)	0 (0.0)	0	0
NUSPEC [11]	6 (19.4)	4 (11.8)	10 (62.5)	6 (37.5)
BITE [12]	1 (0.6)	8 (4.7)	1 (10.0)	9 (90.0)
S-CONNECT [13]	27 (10.2)	34 (13.1)	34 (48.6)	36 (51.4)
SOUVENIR II [14]	10 (7.8)	6 (4.7)	11 (61.1)	7 (38.9)
METAPLUS [15]	39 (25.7)	38 (25.5)	43 (47.3)	48 (52.7)
Total	83 (10.6)	90 (11.6)	99 (48.3)	106 (51.7)
	A–A[a]	C–A[a]	A–A[a]	C–A[a]
SOUVENIR II OLE [16]	10 (10.3) Total: 19 (9.5)	9 (8.7)	11 (52.4) Total: 21 (100.0)	10 (47.6)

[a] A–A/C–A: Active–Active/Control–Active.

Table 3
Overview of all adverse events (AEs) (including serious adverse events) reported in each study.

Study name [reference]	Total number of subjects with at least one (S)AE (% total group subjects)		Total number of (S)AEs (% total (S)AEs)		Number of related[a] (S)AEs including [(S)AE with unknown relationship] (% of total group (S)AEs)		
	Active	Control	Active	Control	Total	Active	Control
EIIC	10 (62.5)	6 (40.0)	14 (60.9)	9 (39.1)	14 (60.9)	9 (64.3)	5 (55.6)
EIIC-RT [10]	13 (65)	10 (55.6)	19 (59.4)	13 (40.6)	29 (90.6)	17 (89.5)	12 (92.3)
NUSPEC [11]	25 (80.6)	21 (61.8)	80 (58.8)	56 (41.2)	35 (25.7)	20 (25.0)	15 (26.8)
BITE [12]	128 (76.2)	128 (74.4)	513 (51.2)	489 (48.8)	370 [2] (36.9)	217 [1] (42.3)	153 [1] (31.3)
S-CONNECT [13]	150 (56.8)	165 (63.5)	458 (50.7)	445 (49.3)	64 [5] (7.1)	34 [5] (7.4)	30 (6.7)
SOUVENIR II [14]	67 (51.9)	78 (60.5)	154 (45.3)	186 (54.7)	55 [1] (16.2)	27 (17.5)	28 [1] (15.1)
METAPLUS [15]	105 (69.1)	105 (70.5)	345 (48.1)	372 (51.9)	96 [1] (13.4)	39 (11.3)	57 [1] (15.3)
Total	498 (63.8)	513 (66.0)	1583 (50.2)	1570 (49.8)	663 [9] (21.0)	363 [6] (22.9)	300 [3] (19.1)
	A–A[b]	C–A[b]	A–A[b]	C–A[b]	Total	A–A[b]	C–A[b]
SOUVENIR II OLE [16]	48 (49.5) Total: 105 (52.2)	57 (54.8)	74 (39.2) Total: 189 (100.0)	115 (60.8)	12 (6.3)	7 (9.5)	5 (4.3)

[a] The relationship of the (S)AE to the study product is assessed by the investigator as being possibly, probably, definitely or unlikely related. In some cases the relationship has not or could not have been assessed by the investigator. Figures in this table include events possibly, probably or definitely related to the study product and [AE with unknown relationship].
[b] A–A/C–A: Active–Active/Control–Active.

patients. The bleeding-related AEs were mostly of gastrointestinal nature, followed by epistaxis.

No bleeding-related (S)AE was considered related to study product consumption by the investigators. The complete list of bleeding-related (S)AEs is available in Appendix 1.

In all studies, most AEs were of a gastrointestinal nature (diarrhoea, constipation, nausea, flatulence, abdominal distension, abdominal cramp and belching) and of mild severity. The distribution between groups and relationship to study product consumption varied depending on the study. In all studies except

Table 4
Overview of all bleeding-related adverse events (AEs) (including serious adverse events) reported in each study.

Study name [reference]	Total number of subjects with at least one bleeding related event (% total group subjects)				Total number of bleeding-related events (% total (S)AE)			
	SAE		AE		SAE		AE	
	Active group	Control group	Active group	Control group	Active group	Control group	Active group	Control group
EIIC	0 (0.0)	0 (0.0)	1 (6.3)	1 (6.7)	0	0	1 (7.1)	1 (11.1)
EIIC-RT [10]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0	0	0 (0.0)	0 (0.0)
NUSPEC [11]	2 (6.5)	1 (2.9)	2 (6.5)	1 (2.9)	2 (20.0)	1 (16.7)	2 (2.5)	1 (1.8)
BITE [12]	0 (0.0)	0 (0.0)	1 (0.6)	4 (2.3)	0 (0.0)	0 (0.0)	1 (0.2)	4 (0.8)
S-CONNECT [13]	3 (1.1)	2 (0.8)	6 (2.3)	5 (1.9)	3 (8.8)	2 (5.6)	6 (1.3)	5 (1.1)
SOUVENIR II [14]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
METAPLUS [15]	4 (2.6)	2 (1.3)	10 (6.6)	8 (5.4)	4 (9.3)	2 (4.2)	15 (4.3)	8 (2.2)
Total	9 (1.1)	5 (0.6)	20 (2.5)	20 (2.6)	9 (9.1)	5 (4.2)	25 (1.6)	20 (1.3)
	A–A[a]	C–A[a]	A–A[a]	C–A[a]	A–A[a]	C–A[a]	A–A[a]	C–A[a]
SOUVENIR II OLE [16]	0 (0.0) Total: 0 (0.0)	0 (0.0)	0 (0.0) Total: 1 (0.5)	1 (1.0)	0 (0.0) Total: 0 (0.0)	0 (0.0)	0 (0.0) Total: 2 (1.1)	2 (1.7)

[a] A–A/C–A: Active–Active/Control–Active.

Table 5
Overview of bleeding-related (serious) adverse events by usage of antithrombotic agents.

Study name [reference]	Total number of subjects receiving antithrombotics (% of total subjects)		Total number of subjects receiving antithrombotics with a bleeding-related event		Total number of subjects with at least one bleeding related event (AE or SAE)	
	Active	Control	Active	Control	Active	Control
EIIC	3 (18.8)	2 (13.3)	0	0	1	1
EIIC-RT [10]	0 (0.0)	1 (5.6)	0	0	0	0
NUSPEC [11]	12 (38.7)	12 (35.3)	1	1	1	1
BITE [12]	4 (2.4)	1 (0.6)	0	0	1	4
S-CONNECT [13]	143 (54.2)	146 (56.2)	6	5	6	5
SOUVENIR II [14]	46 (35.7)	54 (41.9)	0	0	0	1
Total	208 (33.1)	216 (34.4)	7	6	9	12
	A–A[a]	C–A[a]	A–A[a]	C–A[a]	A–A[a]	C–A[a]
SOUVENIR II OLE [16]	34 (35.1) Total: 71 (35.3)	37 (35.6)	0 Total: 1	1	0 Total: 1	1

No data were available on the concomitant treatments in the METAPLUS study.
[a] A–A/C–A: Active–Active/Control–Active.

METAPLUS, the AEs of a gastrointestinal nature were reported as related (definite, probably or possibly related by the investigator) to the active product.

Especially in the BITE study, AEs for GI system disorders (i.e. flatulence, diarrhoea, abdominal distension, abdominal cramp and belching) were more often a reason for the subjects to withdraw from the study in the active group compared to the control group. This is most likely due to the high amount of fibre in the active product, leading to a daily dose of 15 g of fibres (galactooligosaccharide (GOS), fructooligosaccharide (FOS) and pectin).

Data on concomitant antithrombotic medications could be retrieved for 7 studies out of 8 (see Table 5). No data were available from the tube feeding trial, although considering the type of patient population (ICU) the use of anti-coagulant medication such as heparin, especially in surgical patients, is considered standard care. A total of 433 subjects out of 1256 (34.5%) in the AST population were under antithrombotic treatment during the studies. Treatments were similarly distributed between groups in the 7 considered studies, with a total of 33.1% of the subjects in the active groups receiving antithrombotic medication, and 34.5% in the control groups. Most of the subjects receiving antithrombotic medications were observed in the Souvenaid® studies (S-CONNECT, SOUVENIR II and SOUVENIR II OLE) and NUSPEC study. In the S-CONNECT study, 55.2% of the subjects were using antithrombotics (54.2% in the active group, 56.2% in the control group). In the SOUVENIR II and NUSPEC studies, it was more than one third of the subjects (36.9% of the total subjects in NUSPEC, 38.8% in SOUVENIR II). The main anti-platelet treatments reported were acetylsalicylic acid (83.0% and 66.0% of the subjects on antithrombotic treatment, respectively for the S-CONNECT and SOUVENIR II studies), and vitamin K antagonists (VKA: warfarin, acenocoumarol, fluindione) in 14.5% and 22.0% of the subjects with antithrombotic treatment in the S-CONNECT and SOUVENIR II studies, respectively. Clopidogrel was rarely prescribed, mostly in the S-CONNECT study.

In the EIIC, BITE and SOUVENIR II studies, all bleeding-related (S)AEs occurred in subjects who were not using antithrombotic agents.

In the NUSPEC and S-CONNECT studies, all bleeding-related events occurred in subjects using antithrombotics: 2 and 11 events respectively in NUSPEC and S-CONNECT. In the SOUVENIR II OLE study, the sole bleeding-related event was reported in a subject using antithrombotics in the control group. Therefore, this was considered unrelated to n-3 LC-PUFAs consumption as use of fish oils supplements was an exclusion criterion.

3.2. Coagulation parameters

Platelet count was investigated in 4 out of the 8 studies (EIIC, EIIC-RT, NUSPEC, BITE). At the end of the study period, there were no differences between the Active and control groups for this parameter in all 4 studies (data not shown). In the BITE study, platelet count was significantly lower in the active group vs control group at week 13, but values remained within reference ranges. This difference disappeared at the end of the study (week 52).

The results of the coagulation analyses are presented, per parameter, in Figs. 1–3.

Forest plot representing changes from baseline for each study at indicated time point for the AST population.

The consumption of products containing n-3 LC-PUFAs was not associated with a significant change in PT value at the end of the study period and there was no difference between study groups, whatever the clinical trial considered (Fig. 1).

No difference was seen for the PTT parameter between groups for the change from baseline at the end of study period.

Forest plot representing changes from baseline at indicated time point (AST population).

The consumption of a product containing n-3 LC-PUFAs was not associated with a significant change in PTT value at the end of the study period and there was no difference between groups (Fig. 2).

Forest plot representing change from baseline at indicated time point (AST population).

No association was seen between the consumption of products containing n-3 LC-PUFAs and change in aPTT value at the end of the study period and there was no difference between study groups, whatever the clinical trial considered (Fig. 3).

4. Discussion

This paper addresses the debate about the potential adverse effect on blood coagulation as a consequence of n-3 LC-PUFA supplementation. We assessed the impact of DHA and EPA intake from enteral medical nutrition products on coagulation parameters (PT and (a)PTT) and (S)AEs related to clinical bleeding manifestations in various vulnerable patient populations including subjects on concomitant antithrombotic medication. Primary outcomes of the evaluated studies have been reported previously in several publications. The common feature of all products investigated in these studies is their contribution to a relatively high intake of n-3 LC-PUFAs via the investigational products ($\geq$1.5 g EPA + DHA/day)

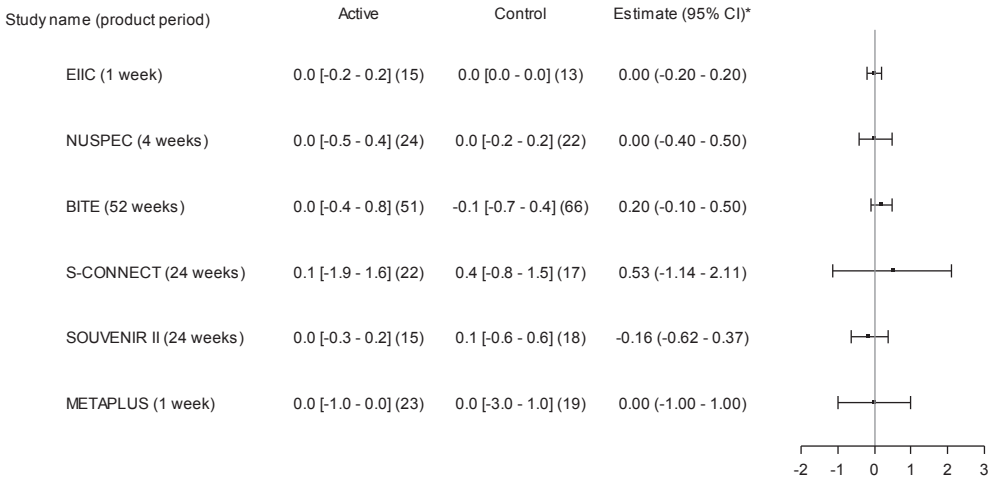

Fig. 1. Treatment difference for Prothrombin Time (PT) change. *The estimate is the Hodges-Lehmann estimate of location shift and the 95% CI is its asymptotic 95% confidence interval.

on top of the diet. These interventions resulted in different n-3 LC-PUFA exposure levels and different durations of exposure, and they included a heterogeneous population according to age, medical history and medical conditions. The administration route also differed (oral versus tube feeding).

This review of 8 clinical studies includes 1561 patients with diverse diseases for the (S)AE analysis including 780 receiving n-3 LC-PUFA. Among all the subjects, 822 had at least one coagulation parameter measured, 408 in the Active groups and 414 in the Control groups. Coagulation is often investigated during intervention studies with fish oils or n-3 LC-PUFAs, but no standardized parameters are defined to enable a comparison of all the studies. Several parameters are reported to assess the coagulation pathway such as bleeding time (BT), PT, (a)PTT, or specific coagulation factor levels in the bloodstream. Moreover, no standardized protocols are currently in place for measuring such parameters, and each laboratory has its own standards and normal values.

Among the coagulation parameters, bleeding time (BT) is often determined by measuring time to haemostasis at the incision edge after a small incision has been made. However, in our studies, BT

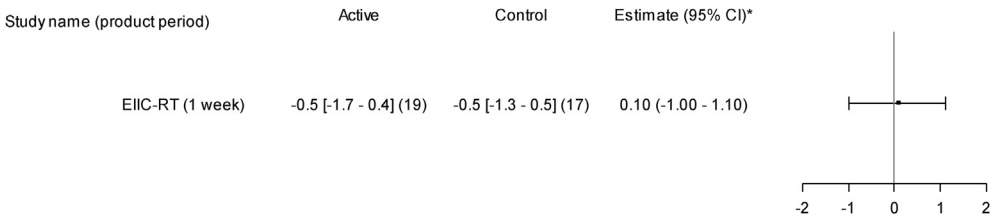

Fig. 2. Treatment difference for Partial Thromboplastin Time (PTT) change. *The estimate is the Hodges-Lehmann estimate of location shift and the 95% CI is its asymptotic 95% confidence interval.

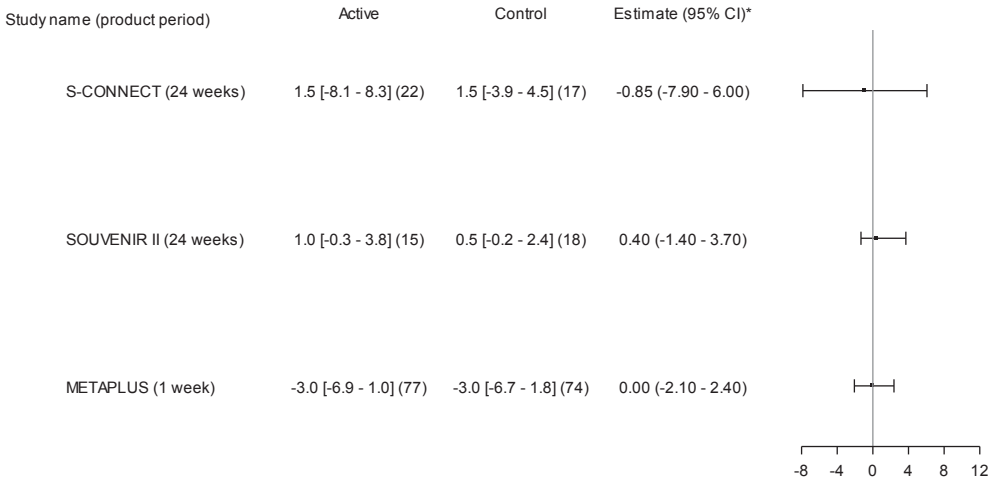

Fig. 3. Treatment difference for activated Partial Thromboplastin Time (aPTT) change. *The estimate is the Hodges-Lehmann estimate of location shift and the 95% CI is its asymptotic 95% confidence interval.

was not used due to its invasive nature and the fact that this parameter is not recommended for patients on anticoagulant treatment which was anticipated to be common in these groups. Furthermore, this method is difficult to standardize which makes these findings difficult to interpret and the procedure less appropriate for multi-country, multi-centre designs that were applicable in most of the studies. Alternatively, PT and (a)PTT were measured and no statistical differences between groups differences were observed for the change from baseline at the end of study period for any of the coagulation parameters investigated.

Findings with BT can be considered questionable in terms of clinical relevance and reliability. For instance, Dyerberg found no change in aPTT, but a longer BT in Inuit compared to Danish control subjects. But most of Inuit subjects had a BT within the normal range [5]. Other studies also report an increase in BT after n-3 LC-PUFA or fish oil ingestion, without clinically relevant bleeding manifestations [18–21], in various populations (healthy and moderately diseased), at various doses of EPA + DHA (from 3.2 g/day up to 15 g/day) and for various periods of time (5 weeks up to 9 months). In contrast, other interventional studies and reviews do not report increases in this parameter in various healthy and unhealthy subjects, including subjects under anticoagulant therapy [18,22–24].

Some publications with fish oils or n-3 LC-PUFAs have also shown no modification of PT or aPTT parameters [5,25,26] as seen in this current study. These previous studies were conducted in a limited number of healthy or diseased subjects (around 10–30 subjects per group), with n-3 LC-PUFAs given orally to healthy subjects or before cardiac surgery. In all these studies, the PT and/or aPTT parameters were investigated and no differences were observed between groups [5,26] or after the n-3 LC-PUFA consumption period [25].

However 2 studies did report an increase in PTT in subjects with hypercholesterolaemia after consumption of n-3 LC-PUFAs at 2 or 4 g/day, for 12 or 9 weeks, respectively [27,28].

In expert opinions, the supplementation of EPA and/or DHA is generally considered safe at doses up to 5 g/day [29] or 5.4 g/day in adults [30], respectively in a healthy population or with anticoagulation treatments. At these doses, no impact on BT can be expected.

N-3 PUFAs are suspected to interact with several elements and pathways of the coagulation, which may not all be covered by the PT and (a)PTT parameters which address the major pathways. For instance interactions of n-3 PUFAs with platelet activating factor (PAF) cannot be detected with these standard parameters. Since these interactions could not be excluded, a specific analysis was conducted on potential clinical manifestations with a focus on the (serious) adverse events (S)AE occurrence and particularly those of a bleeding nature. In this review of studies, no increase in bleeding-related (S)AEs was reported following consumption of n-3 LC-PUFA containing enteral medical nutrition products, even in patients with antithrombotic treatments. The number of subjects using antithrombotics varied a lot between studies. Use was particularly limited in EIIC, EIIC-RT, and BITE studies, and was higher in the NUSPEC study and in studies on patients with Alzheimer's disease. These differences can be explained by the profile of the included subjects. Studies presented here did not aim at studying patients with cardiovascular diseases (CVD) in particular, which is the main population using antithrombotic agents. Here, considering the health status of subjects and their medical treatments, antithrombotic therapies were not set as exclusion criteria and were thus allowed in the studies. Particularly in the Souvenaid studies, a significant proportion of the population received antithrombotic agents, equally distributed within groups, probably because they were also suffering from CVD due to their age (mean age in the studies > 73 years). The most common platelet aggregation inhibitor in the studies was acetylsalicylic acid (aspirin) taken daily at low doses (usually 50–60 mg/day). This is of particular interest in relation to the safety of EPA or DHA on coagulation since both aspirin and n-3 LC-PUFAs interact with cyclooxygenase enzymes.

Apart from antithrombotic agents, other treatments may have an indirect effect on the coagulation efficiency. Antibiotics, chemotherapies, radiotherapies and all drugs that may compromise the gut microflora, and thus reduce the vitamin K synthesis are important to consider. However we are not aware of an existing relation between fish oil consumption and vitamin K production by microbes in the intestine nor did we see fish oil related differences in bleeding events occurrence in our studies.

This lack of effect of n-3 LC-PUFAs on bleeding is in line with other studies. Watson et al. [31] retrospectively studied the risk of bleeding in 182 patients with cardiovascular disease receiving aspirin + clopidogrel in addition to high doses of n-3 LC-PUFAs (mean dose: 3 ± 1.25 g/day). During a mean follow-up period of 33 months, one patient had a major bleeding episode and 4 subjects had a minor one in the treatment group compared to none and 7 respectively in the control group. The difference was not statistically significant. The authors concluded that in their study, the use of high doses of n-3 LC-PUFAs was not associated with an increased risk of bleeding in subjects already receiving the anticoagulant combination of aspirin + clopidogrel. In another study, 551 patients undergoing cardiac surgery (percutaneous transluminal coronary angioplasty) were randomized to receive a daily dietary supplement of ten capsules containing ethyl esters of n-3 LC-PUFAs, providing a total of 4.1 g/day EPA and 2.8 g/day DHA or an equal amount of an ethyl ester of corn oil for 6 months [32]. All patients also received a dose of 325 mg/day of aspirin throughout the 6 months of the study. No significant differences in AEs occurred between the groups, and there were 3% bleeding episodes noted in each group. Although the BT was slightly but significantly increased in the n-3 LC-PUFA-supplemented subjects, all other parameters measured remained within the normal range. Similar investigations were conducted in subjects taking warfarin (VKA) concomitantly to high intakes of n-3 LC-PUFAs (up to 3.3 g/day) [33,34] and no difference in international normalized ratio, a standardized value to express PT time, was observed and no increase in bleeding events was reported. This suggests that subjects could be treated safely with warfarin and fish oil in combination.

One review from Wachira et al. looked at recent publications investigating the effects of n-3 LC-PUFAs on coagulation parameters and bleeding events in a wide variety of clinical settings. The authors concluded there was no support for discontinuing the use of different doses of n-3 LC-PUFAs as a treatment (doses range: 0.84–10 g EPA + DHA/day) before invasive procedures or when given in combination with other agents that affect bleeding [22]. A Cochrane review of 48 randomized controlled trials (involving around 37,000 subjects) and 41 cohort analyses concluded that 0.4–7 g/day EPA + DHA did not lead to any change in clinical bleeding manifestations in adults with or without risk factors for cardiovascular disease [35].

The oil preparations used in the studies reviewed here come from different species of fish with a broad range of fatty acid compositions, containing variable amounts of fatty acids but also sterols, vitamins and other components. It has been described that some minor lipid fractions of cod had anti-PAF action in *in vitro* experiments [36]. However, despite this diverse sourcing, no differences were observed in terms of coagulation parameters. From a general tolerance point of view, products were only reported to lead to some mild to moderate gastrointestinal disturbances but did not change the profile and occurrence of other adverse events.

A limitation that we see is the power of this analysis to determine the safety of n-3 LC-PUFA intake in such diverse populations. Indeed the 8 clinical studies were not primarily designed to assess the safety or tolerance of the products. This is the reason why we collected results from different studies in order to obtain a large and diverse data set from very vulnerable patient groups. This large collection of data in a heterogeneous population is to our opinion one of the strengths of this study. Because we have addressed so many different groups including some taking antithrombotic medications, and with the support of other clinical trials investigating the impact of fish oil on coagulation, we conclude that our findings on the safe use of n-3 LC-PUFAs are applicable to a diverse clinical population including those on concomitant anticoagulant medications as used here.

This analysis of 8 different clinical studies conducted with n-3 LC-PUFA enriched enteral medical nutrition products addresses adverse event occurrence, particularly bleeding events, and laboratory analysis of coagulation parameters. In the populations considered, from moderately to severely diseased, at home or in ICUs, no sign of increased risk of clinical bleeding has been reported. The doses of n-3 LC-PUFAs used ranged from 1.5 to 10.2 g/day. There were no statistically significant changes from baseline for the coagulation parameters (PT, PTT, aPTT). There was no increase in bleeding-related events, even with the concomitant use of platelet aggregation inhibitors such as aspirin, VKA or clopidogrel. The findings of this review support the safe consumption of n-3 LC-PUFAs, even at high doses, in vulnerable and sensitive populations such as subjects with gastrointestinal cancer or subjects in the ICU, since no increased risk of clinical bleeding manifestations has been identified.

Statement of authorship

SJ, JAG and AVH designed, analysed and interpreted the data. SJ drafted the manuscript. JG, AVH, RFW and PCC critically reviewed the paper. All approved the final version of the article.

Funding sources

The studies reviewed were funded by Nutricia Research, The Netherlands.

Disclosure

PCC is an advisor to Nutricia Research. RFW has served as an independent member of Data Monitoring Committees related to clinical studies carried out by Nutricia Research for which the university is financially compensated. His department is receiving grant money from Nutricia Research to perform fundamental research not related to the topic of this paper. SJ is employee of Danone Research, Palaiseau, France. JAG and AVH are employees of Nutricia Research, Utrecht, The Netherlands. All authors comply with the ethical guidelines for authorship and publishing in this journal.

Conflict of interest

No conflicts of interest are present.

Acknowledgements

The authors would like to thank Marion Kaspers and Egbert Biesheuvel for their statistical support.

Appendix 1. List of bleeding related (serious) adverse events by preferred term of the MeDDRA – AST

Adverse event preferred term	Occurrence	AE/SAE	Study	Group
Anal pain and blood loss	1 event for 1 subject	AE	BITE	Control
Application site bleeding	1 event for 1 subject	AE	METAPLUS	Control
Bleeding	1 event for 1 subject	SAE	NUSPEC	Active
Cerebral haemorrhage	1 event for 1 subject	AE	METAPLUS	Control
	4 events for 4 subjects	SAE	METAPLUS	Active
Duodenal ulcer haemorrhagic	1 event for 1 subject	AE	S-CONNECT	Active
Epistaxis	1 event for 1 subject	AE	METAPLUS	Active
	1 event for 1 subject	AE	METAPLUS	Control
	1 event for 1 subject	AE	S-CONNECT	Active
	1 event for 1 subject	AE	S-CONNECT	Control
	2 events for 1 subject	AE	SOUVENIR II OLE	Control
GI haemorrhage	2 events for 2 subjects	AE	METAPLUS	Active
	1 event for 1 subject	AE	METAPLUS	Control
	1 event for 1 subject	AE	SOUVENIR II	Control
	1 event for 1 subject	SAE	METAPLUS	Control
Haematoma	1 event for 1 subject	AE	S-CONNECT	Control
Haematoma neck	1 event for 1 subject	SAE	NUSPEC	Control
Haematuria	2 events for 2 subjects	AE	METAPLUS	Active
	1 event for 1 subject	AE	S-CONNECT	Active
	1 event for 1 subject	AE	S-CONNECT	Control
Haemoperitoneum	1 event for 1 subject	AE	METAPLUS	Active
Haemorrhage intracranial	1 event for 1 subject	SAE	S-CONNECT	Active
Haemorrhage nos	1 event for 1 subject	AE	METAPLUS	Active
	1 event for 1 subject	AE	METAPLUS	Control
	1 event for 1 subject	SAE	METAPLUS	Control
Haemorrhage rectum	1 event for 1 subject	SAE	S-CONNECT	Active
	1 event for 1 subject	SAE	S-CONNECT	Control

(*continued*)

Adverse event preferred term	Occurrence	AE/SAE	Study	Group
Haemorrhoids haemorrhage	1 event for 1 subject	AE	EIIC	Control
Melaena	3 events for 2 subjects	AE	METAPLUS	Active
	1 event for 1 subject	AE	METAPLUS	Control
Metrorrhage	1 event for 1 subject	AE	BITE	Control
Oesophageal haemorrhage	1 event for 1 subject	SAE	NUSPEC	Active
Post-operative haemorrhage	1 event for 1 subject	SAE	S-CONNECT	Active
	1 event for 1 subject	SAE	S-CONNECT	Control
Peri-rectal bleeding	1 event for 1 subject	AE	BITE	Active
Pulmonary haemorrhage	1 event for 1 subject	AE	METAPLUS	Active
Rectal bleeding	2 events for 2 subjects	AE	BITE	Control
	1 event for 1 subject	AE	EIIC	Active

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