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Dose-response effects of omega-3 on platelet aggregation: an observational study

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Abstract

Objective: This study aimed to evaluate the dose-response effects of supplemental omega-3 fatty acids on platelet function in healthy volunteers.

Methods: Twelve healthy volunteers ingested a normal supplemental dose of 1260 mg omega-3 fatty acids daily for 5 days, followed by a high dose of 2520 mg daily for another 5 days. Multiple electrode aggregometry (MEA) with four different agonists was used to measure platelet aggregation before and after the normal- and high-dose regimes. *In vitro* spiking using physiological doses of omega-3 fatty acids was also performed to determine whether MEA is capable of detecting a platelet-inhibiting effect due to omega-3 fatty acids.

Results: There were no differences in platelet aggregation measured by the MEA assay in healthy volunteers after intake of either the normal or high dose of omega-3 fatty acids. In the *in vitro* experiment, a platelet-inhibiting effect of omega-3 fatty acids was shown by an arachidonic acid agonist in MEA .

Conclusions: Supplemental omega-3 fatty acids do not evoke their positive health effects through inhibition of platelet aggregation measurable with MEA.

Keywords

Thrombocyte, platelet aggregation assay, alternative medicine, fish oil, omega-3 fatty acids, arachidonic acid

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Introduction

Fish oil contains omega-3 polyunsaturated fatty acids and is derived from certain oily fish species. Oily fish accumulate omega-3 fatty acids by eating microalgae, plankton, or other small fish. Cold water oily fish, such as salmon, herring, mackerel, anchovies, and sardines, are examples of fish with the highest levels of omega-3 fatty acids per gram. A significant increase in blood serum omega-3 fatty acid levels, especially after eating salmon, has been demonstrated in humans.[1] Omega-3 fatty acids are associated with many positive health effects, including decreased coronary heart disease mortality[2–5] and are also sold as a supplementary naturopathic drug.

Perioperative bleeding can increase after continuous use of omega-3 fatty acid supplements. An experimental study on rats showed that excessive consumption of omega-3 fatty acids could result in a higher risk of bleeding as a complication of surgery.[6] In contrast, although omega-3 fatty acids negatively affect platelet aggregation, they do not contribute to an increased risk of bleeding. Therefore, recommendations state that there is no need to discontinue a fish oil diet before surgery.[7]

Measuring platelet function is a difficult and time-consuming process. However, through the development of point-of-care (POC) techniques, platelet function is easily accessible and is routinely used in some centers to assess perioperative bleeding.[8] We recently investigated the effects of seven different naturopathic drugs, including fish oil on platelet function, using POC instruments.[9] Five healthy volunteers were provided a daily dose of 1260 mg of omega-3 fatty acids for 7 days and blood samples were taken before and after the treatment period. We found decreased platelet aggregation after treatment with omega-3 fatty acids as measured with POC multiple electrode aggregometry (MEA). MEA is used as POC assessment of platelet function in trauma and in perioperative bleeding. Patients who ingest supplemental omega-3 fish oil may be at risk for excessive bleeding in these situations.

The present study aimed to assess whether normal or high doses of omega-3 fatty acids affect platelet function as measured with MEA in a dose-response manner. We hypothesized that a normal dose of omega-3 fatty acids would decrease platelet aggregation and a high dose would enhance this platelet-inhibiting effect.

Materials and methods

Volunteers and omega-3 fatty acid supplementation

This study was approved by the Regional Ethical Review Board, Lund, Sweden (registration number: 2010/482) and all volunteers gave their written consent to participate. The study was conducted in agreement with the Helsinki Declaration and was performed according to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for observational studies.[10] The study was registered as a clinical trial with ClinicalTrials.gov (Identifier: NCT03048981; URL: https://clinicaltrials.gov/show/NCT03048981).

Exclusion criteria were intake of drugs that have an effect on blood coagulation or thrombosis (anti-depressants, non-steroidal anti-inflammatory drugs, acetylsalicylic acid, and selective serotonin reuptake inhibitors), systemic diseases, hemophilia, and smoking. The subjects were carefully instructed in how to consume the supplemental omega-3 fish oil and the importance of following the protocol. Two omega-3 fatty acid capsules (total of 1260 mg) were taken daily for 5 days. Four capsules (total of 2520 mg) were then taken daily for another 5 consecutive days

in the form of Pharbio's Omega-3 Forte fish oil capsules (Pharbio Medical International AB, Stockholm, Sweden) (Figure 1). Details of the Pharbio Omega-3 Forte® capsule contents are provided in Table 1.

Blood sampling

Venous blood was sampled from a brachial vein using a BD Vacutainer™ Blood Transfer Device (BD Vacutainer Systems, Franklin Lakes, NJ, USA) before ingestion of omega-3 fatty acids, after 5 days (normal-dose period), and after 10 days (high-dose period). The samples were collected between 2 and 4 hours after final ingestion of supplemental omega-3 fatty acids in 3.0-mL hirudin blood tubes (Dynabyte GmbH, Munich, Germany).

Platelet aggregation

All blood samples were analyzed using MEA (Multiplate Analyzer®; Roche Diagnostics Scandinavia AB, Bromma, Sweden) 30 to 120 minutes after sampling. MEA measures platelet aggregation using impedance aggregometry. All analyses were performed in duplicate at 37°C. Test variability for the Multiplate Analyzer ranged from 2% to 8% in the study laboratory.[11] The hirudin blood sample (300 µL) was added to a disposable test cell and incubated at a temperature of 37°C with an equal amount of 0.9% saline solution for 3 minutes. Thereafter, 20 µL of a platelet agonist was added and platelet aggregation commenced. The electrical current was gradually broken by the increasing number of platelets aggregating on the positive and negative electrodes. The increase in electrical impedance was plotted on a graph and the area under the curve (AUC) (without a defined unit) was used to quantify the aggregation.

The exact mechanisms of action that lead to platelet inhibition after omega-3 fish oil ingestion are largely unknown. Therefore, we used available agonists provided at the time of the experiment. The activators that we used were adenosine diphosphate (ADP) (platelet aggregation in response to ADP, final concentration of 6.5 µM), thrombin receptor agonist peptide (TRAP) (platelet aggregation in response to TRAP, final

Table 1. Contents of fish oil capsules according to the manufacturer

Content	One capsule
Omega-3-fatty acids	630 mg
EPA	300 mg
DHA	200 mg
DPA	30 mg
Other omega-3 fatty acids	100 mg

EPA, DHA, and DPA are omega-3 polyunsaturated fatty acids that are commonly found in marine oils. They are the main components of fish oil capsules.
EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, DPA = docosapentaenoic acid

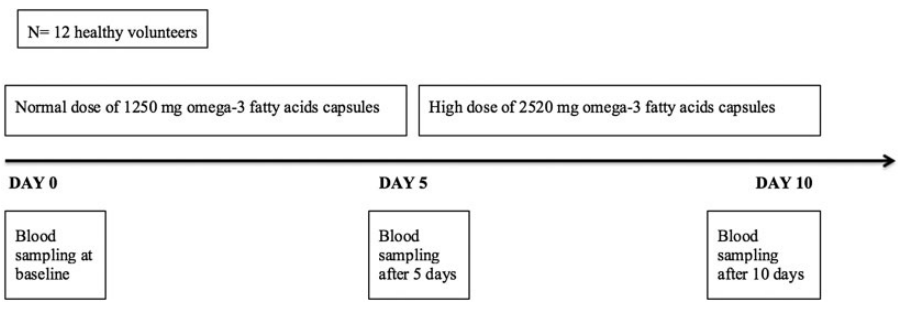

Figure 1. CONSORT flow diagram

concentration of 32 μM), ASPI (platelet aggregation in response to an arachidonic acid agonist, final concentration of 0.5 mM), and ristocetin (RISTO) (platelet aggregation in response to a high concentration of RISTO agonist, final concentration of 0.77 mg/mL). Normal reference ranges for the AUC of MEA were 53 to 122, 94 to 156, 74 to 136, and 90 to 201 for the different agonists of ADP, TRAP, ASPI, and RISTO, respectively.

Omega-3 in vitro testing on volunteer blood

The *in vitro* effect on MEA was investigated using two different doses of omega-3 fatty acids (5 μL = 70 μg for the normal dose and 10 μL = 140 μg for the high dose). Omega-3 fatty acids were extracted from a fish oil capsule using a sterile needle and syringe to avoid contact with air. The extract was added directly to sealed tubes with 0.3 mL of a hirudin-treated blood sample provided by one of the volunteers. These dosages corresponded to an intake of two capsules (1260 mg) and four capsules (2520 mg) of omega-3 fatty acids if fully absorbed from the gut into the blood.

Statistical analysis

The sample size was calculated using MEA data from a previous study.[9] The ADP assay showed a decreased AUC (by 13 ± 12) (mean ± standard deviation [SD]) after 7 days of omega-3 fatty acid intake. To show the same decrease with 90% power and a 5% alpha risk of error after ingestion of a normal dose, a sample size of 10 was required. All of the variables were tested for normality and were found to be non-parametric. The Wilcoxon signed-rank test was used for comparison of before and after values. After compensating for multiple testing of two different doses of omega-3 fatty acids with a modified Bonferroni correction, $P < 0.025$ was considered significant. Statistical analyses were performed using GraphPad Prism 7.00 (GraphPad Software, La Jolla, CA, USA).

Results

Twelve Caucasian men aged between 21 and 64 years were included. All of the participants completed the study without any reported adverse events. There were no differences in platelet aggregation as measured by the MEA assays after intake of either normal doses or high doses of omega-3 fatty acids by healthy volunteers (Figure 2).

In vitro titration showed a clear dose-dependent decrease in platelet aggregation when the ASPI agonist was used for MEA. There was no such effect when using the ADP, TRAP, or RISTO agonists (Figure 3).

Discussion

This study showed that omega-3 fatty acid intake did not affect MEA platelet aggregation in healthy volunteers either at the normal recommended daily supplementary dose of 1260 mg for 5 days or at a double dose for the next 5 consecutive days. The present study is important because the use of naturopathic medicines is increasing. Additionally, the effect of these medicines on platelets should be considered when treating patients with bleeding who may be monitored with instruments, such as the Multiplate.

These results add to the controversy concerning what effect omega-3 fish oils have on platelets and coagulation. Fish oil has been shown to reduce stimulated platelet aggregation and increase bleeding time.[12,13] In a recent study, Gong et al.[14] showed that omega-3 fatty acids in combination with low-dose aspirin led to a significant reduction in platelet aggregation as measured with Born aggregometry in a

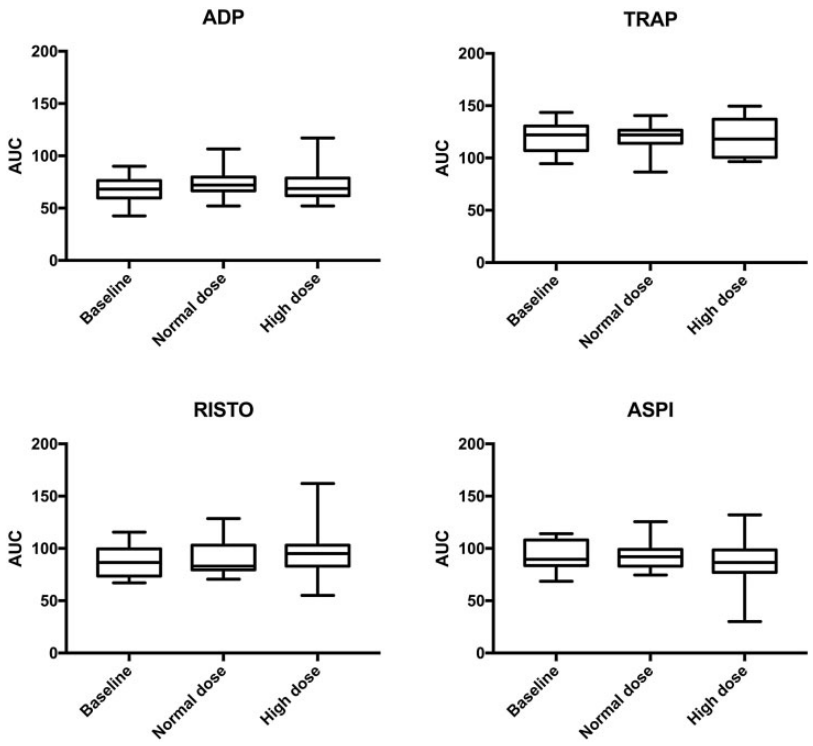

Figure 2. Results of multiple electrode assays. No significant differences were found for ADP, TRAP, ASPI, and high-concentration RISTO agonists. A normal dose of 1260 mg of omega-3 fatty acids was provided daily for 5 days and a high dose of 2520 mg was provided daily for another 5 days. ADP = adenosine diphosphate, TRAP = thrombin receptor activator peptide, ASPI = arachidonic acid agonist, RISTO = ristocetin

mouse model. Their study suggests that omega-3 fatty acids might not be powerful enough to inhibit platelet aggregation by themselves, but may enhance the effect of other platelet inhibitors. Gajos et al.[2] supported this theory. These authors showed that omega-3 fatty acids in combination with aspirin could affect the platelet response to clopidogrel using Born aggregometry. However, *in vitro* platelet adhesiveness was effectively reduced without interaction of other drugs in another study using laminar flow chambers after 6 g of omega-3 fish oil was provided for 25 days.[15] Several studies do not support the findings that omega-3 fatty acids inhibit platelet aggregation.[16–20] Our results are in line with a recently published prospective, double-blind, placebo-controlled, randomized study by Poreba et al.[21] They demonstrated that high doses of omega-3 fatty acids did not affect coagulation in patients with atherosclerosis and type-2 diabetes or platelet function as measured with Born aggregometry. Furthermore, high doses of omega-3 fatty acids did not improve metabolic status or inflammation markers in the same cohort.

In the present study, *in vitro* testing showed that omega-3 fatty acids decreased platelet aggregation with the ASPI agonist in a dose-response pattern (Figure 3). This finding is in line with several previous studies[2,12–15] and implies that the effect of

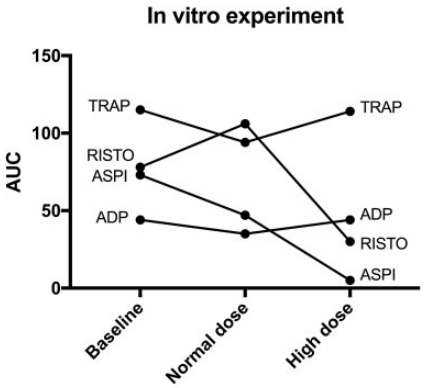

Figure 3. *In vitro* effects of adding two different doses of omega-3 fatty acids. A normal dose (70 μg) and high dose (140 μg) were added directly to a hirudin-treated blood sample that was provided by one of the volunteers. MEA assays were performed once using ADP, TRAP, ASPI, and high-concentration RISTO agonists. The results of the assays are indicated by filled circles.
ADP = adenosine diphosphate, TRAP = thrombin receptor activator peptide, ASPI = arachidonic acid agonist, RISTO = ristocetin.

omega-3 fish oils on platelets may be measurable using MEA.

In contrast to our previous study,[9] the present *in vivo* findings did not show that omega-3 fatty acids have an effect on platelet aggregation. The reason for the different results between studies may be as follows. One reason may be that the previous study was only a pilot study with the risk of chance findings. Additionally, genetic polymorphisms in platelet receptors, as well as coagulation proteins, such as fibrinogen and cytokines, can interact with the effects of omega-3 fatty acids *in vivo*.[22] Natural differences between individuals also need to be taken into account because age, sex, and the level of physical activity can all affect platelet function.[23,24]

Hemostasis is a highly complex system involving multiple components that cannot be completely monitored using current laboratory tests. Our selected method, platelet aggregation analysis, measures only a part of total hemostasis, namely platelet aggregation, which reflects the contribution of platelets in primary hemostasis. However, coagulation and platelet adhesion were not measured in the present study. Therefore, inhibition of the coagulation cascade or platelet adhesion may be a plausible explanation for the putative increased risk of bleeding after ingestion of omega-3 fatty acids.

The American Heart Association suggests that omega-3 fatty acids provide beneficial effects to patients with cardiovascular disease or individuals at risk of developing cardiovascular disease[25] The US National Institutes of Health list some potential health benefits of omega-3 fatty acids as the ability to counteract hypertension, hypertriglyceridemia, and secondary cardiovascular disease.[26] The National Institutes of Health do not definitively suggest that supplemental omega-3 fatty acids are associated with a risk of bleeding. However, the NIH advises caution regarding the simultaneous use of supplemental omega-3 fatty acids and anticoagulants or non-steroidal anti-inflammatory drugs. The Swedish Medical Products Agency suggests that individuals preparing for surgery should inform their doctor about any supplements that they are using.[27]

Whether omega-3 fatty acids inhibit platelet aggregation is an area of controversy concerning supplemental omega-3 fatty acids. One study suggested that the effects of fish oil can benefit patients with atrial fibrillation,[28] but another study reported that fish oil does not provide any major beneficial effects in this situation.[29] Convincing evidence that omega-3 fatty acids can help prevent ventricular arrhythmia was not found in another study.[30] Furthermore, consumption of omega-3 fatty acids was not found to lower the overall risk of myocardial infarction, stroke, cardiac death, or all-cause mortality,

according to a meta-study on 68,680 patients.[31] However, this finding was contradicted by Miza et al. who demonstrated a 7% lower risk of coronary heart disease mortality with consumption of omega-3 fatty acids.[4] Therefore, whether supplemental omega-3 fatty acids prevent myocardial infarction and stroke is still debatable.[32]

Limitations

There are some limitations to this study. The agonists in this study were not tested in a dose-response with different concentrations. Therefore, we were not able to address the question of whether omega-3 fatty acids are able to inhibit platelet aggregation at lower agonist concentrations. Furthermore, the treatment period of 5 to 10 days may not have been sufficient for omega-3 fatty acids to be incorporated into platelets. This finding is in agreement with a study by Thorngren et al.[33] in which omega-3 fatty acids were present in the cell membranes of platelets and were suggested to reduce interaction with the vessel wall. However, von Schacky et al.[34] showed that omega-3 fatty acids were incorporated into the cell membrane of megakaryocytes only 6 days after the start of ingesting omega-3 fatty acids. This previous finding supports the possibility that the 10 days used in the present study should have been sufficient to affect platelets. Nevertheless, in future studies, the time of intervention needs to be increased and omega-3-fatty acid content of platelets needs to be measured before and after the treatment period. A further limitation is the specificity of the method used. As mentioned above, there are no methods that precisely evaluate the total *in vivo* hemostasis. This study focused on a platelet function assay, which only measures a small part of normal hemostasis. Therefore, we cannot rule out that omega-3 fatty acids affect other aspects of coagulation and platelet function.

Conclusion

The findings of this study lead to new questions about the hemostatic effects of supplemental omega-3 fatty acids. Differences in platelet aggregation after ingestion of either normal or high doses of supplemental omega-3 fatty acids in healthy volunteers as measured with MEA could not be shown in the current study. Further studies, including clinical evaluation, as well as other instruments for assessment of coagulation and platelet function, are required to confirm these results. More studies are also required to confirm the clinical significance of omega-3 fatty acids on health and as a risk factor for increased bleeding during surgery.

Declaration of conflicting interests

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