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ORIGINAL ARTICLE

Influence of different supplementation on platelet aggregation in patients with rheumatoid arthritis

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Abstract

Introduction Long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs; eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) have been reported to reduce platelet aggregation. Our aim was to prospectively assess the potential influence of different supplementation omega-3 PUFA on the antiplatelet effects in rheumatoid arthritis (RA) patients.

Methods The study included 60 patients with RA at the Department of Rheumatology, Clinical Center Kragujevac. Patients were divided into three groups depending on who used concentrated fish oil only or concentrated fish oil in combination with evening primrose oil or control group without supplementation in a period of 3 months. Platelet aggregation was measured using the multiplate analyzer and expressed through the value of adenosine diphosphate (ADP) test, aranchidonic acid–induced aggregation (ASPI) test, thrombin receptor–activating peptide (TRAP) test (to assess baseline platelet aggregation), and the ratio of ADP/TRAP and ASPI/TRAP representing the degree of inhibition of platelet aggregation compared to the basal value. The platelet function analysis in whole blood was performed 18–24 h before starting supplementation and after 90 days. Considerations were taken in the representation of demographic, clinical characteristics, and laboratory parameters between the groups.

Results Patients who used concentrated fish oil only had a significantly lower value of the ratio of ADP/TRAP (0.68 ± 0.20) compared to patients without supplementation (0.83 ± 0.12; $p = 0.008$), while there was no statistically significant difference in values of other laboratory parameters of platelet function between other groups.

Conclusions Co-administration of supplementation-concentrated fish oil may reduce platelet aggregation in adults with RA.

Key Points

- *Omega-3 PUFAs are essential for health and are known to possess anti-inflammatory properties, improving cardiovascular health as well as benefiting inflammatory diseases..*
- *In this paper, we report on anti-aggregation effects n-3 PUFAs and -linolenic acid in RA.*
- *The risk of cardiovascular morbidity and mortality is increased in RA, and dietary supplementation of n-3 PUFA may have preventive potential for the cardiovascular management in rheumatoid arthritis.*

Keywords Fatty acids · Platelet aggregation · Rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease that affects the joints, leading to cartilage and bone destruction. The worldwide prevalence of this chronic disease ranges between 0.5 and 1.0%. In recent years, attention has been drawn to the similarity between atherosclerotic inflammatory processes and inflammatory changes in the course of systemic connective tissue diseases, in particular RA [1]. The risk of cardiovascular (CV) disease and mortality is increased in RA [2]. Although traditional CV risk factors may not explain the excess CV risk in RA, they do play an important role and should not be neglected when it comes to CV risk prevention. Since there are no CV risk assessment models for RA specifically, the national guidelines for CV risk management can best be used to determine CV risk and treatment, as advised by the European League Against Rheumatism (EULAR) guidelines for CV risk management in RA [3].

Omega-3 polyunsaturated fatty (PUFA) acids are essential for health and are known to possess anti-inflammatory properties, improving cardiovascular health as well as benefiting inflammatory diseases [1]. Studies support the efficacy of omega-3 PUFA in reducing pain, number of tender joints, duration of morning stiffness, disease activity, use of non-steroidal anti-inflammatory drugs, and improving physical performance in RA patients [4]. The dose of n-3 PUFAs used in these trials has typically been high, between about 1 and 7 g day and averaging about 3.5 g day [5]. Omega-3 PUFA can improve heart rate variability, lower heart rate and blood pressure, improve endothelial function, decrease platelet aggregation, and lower blood triglyceride levels in healthy subjects or CV patients [6].

Activated platelets contribute to plaque formation within blood vessels in the early and late stages of atherogenesis, and therefore, they have been proposed as a risk factor for cardiovascular disease [7]. Clinical studies have demonstrated that activation of circulating platelets occurs in patients with RA. Most studies evidenced omega-PUFA antithrombotic effects [8–12] with no excess in clinically significant bleeding risk [13]. Major (n-3) PUFA includes alpha-linoleic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). ALA is the plant-derived (n-3) PUFA, found in certain seeds, nuts, and their oils. EPA and DHA are the major long-chain (n-3) PUFA derived from seafood consumption [4]. The effect of different supplementation omega-3 PUFA on platelet aggregation in RA patients has not been studied yet.

Currently, there are numbers of platelet (PLT) tests available. Whole blood impedance aggregometry (WBA) has a high sensitivity. The method is based on PLT activation stimulated in vitro in different assays, including arachidonic acid (AA), adenosine diphosphate (ADP), and thrombin receptor–activating peptide (TRAP) [12].

The aim of our study was to examine the influence of different supplementation omega-3 PUFA on platelet aggregation induced by ADP, AA, and TRAP in patients with RA.

Methods

The study group included 60 female patients (mean age 63.1 ± 9.6 years) with RA. The study was prospective,randomized-controlled. Patients were recruited into the study after routine control from the outpatient unit of the Rheumatology Department at the Clinical Center Kragujevac, Serbia, in 2014. RA was diagnosed according to the American College of Rheumatology 2010 revised criteria [13]. The mean duration of the disease was 59 months, SD ± 60 (12–180 months). All patients were on identical rheumatic therapy. Patients treated with low doses of steroids (< 10 mg/day), oral methotrexate (mean dose 15 mg/week) with folic acid 10 mg/week, and non-steroidal anti-inflammatory drugs were given only occasionally.

They were randomly assigned to 3 groups of 20 patients. The first group receive daily after meals 5000 mg of omega-3 PUFA (5 gel capsules Omega-3 Cardio®, Natural Wealth, NBTY Inc.,New York, U.A, in which 1 gel capsule contains 1000 mg of concentrated fish oil with 300 mg of docosahexaenoic acid (DHA), 200 mg of eicosapentaenoic acid (EPA), and 100 mg of other omega-3 fatty acids), the second group receive daily after meals 2 gel capsules Omega-3 Cardio® and 2 gel capsules Evening Primrose Oil® (Natural Wealth, NBTY Inc., New York,USA, in which 1 gel capsule contains 1300 mg of evening primrose oil (EPO) (Oenotherabiennis, seed) with linoleic acid 949 mg and gamma linolenic acid 117 mg), and the third group receive only previous described rheumatologic therapy in the period of 3 months.

Clinical assessment of rheumatology was based on disease activity score (DAS) 28. DAS 28 involves the number of 28 swollen and tender joints, including the proximal interphalangeal joints, metacarpophalangeal joints, wrists, elbows, shoulders, and knees along with erythrocyte sedimentation rate (ESR) and visual analog score (VAS). The VAS was used a 100-mm horizontal line where patients indicated the level the global health assessment by placing a mark on a line between "best" (left end, 0 mm) and "worst" (right end, 100 mm). DAS 28 was calculated using automatic DAS 28 calculator V1.1-beta by Alfons and Michel available at www.umcn.nl.DAS28.

Laboratory testing of platelet reactivity, other laboratory tests, and clinical evaluation were performed on two occasions: before administering omega-3 PUFA and 3 months after randomization.

The following exclusion criteria were used: history of ischemic heart disease, hypertension, diabetes mellitus, smoking habit (in the last 5 years), hyperlipidemia, family

history of cardiovascular disease, premature menopause, chronic renal diseases, deficiency of folic acid, vitamin B_6, vitamin B_{12}, and any other form of arthritis except RA. Clinical evaluation included anthropometric measurements (height, weight). Body mass index (BMI) was calculated as weight/height^2. Written informed consent was obtained from all study participants prior to enrolment. The study was approved by the Ethical Committee, Clinical Center, Kragujevac, Serbia. This study has been conducted in accordance with the principles of the Declaration of Helsinki.

Platelet function monitoring

Platelet aggregability has been measured in heparinized whole blood samples by the method of impedance aggregometry using Multiplate analyzer (Dynabyte, Munchen, Germany). The antiplatelet effect of omega-3 PUFA was assessed in two ways. The first is by direct measurements of platelet aggregability after addition of agonist adenosine phosphate (ADP test) and arachidonate (ASPI test), whereby higher values of these tests indicated a higher residual platelet aggregation and reduced antiplatelet effect of supplementation. The thrombin receptor–activating protein (TRAP) test is used to assess the effect of inhibitors of glycoprotein IIb/IIIa receptors on the platelet aggregability, which is the reason why this test is used for measuring basal platelet aggregability if the patient did not take glycoprotein IIb/IIIa antagonist. The second is by the ratio of ADP/TRAP or ASPI/TRAP (expressed as a percentage), which indicates the percentage of residual platelet activity, compared to the basal value.

Fatty acid extraction and analysis

Venous blood samples were taken after 12 h of fasting at the beginning of the study and after 3 months of supplementation. The modified method by Folch et al., using a chloroform: methanol mixture (2:1 *v*/*v*) as a solvent and 2,6-di-tert-butyl-4-methylphenol (BHT, 10 mg/100 ml) as an antioxidant, was applied to extract total plasma lipids, as described previously [14]. In the next step, the phospholipid (PL) fraction was separated from other lipid subclasses via silica thin-layer chromatography in a neutral solvent system (petrol ether to diethyl ether to acetic acid, 87:12:1 *v*/*v*). Phospholipid FAs then underwent transesterification; the resulting hexane extracts were dried under a stream of nitrogen, and the residue that contained FA methyl esters was dissolved in 10 μl of hexane. Fatty acid analysis was started with the injection of 1 μl of sample into the gas-liquid chromatography device (Shimadzu GC 2014, flame ionization detector, Rtx 2330 column 60 m × 0.25 mm ID, film thickness 0.2 μm, Restek, Bellefonte, PA, USA). Individual FA methyl esters in the samples were identified by comparing sample peak retention times with the standards (Sigma Chemical Co., St. Louis, MO, USA) and PUFA-2 standard mixture (Restek Co., Bellefonte, PA, USA). Phospholipid FA profiles were expressed as the relative percentage of total FAs.

Statistical analysis

Data were analyzed by descriptive and analytical statistics. Depending on the obtained distributions, we used the analysis of variance (ANOVA) or Kruskal-Wallis test for the comparison of groups with different supplementation in relation to the value of ADP, ASPI, and TRAP tests. In order to reduce the family-wise error rate in multiple comparisons, we used the Bonferroni correction, which is incorporated in the post hoc analysis of ANOVA in statistical program SPSS. We also performed contrast tests for post hoc testing of ANOVA results of interest. We also used chi-square ($\chi 2$) test, *t* test, and Mann-Whitney *U* test for the analysis of the influence of different demographic, clinical, and laboratory factors on the platelet aggregation test between the compared groups. To assess the effects of the supplementations, two-way (group and time) repeated measures ANOVA was applied, with the Bonferroni post hoc tests, where appropriate.

Results

Patients were divided into 3 groups from 20 depending on the type of supplements that were used. The first group took 3 months of omega-3 PUFA, the second group took 3 months of omega-3 PUFA + EPO, and a third group did not take supplements. Patients' demographics and clinical characteristics are shown in Table 1. The three groups did not differ in regard to age, body mass index, duration of RA, prevalence of previous symptoms, and clinical signs of RA. Values of laboratory parameters and hemostasis parameters did not significantly differ between the groups that were compared.

The baseline values of PTL function tests assessed 18–24 h before starting supplementation were comparable between the groups (Table 1).

After 12 weeks of supplementation, levels of EPA, DHA, and n-3 PUFA were higher, and the n-6/n-3 ratio was lower in the two supplemented groups than in the control group. AA was higher in group II (fish oil + EPO) than in the other two groups (Table 2).

Table 3 shows the mean, standard deviation, and median values for ADP, ASPI, and TRAP test and the ratio of ADP/TRAP and ASPI/TRAP between the test groups before supplementation. The variables ADP, TRAP, and ADP/TRAP had a normal distribution, so we used ANOVA in order to analyze the differences between three different groups. For all variables, ADP, TRAP, and ADP/TRAP, Levene's test indicated the homogeneity of variances. Between the groups, there was no significant difference, as shown in Table 2.

Table 1 Baseline clinical and laboratory characteristics in patients with rheumatoid arthritis

Patient characteristics	Group I ($n = 20$)	Group II ($n = 20$)	Group III ($n = 20$)	p value
Age ($x \pm$ SD; years)	54 ± 8	57,3 ± 8	59 ± 7.5	0.80[a]
BMI (kg/m^2)	26.48 ± 4.15	26.52 ± 4.37	24.65 ± 6.29	0.313[b]
PTL ($\times 10^3$/μl; mean ± SD)	173 ± 52	186 ± 52	219 ± 53	0.517[b]
Fibrinogen (mean ± SD)	5.38 ± 1.97	4.74 ± 0.91	4.52 ± 0.56	0.869[b]
CRP, mg/l	12.4 ± 8.2	16.0 ± 18.3	12.7 ± 7.2	0.184[b]
Disease duration ($x \pm$ SD; years)	6.6 ± 4	8.1 ± 2.75	7.25 ± 2.6	0.566[b]
Tender joint count	6.25 ± 2	5.45 ± 1.9	5.0 ± 2	0.719[b]
Swollen joint count	1.85 ± 1	1.5 ± 1.6	1.0 ± 1.3	0.363[b]
VAS (global health assessment)	55.7 ± 10.1	58.95 ± 9.1	61.5 ± 8.9	0.302[b]
ESR, mm/h	35 ± 24	36.7 ± 19.2	33.25 ± 17.14	0.674[b]
DAS 28 (ESR)	4.99 ± 0.88	4.76 ± 0.85	4.66 ± 0.80	0.720[b]
HAQ	1.4 ± 0.38	1.36 ± 0.19	1.36 ± 0.23	0.610[b]

Values are expressed in mean ± SD. *Abbreviations: ANOVA* analysis of variance, *NS* not statistically significant, *BMI* body mass index, *PTL* platelets, *CRP* C-reactive protein, *VAS* visual analog scales, *ESR* erythrocyte sedimentation rate, *DAS 28* disease activity score 28, *HAQ* health assessment questionnaire, *w2* chi-square

[a] ANOVA test

[b] w2 test

[c] Significant at $p < .05$

Table 4 shows the mean, standard deviation, and median values for ADP, ASPI, and TRAP test and the ratio of ADP/TRAP and ASPI/TRAP between the groups after 3 months of supplementation. The ANOVA of the variable ADP indicated that differences between the 3 compared groups were not significant ($F3, 327 = 2.502$, $p = 0.057$).

Because of the borderline level of significance, we have decided to make contrast analysis between the group with lowest ADP mean value, in contrast to all other groups combined, and, in the second step, to compare it with the group with highest ADP mean value. Contrast analysis indicated significantly lower mean in group I, than the rest of the 2 groups combined ($t327 = 2121$, $p = 0.035$), and significantly lower mean compared to group III ($t327 = 2.679$, $p = 0.008$), which could indicate that, despite non-significant finding in ANOVA test, a significant difference between group I and III could be present. The variable ASPI did not have a normal distribution, so we performed the Kruskal-Wallis test, which indicated there were no significant differences between the three different groups.

The ANOVA of the variable TRAP did not find significant differences between the three compared groups. There was a significant difference in the ANOVA test of the variable ADP/TRAP between the compared groups for the 3 conditions ($F3,327 = 2.846$, $p = 0.042$). In order to identify between which groups the difference was significant, and to decrease the probability of type 1 error in multiple comparisons, we performed the Bonferroni post hoc analysis, which indicated that the mean value of the ADP/TRAP in group I (0.68 ± 0.20) was significantly lower than the mean value of the ADP/TRAP in group III (0.83 ± 0.12). Post hoc contrast analysis indicated that group I had a significantly lower mean of the ADP/TRAP value, than the rest of the 2 groups combined ($t327 = 2612$, $p = 0.009$), and also significantly lower compared to group III only ($t327 = 2.848$, $p = 0.005$).

The variables ASPI/TRAP did not show normal distribution, so we performed the Kruskal-Wallis test, which indicated there were no significant differences between the three different groups.

Adverse effects

Adverse effects related to the study treatment were mild, and their rate did not differ between the placebo and omega-3 PUFA groups. Mild gastrointestinal discomfort (mild diarrhea, abdominal pain, dyspepsia, or nausea lasting less than 72 h) that did not require any additional intervention was noted in 2 patients in the omega-3 PUFA group.

Discussion

Patients with rheumatoid arthritis (RA) suffer significantly increased CV morbidity and mortality when compared to the general population. Both traditional CV risk factors and high levels of systemic inflammation have been linked to the increased CV risk in RA patients, but significant uncertainty remains regarding the mechanisms through which these factors contribute to CV disease. In addition, ongoing questions remain regarding how best to identify RA patients at high risk for CV disease and what primary and secondary prevention strategies are effective at influencing CV outcome [15].

Table 2 Comparisons of fatty acid distribution in plasma phospholipids after 12 weeks

Fatty acid	Group I	Group II	Group III
20:4 n-6 AA	10.22 ± 1.81	11.29 ± 2.14*#	9.60 ± 2.90
20:5 n-3 EPA	1.01 ± 1.02**	0.49 ± 0.23*#	0.40 ± 0.25
22:5 n-3 DPA	0.58 ± 0.30	0.45 ± 0.16	0.56 ± 0.69
22:6 n-3 DHA	2.74 ± 1.08*	2.22 ± 0.74*	1.83 ± 0.72
n-3	4.55 ± 2.26**	3.33 ± 1.00*	3.09 ± 1.15
n-6	39.05 ± 3.22	40.75 ± 2.86	40.03 ± 2.71
n-6/n-3	10.62 ± 5.07**	13.50 ± 4.81*	14.27 ± 4.44
SFA	46.24 ± 3.97	45.19 ± 2.80	46.26 ± 3.67
MUFA	10.16 ± 1.34	10.67 ± 1.12	10.61 ± 1.74
PUFA	43.13 ± 3.17	44.07 ± 2.96	43.60 ± 4.22

* Significantly different compared groups ($p \leq 0.05$)

** $p \leq 0.01$

Significantly different from group I ($p \leq 0.05$). Assessed by one-way ANOVA followed by Tukey's post hoc test

Data are expressed as the mean ± SD. *NS* not statistically significant, *AA* arachidonic acid, *EPA* eicosapentaenoic acid, *DPA* docosapentaenoic acid, *DHA* docosahexaenoic acid, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

The consumption of omega-3 in combination with standard drugs can improve symptoms of cardiovascular problems. In our study, patients intake capsules with n-3 PUFA or GLA and these resulted in an increased incorporation of the anti-inflammatory eicosanoid precursor FAs (EPA, DHA, DGLA) in plasma phospholipids. The AA/EPA ratio and the n-3 FA index, which represent highly discriminative risk factors for sudden cardiac death [16–18], were significantly improved due to the consumption of 3 g of n-3 PUFA. This is an added benefit because CV mortality is strongly elevated in patients with RA [19, 20].

Despite the abundance of studies concerning omega-3 supplements, evidence is not clear about the benefits of these supplements, with both positive and negative trials [21]. Meta-analysis shows that supplementation with n-3 PUFAs is associated with a significant reduction in ADP-induced platelet aggregation and PAU when using the RPFA VerifyNow® system. Furthermore, there is a trend toward a decrease in collagen- and AA-induced platelet aggregation compared with controls, in the absence of statistical significance [21].

Several uncontrolled studies have reported conflicting results regarding the effects of fish oil supplementation on platelet aggregation; thus, its putative effects remain unclear and controversial. We report for the first time to our knowledge in a controlled study the antiaggregatory effects of EPA, DHA, and LA in RA patients. Process of platelet activation aggregation is influenced by numerous factors. During data collection and analysis, we considered all demographic, clinical, and laboratory factors that could affect the aggregation test, but did not find a significant difference in their distribution between the groups. Values of laboratory parameters including hemostasis parameters and inflammation markers did not significantly differ between the compared groups. In our study, we observed different levels of reduction in ADP-induced platelet aggregation compared to baseline values (ADP/TRAP) between the groups of patients who used omega-3 PUFA and the group of patients without supplementation, which cannot be attributed to other factors.

In patients treated with omega-3 PUFA, we also noted a lower platelet aggregation upon stimulation with ADP at both 0/90 days compared to the control group, but not significant statistically. Thus, the 3-month treatment with omega-3 PUFA resulted in an additional inhibition of platelet aggregation upon stimulation with ADP by nearly 7%.

Nomura et al. clearly showed that platelet activation significantly decreased with EPA treatment in hyperlipidemic and diabetic patients [21]. The 4-week supplementation of omega-3 PUFA reduced the measures of platelet aggregation and activation in healthy subjects showed a recent study [22]. The previous study clearly demonstrated that the addition of omega-3 PUFA to standard dual antiplatelet therapy was associated with a significant reduction of platelet reactivity compared to placebo [23].

As we know, arachidonic acid can produce several eicosanoids through cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) pathways. One important mechanism for the effect of n-3 PUFAs can be attributed to its competing role with arachidonic acid in eicosanoids metabolism; n-3 PUFAs can inhibit the oxidation of arachidonic acid by cyclooxygenase (COX) enzymes, and thus reducing the production of eicosanoid thromboxane A2 (TXA2) and reduced TXA2-mediated platelet activation and aggregation; n-3 PUFAs can be incorporated into membrane phospholipids at the expense of arachidonic acid and thus reduce the production of eicosanoids from arachidonic acid [24, 25].

Omega-3 PUFA were shown to affect properties of the platelet membrane, its receptors, and ion channels. Omega-3 PUFA may also affect metabolic processes related to the hepatic cytochrome P450 (CYP) activity. PUFA and related eicosanoids were shown to be metabolized by the CYP system [24]. Most literature data refer to arachidonic acid which is oxidized, epoxidized, and hydroxylated by the CYP system [25]. The latter epoxide products of arachidonic acid metabolism, also known as oxylipids, play complex and often counter-regulatory autocrine and paracrine roles in the CV system [21]. There are few data in the literature regarding CYP-related metabolism of omega-3 PUFA and interactions with arachidonic acid metabolism or drugs metabolized by the CYP system. As the same CYP isoforms also metabolize arachidonic acid, Fer et al. [26] were able to demonstrate experimentally that administration of EPA and DHA reduces the production of epoxide derivatives of arachidonic acid by

Table 3 Differences in platelet aggregability values among the groups before supplementation

Test		Group I	Group II	Group III	*p* value
ADP	Mean ± SD	600.00 ± 285.0	671.90 ± 255.20	676.20 ± 193.18	0.352[a]
	Median	520.00	698.50	708.00	
ASPI	Mean ± SD	641.70 ± 311,59	667.65 ± 339.76	820.40 ± 278.42	0.733[b]
	Median	641.00	801.00	828,00	
TRAP	Mean ± SD	838.15 ± 314.56	960.60 ± 199.73	864.70 ± 196.82	0.320[a]
	Median	860.50	1007.50	874.00	
ADP/TRAP	Mean ± SD	0.99 ± 0.34	0.70 ± 0.19	0.78 ± 0.15	0.176[b]
	Median	0.77	0.79	0.75	
	Percentage of reduction	99	70	78	
ASPI/TRAP	Mean ± SD	0.89 ± 0.31	0.70 ± 0.35	0.93 ± 0.22	0.534[a]
	Median	0.95	0.85	0.96	
	Percent of reduction	89	70	93	

Abbreviations: *ADP* adenosine diphosphate, *ANOVA* analysis of variance, *SD* standard deviation

[a] ANOVA test

[b] Kruskal-Wallis test

[c] Significant at $P < .05$

80% and 60%, respectively. The role of CYP-related metabolites of omega-3 PUFA was recently highlighted by Arnold et al. [27] who reviewed experimental and clinical studies in this area and found that these metabolites may be partially responsible for the beneficial effects of omega-3 PUFA in the CV system. It has been recently reported that omega-3 PUFA has some other beneficial properties, including decreased thrombin generation, reduction of oxidative stress, and favorable modification of fibrin clot characteristics [25].

Several uncontrolled studies have reported conflicting results regarding the effects of fish oil supplementation on platelet aggregation. Larson et al. [28] demonstrated in ten healthy volunteers using whole-blood impedance aggregometry that omega-3 PUFAs alone were not able to change platelet aggregation. Serebruany et al. evaluated platelet function following 2 weeks of omega-3 fatty acid supplementation in patients with CAD who were taking ASA. Collagen- and AA-induced aggregation were not affected by the treatment [29]; thus, its putative effects remain unclear and controversial.

In a controlled study report that the antiaggregatory effects of EPA and DHA are sex specific [30]. They offer a plausible explanation whereby sex, a non-modifiable determinant of cardiovascular disease, may likely explain the inconsistent results in the fish oil and platelet aggregation literature [31]. Interactions between sex hormones and omega-3 fatty acids exist to differentially reduced platelet aggregation [31, 32]. Our study group consisted exclusively of women, in which we avoid the influence of sex as an independent CV factor on platelet aggregation.

Table 4 Differences in platelet aggregability values among the groups after supplementation

Test		Group I	Group II	Group III	*p* value
ADP	Mean ± SD	560.55 ± 251.32	651.50 ± 204.35	667.50 ± 189.49	0.057[a]
	Median	489.50	704.50	738.00	
ASPI	Mean ± SD	609.80 ± 332.24	662.40 ± 314.08	783.95 ± 309.47	0.480[b]
	Median	611.00	728.00	835.00	
TRAP	Mean ± SD	836.20 ± 242.17	893.00 ± 253.05	804.25 ± 209.83	0.586[a]
	Median	818.00	893.00	827.50	
ADP/TRAP	Mean ± SD	0.68 ± 0.20[c]	0.79 ± 0.14	0.83 ± 0.12[c]	0.042[b]
	Median	0.65	0.78	0.89	
	Percent of reduction	68	79	83	
ASPI/TRAP	Mean ± SD	0.76 ± 0.39	0.80 ± 0.30	0.96 ± 0.23	0.565[a]
	Median	0.91	0.87	0.95	
	Percentage of reduction	76	80	96	

Abbreviations: ADP adenosine diphosphate, *ANOVA* analysis of variance, *SD* standard deviation

[a] ANOVA test

[b] Kruskal-Wallis test

[c] Significant at $p < .05$

In patients with RA, we found significantly lower ADP-induced residual platelet aggregation compared to baseline values in the group of patients who used omega-3PUFA 3 months to the group of patients who did not use supplementation. There was no significant difference in neither ADP nor arachidonic acid-induced platelet aggregation between the groups of patients with RA who used omega-3 PUFA and the patients with RA who used omega-3 PUFA and EPO.

Meta-analysis of 15 randomized controlled trials demonstrates that n-3 PUFA-supplementation is associated with a significant reduction in platelet aggregation when the participants were at poor health status, but not in healthy persons [32].

Our findings did not show that platelet hyperactivity was reduced when EPO was added to the diet. EPO may affect the modulation of hyperaggregability. Thus, the modulation of platelet aggregation percentage using EPO may potentiate the effect of other medications commercially used in CVD.

We can also assume that some of the observed differences in the values of aggregation test (especially regarding ADP test) could not manifest due to the smaller number of patients included in our study, so further research with a larger number of patients is needed. The weakness of this study is the lack of a blinded placebo-controlled group, which would have made the interpretation of the data stronger.

Small study sizes and lack of comparability between methods to assess platelet function currently limit robust evidence on the efficacy of dietary bioactives in specific patient groups. Implementation of uniform point-of-care tests to assess platelet function, and enhanced knowledge of the efficacy by which specific dietary compounds and their metabolites affect platelet function, may enable the identification of functional antiplatelet ingredients that are eligible for a health claim, or combined treatment strategies, including both pharmacological antiplatelet treatment as well as dietary intervention, to tackle CV disease prevention in RA. Further ongoing investigations could be helpful in that regard.

Compliance with ethical standards

Disclosures None.

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