

Differential Effects of Dietary Supplementation with Fish Oil or Soy Lecithin on Human Platelet Adhesion

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Key words

Fatty acid, adhesion, platelet, thrombosis

Summary

To investigate the possible regulating role of ω-6 and of ω-3 fatty acids on platelet adhesiveness, we randomised 60 volunteers into three groups to take 20 ml (equivalent to 0.3 g ω-6, 3.6 g ω-3; ω-6/ω-3 ratio 0.1) per day of a fish oil supplement, or to take 25 g (equivalent to 1.5 g ω-6, 0.5 g ω-3; ω-6/ω-3 ratio 3) per day of a soy lecithin supplement, or to continue on their usual diet without any supplement (control group) for a period of 15 days. Platelet adhesion on fibrinogen-coated 96-well microtitre plates was evaluated in the resting condition and after stimulation with 2 μM ADP or 0.02 U/ml thrombin. Compared to the values before the experimental period, the fish oil group showed a significant reduction in stimulated adhesion (with ADP: from 18.8% to 15.6%, $p < 0.01$; with thrombin: from 24.4% to 20.8%, $p < 0.005$), whereas no difference was noted in the resting condition (from 3.6% to 3.5%, NS). In the soy lecithin group, platelet adhesion was increased in all test conditions (with ADP: from 18.7% to 23.2%, $p < 0.001$; with thrombin: from 24.0% to 29.9%, $p < 0.001$; resting: from 3.5% to 6.6%, $p < 0.001$). No significant changes were observed in the control group. A good correlation was found between platelet adhesion data and the changes in the platelet fatty acid ω-6/ω-3 ratio caused by the different supplementations. Our results indicate an inhibitory effect of fish oil rich in ω-3 fatty acids on stimulated human platelet adhesiveness and a stimulatory effect of soy lecithin rich in ω-6 fatty acids on resting and stimulated adhesion. They suggest moreover that the ω-6/ω-3 ratio is a determinant of platelet adhesion.

Introduction

Polyunsaturated fatty acids of the ω-6 series as well as those of the ω-3 series are known to be essential nutrients in human diets: these are two non-interconvertible classes of fatty acids that constitute a significant part of cell membranes (1). Whereas cellular proteins are genetically determined, the lipid composition of cell membranes depends to a substantial extent on the composition of the diet. Over the past 15-20 years, numerous epidemiological and experimental studies have demonstrated that polyunsaturated fatty acids of the ω-3 series have beneficial effects on coronary artery disease and on chronic inflammatory diseases such as rheumatoid arthritis (2, 3). The effects of ω-3 on haemostasis are still a matter of debate and among the more controversial effects ofω-3 are those on *ex-vivo* platelet function (4). Since platelets are regarded as playing a key role in processes such as thrombogenesis, atherosclerosis and inflammation, investigations have been carried out to evaluate whether ω-3 fatty acids affect platelet number, composition and function (5).

At present there are few data regarding the effects of fatty acids on human platelet adhesion, the earliest event manifested by circulating platelets once the endothelium is injured. Moreover, the studies investigating the relationship between platelet adhesion and ω-3 have yielded conflicting results: whereas Li and Steiner (6, 7) demonstrated an inhibitory effect of dietary fish oil on platelet adhesion, this was not confirmed by Owens and Cave (8). On the other hand, to the best of our knowledge, there are as yet no data in the scientific literature regarding the possible effects of ω-6 fatty acids on human platelet adhesion.

The optimal amount of dietary ω-3 fatty acids required to achieve positive effects, for reducing the severity of inflammatory diseases and of atherosclerotic process, is also unclear, ranging from 3 to 30 g/day (9, 10). Since there is competitive inhibition between ω-3 and ω-6 fatty acids for desaturation and elongation, it has recently been postulated that the effectiveness of dietary ω-3 fatty acids in vascular disease depends not only on the amount of ω-3 but also on the amount of ω-6 fatty acids in the diet (11, 12). This evidence could suggest that the ratio ω-6/ω-3 might be a determinant of platelet response more sensitive than the absolute amount of ω-3 or ω-6 fatty acids.

We designed the present study to investigate, in a homogeneous population of healthy adult subjects, the effects of different supplementations, causing opposite changes in ω-6 and ω-3 fatty acids. The changes in platelet fatty acids after 15-day supplementation were then correlated with a very sensitive parameter of platelet function (13), namely resting and agonist-stimulated adhesion to fibrinogen-coated surfaces.

Patients, Materials and Methods

Experimental Design

A total of 60 normal, healthy volunteers (30 men and 30 women), all non-smokers, aged from 25 to 45 years participated to the study. Three groups of 20 persons each (10 men and 10 women) were randomly formed: the volunteers in the first group took 20 ml (equivalent to 0.3 g ω-6, 3.6 g ω-3; ω-6/ω-3 ratio 0.1) per day of a fish oil supplement (Oil-Check Prep, Elysian); the second group took 25 g (equivalent to 1.5 g ω-6, 0.5 g ω-3; ω-6/ω-3 ratio 3) per day of

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Table 1 The fatty acid composition of fish oil and of soy lecithin used

FISH OIL			SOY LECITHIN		
FA	mg/ml	% total FA	FA	mg/ml	% total FA
C12:0	0.33	0.07	C12:0	0.02	0.01
C14:0	25.14	5.33	C14:0	0.31	0.23
C16:0	74.25	15.74	C16:0	32.68	23.76
C16:1ω7	42.55	9.02	C16:1ω7	0.35	0.25
C18:0	19.74	4.18	C18:0	7.46	5.42
C18:1ω9	98.99	20.99	C18:1ω9	17.73	12.89
C18:2ω6	10.97	2.33	C18:2ω6	59.80	43.48
C18:3ω3	5.00	1.06	C18:3ω3	17.93	13.04
C18:4ω3	21.02	4.46	C18:4ω3	0.03	0.02
C20:0	0.48	0.10	C20:0	0.57	0.41
C20:1ω9	5.45	1.16	C20:1ω9	0.24	0.17
C20:2ω6	1.78	0.38	C20:2ω6	0.30	0.22
C20:4ω6	3.33	0.71	C20:4ω6	0.04	0.03
C20:5ω3	67.07	14.22	C20:5ω3	-	-
C22:0	0.37	0.08	C22:0	-	-
C22:1ω9	5.89	1.25	C22:1ω9	0.07	0.05
C22:6ω3	89.05	18.88	C22:6ω3	-	-
C24:0	0.30	0.06	C24:0	-	-

FA: fatty acids

a soy lecithin supplement (6H12 Soy, Forum Srl); the third group continued the usual diet without any supplement and served as a control group. The fatty acid compositions of the fish oil and soy lecithin used are shown in Table 1. It should be noted that soy lecithin also contains a certain amount of ω-3 (C18:3ω3), but the main fatty acid is C18:2ω6 (43.48%), which is the reason why soy lecithin is used as a source of ω-6 fatty acids. The participants had not taken any drugs for at least three weeks before the experiments. They abstained from all medications for the entire 15-day supplementation period and they were advised to continue their regular diet and their usual activities throughout the study. To monitor the diets, the subjects kept a daily dietary record during the experimental period. Dietary compliance was verified by measuring platelet fatty acid composition at baseline and at the end of the experimental period. Informed consent was obtained from all volunteers participating in this study.

Platelet Separation

Blood samples were collected after overnight fasting. For the adhesion test, platelets were harvested from blood by differential centrifugation. A final volume of 10 ml of blood was drawn by venipuncture in 1.66 ml of anticoagulant solution (15 g/l citric acid, 20 g/l dextrose, 25 g/l sodium citrate) and platelet-rich plasma was obtained by centrifugation at 300 g for 10 min. The platelet-rich plasma was recentrifuged at 700 g for 15 min and platelets were gently suspended (5×10^7 platelets/ml) in a buffer composed of 145 mM NaCl, 5 mM KCl, 10 mM Hepes, 0.5 mM Na_2HPO_4, 6 mM glucose and 0.2% human serum albumin, pH 7.4 (buffer A). The platelet suspension was kept at room temperature and utilized within 1 h. Preliminary experiments showed that within this time platelet adhesion did not change. Ten min before use, platelets were warmed to 37° C.

For the purpose of the platelet fatty acid analysis, 20 ml of venous blood were collected with EDTA-containing vacutainer tubes. Platelets were separated by centrifugation at 20° C for 20 min at 120 g. The resulting platelet-rich plasma was transferred to silicon coated glass tubes; platelets were isolated by centrifugation at 1,000 g for 15 min at 20° C and washed three times in ice-cold NaCl (154 mM) solution containing EDTA (0.1 mM), buffered at pH 7.4. The platelet pellet was resuspended in 500 μl of ice-cold double-distilled water and sonicated, and aliquots were assayed for fatty acid determination and protein measurements.

Fatty Acid Analysis

Fatty acid analysis was performed on aliquots of platelets. Total lipids were extracted with a mixture of isopropanol and chloroform (11:7, v:v) containing 0.45 mM 2,6-di-ter-*p*-cresol (BHT) as antioxidant. A gas chromatographic method (Hewlett Packard 5890 chromatograph, equipped with an FFAP column of 25 m, 0.2 mm internal diameter, 0.33 μm phase thickness) was employed as previously described (14), based on the direct fatty acid transesterification technique (15). Assays were performed in duplicate on each sample. Peak identification and quantification were done with reference commercially available fatty acids (Sigma Chemical Company). As internal standard heptadecanoic acid (Sigma Chemical Company) was used and was added to each sample of platelet lysate before adding the organic solvent mixture for lipid extraction. The heights of the peaks were measured and the quantification was performed with the aid of a Vectra QS/16S PC equipped with HP-3365 Chem Station software (Hewlett Packard). The fatty acid peaks identified and measured were C12:0, C14:0, C16:0, C16:1ω7, C18:0, C18:1ω9, C18:2ω6, C18:3ω3, C18:4ω3, C20:0, C20:1ω9, C20:2ω6, C20:4ω6, C20:5ω3, C22:0, C22:1ω9, C22:6ω3, C24:0, C26:0. The mean recovery of fatty acids obtained by this procedure was 90.6% ± 2.7 (range: 85-94%). The recovery was calculated by so called addition method, which consists in adding to several aliquots of a sample known amounts of each fatty acid. Data in the text are expressed as percentage of each fatty acid moiety or of each fatty acid group with respect to total fatty acids (% of total fatty acids).

Platelet Adhesion

A colorimetric procedure measuring the activity of acid phosphatase was used for the determination of the adhesion of human platelets to fibrinogen-coated culture microplates (13). 96-well microtitre plates were coated overnight with 0.2 mg/ml human fibrinogen (Sigma Chemical Company) in PBS and washed twice with physiological saline. Immediately after coating and washing, the wells were supplemented with 25 μl of either 2 μM ADP or 0.02 U/ml thrombin (final concentration, in buffer A containing 3 mM $CaCl_2$ and 3 mM $MgSO_4$). In these conditions, the method is specific for platelet adhesion that takes place in the absence of any aggregation (13). Plates were then brought to 37° C and 50 μl of the platelet suspension (2.5×10^6 platelets),

Table 2 Calculated intake of energy and nutrients. The data were obtained from the daily records compiled by the studied subjects during the experimental period. The baseline diet (1st day) and the diet at the 16th day of the experimental period are reported. Supplements are not included in the values indicated. SFA: Saturated Fatty Acid; PUFA: PolyUnsaturated Fatty Acid; MUFA: MonoUnsaturated Fatty Acid

NUTRIENT	CONTROL GROUP		FISH OIL GROUP		SOY LECIT GROUP	
	1st DAY	16th DAY	1st DAY	16th DAY	1st DAY	16th DAY
Energy (kj)	8489 ± 357	8542 ± 328	8501 ± 338	8312 ± 412	8576 ± 384	8491 ± 376
Proteins (% of energy)	16.7 ± 1.4	15.6 ± 1.7	16.1 ± 1.5	17.6 ± 2.2	16.6 ± 1.7	16.2 ± 1.5
Carbohydrates (% of energy)	50.1 ± 2.9	52.7 ± 2.5	52.0 ± 2.7	50.1 ± 3.4	51.7 ± 3.0	54.3 ± 3.5
Total fat (% of energy)	33.2 ± 3.1	31.7 ± 3.4	31.9 ± 2.2	32.3 ± 3.1	31.7 ± 2.6	29.5 ± 3.0
SFA (% of energy)	8.9 ± 1.3	8.1 ± 1.3	8.3 ± 0.9	9.4 ± 1.3	9.0 ± 0.8	8.2 ± 1.8
PUFA (% of energy)	3.9 ± 0.4	3.4 ± 0.2	3.9 ± 0.3	3.6 ± 0.5	3.4 ± 0.4	3.0 ± 0.2
MUFA (% of energy)	20.4 ± 1.3	20.1 ± 1.1	19.7 ± 1.3	19.3 ± 0.9	19.3 ± 1.3	18.2 ± 1.5
Cholesterol (mg)	232 ± 21	247 ± 22	244 ± 22	235 ± 29	237 ± 18	241 ± 19
Fiber (g)	25 ± 3	23 ± 2	24 ± 2	26 ± 1.5	23 ± 3	21 ± 1

pre-warmed to 37° C, were added to each well using a multichannel pipette. The incubation was carried out for 60 min and plates were then transferred to the automatic washer (Easy Washer 2, SLT Labs Instruments) and subjected to two washing cycles with PBS at room temperature. After washing, the wells containing adherent platelets were rapidly supplemented with 150 µl of 100 mM citrate buffer, pH 5.4, containing 5 mM *p*-nitrophenyl-phosphate and 0.1% Triton X-100. After incubation at room temperature for 60 min, the reaction was stopped and the colour was developed by the addition of 100 µl/well of 2 N NaOH. The *p*-nitrophenol produced by the reaction was measured with a microplate reader (Reader 400, SLT Labs Instruments) at 405 nm against a platelet-free blank. The percentage of adherent cells was calculated on the basis of a standard curve obtained with a defined number of platelets from the same donor.

Statistical Analysis

The data are presented as means ± standard error of mean (SEM). Differences in baseline values of various parameters between groups were determined by Student's unpaired *t*-test. For each parameter tested the differences between the values before and after the experimental period were calculated within the groups. One-way analysis of variance followed by Dunnett's *t*-test was used for simultaneous, multiple comparisons of the fish oil and soy lecithin groups with the control group. Correlations between ω-6, ω-3, ω-6/ω-3 ratio, saturated fatty acids (SFA), and mono-unsaturated fatty acids (MUFA) with adhesion data were calculated by Spearman Rank Order Correlation test using a computer program (SigmaStat, SPSS). For the correlation analysis between the baseline values, we consider the 60 volunteers all together as an only one experimental group. For the values obtained after the experimental period, the correlation analysis was performed within the groups. $P < 0.05$ was considered statistically significant.

Results

The subjects' estimated mean intakes of nutrients as resulting from the daily food records of the three study groups are shown in Table 2. No statistically significant changes between the nutrient intakes at the end of the experimental period and those of the baseline were found. There was no difference between the supplementation and control groups, indicating that the usual diet was not affected by the daily intake of either 20 of ml oil or 25 g of soy lecithin. The mean body weight of the subjects in all the three groups did not change during the fifteen days of experimental period (data not shown).

	CONTROL GROUP				FISH OIL GROUP					SOY LECITHIN GROUP				
	PRE	POST	VAR		PRE	POST	VAR			PRE	POST	VAR		
	1	2	3	p (1vs2)	4	5	6	p (4vs5)	p (3vs6)	7	8	9	p (7vs8)	p (3vs9)
MUFA	17.72±0.96	17.79±1.11	0.07±0.28	NS	18.07±1.96	18.91±1.81	0.84±0.12	<0.01	<0.005	18.18±1.96	16.91±1.74	-1.27±0.89	<0.01	<0.01
SFA	44.71±1.30	44.25±1.89	-0.46±0.68	NS	44.52±1.60	44.57±1.25	0.05±0.24	NS	NS	45.03±1.26	44.98±1.49	-0.05±0.84	NS	NS
C18:2ω6	5.44±0.28	5.51±0.16	0.07±0.01	NS	5.52±0.31	4.63±0.26	-0.89±0.14	<0.01	<0.01	5.21.±0.36	9.24±0.42	4.21±0.30	<0.005	<0.001
C20:4ω6	28.51±0.58	28.64±0.93	0.13±0.1	NS	28.31±0.67	25.10±0.74	-3.21±0.18	<0.001	<0.001	28.25±0.70	32.17±0.91	3.92±0.32	NS	<0.01
ω-6	34.52±0.67	34.17±0.74	-0.35±0.07	NS	34.37±0.76	30.42±0.70	-3.95±0.73	<0.001	<0.005	33.96±0.70	41.86±0.56	7.90±0.63	<0.001	<0.005
C20:5ω3	0.38±0.06	0.41±0.08	0.03±0.03	NS	0.34±0.02	2.20±0.14	1.86±0.22	<0.005	<0.001	0.33±0.06	0.36±0.05	0.03±0.01	NS	NS
C22:6ω3	1.86±0.10	1.80±0.12	-0.06±0.02	NS	1.85±0.14	3.28±0.26	1.43±0.15	<0.003	<0.001	1.83±0.11	1.78±0.15	-0.05±0.10	NS	NS
ω-3	2.32±0.06	2.30±0.06	-0.02±0.04	NS	2.21±0.03	5.72±0.22	3.51±0.23	<0.001	<0.001	2.26±0.06	2.20±0.04	-0.06±0.05	NS	NS
ω-6/ω-3	14.97±0.27	14.92±0.28	-0.05±0.11	NS	15.55±0.31	5.47±0.24	-10.08±0.45	<0.001	<0.001	15.14±0.28	19.15±0.26	4.01±0.32	<0.001	<0.001

Table 3 Platelet fatty acid analysis obtained from the studies subjects before and after the supplementations. MUFA: MonoUnsaturated Fatty Acid; SFA: Saturated Fatty Acid

Table 4 Resting and agonist-stimulated platelet adhesion on fibrinogen in the different study groups

	CONTROL GROUP				FISH OIL GROUP					SOY LECITHIN GROUP				
	PRE	POST	VAR		PRE	POST	VAR			PRE	POST	VAR		
	1	2	3	p (1vs2)	4	5	6	p (4vs5)	p (3vs6)	7	8	9	p (7vs8)	p (3vs9)
Resting	3.7±0.2	3.6±0.2	-0.1±0.3	NS	3.6±0.2	3.5±0.3	-0.1±0.3	NS	NS	3.5±0.2	6.6±0.3	3.1±0.4	<0.001	<0.001
ADP	18.7±0.3	18.7±0.3	0.0±0.4	NS	18.8±0.3	15.6±0.4	-3.2±0.7	<0.01	<0.01	18.7±0.4	23.2±0.5	4.5±0.7	<0.001	<0.001
Thromb.	24.1±0.4	24.3±0.4	0.2±0.8	NS	24.4±0.4	20.8±0.5	-3.6±0.6	<0.005	<0.005	24.0±0.4	29.9±0.7	5.9±0.7	<0.001	<0.001

Table 5 Correlation analysis of the changes of the ω-6/ω-3 ratio (after-before diet) versus the changes of the adhesion (after-before diet) measured in resting and activated platelets

	CONTROL GROUP		FISH OIL GROUP		SOY LECITHIN GROUP	
	r_s	p	r_s	p	r_s	P
RESTING	-0.128	NS	0.608	<0.01	0.702	<0.001
ADP	0.074	NS	0.644	<0.005	0.623	<0.005
THROMBIN	-0.068	NS	0.502	<0.05	0.494	<0.05

Platelet fatty acids from the study subjects before and after supplementation were analysed (Table 3). As regards the fish oil group, the main differences found after the 15-day period of the investigation as compared to baseline were the following: a reduction in ω-6 fatty acids (34.37 ± 0.76 vs. 30.42 ± 0.70% of total fatty acids, -11.5%; p <0.001) and in ω-6ω-3 ratio (15.55 ± 0.31 vs. 5.47 ± 0.24; - 65%; p <0.001); and an increase in ω-3 (2.21 ± 0.03 vs. 5.72 ± 0.22% of total fatty acids; +159%; p <0.001). A different pattern was found in the soy lecithin group: an increase in ω-6 (33.96 ± 0.70 vs. 41.86 ± 0.56% of total fatty acids; +23%; p <0.001) and in the ω-6/ω-3 ratio (15.14 ± 0.28 vs. 19.15 ± 0.26; +26.5%; p <0.001) whereas there was no statistically significant change in ω-3 after the experimental supplementation. As expected, no changes were found in the control group after the experimental period as compared to baseline.

In all experimental groups the platelet adhesion to fibrinogen-coated plastic wells under different conditions of stimulation was determined. Table 4 shows the data for platelet adhesion on fibrinogen. In the resting condition, in the fish oil group, no difference was noted between baseline and post-experimental supplementation (3.6 ± 0.2 vs. 3.5 ± 0.3% adhesion; -3%; NS), whereas in the soy lecithin group platelet adhesion was significantly increased after supplementation (3.5 ± 0.2 vs. 6.6 ± 0.3% adhesion; +86%; p <0.001). In ADP-stimulated platelets, fish oil supplement reduced platelet adhesion (18.8 ± 0.3 vs. 15.6 ± 0.4% adhesion; -17%; p <0.01), whereas soy lecithin caused an increase (18.7 ± 0.4 vs. 23.2 ± 0.5% adhesion; +24%; p <0.001). In thrombin-stimulated platelets, the fish oil group showed a decrease in platelet adhesion (24.4 ± 0.4 vs. 20.8 ± 0.5% adhesion; -15%; p <0.005),whereas the soy lecithin group showed an increase (24.0 ± 0.4 vs.29.9 ± 0.7; +25%; p <0.001). No differences were noted in the control group in any of the test conditions as compared to baseline.

In order to analyse the possible relationship between fatty acid composition of platelets and their function, we calculated the correlation between ω-6, ω-3, saturated fatty acids (SFA), and mono-unsaturated fatty acids (MUFA) with adhesion data. No significant correlation was found between adhesion data and SFA, MUFA, and ω-6 or ω-3 absolute values. Analysing the baseline values obtained combining all the 60 volunteers participating to the study, we found a statistically significant correlation between ω-6/ω-3 ratios and adhesion data in resting condition (correlation coefficient: 0.874, p <0.001; Fig. 1A) and after ADP (correlation coefficient: 0.716, p <0.001; Fig. 1B) and thrombin stimulation (correlation coefficient: 0.431, p <0.005; Fig. 1C). The effect of the diet was evaluated by analysing the changes of the ω-6/ω-3 ratio (after-before diet) versus the changes of the adhesion (after-before diet) measured in resting and activated platelets. As shown in Table 5, the correlation between these diet-induced changes was statistically significant in both the fish oil and soy lecithin groups, while control group did not show any correlation between the studied parameters as expected.

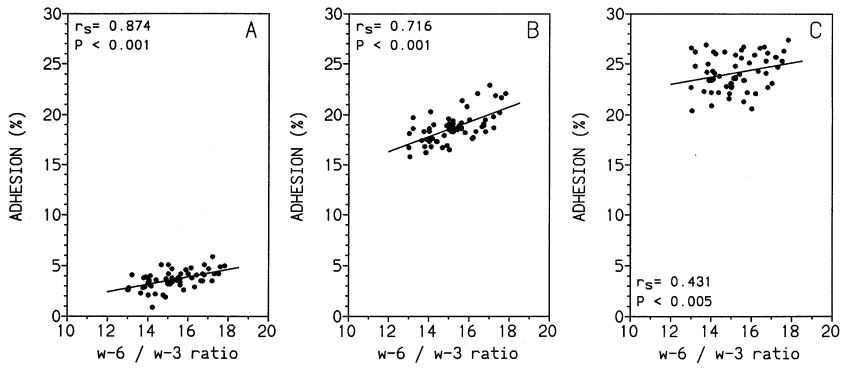

Fig. 1 Correlations between ω-6/ω-3 fatty acids ratio and platelet adhesion in the resting (A), ADP-stimulated (B) and thrombin-stimulated (C) platelets. All the sixty volunteers' baseline values (before diet) were included

Discussion

It is common knowledge that diet may greatly influence health as well as pathological states. Cellular functions and specifically the haemostatic processes are also influenced by individual dietary habit (16). This is also true of human platelet functions as the data on the effects of fatty acids on platelet aggregation and secretion show (5). However, whereas a reasonable amount of data is available on the effects of ω-3 on the later platelet responses in healthy subjects and in patients (5), few data have been reported regarding the effects of fatty acids on human platelet adhesion. Adhesion to the vessel wall may be regarded as the first and crucial step in the complex series of events by which platelets perform their functions in the haemostatic process, involving a series of plasma and subendothelial tissue components that specifically bind to several different membrane glycoproteins (17). Physiological platelet agonists such as ADP, collagen and thrombin activate stimulus-response coupling pathways that may increase both the number and the ligand affinity of specific adhesion receptors (18). While acquired or genetic defects of platelet adhesion may seriously compromise the haemostatic process, an unnecessary increase in adhesiveness may result in a greater risk of vascular disorders. These preliminary remarks indicate the importance of investigating platelet adhesion and the possible influence of different food supplements.

In this experiment, we studied the effects of a period of fish oil or soy lecithin supplementation on human platelet adhesion. For this purpose, platelet adhesion was assayed under static conditions in culture microplates using a colorimetric method (13) which has been shown to be sensitive and versatile in both basic (19, 20) and clinical studies (21). The results of platelet fatty acid analysis in this study, before and after the supplementation period, show good compliance with the experimental design: we can thus assume that the changes in platelet function during the experimental diets are due to supplementation.

Our results indicate an inhibitory effect of diet supplementation with fish oil rich in ω-3 fatty acids on stimulated human platelet adhesiveness and a stimulatory effect of diet supplementation with soy lecithin rich in ω-6 fatty acids on resting and stimulated adhesion. Moreover, our study suggests that the platelet ω-6/ω-3 ratio, rather than the absolute amount of any single fatty acid, is a determinant of platelet adhesion.

It is well known that the manipulation of dietary fatty acids may result in changes in the fatty acid composition of platelets, inducing alterations in platelet reactivity and in their ability to produce eicosanoids (22). Reduced platelet TxA_2 synthesis after fish oil supplementation (23) may inhibit the positive feedback mechanism that normally amplifies primary responses to agonists and results in secretion and ATP release (18). Fish oil would not appear capable of reducing the resting adhesion but it inhibits the platelet response to agonists (ADP and thrombin). It is possible that the lack of inhibition of resting adhesion is due to a short period of diet supplementation (two weeks), though it should be noted that Li and Steiner have reported a drastic reduction in platelet adhesion within 14 days of initiation of fish oil administration (6). Another possibility is that the (very low) basal adhesion occurring in our assay system may be independent of intracellular signal transduction mechanisms, which are affected by fatty acid manipulation.

To the best of our knowledge, no studies have been conducted to date on the effect of soy lecithin (rich in ω-6) on platelet adhesion in man. Over the past few years, the use of soy and other foods rich in ω-6 has increased remarkably in western countries (11). Because of the increased amounts of ω-6 fatty acids in the diet, the eicosanoid metabolic products from arachidonic acid, specifically prostaglandins, thromboxanes, leukotrienes, hydroxy fatty acids and lipoxins, are formed in larger quantities than those formed from ω-3 fatty acids. In this paper, we have shown that soy lecithin induces a marked increase of C18:2ω6 in platelet membranes and a small (albeit not significant) increase of C20:4ω6. On the basis of these data, it is conceivable that these changes in ω-6 platelet fatty acids shift the physiological state of platelets towards a higher responsiveness to stimulation. It is also important to recognise that ω-3 fatty acids are precursors of eicosanoids that in general are less potent than eicosanoids derived from the ω-6 fatty acid family (24). Since ω-6 and ω-3 fatty acids compete for enzymes involved in desaturation and elongation to longer-chain fatty acids (11), the increase of ω-6 in platelet membrane may counteract the inhibitory effect of ω-3 fatty acids. These conclusions are in agreement with data obtained by Boundreau and associates (12) in rats showing that the ratio of ω-3 to ω-6 fatty acids, rather than the absolute amount of ω-3, determines a degree of inhibition of eicosanoid biosynthesis from arachidonic acid.

On the basis of our data, however, the possible involvement of mechanisms other than effects on arachidonic acid metabolism, cannot be entirely ruled out. The percentage increase in adhesion after soy supplementation appears to be particularly marked in unstimulated platelets, where arachidonic acid metabolism is presumably not activated, as shown by the lack of inhibition by non-steroidal anti-inflammatory agents (20, 25). Since ω-6 fatty acids in this study are "carried" by soy lecithin and the ω-3 fatty acids by fish oil, we cannot exclude that the different effects may be related to other compounds besides the differences in fatty acid type of supplementation. The possible changes induced by soy lecithin in membrane adhesion molecules of the integrin or selectin type warrant further investigation.

In conclusion, our results indicate an inhibitory effect of fish oil rich in ω-3 fatty acids on stimulated human platelet adhesiveness and a stimulatory effect of soy lecithin rich in ω-6 fatty acids on resting and stimulated adhesion. They suggest, moreover, that the ω-6/ω-3 ratio is a determinant of platelet adhesion. In the light of these data, when referring to polyunsaturated fatty acids, it is essential to distinguish between ω-6 and ω-3 fatty acids in studying their effects on platelet physiology: simply using the P-S ratio of polyunsaturated fatty acids to saturated fatty acids is inappropriate and inadequate. In addition, it would be difficult to establish the minimum amounts of ω-3 fatty acids capable of improving platelet function without knowing the ratio of ω-6 to ω-3 fatty acids in the diet. When desirable amounts of dietary ω-3 needed to regulate platelet response are determined, not only the absolute amounts of ω-3 but also the amounts of ω-6 fatty acids in the diet should be adjusted.

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