Effect of catfish and salmon diet on platelet phospholipid and blood clotting in healthy men

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The effect of diets containing either catfish or salmon fish on blood clotting and platelet phospholipid fatty acid composition was studied in 17 healthy, young men. The subjects received a control diet for 21 days followed by either catfish diets (n = 9) *or salmon diets* (n = 8) *for an additional 19 days. Catfish and salmon diets prolonged the subjects' bleeding times (by 66% and 57%, respectively) and clotting times (by 25% and 31%, respectively),* $P < 0.05$. *The platelet count in catfish and salmon groups decreased by 7% and 9%, respectively. There were no differences in the prothrombin time (PT) and activated partial thromboplastin time (APTT). A small but significant increase* ($P < 0.05$) *in fibrinogen concentration was observed in catfish (243.7 mg/dL) and salmon diet groups (246.5 mg/dL) compared to the control group (223.9 mg/dL). Diets containing catfish and salmon led to significant incorporation of total* n-3 *polyunsaturated fatty acids, PUFAs (from 5.04% on the control to 6.14% and 10.26%, respectively) and total EPA + DHA (from 3.77% on the control to 4.84% and 8.83%, respectively). The* n-3/n-6 *ratio increased in both groups while the ratio of 20:5/20:4 increased only in the salmon group from 0.05 on the control diet to 0.14. The reduction in arachidonic acid (20:4* n-6*) and total* n-6 *PUFAs was not statistically significant in the catfish group but was significant in the salmon group. The findings suggest that there were minor differences between hemostatic parameters in men fed catfish and salmon. The consumption of either diet led to incorporation of dietary* n-3 *PUFAs into platelet phospholipids, and may result in alteration in platelet and hemostatic function in men.*

Keywords: catfish; salmon; blood clotting; phospholipid

Introduction

It has been suggested that the *n*-3 polyunsaturated fatty acids (PUFAs) found in marine fish and fish oils might help prevent cardiovascular disorders, result in increased amounts of *n*-3 PUFAs and decreased amounts of *n*-6 PUFAs in the serum, and increased bleeding times.[1-8] It was proposed that large quantities of *n*-3 PUFAs, mainly eicosapentaenoic acid (EPA), in the diet protects Eskimos against thrombotic cardiovascular disorders.[1,2] Their intake of monounsaturated and *n*-3 PUFAs is high and that of linoleic and arachidonic acids is low. Both their plasma and platelet lipids have similar fatty acid profiles to that present in their diet. EPA (C20:5 *n*-3) appears to be important among the dietary fatty acids because of its potent antiaggregatory effects.[1,7] It has been suggested that C20:5 in the platelets is converted by the vascular wall tissue to an anti-aggregatory prostacyclin, and may have antithrombotic influence on hemostasis and thrombosis.[2] The antithrombotic influence produced by an altered prostaglandin substrate pool may prolong the bleeding time by reducing platelet aggregation.

The results of intervention studies on other indices of coagulation, such as platelet number, prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen concentration are inconsistent.[4] Changing the dietary fatty acids to resemble those taken by Greenland Eskimos may help the West-

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ern population prevent certain cardiovascular disorders.

To help explain the possible relationship between fish diet and hemostasis and the lack of vascular disease in the Greenland Eskimos, we carried out a short-term feeding trial with catfish and salmon diets. The aim was to determine if catfish is comparable to salmon in preventing thrombotic cardiovascular disorders in healthy young men.

Materials and methods

Subjects

Informed consent was obtained from 17 normal, healthy male students and workers, volunteers who were taking no medication or drugs that would influence hemostatic function. The average age of the subjects was 29 years. The study was approved by the Institutional Review Board for the protection of human subjects in research at Mississippi State University.

Diets and study design

The study started in March 1989 with all subjects receiving controlled menus for the 40-day feeding period. A 5-day cycle menu planned by a registered dietitian and based on the USDA/USHHS Dietary Guidelines and the USDA Daily Food Guide[9,10] was used for all subjects. The diets consisted of fruits, vegetables, rice, bread, cereal, muffins, cookies, juice, milk, margarine, soybean and corn oil, egg, turkey, chicken, beef, mayonnaise, green peas, beans, cheese, and pasta. All subjects were fed a balanced isocaloric control diet containing about 28–33% of calories from fat, 15–22% as protein, and 46–56% as carbohydrate as determined by proximate analysis. The lipid composition of the meals contained about 36% saturated, 30% monounsaturated, 34% polyunsaturated fatty acids, and essentially no *n*-3 PUFAs (20:5 and 22:6). Since sodium level is regarded as one of the most important factors contributing to blood disorders, the sodium content was maintained at or slightly above the 1,000 mg of sodium per 1,000 calories as recommended by the American Heart Association.

After 21 days on the control diet, each subject was randomly assigned to either the catfish diet ($n = 9$) or the salmon diet ($n = 8$) for an additional 19 days. The diets were identical in composition except that the major source of protein was derived from catfish (Delta Pride, Indianola, MS) or chum salmon (Sysco Foods, New Orleans, LA) instead of beef and poultry in the control diet. The fatty acid composition of catfish and salmon used in diet formulations has been reported.[11] The catfish and salmon were prepared by baking, blackening, broiling, or as fish gumbo, and were also served as a 5-day cycle menu.

The average portion of fish served per day to each subject in both groups was about 194 g (uncooked). The overall fatty acid composition of the 5-day menu control and experimental diets is given in *Table 1*. The amount of *n*-3 PUFAs consumed per person was estimated to be about 0.2–0.4 g/day for the catfish diet and 1–2 g/day for the salmon diet. All the diets were well tolerated and very palatable. Subjects' body weights remained relatively constant throughout the study except for one person who lost some weight, initially, but his diet was adjusted accordingly. Three people dropped out from the original 20 subjects due to their inability to remain in the city throughout the study period.

Table 1 Fatty acid composition (%) of the 5-day menu control and experimental diets fed to the subjects

Fatty acids	Control	Catfish	Salmon
14:0	2.98 ± 0.06	3.78 ± 0.08	4.26 ± 1.00
16:0	24.70 ± 1.09	21.40 ± 1.21	21.66 ± 1.26
16:1 *n*-7	2.62 ± 0.50	2.04 ± 0.40	1.40 ± 0.08
18:0	8.50 ± 0.48	8.02 ± 0.62	8.00 ± 0.64
18:1 *n*-9	27.66 ± 0.60	30.62 ± 0.71	27.01 ± 0.65
18:2 *n*-6	30.28 ± 1.00	26.62 ± 1.08	26.09 ± 0.98
20:0	ND[a]	0.18 ± 0.02	0.06 ± 0.03
18:3 *n*-3	3.26 ± 0.43	3.38 ± 0.51	ND
20:1 *n*-9	—	ND	3.54 ± 0.48
20:2 *n*-6	—	0.11 ± 0.03	ND
20:3 *n*-6	—	0.30 ± 0.08	1.02 ± 0.05
20:4 *n*-6	—	1.12 ± 0.07	0.90 ± 0.05
20:5 *n*-3	—	0.66 ± 0.05	1.38 ± 0.04
22:4 *n*-6	—	0.57 ± 0.04	ND
22:5 *n*-3	—	0.48 ± 0.02	0.60 ± 0.02
22:6 *n*-3	—	0.72 ± 0.12	4.08 ± 0.60

Note: Average of triplicate 5-day menu cycle. Percent of the total amount of fatty acids present in the sample.
[a] ND = not detected.

Methods

Blood samples were drawn initially by a certified medical technologist at the Oktibbeha County Hospital, during 21 days on the baseline or control diet and after 19 days on the experimental fish diets. Blood samples were taken in the morning after an overnight fast of 12 hours.

Coagulation studies

Blood for coagulation studies was collected in sodium citrate. All analyses took place within 2 hours of sampling. Platelet-rich plasma (PRP) was obtained after centrifugation at 120*g* for 10 min. Platelet count (PC), white blood corpuscles (WBC), red blood corpuscles (RBC), and hemoglobin (HGB) were determined according to established procedure[12] with a Coulter Counter (Coulter Electronics, Inc., Hialeah, FL, USA). Fibrinogen (FBG) in citrated plasma was determined quantitatively with an automatic DuPont aca IV discrete clinical analyzer[13] based on established research procedures[14,15] using the FBG test pack (Du Pont, Wilmington, DE, USA). Activated partial thromboplastin time (APTT) and prothrombin time (PT) were determined according to established procedure[16] with an MLA Electra 700 automatic coagulation

timer (Medical Laboratory Automation, Inc., Mount Vernon, NY, USA). Bleeding times (BT) were determined by the Duke method.[17] Whole blood clotting time was measured as the time in seconds for blood in a capillary tube to clot while being inverted gently or mixed by hand.

Platelet phospholipids

Platelet-rich plasma was obtained as described above. Platelets were sedimented at 2000*g* for 15 min and total lipids extracted according to the method of Bligh and Dyer.[18] The platelet phospholipids were extracted using Sep-pak silica cartridges (Waters Associates, Milford, MA, USA) according to the method of Bitman et al.[19]. Butylated hydroxytoluene (BHT) was added to reduce lipid oxidation. Phospholipid fatty acids were methylated[20] in the presence of heptadecanoic acid and analyzed by gas-liquid chromatography (GLC) in a Varian 3300 gas chromatograph (Varian Associates, Sunnydale, CA, USA) equipped with 6 ft. × 2 mm i.d. glass column packed with 10% SP-2330 on 100/120 chromsorb WAW (Supelco Inc., Bellefonte, PA, USA). The chromatographic conditions were as follows: column oven temperature 200° C, for 10 min, then rising at 3° C/min to 220° C for 20 min, injection port 250° C, a flame ionization detector at 250° C, and an N_2 carrier gas flow of 20 mL/min. The signals were compared with those obtained with pure standards (Nu-Check-Prep, Elysian, MN; Altech Associates, Inc., State College, PA; and Sigma Chemical Co., St. Louis, MO, all USA). Retention times and peak areas were computed automatically by an on-line Varian 4290A integrator (Varian Associates, Sunnydale, CA, USA).

Statistical analysis was performed using the SAS program.[21] A crossover design was used with each participant serving as his own control. All data were arranged in a completely random design and analyzed by analysis of variance with mean separation by least significant difference ($P < 0.05$).

Results and discussion

Table 1 shows the overall fatty acid composition of the 5-day menu control and experimental fish diets as fed to the subjects. The control diet contained no 20:5 and 22:6 *n*-3 PUFAs. The level of EPA was 0.66% and 1.38%, and DHA 0.72% and 4.08%, respectively, for the catfish and salmon diet groups. Catfish diet contained 30.62% by weight of 18:1 *n*-9 compared to 27.01% and 27.66% in salmon and control diets, respectively. We have reported previously[11] that the sum of EPA and DHA was 50% by weight in salmon and 12.70% in catfish before incorporation into the diet. The ratio of the sum of EPA and DHA in salmon and catfish diets was 4:1, confirming our data on the fatty acid profile of both fish prior to incorporation into the diets.[11] Notably, the catfish diet contained more *n*-6 PUFAs than the salmon diet.

Table 2 Bleeding time, clotting time, and platelet count of young adult males on control and fish diets (mean ± SEM)

Diet	*n*	Bleeding time (sec)	Clotting time (sec)	Platelet count (×10³ cu mm)
Control	17	88.2 ± 15.4[a]	299.1 ± 14.4[a]	261.5 ± 3.3[a]
Catfish	9	146.7 ± 19.6[b]	375.0 ± 21.8[b]	242.2 ± 4.2[b]
Salmon	8	138.8 ± 25.3[b]	391.9 ± 19.8[b]	238.0 ± 5.5[b]

Note: Means followed by the same letter are not significantly different at $P < 0.05$.

Table 3 Prothrombin time (PT), activated partial thromboplastin time (APTT), and plasma fibrinogen (FBG) concentration of young adult males on control and fish diets (mean ± SEM)

Diet	*n*	PT (sec)	APTT (sec)	FBG (mg/dL)
Control	17	11.2 ± 0.1	27.4 ± 0.3	223.9 ± 6.8[a]
Catfish	9	11.3 ± 0.1	28.0 ± 0.3	243.7 ± 11.2[b]
Salmon	8	11.3 ± 0.1	28.0 ± 0.3	246.5 ± 7.4[b]

Note: Means followed by the same letter are not significantly different at $P < 0.05$.

The effects of catfish and salmon diets on hemostasis indices are shown in *Tables 2* and *3*. Bleeding times of the subjects increased from 88.2 to 146.7 and 138.8 sec with the catfish and salmon diets representing a 66% and 57% increase, respectively. Blood clotting time increased from 299.1 to 375.0 sec (25% increase) in the catfish group and to 391.9 sec (31% increase) in the salmon group. The platelet count fell from 261,500 to 242,200 and 238,000/cu mm in subjects consuming the catfish and salmon diets, respectively. There were no differences in bleeding, clotting time, and platelet count (*Table 2*) between subjects fed either catfish or salmon diets. Although the level of *n*-3 PUFAs in catfish is lower than that of salmon, it seems that the amount of EPA present in the catfish diet could be sufficient to increase the observed bleeding times and to elicit antithrombotic effect. The Duke method[17] generally gives shorter bleeding times compared to the standardized Simplate II device (General Diagnostics, Warner-Lambert Co., Morris Plains, NJ, USA). However, on a relative basis, the Duke method can give reliable data. Notably, there were no changes in white blood cells, red blood cells, and hemoglobin values in both groups. Thorngren and Gustafson[4] reported a 42% increase in subject bleeding time after 6 weeks on a fish diet. The increase in subject bleeding times was similar to the longer bleeding times reported by other researchers.[2,4,5] No subject exhibited bleeding and clotting phenomenon. It has been proposed that large quantities of *n*-3 PUFAs, especially EPA (20:5 *n*-3), in the diet of Eskimos were responsible for protecting them against thrombotic cardiovascular disorders.[1,2] Dyerberg and Bang[2] suggested that 20:5 *n*-3 in the platelet was converted by the vascular wall tissue

to an anti-aggregatory prostacyclin. However, there was no absolute correlation between bleeding time and platelet counts.

Table 3 shows the results of extrinsic and intrinsic coagulation cascades, and fibrinogen concentration. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were similar in both the catfish and salmon groups. Fibrinogen concentration of subjects increased significantly ($P < 0.05$) from 223.9 mg/dL on the control diet to 243.7 and 246.5 mg/dL on the catfish and salmon diets, respectively. The increase in fibrinogen concentration reported here is similar to those reported by Dyerberg and Bang[2] between Eskimos and Danes. The Eskimos had higher fibrinogen concentration than did the Danes. The reasons for the differences in platelet counts and fibrinogen concentration were not explained.[2] Our results suggest that the platelet counts decreased as the fibrinogen concentration increased. For example, the salmon group with a platelet count of 238,000/cu mm had the highest fibrinogen concentration of 246.5 mg/dL. However, we did not find any significant difference or correlation between platelet count and fibrinogen changes. Radack et al.[22] reported that *n*-3 and *n*-6 PUFAs were associated with reductions in fibrinogen levels. The observed increase in fibrinogen concentration may be due to a nonspecific response to the change in diet from control to fish diets.

Table 4 summarizes the fatty acid profile of the platelet phospholipids of the control, catfish, and salmon diet groups. Diets containing catfish and salmon led to significant incorporation of total *n*-3 PUFAs and total EPA + DHA into blood platelet ($P < 0.05$). The *n*-3 PUFAs content of the salmon group increased from 5.05 to 10.26% representing more than 1.5 times that of catfish (6.14%). The salmon group had a twofold increase of EPA + DHA (8.83%) compared to the catfish (4.84%) group. The ratio of *n*-3/*n*-6 increased from a control value of 0.12 to 0.15 and 0.27 in the catfish and salmon groups, respectively. The decrease in *n*-6 PUFAs content of the catfish group was not statistically significant. However, the salmon group showed a significant decrease in *n*-6 PUFAs from 41.14 to 37.98%. The high content of EPA (1.74%) in salmon diet may have caused a decrease in the amount of 20:4 *n*-6 released from the platelets due to competitive inhibition of the platelet cyclo-oxygenase by the released 20:5 *n*-3. Part of the explanation could be attributed to the fact that salmon contains less *n*-6 series than catfish.[11] However, it was noted that 20:2 *n*-6 and 22:6 *n*-3 content increased significantly ($P < 0.05$) during both catfish and salmon diets. The ratio of 20:5/20:4 increased from 0.05 to 0.14 in the salmon group with no change in the catfish group. Salmon has a higher concentration of 20:5 *n*-3 than catfish. Goodnight et al.[5] reported an increase in *n*-3 PUFAs in the platelet phospholipids of subjects after fish or fish oil diets. In the present study, there were no significant increases in 20:5 *n*-3 during the catfish diet. Terano et al.[23] reported no change in DHA and 20:4 *n*-6 contents in platelet phospholipid after feeding purified EPA to male subjects. Only minor changes were observed in the concentration of other platelet fatty acids.

Table 4 Fatty acid composition (%) of platelet phospholipids in young adult men on 19-day fish diets

Fatty acids	Control (n = 17)	Catfish (n = 9)	Salmon (n = 8)
14:0	0.44 ± 0.07[a]	0.25 ± 0.06[b]	0.20 ± 0.09[b]
16:0	28.61 ± 1.02[a]	29.23 ± 0.97[a]	29.25 ± 1.24[a]
16:1 *n*-7	0.28 ± 0.16[a]	0.52 ± 0.17[a]	0.38 ± 0.15[a]
18:0	13.31 ± 0.48[a]	12.51 ± 0.48[a]	12.88 ± 0.49[a]
18:1 *n*-9	10.64 ± 0.58[a]	10.41 ± 0.66[a]	9.28 ± 0.57[b]
18:2 *n*-6	24.07 ± 1.24[a]	23.51 ± 1.23[a]	22.30 ± 1.15[a,b]
20:0	0.06 ± 0.03[a]	0.07 ± 0.02[a]	0.06 ± 0.03[a]
18:3 *n*-3	0.03 ± 0.02[a]	0.10 ± 0.02[b]	0.09 ± 0.04[b]
20:1 *n*-9	NS[a] ± 0.02[a]	0.16 ± 0.01[b]	0.11 ± 0.02[b]
20:2 *n*-6	0.15 ± 0.04[a]	0.28 ± 0.03[b]	0.33 ± 0.05[c]
20:3 *n*-6	2.68 ± 0.15[a]	2.76 ± 0.17[a]	2.13 ± 0.14[c]
20:4 *n*-6	13.09 ± 1.50[a]	13.20 ± 1.30[a]	12.26 ± 2.37[b]
20:5 *n*-3	0.64 ± 0.18[a]	0.64 ± 0.16[a]	1.74 ± 0.22[b]
22:4 *n*-6	1.15 ± 0.17[a]	0.80 ± 0.33[b]	0.96 ± 0.43[b]
22:5 *n*-3	1.23 ± 0.18[a]	1.20 ± 0.20[a]	1.35 ± 0.15[a,b]
22:6 *n*-3	3.13 ± 0.22[a]	4.20 ± 0.24[b]	7.09 ± 0.42[c]
Saturated	42.42 ± 1.65[a]	42.10 ± 1.64[a]	42.50 ± 1.66[a]
Monoenes	10.95 ± 1.35[a]	11.10 ± 1.32[a]	9.80 ± 1.19[a]
Polyunsaturated	46.19 ± 0.29[a]	46.70 ± 0.29[a]	48.30 ± 0.29[a]
EPA + DHA[b]	3.77 ± 0.38[a]	4.84 ± 0.36[b]	8.83 ± 0.50[c]
n-3	5.04 ± 0.43[a]	6.14 ± 0.41[b]	10.26 ± 0.60[c]
n-6	41.14 ± 1.48[a]	40.54 ± 1.44[a]	37.98 ± 1.94[c]
n-3/*n*-6	0.12 ± 0.01[a]	0.15 ± 0.01[b]	0.27 ± 0.02[c]
20:5/20:4	0.05 ± 0.01[a]	0.05 ± 0.01[a]	0.14 ± 0.02[b]

Note: Means in the same row followed by the same letter are not significantly different at $P < 0.05$.
[a]NS indicates that the mean value is not significantly different than zero.
[b]EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

Sodium content in the diet is regarded as one of the most important factors contributing to blood circulation disorders and high blood pressure. The benefit of fish diet on prevention of atherosclerosis and thrombosis may be counteracted by high sodium content in some fish. In the present study, the level of sodium in the diet was maintained at or slightly above 1,000 mg per 1 kCal as recommended by the American Heart Association and, therefore, may not have a major effect on the antithrombotic effect of fish diet.

We have demonstrated that feeding young men both marine and freshwater fish in their diets will alter their platelet lipid composition and blood-clotting factors, and may reduce the incidence of thrombosis.

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