Effects of Eicosapentaenoic Acid Intake on Plasma Fibrinolytic and Coagulation Activity by Using Physical Load in the Young

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To assess the effect of eicosapentaenoic acid (EPA) intake on fibrinolysis and coagulation, 30 male subjects, approximately 19–23 y old, were examined for plasma fibrinolytic and coagulation activity by using a bicycle ergometer load (90 W, 20 min) before and after EPA intake of 1.125 g/d for 2 wk. Because of the EPA intake, the fibrinolytic activity was promoted, the plasmin-α2 plasmininhibitor complex (PIC) level was decreased by 16.7%, and the thrombin-antithrombin III complex (TAT) level was increased by 75.4%; conversely, the D-dimer of the fibrin degradation peptide (D-dimer) level did not change from that before EPA intake. By the physical load, 1 h after ingesting the load, the PIC level was significantly decreased by 26.7%, the TAT level was significantly increased by 51.1%, and the D-dimer level was significantly decreased by 24% in comparison with levels before EPA intake. Thus, as determined by the load, a small amount of daily EPA intake clearly decreased fibrinolytic activity and increased coagulation activity. One hour after a physical load, the rate of change of the γ-glutamyl transpeptidase (γ-GTP) level correlated significantly and negatively to the rate of change in the PIC and TAT levels. Thus, EPA intake may affect liver and kidney function. EPA intake decreased systolic blood pressure by 5 mmHg and diastolic blood pressure by 10 mmHg. *Nutrition* 2000;16:11–14. ©Elsevier Science Inc. 2000

Key words: eicosapentaenoic acid, plasma coagulatic and fibrinolytic activity, physical load, young males

INTRODUCTION

There have been reports that intake of eicosapentaenoic acid (EPA) is effective in preventing hypertension and arteriosclerosis, and taking fish oil for preventing adult diseases has become popular.[1–4] It is well known that the incidence of arteriosclerosis in Eskimos is low because of the large amount of fish intake.[5–7] In addition, a large amount of EPA intake causes bleeding as a result of suppression of the coagulation factor due to stimulation of an arachidonic acid cascade in endothelial cells or thrombocytes.[8] In this study, to clarify the effect of a small, daily amount of EPA on plasma coagulation and fibrinolytic activity in the young, the subjects before and after EPA intake were examined to compare concentrations of plasmin-α 2 plasmininhibitor complex (PIC)[9] for the fibrinolytic index, thrombin-antithrombin III complex (TAT)[10,11] for the coagulation indices after the physical load, and the D-dimer of the fibrin degradation peptide (D-dimer)[12] for the coagulation index.

MATERIALS AND METHODS

Seventeen healthy male volunteers, ages 19–23 y, had blood taken before, immediately after, and 1 h after a physical load (90 W, 20 min) measured with a bicycle ergometer in the morning, before breakfast. During the day, the subjects took 15 EPA tablets per day (total EPA intake = 1.125 g) with their normal food intake for 2 wk. The EPA tablet contained 24.48% EPA, 13.19% docosahexaenoic acid, 9.42% oleic acid, and 7.51% palmitic acid. We confirmed that all subjects took their tablets daily. After EPA intake, the subjects had their blood sampled three times, as described earlier. All blood samples were preserved at −20°C until measurement. The TAT level for the coagulation factor was measured by an enzyme immunoassay[10,11] (TAT test, Teijin, Tokyo, Japan), and the PIC level for the fibrinolytic factor was measured by an enzyme immunoassay with a limit criteria of 0.3 μg/mL[9] (PIC test, Teijin, Tokyo, Japan). The D-dimer level for fibrinolytic activity as an index for degradation of fibrin was measured by an enzyme-linked immunosorbent assay with a limit criteria of 30 ng/mL[12] (Dimer test EIA, Fuji Rebio, Tokyo, Japan). The serum levels of fatty acids, triacylglycerol (TG), and total cholesterol (T-chol) were measured with the enzyme method. γ-Glutamyl transpeptidase (γ-GTP), glutamate oxaloacetate transaminase (GOT), glutamic pyruvic transaminase (GPT), and blood urea nitrogen (BUN) were measured with a Hitachi autoanalyzer, and serum glucose was measured by using an enzyme method. Blood pressure was measured in the sitting position with a mercury manometer. Because the distribution of values for coagulation and fibrinolytic activity were widespread and there were values under the limit criteria, we analyzed the data by using the median values for these parameters. Statistical significance was tested with the Wilcoxon method and *t* test, and Pearson's correlation coefficient used for analyzing the relation.

RESULTS

Distribution of Plasma Coagulation and Fibrinolytic Activity Before and After EPA Intake

As shown in Table I, before EPA intake by the physical load, the TAT level decreased significantly from 2.85 to 2.05 ng/mL immediately after the load and tended to increase to 2.25 ng/mL 1 h after

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TABLE I.

DISTRIBUTION OF TAT, PIC, AND D-DIMER BEFORE, IMMEDIATELY AFTER, AND 1 H AFTER A PHYSICAL LOAD BEFORE AND AFTER EPA INTAKE ($N = 17$)

	Before EPA intake								After EPA intake							
	Before			Immediately after			After 1 h		Before			Immediately after			After 1 h	
Item	25 percentile	Median	75 percentile	25 percentile	Median	75 percentile	25 percentile	Median	75 percentile	25 percentile	Median	75 percentile	25 percentile	Median	75 percentile	25 percentile	Median	75 percentile
TAT (ng/mL)	2.2	2.85_a	6.0	1.8	2.05_a	2.6	1.9	2.25_c	3.8	2.0	5.0	13.6	1.9	2.3	4.5	2.4	3.4_c	15.4
PIC (μg/mL)	0.4	0.6_b	0.9	0.7	1.0_b	1.5	0.6	0.75_d	1.0	0.3	0.5	0.6	0.5	0.7	0.8	0.4	0.55_d	0.7
D-dimer (ng/mL)	30.0	30.0	56.0	30.0	31.5	56.0	32.0	39.5_e	58.0	30.0	30.5	39.0	30.0	30.0	51.0	30.0	30.0_e	39.0

$P < 0.05$ for medians with the same subscript.
D-dimer, D-dimer of the fibrin degradation peptide; EPA, eicosapentaenoic acid; PIC, plasmin-α-2 plasmin inhibitor complex; TAT, thrombin-antithrombin III complex.

the load. The PIC level increased significantly from 0.6 to 1.0 μg/mL immediately after the load and tended to decrease to 0.75 μg/mL 1 h after the load. The D-dimer level was under the limit criteria before the load. Immediately after the load, it tended to increase to 31 ng/mL and then to 39.5 ng/mL 1 h after the load. After EPA intake, the TAT level tended to increase by 75.4%, the PIC level tended to decrease by 16.7%, but the D-dimer level did not differ from the level before EPA intake. One hour after the load, the TAT level tended to decrease by 32%, the PIC level tended to increase by 10%, but the D-dimer level did not differ from the level before the load. In addition, the TAT level increased significantly by 51.1%, the PIC level decreased significantly by 26.7%, and the D-dimer level decreased significantly by more 24% in comparison with the level 1 h after the load before EPA intake.

Distribution of Serum Lipid, Biochemical Parameters, and Blood Pressure Due To Physical Exercise Before and After EPA Intake

Before EPA intake (Table II), the TG level was 79.2 mg/dL but tended to decrease to 69.7 mg/dL 1 h after the load. The T-chol level was 159.3 mg/dL and remained at that level 1 h after the load. After EPA intake, the TG level tended to increase by 10.6% in comparison with the level before EPA intake. In addition, the TG level tended to decrease by 19.7% 1 h after the load in comparison with the level before the load. The T-chol level as a result of the load was at the same level as that before EPA intake. The concentrations of γ-GTP, GOT, GPT, BUN, and serum glucose did not change much as a result of the physical load before EPA intake. After EPA intake, γ-GTP tended to increase 15% and GPT tended to decrease 34.3% compared with the levels before EPA intake. In addition, 1 h after the load, those parameters did not change, but the γ-GTP level tended to increase by 14.2% and the GPT level tended to decrease by 27.5% compared with the levels 1 h after the load before EPA intake, but GOT and BUN did not change under these conditions. After EPA intake, the change in systolic blood pressure (SBP) and diastolic blood pressure (DBP) due to the load was the same before and after EPA intake. Also, SBP tended to decrease to 6 mmHg and DBP tended to decrease to 10 mmHg as compared with levels before EPA intake.

Correlation Coefficient Between the Rates of Change of the PIC and TAT Levels, and That of the Other Biochemical Parameters Resulting From the Physical Load Before and After EPA Intake

The correlation coefficient (γ) between the rates of change of the TAT and PIC levels and that of the other biochemical parameters were examined 1 h after the load before and after EPA intake (Table III). The rate of change of blood glucose correlated significantly and negatively to the TAT level ($r = -0.49$, $P < 0.05$) after EPA intake. The rate of change of γ-GTP showed no relation to that of the TAT and PIC levels before EPA intake. However, after EPA intake, it correlated significantly and negatively to the TAT level ($r = -0.72$, $P < 0.01$) and the PIC level ($r = -0.68$, $P < 0.05$), respectively. The rate of change of the BUN level correlated negatively to that of the PIC level before EPA intake, and it changed significantly and positively to the PIC level ($r = 0.68$, $P < 0.05$) after EPA intake. The rate of change of the T-chol level tended to correlate negatively to the PIC level before EPA intake but positively after EPA intake.

DISCUSSION

To clarify the effect of daily EPA intake on the coagulation and fibrinolytic activity in serum, the rate of change in the TAT, PIC, and D-dimer levels of the subjects who had an EPA intake of

TABLE II.

DISTRIBUTION OF SERUM BIOCHEMICAL PARAMETERS AND BLOOD PRESSURE BY PHYSICAL LOAD AND EPA INTAKE BEFORE, IMMEDIATELY AFTER, AND 1 H AFTER THE LOAD (MEAN ± SD; $N = 17$)

	Before EPA intake			After EPA intake		
	Before the load	Immediately after the load	1 h after the load	Before the load	Immediately after the load	1 h after the load
Triacylglycerol (mg/dL)	79.17 ± 35.30	82.00 ± 34.44	69.67 ± 26.54	87.56 ± 37.14	86.44 ± 30.99	70.33 ± 23.42
Total cholesterol (mg/dL)	159.28 ± 26.94	162.89 ± 28.45	157.94 ± 26.88	159.33 ± 24.15	163.56 ± 24.34	160.94 ± 23.92
γ-Glutamyl transpeptidase (IU/L)	13.33 ± 7.88	15.89 ± 8.44	13.28 ± 7.85	15.33 ± 7.63	13.22 ± 7.07	15.17 ± 7.67
Glutamate oxaloacetate transaminase (IU/L)	13.89 ± 8.53	13.44 ± 7.71	13.78 ± 8.32	13.06 ± 7.79	14.33 ± 8.14	13.50 ± 8.64
Glutamic pyruvic transaminase (IU/L)	9.22 ± 11.36	9.06 ± 11.15	9.11 ± 11.49	6.06 ± 6.20	6.94 ± 10.22	6.61 ± 4.93
Blood urea nitrogen (mg/dL)	13.51 ± 2.74	12.88 ± 2.35	12.94 ± 2.30	12.88 ± 3.27	12.85 ± 3.21	12.30 ± 3.07
Serum glucose (mg/dL)	83.61 ± 7.13	81.17 ± 5.90	83.11 ± 3.25	86.39 ± 5.67	81.00 ± 5.65	87.56 ± 5.72
Systoric blood pressure (mmHg)	127.4 ± 8.5	149.8 ± 12.0	120.8 ± 15.0	119.7 ± 12.2	143.8 ± 13.0	117.9 ± 9.7
Diastric blood pressure (mmHg)	81.3 ± 11.8	81.2 ± 11.3	84.1 ± 10.5	72.6 ± 9.8	75.3 ± 10.0	73.7 ± 10.7

EPA, eicosapentaenoic acid.

1.125 g/d for 2 wk was examined by applying a physical load before and after EPA intake. The amount of EPA intake used was rather small when compared with that in the study by Morris et al.,[13] which studied the effect of fish oil on blood pressure and in which subjects were given 6–12 g fish oil/d (50%, ω-3 fatty acids). Coagulation and fibrinolytic activity consist of mutual activities among many factors.[14] In the present study, we examined the TAT level, which is a sensitive index for coagulation activity.[10,11] The PIC level is effective for measuring fibrinolytic activity as an index of the synthesis of plasmin, and the half-life of the PIC level in serum is much longer (6 h).[9] The D-dimer level is an indicator of the activated state of coagulation by showing the degradation of stable fibrin, which is one of the fibrin/fibrinogen degradation products produced by fibrinolysis.[12] After EPA intake, the TAT level tended to increase by 75.4% in comparison with the level before EPA intake; 1 h after the load, the TAT level increased significantly by 51.1% ($P < 0.05$) in comparison with the level before EPA intake. The PIC level tended to decrease by 16.7% in comparison with level before EPA intake; 1 h after of the load, the PIC level decreased significantly by 26.7% ($P < 0.05$) in comparison with the level before EPA intake. The D-dimer level was not changed by EPA intake; however, 1 h after the load, the D-dimer level decreased significantly by 24% ($P < 0.05$) in comparison with the level before EPA intake. Thus, as fibrinolytic activity was promoted by EPA intake, the PIC level showed a tendency to decrease, and the TAT level showed a tendency to increase, with no change occurring for the D-dimer level. However, 1 h after the load, the PIC level decreased significantly, the TAT level increased significantly, and the D-dimer level decreased significantly in comparison with that before EPA intake. Thus, the phenomenon that a small amount of EPA can suppress the coagulation activity could be clearly observed after the physical load.

TABLE III.

CORRELATION COEFFICIENT BETWEEN THE RATE OF CHANGE OF PIC, TAT, AND BIOCHEMICAL PARAMETERS BY EPA INTAKE AT 1 H AFTER A PHYSICAL LOAD ($N = 17$)

	Before EPA intake		After EPA intake	
	PIC	TAT	PIC	TAT
GLU	0.236	−0.199	−0.091	−0.488*
γ-GTP	−0.060	−0.105	−0.678*	−0.716†
BUN	−0.367	0.369	0.683*	0.191
TG	−0.120	−0.142	0.003	−0.224
T-chol	−0.279	0.045	0.271	−0.081

* $P < 0.05$.
† $P < 0.01$.
BUN, blood urea nitrogen; EPA, eicosapentaenoic acid; γ-GTP, γ-glutamyl transpeptidase; GLU, blood glucose; PIC, plasmin-α-2 plasmin inhibitor complex; T-chol, total cholesterol; TAT, thrombin-antithrombin III complex; TG, triacylglycerol.

In 1991, we surveyed plasma coagulation and fibrinolytic activity in fishermen older than 50 y (in Awaji, an island Hyogo prefecture). The TAT, PIC, and D-dimer levels were examined in three groups: those consuming fish less than 7 times per week (19 men), those consuming fish 7–14 times per week (14 men), and those consuming fish more than 15 times per week (15 men). The TAT levels were 2.05, 1.90, and 1.95 ng/mL, respectively. The PIC levels were 0.51, <0.3, and 0.31 ng/dL, respectively, and these levels were intended to show a low level for the group with high fish intake. The D-dimer levels were 43.7, 44.0, and 41.5 ng/mL, respectively, and the D-dimer level of the group with high fish intake showed a rather low level in comparison with that of the group with small fish intake. Thus, men who consumed fish more than 17 times a week may show a suppression in the degree of coagulation activity. Phillipson et al.[7] reported that fish oil intake in patients with type IIb hyperlipemia decreases the TG level by 64% and the T-chol levels by 27%. Also, there have been many studies reporting that the TG and T-chol levels were decreased by EPA intake.[8,15,16] Among those subjects, the TG level tended to increase by 10%, and the T-chol level did not change after EPA intake. The EPA level in the membranes of red cells increased significantly by 74% from 15.5 μg/gHb because of EPA intake.[17]

To analyze the effect of EPA intake on the relation between

coagulation and fibrinolytic activity and other biochemical parameters, γ between the rates of change of the TAT and PIC levels and other parameters were analyzed 1 h after the load. Before EPA intake and after the load, the rate of change of the TAT and PIC levels did not correlate to any of the other parameters. After EPA intake, the rate of change of the TAT and PIC levels correlated significantly and negatively to that of γ-GTP 1 h after the load. Vuorinen et al.[18] reported that γ-GTP may produce a local antithrombotic effect. γ-GTP was distributed mainly in the kidney uriniferous tubule, liver, bilious ducts, and pancreas and was related to amino acid metabolism in the microsomes in cells.[19] Tisdale et al.[20] reported that EPA inhibited the cyclic AMP formation in cells. The rate of change of glucose after EPA intake correlated significantly and negatively to that of the TAT level. In contrast, Fasching et al.[21] reported that the glucose and insulin levels did not change after fish oil was consumed by patients with impaired glucose tolerance. Before EPA intake, the rate of change of the BUN level correlated negatively to the PIC level, but after EPA intake, this relation was changed significantly to a positive one. Thus, EPA may activate kidney function to produce BUN and PIC. The rate of change of the TG level correlated negatively to that of the TAT level ($r = -0.22$) but did not correlate to that of the PIC level after EPA intake. Matsuda[22] reported that the increase in the TG level correlated to the PAI-1 level and that the increment of the TG and PAI-1 levels in serum was one cause of the thrombogenic factors in hyperlipemia patients. The rate of change of the T-chol level correlated negatively to the PIC level ($r = -0.28$) but changed after EPA intake to correlate positively to the PIC level ($r = 0.27$). These relations were similar to the relation of the BUN to the PIC levels. Asano et al.[23] reported that EPA activates the K^+ current in smooth muscle cells and regulates the vascular tone. SBP tended to decrease by 5 mmHg and DBP by 10 mmHg after EPA intake.

SUMMARY

A small EPA amount of 1.125 g taken daily for 2 wk significantly decreased the fibrinolytic activity and significantly increased the coagulation activity 1 h after a physical load, and the rate of change of the PIC and TAT levels correlated significantly and negatively to that of γ-GTP level. SBP and DBP tended to decrease by 5 and 10 mmHg, respectively, after EPA intake.

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