Variable hypocoagulant effect of fish oil intake in humans: modulation of fibrinogen level and thrombin generation

Variable Hypocoagulant Effect of Fish Oil Intake in Humans

Modulation of Fibrinogen Level and Thrombin Generation

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Objective—The beneficial effect of dietary fish oil, rich in omega-3 polyunsaturated fatty acids (PUFAs), on cardiovascular disease is multifactorial and may partly rely on their anticoagulant action. We studied how fish oil intake influenced thrombin generation in plasma and which factors were involved herein.

Methods and Results—Twenty-five healthy males with borderline overweight received 3.0 g omega-3 PUFAs daily for 4 weeks. Fish oil intake reduced plasma triglycerides and lowered platelet integrin activation, as well as plasma levels of fibrinogen and factor V, but had no effect on vitamin K-dependent coagulation factors. Before fish oil intake, thrombin generation (reflecting the coagulant potential) considerably varied between plasmas from individual subjects, which were partly explained by variation in prothrombin, antithrombin, fibrinogen, and factor V levels. Fish oil intake reduced thrombin generation in the presence and absence of platelets. This reduction correlated with the fish oil effect on fibrinogen and factor V levels. Interestingly, the lowering effect of fish oil on thrombin generation and fibrinogen clustered around subjects with high fibrinogen carrying a structural fibrinogen α-chain polymorphism.

Conclusions—Dietary omega-3 PUFAs provoke a hypocoagulant, vitamin K-independent effect in humans, the degree of which may depend on fibrinogen level. **(*Arterioscler Thromb Vasc Biol*. 2004;24:1734-1740.)**

Key Words: coagulation ■ factor V ■ fibrinogen ■ fish oil ■ thrombin generation

Since the 1970s, fish oil has been studied as a nutritional component with antithrombotic potential.[1] The protective effect on thrombosis has been attributed to the omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid, and docosahexaenoic acid, which are abundantly present in fish oil. Early epidemiological and intervention studies pointed to a strong association between consumption of omega-3 PUFAs and a reduced risk of coronary heart disease, even with only 2 fish dishes per week.[2,3] Currently, a daily intake of 0.3 g of omega-3 PUFAs is recommended for healthy adults, and a daily dose up to 1 to 3 g is recommended for patients with coronary heart disease or hypertriglyceridemia.[4]

Despite 30 years of study, the precise mechanisms of action of omega-3 PUFAs are still a matter of debate.[5] Established effects include an altered heart and vessel function, a decreased risk for arrhythmias, and lowering of blood pressure. Many reports also describe effects on plasma hemostatic variables, but usually with high interstudy variation. Best documented is that omega-3 PUFA intake reduces plasma triglycerides levels, whereas plasma cholesterol is decreased in only few studies.[6–8] Part of published studies show, often mild, lowering effects of omega-3 PUFA on platelet activation and bleeding time.[9]

With respect to coagulation, some trials point to a moderate reduction by fish oil of the plasma levels of fibrinogen and coagulation factors V, VII, and X,[10–12] whereas other studies fail to detect this.[5,9] Because some of these factors require vitamin K-dependent carboxylation for coagulant activity, it was suggested that fish oil interferes with the vitamin K action. In rat, we and others have found that high amounts of dietary omega-3 PUFAs reduce the levels of the vitamin K-dependent factors X and prothrombin.[13,14] By continuous measurement of thrombin generation, which provides a highly sensitive method of measuring the coagulant potential of plasma,[15,16] we established that this lowering of coagulation factor levels resulted in hypocoagulant activity.[13] However, because this hypocoagulant effect in rat was not enlarged by vitamin K depletion and was accompanied by a reduction in (vitamin K-independent) factor V, the hypocoagulant effect of fish oil has at least a partially vitamin K-independent cause.[17] How intake of omega-3 PUFAs influences thrombin generation in humans is still unknown.

Received December 24, 2003; revision accepted June 4, 2004.

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***Arterioscler Thromb Vasc Biol.* is available at http://www.atvbaha.org** **DOI: 10.1161/01.ATV.0000137119.28893.0b**

TABLE 1. Effect of Fish Oil on Plasma Lipid Levels and Hemostatic Parameters

	No Treatment	After Fish Oil	ΔNo Treatment (%)	ΔFish Oil (%)
Lipids				
Triglycerides (mmol/L)	1.59±0.23	1.33±0.16	+2.6±8.3	−10.4±5.0**
Total cholesterol (mmol/L)	5.64±0.25	5.70±0.27	+2.5±1.7	+1.1±1.6
LDL cholesterol (mmol/L)	3.91±0.26	4.07±0.27	+2.2±2.9	+5.7±3.4*
Factors				
Fibrinogen (g/L)	3.39±0.11	3.29±0.13	−1.2±1.9	−2.9±2.0*
Prothrombin (%)	101.6±1.9	102.1±2.3	+1.6±0.7*	+0.4±0.9
Factor V (%)	100.8±3.7	97.6±3.8	−0.9±1.6	−3.1±1.5**
Factor VII (%)	102.7±3.0	102.5±3.3	−1.4±2.0	−0.3±1.3
Factor X (%)	103.0±2.2	101.8±2.4	+0.7±0.9	−1.2±0.8
Antithrombin (%)	84.1±1.3	83.6±1.2	+2.0±1.0	−0.4±1.2
Protein C (%)	108.5±2.3	110.4±2.7	+1.6±1.1	+1.8±1.3

Levels or activities (% of normal pooled plasma) were measured 4 weeks before, immediately before, and 4 weeks after fish oil treatment. There were no systematic differences between the two baseline samples. Shown are averaged values before treatment and values after fish oil intake, further percent differences (calculated per subject) between pretreatment values and fish oil effect (Δno treatment and Δfish oil). Mean±SE (n=25). *$P<0.1$ and **$P<0.05$ (Wilcoxon).

Genetic variation in coagulation factors and adhesive platelet glycoprotein is likely to contribute to the risk for arterial and venous thrombosis.[18,19] There is limited evidence that genetic variety may also contribute to the antithrombotic response to nutrition. For instance, the reducing effect of dietary omega-6 fatty acids on lipoprotein levels has been found to differ between carriers of polymorphisms of several genes encoding for apolipoproteins.[20] It is therefore possible that the hypocoagulant effect of omega-3 PUFAs is also sensitive to gene–environmental interactions which, in turn, contribute to the variable outcome of fish oil intervention studies.

In the present study, we investigated the effects of fish oil-derived omega-3 PUFAs on thrombin generation (reflecting the coagulant potential) in a group of subjects with borderline overweight and therefore slightly increased thrombotic risk. Because thrombin generation in plasma is critically dependent on the presence of procoagulant phospholipids,[21] this process was measured in the presence of either phospholipid vesicles or autologous platelets. It appeared that fish oil intervention decreased thrombin generation even in the absence of platelets, along with coagulation factors fibrinogen and factor V. Intriguingly, this hypocoagulant effect was clustered in a subgroup with relatively high baseline levels of fibrinogen who were carrying the 312A polymorphism in the fibrinogen-α gene.

Methods

Please see http://atvb.ahajournals.org for an expanded Methods section.

Results

Effects of Fish Oil Intervention on Coagulation Factor Levels and on Thrombin Generation in the Presence and Absence of Platelets

Twenty-five healthy male subjects, aged 48.5±9.8 years (mean±SD), with borderline overweight (body mass index 29.0±2.5 kg/m^2) participated in the fish oil study. The intervention consisted of intake of capsules with 3.0 g omega-3 PUFAs per day for 4 weeks, which was equivalent to, on average, 32.8 mg omega-3 PUFAs per kg body weight daily. Blood samples were taken 4 weeks before, immediately before, and 4 weeks after fish oil treatment. In baseline blood samples, all 25 subjects had normal counts of platelets ($215\pm9\times10^9$/L), erythrocytes ($5.2\pm0.1\times10^{12}$/L), and leukocytes ($6.6\pm0.4\times10^9$/L). Fish oil intake did not affect these parameters. The intervention resulted in significantly lower levels of plasma triglycerides, which is a common effect of fish oil. In contrast, cholesterol in low-density lipoprotein tended to be increased (borderline significant; Table 1).

After earlier diet studies with rats,[13,17] vitamin K-(in)dependent coagulation factors were measured. In the group of 25 volunteers, fish oil intake reduced plasma levels of the vitamin K-independent coagulation factor V (significant) and of fibrinogen (borderline significant), but not of the vitamin K-dependent factors, prothrombin, factor VII, and factor X (Table 1). Fibrinogen antigen level was significantly reduced after fish oil by −4.1±3.1% (mean±SE; $P=0.03$) when an EIA-based test was used instead of the activity-based Clauss test. Anticoagulant proteins antithrombin and protein C were not altered (Table 1).

To measure platelet activation, platelets in platelet-rich plasma (PRP) were stimulated with the PAR1 thrombin-receptor agonist, SFLLRN. Flow cytometric analysis indicated that fish oil intake significantly lowered SFLLRN-induced activation of αIIbβ3 integrin (−18.4%), but not SFLLRN-provoked exposure of P-selectin, when compared with effects of SFLLRN in pre-intervention PRP samples (Table 2).

As a sensitive way to monitor the coagulant potential under hypocoagulant conditions, we measured thrombin generation in tissue factor-triggered plasma after the thrombogram method.[15] To provide procoagulant phospholipids, plasma was

TABLE 2. Effect of Fish Oil on Platelet Activation and Thrombin Generation

	No Treatment	After Fish Oil	ΔNo Treatment (%)	ΔFish Oil (%)
Platelet activation				
allbβ3 (% pos. cells)	55.4±5.5	45.6±6.2	+8.0±12.2	−18.4±10.6**
P-selectin (% pos. cells)	48.1±2.9	44.6±5.0	+9.2±10.7	−3.0±12.8
Thrombin generation with platelets				
Time-to-peak (min)	25.7±0.6	27.0±0.7	+0.6±2.2	+5.6±2.5*
Thrombin peak (nmol/L)	118±5	101±5	+3.1±5.9	−13.9±3.7**
ETP (nmol/L×min)	1524±50	1369±61	+0.4±3.0	−9.8±3.2**
Thrombin generation with phospholipids				
Time-to-peak (min)	5.6±0.2	5.8±0.2	+1.6±2.0	+4.2±1.7**
Thrombin peak (nmol/L)	305±9	294±9	+2.7±3.1	−3.0±2.3*
ETP (nmol/L×min)	1847±48	1805±50	+3.3±1.6*	−2.1±1.6*
APC resistance				
nAPCsr	1.17±0.16	1.20±0.17	+6.0±4.9	+3.8±4.5

Shown are averaged values before treatment and values after fish oil intake, as well as difference between these (Δno treatment and Δfish oil). Platelets were stimulated with 5 μmol/L SFLLRN and exposure of activated αIIbβ3 integrin and of P-selectin was evaluated. Thrombin generation in plasma was measured as indicated for Figure 1. nAPCsr was determined from thrombin generation in the presence and absence of APC. Mean±SE (n=25). *$P<0.1$ and **$P<0.05$ (Wilcoxon).

supplied with platelets (PRP of standardized platelet count) or with a nonlimiting concentration of 4 μmol/L phospholipids (platelet-free plasma [PFP]/phospholipids). Coagulation in PRP was initiated with a low concentration tissue factor (0.5 pmol/L) sufficient to start intrinsic coagulation and to detect platelet-dependent effects. Coagulation in PFP/phospholipids was triggered with optimal tissue factor (5 pmol/L). The thrombograms were analyzed on the following parameters: time to thrombin peak; peak height (indicative of maximal rate of thrombin formation); and area-under-the-curve or endogenous thrombin potential (ETP), reflecting total activity of thrombin during coagulation. Under these experimental conditions with PRP or PFP/phospholipids, thrombin generation relied on the extrinsic coagulation pathway and had an assay variability <3%.[22]

Before fish oil intervention, thrombin generation curves greatly differed between plasmas from the 25 subjects, as apparent from the high variation in thrombin peak heights and ETP levels (Figure 1A). The *inter*subject variation coefficients of ETP with PRP and PFP/phospholipids (16.5% and 13.1%, respectively) were much higher than *intra*subject variation coefficients (≈4.5%). Typically, ETP levels in the presence and absence of platelets were strongly correlated with $R=0.48$ and $P=0.001$ (Figure 1B). Thus, those subjects whose plasmas displayed relatively high thrombin generation with platelets also had high thrombin generation with phospholipids present (Figure 1A). This indicated that much of the subject-dependent variability in thrombin generation with platelets was caused by variant activity of the coagulation. No differences were seen in thrombogram parameters between 2 plasma samples taken before the intervention (Table 2).

In addition, thrombin generation was measured in the presence and absence of activated protein C (APC) to determine the sensitivity of factor Va toward inactivation by APC. This reaction is sensitive to lipid (steroid) plasma components.[23] The results were expressed as normalized APC sensitivity ratio (nAPCsr), which by definition has a value of 1.0 in normal pooled plasma.[23] Before intervention, plasmas from the 25 subjects displayed variable nAPCsr values, with 2 subjects showing a nAPCsr >2.5 (Figure 1C).

When averaged for all 25 subjects, thrombogram parameters of PRP were significantly altered after 4 weeks of fish oil intake. The intervention significantly prolonged the time-to-peak and decreased the peak height and the ETP by >10% (Table 2). With PFP/phospholipids, fish oil prolonged the time-to-peak and had a smaller (borderline significant) effect on peak height and ETP of ≈3%. Typically, in some, but not in all, subjects, fish oil intake resulted in a decreased thrombin generation (Figure 2). The intervention did not significantly influence nAPCsr, indicating that factor Va inactivation remained unchanged. These data thus indicate that the reducing effect of fish oil on coagulant potential in the presence and absence of platelets is caused by reduced thrombin formation rather than by increased APC-dependent factor Va inactivation or increased thrombin inhibition.

Contribution of Fibrinogen and Factor V to Fish Oil Effect on Thrombin Generation

By multivariate regression analysis, the contribution of plasma (anti)coagulant factors to the large intersubject variation in thrombin generation was evaluated. Comparison of pre-intervention values of the 25 subjects (Table 2) showed that levels of prothrombin and antithrombin were main determinants of ETP with PFP/phospholipids ($R=0.26$, $P=0.042$). This is in agreement with published kinetic data.[24] The levels of only fibrinogen and factor V further contributed to the ETP variation. Together, these 4 factors explained ≈30% of the variation of peak height and ETP ($R=0.31$, $P=0.036$). The vitamin K-dependent factor VII, factor X, and protein C, which were covariants ($P<0.03$), only determined

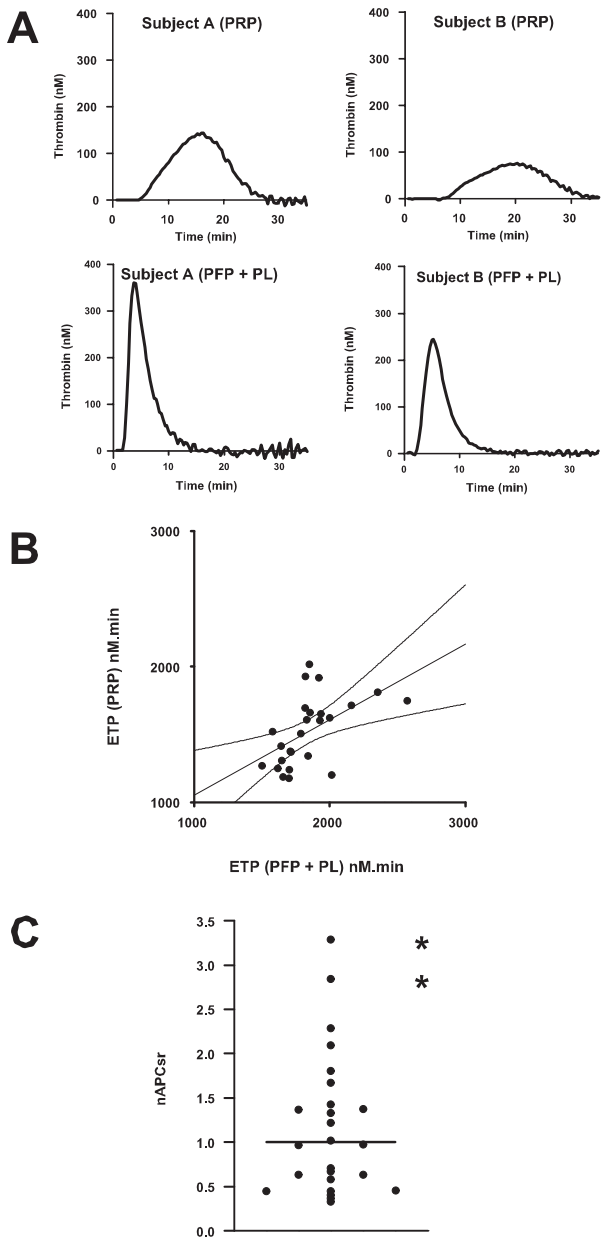

Figure 1. Intersubject variation in thrombin generation. A, Tissue factor-induced thrombin generation in plasma from 2 representative subjects with either 100×10^9 platelets/L (PRP) or 4 μmol/L phospholipids (PFP+PL). Note the high curves for subject *A* in comparison to subject *B*. B, Correlation between ETP levels determined in plasma from all 25 subjects in the presence of platelets and with phospholipids (95% CI is shown, $R=0.48$, $P=0.001$). C, Normalized APC sensitivity ratio (nAPCsr) determined in plasma from the 25 subjects. *Heterozygous factor V^{Leiden} carriers.

the time-to-peak ($P<0.046$) but did not further contribute to peak height and ETP level.

Multiregression analysis of data from all subjects was also used to determine whether the fish oil effect on coagulation factor levels could explain its effect on thrombin generation. For PFP, the fish oil-evoked reduction in ETP significantly correlated with the reduction in fibrinogen or factor V level of $R=0.48$ ($P=0.015$) or $R=0.41$ ($P=0.045$), respectively. The variable decrease in thrombin peak height also correlated with the reduction in factor V ($P=0.041$), whereas the increase in lag-time of thrombin formation correlated with the reduction in fibrinogen ($P=0.021$). Also in PRP, the intervention effects on ETP and factor V level were correlated ($P=0.016$), but not those on ETP and fibrinogen level ($P=0.77$). An indication that variable fibrinogen did contribute to thrombin generation in PRP came from reanalysis of calibrated curves; fish oil effects on thrombogram peak height were correlated with fibrinogen level ($P=0.048$, n=11). However, there was no correlation between the fish oil effect on αIIbβ3 integrin activation and on thrombin generation in PRP (Table 2).

Control experiments were performed to determine whether changes in fibrinogen and factor V could influence thrombogram characteristics. Figure 3 shows that addition of 10% human fibrinogen to normal pooled PFP/phospholipids resulted in increased thrombin generation, as indicated by higher thrombin peak and ETP levels. Partial depletion of fibrinogen but not factor V led to decreased thrombin generation. Complete depletion of factor V but not of fibrinogen abolished thrombin generation.

Genetic Variation in Fibrinogen and Factor V to Hypocoagulant Fish Oil Effect

Given the high variation in thrombograms between subjects, we explored the possibility that genetic variation in fibrinogen and factor V contributed to the variable effects of fish oil intervention on the coagulant potential. As a start, common polymorphisms in fibrinogen-α/β and factor V genes were determined that have been associated with an increased risk of thrombosis. With respect to the fibrinogen-β gene, 11 subjects were carrying the less common allele of all the G-854A, G-455A, and C-148T polymorphisms (1 homozygous), which are known to be in linkage disequilibrium. For these polymorphisms, we did not find associations with ETP or fish oil effects on ETP. In contrast, 10 subjects carrying the 312Ala allele of the fibrinogen-α T312A polymorphism,[25] had higher baseline levels of fibrinogen and a greater reduction in fibrinogen on fish oil consumption ($P=0.008$ and 0.021, respectively) in comparison to the 15 noncarriers (Figure 4A). Fibrinogen reduction in the group of carriers correlated with the decrease in thrombin generation after fish oil ($P=0.048$). Analysis of data from all subjects indicated that the 312Ala carriers were responsible for the fish oil effect on fibrinogen and thrombin generation. In the heterozygote TA group, the average decrease in fibrinogen was 8%, whereas in the homozygote TT group, fibrinogen increased 0.5% (Figure 4B).

Five of 25 subjects carried the less common 1299Arg allele of the A4070G polymorphism of factor V, which is invariably associated with the HR2 haplotype.[26,27] These carriers had lower factor V levels than noncarriers both before and after the intervention period ($P=0.032$ and 0.035, respectively). They had a tendency to greater factor V reduction with fish oil ($P=0.06$) in comparison to noncarriers, which correlated with the reduced thrombin generation (Figure 4C and 4D). Two subjects carried the Gln506 (factor V_{Leiden}) allele of the G1691A factor V polymorphism. They were responsible for the high nAPCsr levels >2.5 (Figure 1C), which is in

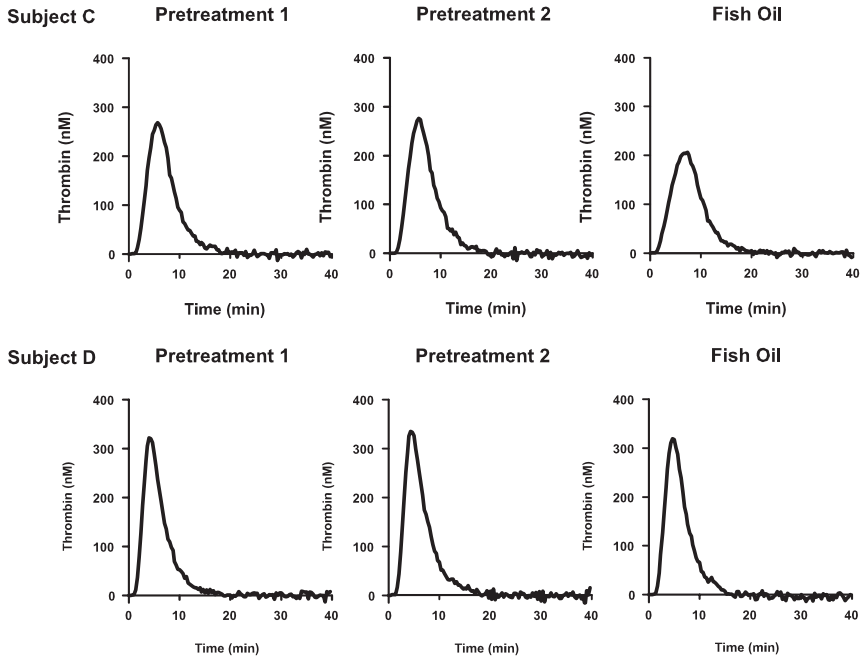

Figure 2. Variable effect of fish oil on thrombin generation. Thrombograms are given of plasma (PFP+PL) before and after fish oil intake from 2 representative subjects with different intervention responses (conditions as in Figure 1). For subject *C,* fish oil resulted in a 20% reduction in ETP, which was accompanied by a 7% reduction in fibrinogen. Fish oil did not alter ETP or fibrinogen in plasma from subject *D*.

agreement with expected APC resistance,[28] but they did not show particular responses to fish oil.

With respect to platelet receptors, 12 subjects carried the high-risk 807T variant of integrin-α2, and 5 subjects carried the Leu33 (Pl^{A2}) variant of the C1565T integrin-β3 polymorphism. Thrombograms of PRP with or without fish oil were not different between carriers and noncarriers ($P>0.073$).

Discussion

The present data are a first report in humans on reduced thrombin generation in response to fish oil intake. This effect was achieved in healthy male volunteers with borderline overweight, consuming 3.0 g fish oil-derived omega-3 PUFAs daily for 4 weeks, and it was accompanied by a reduction in plasma levels of mainly fibrinogen and factor V. In agreement with other studies, fish oil intake lowered the plasma triglyceride concentrations.[6–8] This latter effect pointed to efficacy of the omega-3 PUFA intervention. The intervention led to a small increase in low-density lipoprotein cholesterol, an effect that is also not uncommon after fish oil intervention in normal and hyperlipoproteinemic subjects.[6,8]

The data suggest that the reducing effects of fish oil on tissue factor-induced thrombin generation and on fibrinogen level are causally related. First, variability analysis shows that fibrinogen, in addition to prothrombin, antithrombin, and factor V, is a main coagulation factor contributing to the intersubject variation in thrombogram parameters (peak height and ETP). The enhancing effect of fibrinogen on thrombin generation in situ has earlier been described by Hemker et al.[22] Second, the intervention effect on thrombin generation in individuals significantly correlates with the effects on fibrinogen and factor V level. This holds for thrombograms obtained in the presence and absence of platelets. Third, in vitro modulation of fibrinogen in normal pooled plasma causes similar changes in thrombin generation as the fish oil intervention. Because artificial addition or depletion of factor V in normal plasma did not influence thrombin generation, it is likely that factor V, in the thrombogram variation, is a confounder for another related plasma factor that influences thrombin formation or inactivation. The lack of fish oil effect on nAPCsr suggests that this concerns a coagulant rather than anticoagulant factor.

Earlier studies often failed to measure anticoagulant effects of fish oil consumption,[29] most probably because less sensitive coagulation assays like the prothrombin time were used.[15] However, in some but not all human studies, a reduction in plasma fibrinogen and/or factor V activity was observed in response to fish oil.[9,12] In addition, in rats, feeding of low doses of omega-3 PUFAs lowered factor V levels.[17] With respect to fibrinogen, which is likely an independent cardiovascular risk factor when increased, fish oil has been shown to decrease this factor mainly in subjects with elevated baseline levels.[10,11] The present data agree with this.

At the applied dose of 3.0 g omega-3 PUFAs/d, we did not find an intervention effect on vitamin K-dependent coagulation factors, prothrombin, factor VII, factor X, and protein C. This contradicts to the idea that fish oil can interfere with vitamin K action, as proposed from other studies in which factor VII and factor X were moderately reduced by fish oil.[11,12,30] In rat plasma, we and others have measured reduced levels of prothrombin and factor X, an observation that was compatible with vitamin K antagonistic activity.[13,14] However, this was seen after relatively high doses of fish oil ($\geq$3 energy%), and the hypocoagulant effect was not further affected by vitamin K depletion.[17] This strongly suggests that the main fish oil effect, especially at lower doses, is independent of vitamin K.

Quantitatively, the fish oil-induced decline in thrombin generation (peak level) was greater in the presence (−15%) than in the absence (−3%) of platelets. It is noted that

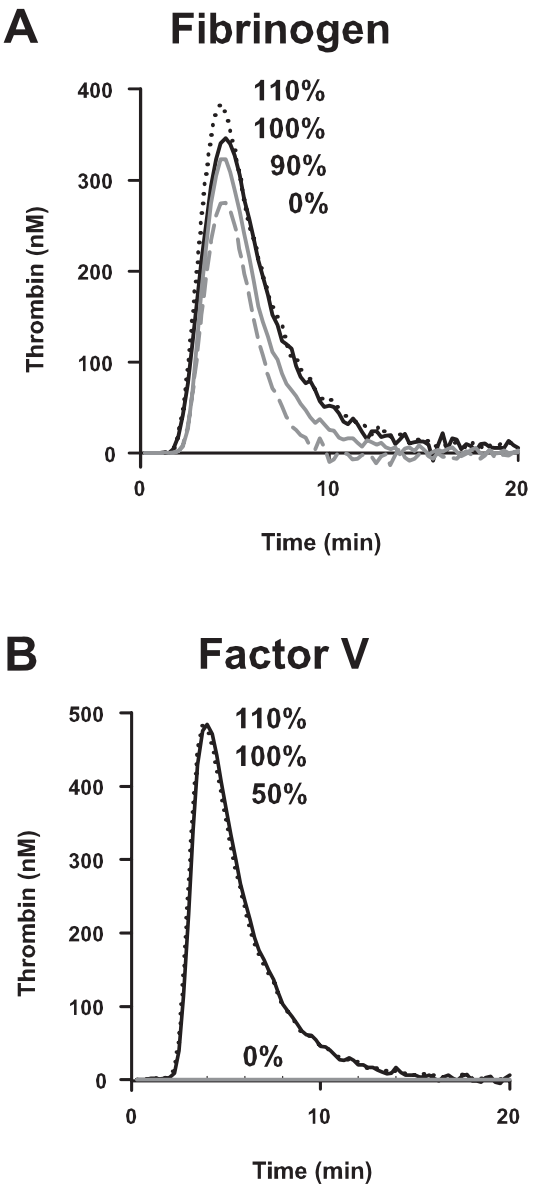

Figure 3. Effect of fibrinogen and factor V on thrombin generation. Normal pooled plasma was prepared with variable concentrations of human fibrinogen or factor V. Tissue factor-induced thrombin generation was measured with 4 μmol/L phospholipids at the following conditions: (A) 0% to 110% fibrinogen and 100% factor V; (B) 0% to 110% factor V and 100% fibrinogen. Curves of nanomolar thrombin concentrations were obtained by using separate calibrator traces for each different sample to eliminate differences in light scatter. Indicated factor levels (compared with normal pooled plasma) were always checked in the reconstituted plasma samples. Data are representative for 3 or more experiments. Thrombin generation curves with plasmas from carriers of 1299Arg and His1299 factor V gave the same factor V dependency.

relations between thrombin generation with platelets and, eg, fibrinogen levels are more difficult to establish because of the high intrasubject variation in the assay with PRP. Fish oil reduced platelet integrin αIIbβ3 activation in response to thrombin-receptor stimulation. This is compatible with the notion that thrombin generation depends on the mutually stimulatory interactions of platelet activation and coagulation,[21] and that integrin αIIbβ3 activation significantly contributes to platelet-dependent thrombin generation.[31] Thus, as proposed earlier,[9] moderate antiplatelet and hypocoagulant effects of fish oil may add in lowering the thrombogram curve. From the present results, we can conclude that the fish oil effect on thrombin generation is enhanced by the presence of platelets, but that the contribution of integrin activation is still unclear.

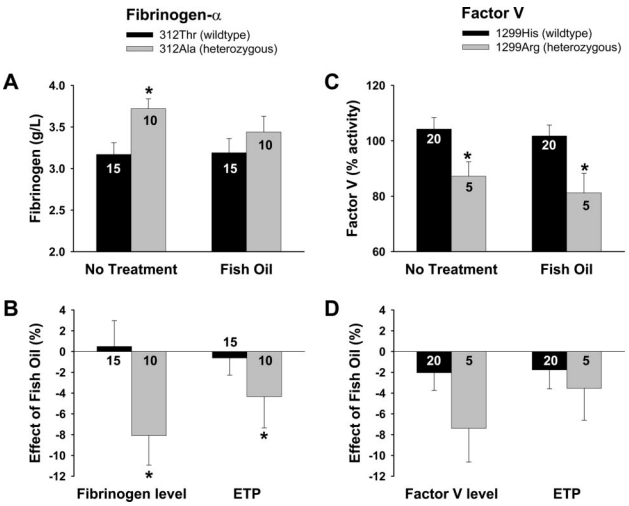

Figure 4. Effect of fish oil on fibrinogen and factor V levels and thrombin generation in plasma/phospholipids. A and B, Carriers of fibrinogen-α 312Ala allele (n=10 heterozygotes/25). C and D, Carriers of factor V 1299Arg allele (n=5 heterozygotes/25). Data (mean±SE) were obtained as described for Tables 1 and 2. When fibrinogen was measured as antigen level, 312Ala carriers also had higher baseline levels than noncarriers (3.70±0.20 versus 2.57±0.19 g/L), and showed a greater reduction after fish oil (P=0.016). *P<0.05 (Mann–Whitney).

As considerable intersubject variation was observed in coagulation factor levels and size of the thrombogram, the 25 volunteers were evaluated on the presence of frequent polymorphisms in fibrinogen and factor V genes with reported increased thrombotic risk. Typically, carriers of fibrinogen α-chain 312Ala variant (n=10/25) had a higher baseline fibrinogen level that was accompanied by stronger reduction in both thrombin generation (ETP) and fibrinogen level with fish oil than noncarriers. In fact, the fibrinogen reduction in carriers explained most of the effect on thrombin generation with or without platelets. In literature, the 312Ala allele influences clot stability[32] and predisposes clots to embolization,[25] but the relation with fibrinogen expression is still unclear. This polymorphism is relatively abundant among whites with an estimated frequency of 35% to 40%.[25] However, there was no difference between carriers of common haplotypes in the promoter region of the β-fibrinogen gene (−854/−148), which in some, but not all, studies is linked to increased fibrinogen expression.[33]

The factor V His1299Arg (A4070G) polymorphism, associated with HR2 haplotype, is related with lower factor V levels.[26] The 5 carriers of 1299Arg factor V had lower factor V levels than the noncarriers, both before and after fish oil supplementation. Carriers tended to respond better to fish oil, but group size was too small to validate this.

In general, limitation, and strength, of this study is that the effect evaluation was analyzed in samples from a limited number of 25 individuals with borderline increased body mass index. This limits the statistical power and precludes the finding of small effects but, when effects are found, these are likely to be biologically and medically significant. The small numbers make it difficult to draw strong conclusions on differences between the polymorphisms. Yet, this report provides a first indication that genetic variation can contribute to a variable hypocoagulant (thromboprotective) fish oil effect.

Considering that the hypocoagulant effect of in fish oil is vitamin K-independent and has a genetic component, it is of interest to speculate on the mechanism of action. In mice, omega-3 PUFAs can downregulate the hepatic expression of the sterol regulatory element-binding protein-1 [34] and of the peroxisome proliferator-activated receptor-α system.[35] Further, peroxisome proliferator-activated receptor-α controls fibrinogen levels in humans.[36] One possibility, therefore, is that omega-3 PUFAs provoke hypocoagulant conditions by influencing transcription systems, in which genetic variation may play a modulatory role. However, fish oil may also act on the translation or posttranslation level, eg, altering hepatic secretion quantitatively or qualitatively.

Acknowledgments

We acknowledge financial support from the Netherlands Organization for Scientific Research (NWO 980-10-018). We thank Pharma Nord (Vejle, Denmark) for supply of fish oil capsules.

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