Long-Chain n-3 Fatty Acids Specifically Affect Rat Coagulation Factors Dependent on Vitamin K
Relation to Peroxidative Stress

Claude Leray, Marie-Louise Wiesel, Monique Freund, Jean-Pierre Cazenave, Christian Gachet

Abstract—Fatty acids of marine origin have been shown to affect blood coagulation in the rat. In an attempt to gain insight into the mechanisms of this phenomenon, we studied the effects of dietary linseed and fish oils on the liver antioxidant status and plasma coagulation parameters in rats on a time-course basis. Dietary enrichment in eicosapentaenoic and docosahexaenoic acids resulted in strong hypocoagulation after only 1 week and a concomitant increase in liver lipid peroxidation and tocopherolquinone content. Enrichment in linolenic acid induced similar increases in lipid peroxidation and tocopherol catabolism but negligible alteration of coagulation. A significant correlation between plasma factor II coagulant activity and liver tocopherolquinone was found in fish oil– but not in linseed oil–fed rats. Although ingestion of tocopherolquinone led to high levels of this compound in the liver, it had only marginal effects on coagulation factors. Thus, it seems unlikely that this vitamin E metabolite could be involved in the lowering of vitamin K–dependent clotting factors through inhibition of γ-glutamylcarboxylase. Rather, our results indicate that the effects of the n-3 fatty acids of fish oil on vitamin K–dependent coagulation factors are specific and independent of liver tocopherolquinone levels.

Key Words: coagulation ■ fish oil ■ linseed oil ■ tocopherolquinone ■ vitamin E

There is increasing evidence that very-long-chain n-3 fatty acids such as eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids of marine origin are efficient in the prevention and treatment of hyperlipidemia, thus favorably influencing atherosclerosis and heart and inflammatory conditions.1–4 Whereas its hypolipidemic effects and capacity to regulate platelet and endothelial functions are well documented,5,6 the impact of fish oil on blood coagulation is still far from clear.7–9 Recently, it was reported that diets enriched in both fish oil and long-chain n-3 fatty acids induced a significant depression of the blood coagulation capacity in rats after some weeks of treatment.10–12 The mechanism of this inhibition is unknown but may reside in either direct effects of n-3 fatty acids on vitamin K– dependent γ-glutamylcarboxylase or indirect effects of various metabolites formed during fatty acid catabolism and/or oxidation.

Vitamin E quinone (tocopherolquinone), an oxidation product of α-tocopherol that is a potent in vitro inhibitor of vitamin K– dependent γ-glutamylcarboxylase,13 could provide a link between the ingestion of n-3 fatty acids and their hypocoagulant effects. Although this has not yet been investigated, the consumption of fish oil may be expected to lead to a higher production of vitamin E quinone, because this nutrient is known to increase the susceptibility of cellular lipids to peroxidation14–17 and simultaneously alter α-tocopherol homeostasis in animals15,16,18 and humans.19–21 On the other hand, whereas α-linolenic acid (18:3n-3), the precursor of the n-3 series, has been shown to share some of the physiological properties of marine n-3 fatty acids,22–26 its effects on blood coagulation remain unexplored.

The aim of the present study was to determine whether all n-3 fatty acids affect coagulation factors and whether this implies the participation of tocopherolquinone. Using a rat model and 2 different lipid sources, linseed oil rich in 18:3n-3 and fish oil rich in EPA and DHA, we designed experiments to examine the specificity of the hypocoagulant response and whether it involved alteration of the antioxidant status through lipid peroxidation. The evolution of liver vitamin E and tocopherolquinone contents and the composition of liver phospholipids, plasma lipids, and several coagulation factors were determined in rats fed the same amounts of vitamin E, cholesterol, and n-3 fatty acids from vegetal or marine origin. A possible direct effect of tocopherolquinone on clotting factors was investigated in rats receiving the pure compound by gavage. Blood coagulation was explored with global tests measuring the activated partial thromboplastin time (APTT) and the prothrombin time (PT) or with more specific tests measuring the coagulant activities of factors II, V, and VII–X. APTT evaluates the endogenous system, including the common terminal section with the exogenous system.

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459
received official approval with regard to the care and use of laboratory animals.

Blood and Tissue Sampling
At each sampling time and in each dietary group, 8 rats were anesthetized after an overnight fast and blood was drawn from the abdominal aorta into 3.15% sodium citrate anticoagulant (1 volume for 9 volumes of blood). Plasma samples were obtained by centrifugation at 10,000g and 4°C, and an aliquot was reserved for clotting tests that were performed immediately; the remainder was stored at −70°C for lipid analyses. Liver samples were removed, rapidly frozen in LN2, and stored at −70°C.

Tocopherol Analyses
Before tocopherol extraction, liver tissue was homogenized in 10 volumes of 100 mmol/L KCl. Tocopherols were extracted from plasma samples (0.5 mL) and liver homogenates (1 mL), and α- and γ-tocopherols and tocopherolquinone were separated and quantified by high-performance liquid chromatography, followed by a postcolumn reactor that reduced tocopherolquinone to the hydroquinone as previously described.28 An electrochemical detector (Coulochem II, ESA) was coupled to an electronic integrator.

Methods

Animals and Diets
Male Wistar rats, aged 6 weeks and weighing 160 g (Iffa Credo, L’Arbresle, France), were maintained on a chow diet (AO4, Usine d’Alimentation Rationnelle) for a 1-week acclimatization period in the animal unit and then transferred to 1 of 2 experimental diets for 4 weeks, all rats being weighed weekly. These diets were enriched in linseed oil (LIN diet) or purified fish oil (MaxEPA diet). Eight groups of 8 animals each were randomly assigned to 1 of the 4 sampling times for each diet. Thus, in each dietary category, 8 rats fed the basal diet were killed at the outset of the experiment, and 8 rats were humanely killed after 1, 2, and 4 weeks of feeding. The 2 semisynthetic diets contained (in g/kg diet) 184 sucrose, 188 casein, 34.4 minerals (mix 102, APAE), 8.8 vitamins (mix 102, APAE), 1.6 D,L-methionine, and 200 lipids. Various mixtures of refined vegetable oils (olive, sunflower, linseed), tristearin, and purified fish oil (MaxEPA, RP Scherrer) were used to obtain the appropriate fatty acid composition (Table 1). Taking into account the amounts provided by natural oils, cholesterol and α-tocopherol were added to keep their concentrations similar (respectively, 250 and 310 mg/kg). Only the LIN diet contained a significant amount of γ-tocopherol (95 mg/kg). The essential fatty acid compositions of the diets differed mainly in the balance between n-3 and n-6 fatty acids and in their peroxidizability index (PI). Preparations were made weekly and stored at −20°C until use, food cups were changed daily, and food and water were freely available.

The direct in vivo effect of tocopherolquinone was studied in 2 groups of 5 rats receiving the chow diet and the pure compound by gavage (20 mg dissolved in 0.5 mL of olive oil) or olive oil only (controls) once a day for 4 days. In both groups, blood and liver were sampled on the fifth day. The dose of 20 mg was chosen after preliminary experiments with 10 mg, which gave no variations in blood coagulation factor activities. Tocopherolquinone was prepared29 by oxidation of α-tocopherol with FeCl3, and its purity was checked by high-performance liquid chromatography.29 The protocol

### TABLE 1. Lipid Composition of the Diets and Their Fatty Acid Profiles (Weight Percent)

<table>
<thead>
<tr>
<th></th>
<th>AO4 Linseed Oil</th>
<th>MaxEPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sat</td>
<td>21.0</td>
<td>31.6</td>
</tr>
<tr>
<td>Sum n-9</td>
<td>20.5</td>
<td>32.0</td>
</tr>
<tr>
<td>Sum n-7</td>
<td>2.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Sum n-6</td>
<td>49.3</td>
<td>9.2</td>
</tr>
<tr>
<td>Sum n-3</td>
<td>6.5</td>
<td>25.9</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>49.2</td>
<td>9.2</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>4.5</td>
<td>25.9</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.8</td>
<td>0.0</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>1.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Unsat/sat</td>
<td>3.8</td>
<td>2.2</td>
</tr>
<tr>
<td>DBI</td>
<td>147</td>
<td>129</td>
</tr>
<tr>
<td>PI</td>
<td>68</td>
<td>61</td>
</tr>
<tr>
<td>α-Tocopherol, mg/kg</td>
<td>30</td>
<td>310</td>
</tr>
<tr>
<td>γ-Tocopherol, mg/kg</td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>Cholesterol, mg/kg</td>
<td>0</td>
<td>250</td>
</tr>
</tbody>
</table>

AO4 was the basal chow diet. Sat indicates sum of the saturated fatty acids; Sum n-9, sum of the n-9 fatty acids; Sum n-7, sum of the n-7 fatty acids; Sum n-6, sum of the n-6 fatty acids; Sum n-3, sum of the n-3 fatty acids; and Unsat/sat, ratio of unsaturated to saturated fatty acids. DBI and PI were determined as defined in Methods. Only the polyunsaturated fatty acids that are relevant to the present study are presented.

(factors I, II, V, and X), whereas the PT (or Quick test) reveals disorders in the exogenous system.27

### TABLE 2. Effects of Dietary Lipids on Plasma Concentrations of Triglycerides, Cholesterol, Coagulation Factors, and Fibrinogen

<table>
<thead>
<tr>
<th></th>
<th>Linseed Oil Diet (LIN)</th>
<th>MaxEPA Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma triglycerides, mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 w</td>
<td>0.80±0.07</td>
<td>0.80±0.11</td>
</tr>
<tr>
<td>1 w</td>
<td>0.36±0.03†</td>
<td>0.35±0.04†</td>
</tr>
<tr>
<td>2 w</td>
<td>0.27±0.03†</td>
<td>0.34±0.04†</td>
</tr>
<tr>
<td>4 w</td>
<td>0.41±0.04†</td>
<td>0.31±0.05†</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 w</td>
<td>1.33±0.06</td>
<td>1.48±0.11</td>
</tr>
<tr>
<td>1 w</td>
<td>1.02±0.05†</td>
<td>0.84±0.04†</td>
</tr>
<tr>
<td>2 w</td>
<td>1.09±0.06*</td>
<td>1.03±0.04†</td>
</tr>
<tr>
<td>4 w</td>
<td>1.05±0.06*</td>
<td>1.06±0.10†</td>
</tr>
<tr>
<td>Plasma factor II, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 w</td>
<td>99.9±5.6</td>
<td>100.4±2.8</td>
</tr>
<tr>
<td>1 w</td>
<td>103.0±8.5</td>
<td>28.5±6.4†</td>
</tr>
<tr>
<td>2 w</td>
<td>102.0±7.2</td>
<td>24.7±9.8†</td>
</tr>
<tr>
<td>4 w</td>
<td>90.0±7.4</td>
<td>18.3±3.4†</td>
</tr>
<tr>
<td>Plasma factor VII-X, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 w</td>
<td>98.8±3.3</td>
<td>97.7±3.4</td>
</tr>
<tr>
<td>1 w</td>
<td>101.0±4.8</td>
<td>50.2±7.6†</td>
</tr>
<tr>
<td>2 w</td>
<td>103.5±6.4</td>
<td>50.1±13.0†</td>
</tr>
<tr>
<td>4 w</td>
<td>100.5±6.4</td>
<td>38.0±6.6†</td>
</tr>
<tr>
<td>Plasma factor V, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 w</td>
<td>96.0±7.2</td>
<td>100.3±3.8</td>
</tr>
<tr>
<td>1 w</td>
<td>109.4±8.3</td>
<td>98.9±3.2</td>
</tr>
<tr>
<td>2 w</td>
<td>101.4±4.6</td>
<td>110.4±5.2</td>
</tr>
<tr>
<td>4 w</td>
<td>99.4±4.0</td>
<td>90.2±3.8</td>
</tr>
<tr>
<td>Plasma fibrinogen, g/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 w</td>
<td>1.92±0.07</td>
<td>2.04±0.05</td>
</tr>
<tr>
<td>1 w</td>
<td>2.07±0.04</td>
<td>1.93±0.04</td>
</tr>
<tr>
<td>2 w</td>
<td>1.99±0.13</td>
<td>2.13±0.05</td>
</tr>
<tr>
<td>4 w</td>
<td>2.02±0.07</td>
<td>2.14±0.06</td>
</tr>
</tbody>
</table>

Results are means±SEM (n=8). Comparisons were made with the Dunnet test and significant differences are indicated with the control group (time 0 w). *P<0.05, †P<0.01.

(For the tables and figures, please refer to the original document.)
Coagulation
Fibrinogen was determined from the thrombin clotting time.30 The APTT and PT were measured as the clotting time after addition of cephalin, contact-phase activator, and calcium reagent (PTT-A, Stago) or of tissue factor (Neoplastine IS C10, Stago), respectively. Coagulant activities of factors II, V, and VII–X were determined in 1-stage clotting assays with human factor–deficient plasmas (Stago) or of tissue factor (Neoplastine IS C10, Stago), respectively. Fibrinogen was determined from the thrombin clotting time.30 The clotting time after addition of cephalin, contact-phase activator, and calcium reagent (PTT-A, Stago) or of tissue factor (Neoplastine IS C10, Stago), respectively. Coagulant activities of factors II, V, and VII–X were determined in 1-stage clotting assays with human factor–deficient plasmas (Stago) or of tissue factor (Neoplastine IS C10, Stago), respectively. Fibrinogen was determined from the thrombin clotting time.30

Lipid Peroxidation
Lipid peroxidation was estimated as thiobarbituric acid–reactive substances (TBARS) in liver homogenates (0.1 mL) after separation of the malonaldehyde–thiobarbituric acid complex by high-performance liquid chromatography with fluorescence detection.34

Statistical Analyses
One-way ANOVA was applied to the data, and Dunnett’s test was used to compare the means at each time point (1, 2, and 4 weeks) with those of the control animals (time = 0 weeks). The Mann-Whitney test was used to compare the means between dietary groups. Simple correlations between parameters were calculated by linear regression analysis.

Results
During the experiment, no significant differences were observed in the growth rates (≈33% after 4 weeks) between the 2 dietary groups.

Plasma Lipids
The effects of the 2 n-3 fatty acid–enriched diets on plasma triglycerides and cholesterol are shown in Table 2. Concentrations of triglycerides dropped significantly and similarly by ≈55% in the 2 dietary groups after the first week and thereafter remained nearly steady. In contrast, when choles-
Liver tocopherol also decreased significantly after 1 week, the decrease was about 2 times greater in the MaxEPA group than in the LIN group (43% vs 25%). Cholesterol subsequently tended to rise progressively, to reach ~75% of its initial level after 4 weeks in both dietary groups. Thus, without drawing conclusions as to their effects over a longer period of time, the 2 diets appeared in our experiments to similarly influence plasma lipids.

Blood Coagulation Factors
No significant changes in coagulation variables could be detected in the LIN group (Table 2). In the MaxEPA group, factor II and factor VII–X activities decreased significantly after 1 week (by ~70% and 50%, respectively) and remained stable thereafter (Table 2). In the same dietary group, APTT increased significantly by 47% (P<0.05) but only after 4 weeks, while no modifications were observed in fibrinogen or factor V (Table 2).

Fatty Acid Composition of Liver Phospholipids
The effects of the 2 diets on the fatty acid composition of liver phospholipids are shown in Table 3. The 2 control groups displayed similar fatty acid compositions except for 20:4n-6, which had levels 24% lower in the LIN oil group than in the MaxEPA group. The changes in liver phospholipid fatty acids were similar to those described for total liver fatty acids, which had levels 24% lower in the LIN group than in the MaxEPA group. It is noteworthy of note that in contrast to linseed oil, fish oil was able to induce time-course changes in both 22:6n-3 and 20:5n-3, comparable to those of the vitamin K–dependent coagulation factors. In both groups, the decrease in n-6 fatty acids resulted in similar low levels of arachidonic acid, but linoleic acid significantly diminished in the MaxEPA group only. The DBI and PI increased significantly during the experiment and reached comparable values after 4 weeks in the 2 dietary groups. These parameters would therefore seem to be more dependent on dietary n-3 fatty acids than on other unsaturated fatty acids.

Liver Tocopherols
Despite similar intakes, liver α-tocopherol was about twice as high in the LIN group as in the MaxEPA group from the first to the fourth week (Table 4), probably due to a higher rate of antioxidant utilization in the MaxEPA group. Although the diets contained different amounts of γ-tocopherol (Table 1), its levels remained low throughout the experiment (1 to 2 nmol/g of liver; data not shown). In contrast, liver tocopherolquinone followed a parallel time course in the 2 dietary groups, with an increase of ~80% at 1 and 2 weeks and 160% to 200% at 4 weeks (Table 4). This similar tocopherolquinone production is consistent with the comparable enrichment of liver membranes in polyunsaturated fatty acids.

Lipid Peroxidation
The changes in liver TBARS in the LIN and MaxEPA groups are shown in Table 4. At 1 and 2 weeks, TBARS were, respectively, 1.5 and 2 times higher in the MaxEPA group than in the LIN group (P<0.01). At 4 weeks, TBARS were 4 to 5 times greater than at time 0 (P<0.01) and 25% higher in the LIN group than in the MaxEPA group (P<0.01).

Tocopherolquinone Treatment
Administration of tocopherolquinone (20 mg) to rats by gavage for 4 days did not affect PT but did prolong APTT by ~24% (the Figure, panel A). Factor II and factor VII–X...
activities decreased by ≈34% and 24%, respectively, in the treated group compared with the control group, whereas factor V activity and plasma fibrinogen remained unchanged (data not shown). At the end of treatment, very high levels of both the oxidized and reduced forms of tocopherolquinone were found in the liver, whereas α-tocopherol levels were similar in the control and treated groups (the Figure, panel B). Thus, despite an 8-fold higher concentration of tocopherolquinone in these rats compared with those that were fed diets rich in n-3 fatty acids, the effect was half that observed after fish oil feeding. Previous experiments with 10 mg tocopherolquinone instead of 20 mg failed to show significant differences in the activities of coagulation factors (data not shown).

Discussion

A range of physiological effects have been ascribed to n-3 polyunsaturated fatty acids, in particular, to those found in marine oils. Fish oil is known to lower plasma triglycerides, whereas its influence on cholesterol levels is far less well characterized. Hemostasias was at the origin of the physiological investigations of n-3 fatty acids conducted in the early 1970s, although the effects described were mainly discovered at the level of platelet functions.

More recently, various dietary intervention studies in humans revealed either no change or a small decrease in the activity of clotting factors. In the rat, coagulation assays revealed transient hypocoagulation after feeding for 13 weeks with fish oil or for 2 weeks with DHA. The vitamin K–dependent coagulation factors II and VII–X were found to be strongly depressed after 6 to 10 weeks of feeding with fish oil, but no precise mechanism could be defined.

In accordance with previous reports, a diet rich in 18:3n-3 or long-chain fatty acids decreased plasma cholesterol and triglyceride levels. It is worthy of note that these changes followed a similar time course for diets enriched in linseed or fish oil. The effects of fish oil on coagulation factors II and VII–X were already clearly apparent after 1 week of feeding and only slightly more pronounced thereafter, whereas PT and APTT were little affected. A comparable lack of sensitivity of these coagulation tests has been reported in other experiments on rats. In contrast to fish oil, linseed oil had no influence on blood coagulation, and even a diet containing 58% 18:3n-3 in its lipid component (pure linseed oil) had no effect after 4 weeks (data not shown). These results indicate that dietary 18:3n-3 and long-chain fatty acids act differently on the activity of vitamin K–dependent clotting factors in the rat.

As expected from previous nutritional studies in animals and humans, and from human nutrition studies, the liver α-tocopherol content of rats fed fish oil was half that of rats fed linseed oil, despite similar dietary intakes. This could reflect the larger amounts of α-tocopherol required to protect cellular membranes enriched in fatty acids with a higher DHQ. Because the intake of γ-tocopherol was greater in the LIN than in the MaxEPA diet, an influence of this compound on liver peroxidation status and α-tocopherol content cannot be entirely excluded. However, its effect should be marginal, because similar low γ-tocopherol levels were observed in the 2 dietary groups at each sampling time. These conclusions are supported by a report that the in vivo antioxidant activity of γ-tocopherol is ≈30% that of α-tocopherol. The point merits further investigation in view of the high efficiency of γ-tocopherol in protecting lipids against nitric oxide–initiated peroxidation. Nevertheless, if it is largely accepted that the tocopherol content of membranes determines their susceptibility to damage by peroxidizing agents, then the relationships between this content and the intensity of oxidative stress are not yet clearly established. Thus, recent studies have shown that tocopherol concentrations in various tissues are somewhat dependent on the type of dietary fat and that tocopherol consumption may take place before any lipid peroxidation.

As in earlier studies, we found that feeding with n-3 fatty acids was associated with a net increase in lipid peroxidation, as indicated by a rise in TBARS values after 1 week. The peroxidation status of the liver was more strongly affected in rats fed fish oil than in those fed linseed oil during the first 2 weeks, whereas linseed oil induced the highest levels of TBARS after 4 weeks (P < 0.005). Unexpectedly, and unrelated to their α-tocopherol contents, consumption of either of the 2 oils led to similar levels of tocopherolquinone. This α-tocopherol metabolite may nevertheless be considered a valid indicator of liver lipid peroxidation, because in our experiments its concentration was strongly correlated with concentrations of TBARS (r = 0.45, P = 0.003, n = 64) and the lipid PI (r = 0.43, P = 0.015, n = 32).

Previous studies on various supplies of dietary fatty acids have pointed to a close relationship between tocopherols and long-chain n-3 fatty acids. Thus, it was demonstrated that more tissue α-tocopherol was required to protect rat liver membranes when they were enriched in n-3 fatty acids and that the antioxidant efficiency was lower for n-3 than for n-6 fatty acids in monkey liver. In our in vivo experiments, production of tocopherolquinone was likewise strongly correlated with the sum of liver long-chain n-3 fatty acids, not only in each dietary group but also in all pooled animals (r = 0.57, P = 0.001, n = 32). A similar highly significant correlation was observed between levels of TBARS and the sum of n-3 fatty acids. The unexpected comparable production of tocopherolquinone in the 2 dietary groups was therefore probably linked to comparable antioxidant mechanisms in liver membranes having similar n-3 fatty acid contents. In fact, the LIN diet enriched liver membranes mainly in EPA without altering their DHA content, whereas the MaxEPA diet markedly increased the concentrations of both fatty acids. Similar results have been previously reported in rats and were attributed to specific fatty acid metabolism based on the high affinity of Δ6-desaturase for linolenic acid, together with poor conversion of EPA to DHA and efficient acylation of preformed EPA and DHA in hepatic phospholipids. Consistent with earlier reports, we found that feeding of n-3 fatty acids led to a global reduction of arachidonic acid in liver phospholipids whatever the diet, except for a transient rise observed in rats fed the linseed oil after 1 week. In the absence of comparable literature data, no metabolic explanation can yet be proposed for this isolated observation, which deserves further investigation.

One current hypothesis of the mechanism of action of n-3 fatty acids on vitamin K–dependent clotting factors is based on experiments in which tocopherolquinone was added to liver microsome preparations. In rats fed fish oil, the
activity of vitamin K–dependent coagulation factor II was indeed significantly correlated with liver tocopherolquinone ($r = -0.59, P=0.0005, n=32$). This correlation is consistent with the simultaneous increase in lipid peroxidation and decrease in blood coagulability. However, no such correlation could be detected in rats fed linseed oil. Thus, a somewhat unexpected finding was that although the diet rich in linolenic acid increased lipid peroxidation and induced a concomitant generation of tocopherolquinone, it did not alter plasma coagulation factors. Moreover, when tocopherolquinone was administered to rats by gavage, it had minimal inhibitory effects on blood coagulation, despite liver levels of tocopherolquinone that were 8 times higher than those measured in fish oil–fed rats. Therefore, even if this pharmacological property might result from partial inhibition of γ-glutamylcarboxylase as observed in experiments with rat liver microsomes, it cannot account for the dietary properties of the n-3 fatty acids of fish oil.

The observation of rapid restoration of factor II and factor VII–X activities in MaxEPA-treated rats after injection of vitamin K points to a close interaction between vitamin K and carboxylase in the regulatory mechanism. A recent demonstration that the vitamin K cycle can act as a potent antioxidant system and that microsomal vitamin K epoxide reductase is strongly inhibited by membrane lipid peroxidation further highlights the complexity of the possible connections between lipid peroxidation and blood coagulation. Thus, an increase in fatty acid hydroperoxide production and simultaneous enrichment of liver membranes in DHA, as found in fish oil–fed rats, might contribute to retard the vitamin K cycle and hence, lower the levels of circulating coagulation factors. The mechanism underlying the involvement of DHA is far from clear, although this fatty acid has been reported to have specific effects on aortic prostacyclin production or in hepatic cholesterol metabolism.

In conclusion, dietary supplementation with fish oil is generally regarded as having several beneficial effects on cardiovascular disease in humans, and various potential mechanisms could be evaluated in a rat model. Dietary experiments enabled us to demonstrate that the anticoagulant activity of n-3 fatty acids thought to be mediated by inhibition of vitamin K–dependent coagulation factors is specific to the long-chain components and is not shared by the linolenic acid present in some vegetable oils. It is possible that high oxidative stress and enrichment of liver membranes in DHA, such as that observed with fish oil diets, specifically inhibits γ-glutamylcarboxylase and consequently lowers vitamin K–dependent clotting factors. Worthy of consideration for future studies are questions relating to the modulation of liver γ-glutamylcarboxylase activity by dietary lipids, lipid peroxides, and antioxidants.

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References


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