Associations of Fish Intake and Dietary n-3 Polyunsaturated Fatty Acids With a Hypocoagulable Profile

The Atherosclerosis Risk in Communities (ARIC) Study

Eyal Shahar, Aaron R. Folsom, Kenneth K. Wu, Barbara H. Dennis, Tomoko Shimakawa, Maureen G. Conlan, C.E. Davis, and O. Dale Williams, for the ARIC Study Investigators

Recent epidemiological evidence indicates that the hemostatic profile is an important predictor of cardiovascular disease, yet its dietary determinants are not well established. An important question is whether dietary fatty acid intake influences blood levels of coagulation proteins. We examined potential dietary determinants of six hemostatic factors—fibrinogen, factor VII, factor VIII, von Willebrand factor (vWF), protein C, and antithrombin III—in four population-based samples totaling over 15,000 participants, blacks and whites, in the Atherosclerosis Risk in Communities (ARIC) Study. Usual dietary intake was assessed by a food frequency questionnaire. Cross-sectional associations were explored using multiple linear regression analysis, adjusting for gender, race, age, body mass index, smoking status, alcohol use, diabetes, and field center. Dietary intake of n-3 polyunsaturated fatty acids (PUFAs) showed negative associations with fibrinogen, factor VIII, and vWF (blacks and whites) and a positive association with protein C (whites only). Fish intake, the major source of dietary n-3 PUFAs, was similarly related to the hemostatic profile: a 1 serving per day greater fish intake was associated with the following predicted differences (95% confidence interval): fibrinogen, −2.9 mg/dL (−6.3, 0.5); factor VIII, −3.3% (−5.4, −1.3); vWF, −2.7% (−5.2, −0.1) (blacks and whites); and protein C, +0.07 μg/mL (0.03, 0.11) (whites only). Other nutrients or foods were variably associated with the hemostatic factors. These population-based associations, although cross-sectional, suggest that increases in n-3 PUFA intake from fish may modify the blood levels of several coagulation factors. (Arteriosclerosis and Thrombosis 1993;13:1205-1212)

KEY WORDS • blood coagulation factors • fibrinogen • factor VIII • von Willebrand factor • protein C

Recent epidemiological evidence indicates that hemostatic factors play an important role in cardiovascular disease. Two coagulation proteins, fibrinogen and factor VII, have been shown in prospective studies to be independent predictors of cardiovascular events.1-3 The contribution of other coagulation factors such as factor VIII, antithrombin III (AT-III), and protein C to cardiovascular disease has not been established prospectively but is quite plausible in view of their well-known roles in thrombogenesis.

Dietary determinants of the hemostatic profile have attracted scientific attention over the last decade, with specific interest in the possible influence of various forms of fat intake.4 It was hypothesized that the reduced platelet aggregability in Greenland Eskimos might be attributed to their unique diet, which is largely composed of marine fish rich in n-3 polyunsaturated fatty acids (PUFAs).5 Different levels of coagulation proteins across population samples of Japanese and Caucasians may be related to dietary habits that presumably differ in quantity and type of fat consumption.6 Vegetarians, whose total fat intake is low, also reportedly have alterations of the hemostatic profile.7 Several prospective studies have suggested that fish intake is inversely associated with coronary heart disease.8-11 Inspired by these observations, numerous studies have examined the effect of fish oil supplements on a variety of blood constituents. Effects on platelet function are well established.12 Effects on the coagulation system, principally fibrinogen and AT-III, however, have been inconsistent. Several investigators reported a lowering of fibrinogen with fish oil supplementation,13-20 but others did not corroborate this finding.21-28 Conflicting results have also been reported for AT-III, ranging from a lowering21,28,29 to an elevating22,26 to no effect at all.15,16 Few data are available on other important hemostatic factors. In addition, inferences from these supplementation studies to the usual diet of populations are limited, since the vast majority of such studies have

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used supplemental doses that exceeded by several fold the typical n-3 PUFA content of the Western diet. Only a few attempts have been made to explore the associations between Western dietary habits and hemostatic factors at the population level.\textsuperscript{31-33} We had the opportunity to pursue these issues in a large population-based sample of participants in the Atherosclerosis Risk in Communities (ARIC) Study.

Methods

Study Design

The overall design and operational characteristics of the ARIC Study, which is a longitudinal investigation of the etiology and natural history of atherosclerosis, were described earlier.\textsuperscript{34} The ARIC Study includes two components: a community surveillance component, monitoring the occurrence of hospitalized myocardial infarction and coronary heart disease death in four US communities, and a longitudinal cohort component established in these communities.

Cohort Component

Cohorts of about 4000 persons aged 45 to 64 years and including both men and women were recruited between 1986 and 1989 in four US communities: Forsyth Co, NC; Jackson, Miss; a collection of suburbs of Minneapolis, Minn; and Washington Co, Md. Each cohort was a probability sample of age-eligible residents of the local community, except that only black subjects were eligible for selection in Jackson, Miss.

This component of the study involves repeated comprehensive clinic examinations of the members of the cohort at 3-year intervals. The baseline evaluation undertaken between 1986 and 1989 included medical history, dietary questionnaire, anthropometric and blood pressure measurements, blood sampling for lipid and hemostatic profiles, pulmonary function studies, and B-mode ultrasound scanning of the carotid arteries. Cross-sectional data from that baseline examination were used for the present analysis.

Assessment of Diet

Usual dietary habits, defined as the average intake over the last year, were estimated in ARIC by a semiquantitative food-frequency questionnaire, a modified version of a 61-item instrument developed and validated by Willett et al.\textsuperscript{35} Four principal modifications were made: (1) A few items were split into detailed subcategories, for example, a single question addressing fish consumption was separated into three specific fish servings of the food items within the group were summed, regardless of differences in portion sizes among component foods. Alcoholic beverage consumption was evaluated by asking participants to report their average weekly servings of wine, beer, and hard liquor.

Daily nutrient intake was calculated by multiplying the nutrient content of the specified portion of each food item by the frequency of its daily consumption and summing over all items. Nutrient values for each item were obtained from a nutrient database.\textsuperscript{36} Nutrient values for total fat were obtained by summing the values of animal fat and vegetable fat.

Hemostatic Factor Measurements

Participants were asked to fast for 12 hours before the clinic visit. A blood sample was drawn from an antecubital vein with minimal stasis. The specimen for analysis of hemostatic factors was collected into 4.5-mL vacuum tubes containing 1/10th volume of 3.8\% sodium citrate. After centrifugation at the field center laboratory at 3000g for 10 minutes at 4°C, supernatant aliquots were stored at -70°C until final analysis. Samples were shipped to the Central Hemostasis Laboratory at the University of Texas at Houston, where they were assayed in a blinded fashion within 2 weeks of blood collection. Detailed descriptions of the blood collection and processing techniques have been published.\textsuperscript{37}

Six hemostatic factors—fibrinogen, factor VII, factor VIII, von Willebrand factor (vWF), protein C, and AT-III—were measured. Plasma fibrinogen level was measured by the thrombin time titration method described by Clauss.\textsuperscript{38} Reagents for the fibrinogen assay were obtained from General Diagnostics (Organon-Teknika Co, Morris Plains, NJ). Factor VII and factor VIII coagulant activity were measured by determining the ability of the testing sample to correct the clotting time of human factor VII- or factor VIII-deficient plasma obtained from George King Biomedical, Inc (Overland Park, Kan). The plasma levels were then expressed as percent activity by relating the clotting time to a calibration curve constructed for each batch of samples. vWF and protein C were measured by enzyme-linked immunosorbent assays using kits from American Bioproducts ( Parsippany, NJ). AT-III activity was measured by the chromogenic substrate method.\textsuperscript{39} Different batches of Universal Coagulation Reference (Thrombost, Pacific Hemostasis) were used for calibration and quality control of hemostasis assays. Reliability coefficients estimated from split specimens sent 1 week apart to the laboratory were AT-III, 0.42; protein C, 0.56; vWF, 0.68; fibrinogen, 0.72; factor VII, 0.78; and factor VIII, 0.86.\textsuperscript{39} These coefficients are generally higher than those reported in other studies.\textsuperscript{39}

Other Measurements

Body mass index was computed from height and weight (in kilograms per meter squared). Height was measured to the nearest centimeter using a vertical metal rule. Weight was measured on a calibrated scale
levels of hemostatic factors were compared across quartiles of nutrient or food intake.

Initially, energy intake was considered as a potential confounder in this analysis. Univariately, total energy intake was inversely related to most of the hemostatic factors. However, when these relationships were adjusted for the predetermined covariates, only a weak association \((P<.05)\) of calories with \(\text{vWf} \) remained. We concluded that energy intake was not an independent predictor of the hemostatic factors in question, and its potentially confounding effect was properly taken into account by including other covariates in the models.

The associations between nutrients or foods and the hemostatic factors may differ across race and/or gender strata. To investigate such a possible effect modification, two-way interaction terms involving race or gender were tested in the linear regression models. Whenever a significant \((P<.05)\) interaction was found, race (or gender)-specific associations were examined and only those that were statistically significant \((P<.05)\) are reported. SAS was used for computations.\(^{42}\)

**Results**

The distribution of dietary intake of selected nutrients among the study sample subjects is shown in Table 1. All of the distributions were skewed to the right to some extent. As expected, the contribution of n-3 PUFAs to total fat intake was very small: eicosapentaenoic acid (EPA, 20:5), docosahexaenoic acid (DHA, 22:6), and docosapentaenoic acid (DPA, 22:5) together accounted for only 0.5% of total fat intake. The Pearson correlation coefficients between these three fatty acids ranged from 0.87 (DHA vs DPA) to 0.97 (DHA vs EPA).

Table 2 shows the distribution of fish and other seafood intake. The average consumption of any fish item was only 2 servings per week. Dark meat fish, particularly rich in n-3 PUFAs,\(^{43}\) accounted for less than 25% of total fish intake. Mean±SD levels of the six

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<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean ± SD</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat</td>
<td>60.0 ± 18.0</td>
<td>40.3</td>
<td>55.5</td>
<td>74.3</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>22.0 ± 5.0</td>
<td>14.4</td>
<td>20.2</td>
<td>27.3</td>
</tr>
<tr>
<td>Monounsaturated fat</td>
<td>23.1 ± 5.0</td>
<td>15.2</td>
<td>21.3</td>
<td>28.9</td>
</tr>
<tr>
<td>Polyunsaturated fat</td>
<td>9.0 ± 2.0</td>
<td>5.9</td>
<td>8.2</td>
<td>11.2</td>
</tr>
<tr>
<td>Animal fat</td>
<td>36.2 ± 6.0</td>
<td>23.2</td>
<td>33.4</td>
<td>45.6</td>
</tr>
<tr>
<td>n-3 Fatty acids</td>
<td>0.30 ± 0.1</td>
<td>0.12</td>
<td>0.22</td>
<td>0.39</td>
</tr>
<tr>
<td>Eicosapentaenoic acid</td>
<td>0.09 ± 0.05</td>
<td>0.03</td>
<td>0.07</td>
<td>0.12</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>0.18 ± 0.05</td>
<td>0.08</td>
<td>0.13</td>
<td>0.23</td>
</tr>
<tr>
<td>Docosapentaenoic acid</td>
<td>0.03 ± 0.02</td>
<td>0.01</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.253 ± 0.11</td>
<td>0.162</td>
<td>0.228</td>
<td>0.314</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>17.2 ± 6.0</td>
<td>11.4</td>
<td>15.9</td>
<td>21.3</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>4.3 ± 1.0</td>
<td>2.8</td>
<td>3.9</td>
<td>5.3</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>195.4 ± 51.0</td>
<td>135.3</td>
<td>181.5</td>
<td>239.5</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.288 ± 0.12</td>
<td>0.048</td>
<td>0.168</td>
<td>0.380</td>
</tr>
<tr>
<td>Alcohol</td>
<td>5.6 ± 1.0</td>
<td>0.0</td>
<td>0.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Total energy (kcal/d)</td>
<td>1610 ± 520</td>
<td>1175</td>
<td>1517</td>
<td>1938</td>
</tr>
</tbody>
</table>

**TABLE 1.** Means and Percentiles for Estimated Dietary Intake of Selected Nutrients Among 14 571 Participants in the ARIC Study, 1986-1989

ARIC, Atherosclerosis Risk in Communities. Values are given in grams per day.

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**Statistical Methods**

The cohort that completed the baseline evaluation included 15 800 participants. This report is limited to participants who reported their race as either white or black. We excluded from the analysis 835 participants who reported that they were currently taking "blood thinning" medications. To improve the validity of the dietary data, we excluded 329 subjects whose daily energy intake was below the first percentile or above the 99th percentile of the sex-specific distribution, on the assumption that the information provided was less valid. These values were 591 and 4173 kcal/d for men and 488 and 3608 kcal/d for women. We also excluded a few subjects with 10 or more missing responses on the questionnaire. These exclusions left 14 571 subjects with data available for this report. We did not exclude aspirin users because hemostatic factor levels were unrelated to aspirin usage.

Nutrient and food intake was described by computing means and percentiles for the final sample. Potential associations between nutrient or food intake and hemostatic factors were explored using linear regression models with the hemostatic factor as the dependent variable and the nutrient or food as a predictor. Each model included the following predetermined list of covariates: age, gender, race, body mass index, smoking status, alcohol use, diabetes, and field center. All are known to be associated with the hemostatic profile in the ARIC Study,\(^{40,41}\) and their relation to dietary habits is very plausible. The data were also analyzed by analysis of covariance. In this approach, adjusted mean

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TABLE 2. Means and Percentiles for Dietary Fish and Other Seafood Intake Among 14,571 Participants in the ARIC Study, 1986-1989

<table>
<thead>
<tr>
<th>Food item (serving size)</th>
<th>Mean 25th 50th 75th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any fish</td>
<td>2.0  0.7  1.4  2.4</td>
</tr>
<tr>
<td>Canned tuna fish (3-4 oz)</td>
<td>0.9  0.0  0.5  1.0</td>
</tr>
<tr>
<td>Dark meat fish (3-5 oz)*</td>
<td>0.4  0.0  0.0  0.5</td>
</tr>
<tr>
<td>Other fish (3-5 oz)†</td>
<td>0.3  0.0  0.5  1.0</td>
</tr>
<tr>
<td>Shrimp, lobster, scallops</td>
<td>0.3  0.0  0.0  0.5</td>
</tr>
</tbody>
</table>

ARIC, Atherosclerosis Risk in Communities. Values are given in servings per week.
*For example, salmon, mackerel, swordfish, sardines, bluefish.
†For example, cod, perch, catfish.

hemostatic factors were fibrinogen, 303±65 mg/dL; factor VII, 119±29%; factor VIII, 131±39%; AT-III, 111±22%; protein C, 3.17±0.62 μg/mL; and vWF, 118±48%.

Three n-3 PUFA, namely EPA, DHA, and DPA, showed negative associations with plasma levels of fibrinogen, factor VIII, and vWF and positive associations with protein C level (Table 3). These relationships were almost always consistent in direction and usually consistent in magnitude across the four race and sex strata. However, the associations of EPA and DHA with protein C were limited to whites. There was no association of n-3 PUFA with these factors were strong but should be interpreted cautiously in light of the extremely low consumption of DPA among the study population. This limitation of DPA is reflected in the wide confidence limits of the predicted differences. Linoleic acid (18:3), the parent compound of n-3 PUFAs, was essentially unrelated to the hemostatic factors (data not shown).

Since fish is the major source of dietary n-3 PUFA, we further explored the relationships at the food-item level. Table 4 shows the predicted differences in fibrinogen, factor VIII, vWF, and protein C levels associated with a 1 serving per day greater intake of different fish items. The findings paralleled those for the nutrient data, but most associations (P<.05) were limited to the “other fish” item and the “any fish” variable. As before, the relationships were almost always consistent across the four race and sex strata, although the relations with protein C were again restricted to whites.

In another analytic approach, participants were categorized according to quartiles of fish consumption. Adjusted mean levels of the hemostatic factors were computed for each quartile from an analysis of covariance model. Although a graded relationship was not always apparent, participants in the upper consumption quartile had the lowest levels of fibrinogen, factor VIII, and vWF and the highest protein C level (Figure).

Arachidonic acid (20:4), an n-6 PUFA whose physiology and metabolism sometimes compete with those of the n-3 PUFAs, was not associated with any hemostatic factor (data not shown). Likewise, neither total fat, total saturated fatty acids, nor total monounsaturated fatty acids were associated (P<.05) with any hemostatic factor in the study sample. However, among white men (n=4834) total fat intake as well as saturated fatty acid and total energy intake were positively albeit weakly related to factor VII. A 10-g/d greater fat intake predicted a 0.3% higher factor VII level (95% confidence interval, 0.1% to 0.5%).

Associations of several other nutrients with hemostatic factors are presented in Table 5. Statistically significant (P<.05) associations are identified. Cholesterol intake was positively associated with fibrinogen, factor VII, factor VIII, and vWF, although most of these associations were restricted to men. Animal fat intake showed a similar pattern. The data suggested modest inverse relationships of crude fiber intake with factor VII and factor VIII levels. Similar associations with factor VII and factor VIII were found for total fiber intake and consumption of all fruits and all vegetables (data not shown).

Caffeine was inversely related to factor VII and factor VIII (blacks and whites) and to vWF (whites only). Alcohol intake was negatively associated with plasma fibrinogen in men and women and with factor VIII and vWF in women only (Table 5). Of note, ARIC data did not suggest any important dietary determinants of AT-III.

Table 3. Predicted Differences and 95% Confidence Intervals* in Hemostatic Factor Levels Associated With a 100-mg Greater Daily Intake of n-3 Polyunsaturated Fatty Acids

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Hemostatic factor</th>
<th>Fibrinogen (mg/dL)</th>
<th>Factor VII (%)</th>
<th>Factor VIII (%)</th>
<th>von Willebrand factor (%)</th>
<th>Protein C (μg/mL)</th>
<th>Antithrombin III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eicosapentaenoic acid</td>
<td>-1.1 (-2.2, -0.1)†</td>
<td>0.2 (-0.3, 0.7)</td>
<td>-0.9 (-1.6, -0.3)†</td>
<td>-0.9 (-1.7, -0.1)†</td>
<td>0.02 (0.01, 0.04)††</td>
<td>0.0 (-0.4, 0.4)</td>
<td></td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>-0.6 (-1.2, 0.0)</td>
<td>0.1 (-0.2, 0.4)</td>
<td>-0.6 (-0.9, -0.2)†</td>
<td>-0.5 (-0.9, 0.0)</td>
<td>0.01 (0.01, 0.02)††</td>
<td>0.0 (-0.2, 0.2)</td>
<td></td>
</tr>
<tr>
<td>Docosapentaenoic acid</td>
<td>-3.9 (-7.6, -0.2)†</td>
<td>0.5 (-1.2, 2.2)</td>
<td>-3.6 (-5.8, -1.4)†</td>
<td>-3.7 (-6.4, -0.9)†</td>
<td>0.05 (0.02, 0.09)†</td>
<td>-0.4 (-1.7, 0.9)</td>
<td></td>
</tr>
</tbody>
</table>

*From linear regression adjusted for age, gender, race, body mass index, smoking status, alcohol use, diabetes, and field center.
†P<.05.
‡Whites only.
Table 4. Predicted Differences and 95% Confidence Intervals* in Hemostatic Factor Levels Associated With a 1-Serving Greater Daily Intake of Fish and Other Seafood

<table>
<thead>
<tr>
<th>Food item</th>
<th>Fibrinogen (mg/dL)</th>
<th>Factor VII (%)</th>
<th>Factor VIII (%)</th>
<th>von Willebrand factor (%)</th>
<th>Protein C (µg/mL)</th>
<th>Antithrombin III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any fish</td>
<td>-2.9 (-6.3, 0.5)</td>
<td>0.5 (-1.1, 2.0)</td>
<td>-3.3 (-5.4, -1.3)</td>
<td>-2.7 (-5.2, -0.1)†</td>
<td>0.07 (0.03, 0.11)‡</td>
<td>-0.3 (-1.4, 0.9)</td>
</tr>
<tr>
<td>Canned tuna fish (3-4 oz)</td>
<td>-1.3 (-6.7, 4.0)</td>
<td>0.2 (-2.3, 2.7)</td>
<td>-3.0 (-6.2, 0.2)</td>
<td>-0.8 (-4.8, 3.2)</td>
<td>0.04 (-0.01, 0.10)</td>
<td>-0.5 (-2.3, 1.4)</td>
</tr>
<tr>
<td>Dark meat fish (3-5 oz)</td>
<td>-9.2 (-18.7, 0.3)</td>
<td>1.6 (-2.7, 5.9)</td>
<td>-3.4 (-9.1, 2.4)</td>
<td>-5.7 (-12.8, 1.4)</td>
<td>0.19 (0.07, 0.31)‡</td>
<td>1.7 (-1.5, 5.0)</td>
</tr>
<tr>
<td>Other fish (3-5 oz)</td>
<td>-5.1 (-12.2, 2.0)</td>
<td>0.6 (-2.7, 3.9)</td>
<td>-7.1 (-11.4, -2.8)†</td>
<td>-6.9 (-12.3, -1.6)†</td>
<td>0.08 (0.01, 0.16)‡</td>
<td>-1.3 (-3.8, 1.2)</td>
</tr>
<tr>
<td>Shrimp, lobster, scallops</td>
<td>-6.4 (-20.3, 7.4)</td>
<td>1.7 (-4.5, 7.9)</td>
<td>-4.7 (-13.0, 3.6)</td>
<td>-4.5 (-14.9, 5.9)</td>
<td>-0.11 (-0.25, 0.02)</td>
<td>-4.4 (-9.1, 0.3)</td>
</tr>
</tbody>
</table>

*From linear regression adjusted for age, gender, race, body mass index, smoking status, alcohol use, diabetes, and field center. ‡P<.05.

Table data reported here provide insights into these relationships at the population level. Dietary n-3 PUFAs, derived primarily from fish, were found to be negatively associated with the three hemostatic factors fibrinogen, factor VIII, and vWF. They were positively associated with the coagulation inhibitor protein C, although the clinical implication of this finding is uncertain. Heterozygous protein C deficiency did not seem to confer an increased risk of venous thrombosis, and the role of protein C in arterial thrombosis is controversial. Overall, the observed relationships with the four factors suggest an association of these fatty acids with reduced blood coagulability. The inverse relationship with fibrinogen, albeit weak, could be considered favorable with respect to the risk of cardiovascular disease.

Numerous human feeding studies but not all have reported a lowering of plasma fibrinogen after supplementation of the diet with various doses of fish oil. A dose-response relation has also been suggested. Few feeding studies have examined the effect of n-3 PUFAs on vWF or factor VIII. Lowering of the vWF level was reported in two trials; a single study reported lowering of the factor VIII level. To our knowledge, this is the first report corroborating these associations within a population-based sample consuming a Western diet.

A positive association of n-3 PUFA intake with protein C has not been reported previously. Three feeding studies did not find any effect, and another suggested that EPA and DHA feeding lowered protein C level. The associations in this study were statistically significant but were limited to whites. We suspect that the race difference may be due to the smaller sample size in blacks. Reanalysis by the four

Adjusted mean (SE) hemostatic factor levels according to quartile of fish consumption. Mean levels were adjusted for gender, race, age, body mass index, smoking status, alcohol use, diabetes, and field center using analysis of covariance. Protein C results are for whites only.
TABLE 5. Predicted Differences and 95% Confidence Intervals* in Hemostatic Factor Levels Associated With a Specified Unit Greater Daily Intake of Various Nutrients

<table>
<thead>
<tr>
<th>Nutrient (unit)</th>
<th>Fibrinogen (mg/dL)</th>
<th>Factor VII (%)</th>
<th>Factor VIII (%)</th>
<th>von Willebrand factor (%)</th>
<th>Protein C (µg/mL)</th>
<th>Antithrombin III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (100 mg)</td>
<td>0.9 (0.1, 1.7)†</td>
<td>0.7 (0.2, 1.1)††</td>
<td>0.6 (0.0, 1.2)††</td>
<td>0.9 (0.2, 1.7)††</td>
<td>0.00 (-0.01, 0.01)</td>
<td>0.1 (-0.2, 0.3)</td>
</tr>
<tr>
<td>Animal fat (10 g)</td>
<td>0.7 (0.1, 1.4)§§</td>
<td>0.4 (0.1, 0.7)††</td>
<td>0.1 (-0.3, 0.4)</td>
<td>0.8 (0.2, 1.3)††</td>
<td>0.00 (-0.01, 0.01)</td>
<td>0.1 (-0.1, 0.3)</td>
</tr>
<tr>
<td>Crude fiber (1 g)</td>
<td>0.0 (-0.5, 0.5)</td>
<td>-0.3 (-0.6, -0.1)†</td>
<td>-0.5 (-0.7, -0.2)†</td>
<td>-0.1 (-0.4, 0.3)</td>
<td>0.00 (-0.01, 0.00)</td>
<td>-0.1 (-0.3, 0.0)</td>
</tr>
<tr>
<td>Caffeine (100 mg)</td>
<td>0.3 (-0.1, 0.7)</td>
<td>-0.2 (-0.4, -0.1)†</td>
<td>-0.4 (-0.7, -0.2)†</td>
<td>-0.6 (-0.9, -0.3)§§</td>
<td>0.00 (-0.01, 0.00)</td>
<td>0.1 (-0.1, 0.2)</td>
</tr>
<tr>
<td>Alcohol (1 g)</td>
<td>-2.5 (-3.3, -1.6)†</td>
<td>0.2 (-0.2, 0.5)</td>
<td>-2.8 (-4.1, -1.6)††</td>
<td>-4.1 (-5.6, -2.5)††</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.0 (0.0, 0.0)</td>
</tr>
</tbody>
</table>

*From linear regression adjusted for age, gender, race, body mass index, smoking status, alcohol use, diabetes, and field center. Alcohol use was not included as a covariate in models for alcohol intake. Gender (or race)-specific models did not include the gender (or race) variable.
†P<.05.
§Men only.
§§Whites only.
||Women only.

race and sex strata revealed that the associations were similar for white men, white women, and black women but not for black men. The latter group contained by far the smallest number of participants (n=1501).

Intake of fish, particularly dark meat fish, is the main source of dietary n-3 PUFAs and is strongly correlated with plasma levels of EPA and DHA. Dietary n-3 PUFAs, as estimated by food-frequency questionnaires similar to the ARIC dietary questionnaire, also correlate with the levels of these fatty acids in plasma phospholipids. Not surprisingly, the relations with hemostatic factors were consistent for both fish consumption and n-3 PUFA intake. Statistical significance (P<.05) for the most part, however, was limited to the “other fish” and “any fish” variables rather than to the “dark meat fish” item. Although “dark meat fish” are rich in n-3 PUFAs, participants reported to have consumed them far less frequently than “other fish” (Table 2). The contribution of the “other fish” category to total EPA, DHA, and DPA intake was as great as that of “dark meat fish” (about 30%) and was particularly substantial to DPA intake (about 50%). A relatively small contribution of “other seafood,” also known to be rich in n-3 PUFAs, to n-3 PUFA intake might explain the lack of any significant association of this item with the hemostatic factors.

ARIC data did not suggest any relation between dietary n-3 PUFAs and AT-III. Although Eskimos, who consume large amounts of EPA and DHA, reportedly have varied results of human feeding studies have varied. A rather large variability in the laboratory measurement of AT-III may account for the inconsistencies. The chromogenic substrate method that was used to measure AT-III in this and many other studies did not prove to be highly reliable; the reliability coefficient in ARIC was only 0.42, the lowest of all the six hemostatic factors measured.

Cross-cultural and prospective studies have suggested an inverse relationship between fish intake and coronary heart disease. Suggested mechanisms include an improved lipid profile, decreased platelet aggregability, and enhanced fibrinolytic activity. Another potential mechanism may be hypocoagulability. Plasma fibrinogen level has been reported to be an independent predictor of cardiovascular events; factor VIII plays an important role in the intrinsic coagulation pathway; and vWF regulates platelet adhesion to the vessel wall. All of these factors were inversely related to fish intake.

The biological mechanisms underlying the associations remain to be elucidated. Both fibrinogen and protein C are synthesized by hepatocytes, whereas vWF is synthesized in extraplastic sites, mostly in endothelial cells. The exact site of synthesis of the active component of factor VIII is unknown. Recently it was reported that fibrinogen production by hepatoma cells was inhibited in fish oil media. Animal studies suggest that ingestion of EPA and DHA increases their content in liver mitochondrial and microsomal membranes and renders the latter more susceptible to lipid peroxidation. The resultant alterations in cellular membranes may influence the production of fibrinogen and protein C in the hepatocyte. The effect of n-3 PUFAs on vWF synthesis in endothelial cells has not been studied. However, fish oils were found to modulate production of other substances by endothelial cells, such as platelet-derived growth factor–like protein and endothelium-derived relaxing factor.

With the possible exception of animal fat intake, other dietary fats generally did not prove to be important determinants of the hemostatic factors in question. Dietary cholesterol, however, was positively associated with plasma fibrinogen, a finding consistent with a previous report of higher fibrinogen levels in subjects on a habitual high-cholesterol diet. Two feeding studies suggested that total fat intake is positively related to factor VII coagulant activity, but a more recent report did not corroborate this finding. ARIC data showed only a weak association between total fat intake and factor VII, which was restricted to white men. The single other population-based study reporting this relation was conducted in white men as well. However, it is uncertain whether fat
consumption per se or total energy intake primarily accounted for this association. Both were related to factor VII level in ARIC white men and, as expected, were highly intercorrelated \((r=9)\). Adjustment for calorific intake using the nutrient residual method\(^6\) suggested that total energy intake rather than fat consumption was the main determinant of factor VII.

Several limitations of this study should be mentioned. First, cross-sectional relationships do not necessarily imply causal associations. Fish intake, for example, may be a surrogate measure for a yet-undetermined factor that influences the hemostatic profile. Nonetheless, the causal nature of many of the associations revealed is supported by previous experimental evidence. Second, n-3 PUFAs were highly intercorrelated in this data set. Theoretically, only one of them might have been causally related to the hemostatic profile, whereas the other two might have been merely surrogate measurements. Finally, the range of n-3 PUFA consumption among the study participants was remarkably narrow. Predictions outside this range must be made cautiously.

Many of the observed associations were weak. However, it should be remembered that the strong association between dietary lipids and serum cholesterol level demonstrated consistently in feeding studies is undetectable in most cross-sectional studies.\(^6\) Dietary habits as well as hemostatic factors are measured with considerable error, which is likely to attenuate existing associations. Thus, the true magnitude of the relationships between dietary habits and hemostatic factors could be greater than apparent from these analyses. Based on risk estimates from the Northwick Park Heart Study,\(^2\) increasing fish consumption to 1 serving per day might reduce plasma fibrinogen and thereby lower the risk of ischemic heart disease by 1% to 2%. The true risk reduction could be greater, given the attenuation effect of measurement error. The clinical significance of the observed relationships with factor VIII, vWF, and protein C is yet to be determined.

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References

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