Association of Dietary Fish and n-3 Fatty Acid Intake With Hemostatic Factors in the Coronary Artery Risk Development in Young Adults (CARDIA) Study

Sujata L. Archer, David Green, Maryann Chamberlain, Alan R. Dyer, Kiang Liu

Abstract—Hemostatic factors play an important role in the complications of ischemic heart and vessel disease. Dietary fats such as n-3 fatty acids have been shown to possibly influence hemostatic factors. However, most studies reporting an inverse association between cardiovascular disease and fish and n-3 fatty acid consumption used supplemental doses of fish oil or intakes exceeding the typical amount consumed by the US population. This report examined the associations of usual intakes of fish, linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid with fibrinogen, factor VII, factor VIII, and von Willebrand factor in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. The analyses reported here included 1672 black and white men and women aged 24 to 42 years in 1992 to 1993. After adjustment for age, body mass index, diabetes, number of cigarettes smoked per day, race, and energy and alcohol consumption, no significant associations were observed between those who consumed no fish versus those who consumed the highest level of dietary fish with respect to fibrinogen, factor VIII, or von Willebrand factor for any race-sex group. Comparisons of tertile 1 versus tertile 3 for dietary linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid were also not significantly associated with fibrinogen, factor VII, factor VIII, or von Willebrand factor for any race-sex group. These data suggest that customary intakes of fish and n-3 fatty acids in populations that generally do not consume large amounts of these food items are not associated with these hemostatic factors. (Arterioscler Thromb Vasc Biol. 1998;18:1119-1123.)

Key Words: hemostatic factors ▪ dietary fish ▪ linolenic acid ▪ eicosapentaenoic acid ▪ docosahexaenoic acid

Epidemiological studies conducted more than a decade ago revealed positive associations of fibrinogen and FVII with ischemic heart disease.1-4 Findings from these early studies, including the Northwick Park Heart Study and the Framingham Study, have subsequently been confirmed by more recent investigations.5-7

Dietary fats have been shown to influence the plasma concentration and activity of hemostatic factors.8,9 The n-3 fatty acids displace arachidonic acid from platelet phospholipid stores, thereby decreasing the available substrate for thromboxane A2 synthesis.10 This then reduces the ability of thromboxane A2 to induce platelet aggregation. Hence, the n-3 fatty acids appear to exert an antiatheromatous effect. Studies have shown a low prevalence of atherosclerosis among Eskimos in Greenland, whose dietary fat composition is very different from that of the Western diet, since they consume more seafood rich in the long-chain n-3 fatty acids EPA (20:5n-3) and DHA (22:6n-3).11,12

Observational and feeding studies of dietary influences, specifically of n-3 fatty acids in relation to hemostatic profiles, have provided conflicting results. Some studies have reported that fish or fish oil intake has inverse associations with fibrinogen, FVIII, and vWF.13-16 However, other studies found no association of dietary fish or fish oil intake with coagulation factors.17,18 Adipose tissue analysis conducted as a marker of dietary lipids also did not show any relationship between n-3 fatty acids and fibrinogen.19

Despite the large number of studies on supplementation with fish oil and n-3 fatty acids on hemostatic factors, data on the relationships of habitual fish consumption or different n-3 fatty acid intakes and hemostatic factors in epidemiological studies are sparse. Much less is known about α-linolenic acid, the n-3 fatty acid of plant origin from which EPA can be derived via elongation and desaturation. Furthermore, there is a paucity of data on such associations within specific race and sex groups. The purpose of these analyses was to investigate the relationships between fish, linolenic acid (18:3n-3), EPA, and DHA consumption and plasma concentrations of hemostatic factors, including FVII, FVIII, and vWF, as well as fibrinogen in a young cohort of black and white men and women from the Coronary Artery Risk Development in Young Adults Study (CARDIA).
Methods

CARDIA is a prospective multicenter study investigating the evolution of cardiovascular disease risk factors in young adults. At baseline (1985 to 1986), a cohort of 5115 black men, white men, black women, and white women were recruited from 4 centers—Chicago, Ill; Minneapolis, Minn; Birmingham, Ala; and Oakland, Calif. For this investigation of hemostatic risk factors, a subgroup of 1894 participants from the Chicago and Minneapolis centers was studied during year 7 (1992 to 1993). After exclusions, 1672 participants were included in these analyses: 293 black men, 481 white men, 381 black women, and 508 white women. Participants were excluded if they were pregnant or had extremely low or high energy intake as an indicator of unreliable dietary interviews (cut-off point for men was ±3.3 and ±33 MJ and for women ±2.5 and ±25 MJ). Minimum and maximum cutoffs of energy intake account for underreporting or overreporting of habitual energy intake below or above that which a person may not be able to function in a normal lifestyle.20

Dietary intake data were obtained from a diet history questionnaire developed for the CARDIA study at baseline and readministered at year 7.21 Study participants were asked to recall their usual dietary intake by using the previous 30 days as the time frame. Participants were asked to report on the frequency, amount, and method of food preparation for this period. Total fish and linolenic acid, EPA, and DHA intakes of plant and animal origins were used for analyses. Total fish intake was the sum of lean fish (2% fat), medium-fat fish (6% fat), and high-fat fish (12% fat). Examples of some of the lean fish included in the diet history questionnaire were cod, flounder, and haddock; medium-fat fish included albacore, bass, and catfish; and high-fat fish included salmon, eel, and pompano. Validity and quality-control issues concerning the administration of the diet-history questionnaire have been described previously.22,23 In brief, 12 nutrients were selected for evaluating the reliability and energy-adjusted validity of the CARDIA diet history. A total of 128 participants recruited from 4 CARDIA centers were recruited and divided into groups A, B, and C. Participants from cohort A completed 2 dietary histories 1 week apart. They then provided 7 randomly scheduled 24-hour recalls within the next 28 days. At the end of the 28 days they completed a third dietary history. Participants in cohort B completed a dietary history and then provided 7 random 24-hour recalls within 28 days and in the end completed another dietary history. Participants in cohort C completed 7 24-hour recalls randomly scheduled over 28 days and then completed 1 dietary history. Data from the 3 cohorts were pooled in 2 different ways to examine comparative validity. The first analysis pooled the first histories from the 3 cohorts. The second analysis was based on the last dietary history for each cohort. Analyses for comparative validity were based on comparisons of values from the pooled first or last dietary histories and the mean of the 7 random 24-hour recalls. Mean nutrient values from the dietary histories were higher than those estimated from a mean of 7 24-hour recalls. Correlation coefficients for logarithmically transformed nutrient values and energy-adjusted nutrient values from the 2 dietary histories ranged from 0.50 to 0.80 for whites and 0.30 to 0.70 for blacks. The mean nutrient values were higher for the last dietary history than they were from the mean of 7 24-hour recalls.21 Nutrients from the food items were calculated by using the University of Minnesota Nutrition Coordinating Center Nutrient Database tape.24

Blood was drawn after an overnight fast. Methods for blood collection and coagulation assays have been described elsewhere.4 In brief, blood was drawn from a large antecubital vein, the tourniquet was removed, and samples were collected for blood chemistry analyses. Five milliliters was then placed into each of 2 Vacutainer tubes containing 3.8% sodium citrate, mixed by repeated inversion, and spun at 3000g for 20 minutes at 4°C in a refrigerated centrifuge. This centrifugation was performed within 10 minutes of collection, and within 1 hour the plasma was placed in a −70°C freezer. After storage for a maximum of 4 months, samples were analyzed. Fibrinogen was assessed by the Clauss method with the use of reagents from the Dade Division, Baxter Healthcare Corp. The assay was calibrated with standard normal plasma (SNP reagent, Dade), and the results were calculated by using the data management system of the MLA Electra 800 clot timer. FVII and FVIII coagulant activities were assayed by a 1-stage system using reagents from Pacific Hemostasis and George King Biomedical, Inc, respectively.25 The standard reference plasma was from Curtin Matheson Scientific (universal reference plasma), and the results were calculated as a percentage of the standard by using the data management system of the MLA Electra 800. von Willebrand antigen was measured by an ELISA assay obtained from American Bioproducts Co.26 A standard curve was prepared with universal reference plasma, and the results were reported as a percentage of the standard. The technical error as a percent of the mean was 5.6% for fibrinogen, 5.0% for FVII, 6.0% for FVIII, and 7.6% for vWF.

Body weight was measured to the nearest 0.2 kg with a calibrated scale while the subjects were light clothing. Height was measured without shoes to the nearest 0.5 cm with a vertical ruler. Self-reported alcohol intake was converted to milliliters of absolute alcohol per week.27 Smoking status was obtained from a self-reported smoking history questionnaire.28,29 Categorical variables for fish, linolenic acid, EPA, and DHA intake were created to assess whether there was a graded association of these foodstuffs with hemostatic factors. Those persons with a >0 intake of fish were divided into tertiles by using cutoff points for each sex. Comparisons were made between those who did not consume any fish versus those in the highest tertile of fish consumption. ANCOVAs were done to examine...
associations of dietary fish, linolenic acid, EPA, and DHA with hemostatic factors. The covariates included in the regression models were those that had shown a significant association with the hemostatic factors for any of the race and sex groups. These were age, BMI, diabetes, cigarettes smoked per day, race, and energy and alcohol intakes. Version 6.08 of the SAS software package (SAS Institute) was used for all analyses.

## Results

Fifty-nine percent of all participants consumed some fish. Men consumed an average of 10.7 g/d and women 8.4 g/d. Both men and women consumed lean fish the most (4.8 g/d) followed by medium-fat fish (3.5 g/d) and high-fat fish (1.2 g/d) (data not shown). The means of selected nutrients and lifestyle variables are shown in Table 1.

ANOVA with adjustment for age, BMI, diabetes, cigarettes/d, race, and energy and alcohol intakes showed no significant differences in mean levels of fibrinogen, FVIII, and vWF between those who consumed no fish versus those in the highest fish-intake tertile (Table 2). Only among men was there a significant but nongraded difference in FVII among those who did not consume any fish versus those in the upper tertile of intake (Table 2). Further analyses to assess whether any race differences existed in the associations of fish consumption with hemostatic factors showed no significant associations between fish consumption and FVII, FVIII, fibrinogen, and vWF for any race-sex group (data not shown). Table 3 shows the association of n-3 fatty acids with hemostatic factors. There were no statistically significant differences between the highest and lowest tertiles for linolenic acid, EPA, or DHA in any group. Nonsignificant positive associations with linolenic acid intake were observed for FVII in men and for vWF among men and women. Dietary EPA showed a nonsignificant inverse association with vWF in men. ANCOVA using sex-based tertile cutoff points for each race group also showed no significant associations of linolenic acid, EPA, and DHA intake with FVII, fibrinogen, FVIII, and vWF among black and white men and women (data not shown).

### Table 2. Adjusted Means of Hemostatic Factors by Dietary Fish Intake Tertiles*

<table>
<thead>
<tr>
<th></th>
<th>No Fish</th>
<th>Some Fish</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>Tertile 1</td>
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<tr>
<td></td>
<td></td>
<td>Tertile 3</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>351</td>
<td>143</td>
</tr>
<tr>
<td>Mean fish, g/d</td>
<td>0</td>
<td>4.5</td>
</tr>
<tr>
<td>FVII, %</td>
<td>75</td>
<td>73</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>FVIII, %</td>
<td>104</td>
<td>103</td>
</tr>
<tr>
<td>vWF, %</td>
<td>115</td>
<td>115</td>
</tr>
</tbody>
</table>

Women

<table>
<thead>
<tr>
<th></th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>420</td>
<td>169</td>
<td>144</td>
</tr>
<tr>
<td>Mean fish, g/d</td>
<td>0</td>
<td>4.2</td>
<td>9.8</td>
</tr>
<tr>
<td>FVII, %</td>
<td>77</td>
<td>78</td>
<td>76</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>2.7</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>FVIII, %</td>
<td>106</td>
<td>104</td>
<td>106</td>
</tr>
<tr>
<td>vWF, %</td>
<td>112</td>
<td>111</td>
<td>107</td>
</tr>
</tbody>
</table>

*Model adjusted for age, BMI, diabetes, cigarettes/d, race, and energy and alcohol intakes.
†P<0.05 for comparison of “no fish” vs “tertile 3.”

### Table 3. Adjusted Means of Hemostatic Factors by Dietary n-3 Fatty Acid Tertiles*

<table>
<thead>
<tr>
<th></th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>266</td>
<td>260</td>
<td>257</td>
</tr>
<tr>
<td>Mean 18:3, g/d</td>
<td>1.2</td>
<td>2.1</td>
<td>3.8</td>
</tr>
<tr>
<td>FVII, %</td>
<td>74</td>
<td>74</td>
<td>76</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>FVIII, %</td>
<td>102</td>
<td>104</td>
<td>104</td>
</tr>
<tr>
<td>vWF, %</td>
<td>111</td>
<td>113</td>
<td>118</td>
</tr>
</tbody>
</table>

Women

<table>
<thead>
<tr>
<th></th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>300</td>
<td>294</td>
<td>295</td>
</tr>
<tr>
<td>Mean 18:3, g/d</td>
<td>0.86</td>
<td>1.5</td>
<td>2.8</td>
</tr>
<tr>
<td>FVII, %</td>
<td>77</td>
<td>77</td>
<td>76</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>2.7</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>FVIII, %</td>
<td>105</td>
<td>105</td>
<td>104</td>
</tr>
<tr>
<td>vWF, %</td>
<td>108</td>
<td>110</td>
<td>112</td>
</tr>
</tbody>
</table>

*Model adjusted for age, BMI, diabetes, cigarettes/d, race, and energy and alcohol intakes.
18:3 indicates linolenic acid.
Discussion
This study examined the relationships of usual intakes of fish, linolenic acid, EPA, and DHA with FVII, FVIII, fibrinogen, and vWF in a cohort of black and white young men and women. Results from multivariate analyses indicated that in these young adults, usual intake of fish was not associated with FVIII, fibrinogen, or vWF. Dietary linolenic acid, EPA, and DHA were also not associated with these hemostatic factors and FVII. These findings were observed consistently for each race and sex group.

Most studies investigating these relationships have been feeding studies with fish and fish oil supplementation, which have reported conflicting results in their associations with hemostatic factors. An intervention trial was conducted in which fatty fish such as salmon, herring, and mackerel was fed for 10 days, followed by normal diets at home for the next 18 days and finally a 10-day meat diet. No effect of the fish diet was seen on fibrinogen and FVII levels.13 Emes et al30 reported no significant changes from 0 to 6 weeks in fibrinogen levels in a group consuming fish paste versus meat paste. Muller et al31 fed 135 g/d of fish or meat paste for 6 weeks. Fish consumption did not have a significant effect on FVII, activated FVII, vWF, and fibrinogen. A feeding study with fish oil for 6 weeks (amounts ranged from 1.3 to 9.0 g/d) reported a decrease in levels of fibrinogen and vWF with higher n-3 fatty acid levels.32 Another study included 40 patients who were assigned 3 g/d of EPA, DHA, or vegetable oil for 16 weeks. Blood samples were drawn at 3, 8, and 16 weeks. No changes were seen in FVII clotting activity.33 Toft et al34 examined the influence of dietary fish oil supplements on fibrinolytic function in persons with untreated essential hypertension. Daily supplementation with 4 g of n-3 fatty acids did not influence fibrinolytic activity. A greater increase in fibrinogen levels was observed during intake of fish oil compared with corn oil (27% versus 18%). The Physicians’ Health Study, which included middle-aged persons who were of a high socioeconomic status, reported that only 2% of the participants took fish oil capsules.35 In the CARDIA study, although fish oil consumption was not assessed (due to the young age and varying socioeconomic status of the participants), we believe that the amounts consumed are likely to be very limited and thus would not have had a significant impact on the results. The method of fish preparation, such as breaded fish or fish sticks, could have influenced the results. However, in CARDIA only 27% of the cohort reported consuming breaded fish or fish sticks.

Very few epidemiological studies have examined the associations of usual fish and n-3 fatty acid intakes with hemostatic factors. Similar to our findings, the Atherosclerosis Risk in Communities (ARIC) study did not observe any association between dietary linolenic acid and the hemostatic factors examined.36 However, the ARIC study reported inverse associations for other n-3 fatty acids with fibrinogen, FVIII, and vWF. Differences in the results between the ARIC and CARDIA studies may be attributed to several factors. Although both studies assessed diet by using a food-frequency questionnaire, ARIC used a semiquantitative version that had 66 food items to recall for the past year, whereas CARDIA had a quantitative version with ~300 food items to recall over the previous 30 days. There was also a different nutrient ingestion pattern in ARIC compared with CARDIA. ARIC reported higher EPA and DHA intakes but lower total, saturated, and monounsaturated fatty acids; dietary fiber; and energy intake. The mean hemostatic factor levels were lower in the CARDIA cohort and could be related to the younger age (the CARDIA participants were aged 24 to 42 at year 7 versus 45 to 64 for ARIC at baseline). Despite attenuation due to measurement errors that accompany any dietary tool, these results raise the question of whether a usual dietary intake of n-3 fatty acids has an impact on hemostatic factors.

For the present report we included results on the association of hemostatic factors with linolenic acid. Although linolenic acid accounts for 96% of total n-3 fatty acids, it is often not included in reports of n-3 fatty acids. Although fish on average accounts for 90% of EPA and 75% of DHA intake, these nutrients can also be obtained through other sources.36 Poultry has some EPA and DHA due to the fishmeal component in the diet. The contribution of these nutrients from poultry has risen over the years. Organ meats also have a small quantity of DHA. Therefore, we believe that by including total n-3 fatty acids from plant, marine, and other animal sources, we have used a very comprehensive source of these nutrients.

Many earlier studies were based on results from populations that primarily consumed fatty fish, which is not eaten frequently by the majority of the US population due to the cost and availability of such fish. Most of the fish consumed by the CARDIA participants was lean fish, followed by medium- and high-fat fish, with a mean total consumption by both men and women of 9.5 g/d. Ascherio et al37 reported no substantial reduction in the risk of coronary heart disease among men who increased their fish intake from 1 to 2 servings/wk to 5 to 6 servings/wk.37 Many studies reporting the beneficial effects of fish consumption were also from populations that consumed large quantities of fish. In a recent study Daviglus et al38 reported an inverse association between fish consumption and death from coronary heart disease for men who consumed 35 g or more of fish daily compared with those who consumed no fish. In the Zutphen Study, habitual fish consumers ate on average between 29.4 and 37.4 g of fish per day. The Continuing Survey of Food Intakes by Individuals (1989 to 1991) in the United States reported an overall mean intake of 13 g/d of fish, with individuals residing in the Midwest region consuming 10 g/d.39 The CARDIA participants for these analyses were from Chicago and Minneapolis, and their intakes appear comparable to the mean intake of fish in the Midwestern region.

In conclusion, data from the CARDIA study provide no evidence of an association for the intake of fish with fibrinogen, FVIII, and vWF for any race-sex group. Dietary linolenic acid, EPA, and DHA also showed no association with FVII, FVIII, fibrinogen, and vWF for any race-sex group. These data demonstrate that habitual fish intake in populations that do not consume large amounts of fish has no beneficial effect on hemostatic factors. The influence of dietary fish and n-3 fatty acids on hemostatic factors is still unresolved. More research is needed to explore the mecha-
nisms involved in the reported inverse associations of dietary fish and n-3 fatty acids with coronary heart disease.

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References
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