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REGULAR ARTICLE

Haemostasis in Relation to Dietary Fat as Estimated by Erythrocyte Fatty Acid Composition: The Prime Study


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Abstract

We investigated whether haemostatic variables were related with dietary fatty acid composition as estimated by the fatty acid content of erythrocytes. Subjects were a subsample (n = 283) of the participants in the Prospective Epidemiological Study of Myocardial Infarction (PRIME) Study. Factor VII, fibrinogen, tissue-type plasminogen activator antigen (tPA-ag), plasminogen activator inhibitor type 1 (PAI-1), D-dimer and von Willebrand factor (vWF) were measured and the fatty acid composition was determined in the phospholipids of total erythrocytes by gas chromatography. Statistical analyses were performed using multiple linear regression analyses with adjustment for age, center and body mass index. tPA-ag was significantly related to the n-3 fatty acids derived from fish. This was reflected in an inverse association of all n – 3 fatty acids combined with tPA-ag (β = –0.37 ng/ml/%, 95% confidence intervals: –0.45, –0.29, P < .01). Positive and significant associations of D-dimer with arachidonic and eicosamonoenoic acid were observed (P < .01). No relationships were found between fatty acids and fibrinogen, vWF, PAI-1 or factor VII. The results of this study suggest that consumption of n-3 fatty acids derived from fish may favourably influence tPA-ag.


Abbreviations: tPA-ag, tissue-type plasminogen activator antigen; PAI-1, plasminogen activator inhibitor type 1; vWF, von Willebrand factor.

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to the prevention of cardiovascular disease. Although the relation between dietary fat intake and the haemostatic system has been evaluated previously, former studies have not provided conclusive evidence. Factor VII seems to be affected by total fat intake, but probably not by the fatty acid composition of the diet [16]. Fibrinogen may be related to fish oil intake, although the results of previous studies are inconclusive [17–19]. Limited data are available on associations of PAI-1 and tPA with fatty acid composition of the diet. Studies have observed an increase in PAI-1 after a diet rich in n-3 polyunsaturated fatty acids [18], a decrease after a monounsaturated fat rich diet [20], and a decrease in tPA-ag after fish supplementation [21], while others did not observe changes in PAI-1 or in tPA-ag after dietary intervention [19,22–25]. Studies on the intake of n-3 fatty acids and vWF concentration were not able to show an effect of these fatty acids [18,26–29]. Other studies have shown a lower concentration of vWF after a saturated or monounsaturated fat rich diet compared with an n-6 or n-3 fatty-acid-rich diet [24,30]. The only intervention study that included measurement of D-dimer did not show any effects of different types of fat [31].

The fatty acid composition of the diet is not easy to assess. Measurement of erythrocyte fatty acid composition, however, is a reliable biological marker of the fatty acid composition of the diet over the last couple of months [32,33]. In the Prospective Epidemiological Study of Myocardial Infarction (PRIME) Study, several haemostatic factors and the phospholipid fatty acid composition of erythrocytes were measured in middle-aged men. We used the cross-sectional data from this study to investigate the relationship of factor VII:C and factor VII:ag, fibrinogen, tPA-ag, PAI-ag, PAI-1, D-dimer and vWF to dietary fat intake as estimated by the phospholipid fatty acid composition of the erythrocytes.

1. Methods

Subjects were participants in the PRIME Study. The PRIME Study is a prospective study of myocardial infarction based in three World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) field centers in France (Lille, Strasbourg and Toulouse) and Northern Ireland (Belfast) [34]. In each center, more than 2500 subjects, all men aged between 50 and 59 years, are being followed (total 10,592). All participants gave informed consent and the local ethical committees approved the study. Erythrocyte fatty acids, FVII-ag, tPa-ag, PAI-ag, D-dimer and vWF were measured in a random subsample from each center. Subjects with cardiovascular disease (defined as a self-reported history of physician-diagnosed myocardial infarction or angina pectoris, or a self-reported history of stroke or intermittent claudication) were excluded (6 in Northern Ireland, 22 in France). The present study included 57 subjects from Northern Ireland and 226 from France (total 283).

Information on smoking and physical activity were obtained by means of a self-administered questionnaire. Physical activity during leisure time was coded as 1 (low level) to 4 (high level corresponding to intense physical activity for at least 20 min a week). The percentage of subjects having a high level of physical activity was used in the statistical analysis. Weight and height were measured with subjects in light clothing and the body mass index was calculated as weight (kg) divided by height squared (m²).

Subjects were asked to fast for at least 10 h before blood collection. Venous blood (9 vol) was collected between 9 and 10 AM into siliconised vacutainer tubes (Vacutainer, Becton Dickinson) containing 0.11 M trisodium citrate (1 vol). Platelet-poor plasma was obtained by centrifugation at 4500 × g and 4°C (PAI-1 measurement) or 20°C (fibrinogen, FVII, vWF and D-dimer) for 15 min as previously described [15]. Without delay, aliquots of plasma were transferred into plastic tubes and frozen on-site to −80°C, and then shipped in batches to the central laboratory in Lille.

Haemostatic variables were measured in the central haemostasis laboratory of la Timone Hospital at Marseille, France [15]. All the haemostatic tests were performed in duplicate between January 1992 and January 1993. Accuracy and precision of haemostatic assays were assured by a strict internal quality control program. A single batch of normal plasma pool prepared from 50
healthy subjects was used as control material. For each assay, two to four controls were included in each run of PRIME Study samples. Fibrinogen was measured according to the method of Clauss [35] with reagents (Fibriprest automate) and reference material obtained from Diagnostica Stago (Asnières, France). Factor VII:C was assayed in a regular one-stage system using rabbit brain thromboplastin obtained from Diagnostic Reagents Limited (Oxon, England). Factor VII-deficient substrate plasma was prepared from absorbed bovine plasma and a concentrate of human factors IX, X and II as described [36]. Factor VII international standard was obtained from National Institute for Biological Standard and Control (NIBSC, Hertfordshire, England). Results were expressed as a percentage of reference plasma.

PAI-1 activity was measured by a two-stage amidolytic method using a commercially available kit (Spectrolyse/TM/Fibrin, Bio-pool, Umeå, Sweden). Commercially available kits based on ELISA methods were used for determination of factor VII antigen (Asserachrom FVIIag, Diagnostica Stago), D-dimer (Fibrinostika FBDP, Organon Teknika, Fresnes, France), vWf (Asserachrom vWf, Diagnostica Stago), PAI-1 antigen (Tint Elize PAI1 Biopool) and tPA-ag (Tint Elize tPA, Biopool). Analysis of internal quality control data showed that laboratory coefficients of variation ranged from 4.3% for fibrinogen to 9.5% for PAI-1 activity.

Plasma prepared with EDTA was used for the analysis of lipids. Plasma total cholesterol and triglycerides were measured by enzymatic methods using reagents obtained from Boehringer Mannhein (Mannheim, Germany). High-density lipoprotein (HDL) cholesterol was measured after sodium phosphotungstatemagnesium chloride precipitation.

To measure phospholipid fatty acid composition in erythrocytes, 5 ml of blood was centrifuged at 2000 rpm for 15 min to isolate the red blood cells. The cell pellet was washed two times with 10 ml phosphate buffer each containing 0.1 mol/1 NaH2PO4 (5/1 vol/vol) and then homogenized in buffer diluted twofold. The precipitate was stored in tubes with little air for 3 months at −80°C before fatty acid measurement was performed. Lipids were extracted according to the Folch method. The lipid extract was dried under nitrogen, dissolved in heptane and separated by thin-layer chromatography using petroleum ether/ether/acetic acid (90:10:5 by volume). Phospholipids were identified by simultaneous migration of standards. Phospholipids were scraped, hydrolyzed and the resulting fatty acids methylated by treatment with methanolic H2SO4 for 2 h at 70°C. The fatty acid methyl esters were extracted with heptane and analyzed by gas chromatography on a Varian 3400 chromatograph (Palo Alto, CA) equipped with a flame ionization detector and a capillary column. A gradient temperature from 160°C to 210°C at 1.5°C min−1 and a N2 pressure of 85 kPA were used. Identification of fatty acids was achieved using commercially available fatty acid methyl esters. A quality control made by ox red cells was added at the beginning of every series.

Any differences between the four centers were tested by analysis of variance. The relationships of factor VII:C, factor VII:Ag, PAI-1-act, PAI-1-ag, vWf, tPA-ag and D-dimer with the fatty acid composition of the erythrocytes in all subjects were evaluated using multiple linear regression analyses. Adjustments were made for age, center, body mass index, blood lipids, smoking and physical activity by inclusion of these variables in the regression model. A large number of statistical tests were made and only type I errors less than 0.01 were considered.

2. Results

Age, smoking habits, physical activity and body mass index, blood lipids and haemostatic factors are shown in Table 1. Only serum-triglycerides, HDL-cholesterol and Factor VII:Ag differed between the centers.

More than a quarter of the erythrocyte fatty acids consisted of palmitic acid (Table 2). Stearic acid, oleic acid, linoleic acid and arachidonic acid each contributed about 10–15% to the fatty acid composition. This was similar for Belfast and the centers in France. Except for myristic, palmitoleic, linoleic, γ-linolenic, arachidic, eicosapentaenoic and eicosadienoic acid, the concentration of fatty acids differed between the centers.

Total n–3 fatty acids combined was inversely associated with tPA-ag (regression coefficient...
This means that an absolute increase of 10% in n−3 fatty acids corresponds to a decrease in tPA-ag of 3.7 ng/ml (26%). This was reflected in an inverse association between tPA-ag and docosahexaenoic acid (P < .01). There was also a nonsignificant trend (P < .05) to the decrease in tPA-ag with the increase in eicosapentaenoic and docosapentae-

\[ \beta = -0.37 \text{ ng/ml/\%}, \quad 95\% \text{ confidence interval: } -0.45, -0.29 \quad (P < .01)\]

This means that an absolute increase of 10% in n−3 fatty acids corresponds to a decrease in tPA-ag of 3.7 ng/ml (26%). This was reflected in an inverse association between tPA-ag and docosahexaenoic acid (P < .01). There was also a nonsignificant trend (P < .05) to the decrease in tPA-ag with the increase in eicosapentaenoic and docosapentae-

Table 2. Mean (S.D.) levels (% of total) of erythrocyte fatty acids for the total population and by center: the PRIME Study

<table>
<thead>
<tr>
<th>Fatty acids (C20:0)</th>
<th>Total group</th>
<th>Belfast</th>
<th>Lille</th>
<th>Strasbourg</th>
<th>Toulouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic (C14:0)</td>
<td>0.54 (0.27)</td>
<td>0.53 (0.24)</td>
<td>0.59 (0.33)</td>
<td>0.49 (0.21)</td>
<td>0.50 (0.24)</td>
</tr>
<tr>
<td>Palmitic (C16:0)</td>
<td>28.3 (5.52)</td>
<td>28.0 (5.58)</td>
<td>28.9 (5.39)</td>
<td>26.0 (3.74)</td>
<td>29.2 (6.30) *</td>
</tr>
<tr>
<td>Palmitoleic (C16:1)</td>
<td>0.48 (0.52)</td>
<td>0.43 (0.44)</td>
<td>0.49 (0.64)</td>
<td>0.53 (0.47)</td>
<td>0.47 (0.44)</td>
</tr>
<tr>
<td>Stearic (C18:0)</td>
<td>11.9 (3.01)</td>
<td>11.3 (3.07)</td>
<td>12.5 (2.94)</td>
<td>10.1 (1.40)</td>
<td>12.8 (3.29) *</td>
</tr>
<tr>
<td>Oleic (C18:1)</td>
<td>15.8 (2.20)</td>
<td>17.0 (2.21)</td>
<td>15.3 (1.99)</td>
<td>15.4 (1.63)</td>
<td>15.8 (2.51) *</td>
</tr>
<tr>
<td>Linoleic (C18:2, n−6)</td>
<td>11.8 (2.66)</td>
<td>12.0 (2.39)</td>
<td>11.7 (2.79)</td>
<td>12.2 (1.90)</td>
<td>11.6 (3.11)</td>
</tr>
<tr>
<td>γ-linolenic (C18:3, n−6)</td>
<td>0.16 (0.31)</td>
<td>0.28 (0.44)</td>
<td>0.14 (0.27)</td>
<td>0.20 (0.29)</td>
<td>0.09 (0.19)</td>
</tr>
<tr>
<td>α-linolenic (C18:3, n−3)</td>
<td>0.17 (0.21)</td>
<td>0.23 (0.23)</td>
<td>0.15 (0.21)</td>
<td>0.22 (0.24)</td>
<td>0.10 (0.12) *</td>
</tr>
<tr>
<td>Arachidic (C20:0)</td>
<td>0.49 (0.42)</td>
<td>0.56 (0.48)</td>
<td>0.49 (0.45)</td>
<td>0.51 (0.41)</td>
<td>0.42 (0.30)</td>
</tr>
<tr>
<td>Eicosamonoenoic (C20:1)</td>
<td>0.38 (0.37)</td>
<td>0.50 (0.43)</td>
<td>0.34 (0.36)</td>
<td>0.43 (0.45)</td>
<td>0.321 (0.23)</td>
</tr>
<tr>
<td>Eicosadienoic (C20:2, n−9)</td>
<td>0.41 (0.41)</td>
<td>0.44 (0.43)</td>
<td>0.38 (0.40)</td>
<td>0.53 (0.53)</td>
<td>0.34 (0.28)</td>
</tr>
<tr>
<td>Arachidonic (C20:4, n−6)</td>
<td>10.4 (3.66)</td>
<td>10.0 (3.51)</td>
<td>10.3 (3.59)</td>
<td>12.0 (2.49)</td>
<td>9.66 (4.26) *</td>
</tr>
<tr>
<td>Eicosapentaenoic (C20:5, n−3)</td>
<td>0.76 (0.52)</td>
<td>0.95 (0.57)</td>
<td>0.69 (0.46)</td>
<td>0.95 (0.43)</td>
<td>0.58 (0.54) *</td>
</tr>
<tr>
<td>Docosatetraenoic (C22:4, n−6)</td>
<td>2.04 (0.82)</td>
<td>1.87 (0.73)</td>
<td>1.98 (0.81)</td>
<td>2.52 (0.59)</td>
<td>1.91 (0.93)</td>
</tr>
<tr>
<td>Docosapentaenoic (C22:5, n−6)</td>
<td>0.50 (0.29)</td>
<td>0.39 (0.19)</td>
<td>0.58 (0.35)</td>
<td>0.39 (0.16)</td>
<td>0.57 (0.30) *</td>
</tr>
<tr>
<td>Docosapentaenoic (C22:5, n−3)</td>
<td>1.48 (0.68)</td>
<td>1.67 (0.79)</td>
<td>1.45 (0.66)</td>
<td>1.72 (0.41)</td>
<td>1.19 (0.656) *</td>
</tr>
<tr>
<td>Docosahexaenoic (C22:6, n−3)</td>
<td>2.85 (1.46)</td>
<td>3.00 (1.54)</td>
<td>2.84 (1.45)</td>
<td>3.21 (1.05)</td>
<td>2.48 (1.61) *</td>
</tr>
</tbody>
</table>

* P < .01; for any difference between the centers.
Table 3. Linear regression analyses [regression coefficients (95% confidence interval)] with tPA-ag, D-dimer and vWf as dependent variables and erythrocyte fatty acids as independent variables, adjusted for age, center and body mass index: the PRIME Study

<table>
<thead>
<tr>
<th>Fatty acids (%)</th>
<th>tPA-ag (ng/ml/%)</th>
<th>D-dimer (ng/ml/%)</th>
<th>vWf (IU/ml/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic (C14:0)</td>
<td>0.11 (−2.46, 2.68)</td>
<td>112 (16, 208)</td>
<td>−0.02 (−0.22, 0.18)</td>
</tr>
<tr>
<td>Palmitic (C16:0)</td>
<td>0.13 (0.005, 0.26)</td>
<td>−2.1 (−6.9, 2.7)</td>
<td>0.005 (−0.005, 0.015)</td>
</tr>
<tr>
<td>Palmitoleic (C16:1)</td>
<td>−0.28 (−1.61, 1.05)</td>
<td>36 (−13, 85)</td>
<td>0.03 (−0.07, 0.13)</td>
</tr>
<tr>
<td>Stearic (C18:0)</td>
<td>0.05 (−0.20, 0.30)</td>
<td>−7.6 (−16.8, 1.6)</td>
<td>0.007 (−0.01, 0.02)</td>
</tr>
<tr>
<td>Oleic (C18:1)</td>
<td>0.25 (−0.08, 0.58)</td>
<td>2.2 (−10.1, 14.5)</td>
<td>0.03 (0.01, 0.05)</td>
</tr>
<tr>
<td>Linoleic (C18:2, n−6)</td>
<td>−0.20 (−0.47, 0.07)</td>
<td>−3.8 (−14.0, 6.4)</td>
<td>−0.006 (−0.03, 0.01)</td>
</tr>
<tr>
<td>γ-linolenic (C18:3, n−6)</td>
<td>0.21 (−2.10, 2.52)</td>
<td>99 (13, 185)</td>
<td>−0.009 (−0.19, 0.17)</td>
</tr>
<tr>
<td>α-linolenic (C18:3, n−3)</td>
<td>−0.49 (−3.96, 2.98)</td>
<td>167 (38, 296)</td>
<td>−0.04 (−0.29, 0.21)</td>
</tr>
<tr>
<td>Arachidonic (C20:4)</td>
<td>−0.84 (−2.51, 0.83)</td>
<td>94 (31, 157)</td>
<td>−0.01 (−0.13, 0.11)</td>
</tr>
<tr>
<td>Eicosapentaenoic (C20:5)</td>
<td>−0.19 (−2.09, 1.71)</td>
<td>116 (45, 187)</td>
<td>−0.005 (−0.14, 0.13)</td>
</tr>
<tr>
<td>Eicosadienoic (C20:2, n−9)</td>
<td>−0.77 (−2.48, 0.94)</td>
<td>80 (17, 143)</td>
<td>0.005 (−0.11, 0.12)</td>
</tr>
<tr>
<td>Arachidonic (C20:4, n−6)</td>
<td>−0.10 (−0.30, 0.10)</td>
<td>4.3 (−3.0, 11.6)</td>
<td>−0.007 (−0.02, 0.02)</td>
</tr>
<tr>
<td>Docosapentaenoic (C22:5, n−3)</td>
<td>−1.46 (−2.85, −0.07)</td>
<td>29 (−24, 82)</td>
<td>−0.09 (−0.19, 0.008)</td>
</tr>
<tr>
<td>Docosatetraenoic (C22:4, n−6)</td>
<td>0.14 (−0.74, 1.02)</td>
<td>17 (−16, 50)</td>
<td>−0.02 (−0.08, 0.04)</td>
</tr>
<tr>
<td>Docosapentaenoic (C22:5, n−6)</td>
<td>2.45 (−0.02, 4.92)</td>
<td>−62 (−156, 32)</td>
<td>−0.07 (−0.25, 0.11)</td>
</tr>
<tr>
<td>Docosapentaenoic (C22:5, n−3)</td>
<td>−1.10 (−2.08, −0.12)</td>
<td>−5.0 (−45.2, 35.2)</td>
<td>−0.07 (−0.15, 0.008)</td>
</tr>
<tr>
<td>Docosahexaenoic (C22:6, n−3)</td>
<td>−0.70 (−1.17, −0.23)</td>
<td>−14 (−32, 4)</td>
<td>−0.04 (−0.08, −0.01)</td>
</tr>
</tbody>
</table>

* P < 0.01.

and fibrinogen, PAI-1 activity and antigen, FVII:C or FVII:Ag.

3. Discussion

In this cross-sectional study, we have observed an inverse associations of tPA-ag with n−3 fatty acids. D-dimer was positively associated with arachidic and eicosapentaenoic acid. The mean levels of PAI-1-ag and tPA-ag were somewhat high compared to the mean levels in other studies [26,27,31,37]. Analysis of internal quality control data showed that laboratory coefficients of variation were all below 10%. Furthermore, consistent associations of PAI-1-ag and tPA-ag with triglycerides levels and body mass index were observed in the PRIME Study as previously described. Therefore, it is unlikely that these relatively high mean levels of PAI-1 and tPA have influenced the results of the regression analyses.

The fatty acid composition in erythrocytes have been found to be a reliable marker of the habitual fatty acid intake during the last few months [32,33]. Monounsaturated and saturated fat are reflected in the oleic acid content of erythrocytes when saturated fat intake is high, while polyunsaturated fat and linoleic acid intake are reflected in the linoleic acid content of erythrocytes [38]. The eicosapentaenoic acid and docosahexaenoic acid intake are directly reflected by their contents in erythrocytes [39]. Although the present study was cross-sectional, we may speculate that the relationships we observed between fatty acid contents and tPA-ag, vWf and D-dimer may reflect the effect of dietary fat intake on these haemostatic factors. On the other hand, typical patterns in dietary fat consumption may also reflect a certain lifestyle, which may affect haemostatic factors as well.

At least four other studies have investigated the association between fatty acid composition and haemostatic factors. Folsom et al. [40] measured factor VII:C in 1059 men from the ARIC Study. Factor VII:C was positively associated with myristic, stearic and palmitoleic acid, while it was inversely related with linoleic and α-linolenic acid. Salomaa et al. [41] did not observe a relation between factor VII:C and fatty acid composition in about 330 middle-aged Finnish men, while an inverse relation between fibrinogen and linoleic acid was found. Furthermore, they observed a 9% higher fibrinogen concentration in subjects with a high palmitic/low linoleic content compared to those who had the opposite pattern. Cigolini et al. [37,42] evaluated in two studies in 94 healthy
middle-aged Italian men the relation of fibrinogen and PAI-1 with fatty acid composition in adipose tissue, which reflects dietary fat intake of the past years [33]. After adjustment for serum-triglycerides, PAI-1-act and PAI-1-ag were both inversely related to ω-linolenic acid, while no relation was observed between fibrinogen and fatty acid composition. In our population of 283 middle-aged men from France and Northern Ireland, we did not observe any relation between fibrinogen, factor VII or PAI-1 and fatty acid composition. A difference between our study and the studies of Salomaa et al. [41] and Folsom et al. [40] is the measurement of fatty acids. In our study, the phospholipid fatty acid composition of total erythrocytes was measured, while Salomaa et al. and Folsom et al. measured the phospholipid fatty acid composition of serum and plasma, respectively. These pools of fatty acids may not be completely comparable.

In the present study, tPA-ag were inversely related to \( n - 3 \) fatty acids derived mostly from fish oil (eicosapentaenoic, docosapentaenoic and docosahexaenoic acids), but not with \( n - 3 \) fatty acids from vegetable origin (α-linolenic acid). This is in line with a large intervention study in diabetic subjects that showed a decrease in tPA-ag after fish supplementation [21]. However, other intervention studies failed to show an effect of fish (oil) consumption on tPA-ag [19,21,22,25,43]. With regard to PAI-1 levels, increase or no substantial change with intake of \( n - 3 \) fatty acids from fish oil has been reported [19,21,22,25,43]. Our data are consistent with these negative findings.

Our data show no significant association between vWf and fatty acid composition. This is in agreement with previous reports. In a cross-sectional study by Archer et al. [29], a nonstatistically significant positive association between vWf and linolenic acid and an inverse one between vWf and eicosapentaenoic acid was found. Another cross-sectional study showed a lower vWf concentration in subjects who took fish oil capsules [44]. The relationship between vWf and consumption of \( n - 3 \) fatty acids was, however, not confirmed in intervention studies [18,24,28].

Plasma concentration of D-dimer is a marker of the formation of crosslinked fibrin due to activation of blood coagulation and thrombin formation and subsequent clot lysis, due to activation of endogenous fibrinolysis and plasmin formation. D-dimer may thus be a useful marker of thrombogenic potential [45]. The relationship of D-dimer with fatty acid composition was strong but not consistent. It seemed that D-dimer was preferably related to fatty acids with 18 or 20 carbons, but stearic, oleic and linoleic (all C18), and arachidonic and eicosapentaenoic acid (both C20) did not show an association with D-dimer. Furthermore, most of the fatty acids are correlated with each other, and it is possible that one fatty acid, which is related to D-dimer, may in part explain the relationship of another fatty acid also related to D-dimer. Therefore, the observation of positive associations between the other C18 and C20 fatty acids and D-dimer warrants further research in order to explore the exact effects of fatty acid intake on D-dimer. Furthermore, the clinical relevance of associations between quantitatively very minor fatty acids and D-dimer remains questionable.

A high intake of fish has been found to be protective for coronary heart disease in some populations[46]. Fish fatty acids were related to tPA-ag, a marker of endothelial cell damage [47,48]. Further studies have to clarify whether a high consumption of fish directly influences the condition of the vessel wall, or whether its putative beneficial effect is mediated by other factors.

In conclusion, the results of this study suggest that the consumption of \( n - 3 \) fatty acids derived from fish may favourably influence tPA-ag.

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References


32. Ma JM, Folsom AR, Shahar E, Eckfeldt JH.


