Fasting Blood Coagulation and Fibrinolysis of Young Adults Unchanged by Reduction in Dietary Fat Content

Peter Marckmann, Brittmarie Sandström, and Jørgen Jespersen

Low-fat, high-fiber diets may influence the variables of blood coagulation and fibrinolysis associated with cardiovascular morbidity. Dietary fat content has been suggested as the important determinant. This hypothesis was tested in a strictly controlled dietary study of 13 healthy individuals. They were fed two experimental diets in a 2×2-week crossover trial. The diets differed in fat content (39% versus 31% of total energy), whereas the fatty acid composition and the fiber content were virtually identical. We observed no significant differences between diets in terms of fasting plasma levels of factor VII coagulant activity, fibrinogen, euglobulin fibrinolytic activity, tissue-type plasminogen activator (t-PA) activity, t-PA antigen, plasminogen activator inhibitor type 1 (PAI-1) antigen, or PAI activity. Serum levels of total cholesterol, high density lipoprotein cholesterol, and triglycerides were also unaffected. In conclusion, a moderate reduction in dietary fat intake, at a fixed fatty acid composition and dietary fiber intake, did not significantly influence blood coagulation, fibrinolysis, or blood lipids in the fasting state. (Arteriosclerosis and Thrombosis 1992;12:201-205)

Epidemiological and clinical studies have demonstrated the variables of blood coagulation and fibrinolysis to be independent risk markers of arterial disease.1-4 The observed associations could be causal and reflect an increased atherothrombotic tendency of individuals with high coagulant and/or low fibrinolytic capacity. The recognition of considerable dietary effects on coagulation and fibrinolysis5-8 is therefore highly relevant with respect to primary and secondary prevention of ischemic heart disease. Attempts have been made to identify the specific dietary characteristic(s) affecting the hemostatic system, and these have indicated that the total dietary fat content may be influential.9-11 However, controlled dietary trials supporting this suggestion are lacking. Therefore, we conducted a trial primarily designed to elucidate the effect of a change in dietary fat content. It was a randomized crossover study with a high degree of dietary control, in which a typical Danish diet (fat content, 39% of energy) was compared with a diet with a fat content at the officially recommended level (31% of energy).12 The fat quality and the fiber content of the two diets did not differ. Here, we present the observed effects on coagulation, fibrinolysis, and blood lipids.

Methods

Participants

The study included seven women and six men aged 21–37 years (median age, 26 years) who were all healthy. None were taking any medication, including contraceptives. The occasional use of vitamins and analgesics reported by some participants was totally avoided during the study. Three were smokers, but they abstained from smoking during the study. The habitual diets of the participants were assessed by a 7-day weighed-food record, and the nutrient content was calculated with reference to the Danish nutrient data base13 (Table 1).

The participants weighed 58–76 kg (median, 62 kg), and their body mass index ranged from 20.0 to 24.6 kg/m² (median, 21.7 kg/m²). All had normal systolic and diastolic blood pressures (below 150 and 85 mm Hg, respectively). All women had regular menstrual cycles of 23–29 days (median, 28 days). Blood lipids and coagulation and fibrinolytic variables were determined at study entry. An acute-phase protein, the C-reactive protein, was assessed in serum samples at entry and twice during the study to detect signs of acute disease. None showed patholog-
Two experimental diets were served for 2 weeks each in a randomized crossover design. The experimental periods were separated by a 2-week wash-out period in which the participants returned to their habitual diets. Blood samples were taken at study entry and at the end of each experimental period. The stored samples were analyzed in one run at the end of the study, and the results were pooled according to diet.

Statistics

The effect of the experimental diets on the measured variables was compared by Wilcoxon’s matched-pairs signed-rank test. Correlation analysis was also performed nonparametrically (Spearman’s rank correlation). A 5% significance level was chosen.

Experimental Diets

The experimental diets (A and B) consisted of three daily main meals and an evening snack in repeated 7-day menus. They were served according to individual energy needs as estimated from height, weight, gender, and habitual physical activity. All food items used were weighed on precision scales, and all meals were prepared in our metabolic kitchen. On weekdays, lunch and dinner were served and eaten while the subjects were under observation at the department. Breakfast and weekend meals were supplied prepackaged to be eaten at home. The participants were allowed to drink water, tea, and coffee without additives in free amounts during the experimental periods.

Diet A corresponded to the average Danish diet as characterized in a recent national survey with regard to food items as well as nutrient composition. The only exception was the exclusion of alcohol from the experimental diet. Diet B was a low-fat version of diet A. The dairy and meat products were leaner, and less added fat (margarine and oils) was used. The removed fat was replaced by food items, primarily juices and fruits, rich in carbohydrates but low in fiber. Diets A and B were virtually identical with regard to their fatty acid composition and dietary fiber content. The mean daily intakes of protein, fat, fatty acids, carbohydrates, and dietary fiber, as assessed from chemical analyses of pooled duplicate portions of the 7-day menus, and the dietary contents of mono- and disaccharides and cholesterol, as calculated with reference to the Danish nutrient data base, are presented in Table 2. The difference in fat content between diets A and B (39.4 versus 31.1 energy percent, respectively) was counterbalanced by the content of carbohydrates (46.6 versus 54.3 energy percent), primarily mono- and disaccharides (16.0 versus 23.5 energy percent).

Blood Sampling and Analysis

Venous blood samples were collected, with minimal stasis, from fasting individuals in the morning (between 8 and 10 AM) after 10 minutes of supine rest. The participants had abstained from alcohol for at least 24 hours and from heavy physical activity for at least 36 hours. Siliconized, evacuated tubes and 20-gauge needles were used.

### Table 1. Energy Intake and Nutrient Content of the Habitual Diets of 11 Participants

<table>
<thead>
<tr>
<th>Energy</th>
<th>MJ/day</th>
<th>(8.8-14.3)</th>
<th>Kcal/day</th>
<th>2,703 (2,102-3,402)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (% of energy)</td>
<td>14 (12-17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat Percent of energy</td>
<td>35 (29-37)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFAs</td>
<td>15 (9-20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUFAs</td>
<td>5 (3-7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUFASFA</td>
<td>0.32 (0.27-0.45)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Carbohydrates Percent of energy

| Alcohol (% of energy) | 0 (0-10) |
| Cholesterol (mg/MJ) | 41.1 (25.9-68.2) |
| Dietary fiber (g/MJ) | 3.1 (2.5-3.7) |

Values are mean and (range). SFAs, saturated fatty acids; PUFAs, polyunsaturated fatty acids; PUFASFA, polyunsaturated to saturated fat ratio.

Two of the 13 participants did not complete the food record.

### Table 2. Average Nutrient Content of Experimental Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Protein (% of energy)</th>
<th>14.0</th>
<th>14.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>Percent of energy</td>
<td>39.4</td>
<td>31.1</td>
</tr>
<tr>
<td>SFAs</td>
<td>15.4</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>Palmitic</td>
<td>7.7</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Stearic</td>
<td>2.7</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>MFAs</td>
<td>11.8</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>Oleic</td>
<td>11.4</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>PUFAs</td>
<td>6.0</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Linoleic</td>
<td>5.2</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>SFA/MFA/PUFA</td>
<td>2.6:2.0:1.0</td>
<td>2.9:1.8:1.0</td>
<td></td>
</tr>
<tr>
<td>PUFASFA</td>
<td>0.39</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates (% of energy)</td>
<td>46.6</td>
<td>54.3</td>
<td></td>
</tr>
<tr>
<td>Mono- and disaccharides*</td>
<td>16.0</td>
<td>23.5</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/MJ)*</td>
<td>36.2</td>
<td>31.1</td>
<td></td>
</tr>
<tr>
<td>Dietary fiber (g/MJ)</td>
<td>2.3</td>
<td>2.2</td>
<td></td>
</tr>
</tbody>
</table>

Values based on the Danish nutrient data base. All other values were obtained from chemical analysis of duplicate food portions.

FAs, fatty acids; S, saturated; M, monounsaturated; P, polyunsaturated; SFA/MFA/PUFA, S to M to P fat ratios; PUFASFA, P to S fat ratio.

Statistics

The effect of the experimental diets on the measured variables was compared by Wilcoxon's matched-pairs signed-rank test. Correlation analysis was also performed nonparametrically (Spearman's rank correlation). A 5% significance level was chosen.

Experimental Diets

The experimental diets (A and B) consisted of three daily main meals and an evening snack in repeated 7-day menus. They were served according to individual energy needs as estimated from height, weight, gender, and habitual physical activity. All food items used were weighed on precision scales, and all meals were prepared in our metabolic kitchen. On weekdays, lunch and dinner were served and eaten while the subjects were under observation at the department. Breakfast and weekend meals were supplied prepackaged to be eaten at home. The participants were allowed to drink water, tea, and coffee without additives in free amounts during the experimental periods.
The first 10 ml of blood was collected in tubes without additives for lipid analysis. Subsequently, we collected 5 ml in a citrated tube (Vacutainer 606608, Becton Dickinson, Meylan-Cedex, France) for factor VII coagulant activity (FVIIc) analysis, 5 ml in a tube with citrate and e-aminocaproic acid (Venoject VT-050CA, Terumo Europe, Leuven, Belgium) for determination of fibrinogen, and 2×5 ml in precooled citrated tubes (Vacutainer 606608) for fibrinolytic assays. All samples were spun for 15 minutes at 3,000g. The precooled tubes were spun at 3°C, the others at 18°C. The separated plasma was pipetted into plastic vials, then rapidly frozen at −50°C within 2 hours and stored at −80°C. Serum for lipid analysis was kept at −20°C. Analysis was performed in duplicate and, in one series for each participant, within 6 months of sampling.

Plasma FVIIc was measured in a one-stage clotting assay with human factor VII–deficient plasma (Sigma Chemical Co., St. Louis, Mo.) and human thromboplastin. Results are expressed relative to a reference plasma pool (percent). The plasma concentration (micromoles per liter) of clottable fibrinogen was determined by a modified Clauss assay.16 The euglobulin fraction of plasma (precipitated at pH 5.9, dilution 1:9) was applied on plasminogen-rich fibrin plates without or in the presence of excess Cl− and, in one series for each participant, within 6 months of sampling.

Plasma FVIIc was measured in a one-stage clotting assay with human factor VII–deficient plasma (Sigma Chemical Co., St. Louis, Mo.) and human thromboplastin. Results are expressed relative to a reference plasma pool (percent). The plasma concentration (micromoles per liter) of clottable fibrinogen was determined by a modified Clauss assay.16 The euglobulin fraction of plasma (precipitated at pH 5.9, dilution 1:9) was applied on plasminogen-rich fibrin plates without or in the presence of excess Cl− and, in one series for each participant, within 6 months of sampling.

The total plasma antigen concentrations (nanograms per milliliter) of t-PA and PAI-1 were determined by enzyme-linked immunosorbent assay methods (Biopool Imulyse 5 t-PA and TintElize PAI-1; Biopool, Umeå, Sweden). Plasma PAI activity was determined by an amidolytic assay and expressed as international units t-PA per milliliter.18 Enzymatic methods were used to assess serum concentrations (millimoles per liter) of total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides (Boehringer Mannheim GmbH, Mannheim, FRG). Serum C-reactive protein (milligrams per liter) was assessed by an immunochemical method (Orion Diagnostica, Espoo, Finland).

**Results**

The participants’ fasting body weights were monitored during the study. None of the diets were associated with a significant change in body weight (median change, 0.0 kg; range, −1.4 to 2.1 kg).

Blood samples taken at study entry showed the participants to be at low risk for cardiovascular disease, exhibiting a low median PAI-1 antigen concentration and PAI activity, low levels of serum total cholesterol and triglycerides, and a median HDL cholesterol close to 1.50 mmol/l (Table 3). The coagulation factors FVIIc and fibrinogen showed no significant deviations from baseline values during the experiment and did not differ between the two diets (Table 3). However, plasma fibrinogen tended to be higher on the low-fat diet B (p=0.06). Both experimental diets were associated with an insignificant increase in the median plasma PAI-1 antigen concentration and PAI activity compared with baseline values and a concomitant decrease in median plasma euglobulin fibrinolytic activity (baseline versus diet A: p=0.03) and t-PA activity (Table 3). No significant differences in fibrinolytic variables were noted between diets A and B (Table 3).

Serum concentrations of total cholesterol, HDL cholesterol, and triglycerides stayed close to habitual levels during the intervention periods (Table 3). Serum triglycerides tended to be higher on the low-fat diet B than on diet A, but the difference did not reach statistical significance (p=0.08).

The baseline values were analyzed for associations between serum total cholesterol, triglyceride levels, and hemostatic variables and for mutual associations between fibrinolytic variables. The blood lipids showed no significant associations with the hemostatic variables. Plasma PAI-1 antigen concentrations correlated significantly with plasma euglobulin fibrinolytic activity (r = −0.72, p=0.009), t-PA activity
(r_s = -0.77, p = 0.004), and PAI activity (r_s = 0.71, p = 0.01). Similarly, PAI activity and measures of plasma fibrinolytic activity were inversely associated. Plasma t-PA antigen concentrations showed no significant association with euglobulin fibrinolytic activity (r_s = 0.55, p = 0.07), t-PA activity (r_s = 0.48, p = 0.11), or plasma PAI-1 antigen levels (r_s = -0.43, p = 0.14).

**Discussion**

The present strictly controlled crossover trial studied dietary effects on blood coagulation and fibrinolysis. Furthermore, blood lipids were monitored. Our study thus gives a comprehensive impression of the short-term effect of a change in the dietary fat content from 39 to 31 energy percent at a fixed fatty acid composition and a fixed dietary fiber intake on the cardiovascular risk profile of healthy individuals.

We reported earlier that FVIIc and factor VII protein concentrations decreased from habitual levels in young students fed a low-fat, high-fiber diet for 2 weeks. This observation indicated that the dietary fat quantity might be an important determinant of factor VII levels, as had been earlier stated by Miller et al. In the present study, this hypothesis was tested and could be rejected: FVIIc was virtually unaffected by the change in total fat content from 39% to 31% of energy. The contrasting results of Miller et al may be explained by their use of experimental diets that had an extremely high or very low fat content and that differed substantially with regard to several other nutrients, as well as energy content.

In our earlier study, we showed that FVIIc changed irrespective of serum cholesterol, and furthermore, we could demonstrate that a change in fat quality does not influence FVIIc. Similar observations were recently reported from a study by Miller et al. Taken together, results of the present and earlier dietary experiments could indicate that concomitant changes in several dietary characteristics, for example, a decrease in total fat and an increase in dietary fiber, are necessary to provoke factor VII changes.

Fibrinolytic variables may be favorably modified by low-fat, high-fiber diets. However, the single dietary characteristic causing such modifications is unknown. According to our findings, it is unlikely that an isolated reduction in the amount of dietary fat could be influential. Plasma euglobulin fibrinolytic activity, t-PA or PAI activity, or the antigen concentrations of PAI-1 and t-PA did not differ on the two experimental diets. We have shown earlier that consumption of fish affects the t-PA–PAI system, and corresponding results have been obtained by others. It is therefore tempting to speculate that the fatty acid composition plays a role in the dietary modification of fibrinolysis. This could help explain the results of the earlier studies. However, specifically designed dietary trials are needed to confirm this suggestion.

The dietary fat quality has a strong and well-known effect on blood lipids. In the present study, it was demonstrated that a change in the quantity of dietary fat has virtually no effect on serum total cholesterol and HDL cholesterol concentrations at the levels of fat content studied. This is in good agreement with the findings in comparable trials. However, in the studies of Weisweiler et al and Jones et al, serum triglycerides were found to increase as volunteers were shifted from high-fat to low-fat diets. There was no such significant change in our study, although the triglyceride level did tend to be slightly higher on the low-fat diet than on the high-fat diet. The statistical insignificance of our finding may be due to the lower initial triglyceride levels of our study participants compared with those of Weisweiler’s volunteers and to the smaller change in total fat content studied here compared with that studied by Jones et al.

The inverse correlation between plasma PAI-1 antigen levels and plasma euglobulin and t-PA activity reported in the present study underlines the well-known prime importance of PAI-1 as an inhibitor of plasma fibrinolytic activity. We were unable to demonstrate the associations between serum triglyceride and plasma levels of FVIIc and PAI-1 antigen observed in other studies. This may be explained by the narrow range of blood lipid values in our study population.

In conclusion, our trial showed that a reduction in dietary fat from 39 to 31 energy percent at a fixed fatty acid composition and a fixed dietary fiber intake was not associated with changes in blood coagulation, fibrinolysis, or blood lipids in the fasting state.

**Acknowledgments**

We thank our expert dietitian, Hanne Jensen, and the staff of the metabolic kitchen and our laboratories for the excellence of their work. The Central Laboratory A of the National Food Agency of Denmark analyzed the food samples.

**References**

Marckmann et al. Dietary Fat and Risk Markers of IHD 205


**KEY WORDS** • dietary fat • factor VII • fibrinolysis • serum cholesterol • fat quantity • diet
Fasting blood coagulation and fibrinolysis of young adults unchanged by reduction in
dietary fat content.

P Marckmann, B Sandström and J Jespersen

doi: 10.1161/01.ATV.12.2.201

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/12/2/201

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright
Clearance Center, not the Editorial Office. Once the online version of the published article for which permission
is being requested is located, click Request Permissions in the middle column of the Web page under Services.
Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online
at:
http://atvb.ahajournals.org//subscriptions/