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. 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 fibrinolysis 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. 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). 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 base (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-
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 prepacked to be eaten at home. The participants were allowed to drink water, tea, and coffee without additives in free amounts during the experimental periods.

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.

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>Kcal/day</th>
<th>Protein (% of energy)</th>
<th>Fat Percent of energy</th>
<th>SFA</th>
<th>PUFA</th>
<th>PUFAs</th>
<th>Carbohydrates Percent of energy</th>
<th>Alcohol (g/MJ)</th>
<th>Cholesterol (mg/MJ)</th>
<th>Dietary fiber (g/MJ)</th>
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<tr>
<td></td>
<td>11.3</td>
<td>(8.8-14.3)</td>
<td>(12-17)</td>
<td>35 (29-37)</td>
<td>15</td>
<td>5</td>
<td>0.32</td>
<td>48 (37-57)</td>
<td>4 (0-10)</td>
<td>41.1 (25.9-68.2)</td>
<td>3.1 (2.5-3.7)</td>
</tr>
<tr>
<td></td>
<td>(2,703</td>
<td>(2,102-3,402)</td>
<td></td>
<td>(9-20)</td>
<td>(3-7)</td>
<td>(0.27-0.45)</td>
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</table>

Values are mean and (range).

*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>Fat Percent of energy</th>
<th>SFA</th>
<th>PUFA</th>
<th>PUFAs</th>
<th>Carbohydrates (% of energy)</th>
<th>Cholesterol (mg/MJ)</th>
<th>Dietary fiber (g/MJ)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>14.0</td>
<td>14.7</td>
<td></td>
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</table>

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

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.
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−-inactivator to assess euglobulin and t-PA fibrinolytic activity (r2= -0.72, p=0.009), t-PA activity

**Table 3. Blood Lipids and Hemostatic Variables of 13 Healthy Individuals at Study Entry and After 2 Weeks on Diet A (39% of Energy From Fat) and Diet B (31% of Energy From Fat)**

<table>
<thead>
<tr>
<th></th>
<th>Study entry</th>
<th>Diet A</th>
<th>Diet B</th>
</tr>
</thead>
<tbody>
<tr>
<td>S total cholesterol (mmol/l)</td>
<td>4.22 (3.31–5.38)</td>
<td>4.39 (3.31–5.22)</td>
<td>4.35 (3.29–5.47)</td>
</tr>
<tr>
<td>S HDL cholesterol (mmol/l)</td>
<td>1.46 (0.81–1.83)</td>
<td>1.42 (0.73–1.70)</td>
<td>1.31 (0.71–1.83)</td>
</tr>
<tr>
<td>S triglyceride (mmol/l)</td>
<td>0.71 (0.50–1.61)</td>
<td>0.65 (0.52–1.20)</td>
<td>0.71 (0.48–1.29)</td>
</tr>
<tr>
<td>P FVIIc (%)</td>
<td>107 (92–139)</td>
<td>108 (89–135)</td>
<td>108 (83–130)</td>
</tr>
<tr>
<td>P fibrinogen (µmol/l)</td>
<td>6.3 (5.0–8.7)</td>
<td>5.8 (5.0–8.2)</td>
<td>6.3 (5.1–8.9)</td>
</tr>
<tr>
<td>P EF activity (mIU/ml)</td>
<td>1,698 (742–2,885)</td>
<td>1,031 (350–2,750)*</td>
<td>1,187 (588–2,607)*</td>
</tr>
<tr>
<td>P t-PA activity (mIU/ml)</td>
<td>429 (78–803)*</td>
<td>214 (0–1,021)*</td>
<td>158 (59–955)*</td>
</tr>
<tr>
<td>P t-PA antigen (ng/ml)</td>
<td>2.3 (1.4–5.9)</td>
<td>2.7 (0.1–5.4)*</td>
<td>2.4 (0.9–7.0)*</td>
</tr>
<tr>
<td>P PAI-1 antigen (ng/ml)</td>
<td>3.4 (1.6–9.1)</td>
<td>3.8 (1.5–17.1)*</td>
<td>5.1 (0.6–11.1)*</td>
</tr>
<tr>
<td>P PAI activity (IU/ml)</td>
<td>2.7 (0.0–8.1)*</td>
<td>3.9 (0.0–17.4)*</td>
<td>4.7 (0.0–9.6)*</td>
</tr>
</tbody>
</table>

Values are median and (range).
S, serum; HDL, high density lipoprotein; P, plasma; FVIIc, factor VII coagulant activity; EF, euglobulin fibrinolytic; t-PA, tissue-type plasminogen activator; PAI, plasminogen activator inhibitor.

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 (r2= -0.72, p=0.009), t-PA activity

**Results**

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**R**
factor VII levels, as had been earlier stated by Miller
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.9 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 laborato-
ries for the excellence of their work. The Central
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mark analyzed the food samples.

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Discussion
The present strictly controlled crossover trial studied
dietary effects on blood coagulation and fibrino-
lysis. 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 lev-
els in young students fed a low-fat, high-fiber diet for
2 weeks.10 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.9 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%
percent of dietary fat. Therefore, dietary fat from
39 to 31 percent at a fixed fatty acid composition
and a fixed dietary fiber intake were not sufficient
to provoke factor VII changes.

Fibrinolytic variables may be favorably modified by
low-fat, high-fiber diets.5–8 However, the single di-
etary 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 fibrino-
lytic 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
in our earlier study, we showed that FVIIc changed irrespective of serum cholesterol, and fur-
thermore we could demonstrate that a change in fat
gut quality does not influence FVIIc.10 Similar observa-
tions were recently reported from a study by Miller et
al.19 Taken together, results of the present and
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**KEY WORDS** • dietary fat • factor VII • fibrinolysis • serum cholesterol • fat quantity • diet
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