Abstract We periodically obtained blood samples from mildly hypercholesterolemic, but otherwise healthy, premenopausal women who were recruited to participate in a study of a long-term, cholesterol-lowering diet. All meals were prepared and most meals were consumed in the study center dining facility. Tests performed on blood samples included fibrinogen, cholesterol, factor VII coagulant activity (VII), and other measures of factor VII. We found that when women switched from a typical American diet (37% fat, polyunsaturated fatty acid to saturated fatty acid [P/S] ratio 0.5, 300 mg cholesterol/d) to a diet lower in fat and cholesterol (American Heart Association phase 2 diet: 30% fat, P/S ratio of 1, 150 to 200 mg cholesterol/d) and maintained that diet for 20 weeks, their plasma cholesterol levels decreased by approximately 6% after 4 weeks and remained at that level until study termination. Likewise, VII, decreased by approximately 11% while factor VII antigen, total factor VII activity, and fibrinogen concentration did not change appreciably from baseline values. Our results show that premenopausal women benefit from a diet lower in total and saturated fat by a reduction in blood cholesterol and VII. Extrapolation from data on men in the Northwick Park Heart Study indicates that the 11% decrease in VII, activity would correspond to an approximately 30% decrease in risk of mortality from coronary heart disease.

Key Words • women • diet • fat • factor VII • cardiovascular disease

In many affluent societies, coronary heart disease (CHD) is the major cause of mortality in men and women, accounting for about 265,000 deaths in men and 245,000 deaths in women per year in the United States during 1987 to 1988.1 The incidence of the disease is much lower in women than in men, especially at younger ages,2 possibly owing to hormonal differences between the sexes until the time of menopause.3

An elevated plasma total cholesterol concentration is a risk factor for CHD in both sexes, although at equivalent concentrations the risk in women is considerably less than in men.4,5 These findings led to the assumption that in both sexes, lowering plasma cholesterol levels would decrease the risk of CHD. Intervention studies designed to test this hypothesis have focused on men because of the sex difference in incidence rates. The reduction of high plasma cholesterol concentrations by pharmacological methods has been shown to reduce the death rate from CHD in men,6-10 but this approach remains to be tested rigorously in women. Nevertheless, the National Cholesterol Education Program (NCEP) and American Heart Association (AHA) guidelines for plasma cholesterol reduction by dietary modification as a first step are being applied equally to men and women in all hypercholesterolemic populations.

A high plasma cholesterol concentration is believed to increase the risk of CHD by accelerating the development of atheroma in the coronary arteries. In most cases the major, acute, clinical presentations of CHD, ie, unstable angina pectoris, acute myocardial infarction, and sudden cardiac death, are not due to atheroma per se but to thrombotic occlusion of a coronary artery at the site of fissuring or rupture of an atheromatous plaque. There is epidemiological evidence that the extent and severity of this major complication of coronary atheroma are related to blood coagulability. Thus, several prospective surveys have shown a raised plasma fibrinogen concentration to be associated with an increased risk for CHD in men11-13 and women.12 In the Northwick Park Heart Study,11 middle-aged men with high factor VII coagulant activity (VII) were shown to be at increased risk for CHD, but similar studies have yet to be conducted in women. Both NCEP and AHA guidelines recommend a reduction in total fat intake to about 30% of dietary energy, and there is limited experimental evidence to indicate that such dietary modification will be accompanied by a rapid decrease in VII,14-16 provided that the dietary change also induces a reduction in plasma triglyceride levels.17 Many studies have shown VII, to be positively associated with plasma triglyceride levels in both sexes.18-20 On this account, we periodically measured VII, factor VII antigen concent-
traction (VII: Ag), total factor VII activity, plasma fibrinogen, and plasma cholesterol levels in mildly hypercholesterolemic women who participated in a long-term, tightly controlled feeding study of a nationally recommended cholesterol-lowering diet.

**Methods**

**Subjects**

Fremenopausal women aged 20 or older were screened for plasma cholesterol concentration. Women with total cholesterol values ≥50th percentile on the basis of age-adjusted tables were evaluated further for inclusion in the study. Exclusion criteria were previous hysterectomy, known heart disease, hypertension, pregnancy, irregular menstrual cycles or amenorrhea, smoking, food allergy, medication usage, or conditions that could affect lipid metabolism, such as diabetes or abnormal thyroid function. Women who used oral contraceptives were allowed into the study, with the condition that the type and dosage of medication remain unchanged throughout the study. Only those women who agreed to refrain from ingestion of aspirin or aspirin-like drugs (eg, ibuprofen) and alcoholic beverages during the study period were considered eligible to participate and were provided with a list of common agents containing aspirin and aspirin-like drugs. Those who agreed to participate in the study signed informed-consent forms approved by the Institutional Review Board of the University of Illinois at Chicago and underwent thorough physical examinations, which included an SMAC 20, hematology, urinalysis, Pap smears, and pregnancy testing before acceptance into the study. Twenty-four women started the study and completed the entire protocol with acceptable compliance. Seven subjects dropped out during the first 4 weeks of the study (during the baseline period) because of an inability to comply with the time-consuming requirements of the study schedule.

**Diet and Diet Management**

Diet composition and management have been described previously. Diets were developed by using commercially available foods and methods of preparation that could be implemented by the public. The diets were prepared and fed to participants at the Nutrition and Metabolism Research Laboratory of the Department of Nutrition and Medical Dietetics, University of Illinois at Chicago, under supervision of the study staff. To stabilize nutritional intake and establish a common baseline, during the first 4 weeks of the study the women consumed a diet that was representative of the typical American diet (TAD) for women. This diet consisted of 37% fat, 300 mg cholesterol/d (at 8300 kJ intake), and a polyunsaturated to saturated fat (P/S) ratio of 0.5. During the remaining 20 weeks, participants were fed a diet in accordance with phase 2 of the AHA diet. Daily meals for the phase 2 diet were designed to maintain a daily consumption of 30% total fat, 150 mg cholesterol/d, and a P/S ratio of 1. Diet composition was calculated by using the Minneapolis Nutrition Coding Center Data Base and confirmed by proximate chemical analysis (Hazelton Laboratories). Selected lipid and fatty acid contents are listed in Table 1. Both diets met or exceeded all recommended daily allowances for vitamins and minerals. Four basic daily diets of 7100, 8300, 9500, and 11 700 kJ were constructed.

Body weights were recorded and graphed daily, and subjects were changed to different energy levels if their weight deviated from their previous weight by 1.1 kg for more than 3 days. For discretionary intake, cookies, standardized by weight and meeting the diet specifications for fat, carbohydrate, protein, cholesterol, and P/S ratio, could be consumed up to 850 kJ/d, and the amounts consumed were recorded daily. Meal plans were based on a 4-day-cycle menu, and diets were served as three meals per day. Generally, two of the three meals were consumed at the laboratory dining room each day, 6 days per week. The remaining meals were packaged for consumption away from the laboratory dining center. Two cups of coffee or sugar-free caffeinated soda were allowed each day as well as ad libitum sugar-free, non–caffeine-containing beverages and chewing gum. Up to one package per day of LifeSavers (Planters LifeSavers Co) was also allowed. Unlike the participants in a previous study who consumed a 20% fat diet and who lost weight despite attempts to maintain body weight, the participants in this study, who consumed the 30% fat diet, were able to maintain body weight over the study period.

Dietary compliance was evaluated by measuring plate waste and by daily self-report. Each day, subjects completed a form that listed the types and amounts of added (nonstudy) food or beverage consumed and of study food not eaten, activity levels, medication used, and illnesses during the previous 24 hours. These forms were reviewed weekly by the study coordinator. Every subject reported occasional infractions, and there were occasional reports of consumption of an alcoholic beverage; however, in no case was the consumption of nonstudy food or beverage accounted for 3% or more of the total energy consumed. A participant was deemed noncompliant if nonstudy foods accounted for 3% or more of the total energy consumed. It should be noted that changes in alcohol consumption could influence some of the analyses. However, this population had been prescreened as a nondrinking population. In addition, we believe that the participants accurately recorded their alcohol intake along with other nonstudy foods that they had freely reported.

**Laboratory Analyses**

After 3 and 4 weeks on the TAD and after 4, 16, and 20 weeks on the phase 2 diet, venous blood samples for platelet and clotting factor measurements were collected into tubes containing citrate anticoagulant (1 part to 9 parts blood) before breakfast and after a 12- to 14-hour fast. Values for

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### Table 1. Selected Fatty Acid Intake for Control and Phase 2 Diets at the 8300-kJ Level of Consumption

<table>
<thead>
<tr>
<th>Constituent and Amount</th>
<th>Control Diet</th>
<th>Phase 2 Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mg</td>
<td>300.56</td>
<td>163.04</td>
</tr>
<tr>
<td>Total SFAs, g</td>
<td>35.15</td>
<td>19.88</td>
</tr>
<tr>
<td>Total MUFA s, g</td>
<td>25.33</td>
<td>20.06</td>
</tr>
<tr>
<td>Total PUFA s, g</td>
<td>14.39</td>
<td>17.05</td>
</tr>
<tr>
<td>Percent calories from SFAs</td>
<td>16.40</td>
<td>9.32</td>
</tr>
<tr>
<td>Percent calories from MUFA s</td>
<td>11.82</td>
<td>9.41</td>
</tr>
<tr>
<td>Percent calories from PUFAs</td>
<td>6.71</td>
<td>7.99</td>
</tr>
<tr>
<td>SFA 16:0 (palmitic), g</td>
<td>17.28</td>
<td>9.87</td>
</tr>
<tr>
<td>SFA 18:0 (stearic), g</td>
<td>7.32</td>
<td>4.24</td>
</tr>
<tr>
<td>MUFA 18:1 (oleic), g</td>
<td>22.83</td>
<td>18.78</td>
</tr>
<tr>
<td>PUFA 18:2 (linoleic), g</td>
<td>12.30</td>
<td>15.05</td>
</tr>
<tr>
<td>PUFA 18:3 (linolenic), g</td>
<td>1.55</td>
<td>1.44</td>
</tr>
<tr>
<td>PUFA 20:4 (arachidonic), g</td>
<td>0.12</td>
<td>0.13</td>
</tr>
<tr>
<td>PUFA 20:5 (EPA), g</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>PUFA 22:5, g</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>PUFA 22:6 (DHA), g</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>P/S</td>
<td>0.41</td>
<td>0.36</td>
</tr>
</tbody>
</table>

SFA(s) indicates saturated fatty acid(s); MUFA(s), monounsaturated fatty acid(s); PUFA(s), polyunsaturated fatty acid(s); EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; and P/S, polyunsaturated to saturated fatty acid ratio.
TABLE 2. Fibrinogen, Cholesterol, Triglyceride, and Factor VII Concentration in Plasma of Premenopausal Women Following the American Heart Association Phase 2 Diet

<table>
<thead>
<tr>
<th>Time of Measurement</th>
<th>Fbg, mg/dL</th>
<th>VII:Ag, %*</th>
<th>VIIit, %*</th>
<th>VIIlc, %*</th>
<th>Chol, mg/dL</th>
<th>Net TRG, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>321±18</td>
<td>83.3±3.3</td>
<td>99.6±6.7</td>
<td>79.8±5.6</td>
<td>200±6.8</td>
<td>105.3±15</td>
</tr>
<tr>
<td>4 Weeks</td>
<td>325±16</td>
<td>82.8±3.3</td>
<td>94.6±5.5</td>
<td>71.1±6.6</td>
<td>192±6.8</td>
<td>105.6±15</td>
</tr>
<tr>
<td>16 Weeks</td>
<td>325±19</td>
<td>82.9±3.1</td>
<td>88.1±7.3</td>
<td>69.9±5.4</td>
<td>187±5.8†</td>
<td>114.0±14</td>
</tr>
<tr>
<td>20 Weeks</td>
<td>314±16</td>
<td>81.3±4.4</td>
<td>94.7±6.6</td>
<td>67.7±6.3‡</td>
<td>190±6.6</td>
<td>110.8±16</td>
</tr>
</tbody>
</table>

Fbg indicates fibrinogen; VII:Ag, total factor VII antigen; VIIit, total factor VII activity; VIIlc, factor VII coagulant activity; Chol, cholesterol; and TRG, triglyceride. Data are expressed as mean±SE.

*Percent of that in standard plasma.
†P≤.005, ‡P≤.001.

Statistical Methods

Data were analyzed for differences by ANOVA. Results of analyses were considered statistically significant at P≤.05.

Results

Of the 22 women with baseline values, 17 women completed the entire 24-week study, and we obtained all required blood samples and complete data on 16 participants. Of these 16, data from one participant were not used because of 2 of 5 samples appeared to have been partially activated during handling, and data from a second were not used because the participant was particularly difficult to phlebotomize and thus, sample integrity could not be ensured. Therefore, complete datasets were analyzed for 14 women, none of whom were taking oral contraceptives.

Results are shown in Table 2 and Figs 1 and 2. The mean baseline fibrinogen concentration (average of measurements after 3 and 4 weeks on the TAD) was 321 mg/dL. When compared with baseline values, no change was detected after 4, 16, or 20 weeks on the phase 2 diet. Similar results were obtained for VII:Ag, with the mean values for the phase 2 diet not differing significantly from baseline values. Although there appears to be a trend to lower total factor VII activity levels at 4 and 16 weeks on the phase 2 diet, this was not sustained at 20 weeks. In addition, when all total factor VII activity values were combined and compared with baseline, there was no significant difference. In contrast, VIIlc on the phase 2 diet decreased steadily, from a mean of 79.8% of standard on the TAD to 67.7% of standard at...
completion of the study. The difference from baseline approached significance at 4 and 16 weeks and was significant by 20 weeks. When all VIIc values obtained during the phase 2 diet were combined and compared with the baseline level, the difference was highly significant (P<.0001). Results are also shown in Fig 2, in which VIIc at each time point is expressed in terms of its percent change from baseline. Most of the reduction in VIIc occurred within the first 4 weeks on the phase 2 diet. Although the number of subjects is small and outlying values can disproportionately affect parametric statistics, the small standard errors in Table 2 would seem to exclude any serious skewing or outlier effects.

Although total plasma cholesterol decreased and remained so throughout the phase 2 diet, the difference from baseline was significant only at 16 weeks (P<.005; Table 1 and Fig 2). If, as for VIIc, all cholesterol values on the phase 2 diet are combined and compared with baseline, the difference becomes highly significant (P<.001, data not shown), although the magnitude of the decrease is not as large as for VIIc. Note that in the larger cohort of women who began the study, the decrease in cholesterol was approximately the same and did not reach significance at each of the three time points when measurements were made (data not shown).

**Discussion**

In agreement with previous studies, no relation was observed between plasma fibrinogen concentration and dietary fat intake. For the premenopausal women in this study, fibrinogen levels did not change significantly over the entire 6 months of observation. Similarly, no change was observed in VIIc:Ag on adoption of a lower-fat diet, and although the functional factor VII level was slightly lower on the phase 2 than on the baseline diet, the reduction was not statistically significant. In contrast, VIIc was significantly reduced within 4 weeks of commencement of the phase 2 diet and remained at this lower level until termination of the study. Laboratory drift could be excluded as a reason for the changes in VIIc observed, because samples collected during the entire study were analyzed in randomized order on one day.

In the absence of significant changes in VIIc:Ag or functional factor VII levels, the decrease in VIIc on the phase 2 diet was most likely due to a reduction in the activity state of circulating factor VII. In healthy adults, factor VII circulates at a concentration of about 450 ng/mL with a half-life of less than 5 hours. About 4 ng/mL is present as the active enzyme factor VIIa and the remainder exists as its zymogen precursor. Coagulant activity is expressed when factor VIIa forms a complex with its cofactor, TF, a surface-bound protein beneath the vascular endothelium that is present on many cell types. The factor VIIa-TF complex then cleaves factors IX and X to their active enzymes, thereby initiating the extrinsic coagulation pathway. Small reductions in factor VIIa levels during the phase 2 diet period could explain the findings of the present study, but confirmation of this possibility requires direct measurement of factor VIIa levels with a new assay that was not available when the work was undertaken.

As shown in Table 2, the change from a 37% fat diet to one containing 30% fat with increased carbohydrate was associated not only with decreases in VIIc and blood cholesterol but also with a 3% to 11% increase (not statistically significant) in fasting triglyceride concentration as has been reported previously. This reduction in VIIc, in the presence of an elevation in fasting triglyceride level, also observed in an earlier study, seems to conflict with the positive relation between these variables that is found consistently in cross-sectional observational studies. However, both VIIc and fasting triglyceride levels are positively associated with the triglyceridemic response to a fatty test meal so that the cross-sectional positive relation may simply reflect this mutual association, even when measurements are made on fasting morning samples. After a heavy evening meal, true fasting levels of triglyceride are not achieved until the last few hours of the night, and the half-life of circulating factor VIIa is about 2 to 3 hours; hence, the effects of postprandial lipemia on VIIc can be carried through into the fasting state. Indeed, in the absence of a fatty breakfast, this effect is seen as a continuing decline in VIIc throughout the morning. Reductions in postprandial triglyceride levels, whether a result of decreased fat intake (as in this study) or efficient clearance of exogenous triglyceride in individuals with low fasting triglyceride levels, could explain the relation of VIIc with triglyceride concentrations that has been observed in this intervention and other observational studies.

The mechanism whereby dietary fat intake influences VIIc and the activity state of factor VII is uncertain. Factor VII can be converted into its active enzyme (factor VIIa) by factor XIa of the contact system and factor Xa of the intrinsic coagulation pathway, which cleave the single-chain zymogen to the two-chain enzyme without a requirement for TF. In addition, factor Xa can activate factor VII in the presence of phospholipid and calcium, a process that is markedly enhanced by TF. In vitro, the contact coagulation system is activated when citrated plasma is exposed to negatively charged surfaces, such as kaolin, glass, or sulfatide vesicles, which promote generation of factor XIIa. In turn, factor XIIa converts prekallikrein to kallikrein and factor XI to factor XIa, both of which can activate factor IX of the intrinsic coagulation pathway. Thus, contact-surface activation of the hemostatic system will increase VIIc by promoting factor VII activation.
In this regard, long-chain saturated fatty acids in micellar form also provide a potent contact surface for in vitro activation of purified factor XII, whereas short-chain saturated fatty acids and unsaturated fatty acids are ineffective in this regard.53 Incubation of citrated plasma with micellar stearic acid at low temperature (to suppress the activity of C1 inhibitor against factor XIIa and kalikrein)24 leads to generation of factor XIIa and activation of factor VII.54 Furthermore, incubation of triglyceride-rich plasma with lipoprotein lipase to release free fatty acids causes a prompt and substantial activation of factor XII.54 These effects raise the possibility that the phase 2 diet induced a reduction in VII, because of its relatively low content of long-chain saturated fatty acids. Two short-term experiments, of 715 and 1416 days' duration, failed to demonstrate an effect of dietary fat composition on VII, independent of the effect associated with total fat intake. However, neither study may have had the power or longevity to induce sufficient changes in plasma free fatty acid levels in response to manipulations in dietary fat composition.

In summary, we have shown that a long-term AHA phase 2 diet, with reduced total fat, saturated fatty acid, and cholesterol contents relative to a TAD, was associated with a sustained decrease in VII, over the entire 5-month duration of the phase 2 diet in premenopausal women. The results of the Northwick Park Heart Study,11 if applicable to women, would suggest that the observed 12% reduction in VII, (average reduction over the entire study) represents an approximately 30% reduction in risk of mortality due to CHD.

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