Effects of Dietary Fat Modification on Fibrinogen, Factor VII, and Plasminogen Activator Inhibitor-1 Activity in Subjects With Impaired Glucose Tolerance

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Our aim was to assess the impact of a monounsaturated fat–enriched (Mono) diet and a diet recommended by the National Cholesterol Education Program (NCEP) on plasma levels of fibrinogen and activities of factor VII (FVII:C) and plasminogen activator inhibitor-1 (PAI-1) and the impact of genetic polymorphisms of these variables (*Hae*III, *Msp*I, and 4G/5G polymorphisms, respectively) in 28 subjects with impaired glucose tolerance ([IGT] 17 men and 11 women; mean age, 55.6 ± 5.5 years). A diet rich in fat and saturated fatty acids served as a baseline diet for 3 weeks. Thereafter, subjects were randomized for the next 8 weeks to either the Mono diet (n = 12) or NCEP diet (n = 16). Fibrinogen levels or PAI-1 activities did not change with either of the diets, but fibrinogen levels were higher (3.4 ± 0.5 *v* 4.0 ± 0.6 g/L, *P* = .007 at baseline) throughout the study in heterozygous subjects with respect to *Hae*III polymorphism. This polymorphism and age accounted for 38% of the variation of fibrinogen levels. *Msp*I polymorphism together with body mass index explained 51% of the variation of FVII:C, which was higher in subjects with the M1M1 genotype compared with M1M2/M2M2 genotypes (127% ± 21% *v* 90% ± 12%, *P* < .001). FVII:C showed a decrease with the NCEP diet (*P* < .05), but the decline was confined to M1M1 subjects. PAI-1 activity did not differ significantly between the genotypes. The insulin sensitivity index (S_I) obtained by the minimal model method was the main explanatory variable of PAI-1 activity. To conclude, despite good compliance, the fat-modified diet did not alter plasma levels of fibrinogen or PAI-1 in white subjects with IGT. FVII:C levels decreased with the NCEP diet, but this was confined to subjects with the M1M1 genotype.

PROSPECTIVE STUDIES have shown that markers of increased blood coagulability such as fibrinogen and factor VII are independent predictors of ischemic heart disease and other cardiovascular events.[1-4] Furthermore, impaired fibrinolytic function measured as elevated plasma plasminogen activator inhibitor-1 (PAI-1) activity is frequently associated with arterial thrombosis,[5-6] and studies on high-risk individuals have shown that reduced fibrinolytic capacity and increased PAI-1 levels are predictors of myocardial infarction.[3,7-9]

The clustering of cardiovascular risk factors such as impaired glucose tolerance (IGT), dyslipidemia (low high-density lipoprotein [HDL] cholesterol and elevated triglyceride serum levels), obesity, and hyperinsulinemia has been termed the insulin resistance syndrome (IRS).[10] Several studies have confirmed that multiple derangements in blood coagulability and the fibrinolytic system are associated with hyperinsulinemia and impaired insulin action,[3,11-15] especially PAI-1 activity. Subjects with IGT have defects in insulin action and associated abnormalities.[10] Therefore, they constitute a good model for studying features of IRS, since the metabolic state is not as severely disturbed as in overt non–insulin-dependent diabetes mellitus (NIDDM).

Levels of fibrinogen, factor VII coagulant activity (FVII:C), and PAI-1 are determined by genetic and environmental factors. Environmental determinants of fibrinogen levels are smoking[16] (elevating) and regular exercise[17] (decreasing), but genetic factors, eg, *Hae*III polymorphism, were the main predictors of fibrinogen levels after smoking in the study by Thomas et al.[18] Dietary interventions have in general had a relatively weak influence on fibrinogen levels.[16]

The decrease in total dietary fat intake has been shown to decrease FVII:C,[19-23] but the effects of dietary fat composition and responses in relation to genetic factors are still relatively weakly known.

PAI-1 levels have been related to hyperinsulinemia, hypertriglyceridemia, and decreased insulin action, and therapeutic measures that decrease serum triglycerides have also decreased PAI-1 activity.[5-6,11-15] However, the role of modifying dietary fat intake with respect to PAI-1 levels is not known, since nearly all dietary studies have also been accompanied by weight loss. Recent cross-sectional studies have suggested that PAI-1 genotype modifies triglyceride regulation.[15,24]

Our aims were to study (1) whether two dietary fat modifications, monounsaturated fat–enriched (Mono) or reduced-fat, polyunsaturated fat–enriched diets, have divergent effects on plasma fibrinogen concentration, FVII:C, and PAI-1 levels; (2) whether the *Hae*III polymorphism of β-fibrinogen, *Msp*I polymorphism of FVII, and 4G/5G polymorphism of PAI-1 have an effect on the respective plasma levels during qualitative dietary fat modification; and (3) the factors associated with fibrinogen, FVII:C, and PAI-1 levels in subjects with IGT.

SUBJECTS AND METHODS

Subjects

Subjects were recruited from a Finnish FinMonica Survey[25] in which an oral glucose tolerance test (OGTT) with a 75-g glucose load was performed on 225 subjects from the Kuopio area in Eastern Finland. The primary criterion for inclusion into the present study was IGT according to the World Health Organization criterion (fasting plasma glucose <7.8 mmol/L and 2-hour postload glucose 7.8 to 11.0 mmol/L).[26] A total of 41 subjects with IGT were screened, and 31 subjects actually participated in the study. For three subjects, hemostatic factors were not measured, and so this study includes 28 IGT subjects (17 men and 11 women). None of the subjects had a history of thyroid,

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kidney, or liver disease or previously diagnosed diabetes or were on lipid-lowering medication, and only two were smokers. At entry to the study, altogether nine subjects had drug treatment for hypertension, two had a history of coronary heart disease, three used β-blockers, and three used diuretics. Two of the women used postmenopausal hormone-replacement therapy. All medication was kept unchanged during the study. Two smokers continued their habit throughout the study.

Study Design

Four weeks before the study (−4 weeks), the subjects underwent the second OGTT and clinical examination including medical history, medication, and basic laboratory measurements (routine hematology, serum thyrotropin, urinary albumin, liver enzymes, and serum creatinine). Subjects meeting the eligibility criterion started the 3-week period during which all subjects followed a similar baseline diet. Afterward, subjects were randomized to either of the test diets. Subjects visited the research unit at baseline (week 0) and at 2, 4, and 8 weeks. Hemostatic factors were examined at −4 weeks, baseline, and 8 weeks. Body weight, percent body fat, blood pressure, and fasting serum lipids were determined at each visit. Percent body fat was measured with an infrared densitometric assay (Futrex 5000; Trebor et Futrex Europe SA, La Garenne, Colombes, France). Intravenous glucose tolerance tests (IVGTTs) were performed and the fatty acid composition of serum triglycerides were determined at baseline and at 8 weeks. Each subject provided informed consent, and the study was approved by the Ethics Committee of the University of Kuopio.

Diets

The composition of the baseline diet for the first 3 weeks was proposed to be 36/18:12:6 percent of total energy (E%), indicating the amount of total fat/saturated:monounsaturated:polyunsaturated fat. The test diets were (1) a generally recommended diet (*National Cholesterol Education Program [NCEP] step 1 diet*)[27] (30/10:10:10 E%) or (2) a diet enriched with monounsaturated fat (Mono, 36/10:18:8 E%). Fatty acid modification was made using different kinds of margarines and oils: butter and low–erucic acid rapeseed oil (LEAR) during the baseline diet, sunflower oil and margarine based on sunflower oil for the NCEP1 diet, and LEAR oil and margarine based on LEAR oil for the Mono diet. In addition, salad dressing made of Trisun oil (high–oleic acid sunflower oil, provided by van den Bergh Foods, Helsinki, Finland) was used to increase the amount of oleic acid in the Mono diet. Dietary fats and salad dressings were provided free of charge, but subjects were not aware of the fatty acid composition. Diets were planned to be isocaloric. Individual caloric intake was estimated using a 4-day food record kept by the subjects before the first visit (−4 weeks). A dietitian provided detailed written and oral instructions on the dietary regimen, specifying the amount and quality of all food items by food groups.

Compliance with the diets was monitored with 4-day food records before the baseline diet, and this was repeated three times before the visits during the experimental diets (altogether 12 days). Calculations for nutrient intake were made using the Nutrica software package for nutrient intake analysis.[28] The fatty acid composition of serum triglycerides was used as an objective marker of compliance with the diets.

IVGTT

For the fasting blood samples and IVGTT, an intravenous catheter was inserted into an antecubital vein on both sides. The modified minimal model method was used as described elsewhere.[29-30] The computer program MINMOD[31] was used for calculation of glucose effectiveness (S_G) and insulin sensitivity index (S_I) from plasma glucose and insulin values obtained during the IVGTT. The area under the insulin curve from 0 to 19 minutes during the IVGTT was calculated to reflect first-phase insulin secretion (ie, before injection of exogenous insulin).

Laboratory Measurements

All samples were taken in the morning between 7 AM and 12 noon after an overnight fast.

Plasma glucose was analyzed by the glucose oxidase method (Glucose Auto & Stat, Model GA-110; Daiichi, Kyoto, Japan) and plasma insulin by a radioimmunoassay method (Phadeseph Insulin RIA 100; Pharmacia Diagnostics, Uppsala, Sweden). Hemoglobin A_{1c} was assayed by liquid cation-exchange chromatography (normal range, 4.0% to 6.0%).

Lipoproteins were separated by ultracentrifugation at density 1.006 to remove very–low-density lipoproteins (VLDLs), followed by precipitation of the infranatant fraction. HDLs were separated with dextran sulfate and magnesium chloride.[32] Low-density lipoprotein (LDL) cholesterol was calculated as the difference between the cholesterol concentration in the infranatant and HDL. Enzymatic colorimetric methods with commercial kits (Monotest Cholesterol and Triglyceride GPO-PAP; Boehringer, Mannheim, Germany) were used for determination of cholesterol and triglycerides from the whole serum and separated lipoprotein fractions. Serum lipoprotein(a) concentration was measured by immunoradiometric assay using Pharmacia reagents.

In the analysis of fatty acid composition of serum triglycerides, serum samples were extracted with chloroform:methanol (2:1) and the lipid classes were separated by solid-phase extraction with an aminopropyl column.[33] Fatty acids were analyzed with a Carlo Erba Vega 6130 gas chromatograph (Carlo Erba Instruments, Milan, Italy) equipped with an NB-351 silica capillary column (HNU-Nordion, Helsinki, Finland).

Hemostatic Factors

Venous blood samples for determination of hemostatic factors were drawn into trisodium citrate tubes between 7 and 9 AM after a 12-hour overnight fast. The collected blood was centrifuged within 30 minutes at 1,400 × *g* for 30 minutes at room temperature, frozen quickly in aliquots, and stored at −70°C.

Fibrinogen level was measured with an ACL 300 R coagulometer (Instrumentation Laboratory, Milan, Italy) from the light scattered by the clot during the prothrombin time assay (PT-Fibrinogen; Instrumentation Laboratory). A single lot of Instrumentation Laboratory calibration plasma (Lexington, MA) was used as a standard throughout the study. The intraassay precision is 3.6% and interassay precision 2.3%. The samples were measured in duplicate. The duplicates varied by less than 10%, or the analysis was repeated using a split sample.

FVII:C level was measured with the one-stage method using rabbit brain thromboplastin (Thromboplastin IS; Baxter Dade, Deerfield, IL) and human immunodepleted FVII-deficient plasma (Behring, Marburg, Germany). The assays were performed with the ACL 300 R coagulometer. A frozen plasma pool was used as a standard. The intraassay precision was 2.4% and interassay precision 3.9%.

PAI-1 activity was measured with a chromogenic method (Coatest PAI; Chromogenix, Mölndal, Sweden). The detection limit is 5 AU/mL and interassay precision 5.5%.

Genetic Analysis

DNA was extracted by the Triton X-100 lysis method.[34] Analysis of the *Hae*III polymorphic site at −453 bp from the start of transcription of the β-fibrinogen gene was performed according to the method used by Thomas et al.[18] *Msp*I polymorphism of FVII, which leads to substitution of an arginine residue by glutamine in the protein, was determined as described by Green et al.[35] The amplified products were digested with *Hae*III and *Msp*I enzymes, respectively. The polymorphic DNA fragments were separated on a 2% agarose gel by electrophoresis. The 4G (deletion)/5G (insertion) polymorphism of PAI-1 was analyzed as described by Dawson et al.[36] Genotyping of the amplified products was

performed by hybridization with allele-specific [γ-^{32}P]ATP-labeled oligonucleotide probes.[36] To confirm the genotyping results, random polymerase chain reaction products were sequenced.[37]

Statistical Analysis

Normal distribution and homogeneity of variance were checked before further analyses. If a variable was not normally distributed, statistical analysis was made after logarithmic transformation. Differences in the means between groups were analyzed with the Student *t* test, and time-related changes (baseline *v* 8 weeks) within groups by paired *t* test. Spearman's or partial correlation coefficients were calculated between selected variables. Adjustments for confounding factors were made by analysis of covariance (ANCOVA) or multiple linear regression analysis. All statistical analyses were performed with the SPSS/PC+ statistical program.[38] Results are presented as the mean ± SD. If not otherwise indicated, the variables reported refer to baseline levels (0 weeks, the period after the standard baseline diet).

RESULTS

Baseline Characteristics

Baseline characteristics of the study subjects are listed in Table 1. Body weight remained stable in both groups (84.8 ± 7.4 *v* 83.8 ± 7.1 kg for the NCEP group and 78.2 ± 11.4 *v* 77.5 ± 11.3 kg for the Mono group, baseline *v* 8 weeks).

Dietary Influences

According to the food records, compliance with both diets was good (Table 2). Results on the fatty acid composition of serum triglycerides were in accordance with nutrient analyses from food records, and indicated that the goals for fatty acid composition of the test diets were well achieved (Table 3). At the end of the study, the diet groups differed from each other with respect to the content of stearic acid in serum triglycerides being lower in the Mono group, whereas oleic and arachidonic acid contents were higher in the Mono group. As expected from dietary changes, serum lipid and lipoproteins improved markedly between baseline and 8 weeks on both diets (total cholesterol, Mono 11% and NCEP 6%; LDL cholesterol, Mono 9% and NCEP 3%; VLDL cholesterol, Mono 18% and NCEP 4%; HDL cholesterol, Mono 0% and NCEP 1%; and total triglycerides, Mono 22% and NCEP 16%). Detailed data on serum lipids by group will be reported elsewhere.[39]

Table 1. Characteristics of the Subjects at the Time of Entry to the Study (-4 weeks) (mean ± SD)

Characteristic	Value
Sex (men/women)	17/11
Age (yr)	55.6 ± 5.5
Body mass index (kg/m²)	30.1 ± 2.6
Waist to hip ratio	0.96 ± 0.08
Plasma glucose (mmol/L)*	
0 h	6.5 ± 1.0
2 h	8.9 ± 2.4
Hemoglobin A_{1c} (%)	5.3 ± 0.5
Fasting insulin (pmol/L)†	91.8 ± 54.0
Serum cholesterol (mmol/L)	6.54 ± 0.94
HDL cholesterol (mmol/L)	1.23 ± 0.25
Serum triglycerides (mmol/L)	2.74 ± 2.25
Systolic blood pressure (mm Hg)	144 ± 15
Diastolic blood pressure (mm Hg)	91 ± 10

*Based on OGTT before the study.
†Measured at baseline (0 weeks).

Table 2. Nutrient Intake on the Basis of Food Records During the Study (mean ± SD)

Nutrient	Baseline Diet (n = 28)	NCEP (n = 16)	Mono (n = 12)
Energy (MJ/d)	7.6 ± 2.5	7.2 ± 2.1	7.6 ± 2.2
Fat (E%)	37 ± 5	34 ± 5	40 ± 3
Fatty acids (E%)			
Saturated	18 ± 3	11 ± 2	11 ± 1
Monounsaturated	11 ± 2	10 ± 2	18 ± 2
Polyunsaturated	5 ± 2	10 ± 2	8 ± 1
Carbohydrates (E%)	44 ± 7	46 ± 5	43 ± 5
Protein (E%)	17 ± 3	18 ± 3	17 ± 3
Alcohol (E%)	2 ± 4	2 ± 3	1 ± 4
Fiber (g/MJ)	3.0 ± 0.8	3.1 ± 0.5	2.8 ± 0.5
Cholesterol (mg/MJ)	41 ± 9	30 ± 7	28 ± 5

Abbreviation: E%, percent of energy.

The responses of hemostatic factors and PAI-1 to the two experimental diets are outlined in Fig 1. Regarding fibrinogen concentration and PAI activity, no differences between the diet groups or time-related changes (diet groups combined) were observed, whereas FVII:C decreased (from 119% ± 24% at 0 weeks to 112% ± 20% at 8 weeks, $P < .05$). Baseline levels of FVII:C were higher in the NCEP group than in the Mono group, but by 8 weeks this difference had vanished due to a slight decrease in FVII:C in the NCEP group. Women had higher FVII:C than men at baseline (132% ± 24% *v* 110% ± 21%, $P < .05$), but no significant differences in this respect were found for fibrinogen or PAI-1 levels (data not shown). Furthermore, there were no significant correlations between fibrinogen, FVII:C, and PAI-1.

Genetic Determinants of Hemostatic Factors

Both diet groups were combined to explore the effects of genetic polymorphism and other variables on hemostatic factors and PAI-1 (Fig 2).

PAI-1 activity did not differ significantly among the three

Table 3. Fatty Acid Composition (% of total molar mass) of Serum Triglycerides in the NCEP and Mono Diet Groups During the Study (mean ± SD)

Fatty Acid	NCEP (n = 16)		Mono (n = 12)		
	0 Weeks	8 Weeks	0 Weeks	8 Weeks	P*
14:0	3.3 ± 0.9	2.6 ± 0.6†	3.6 ± 0.9	2.8 ± 1.1§	
16:0	28.7 ± 4.0	27.4 ± 3.3	29.4 ± 3.4	25.0 ± 4.6‡	
16:1	6.7 ± 2.9	5.9 ± 1.5	5.8 ± 1.3	5.2 ± 1.6	
18:0	4.1 ± 1.0	3.8 ± 0.5	4.0 ± 0.5	3.1 ± 1.0§	.02
18:1	38.2 ± 3.2	35.7 ± 2.5†	37.6 ± 2.7	40.4 ± 7.1‡	.02
18:2(ω-6)	13.5 ± 4.1	18.7 ± 5.0‡	14.5 ± 3.6	17.2 ± 1.8	
18:3(ω-6)	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.2	
18:3(ω-3)	1.3 ± 0.5	1.2 ± 0.5	1.6 ± 0.6	2.1 ± 0.7	
20:4(ω-6)	1.3 ± 0.6	1.5 ± 0.8	1.1 ± 0.4	1.0 ± 0.2	.002
20:5(ω-3)	0.5 ± 0.3	0.6 ± 0.5	0.4 ± 0.2	0.6 ± 0.5	.03
22:6(ω-3)	1.4 ± 0.8	1.5 ± 1.2	1.2 ± 0.9	1.1 ± 0.9	

*Between-group difference at 8 weeks.
†$P < .05$ within-group difference.
‡$P < .001$.
§$P < .01$.

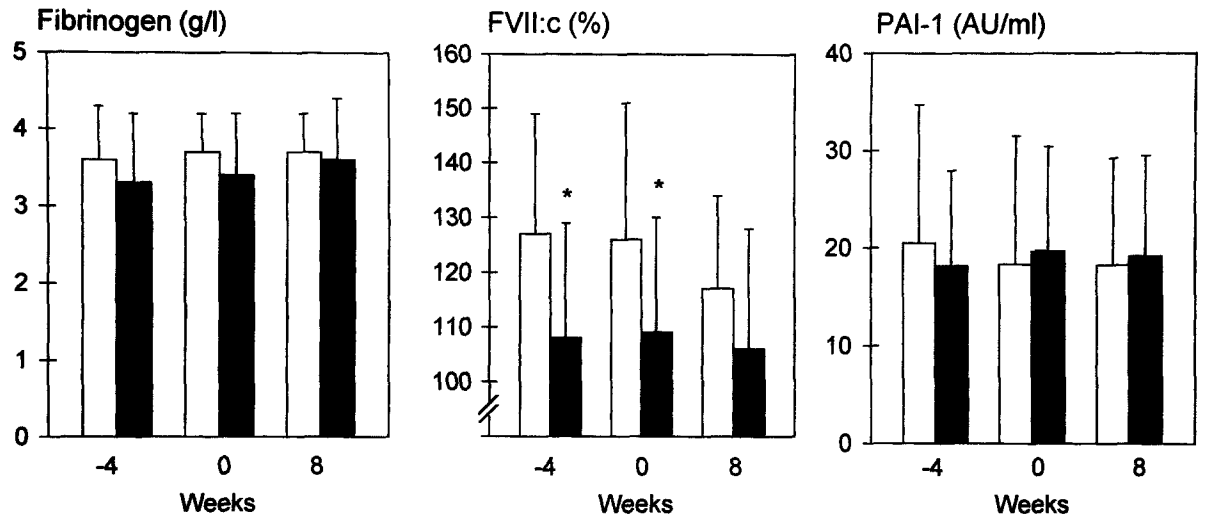

Fig 1. Fibrinogen, FVII:C, and PAI-1 levels during the study according to diet group (mean ± SD). (□) NCEP (n = 16); (■) Mono (n = 12). *$P <$ .05, NCEP *v* Mono.

genotypes and did not respond to either dietary fat modification (Fig 2).

Fibrinogen concentration was consistently higher in subjects heterozygous for *Hae*III polymorphism ($P < .05$ at −4 weeks and baseline, and $P = .08$ at 8 weeks), but the genotype did not contribute to the diet response. After controlling for the effects of age, sex, and body mass index, *Hae*III polymorphism was the only statistically significant explanatory variable for fibrinogen concentration (ANCOVA, F = 14.7, $P = .001$). *Hae*III polymorphism and age accounted for 38% of the variation in fibrinogen concentration according to the stepwise multiple regression analyses when the other factors in the model were sex and body mass index.

FVII:C (Fig 2) was higher in subjects homozygous for the M1 allele at all time points (eg, at 0 weeks, F = 21.68, $P < .001$, adjusted for age, sex, and body mass index), and genetic polymorphism and body mass index explained 51% of the variation of FVII:C. Furthermore, homozygous subjects responded to dietary fat modification by decreasing FVII:C, but in heterozygous subjects FVII:C did not respond to dietary fat

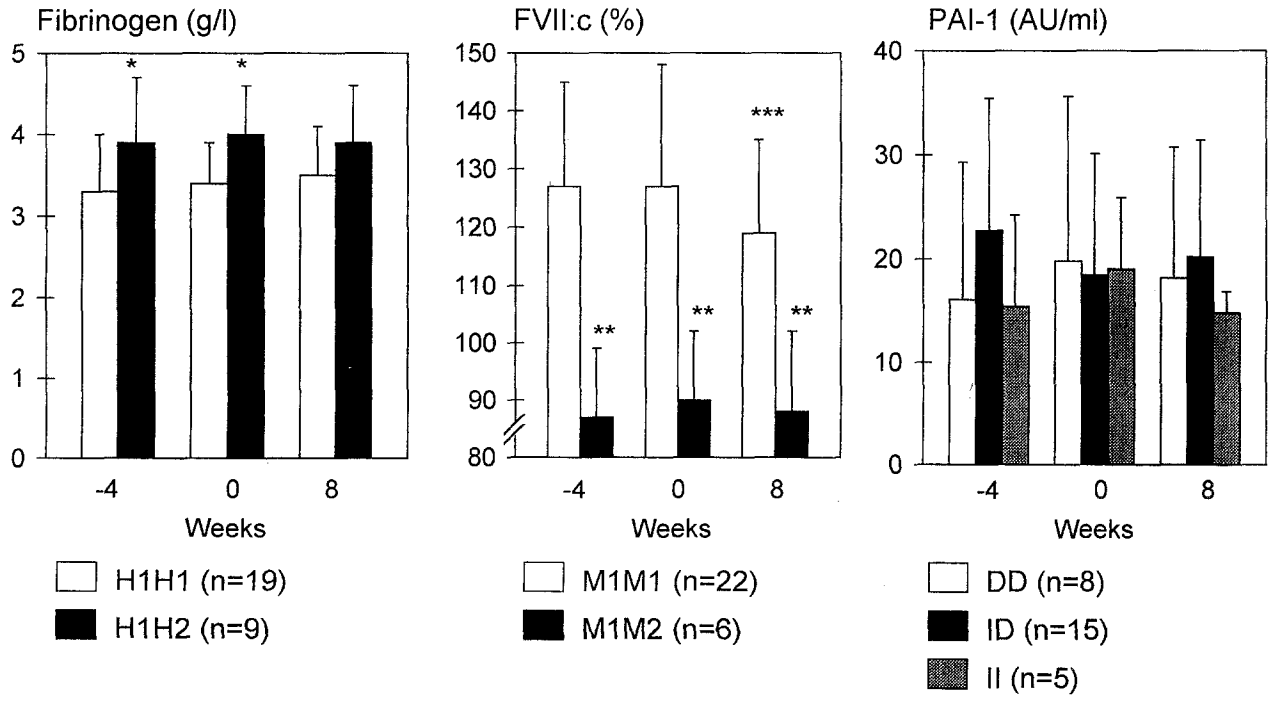

Fig 2. Fibrinogen concentration by *Hae*III polymorphism (H1H1 *v* H1H2), FVII:C (%) by *Msp*I polymorphism (M1M1 *v* M1M2), and PAI-1 activity by DD/II polymorphism (deletion/insertion or 4G/5G polymorphism) during the study (mean ± SD). *$P <$ .05, **$P <$ .001: H1H1 *v* H1H2 or M1M1 *v* M1M2. ***$P <$ .01, change within M1M1 group.

modification. The NCEP diet caused approximately a 10% reduction and the Mono diet a 4% reduction in FVII:C in subjects with M1M1, whereas in those with the M2 allele virtually no decrease (1.8% to 2.0%) was observed in either of the diet groups.

Impact of Anthropometric and Metabolic Variables on Hemostatic Factors

We examined correlations of the fibrinogen concentration, FVII:C, and PAI activity with age, body mass index, waist to hip ratio, S_G, S_i, first-phase insulin secretion (insulin area 0 to 19 minutes), fasting plasma insulin and glucose, serum and lipoprotein lipids, and lipoprotein(a) both at baseline and after dietary intervention (both diet groups combined; Table 4). Fibrinogen at baseline showed a peculiar negative correlation with total and VLDL triglycerides and VLDL cholesterol, but at the end of the study these relationships were no longer statistically significant. FVII:C at baseline showed a correlation with S_G of borderline significance ($P = .051$) and correlated significantly also with age (inversely) and VLDL cholesterol and LDL triglycerides, but only the correlation between LDL triglycerides and FVII:C remained statistically significant at 8 weeks. However, when adjusted (by partial correlation) for age, sex, and body mass index, the statistically independent correlation between FVII:C and LDL triglycerides was reduced to a nonsignificant level.

PAI-1 correlated inversely with age and positively with body mass index. PAI-1 also showed a strong and constant (inverse) correlation with S_i and fasting insulin level (positive). Furthermore, PAI-1 correlated with serum triglycerides and fasting glucose at baseline. No statistically significant correlations were found between the changes in clotting factors and PAI-1 and variables indicated in Table 4.

Table 4. Correlation Coefficients Between Fibrinogen, FVII:C, and PAI-1 With Age, Body Mass Index, Waist to Hip Ratio, S_i, S_G, Insulin Area (0 to 19 minutes), Fasting Insulin and Glucose, Lipids, Lipoproteins, and Lipoprotein (a) at Baseline and at 8 Weeks

	Fibrinogen		FVII:C		PAI-1	
	Baseline	8 Weeks	Baseline	8 Weeks	Baseline	8 Weeks
Age	.363	−.105	−.423*	.093	−.562†	−.352
Body mass index	−.315	−.408*	.130	.082	.452	.678‡
Waist to hip ratio	−.114	−.057	−.321	−.222	.220	.059
S_i	.371	.163	−.022	−.017	−.568†	−.689‡
S_G	−.357	−.076	.395*	.069	−.010	.021
Insulin area	−.196	−.222	.139	.004	.228	.253
Fasting insulin	−.199	−.023	.130	.068	.534†	.476*
Fasting glucose	.039	.194	.091	.335	.465*	.357
Cholesterol						
Total	−.240	−.043	.300	.431*	.001	−.047
LDL	−.157	−.035	−.085	.144	.026	.019
VLDL	−.437*	−.244	.438*	.131	.188	.152
HDL	.474*	.197	.142	.013	−.227	−.246
Triglycerides						
Total	−.509†	−.266	.363	.280	.387*	.271
VLDL	−.565†	−.327	.354	.250	.365	.307
LDL	−.304	.046	.388*	.463*	.187	−.080
HDL	−.111	.106	.372	.031	.138	−.128
Lipoprotein (a)	.039	.194	.091	.335	.465*	.357

*$P < .05$.
†$P < .01$.
‡$P < .001$.

Since PAI-1 activity in univariate analyses showed a constant significant inverse association with S_i, we explored this association in multiple stepwise linear regression analyses adjusting for the effects of age, sex, body mass index, systolic blood pressure, fasting plasma glucose, serum total triglycerides, and PAI-1 4G/5G genotype. Statistically independent explanatory variables for PAI-1 activity were S_i ($\beta = -0.608$, $T = -4.89$, $P < .001$), age ($\beta = -0.606$, $T = -4.70$, $P < .001$), and serum total triglycerides ($\beta = -0.297$, $T = -2.24$, $P = .036$), accounting altogether for 70% of the variation in PAI-1 activity.

DISCUSSION

The primary aim of the present study was to compare the effects of two isocaloric diets on serum lipids, lipoproteins, and insulin sensitivity in IGT subjects. One diet was enriched with monounsaturated fat, and the other had reduced fat but was enriched with polyunsaturated fat. The second important goal of our study was to determine whether these dietary modifications have divergent effects on clotting factors and fibrinolytic activity. The 37% of energy intake in the baseline diet came from dietary fat, mostly from saturated fatty acids (18%). Adherence to the qualitative changes was verified by the fatty acid composition of serum triglycerides. The total amount of fat was 4% higher than the goal in the NCEP diet, and therefore it is not a low-fat diet, but in terms of fatty acid composition it resembled the generally recommended NCEP step 1 diet. Thus, in the test diets, only the amount and quality of fat were modified, and test diets showed similar improvements in the lipid profile.[39] Therefore, it is reasonable to assume that the changes observed were mainly due to the fat modification of the diets. Regarding FVII:C, the groups differed at baseline, due to a higher frequency of M1 homozygosity in the NCEP group.

In this study, dietary fat modification did not change fibrinogen concentration or PAI-1 activity, whereas FVII:C showed a decrease. Furthermore, we confirmed that fibrinogen concentration and FVII:C are significantly related to *Hae*III and *Msp*I polymorphism, respectively. The intriguing but preliminary (due to the small number of subjects) finding was that the favorable response to dietary fat modification regarding FVII:C was restricted to the M1M1 genotype in white IGT subjects. Whether this applies to other groups remains to be demonstrated. Furthermore, in subjects with IGT, insulin sensitivity is the main determinant of PAI-1 activity, whereas modification of dietary fatty acid composition had no significant effect on PAI-1 activity.

Important results of this study include the demonstration of the independent association of *Hae*III polymorphism with fibrinogen concentration in subjects with IGT, a state preceding NIDDM. Patients with NIDDM have, in general, higher plasma fibrinogen levels than nondiabetic subjects,[16] and it is known that many environmental factors such as smoking, obesity, trauma, and use of oral contraceptives in premenopausal women and hormone-replacement therapy in postmenopausal women influence plasma fibrinogen levels,[16,40] whereas the genetic regulation of fibrinogen level is poorly known. Several polymorphisms in the fibrinogen gene have been identified, and the two most interesting have been one identified in the 5′-flanking region upstream of the start of the transcription site of the gene and the other a guanine to adenine substitution at position −455 (G/A^{-455}, *Hae*III),[18] which was analyzed in this study. In healthy middle-aged men, this polymorphism has been de-

scribed to be, after smoking, the most important determinant of fibrinogen levels.[18] To our knowledge, this is the first study to demonstrate the strong independent association of *Hae*III polymorphism with fibrinogen concentration in subjects with IGT, accounting for 38% of its variation. Regarding responses to fat modification, in line with previous studies,[16] no significant changes in fibrinogen levels were observed. Some observational and intervention studies have found correlations, albeit relatively weak, between plasma fibrinogen and serum lipids, lipoproteins, or fasting insulin, but the relationship of these risk factors to fibrinogen has been inconsistent.[6,16] However, recently, in the large Postmenopausal Estrogen/Progestin Interventions (PEPI) Study, a statistically independent relationship between fibrinogen and HDL cholesterol and its subfractions and apolipoprotein A-1 (inverse) and LDL cholesterol and apolipoprotein B (direct) was observed,[41] but possible mediators of this association are not known. In the PEPI Study, a statistically significant correlation ($r = .16$) between fibrinogen and serum triglycerides was also observed in univariate analysis, but after adjustment for other risk factors this relationship was not seen, suggesting that this relationship is mediated via other, primarily life-style, risk factors.

High FVII:C has been reported to be a risk factor for coronary heart disease events independent of serum cholesterol and fibrinogen in the Northwick Park Heart Study.[1] Factor VII is the first enzyme in the extrinsic pathway of blood coagulation, being one of the vitamin K–dependent clotting factors, and it is synthesized principally in the liver and secreted as a single-chain glycoprotein zymogen of M_r 48,000.

Green et al[35] described a polymorphism after *Msp*I digestion in exon 8 of the FVII gene. In this variant, a single base change from guanine to adenine in the codon for amino acid 353 leads to the replacement of Arg by Gln designated as Gln_{353}. Healthy white subjects with the Gln_{353} allele had approximately 20% lower FVII:C levels than those without it. The same group confirmed these findings in populations of Afro-Caribbean and Asian-Indian origin.[42] How this Gln_{353} amino acid substitution leads to reduced FVII:C levels is not exactly known, but it is likely to alter the conformational structure of the protein, leading to increased catabolism and/or reduced synthesis.[6] Regarding FVII:C, our groups differed at baseline, which was due to a higher frequency of M1 homozygosity in the NCEP group. Also, our study subjects with the Gln_{353} allele had greater than 20% lower FVII levels after the baseline diet.

In previous studies, reducing fat intake has constantly reduced FVII:C,[21-23] but fish oil supplementation has increased FVII:C in diabetic patients.[20] Modification of the quality of fat has been regarded as having little influence on either fasting or postprandial FVII:C. However, in this study a significant reduction in FVII:C was seen. Interestingly, the decrease in FVII:C after fat modification was confined to those with the M1M1 genotype, whereas in heterozygous M1M2-individuals no decrease was observed. Since this finding is based on a small number of subjects, it should be confirmed in other studies. After the test diets, FVII:C levels did not differ statistically between the genotypes. If we extrapolate from the findings of the Northwick Park Heart Study,[1] where an elevation in FVII:C of 5.3% and 10.2% was found in men who developed or died, respectively, from ischemic heart disease compared with those without these events, they suggest that simple dietary changes like fat modification may provide marked health benefits for high-risk groups like those with IGT or IRS.

In our study, FVII:C at baseline showed a significant correlation with high VLDL cholesterol and LDL triglycerides. These increased concentrations most likely represent disturbed catabolism of VLDL particles (remnant particles),[43] and these have been shown to be the significant predictors of cardiovascular mortality in patients with NIDDM.[44] The findings in this study are in accordance with the concept that the effects of dietary lipids on FVII:C are mediated through activation of the intrinsic coagulation pathway by large negatively charged plasma lipoprotein particles such as VLDL and chylomicrons.[6]

PAI-1 activity did not change during the diet periods. A reduction in PAI-1 levels can be achieved by significant weight loss and by considerable fat restriction in the long-term.[23,45] Thus, it seems that mere fat modification without significant weight loss is not able to alter PAI-1 activity in subjects with IGT, at least during a 2-month period. Previous studies in patients with NIDDM have indicated that PAI-1 activity tends to be lower with the presence of the 5G allele,[15,46] but in this study this relationship was not observed.

In our study, PAI-1 activity correlated strongly with S_i, and this association remained significant after multivariate analyses including fasting insulin. S_i, serum triglycerides, and age accounted for 70% of the variation of PAI-1 activity in the Finnish IGT subjects. Therefore, our findings confirm previous findings of the association of PAI-1 activity with insulin resistance measured either by fasting insulin[5,11] or more directly by the euglycemic-hyperinsulinemic clamp,[12-14] and support the concept that elevated PAI-1 activity is an integral part of IRS.

To conclude, despite good compliance, the fat-modified diets did not alter plasma levels of fibrinogen or PAI-1 in white subjects with IGT, whereas FVII:C levels decreased. The decrease in FVII:C was confined to subjects with the M1M1 genotype.

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