Several epidemiological studies have found that the plasma fibrinogen level is a risk factor for ischemic heart disease (IHD), similar in importance to the serum cholesterol level. A family history of IHD is also a significant risk factor for IHD, statistically independent of the serum cholesterol level. Whether the familial risk for IHD is related to genetic control of the fibrinogen level is unknown. Estimates of the genetic contribution to the variance in plasma fibrinogen levels vary markedly. We previously found elevated levels of cholesterol and factor VII in young subjects with a familial history of premature IHD. In the present study we chose to measure fibrinogen, factor VII antigen, and total cholesterol levels in 43 asymptomatic first-degree relatives (<50 years old) of patients with premature IHD and in 43 age- and sex-matched asymptomatic young adults at low risk of IHD. No subjects in either group were smokers. The mean plasma fibrinogen level of the high-risk group (259 mg/dL) did not differ significantly from that of the low-risk group (250 mg/dL; p>0.4). In contrast, the high-risk group had significantly higher mean factor VII antigen (p<0.001) and mean serum cholesterol (p<0.0001) than the low-risk group. These data argue against the hypothesis that genetic determination of the plasma fibrinogen level is a common pathophysiological mechanism responsible for familial risk of IHD.

**Keywords** • fibrinogen • factor VII • cholesterol

The plasma fibrinogen level has been found to be an independent risk factor for the development of ischemic heart disease (IHD) in several prospective studies. Factors that have been shown to influence fibrinogen levels include age, gender, smoking, stress, relative body weight, blood pressure, lipid levels, and diabetes as well as the severity of existing coronary artery disease. Factor VII, cholesterol, and fibrinogen were also shown to be risk factors for the development and/or progression of IHD.

We previously found that older patients at high risk of IHD (mean age, 51 years for the combined series of 30 subjects from two studies) as well as younger first-degree relatives of those with premature IHD (mean age, 35 years) had elevated levels of factor VII and cholesterol when compared with age-matched subjects at low risk for IHD.

We hypothesized that the familial risk of IHD in some cases might be due to elevated fibrinogen levels.

Since elevated factor VII and cholesterol levels were detectable at a relatively early age in subjects with a family history of premature IHD, we hypothesized that elevated fibrinogen levels might also be detectable in this high-risk group at an early age. To test this hypothesis, we designed a controlled, prospective, case-comparison study to measure plasma fibrinogen, plasma factor VII, and serum cholesterol values in high-risk subjects <50 years old with a family history of premature IHD and in age- and sex-matched subjects at low risk of IHD.

**Methods**

The protocol for obtaining blood samples was approved by the Committee on Research Involving Human Subjects at the State University of New York at Stony Brook and is in accordance with the principles of the Declaration of Helsinki. Data were collected for each subject regarding age, gender, height, weight, medication usage, and risk factors for IHD. Although we did not specifically collect data on the racial or ethnic backgrounds of our subjects nor exclude them from participating in this study on that basis, virtually all of our donors were Caucasian. A small number of donors were Asian. Relative body weight was calculated by dividing the measured weight by the table weight, as predicted by height and sex, from 1983 Metropolitan Life Insurance Tables.

A group of 43 asymptomatic young adults (<50 years old) at low risk of IHD were recruited for this study.
consisting of 27 men and 16 women (mean age, 32 years). All subjects were nonsmokers who had no family history of premature IHD (defined as evidence of IHD before age 55 in male or before age 60 in female parents or siblings). The low-risk subjects had no known risk factors for IHD except for male sex or obesity (in six subjects). Two subjects were taking oral medication (one a nonsteroidal anti-inflammatory drug [NSAID] and one an antibiotic).

A group of 43 young adults of comparable age and sex (26 men and 17 women with a mean age of 34 years) at high risk of IHD was also recruited. Each subject had a family history of premature IHD (as defined above) in a first-degree relative (i.e., parent or sibling). None of the subjects were smokers. Eight subjects were taking oral medications, including β-blockers (three), NSAIDs (two), antihistamine (one), captopril (one), and low-dose estrogen/progestin replacement (one).

Citrated blood samples from fasting donors were collected by a double-syringe technique, as previously reported. Serum total cholesterol levels were assayed from the contents of the first syringe. Plasma was prepared from the contents of the second syringe (in 0.1 volume of 3.8% sodium citrate) by centrifugation at 3,000 g for 20 minutes at 4°C. Samples were kept at room temperature before centrifugation to avoid cold activation. Plasma was divided into aliquots, stored at −70°C, and used for the factor VII and fibrinogen assays within 1 month.

Assays

Serum total cholesterol levels were analyzed by DART reagents on a DACOS analyzer (Coulter Diagnostics, Hialeah, Fla.) according to the manufacturer's directions. Results were calculated in milligrams per deciliter.

Factor VII antigen was measured by an enzyme immunoassay kit (Diagnostica Stago, Asnieres-sur-Seine, France), which was purchased from American Bioproducts Company (Parsippany, N.J.), and performed as previously reported. The interassay coefficient of variation was 4%.

Fibrinogen levels were measured by a one-stage clotting assay performed on a mechanical timer (BBL, Cockeysville, Md.) with General Diagnostics Fibriquick reagents (Organon-Teknika, Durham, N.C.). The interassay coefficient of variation was 11%.

Reference pooled normal plasma was prepared in our laboratory from healthy male and female volunteers who were taking no medication and was prepared and stored under the same conditions as those used for the subject samples of this study. In addition, a single donor's plasma was assayed with each set of factor VII and fibrinogen assays to serve as a daily internal control.

Statistics

Results of fibrinogen assays and factor VII enzyme immunoassays were analyzed on a personal computer (Dell System 200, Austin, Tex.) by logarithmic transformation of the data in the parlin program for parallel-line analysis of bioassays as previously described. Significant differences in factor VII antigen, fibrinogen, or cholesterol levels of low-risk versus high-risk subject groups were determined by a two-tailed Student's t test for independent samples using CSS:STATISTICA (Statsoft, Tulsa, Okla.) software.

Results

Although the high- and low-risk subject groups were matched by number of subjects, age, and sex, there was a difference in the mean relative body weights (1.14 versus 1.06) of these two groups (Table 1). The high-risk group contained 13 of 43 subjects who were obese (i.e., had a relative body weight >1.2). In contrast, the low-risk group contained only six of 43 subjects who were obese.

There was no significant difference in the mean fibrinogen level between the high-risk and low-risk groups (259 versus 250 mg/dL, p>0.4; Table 2). In contrast, mean factor VII antigen levels were significantly higher in the high-risk than in the low-risk group (1.15 versus 0.93 units/mL, p<0.001; Table 2). Mean serum cholesterol levels were also significantly elevated in the high- versus the low-risk group (225 versus 181 mg/dL, p<0.0001; Table 2).

Discussion

We have demonstrated a statistically significant elevation of factor VII antigen and cholesterol levels in...
subjects at high risk for IHD in this study, thus confirming our previous reports. In contrast, there was no statistically significant difference in the mean fibrinogen level of the high-risk versus the low-risk group. Since our sample size was relatively small, there is always the possibility of making a type II error, i.e., accepting a false null hypothesis regarding differences in fibrinogen levels in our low-risk versus high-risk group. However, the clear differences in factor VII antigen and serum cholesterol levels that we observed between these two groups would tend to argue against our missing a difference of similar magnitude in fibrinogen levels. The slightly higher mean fibrinogen level (259 mg/dL) in the high-risk group compared with the group of low-risk subjects (250 mg/dL) could be entirely accounted for by the higher proportion of high-risk subjects in our study group who were obese (13 of 43) compared with our low-risk subject group (six of 43), given the known relation of fibrinogen to weight.

Recent reports of the genetic contribution to the variance in plasma fibrinogen concentrations differ markedly. Thomas et al found that variation in the promoter region of the β-fibrinogen gene accounted for 31% of the variance in fibrinogen levels of 292 men with no history of IHD. Humphries et al reported that the B2 allele of the gene for the β-chain of fibrinogen was associated with higher fibrinogen levels. They calculated that genetic variation at the fibrinogen locus accounted for 15% of the total phenotypic variance in fibrinogen levels in a group of 91 men and women (mean age, 46 years). Nevertheless, Monsalve et al found no difference in the B2 allele frequency in 180 patients with either peripheral arterial disease or coronary artery disease compared with 104 healthy control subjects. Furthermore, Berg and Kierulf found no association between plasma fibrinogen concentration and any genotype for either of two fibrinogen polymorphisms (one at an α-chain locus and the other at the β-chain locus) in unrelated healthy men and women. Using the β-chain probe of Humphries et al, they also reported a low estimate of heritability of fibrinogen level in a group of monozygotic twins.

Hamsten et al measured fibrinogen levels in young men (<45 years) after confirmed myocardial infarction and in their first-degree relatives and compared them with 85 families randomly selected from the general population. They performed path analysis of the data. Path analysis is a model that analyzes correlations of variables with environmental and genetic inheritance in families. In this model, the environment is assumed to act additively (but not interactively) with the genotype to produce a phenotype. The age- and sex-adjusted plasma fibrinogen level was the phenotype of that study. The authors concluded that 51% of the variance of plasma fibrinogen level may be accounted for by genetic heritability versus 3% of the variance accounted for by the combined effects of obesity and smoking. Some investigators questioned the validity of the application of path analysis to those data. Fowkes et al studied 121 male and female subjects with peripheral arterial disease and compared them with 126 healthy control subjects matched for age and sex (55–74 years). They reported that genetic polymorphisms at the α, β, and γ loci, including the B2 allele of the β-chain gene, were not significantly related to fibrinogen concentrations, although they found higher mean fibrinogen levels in the group of subjects with peripheral arterial disease than in the control group. The frequency of the B2 allele in the peripheral arterial disease group (0.197) was significantly higher than in the control group (0.097), suggesting that this fibrinogen genotype was associated with a risk of peripheral atherosclerosis in some way. The relation of fibrinogen level to risk of IHD was established in middle-aged and older adults. Clearly, more studies are needed to determine whether a familial predisposition toward increased fibrinogen levels in middle age contributes to the overall incidence of IHD, and they should be carefully designed to account for the effect of body weight and other variables known to influence fibrinogen levels. There may also be considerable genetic variation in different ethnic groups and nationalities. Our data argue against the hypothesis that genetic control of fibrinogen level is a common pathophysiological mechanism expressed in young adults with a familial risk of premature IHD in an American population. We conclude that it would be premature to measure fibrinogen levels routinely in healthy young adults with a family history of IHD in an attempt to assess their risk for this disease.

Acknowledgments

We wish to thank Jolyon Jesty, DPhil, for the PARLIN program and Barbara Milano and Majia Smits for expert secretarial assistance.

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Plasma fibrinogen level is not elevated in young adults from families with premature ischemic heart disease.
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doi: 10.1161/01.ATV.13.6.800
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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