Selected genetic polymorphisms and plasma coagulation factor VII changes with exercise training

Ioana A. Ghii,1 Robert E. Ferrell,2 Onanong Kulaputana,1 Dana A. Phares,1 and James M. Hagberg1
1Department of Kinesiology, University of Maryland, College Park, Maryland 20742-2611; and 2Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pennsylvania 15261

Submitted 18 August 2003; accepted in final form 30 October 2003

Ghiu, Ioana A., Robert E. Ferrell, Onanong Kulaputana, Dana A. Phares, and James M. Hagberg. Selected genetic polymorphisms and plasma coagulation factor VII changes with exercise training. J Appl Physiol 96: 985–990, 2004; 10.1152/japplphysiol.00877.2003.—We assessed the effects of coagulation factor VII (FVII) gene polymorphisms, lipid-related polymorphisms, and exercise training-induced plasma lipoprotein lipid changes on FVII level changes with exercise training in middle- to older-aged men and women. Forty-six healthy sedentary men and women were stabilized on a low-fat diet and then underwent baseline testing, 6 mo of endurance exercise training, and final testing. Plasma FVII-Ag levels decreased with exercise training (106.7 ± 1.4 vs. 104.2 ± 1.6%, P = 0.005). There were no significant differences in FVII-Ag changes with exercise training between −323 (0/10 bp)/−401 (G/T) haplotype or −402 (G/A) genotype groups. FVII-Ag changes with training were not correlated with changes in plasma lipoprotein lipids. In linear regression analyses, FVII-Ag changes with training remained significant after adjusting for training-induced plasma lipoprotein lipid changes (P = 0.01). FVII changes with training were associated with apolipoprotein E genotype (P = 0.012); this relationship was still evident after adjusting for training-induced plasma lipoprotein lipid changes (P = 0.047). FVII changes with training also were significantly associated with human ATPase binding cassette-1 genotype (P = 0.018); this relationship persisted after accounting for the effect of the training-induced plasma lipoprotein lipid changes (P = 0.045). We conclude that plasma FVII-Ag changes with exercise training are more closely related to selected lipid-related genotypes than FVII gene promoter variants.

plasma lipoprotein lipids; haplotype; genotype

THROMBOGENIC FACTORS ARE INVOLVED in the initiation of early atherosclerotic lesions (28). Furthermore, thrombogenic factors contribute to the progression of the plaque growth, and they may lead to deleterious clinical events if a hypercoagulable state is present at the time of plaque fissuring or rupture (28). Factor VII (FVII) plays an important role in the initial activation of the coagulation cascade, when it complexes with its natural cofactor and receptor, tissue factor, released from a leaking or ruptured atheromatous plaque. This cascade, also commonly known as the extrinsic pathway of blood coagulation, is believed to contribute significantly to acute thrombin generation. Many, but not all, studies have found a positive association between plasma FVII levels and cardiovascular (CV) disease events (14, 16, 19, 22, 23, 27, 34, 40).

Exercise training is associated with a considerable reduction in CV mortality and morbidity, but its impact on the hemostatic system has not been completely clarified. From cross-sectional studies, it is clear that higher habitual physical activity levels are associated with lower plasma FVII levels (8, 11, 17, 44). However, the results of exercise training intervention studies are, at best, equivocal (6, 15, 18, 43, 46, 48). Several design and measurement differences between the cross-sectional and longitudinal studies may contribute to the lack of consistency between their results.

Approximately one-third of the variation in plasma FVII levels is due to genetic factors (7). Common polymorphisms in the promoter region at the FVII gene locus (−323 0/10 bp, −401 G/T, −402 G/A) are considered likely candidates because they may modulate FVII gene transcription, thus influencing plasma FVII protein levels. It is known that these FVII promoter variants affect plasma FVII levels (14, 25, 37, 42). However, it is also possible that these variants may affect plasma FVII responses to environmental stimuli. Therefore, we hypothesized that these promoter variants would affect the response of plasma FVII levels to exercise training.

There is also substantial evidence documenting significant relationships between plasma FVII levels and plasma lipoprotein lipid levels (13, 20, 26, 30, 35, 38). Because plasma lipoprotein lipid profiles are partially genetically determined and also are generally improved with endurance exercise training, we sought to assess the effect of lipid-related genotypes and training-induced changes in plasma lipoprotein lipid profiles on the changes in plasma FVII levels with exercise training. We hypothesized that the exercise training-induced changes in plasma FVII levels would be associated with common polymorphic lipid-related genotypes but that these associations would not be evident after accounting for the effects of the training-induced improvements in plasma lipoprotein lipid profiles.

METHODS

Interested individuals underwent a screening telephone interview to assess their eligibility. Initial eligibility criteria included the following: age 50–75 yr, nonsmoker, nonobligatory, currently sedentary (<20 min of continuous aerobic exercise, ≤2 times/wk), body mass index (BMI) <37 kg/m², and, if female, postmenopause by >2 yr. Women on hormone replacement therapy (HRT) continued their therapy for the duration of the study; those not on HRT remained off for the duration of the study. Seven women were not on HRT, and 21 women were on HRT, with virtually all of them on combinations of estrogen and progesterone. In addition, the use of medications affecting lipid or glucose metabolism was an exclusion criterion. Subjects meeting these criteria were scheduled for screening visits to determine their eligibility. Initial eligibility criteria included the following: age 50–75 yr, nonsmoker, nonobligatory, currently sedentary (<20 min of continuous aerobic exercise, ≤2 times/wk), body mass index (BMI) <37 kg/m², and, if female, postmenopause by >2 yr. Women on hormone replacement therapy (HRT) continued their therapy for the duration of the study; those not on HRT remained off for the duration of the study.
eligibility for participation. The Institutional Review Board of the University of Maryland College Park approved the study.

During their first screening visit, health history and physical activity questionnaires completed by the subject were reviewed to verify the information obtained during telephone screening. After all questions and concerns regarding the study were addressed, subjects provided their written, informed consent to participate in the study. Height and weight were measured to verify BMI <37 kg/m². A blood sample was also collected to isolate leukocyte DNA for genotyping [32].

The second screening visit consisted of fasting blood sampling and a 2-h postprandial glucose assessment. Only subjects with fasting glucose <126 mg/dl and 2-h postprandial glucose <200 mg/dl who were healthy based on the blood chemistry panel and had a fasting total cholesterol level >200 mg/dl and/or an HDL cholesterol <35 mg/dl continued to qualify for participation in the study.

The final screening visit was to ensure that subjects were free from detectable CV disease, pulmonary disease, or other chronic conditions that would prohibit their participation in a vigorous exercise training program. The subjects underwent a Bruce protocol treadmill exercise test with blood pressure, heart rate, and ECG monitoring [45]. The test was terminated at subjective exhaustion or on signs or symptoms of CV decompensation [4]. Subjects who exhibited signs or symptoms of CV disease were excluded from the study.

After screening, the subjects underwent a 6-wk dietary stabilization program based on the American Heart Association Step 1 Diet [5]; the subjects maintained this diet throughout the study. To monitor their diet, 7-day food records were administered before and during the exercise intervention to document adherence to the dietary guidelines. After completing dietary stabilization, subjects underwent baseline tests that included blood samples for plasma FVII antigen (FVII-Ag) levels and plasma lipoprotein lipid profiles and measurement of maximal oxygen consumption (VO$_2$ max).

Blood samples for FVII-Ag levels were collected in the morning after a 12-h overnight fast. To avoid the effect of circadian variation on FVII levels, all blood samples were drawn between 6:30 and 9:30 AM. To proceed with the blood draw, subjects had to be free from any inflammation for >2 wk. Blood was collected through an atrumatic venipuncture using a 21-gauge butterfly needle. The butterfly needle was connected to a vacutainer tube containing citrate anticoagulant. The first 5 ml of blood were discarded. After the blood collection, the tube was gently inturned four to five times to ensure mixing of the blood with the anticoagulant. The blood from the vacutainer tube was transferred to a plastic tube within 15 min of collection and centrifuged at 10,000 rpm for 20 min at 4°C. After centrifugation, plasma was separated into aliquots and stored at -80°C until analysis. We measured total plasma FVII antigen concentrations as a marker for the chronic state of the coagulation system, because FVII-Ag levels are not as responsive as other measures to acute events that stimulate coagulation [8]. Plasma FVII-Ag levels were analyzed using an ELISA (Asserachrom VII: Ag, Stago Diagnostica, Ansieres, France) [41]. The samples from before and after training for each subject were run on the same ELISA plate, and all plates used in this study came from the same production lot. The intra- and interassay coefficients of variation for the FVII-Ag levels were 1.9 and 2.1%, respectively.

Genotyping for the −401 G/T and −402 G/A polymorphisms was performed as described by van’t Hooft et al. [42], and typing of the −323 0/10-bp polymorphism was performed as described by Dell’Acqua et al. [12]. We also typed the R53Q polymorphism, which codes for a substitution of a glutamine for an arginine at position 535 in a catalytic domain in exon 8. However, as expected, nearly all of our subjects were homozygotes for the common allele, and this variant was not considered further in the statistical analyses. The two alcohol-related polymorphisms were typed using previously described techniques: apolipoprotein E (apoE) [21], lipoprotein lipase Pvu II and Hind III [2, 3, 36], hepatic lipase (HL) [47], endothelial lipase (R. E. Ferrell, unpublished observations), human ATPase binding cassette-1 (hABC1) [10], fatty acid binding protein-2 (1), cholesterol ester transfer protein [45], and IL-6 [9]. These variants were typed because of the previously demonstrated close relationship between plasma lipoprotein lipid levels and FVII levels and because a number of plasma lipoprotein lipid levels have previously been found to be associated with all of these lipid metabolism-related variants. All genotyping was performed at the Department of Human Genetics at the University of Pittsburgh, Pittsburgh, PA, or the Division of Endocrinology, Diabetes, and Nutrition, University of Maryland School of Medicine, Baltimore, MD. Plasma lipoprotein lipid levels were measured on blood samples drawn after an overnight fast; they were analyzed using an Hitachi 717 autoanalyzer in the Baltimore Veterans Affairs Medical Center Clinical Chemistry Laboratory. All plasma lipoprotein lipid levels represent the averages of two samples drawn on different days.

VO$_2$ max was measured during a modified treadmill protocol. A warm-up was used to determine the treadmill speed that elicited 70% of peak heart rate from the screening treadmill exercise test; this speed was used during the entire test. After 2 min of exercise on a level grade, the treadmill incline was increased to 4%. The treadmill grade was then increased 2% every 2 min until the subject was unable to continue due to exhaustion, or the test was terminated by the physician. VO$_2$ consumption was measured throughout this test [45]. A true VO$_2$ max was considered to have been achieved if standard physiological criteria were exceeded [45].

The training program consisted of endurance exercise three times per week for 6 mo under the direct supervision of study personnel. In the first week, subjects exercised for 20 min at 50% VO$_2$ max; training intensity and duration were gradually increased so that subjects exercised for 40 min at 70% VO$_2$ max for the last 3–4 mo of training [45]. Exercise intensity was monitored via electronic heart rate monitors. After 10 wk of exercise, subjects added a lower intensity bout of exercise during the weekend for the remainder of the study.

After completing 6 mo of exercise training, each subject repeated the measurements of plasma FVII levels, plasma lipoprotein lipid profiles, and VO$_2$ max. To avoid the acute effects of exercise, blood samples during final testing were drawn 24–36 h after the last bout of exercise.

All data are presented as means ± SE. The exercise training-induced changes in plasma FVII-Ag levels, lipoprotein lipid levels, and VO$_2$ max were compared using paired t-tests. Initial subject characteristics and plasma FVII-Ag levels were compared between genotype groups at the three FVII loci using ANOVAs. Changes in FVII-Ag levels with exercise training between FVII promoter and lipid-related genotype groups also were compared between carriers and noncarriers of the least frequent allele using ANOVAs. Multiple variable linear regression ANOVA models were used to test 1) the effect of exercise training on plasma FVII-Ag levels after adjusting for exercise training-induced changes in plasma lipoprotein lipid levels and 2) the effect of lipid-related genotypes on plasma FVII level changes with exercise training before and after adjusting for exercise training-induced changes in plasma lipoprotein lipid levels. The associations between FVII-Ag levels, plasma lipoprotein lipid levels, and BMI were measured by bivariate correlations. A $P < 0.05$ was considered statistically significant. All statistical procedures were analyzed on SPSS 10.0 software (SPSS, Chicago, IL).

RESULTS

Forty-six subjects completed the 6-mo exercise training intervention. There were no differences in age, body weight, or BMI between men and women at baseline (Table 1). As expected, the men had a higher VO$_2$ max at baseline than women. There was no difference in baseline plasma FVII-Ag levels between genders.

The exercise training intervention resulted in a 24% increase in VO$_2$ max (25.0 ± 0.7 vs. 31.1 ± 0.3 ml·kg$^{-1}$·min$^{-1}$, $P <$
0.0001), documenting the substantial stimulus provided by the exercise training program. In addition, body weight and BMI both decreased minimally, but significantly, with exercise training in the total population (80.7 ± 1.9 vs. 78.8 ± 1.8 kg and 28.0 ± 0.6 vs. 27.6 ± 0.6 kg/m², respectively; both P < 0.0001). Plasma FVII-Ag levels decreased by 2.5% (P = 0.005) with 6 mo of endurance exercise training (106.7 ± 1.4 vs. 104.2 ± 1.6%). The changes in FVII-Ag levels with training were not affected by gender. In women, baseline FVII-Ag levels were not different between those on (n = 21) and those not on HRT (n = 7) (101.4 ± 3.5 vs. 107.0 ± 2.2%, respectively; P = 0.1). After exercise training, there was somewhat of a stronger trend toward differences in plasma FVII-Ag levels between women on and not on HRT (96.3 ± 1.3 vs. 107.3 ± 1.4%).

Effect of FVII genotypes. The −323/0/10-bp and the −401 G/T allele frequencies were comparable to those reported previously (14,7). As previously reported (14), were in Hardy-Weinberg equilibrium, and G/T allele frequencies were comparable to those reported previously (14). Factor VII levels, % 106.7 ± 0.8, no. of subjects. BMI, body mass index; V02max, maximal oxygen consumption.

Table 1. Baseline physical characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>46</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>Age, yr</td>
<td>58.0 ± 0.9</td>
<td>57.3 ± 1.7</td>
<td>58.5 ± 1.1</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>80.7 ± 1.9</td>
<td>85.3 ± 3.2</td>
<td>78.0 ± 2.2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.0 ± 0.6</td>
<td>26.6 ± 1.0</td>
<td>28.7 ± 1.0</td>
</tr>
<tr>
<td>V02max, ml/kg ⋅ min⁻¹</td>
<td>25.0 ± 0.7</td>
<td>29.2 ± 1.3</td>
<td>22.6 ± 0.6</td>
</tr>
<tr>
<td>Factor VII levels, %</td>
<td>106.7 ± 1.4</td>
<td>106.2 ± 2.1</td>
<td>107.3 ± 1.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. BMI, body mass index; V02max, maximal oxygen consumption.

Table 2. Genotype and allele frequencies in the study population

<table>
<thead>
<tr>
<th></th>
<th>−323 (0/10 bp)−401 (G/T) Polymorphisms</th>
<th></th>
<th>−402 (G/A) Polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Allele frequency (%)</td>
<td>Genotype frequency (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0/0G)</td>
<td>(10/T)</td>
<td>0/0GG</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>0.88</td>
<td>0.12</td>
</tr>
<tr>
<td>Men</td>
<td>18</td>
<td>0.93</td>
<td>0.07</td>
</tr>
<tr>
<td>Women</td>
<td>28</td>
<td>0.84</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. *P < 0.05 represents significant difference with exercise training within group. †Probability for the t-test comparing genotype groups.
Changes in plasma FVII-Ag levels with exercise training were associated with apoE genotype \((P = 0.012)\), with apoE4 individuals reducing FVII-Ag levels the most with training and apoE2 and apoE3 individuals not changing FVII-Ag levels with training (Table 6). The plasma FVII-Ag changes with exercise training remained associated with apoE genotype after adjusting for the exercise training-induced changes in plasma total cholesterol, HDL cholesterol, and TG levels \((P = 0.047)\).

Plasma FVII-Ag changes with exercise training also were significantly associated with hABC1 genotype \((P = 0.018)\), such that carriers of the T allele decreased FVII-Ag levels the most with exercise training, whereas CC homozygotes did not change plasma FVII-Ag levels with training (Table 7). The association between training-induced changes in plasma FVII-Ag levels and hABC1 genotype also remained significant, after accounting for the effect of the exercise training-induced plasma total cholesterol, HDL cholesterol, and TG changes \((P = 0.045)\). Plasma FVII-Ag changes with exercise training were not associated, independently or after adjusting for plasma lipoprotein lipid changes with exercise training, with the other lipid-related genotypes (lipoprotein lipase \(Pvu\) II and \(Hind\) III, hepatic lipase, endothelial lipase, IL-6, fatty acid binding protein-2, and cholesterol ester transfer protein).

**DISCUSSION**

Physical activity is associated with a significant reduction in CV morbidity and mortality in middle- to older-age individuals, a benefit that may be partly due to a reduced thrombogenic potential. We found that the FVII-Ag changes with exercise training were significantly associated with two lipid-related genotypes, apoE and hABC1, and that both of these relationships persisted after accounting for the effects of exercise training on plasma lipoprotein lipids. On the other hand, FVII-Ag changes with exercise training were not affected by three common FVII polymorphic gene variants.

This study was not designed to provide definitive evidence about overall training-induced FVII-Ag changes, as no control group was included. However, we optimized the assessment of plasma FVII-Ag levels as a function of apoE genotype and exercise training.

<table>
<thead>
<tr>
<th>apoE</th>
<th>apoE3</th>
<th>apoE4</th>
<th>(P ) Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>99.7±4.0</td>
<td>107.1±2.8</td>
<td>105.5±3.4</td>
</tr>
<tr>
<td>After training</td>
<td>100.6±4.4</td>
<td>106.0±2.2</td>
<td>99.2±3.7</td>
</tr>
<tr>
<td>Change with training</td>
<td>0.9±2.1</td>
<td>-1.1±1.1</td>
<td>-6.3±1.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. apoE, apolipoprotein E. †*P < 0.05 represents significant difference with exercise training within group. †Probability for the ANOVA comparing genotype groups.
induced changes in FVII-Ag levels. However, we found no difference in FVII-Ag responses to exercise training between $-323 \ (0/10 \text{ bp})/ -401 \ (G/T)$ haplotype groups or $-402 \ (G/A)$ genotype groups, the three FVII gene promoter variants that we had hypothesized might modulate the effect of exercise training on FVII-Ag levels. Whereas, because of the sample size, our results do not definitively prove that FVII gene variants do not affect exercise training-induced changes in FVII-Ag levels, they do provide strong evidence that two lipid-related genotypes are much more strongly associated with training-induced FVII-Ag level changes.

Our second primary purpose, because of the known relationships between plasma lipoprotein lipids and FVII levels (8, 16, 20, 29, 31, 32, 35, 39), was to assess the relationships between FVII changes with exercise training, training-induced plasma lipoprotein lipid changes, and lipid-related genotypes. We found that higher baseline FVII levels were associated with higher levels of total cholesterol, LDL cholesterol, and TG, and higher BMI, as has been shown by others (8, 16, 20, 39). The correlations were different between genders, also as previously reported (20, 30). These associations were still present, although weaker, after training. However, there were no significant correlations between plasma FVII-Ag changes and lipoprotein lipid changes. Moreover, in linear regressions, training-induced FVII-Ag changes were independent of training-induced plasma lipoprotein lipid changes. These findings contradict previous studies (6, 8) that reported that FVII-Ag changes with training were the result of plasma lipoprotein lipid changes. Our results suggest an independent beneficial effect of exercise training on plasma FVII-Ag levels. The differences between our and previous results may be because our study was designed to assess the effect of exercise itself, independent of dietary changes or substantial changes in body weight. Therefore, it is reasonable to conclude that our results also provide strong evidence for an independent association between training-induced FVII-Ag changes and two lipid-related genotypes. Our results indicate that individuals with a “responsive” genotype (apoE4 or hABC1 T allele carriers) reduced their FVII-Ag levels substantially with exercise training, averaged 3.3 and 6.5% reductions, respectively. On the other hand, the apoE2 and hABC1 CC groups, i.e., “nonresponsive” genotype groups, actually increased their FVII-Ag levels by 0.9 and 0.7%, respectively, with training. Thus the response differences between the “responsive” and nonresponsive genotype groups was substantial, amounting to 4–7%. These genotype-dependent relationships were still evident after accounting for the effect of exercise training-induced lipoprotein lipid changes. Interpreting the mechanistic effects of these lipid-related variants on differential FVII-Ag changes with training is difficult. These markers may be in linkage disequilibrium with other variants in the same chromosomal region, which may have a direct impact on FVII-Ag levels. However, the FVII gene is on chromosome 13, whereas the apoE and hABC1 genes are on chromosomes 19 and 9, respectively. Also, there are no other known genes related to coagulation linked to the apoE or hABC1 genes, nor are there any genes linked to the FVII gene that are known to regulate the function of the apoE or hABC1 genes.

In conclusion, we found a significant beneficial effect of endurance exercise training on FVII-Ag levels in a middle- to older-aged population. Because FVII is an independent CV disease risk factor, our results support the possibility of reducing a person’s thrombotic risk by changing his or her physical activity status. FVII gene promoter polymorphisms have been associated with plasma FVII-Ag levels. However, our results indicate that training-induced plasma FVII-Ag changes are not associated with these markers. On the other hand, training-induced FVII-Ag changes were associated with two lipid-related genotypes, apoE and hABC1, and these associations were still evident after adjusting for the effect of training-induced plasma lipoprotein lipid changes.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Dr. Andrew Goldberg, Geriatric Research, Education, and Clinical Center, Baltimore Veterans Affairs Medical Center and the Division of Gerontology, University of Maryland School of Medicine, Baltimore, MD, in the measurement of plasma lipoprotein lipid levels, and Dr. Alan Shuldiner, Division of Endocrinology, Diabetes, and Nutrition, University of Maryland School of Medicine, Baltimore, MD, in the assessment of selected genotypes.

GRANTS

I. M. Hagberg was supported by National Institutes of Health (NIH) Grants AG-15389 and AG-17474. R. E. Ferrell was supported by NIH Grant DK-46204.

REFERENCES


