ABSTRACT

Title of Thesis : INFLUENCE OF LIPOPROTEIN LIPIDS AND
APOLIPOPROTEIN E GENE POLYMORPHISMS ON
COAGULATION FACTOR VIII CHANGES WITH SIX
MONTHS OF AEROBIC EXERCISE TRAINING.

Rakesh Gopinathannair, Master of Arts, 2004.

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Elevated plasma factor VIII antigen (FVIII:Ag) level is an independent risk factor for coronary artery disease. Aerobic training improves cardiovascular risk and improvement in coagulation profile might be a potential contributory mechanism. Available evidence suggests that plasma lipoprotein-lipid levels and lipid-related genotypes might have a regulatory effect on plasma FVIII:Ag levels. We assessed the effects of APO E gene polymorphisms and plasma lipoprotein-lipid changes on plasma FVIII:Ag changes with 6 mo of standardized aerobic training in 44 sedentary, 50-75 year old men and women with different APO E genotypes. Plasma FVIII:Ag levels, lipoprotein-lipid levels, VO_2 max, and intra-abdominal fat (CTIA) were estimated before and after 6 mo of training . Plasma FVIII:Ag levels showed an increase of 3.5% ($152.5\pm6.7\%$ to $156.0\pm6.1\%$, P=0.290) with exercise training. FVIII:Ag levels were positively correlated to CTIA at baseline (r= 0.30) and after training (r=0.37). There was no significant association between FVIII:Ag levels and APO E genotype, before and after covarying for training-induced changes in plasma

lipoprotein-lipids. In conclusion, the effect of regular aerobic exercise training on plasma FVIII:Ag levels appears small and clinically insignificant when compared to the clear and beneficial effects on lipoprotein-lipid profile and body composition. Truncal obesity may be a significant factor modulating baseline plasma FVIII:Ag levels and their response to training.

INFLUENCE OF LIPOPROTEIN LIPIDS AND APOLIPOPROTEIN E
GENE POLYMORPHISMS ON COAGULATION FACTOR VIII
CHANGES WITH SIX MONTHS OF AEROBIC EXERCISE TRAINING

by

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Master of Arts
2004

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INTRODUCTION

Cardiovascular disease (CVD) continues to be the major cause of mortality and morbidity in the western world (1). Atherosclerosis, the most common cause of cardiovascular disease, involves two distinct pathologic processes - atherogenesis during the early stages and atherothrombosis in the advanced stages (2,3). Severe clinical complications of atherosclerosis, including myocardial infarction, ischemic stroke and sudden cardiac death, are caused by thrombus formation primarily on ruptured advanced atherosclerotic plaques, which finally leads to occlusion of the vessel lumen (4,5). Such thrombus formation and the subsequent ischemic event are augmented when a hypercoagulable state is present at the time of plaque fissuring or rupture (6). Aging is also associated with adverse changes in coagulation, contributing to a thrombophilic state (7,8). Thus, predisposition to coagulation and the means to improve this predisposition are important with respect to reducing the risk of acute ischemic events.

Even though the tissue factor dependent extrinsic coagulation pathway is responsible for the initial generation of thrombin in atherosclerosis, the intrinsic pathway catalyses thrombin formation approximately 50-fold more efficiently, and has been shown to account for the dramatic increase in thrombogenicity of the atherosclerotic lesion in the course of its progression (9,10). Coagulation factor VIII (FVIII) plays an integral role in the activation of the intrinsic pathway of coagulation. FVIII is a glycoprotein synthesized in the liver in the inactive single chain form. It circulates in blood bound to an endothelium-synthesized protein, Von-Willebrand factor (VWF), which protects FVIII from premature degradation and also enables the attachment of platelets to sub-endothelial collagen, thereby starting the coagulation process. The activation of FVIII to

FVIIIa occurs in the presence of minute quantities of thrombin. The activated form of FVIII, FVIIIa, acts as the cofactor in the activation of factor X, in the presence of factor IXa, phospholipids and calcium (the Tenase complex). Activated factor Xa converts prothrombin to thrombin, leading to formation of the fibrin clot. As the thrombin concentration increases, it exerts a positive feedback on FVIII, thus forming more thrombin and amplifying the coagulation cascade (11). Thus, by virtue of its central role in the intrinsic pathway and the positive feedback loop with thrombin, FVIII can be considered as a sensitive and representative marker for changes within the coagulation cascade.

Several studies have established a clear association between FVIII levels and thrombotic phenomena. High FVIII levels have been shown to be an independent risk factor for CVD (12,13), coronary artery disease (14-17), myocardial infarction (18), venous thrombosis (19-21), and ischemic stroke (22,23). This association is further strengthened by the low cardiovascular mortality seen in patients with Hemophilia A, which is caused by FVIII deficiency (24). Studies have shown that baseline FVIII levels increase with increasing age (25), giving support to the increased incidence of ischemic events in the elderly.

Even though it is well known that aerobic exercise training improves cardiovascular (CV) and ischemic risk (26,27), the pathways and mechanisms by which regular physical activity achieves this are not fully understood. Improvement in coagulation profile might be a potential mechanism for training-induced CVD risk reduction (28). The results of the few studies that have assessed the effect of exercise training on FVIII levels have been far from consistent. The majority of cross-sectional studies observed a significant inverse

dose-response relationship between physical activity and FVIII levels (25,28,29) though some studies showed no such differences (30,31). Longitudinal exercise intervention studies that assessed FVIII levels before and after training also show mixed results, with studies showing no change (32,33,37), or a significant decrease in plasma FVIII levels (35,36). Altogether, research on training effects on coagulation is minimal and inconclusive. Employing different durations and intensities of exercise training and failing to account for potential confounding variables like age, race, lipoprotein lipid levels, diurnal variation, inflammatory conditions, and body composition (38) may be the reasons for these inconsistent results. Design and measurement variations between cross-sectional and longitudinal studies might also have contributed to this fragmented picture. The well-documented association of high FVIII levels with ischemic events coupled with the high incidence of these events in the older age group makes it worthwhile to undertake a well-designed training study to elucidate the effect of exercise training on plasma FVIII antigen (FVIII:Ag) levels in middle-to older-aged people with known CVD risk factors. A previous study from our laboratory has shown significant reductions in Factor VII:Ag levels with six months of training (unpublished data). Since the intrinsic pathway makes an equally significant contribution to the thromobogenicity of the atherosclerotic plaque (10), assessing FVIII:Ag levels will enable us to develop a broader understanding of the mechanisms underlying coagulation system adaptations with exercise training.

Aerobic training has also been shown to reduce platelet aggregation (39) and platelet adhesion at rest (40). This response might be due to alterations in the endothelium following training. FVIII and VWF have long been considered as potential markers of

endothelial damage (34,42). Because of the high correlation between levels of FVIII and VWF (25), measuring FVIII levels will also give us insight into the endothelial changes that occur with exercise training. Thus, the primary purpose of our study is to assess the effect of six months of standardized, supervised aerobic exercise training on plasma FVIII: Ag levels in sedentary, 50-75 year old men and women.

The molecular basis of high FVIII levels is only partially known and consists of both genetic and acquired factors (38). It has been estimated that 57% of the inter-individual variation in plasma FVIII and 66% of the variation in VWF levels are genetically determined (44). Higher FVIII levels are associated with aging, female sex, black race, non-O blood group, unfavorable lipid profile, and body composition (25,38). Obesity and increased visceral fat were positively associated whereas hormone replacement therapy (HRT) in women showed a negative association with FVIII levels (25,76,73). Apart from the ABO blood group gene locus, which is estimated to account for ~30 % of FVIII genetic variability (44), no other genetic markers have yet been identified that are associated with high plasma FVIII levels. Therefore, it is possible that FVIII levels might be regulated through genes influencing other related metabolic pathways.

Alterations in plasma lipoprotein-lipid levels have been proposed to influence thrombosis by modifying the activity of coagulation proteins (45) and platelets (47). A number of studies have shown significant associations between plasma lipoprotein lipids and FVIII levels (12,25,48-50), suggesting some form of coordinate regulation. Exercise training has been shown to positively affect many of the components of the plasma lipoprotein lipid profile that influence a person's CV disease risk (27,52). These associations suggest

that changes in plasma lipoprotein lipid levels might be a potential mechanism underlying the adaptation of the coagulation system with exercise training.

Apolipoprotein E (apo E for protein; APO E for gene) is a protein that plays an important role in lipid transport and metabolism (53). Polymorphisms in the APO E gene contribute to 7% of the inter-individual variability in plasma total cholesterol and LDL cholesterol (54). Three common alleles, E2, E3 and E4 exist, with allele frequencies in Caucasians of 0.08, 0.77 and 0.15, respectively. Total and LDL cholesterol levels were lowest in individuals with the E2 allele, intermediate in those with E3 alleles, and were highest in persons with the E4 allele, while the reverse has been shown with HDL cholesterol levels (53). This genotype-dependent variability in lipid levels appears constant among different populations (55). Studies have shown that homozygous carriers of the APO E2 allele develop hypertriglyceridemia (89), which in turn is associated with high plasma FVIII levels (25). Carriers of APO E4 have been shown to be more likely to develop atherosclerosis (57,58), coronary artery calcification (59) and ischemic cerebrovascular disease (60). It has also been shown that the E4 allele enhances the early stages of carotid atherosclerosis independent of total and LDL cholesterol serum levels (58). The isoform specific antioxidant property of APO E has been suggested as the cause for the atherogenic nature of APO E4 (61).

APO E genotype has been shown to interact with exercise training to affect the training – induced changes in lipid profile, with E2 and E3 carriers improving their lipid profile to a greater extent than E4 carriers (53,62). A previous study from our laboratory showed significant association between APO E genotype and training-induced changes in plasma Factor VII:Ag levels. These effects persisted even after accounting for the effects of

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exercise training on plasma lipoprotein-lipid levels (unpublished data). Thus APO E genotype, through its effects on lipoprotein lipid levels and coagulation, might be a candidate gene that influences FVIII level changes with exercise training. Since plasma lipoprotein-lipid levels are partially genetically determined, improve with exercise training, and also are significantly associated with FVIII levels, we sought to assess the effect of APO E gene polymorphisms and training-induced changes in plasma lipoprotein-lipid profiles on the changes in plasma FVIII:Ag levels with six months of aerobic exercise training.

PURPOSE

The primary purpose of the study is to examine the effect of six months of standardized, aerobic exercise training on plasma coagulation factor VIII:Ag levels in sedentary, 50-75 year old men and women. A secondary aim is to assess the influence of apolipoprotein E gene polymorphisms and training-induced changes in plasma lipoprotein-lipid levels on the changes in factor VIII:Ag levels with exercise training.

HYPOTHESIS 1

Six months of standardized, aerobic exercise training will result in a significant reduction in plasma factor VIII:Ag levels.

HYPOTHESIS 2

Exercise training-induced changes in plasma factor VIII:Ag levels will be associated with apolipoprotein E gene polymorphisms, with carriers of E2 and E3 alleles demonstrating a greater absolute decrease in factor VIII:Ag levels, compared to carriers of the E4 allele. These changes will still be evident after accounting for the lipoprotein-lipid changes with training.

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METHODS

SUBJECTS

Subjects responding to print and media advertisements were contacted by telephone to determine their eligibility. Subjects were sedentary men and women, 50 to 75 years of age, living in and around the Washington DC area. They were non-smokers, non-diabetic, and free of heart, liver, kidney and chronic lung disease. They had a body mass index (BMI) <37 kg/m^2 and did not have any medical condition that precluded vigorous exercise. Subjects were either normotensive or hypertensive (blood pressure <160/90), were not on medications affecting lipid metabolism and hemostasis, and had at least one lipid abnormality (total cholesterol > 200 mg/dl, LDL-C > 130 mg/dl, HDL-C < 40 mg/dl, or Triglycerides >200 mg/dl). All female subjects were post menopausal (absence of menses for >2 years) and those subjects taking hormone replacement therapy were asked to continue on the same regimen until the end of the study. Suitable subjects who met these initial criteria were scheduled for screening visits to determine their eligibility for participation. The study was approved by the Institutional Review Boards of the University of Maryland and the University of Pittsburgh.

SCREENING VISIT 1

During screening visit 1, written informed consent was obtained from the subjects and their health history questionnaires were reviewed to ensure qualification for the study. Height and weight were measured to ensure BMI < 37 kg/m^2. Blood pressure measurements were taken and blood samples were collected for genotyping, blood chemistry analysis, and for assessment of fasting blood glucose. This was followed by a 2-hr oral glucose tolerance test (OGTT) to rule out diabetes mellitus. Subjects were

excluded from the study if they had one of the following: a hematocrit < 35, systolic or diastolic blood pressure of >159 or >99, respectively (63), any evidence of renal or liver disease from the chemistry panel, not meeting one of the lipid abnormalities mentioned above, or any evidence of glucose intolerance (fasting glucose >126 mg/dl; 2-hr post-prandial glucose > 200 mg/dl).

SCREENING VISIT 2

Recruited subjects were given a physical examination by a qualified physician and underwent a maximal treadmill exercise test using the Bruce protocol (64) to screen for cardiovascular (CV) disease. The test was terminated when the subject could no longer continue or CV signs and symptoms appeared (65). Blood pressure, heart rate, and ECG were recorded before the test, at the end of each exercise stage and every 2 min for 6 min after exercise. Subjects who had < 2 mV ST-segment depression and no CV signs or symptoms during this test were included in the study. During this test, VO_2 was measured with a validated customized on-line system (Marquette respiratory mass spectrometer, Rayfield mixing chamber, VMM ventilatory turbine, 486 PC computer).

STUDY DESIGN

Qualified subjects participated in a 24-week aerobic exercise-training program as described under Exercise Training Intervention. Prior to starting the training, each subject underwent a 6-week dietary stabilization phase. Upon completion of dietary stabilization, and before the start of exercise training, subjects underwent baseline testing for assessment of their FVIII levels, lipid profile, body composition, and VO_2 max. Final testing, which was a repeat of the baseline tests, was conducted at the end of the 24-week exercise training phase.

GENOTYPING

 The genotyping was done at the Department of Human Genetics at the University of Pittsburgh. The genotyping for the APO E polymorphisms was done using the method previously described by Hagberg et al (66). High molecular weight genomic DNA was isolated from lymphocytes in ethylenediamine tetra-acetic acid (EDTA) anti-coagulated blood using the Puregene protocol (Gentra Systems). The genomic DNA was amplified by the standard polymerase chain reaction (PCR) method using amplification primers flanking the variable site. The amplified product was then digested with the Hhal restriction enzyme for APO E and the digested DNA separated on polyacrylamide gel followed by staining with ethidium bromide and visualization under UV light. Test samples were genotyped by direct comparison to sequence-verified controls of known genotype run on the same gels. Two independent observers scored the gels. Subjects were categorized as APO E2 if they had at least one APO E2 allele, APO E4 if they had at least one APO E4 allele, and APO E3 if they had two APO E3 alleles. Allele frequencies were estimated by gene counting.

DIETARY STABILIZATION PHASE

Qualified subjects then entered the dietary stabilization phase of the study. The subjects were maintained on the American Heart Association (AHA) 'Dietary Guidelines for the General Population' (67) throughout the study. The purpose of this diet was to keep the subjects weight stable during the intervention period in order to assess the independent effects of exercise training on plasma FVIII:Ag levels. To monitor their diet and to assess compliance, the subjects were required to complete a 7-day food record before the exercise intervention, at week 8, at week 16 and at the end of the training period. Subjects

were instructed to weigh themselves every week and to record their weight. They were not allowed to gain or lose > 5% of their baseline weight over the study period. The collected food records were analyzed for total caloric intake and the % of calories from fat, carbohydrate and protein using the Computrition software.

BASELINE TESTING

After completing the dietary stabilization phase of the study, subjects underwent baseline testing for measurement of FVIII:Ag levels. Other baseline tests measured plasma total cholesterol (TC), LDL cholesterol, HDL cholesterol, triglycerides (TG), percent total body fat (%TBF), intra-abdominal fat (CTIA), and VO_2 max.

BLOOD SAMPLE COLLECTION: Since we wanted to measure the coagulation profile in a resting state before and after training, we chose to measure factor VIII:Ag levels as the representative marker as it has been shown to be less susceptible than factor VIII coagulant activity (FVIII:C) to acute activation of the coagulation system (38). Also, it has been shown previously that the change in levels of FVIII:Ag were proportionate with the change in levels of FVIII:C (68).

All blood samples for FVIII:Ag level measurements were collected in the morning after a 12-hour overnight fast. To avoid the effect of diurnal variation on FVIII levels, all blood samples were drawn between 7 am and 9.30 am. To avoid a possible influence of inflammation on plasma FVIII levels, subjects were asked about any prior acute inflammations or infections in the previous month. In order to proceed with the blood draw, the subjects had to be free from any inflammation for at least two weeks. The blood was collected through an atraumatic venipuncture using a 21 G butterfly needle that was connected to a vacutainer 5 ml blue-top tube containing sodium citrate as anticoagulant.

The first 5 ml of blood was discarded. After blood collection, the tube was gently inverted 4-5 times to ensure adequate mixing of blood with the anticoagulant. The blood from the vacutainer tube was transferred to a plastic tube within 15 minutes of collection and centrifuged at 10,000 rpm for 20 minutes at 4° C. After centrifugation, plasma was separated into 250 µL aliquots and stored at -80° C until analysis.

Assay analysis for plasma levels of FVIII:Ag was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Asserachrom FVIIIC:Ag, Diagnostica Stago Inc., Parsippany, NJ). All assays for FVIII:Ag estimation were performed on the same day to reduce any between-day variability. Blood samples from each individual obtained before and after training were tested simultaneously in one run to eliminate any intra-assay variation. The coefficient of variation for FVIII:Ag measurement by ELISA in our laboratory was 4.8%.

Plasma lipoprotein-lipid levels were measured on blood samples drawn after an overnight fast; they were analyzed using a Hitachi 717 autoanalyzer at the Baltimore VA Medical Center Clinical Chemistry Laboratory.

BODY COMPOSITION MEASUREMENTS

PERCENT TOTAL BODY FAT (%TBF)

Percent total body fat was measured using the Dual Energy X-ray Absorptiometry (DEXA) scan (Model DPX-L, Lunar Corp, Madison, WI). The underlying theory of the DEXA scan has been described previously in detail (91). The medium scan mode was used, with the slow scan mode employed if warranted by the subject's thickness. The same scan mode was used in an individual subject throughout the study. Scan times usually were 0-30 min in the medium mode and 40-50 minutes in the slow mode. Scanner

calibration was performed daily with the manufacturer's quality assurance standard. Data analyses were performed by a single investigator to eliminate inter-observer variability.

INTRA-ABDOMINAL FAT (CTIA)

Intra-abdominal fat was measured using computerized tomography (CT) scan of the abdomen. A single slice CT scan was taken midway between L4 and L5 vertebrae using a GE Hi-Light CT scanner. Subjects were scanned in the supine position with the arms extended over the head to minimize artifact. Scanning was performed at 120 Kv with a 10 mm slice thickness and a 2 sec scan frame. Visceral and sub-cutaneous adipose tissue areas were delineated by encircling the abdominal muscular wall. Both adipose tissue areas were calculated from the pixel distribution with attenuation values between -190 and -30 Hounsfield units. A single investigator performed all data acquisition and analyses to eliminate inter-tester variability.

VO$_2$ MAX TEST

All subjects underwent a maximal treadmill exercise test to assess VO$_2$ max using a protocol that was used in previous studies on sedentary older women (69). The test started at 70% of the peak heart rate achieved on the subject's screening exercise test and treadmill grade was increased by 2% every 2 min. Blood pressure, heart rate, and ECG were monitored and the test was terminated when the subject could no longer continue. VO$_2$ was measured throughout this test and standard criteria were used to determine if VO$_2$ max had been achieved (69). Measured VO$_2$ max was used to calculate intensity ranges for the exercise training intervention.

EXERCISE TRAINING

All subjects underwent 3 exercise training session/wk supervised by study personnel. The training program lasted 6 months to ensure that adequate time was allowed for training-induced changes in the coagulation profile and FVIII:Ag. The training program was the same as used by Hagberg et al (70). Subjects were taught to exercise within their prescribed target heart rate using heart rate monitors with a wristwatch digital display (Polar, Electro Oy, Finland). Exercise sessions started and concluded with appropriate warm up and cool down activities. Initial training sessions consisted of 20 min of exercise at 50% VO_2 max. Training duration was increased by 5 min every week until 40 min of exercise at 50 % VO_2 max were completed for 3 consecutive sessions. Training intensity was then increased by 5 % VO_2 max every week until an intensity of 70 % VO_2 max was achieved. At the completion of 10 weeks of exercise, the subjects were instructed to undertake a fourth day of unsupervised exercise (during the weekend) keeping within their heart rate range and to record their heart rate when they came to exercise next week. Increases in training duration or intensity occurred only if the subject completed their exercise prescription for 3 consecutive sessions without CV signs or symptoms or undue fatigue. Exercise modes included treadmill walking, stair stepping, bicycle, cross-country ski and rowing ergometry. Subjects recorded exercise heart rate, duration and mode information for all supervised and unsupervised training sessions. Blood pressure was measured weekly throughout the exercise training intervention. If a subject's systolic or diastolic BP was consistently >159 or >99 respectively, they were not allowed to exercise and were referred to their primary care physician. Adherence to

exercise training was assessed by inspecting training log exercise intensity, duration and frequency data.

<u>FINAL TESTING</u>

The final testing was a repetition of the baseline tests. Subjects were studied in the normal physiological state. To avoid the acute effects of exercise, data collection during final testing was done 24-36 hours after the last bout of exercise.

<u>STATISTICAL ANALYSIS</u>

All data are presented as mean ± SE.

Hypothesis 1 - *Six months of standardized, aerobic exercise training will result in a significant reduction in plasma Factor VIII:Ag levels.*

The training-induced changes in plasma FVIII:Ag levels, lipoprotein lipid levels, and VO_2 max were compared using Student's t-tests. Analysis of covariance (ANCOVA) models were used to assess the independent effect of aerobic exercise training on plasma FVIII:Ag levels, after covarying for age, sex, race, body composition and HRT use.

Hypothesis 2 - *Exercise training-induced changes in plasma Factor VIII:Ag levels will be associated with apolipoprotein E gene polymorphisms, with carriers of E2 and E3 alleles demonstrating a greater absolute decrease in Factor VIII:Ag levels, compared to carriers of the E4 allele. These changes will still be evident after accounting for the lipoprotein-lipid changes with training.*

Baseline subject characteristics, lipoprotein lipid levels and FVIII:Ag levels were compared among the 3 APO E genotype groups using analysis of variance (ANOVA). ANCOVA was used to compare the effect of 6 months of endurance exercise training on FVIII:Ag levels among the 3 APO E genotype groups, before and after covarying for

training-induced changes in plasma lipoprotein-lipid levels. Least significant difference *post hoc* analysis was used when needed. When a significant interaction effect with exercise training was noted, stepwise regression procedures were used to determine the proportion of the explained variability that could be attributed to the APO E gene polymorphism.

Forty-four viable blood samples were available for use. With a medium effect size of 0.25 at an α of 0.05, a power of 0.65 was available for the analysis. At a higher effect size of 0.40, the power available to test the hypotheses was 96% (71). Descriptive statistics and ANCOVA models were generated using the SPSS 11.0 software (72). Statistical significance was set at $P \leq 0.05$.

REVIEW OF LITERATURE

INTRINSIC COAGULATION PATHWAY AND ATHEROSCLEROSIS

Cardiovascular disease (CVD) is the major cause of mortality and morbidity in the United States (1). CVD is a multi-factorial entity influenced by both genetic and environmental factors. Atherosclerosis continues to be the most common cause of CVD in the western world. Studies have indicated the significance of thrombus formation and hypercoagulability in causing atherosclerosis-related events. In his review article, Libby describes the progress of atherosclerosis from an early stage of atherogenesis to an advanced stage of atherothrombosis, which predisposes to the acute thrombotic event (3). The Bruneck Study confirmed atherothrombosis to be a key mechanism in the development of advanced stenotic atherosclerosis and showed that the risk profile of advanced atherogenesis included markers of enhanced prothrombotic capacity, attenuated fibrinolysis and clinical conditions that interfered with coagulation (2). Presence of a hypercoagulable state at the time of plaque fissuring can accelerate thrombus formation and resultant occlusion of vessel lumen (4,5,6).

For a long time, the major role in determining thrombogenicity of human atherosclerotic lesions has been ascribed to the extrinsic, tissue factor-dependent pathway of blood coagulation (92). Mann in 1999 showed that even though the extrinsic pathway is responsible for the initial thrombin generation in atherosclerosis, the intrinsic pathway catalyses thrombin formation approximately 50-fold more efficiently, thereby accounting for the dramatic increase in thrombogenicity of atherosclerotic lesion (9). To confirm the importance of the intrinsic pathway in atherosclerosis, Ananyeva et al in 2002 investigated whether in vitro exposure of major cell constituents of the vessel wall to

oxidized LDL alters their ability to support the activity of the FVIII-dependent tenase complex and subsequent thrombin formation. They showed that incubation of smooth muscle cells (SMC) and macrophages with oxidized LDL for 12 hours led to a 5-fold increase in FVIII binding, which accounted for a 10-20 fold increase in the maximal rates of thrombin formation. The increased ability of oxidized LDL-treated cells to support activity of the intrinsic tenase complex correlated with the level of FVIII bound to their surface. These results indicated the presence of FVIII in human atherosclerotic lesions. This study also pointed to the fact that once the endothelial integrity is breached, there is a dramatic increase in plaque thrombogenicity and most of that is accounted for by the FVIII-dependent intrinsic coagulation pathway (10).

FACTOR VIII (ANTIHEMOPHILIC FACTOR A)

Coagulation factor VIII (FVIII) plays a central role in the intrinsic pathway of coagulation. FVIII is a glycoprotein synthesized in the liver in the inactive single chain form. It circulates in blood bound to an endothelium-synthesized protein, Von-Willebrand factor (VWF), which protects FVIII from premature degradation and also enables the attachment of platelets to sub-endothelial collagen. The VWF is a very large multimer and occurs in a large molar excess over FVIII. The concentration of FVIII in human plasma is 100-200 ng/ml, or about 0.5 Nm, and its molecular mass is estimated to be around 250 kDa. The gene for FVIII covers 186 kbp and possesses 26 exons. The exon sequences account for only 9 kbp of the gene's 186 kbp. The gene for FVIII is located on the X chromosome, adjacent to the site denoted Xq27.3. FVIII consists of three kinds of domains, termed A, B and C. The structure of FVIII can thus be described as A1-A2-B-A3-C1-C2 and it shows homology with two other plasma proteins- ceruloplasmin and

factor V. The C domain of FVIII participates in the interaction with VWF. The activation of FVIII to FVIIIa occurs in the presence of minute quantities of thrombin. Thrombin-activated FVIII (FVIIIa) acts as the non-enzyme cofactor in the activation of factor X, in the presence of factor IXa (the actual catalyst), phospholipids and calcium (the tenase complex). Activated factor X (Xa) converts prothrombin to thrombin, leading to formation of the fibrin clot. Increased thrombin concentration exerts a positive feedback on FVIII, thus forming more thrombin and amplifying the coagulation process (11). Once the co-factor role of FVIIIa is completed, it is inactivated proteolytically by protein C.

FVIII LEVELS AND THROMBOTIC RISK

The notion that FVIII levels may be involved in the pathogenesis of arterial thrombosis came to the forefront with the finding of Rosendaal et al in 1989 that mortality due to ischemic heart disease is much lower in patients with Hemophilia A, which is caused by FVIII deficiency, when compared to the general male population (24).

Since then, several studies have identified elevated levels of FVIII and/or VWF as an independent risk factor for CVD, venous thrombosis and ischemic stroke. In the Northwick Park Heart Study (NPHS), a prospective study involving 1500 white men aged 40-64 years, it was shown after 16.1 years of follow-up that an increase of 1 SD of FVIII:C significantly raised the incidence of fatal coronary disease by 28% (93). The Cardiovascular Health Study (CHS), a population-based cohort study of 5,201 subjects over the age of 65 years, looked at the prospective association of fibrinogen and FVIII with CVD and mortality in the elderly. The study results showed that FVIII was significantly associated with coronary heart disease events (RR=1.5), and mortality

(RR=1.8) in men. High FVIII values were also found in people who died of CVD (13). Pan et al, in 1997, in a study of 147 Chinese subjects using carotid dopplers, found that the highest tertile of FVIII:C levels (>150 ng/ml) was associated with an odds ratio of 3.35 for carotid atherosclerosis when compared with the lowest tertile (<120 ng/ml) (12). Jansson et al, in 1992, in a study of 123 myocardial infarction (MI) survivors over 4.9 years, showed that a high concentration of VWF was associated with an increased risk for re-infarction and mortality in survivors of myocardial infarction (18).Yamauchi et al studied FVIII levels in coronary artery disease (CAD) of different severities vs. healthy controls and found that double and triple-vessel CAD patients had significantly higher FVIII levels than controls (15). Rice and Grant in 1998 reported similar findings when they showed that FVIII:Ag levels were higher in patients with MI and with angiographic evidence of atheroma than controls. They concluded that FVIII:Ag had an independent association with MI (14).

The Atherosclerosis Risk in Communities (ARIC) Study examined 14,477, middle to older-aged adults, who were free of CAD, and prospectively evaluated the association between multiple hemostatic factors and CAD incidence. Coronary disease risk factors and several plasma hemostatic factors were measured, and incidence of CAD was ascertained during an average follow-up of 5.2 years. Age- and race-adjusted relative risks of CAD were significantly higher in women with FVIII:C values >141% of standard (highest tertile) when compared to women with FVIII:C values <111% of standard (lowest tertile) (RR= 1.25) (17). But after correction for other CVD risk factors, this association was eliminated in the ARIC study. In contrast, the Caerphilly Heart Study in 1999 showed that, after adjusting for major ischemic heart disease (IHD) risk factors and

for baseline IHD, FVIII was still significantly associated with major incident IHD with an odds ratio of 1.3 (95% CI = 1.08-1.60) (94).

Regarding the risk of stroke, the ARIC study showed that per SD increase in FVIII, the risk increased 1.34 fold (95% CI = 1.2 -1.5) (22). The CHS also showed significant association between FVIII levels and ischemic stroke (RR=1.4) (13). In a case-control study to identify risk factors of cryptogenic brain infarction, Karttunen et al found that a high baseline FVIII level was associated with brain infarction of unknown etiology (OR-3.6, 95% CI = 1.1-12.2, P = 0.04) (23).

In a large population-based study case-control study on venous thrombosis (the Leiden Thrombophilia Study), Koster et al identified FVIII:C as an independent risk factor for venous thrombosis. The study also showed a clear dose-response relationship between FVIII levels and risk of venous thrombosis. The adjusted RR for FVIII:C levels >150 ng/ml compared with levels <100 ng/ml was 4.8 (95% CI = 2.3-10.0) (19). In studying the association of FVIII with recurrence of venous thrombosis, Kraaijenhagen et al found FVIII levels > 150 ng/ml in 57% of patients with recurrent venous thrombosis (21). The Leiden Thrombophilia study estimated the population-attributable risk for FVIII levels > 150 ng/ml as ~ 16%. This means that 16% of all deep-vein thromboses in the population may be due to high FVIII levels. With high FVIII levels being present in ~25% of the population, this constitutes an important prothrombotic risk factor.

From these available data, it can be concluded that FVIII and VWF levels are associated with an independent increased risk for arterial thrombosis as well as venous thrombosis. In the Caerphilly Heart Study, ~ 9% of people had FVIII levels >123 ng/ml, with an associated RR of 1.9 and an attributable risk of 4% (94). This tends to imply that 4% of

all arterial thrombotic events in the general population results from high FVIII levels, assuming a causal relationship between FVIII levels and arterial thrombosis.

EXERCISE TRAINING AND FACTOR VIII

It is well known that regular aerobic exercise training improves cardiovascular (CV) and ischemic risk and results in a significant reduction of CV mortality and morbidity. The pathways and mechanisms by which regular physical activity achieves this are not fully understood. Wood et al in 1988 showed that regular exercise training increased HDL cholesterol levels and reduced TG levels, thereby reducing CVD risk (26). Since then, further research has showed the beneficial effect of regular physical activity on various CVD risk factors such as insulin resistance, obesity, high blood pressure, and elevated lipoprotein-lipid levels. All these beneficial effects were explained by the effect of exercise training in delaying the development and progression of atherosclerotic disease. Further studies, however, established that severe clinical complications of atherosclerosis are caused by thrombus formation, primarily on ruptured advanced atherosclerotic plaques (4,5). This turned attention to the fact that attenuating thrombus formation by affecting the coagulation cascade may be a potential mechanism for exercise training-induced CVD risk reduction. Wannamethee et al in 2002 suggested that the benefit of physical activity may partly be due to reductions in several hemostatic and inflammatory markers (28). FVIII and VWF play a central role in the coagulation cascade and have long been considered as potential markers of endothelial damage (42,43).

The results of the few studies that have assessed the effect of exercise training on FVIII levels have been far from consistent.

Effect of Acute Exercise on FVIII levels

Acute exhaustive exercise is known to induce an increase in both coagulation and fibriniolytic activity. Exercise bouts of varied intensity and duration have all induced significant increases in FVIII levels. Additionally, increases in FVIII coagulant activity and antigen have been positively associated with exercise intensity and this increase persists into recovery (95,96). The mechanism by which exercise increases FVIII is not fully understood. The stimulus responsible for exercise-induced increases in FVIII seems to be mediated via the [beta]-adrenergic receptor pathway because [beta] blockade blunts this increase (97).

Cross-sectional Studies

The ARIC study prospectively evaluated the association between multiple hemostatic factors and CAD incidence. The ARIC cohort consisted of ~15,000 people selected from four US communities. The participants were 45-64 years at baseline and consisted of both Caucasians and African-Americans. Baseline FVIII:C was measured using a one-stage clotting assay using FVIII deficient plasma. Physical activity status was assessed at baseline by interview using a standard questionnaire. Three indices, ranging from 1 (low) to 5 (high), were derived for physical activity at work, during leisure time, and in sports. Both FVIII and VWF showed a significant negative correlation with physical activity scores, with well-trained individuals (sport activity) having the lowest FVIII level among the participants (25). Wannamethee et al in 2002 examined the relationship between physical activity and hemostatic variables as part of the British Regional Heart Study, a prospective study of CVD involving 7735, 40-59 year old men. At initial screening and at reexamination, the men were asked to indicate their usual pattern of physical activity,

under the headings of regular walking or cycling, recreational activity, and sporting (vigorous) activity. FVIII levels were measured in over 4000 people at baseline and after 20 years. In age-adjusted analyses, physical activity showed a significant inverse association with FVIII and VWF at baseline and after 20 years. This inverse association with physical activity persisted after adjustment for age, BMI, smoking, and preexisting CVD. Those subjects who had been at least lightly active at baseline but were no longer active at re-examination showed FVIII levels similar to those who had remained inactive. Those subjects who became active showed FVIII levels similar to those who remained continuously active (28). Geffken et al investigated the cross-sectional association between self-reported physical activity and markers of coagulation in a healthy elderly population as part of the CHS. They found that, compared with persons in the lowest quartile, those in the highest quartile of physical activity had a 3% reduction in FVIII:C after adjustment for gender and other CVD risk factors (29).

In contrast, Ferguson et al in 1987 measured partial thromboplastin time (APTT) as an overall measure of blood coagulability and found no significant difference among sedentary individuals, joggers, or marathon runners at rest (30). Watts compared resting FVIII:C and VWF:Ag levels in 100 athletes vs. 25 non-exercising controls. The athletes had significantly lower VWF:Ag levels whereas FVIII:C levels were not significantly different between athletes and non-exercisers (31). This contrasting finding between VWF and FVIII levels may be due to differences in sensitivity and specificity of the FVIII:C and VWF:Ag assays employed in the study.

To summarize, a majority of the cross-sectional studies observed a significant inverse dose-response relationship between physical activity and FVIII levels and suggests that increased physical activity is associated with a hypocoagulable, beneficial state.

Longitudinal Training Studies

Longitudinal exercise intervention studies that assessed FVIII levels before and after training also show mixed results, with studies showing no change (32,33,37), or a significant decrease in plasma FVIII levels (35,36).

Ponjee, Janssen, and van Wersch in 1993 studied the effect of long-term exercise training on blood coagulation. The subjects were 20 sedentary males (median age – 39 years) and 15 sedentary females (median age – 35 years). They trained 3-4 times a week with increasing intensity for 9 months. The training intensity was kept at 70-80% of maximal heart rate. After 24 and 36 weeks, all subjects ran a 15 km and a half-marathon (21 km) race. Blood samples were drawn before the training program, 5 days before both races and 5 days after the half-marathon run. VWF:Ag was measured using an ELISA method and FVIII:C was measured using a single-stage clotting assay. Plasma FVIII:C and VWF:Ag levels did not change significantly during the training program (33). These study results agreed with the previous finding of Watts that resting plasma FVIII:C and VWF levels in endurance-trained athletes did not differ from controls. The Ponjee et al study investigated younger, healthy people without any metabolic CVD risk factors. The female subjects were premenopausal and had much lesser CVD risk compared to postmenopausal females. So assessing training-induced changes in FVIII levels in an age group with a balanced hemostatic function probably would not have made a significant change. Also the Ponjee et al study did not account for diet or training-induced lipid and

body composition changes, which have been shown to be determinants of FVIII levels (25,50).

Boman et al, 1994, measured the impact of long-term, heavy exercise on VWF levels in 18-55 yr old well-trained healthy men on two food regimens having 30 or 40 percent of fat, before and after a 14-day skiing tour in the Swedish mountains. The participants skied 12-30 km/day carrying a 25-30 kg backpack, with external temperatures ranging between -10° C and -25° C. Citrated plasma was obtained before and after 1 and 2 weeks of exercise. The study did not find any significant change in plasma VWF levels after 2 weeks of intense exercise. The study found a positive correlation between daily dietary fiber and plasma VWF levels, but did not find any relation with fat content of food or energy consumption (32). The study included well-trained individuals over a very wide age group. Since FVIII and VWF show a definite increase with each decade of aging, studying a wider age group makes the findings less representative. Also the training period (2 weeks) as well as the extreme environmental conditions under which the intervention was completed may not be the ideal way to induce training-adaptations in the coagulation system.

Van den Burg et al in 2000 studied the influence of age on training-induced changes in resting and stimulated hemostatic potential in three age categories (Cat I-III; 20-30 yrs, 35-45 yrs, and 50-60 yrs, respectively) of sedentary men before and after 12 weeks of training. Participants exercised twice a week for 1 hr at a constant submaximal level. During each training session, the work rate was adjusted for each individual to maintain a heart rate corresponding to 60-70% of VO_2 max. Anthropometric measurements and a VO_2 max test were performed before the start and after 12 weeks of training. Blood

samples were obtained before and after training and FVIII:C and VWF:Ag levels were measured using an autoanalyzer. Thirty-nine participants (Cat I: $n = 13$; Cat II: $n = 13$; Cat III: $n = 13$) completed the training program. No effect of training was observed on basal (resting) or submaximal exercise (70% VO_2 max) plasma levels of FVIII:C and VWF:Ag across the three different age groups (37). Even though the study applied corrections for changes in body composition observed with training, there was no control over dietary habits of the participants. Also training-induced changes in lipoprotein-lipid levels were not taken into account during analysis. The sample size also may have been too small for each particular age group in order to elicit a significant difference with training.

Dudaev, Diukov, and Borodkin in 1988 studied the effect of exercise training on coagulation in CAD patients and found that intensive structured exercise reduced thrombinemia in CAD patients by reducing FVIII levels (36). Suzuki et al in 1992 examined hemostatic parameters in 56 post-MI patients before and after one month of systematic physical training and in 30 control post-MI patients who did not undergo such training. The training program consisted of two 40-min sessions in the morning and afternoon, 6 days/wk, with a target heart rate set at 75% of maximal heart rate. They found that the levels of FVIII:C and VWF:Ag were significantly decreased and APTT prolonged in the group with physical training ($p < 0.05$), while values were unchanged in the control group. The study concluded that physical training appears to induce a suppression of the coagulation system in patients in the recovery phase of MI (35).

These meager results suggest that FVIII:Ag and FVIII:C levels at rest decrease with training in patients with CAD, but not in normal healthy subjects. However, research on

training effects on coagulation is minimal and inconclusive. After reviewing the existing literature, it is difficult to reach any valid conclusions regarding the actual effects of physical training on blood coagulation. Design and measurement variations, employing different modes of exercise training, failing to account for potential confounding variables like age, race, lipoprotein-lipid levels, inflammatory conditions and body composition, may all have contributed to this fragmented picture. Also, most of the training studies measured FVIII:C using the one-stage clotting assay method. This method has two disadvantages. FVIII:C is more prone to be altered by accidental activation of coagulation either during blood collection, storage or during exercise training when compared to FVIII:Ag. Secondly, the one-stage clotting assay, in spite of having the advantage of simplicity, can give falsely high results if extreme care is not taken during blood collection and storage.

The well-documented association of high FVIII levels with thrombotic events coupled with the high prevalence of elevated FVIII levels in the older age group makes it important to do a well-designed training study to elucidate clearly the effect of exercise training on plasma FVIII antigen (FVIII:Ag) levels in middle-to older-aged people with known CVD risk factors.

Ruaramaa et al in 1986 assessed the influence of regular moderate-intensity physical exercise (brisk walking to slow jogging) on platelet aggregation in a population-based sample of middle-aged, overweight, mildly hypertensive men in eastern Finland. A significant inhibition of platelet aggregation from 27% to 36% was observed in the men taking regular aerobic exercise (38). On a similar front, Wang in 1995 investigated the effects of training and detraining on platelet adhesiveness. Healthy male subjects were

trained on a bicycle ergometer at about 60% of maximal oxygen consumption for 30 minutes per day, 5 days/wk for 8 weeks, and then de-conditioned for 12 weeks. They found that platelet adhesiveness at rest was reduced by exercise training but reverted back to the pretraining state after de-conditioning (39). These responses might be due to alterations in the endothelium following training.

FVIII and VWF have been considered as potential markers of endothelial damage. At 1 week after coronary artery bypass grafting, there was a significantly disproportionate rise in VWF:Ag, which suggested a degree of endothelial damage (42). Kahaleh, Osborn, and LeRoy evaluated VWF levels in 17 patients with scleroderma, a connective tissue disorder affecting the vasculature, and found significantly higher levels of VWF in patients with scleroderma compared with healthy controls (34). The observed increase of VWF in scleroderma may reflect in-vivo endothelial injury and regeneration. Because of the high correlation between levels of FVIII and VWF (25), measuring FVIII levels will also provide insights into the endothelial changes that occur with exercise training. Thus the primary purpose of our study is to assess the effect of six months of standardized, supervised aerobic exercise training on plasma FVIII: Ag levels in sedentary, 50-75 year old men and women.

DETERMINANTS OF HIGH FACTOR VIII LEVELS

The molecular basis of high FVIII levels is not fully understood. Available literature suggests that both genetic and acquired factors are involved in the regulation of plasma FVIII levels (38).

GENETIC DETERMINANTS

Results from a familial aggregation study by Kamphuisen et al have indicated the presence of a strong familial influence on FVIII and VWF levels (78). Orstavik et al determined FVIII and VWF:Ag levels in 74 monozygotic and 84 like-sexed dizygotic twin pairs and found that the variance of FVIII and VWF:Ag levels were smaller within twin pairs than between these pairs. They estimated that 57% of the total variation in plasma FVIII levels and 66% of the variation in VWF levels were genetically determined (44). Souto et al in 2000 analyzed the genetic influences on FVIII levels in the families of the Genetic Analysis of Idiopathic Thrombosis (GAIT) study and reported a heritability of 40% for FVIII levels (79).

Approximately 30 % of the genetic variability in FVIII levels was accounted for by the ABO blood group gene locus (44). The concentration of FVIII:Ag and VWF:Ag varied among ABO blood types, being lowest in type O individuals, higher in AA and BB individuals, and highest in AB individuals (44). The effect of the ABO locus on FVIII:Ag was found to be mediated through VWF:Ag (44,78). Blood group A, B, and H(O) oligosaccharide structures have been identified on the VWF molecule (80), which may affect the clearance of VWF and, thus, of the VWF/factor VIII complex, resulting in altered FVIII levels (81).

FVIII levels are influenced by sex, with women having higher values across all age groups compared to men. This may either be due to a different body fat distribution in women or may reflect an effect of female sex hormones on the synthesis and release of FVIII and/or VWF (25). Race also has a significant effect with African-Americans having much higher plasma FVIII levels compared to Caucasians. The results from the

ARIC study indicate that the black race is associated with a 17% increase in FVIII levels in men and a 15% increase in women (25)

So far, no specific genetic abnormality in the FVIII gene or VWF has been identified that explains the higher levels or increased risk of venous and/or arterial thrombosis. No sequence variations were found in the promoter and 3' terminus of the FVIII gene in 56 patients with venous thrombosis and high FVIII (>150 ng/ml) levels (83). Also, no clear associations were found between VWF or FVIII:Ag levels and polymorphisms in the promoter and FVIII-binding region of the VWF gene (38). So apart from the ABO blood group, no gene variations have been identified that are associated with high plasma FVIII levels. Therefore, it is possible that FVIII levels might be regulated through genes influencing other related metabolic pathways. One piece of supporting evidence is the study by O'Donnell et al which showed that only 50% of the persistently high FVIII levels are associated with high VWF:Ag levels, indicating that changes in VWF such as those caused by the difference in blood group, may not always be responsible for high FVIII levels (82).

OTHER DETERMINANTS OF HIGH FVIII LEVELS

FVIII levels increase with age, with an average rise of 5 to 6 ng/ml per decade (25,78). Diabetes and high serum insulin levels are also significantly associated with increased FVIII levels (25,49). FVIII levels rise as an acute phase reactant in inflammatory conditions, surgery, malignancy, liver disease, and renal disease (84). In postmenopausal women, several studies have shown a significant reduction in FVIII levels with long-term use of HRT and have proposed that reduced FVIII levels may play an important role in the cardio-protective effects of HRT (25,73,74,79). Other studies assessing HRT effects

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on coagulation did not find any significant change in FVIII levels and suggested pro-thrombotic effects for HRT use on account of the major reductions in natural anticoagulants anti-thrombin III, protein C, and protein S (85,86). In summary, prolonged HRT use may reduce FVIII levels. Since it also results in resistance to protein C and protein S, the level of cardioprotection appears questionable. So far, no studies have examined the interactive effects of exercise training and HRT use on plasma FVIII levels.

Plasma lipoprotein-lipid levels and body composition are the two major metabolic factors closely related to plasma FVIII levels, and their associations with FVIII are discussed below.

FVIII/VWF, OBESITY AND BODY FAT DISTRIBUTION

Iso et al in 1989 compared the relationship of FVIII:C levels to known CVD risk factors in Japanese and Caucasian subjects and found no significant correlations between BMI and FVIII levels in either ethnic group (50). The ARIC study showed a positive association between BMI, waist-to-hip ratio and FVIII levels both in men and in women. Sex-specific linear regression analysis showed that for every 5 kg/m^2 change in BMI, FVIII levels changed by 1.4% in men and 2.8% in women (25). Blann et al compared VWF levels in hyperlipidemic patients to levels in asymptomatic controls. In stepwise multivariate analysis, BMI was found to be an independent predictor of VWF levels (41). Abdollahi, Cushman, and Rosendaal in 2003 investigated the associations of obesity with clotting factor levels to identify possible mechanisms of venous thrombosis. They found that individuals with BMI >30 kg/m^2 had significantly higher levels of FVIII levels (43). In a group of premenopausal women, De Pergola et al found higher plasma

concentrations of VWF:Ag and activity in obese subjects compared to age-matched non-obese women (46). Blann et al found no relationship between VWF and waist-to-hip ratio used as a measure of body fat distribution (43). In contrast, in the study by De Pergola et al, plasma VWF:Ag correlated significantly with waist circumference, independent of other metabolic and non-metabolic variables (46). Cigolini et al reported that in a cohort of 38-year-old men with a wide range of fatness and fat distribution, those with more visceral fat had significantly higher levels of FVIII:C (76).

In summary, the available literature indicates that both FVIII and VWF appear to be significantly associated with obesity and truncal fat distribution.

ASSOCIATION BETWEEN LIPOPROTEIN-LIPID LEVELS AND FVIII

Iso et al in 1989 examined FVIII:C, VWF levels, and lipoprotein-lipid levels across four different populations; rural Japanese, urban Japanese, Japanese Americans, and Caucasian Americans, to understand the lower CAD mortality rates in Japanese population. They found that the serum TC, LDL cholesterol, and the ratio of TC to HDL cholesterol were lowest in rural Japanese and progressively higher in urban Japanese, Japanese Americans, and Caucasian Americans. HDL levels were significantly higher in rural and urban Japanese than in Japanese and Caucasian Americans. FVIII:C levels were significantly lower in rural Japanese compared to Japanese and Caucasian Americans. Significant positive correlations were found between FVIII levels and TC and TG levels. Moreover, FVIII:C levels were no longer significantly different between populations after controlling for either TC or TG levels (50). The ARIC prospective trial in 1993 reported the associations of FVIII and VWF with known CVD risk factors in their

preliminary cross-sectional analysis. FVIII and VWF were positively associated with TG, negatively associated with HDL cholesterol, and did not show any significant correlation with LDL cholesterol. After multivariate analysis, a significant positive association persisted for plasma TG levels (25).

Cushman et al in 1996 reported the associations of fibrinogen and FVIII with CVD risk factors as part of the baseline analysis of the CHS and found that FVIII had significant age-adjusted bivariate associations with TC, LDL cholesterol, and TG. HDL cholesterol showed a significant negative association with FVIII levels. However, in multivariate regression analysis, only LDL cholesterol in men independently correlated with FVIII levels. The study results suggested that FVIII levels may not be influenced by lifestyle changes in this age group and hypothesized that the high FVIII levels may be the result of enhanced inflammation associated with atherosclerosis since FVIII is an acute phase reactant (49). Pan et al in 1997 investigated the associations between carotid atherosclerosis, dyslipidemia, and high FVIII activity levels in a Chinese population. He found that subjects with carotid plaques had higher values of FVIII levels, waist-to-hip ratio, TC, and TG. FVIII levels were significantly correlated with TC and TG levels. In multivariate logistic regression analysis including TC, TG, and FVIII in the model, the degree of association between FVIII and carotid atherosclerosis was attenuated, suggesting that the role of FVIII in carotid atherosclerosis may be explained in part by high TC or TG values (12).

In one longitudinal training study, Ponjee et al measured the concentrations of fibrinogen and lipoprotein-lipids before and after a nine-month aerobic training program. The study

did not find any correlation between training-induced changes in fibrinogen and lipoprotein-lipid levels (51).

In conclusion, there is general consensus in literature that a significant correlation between FVIII levels and plasma lipoprotein-lipid levels exists. Both FVIII:Ag, as well as FVIII:C, have been positively correlated with plasma TC and plasma TG levels. On the other hand, a majority of these studies are cross-sectional studies with only one-time measurement. To date, there are no longitudinal studies which have looked at the association between FVIII levels and lipoprotein-lipid levels before and after aerobic training.

Minimally oxidized LDL cholesterol has been proposed to promote thrombosis in atherosclerotic lesions by modifying the activity of coagulation proteins (45). Di Minno et al found significantly increased platelet adhesion and aggregation in patients with familial hypercholesterolemia (47). VWF, the carrier protein of FVIII in plasma, showed a significant reduction with a low fat diet as well as statin therapy in atherosclerotic patients. Furthermore, reductions in VWF levels were significantly correlated with changes in TC, which indicates that endothelial changes are associated with alterations in lipoprotein-lipid levels (48,56). One of the possible mechanisms that could explain the positive association between lipid levels and FVIII is that hyperlipidemia may induce endothelial changes and high shear forces that can cause increased VWF release. Since FVIII requires VWF for stability, this in turn results in an elevated FVIII level (25,38). At the same time, elevated FVIII levels may stimulate the formation of thrombin and, thus, result in increased platelet activation and fibrin formation; processes that may contribute

to the development of large occlusive thrombi from the microthrombi initially formed on the damaged endothelium (38).

Thus, changes in lipids appear to have significant effects on factors influencing acute thrombosis in the setting of atherosclerosis. Also, as mentioned before, a number of studies have shown significant associations between plasma lipoprotein lipids and FVIII levels, suggesting a possible role of lipids and lipid-related genotypes in synthesis and regulation of FVIII and/or VWF.

EFFECT OF EXERCISE TRAINING ON ASSOCIATIONS BETWEEN APO E GENOTYPE, LIPOPROTEIN-LIPIDS, AND COAGULATION FACTORS

Exercise training has been shown to reduce CVD risk by improving the plasma lipoprotein lipid profile (27,52). Wood and Stefanick (27) reported that long-term endurance exercise training lasting longer than 12 weeks generally results in ~10 mg/dl reductions in plasma total and LDL cholesterol, and a 5 mg/dl increase in HDL cholesterol. But considerable inter-individual differences exist in plasma lipoprotein-lipid responses to exercise training (53). It has been suggested that genetic factors may be involved in mediating plasma lipoprotein-lipid responses to various stimuli (53). Apolipoprotein E is a 299 amino acid protein encoded by the APO E gene on chromosome 19q13.2. Three major apo E isoforms are coded by three alleles at the APO E locus, designated E2, E3, and E4, giving rise to six common phenotypes. The most common isoform, E3, is characterized by a cysteine at amino acid residue 112 and an arginine at residue 158. The E2 isoform has a cysteine at residues 112 and 158, whereas the E4 allele product has an arginine at residues 112 and 158. Apo E is a constituent of TG-rich chylomicrons and very-low-density lipoprotein particles and mediates the

interaction of chylomicron remnants and intermediate density lipoprotein particles with lipoprotein receptors. In Caucasians, the allele frequency ranges between 0.7-0.8 for the E3 allele, between 0.10-0.15 for the E4 allele, and between 0.05-0.10 for the E2 allele (53).

The APO E locus contributes to determining the variation in plasma cholesterol levels of healthy and diseased populations. It also influences the expression of hyperlipidemia and appears to modulate the susceptibility to atherosclerosis in a complex multi-factorial interaction. Polymorphisms in the APO E gene contribute ~7 % of the inter-individual variability in plasma TC and LDL cholesterol (54). LDL cholesterol levels are least in individuals with the E2 allele, intermediate in those with E3 allele, and are highest in those with at least one E4 allele. It has been shown that APO E2 allele promotes hypertriglyceridemia (89), which is an important determinant of plasma FVIII levels. Hallman et al analyzed the effect of ethnicity and APO E genotype on lipoprotein-lipid levels and found that the effects of APO E genotype on lipoprotein-lipid levels did not differ significantly among different ethnic groups (55). Hixson, in 1991, investigated the effect of APO E polymorphisms on atherosclerosis in young males. He found that E2E3 heterozygotes had the least atherosclerotic involvement of thoracic aorta, E3E4 had the greatest involvement, and E3E3 had intermediate involvement. APO E genotype accounted for 5.7% in whites and 5.9% in blacks of the observed variation in lesions in the thoracic aorta. Adjusting for cholesterol levels did not significantly change the genotype effect, suggesting that the effect of APO E4 on arterial lesions may not be mediated entirely by changes in serum cholesterol concentrations (57). The MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) project suggested

that an increase of 0.01 in the relative frequency of the E4 allele would increase the CAD death rate by 24.5 per 100,000 (87). Gerdes et al examined the relation between APO E genotype and a major coronary event or death in 966 Finnish survivors of MI as part of the Scandinavian Simvastatin Survival Study. After a 5.5 year follow-up, they concluded that MI survivors carrying the E4 allele have a nearly 2-fold increased risk of dying compared with other patients (88). Cattin et al studied the contribution of the APO E polymorphism to carotid intima-media thickness in middle-aged healthy subjects and concluded that the E4 allele enhanced the early stages of carotid atherosclerosis independent of total and LDL cholesterol levels (58). Kardia et al in 1999 suggested that APO E gene polymorphisms may play a role in determining the contribution of established risk factors to risk of coronary artery calcification (59)

In a meta-analysis on the association between APO E genotype and ischemic cerebrovascular disease, McCarron, Delong, and Alberts reported that carriers of the E4 allele are more frequent among patients with ischemic stroke compared with control subjects (60). The mechanism underlying the excess of the E4 allele in both CAD and ischemic stroke is unclear. It has been proposed that the association may result from the increased production and decreased peripheral catabolism of LDL particles. However, previous studies show that the association of the E4 allele with atherosclerotic CVD persisted after adjustment for LDL cholesterol levels (57,58), suggesting that the APOE E4 effect is independent of lipid levels. The isoform-specific antioxidant property of APO E may account for the atherogenic nature of APO E4 (61). According to this hypothesis the increased antioxidant activity of APO E2 might protect the individual from clinical manifestations of atherosclerosis.

APO E genotype has been shown to interact with exercise training to affect the training-induced changes in lipid profile, with E2 and E3 carriers improving their lipid profile to a greater extent than E4 carriers (53,62). In a cross-sectional study in 1996, Taimela et al investigated the interactive effect of APO E genotype and physical activity on lipid profile. They assessed the plasma lipoprotein-lipid profiles of ~1,500 Finnish children and young adults aged 9–24 yr in whom leisure-time physical activity was assessed by a standard questionnaire. The study found that in males, in addition to physical activity levels affecting plasma lipoprotein-lipid profiles, there was an interaction effect between APO E phenotype and physical activity on plasma lipoprotein-lipid levels. In APO E4/4 men physical activity levels did not affect plasma lipoprotein-lipid levels, whereas in APO E3/4 and 3/3 men, there was an inverse effect of physical activity level on plasma TC and LDL cholesterol and a positive effect on HDL cholesterol/TC ratio (62). Hagberg et al in 1999 undertook the first longitudinal exercise intervention study assessing the impact of APO E genotype on plasma lipoprotein-lipid responses to exercise training and found that middle-aged and older APO E2 genotype men had larger overall plasma lipoprotein-lipid profile improvements with prolonged endurance exercise training than otherwise comparable APO E3 and E4 genotype men (66). The differences in plasma HDL cholesterol increases with exercise training among APO E genotype groups remained significant after controlling for body weight changes, whereas the reduction in plasma TG in the APO E2 genotype men tended to be greater than in the APO E4 men after controlling for changes in body weight (p= 0.09) (66). The longitudinal intervention data provide strong evidence that the plasma lipoprotein-lipid profiles of APO E2 individuals may be affected most by prolonged endurance exercise training.

The direct and indirect effects of APO E genotype on coagulation have not been studied in detail. One previous study showed that in renal failure patients, Vitamin K (required for clotting factors II,VII,IX, and X) concentrations were related to APO E genotype, being greatest in E2 carriers and least in E4 carriers (90). In studying the effects of APO E genotype and training-induced lipoprotein-lipid changes on plasma FVII levels, Hagberg et al found that changes in plasma FVII:Ag levels with exercise training were associated with APO E genotype (P=0.012) with APO E4 individuals reducing FVII:Ag levels the most with training. The plasma FVII:Ag levels remained associated with APO E genotype after covarying for the training-induced changes in plasma TC, HDL-cholesterol, and TG levels (P=0.047) (unpublished data). Thus, it is possible that APO E genotype might have an independent effect on FVIII levels and also an interactive effect with lipoprotein-lipid changes. Thus, the APO E genotype, through its effects on lipoprotein-lipid levels and coagulation, might be a candidate gene that might influence FVIII levels with exercise training. Therefore, the second purpose of our study is to investigate the relationships between APO E genotype and changes in plasma lipoprotein-lipid levels and FVIII levels with exercise training.

RESULTS

Forty–four subjects completed the 6-month exercise training intervention and had blood samples that were analyzed from before and after training. All these subjects also fulfilled the criteria for dietary compliance (AHA-'Dietary Guidelines for the General Population'), weight stability (gain or loss of weight < 5% of baseline weight), and exercise participation (>75% attendance).

The baseline general physical characteristics of the subjects are shown in Table 1. At baseline, there were no significant differences in age or BMI between men and women. Men had significantly higher body weight (87.5±3.8 kg vs. 78.6±2.4 kg, p=0.043) and VO_2 max (29.3±1.2 ml/kg/min vs. 22.6±0.7 ml/kg/min, p<0.0001) when compared to women. Baseline FVIII:Ag levels and lipid profile are given in Table 2 and body composition measures are presented in Table 3. There was no significant difference in plasma FVIII:Ag levels between genders at baseline (144.7±8.6% vs. 157±9.3%). Women had significantly higher baseline TC values (212.8±5.7 mg/dl vs. 182.5±7.8 mg/dl, p=0.003), higher HDL values (53.1±2.8 mg/dl vs. 38.2±2.8 mg/dl, p=0.001), and higher % TBF (41.7±1.6% vs. 26.8±2.0%, p<0.0001) when compared to men. FVIII:Ag levels at baseline were positively correlated to baseline CTIA for all subjects (r= 0.30; p=0.05). This correlation was also seen after 6 months of exercise training (r=0.37; p=0.02). FVIII:Ag levels were not significantly correlated with any of the lipoprotein-lipid parameters at baseline (data not shown).

The exercise intervention resulted in a 15.6 % increase in VO_2 max (25.0±0.8 ml/kg/min vs. 28.9±1.1 ml/kg/min, p<0.0001), indicating a substantial training stimulus. Plasma FVIII:Ag levels increased by 3.5% (152.5±6.7% vs. 156.0±6.1%, p=0.290) with 6

months of exercise training. The changes in FVIII:Ag levels with training were not affected by age, gender, BMI and HRT use. In women, baseline FVIII:Ag levels were significantly different between those on (n=9) and those not on HRT (n=19) (125.2±11.4% in HRT users vs. 172.0±11.2% in non-users; p=0.016). This baseline difference in FVIII:Ag between HRT and non-HRT women was not sustained after 6 months of training, with a trend for an increase in FVIII:Ag levels in the HRT users (Increase of 13.7± 4.6% in HRT users vs. a decrease of 2.0± 6.2% in non-users, p=0.113).

With training there were significant changes in the plasma lipoprotein-lipid profile as well as body composition parameters (Table 4.) There was a significant reduction in plasma TG levels with training (-17.7±6.5 mg/dl, p=0.009) whereas HDL, LDL, and TC showed a significant increase (4.5±0.7 mg/dl, p< 0.0001; 6.5±3.1 mg/dl, p=0.048; 6.1±3.0 mg/dl, p=0.049, respectively). There were significant reductions in %TBF (-1.5±0.3%, p<0.0001) and CTIA (-14.6±3.3 cm^2, p<0.0001) with 6 mo of exercise training. The FVIII:Ag changes with exercise training did not correlate with changes in any of the plasma lipoprotein-lipid levels and body composition measures with training (data not shown). FVIII:Ag changes with exercise training were not affected by training-induced changes in plasma lipoprotein-lipid levels. FVIII:Ag changes with exercise training were not significantly associated with training-induced changes in CTIA or %TBF.

Among women, HRT users had significantly lower CTIA at baseline compared to non-HRT users (89.8±9.2 cm^2 vs. 122.5±5.8 cm^2, p=0.006). This difference persisted after 6 months of exercise training (80.7±7.2 cm^2 vs. 114.8±6.7 cm^2, p=0.006).

Effect of APO E Genotype: The APO E allele frequencies among the subjects who completed the study are given in Table 5. Of the 44 subjects who finished the exercise training intervention, 18% were APO E2 (n=8), 46% were APO E3 (n=20) and 36% were APO E4 (n=16). The frequencies did not vary significantly between genders.

There were no significant differences in baseline FVIII:Ag levels among APO E genotype groups. APO E2 subjects had higher baseline FVIII:Ag levels compared to E3 and E4 subjects (E2: 165.0±10.9%; E3: 151.3±12.0%; E4: 147.8±9.6%) but the differences did not reach statistical significance. No significant difference in FVIII:Ag changes among APO E genotypes were noted after 6 mo of exercise training (Table 6). There was no gender effect in FVIII:Ag changes with exercise training among genotype groups (data not shown). In women, there was no difference in FVIII:Ag changes among APO E genotypes between HRT users and non-users (data not shown).

At baseline, APO E2 individuals had lower TC, TG, LDL, and higher HDL levels but none of the differences were statistically significant (Table 7). Also, plasma lipoprotein-lipid changes with 6 months of exercise training were not significantly different among APO E genotype groups (Table 7). Training-induced changes in FVIII:Ag levels were not associated with the APOE genotype after co varying for training-induced changes in plasma lipoprotein-lipid levels.

At baseline, there was a significant difference in CTIA among the APO E genotype groups (p=0.028, Table 7). Post-hoc tests revealed that APO E4 individuals had significantly lower CTIA when compared to APO E2. With exercise training, the change in CTIA fat was not significantly different among APO E genotypes (data not shown).

Table 1. Baseline physical characteristics

	Total (n=44)	*Men (n=16)*	*Women (n=28)*
Age (yrs)	58.3±0.9	57.6±1.7	58.6±1.1
Body weight (Kg)	81.9±2.1	87.5±3.8 *	78.6±2.4
BMI (Kg/m^2)	28.1±0.6	27.1±0.9	28.9±0.8
VO$_2$ max (ml/kg/min)	25.0±0.8	29.3±1.2 *	22.6±0.7

Values are mean ± SEM
* Significantly higher vs. women (p<0.05

Table 2. Baseline FVIII: Ag levels and lipid profile

	Total (n=44)	*Men (n=16)*	*Women (n=28)*	*P value #*	*HRT (n=9)*	*Non-HRT (n=19)*	*P Value #*
FVIII:Ag (% of standard)	152.5±6.7	144.7±8.6	157.0±9.3	NS	125.2±11.4	172.0±11.2	0.016
Total Cholesterol (mg/dl)	201.8±5.0	182.5±7.8	212.8±5.7	0.003	201.7±7.0	218.0±7.5	NS
Triglycerides (mg/dl)	157.6±10.8	161.9±17.7	155.1±13.9	NS	155.4±31.0	155±15.1	NS
HDL Cholesterol (mg/dl)	47.7±2.3	38.2±2.8	53.1±2.8	0.001	55.3±5.3	52.1±3.2	NS
LDL Cholesterol (mg/dl)	122.2±4.2	112.4±5.8	127.8±5.4	0.077	115.9±8.3	133.4±6.7	NS

Values are mean ± SEM
t-test for differences between genders or between women on and not on HRT
Significant at P <0.05

Table 3. Baseline body composition measures

	Total	*Men*	*Women*	*P value #*	*HRT*	*Non-HRT*	*P value #*
CT Intra-abdominal fat (n=42)	116.6±6.3	123.5±14.5 (n=15)	112.8±5.6 (n=27)	NS	89.8±9.2 (n=8)	122.5±5.8 (n=19)	0.006
Total Body Fat (%)(n=42)	36.7±1.7	26.8±2.0 (n=14)	41.7±1.6 (n=28)	0.0001	41.5±2.6 (n=9)	41.7±2.0 (n=19)	NS

Values are mean ± SEM
t-test for differences between genders or between women on and not on HRT
Significant at P <0.05

Table 4. Changes in response to six months of exercise training

	Baseline	*Final*	*Change*	*P value*
FVIII:Ag ((% of standard)	152.5±6.7	156.0±6.1	3.5±3.3	0.290
Total Cholesterol (mg/dl)	201.8±5.0	207.9±5.4	6.1±3.0	0.049
Triglycerides (mg/dl)	157.6±10.8	139.9±8.8	-17.7±6.5	0.009
HDL Cholesterol (mg/dl)	47.7±2.3	52.2±4.7	4.5±0.7	<0.0001
LDL Cholesterol (mg/dl)	122.2±4.2	128.7±2.4	6.5±3.1	0.048
VO_2 max (ml/kg/min)	25.0±0.8	28.9±1.1	3.9±0.5	<0.0001
CT Intra-abdominal fat (cm^2)	116.6±6.3	102.0±5.6	-14.6±3.3	<0.0001
Total Body Fat (%)	36.7±1.7	35.2±1.7	-1.5±0.3	<0.0001

Values are mean ± SEM
Significant at P <0.05

Table 5. APO E genotype- Allele frequencies

Group	N	Allele Freq APO E2	Allele Freq APO E3	Allele Freq APO E4
TOTAL	44	0.18 (n=8)	0.46 (n=20)	0.36 (n=16)
MEN	16	0.25 (n=4)	0.44 (n=7)	0.31 (n=5)
WOMEN	28	0.14 (n=4)	0.46 (n=13)	0.39 (n=11)

Table 6. FVIII:Ag levels among APO E genotype groups

	All Genotypes	APO E2	APO E3	APO E4	P-Value #
FVIII:Ag - Baseline	152.5±6.7	165.0±10.9	151.3±12.0	147.8±9.6	0.68
FVIII:Ag - Final	156.0±6.1	166.7±8.5	155.2±9.6	151.8±11.3	0.70
FVIII:Ag - Change	3.5±3.3	1.8±5.0	3.9±5.3	4.0±6.0	0.97

Values are mean ± SEM
One-way ANOVA for comparison between genotype groups

Table 7. Lipoprotein-lipid levels and CTIA fat among APO E genotype groups

	All Genotypes	*APO E2*	*APO E3*	*APO E4*	*P-Value #*
Total Cholesterol (mg/dl) - **BASELINE**	201.8±5.0	176.3±10.0	206.7±7.3	208.4±8.1	0.054
Total Cholesterol (mg/dl)- **FINAL**	207.9±5.4	171.0±7.5	216.0±6.8	216.2±9.3	0.003
Total Cholesterol (mg/dl)- **CHANGE**	6.1±3.0	-5.3±3.2	9.3±5.2	7.8±4.5	NS
Triglycerides (mg/dl)- **BASELINE**	157.6±10.8	131.5±19.2	174.3±19.2	149.7±14.2	NS
Triglycerides (mg/dl)- **FINAL**	139.9±8.8	111.3±19.7	154.4±13.1	136.1±14.1	NS
Triglycerides (mg/dl) - **CHANGE**	-17.7±6.5	-20.3±7.8	-20.0±12.7	-13.6±7.9	NS
HDL Cholesterol (mg/dl) - **BASELINE**	47.7±2.3	50.3±5.0	45.0±3.4	49.8±4.0	NS
HDL Cholesterol (mg/dl) – **FINAL**	52.2±2.5	53.5±4.9	49.6±3.6	54.8±4.6	NS
HDL Cholesterol (mg/dl) - **CHANGE**	4.5±0.7	3.3±0.9	4.6±1.1	5.0±1.4	NS
LDL Cholesterol (mg/dl) - **BASELINE**	122.2±4.2	102.5±7.1	125.5±6.2	127.9±7.1	NS
LDL Cholesterol (mg/dl) – **FINAL**	128.7±4.7	94.9±5.7	136.9±5.9	135.4±8.1	0.002

	All Genotypes	APO E2	APO E3	APO E4	P-Value #
LDL Cholesterol (mg/dl) - **CHANGE**	6.5±3.1	-7.6±2.2	11.4±5.9	7.5±3.9	0.097
CT Intra-abdominal fat (cm^2) – **BASELINE**	116.6±6.3	139.9±20.9	123.0±6.7	96.2±8.8	0.028
CT Intra-abdominal fat (cm^2)- **FINAL**	102.0±5.6	114.9±13.8	107.9±7.1	87.5±9.8	0.138
CT Intra-abdominal fat (cm^2) - **CHANGE**	-14.6±3.3	-25.0 ±10.9	15.0±3.9	-8.7±4.9	NS

Values are mean ± SEM

P <0.05 represents significant difference with exercise training among genotype groups

One-way ANOVA for comparison between genotype groups

Graph 1 - Plasma FVIII:Ag changes between genders

Graph 1 - Plasma FVIII:Ag changes between genders
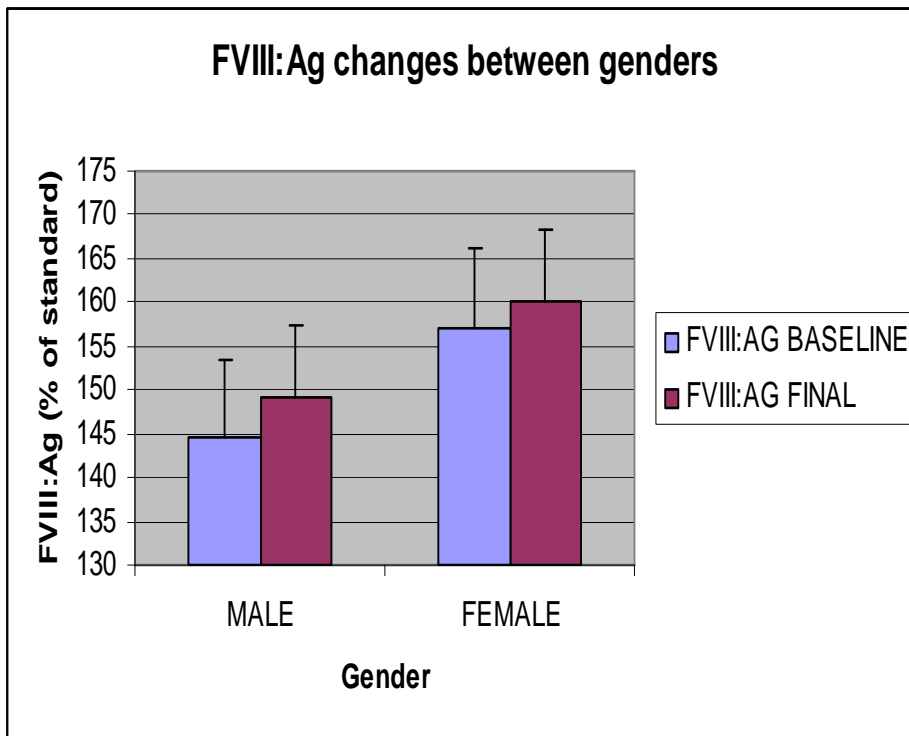
Graph 2- Plasma FVIII:Ag change with HRT use

Plasma FVIII:Ag levels with HRT use

$- p=0.113 and *- p=0.016 b/w HRT users and non-users before and after training
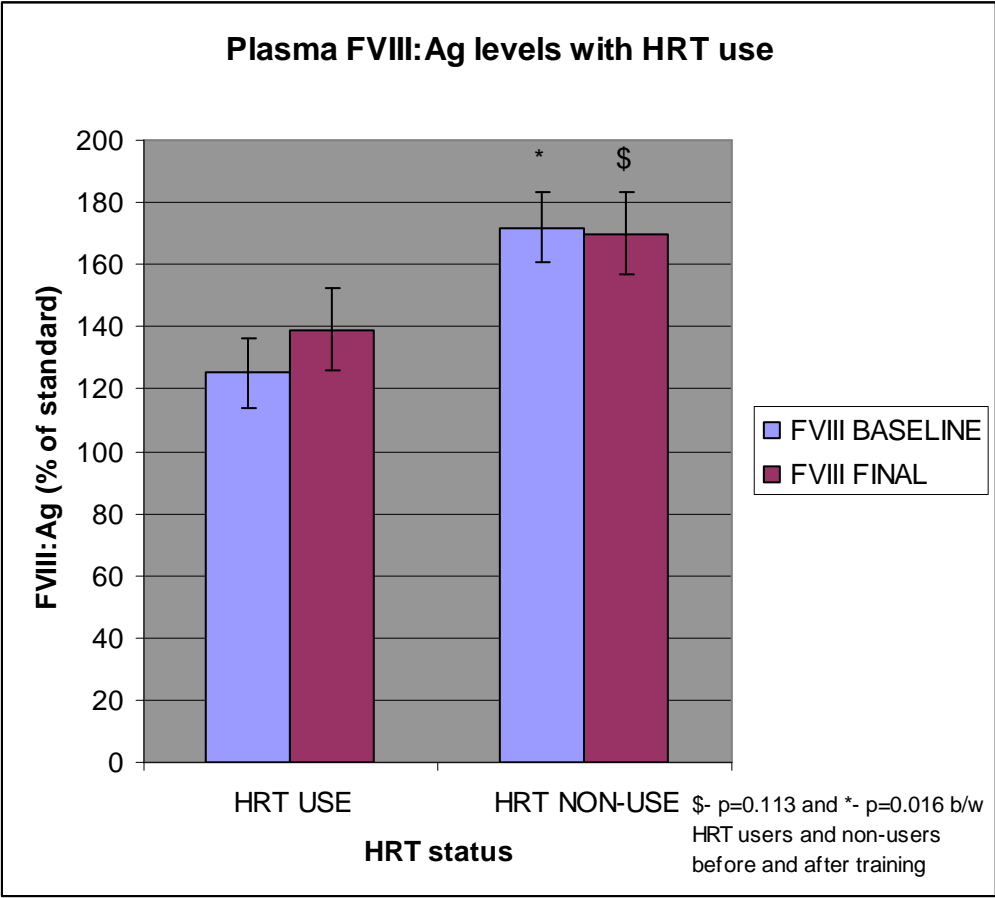
**Graph 3 - Factor VIII:Ag changes among APO E genotype groups –
Baseline and Final**
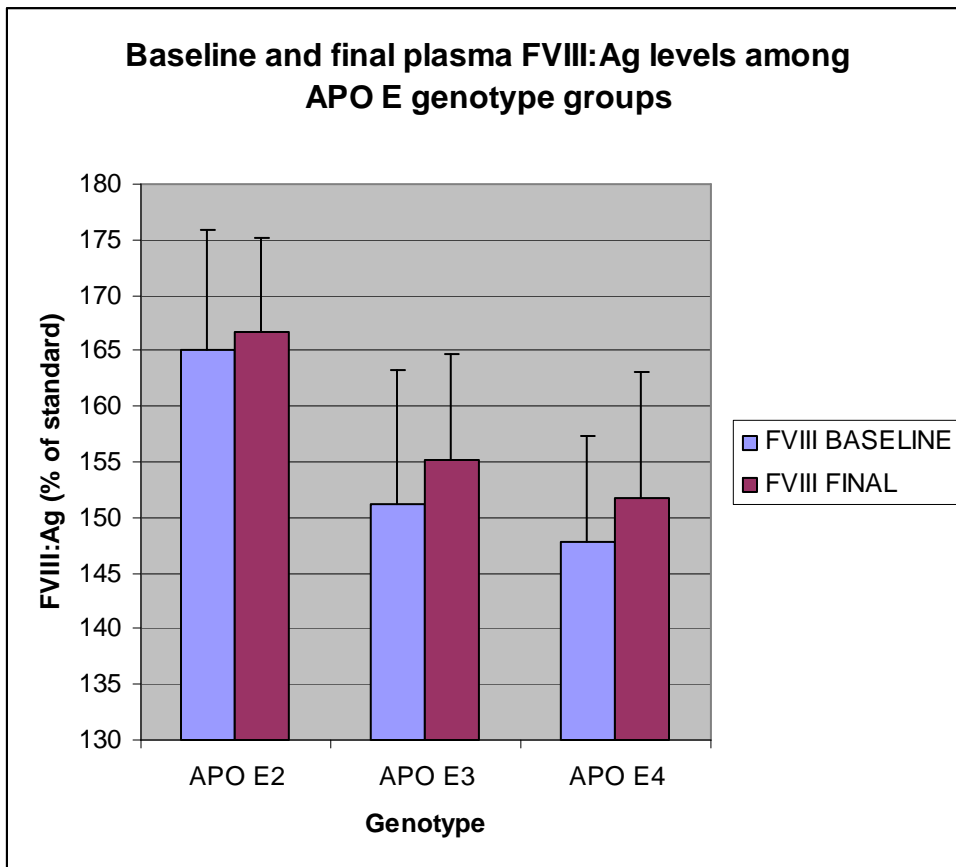
Graph 4 - Baseline and Final CTIA changes among APO E genotype groups

Baseline and final CTIA changes among APO E genotype groups
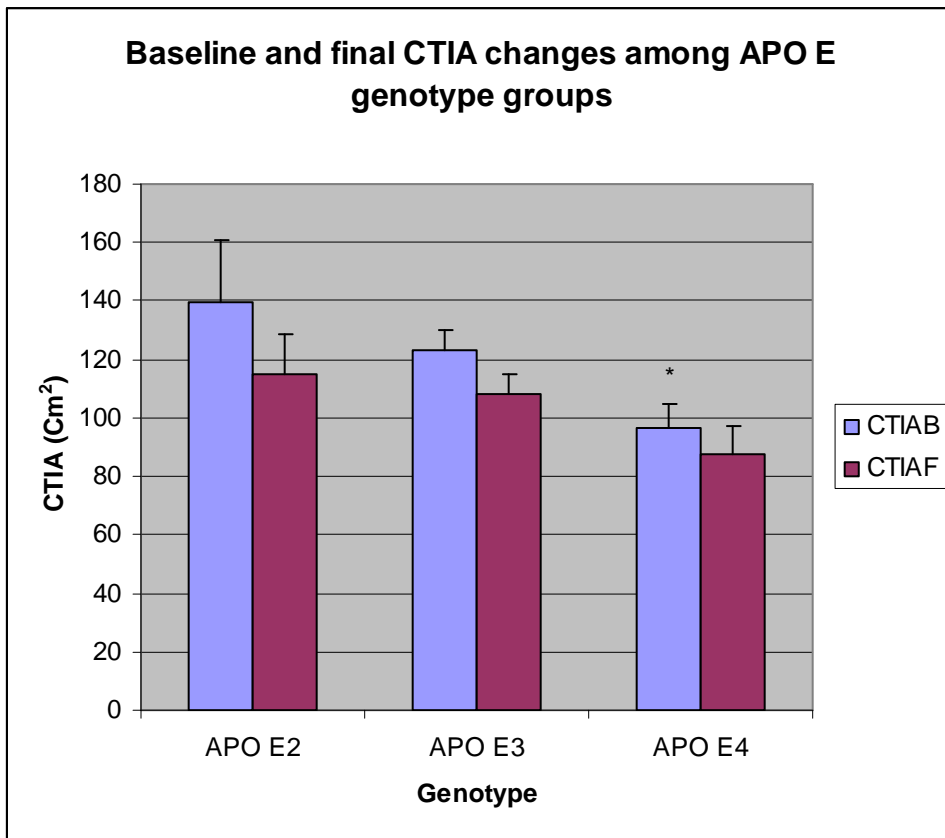
* p=0.028 for difference in CTIA at baseline among APO E genotype groups

Graph 5 – Baseline and Final lipoprotein-lipid levels among APO E genotype groups

Lipoprotein-lipid levels among APO E genotype groups
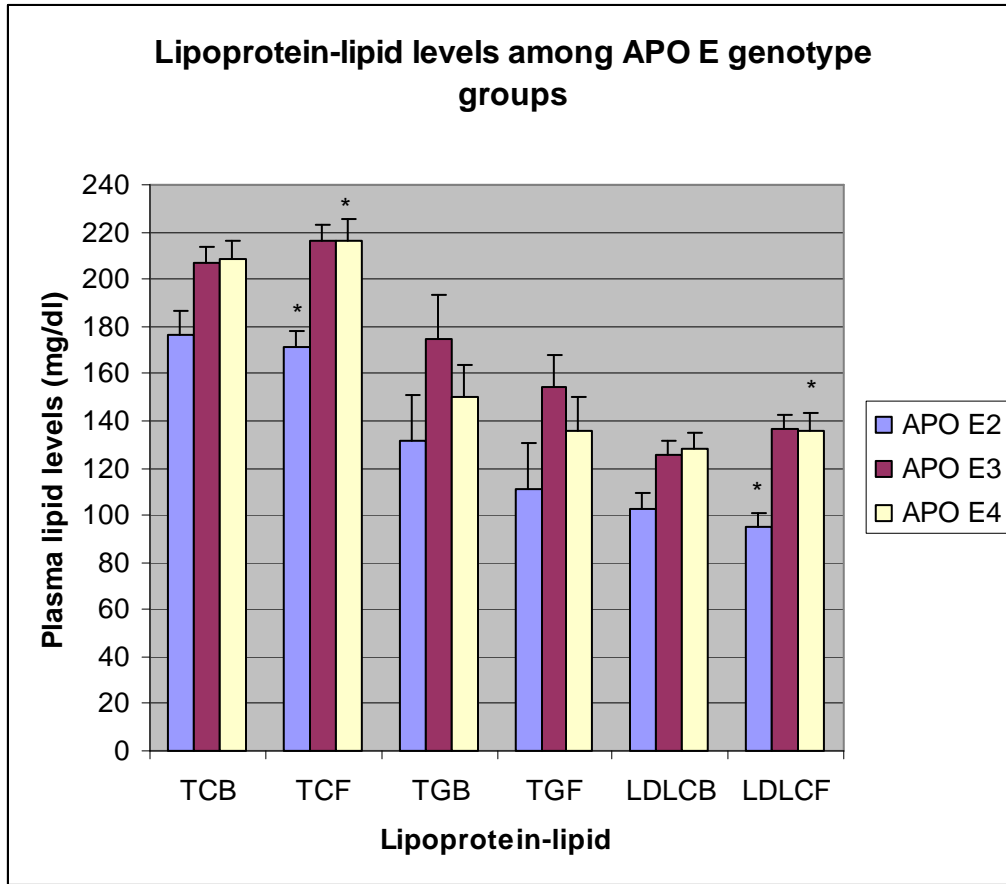
P<0.05 for difference in lipoprotein-lipid levels among APO E genotype groups

TCB- Total cholesterol - baseline
TCF- Total cholesterol – final
TGB- Triglyceride –baseline
TGF- Triglyceride - final
LDLB – LDL cholesterol - baseline
LDLF – LDL cholesterol - final

DISCUSSION

Cardiovascular disease (CVD) continues to be the number one killer of middle to older-aged adults in the United States. Accelerated thrombogenesis is the major trigger for a cardiovascular event. Aging also is associated with unfavorable changes in coagulation components that may play a role in the enhanced risk for thrombotic events (7, 8). It is well known that aerobic exercise training is associated with a considerable reduction in cardiovascular and ischemic risk in middle to older adults (26,27). Available data advocate reduction in thrombogenic potential as a potential mechanism for training-induced CVD risk reduction, thereby supporting the concept that "regular exercise is good for heart and blood vessels". The primary purpose of the present study was to examine the effect of six months of standardized, aerobic exercise training on plasma coagulation factor VIII:Ag levels, a known risk factor for CVD, in older men and women. The study results showed a 3.5% increase in FVIII:Ag levels with 6 months of exercise training; however, this change was not statistically significant. A secondary aim was to assess the influence of Apolipoprotein E gene polymorphisms and training-induced changes in plasma lipoprotein-lipid levels on the changes in FVIII:Ag levels with exercise training. Our study did not show any genotype effect on plasma FVIII:Ag levels with exercise training. With respect to the secondary purpose, ours was the first study to consider a possible gene-exercise interaction on plasma FVIII:Ag levels.

Hypothesis # 1 - *Six months of standardized, aerobic exercise training will result in a significant reduction in plasma Factor VIII:Ag levels.*

There was a significant increase in VO_2 max with 6 months of training, indicating the effectiveness of aerobic training in improving cardio-respiratory fitness. We found that there was no significant change in plasma FVIII:Ag levels with 6 months of aerobic training. Plasma FVIII:Ag levels showed a non-significant increase of 3.5% with exercise training. The changes in FVIII:Ag levels with training were not affected by age, gender, BMI or HRT use. The effect of race on baseline plasma FVIII:Ag levels could not be properly assessed due to large disparity in sample size among whites (n=32) and blacks (n=9). These results are similar to the findings of the three previous studies which reported no change in FVIII levels with training. Boman et al in 1994 measured VWF levels in 18-55 yr old trained healthy men on a low fat diet, before and after a 14-day skiing tour in the Swedish mountains, and found no significant change in plasma VWF levels (32). Ponjee et al in 1993 found no change in plasma FVIII:C after 24 weeks of training (4 days a week) in 20 sedentary men (median age = 39 yrs) and 15 sedentary women (median age = 35 yrs) (33). Van den Burg et al in 2000 found no significant change in FVIII:C and VWF in 50-60 yr old sedentary men after 12 weeks of exercise training (2 days a week for 1 hr) (37). Even though these three previous studies showed the same results, their designs were considerably different in terms of the age group selected, training intensity, training duration, and the selected outcome variable.

The Boman et al study accounted for dietary and body composition changes with training, but studied well-trained individuals over a very wide age group. Since FVIII and VWF levels show a definite increase with each decade of aging, studying a wider age group makes the findings less representative. Also the training period (2 weeks) may not

be enough to induce training-adaptations in either the coagulation and/or fibrinolytic system.

We tried to overcome the inadequacies of the previous studies in designing the present study. Our study included healthy, sedentary, 50-75 year old men and women, who had at least one lipid abnormality according to the NCEP guidelines. Compared to the previous studies, we studied people in a narrow age group (50-75 yrs) as well those who had at least one significant risk factor for thrombotic events. Qualified subjects went through a 6-week dietary stabilization phase, and were tested before and after a 24-week standardized, supervised aerobic exercise training intervention for plasma FVIII:Ag levels, lipid profile, body composition parameters, and VO_2 max.

The study of coagulation factors requires meticulous standardization in sampling and processing. In this study, we tried to minimize these factors as best as we could. Samples were collected between 7-9.30 a.m. to avoid any possible diurnal variation of FVIII:Ag levels. To avoid the acute effect of physical activity, the samples for final testing were taken 24-36 hours after the last bout of exercise. Also, subjects rested for 20 minutes before each blood draw. We also controlled for possible inflammation/infection that might stimulate the coagulation cascade by excluding those subjects who had an infectious/inflammatory event in the 2 weeks prior to sampling. The venipuncture was performed using a 21 ¾ G butterfly needle without a tourniquet, thereby minimizing the false elevation in FVIII levels caused by using a tourniquet and/or catheter. We chose to measure Factor VIII:Ag levels as the representative marker as it has been shown to be less susceptible to acute activation of the coagulation system, either during blood collection and/or storage. We performed all assays for FVIII:Ag estimation on the same

day to reduce any between-day variability. Also, by performing the ELISA method for FVIII:Ag measurement, we were able to minimize the falsely high values that may result from performing the one-stage clotting assay for measuring FVIII coagulant activity (FVIII:C) (38).

Thus, by designing a longitudinal, prospective exercise intervention study with dietary stabilization and strict weight loss/gain criteria, we were able to assess the effect of long term endurance training on plasma FVIII:Ag levels, independent of dietary changes and substantial changes in body weight and body composition. Still, we did not find any significant difference in FVIII:Ag levels with 6 mo of training. But FVIII:Ag levels did show significant inter-individual variations ranging from a 63% decrease to a 40% increase. This suggests a strong influence of genetic and/or metabolic factors on training-induced changes in FVIII levels which requires further exploration. The sample size in our study was higher than the previous longitudinal training studies. Still, it may not have been adequate to elicit a statistically significant difference, considering the volatile nature of changes in coagulation factor levels.

Hypothesis # 2 - *Exercise training-induced changes in plasma Factor VIII:Ag levels will be associated with Apolipoprotein E gene polymorphisms, with carriers of E2 and E3 alleles demonstrating a greater absolute decrease in Factor VIII:Ag levels, compared to carriers of the E4 allele. These changes will still be evident after accounting for the lipoprotein-lipid changes with training.*

With training, there were significant improvements in plasma triglyceride levels, HDL cholesterol levels, CTIA and %TBF. We did not find any significant correlation between FVIII:Ag levels and lipoprotein-lipid levels either at baseline or after 6 months of

training. These results are in contrast to earlier epidemiological studies which found significant associations between FVIII levels and various lipoprotein-lipid components, especially TG and TC (12,25,49,50). Moreover, the changes in FVIII:Ag levels with exercise training were not associated with training-induced changes in lipoprotein-lipid levels or body composition parameters. This lack of association with lipoprotein-lipids could be due to a small sample size in the present study which may have been inadequate to detect such a possible relationship. There is also a possibility that FVIII assays may not be as accurate and sensitive as the lipoprotein-lipid assays and that we may be underestimating the true proportion of variation in FVIII levels explained by the lipoprotein-lipid levels. Our results support the independent role of FVIII in modulating CVD risk and tend to argue against the hypothesis that the role of high FVIII levels in arterial thrombosis may be associated with very high cholesterol or triglyceride values. If future studies can show that lipoproteins are able to support coagulation reactions under physiologically relevant conditions, then this would support the hypothesis that they are thrombotic as well as atherogenic risk factors and would provide a possible mechanism, through direct binding, for the epidemiological association of lipoprotein levels with coagulation factor levels.

A previous study from our laboratory showed a significant association between the lipid-related APO E genotype and training-induced changes in plasma Factor VII:Ag levels, even after covarying for training-induced changes in plasma lipoprotein-lipid levels (unpublished data). This association between APO E genotype and training-induced coagulation factor levels and its independent role as a risk factor for atherosclerosis and stroke prompted us to examine whether APO E genotype played a role in determining the

inter-individual variability of FVIII level responses to training. However, our results showed that plasma FVIII:Ag changes with exercise training were not associated, independently or after covarying for plasma lipoprotein-lipid changes with exercise training, with the APO E genotype. The significant association of lipoprotein-lipids and APO E genotype with FVII:Ag levels, but not with FVIII:Ag levels, suggests that the effect of lipoprotein-lipids, if any, in influencing training-induced coagulation profile is mediated predominantly through the tissue factor-activated extrinsic cascade with minimal involvement of the FVIII-dependent intrinsic coagulation pathway.

One of the interesting findings of the present study was the significant positive correlation of FVIII:Ag levels with CTIA, but not with %TBF, at baseline and after 6 months of exercise training. The insulin resistance syndrome associated with central obesity can cause endothelial dysfunction and elevated FVIII levels, which may explain the lack of association with %TBF. Previous epidemiological studies also found similar associations between FVIII levels and waist-hip ratio and central fat (25,76). It is well known that central obesity, as part of the metabolic syndrome, is a significant CVD risk factor. The increased cardiovascular risk observed in obesity could, in part, be explained by the association between intraabdominal fat, insulin resistance, and components of the coagulation and fibrinolytic systems. Even though diabetic subjects were excluded from our study, reports have shown that insulin resistance may induce endothelial dysfunction, even in non-diabetic subjects (77). The question whether elevated FVIII and VWF levels are true components of the metabolic syndrome needs further investigation.

Exercise training resulted in a significant reduction in CTIA, but not FVIII:Ag levels. This may be because of that fact that effect of training-induced CTIA reduction might

have reflected more on the fibrinolytic system, especially with plasminogen activator-inhibitor levels (PAI-1).

We also found that in women, baseline FVIII:Ag levels were significantly lower in subjects who were using HRT, compared to those not on HRT. This finding is in concordance with the previous studies in literature which showed reductions in FVIII and VWF levels with sustained HRT use (25,73,74,75). This baseline difference in FVIII:Ag between HRT and non-HRT women was not sustained after 6 months of training, with a trend for an increase in FVIII:Ag levels with training in HRT users. We did not find any significant additive effect of HRT and exercise-training on plasma FVIII:Ag levels. Also, women on HRT had significantly lower CTIA with no change in % TBF at baseline and after 6 months of training. Thus, the effect of HRT on baseline and training-induced FVIII:Ag levels may be mediated, in part, through changes in central adiposity. Another explanation for the relative rise in FVIII:Ag levels in the HRT group with training could be training-induced alterations in the vascular endothelium resulting in increased release of inflammatory markers like C- Reactive Protein (CRP) and/or reduced synthesis of anticoagulant proteins like protein C, protein S, and Antithrombin III. Also, it is important to take into account the route of administration of HRT (Oral vs. Transdermal) as well as the components of HRT (Estrogen alone vs. Estrogen-progesterone combination) while interpreting the HRT effect as these factors have been shown to result in varying effects of HRT on coagulation and endothelial function.

Conclusions

1. Based on the evidence obtained from the present study, we conclude that long-term endurance training does not have a significant impact on reduction of

thrombogenic potential through the intrinsic pathway in 50-75 year men and women.

2. We also conclude that intra-abdominal fat, and not plasma lipoprotein lipids, might be a significant factor modulating baseline plasma FVIII:Ag levels and their response to training.

3. In women, HRT use resulted in a considerable reduction in baseline plasma FVIII:Ag levels. The fact that HRT users had a significantly lower CTIA at baseline provides support to the contributory role of CTIA fat on FVIII:Ag levels. There was no significant interactive effect between exercise training and HRT use on FVIII:Ag levels

4. We conclude that changes in lipoprotein-lipid levels did not account for the baseline differences or the training-induced changes in plasma FVIII:Ag levels

5. APO E, a predominant lipid-related genotype, did not have an independent effect on baseline or training-induced plasma FVIII:Ag levels.

6. Considering the fact that truncal obesity had a significant effect on FVIII;Ag changes, the effect of obesity-related genes and their exercise training interactions should be addressed in future studies to obtain a clearer picture of training-induced coagulation profile changes.

Another factor that significantly affects plasma FVIII:Ag level is the ABO blood group gene locus, with non-O blood groups having an elevated FVIII:Ag level (44). In the present study, we did not account for the subject's blood group which might have influenced the overall outcome. Whether the ABO blood group gene is a significant contributor to training-induced changes in FVIII levels should be explored through future

research. It will also be important to know whether the observed training adaptations in the FVIII:Ag levels and their associations with HRT use and intra-abdominal fat do result in changes in thrombin and fibrin formation and/or degradation. Measurement of thrombin-antithrombin complexes, prothrombin fragments 1&2, and factor X generation assays should enhance our understanding of the coagulation profile response to training. The effect of regular sub-maximal aerobic training on coagulation factor levels appears small and clinically insignificant, when compared to the clear and beneficial effects on lipoprotein lipid profile, blood pressure, insulin resistance, and body composition. This view may change as we understand the mechanisms that link the lipid, body composition and hemostatic changes with exercise training.

LIMITATIONS AND DELIMITATIONS

DELIMITATIONS

1. Subjects were sedentary, healthy men and women, aged 50-75 years, without evidence of symptomatic ischemia (no history of myocardial infarction, stroke or peripheral vascular disease).

2. All subjects were recruited from the Washington DC metropolitan area.

3. Female subjects were post-menopausal (absence of menses for >2 years) and those subjects taking hormone replacement therapy were asked to continue on the same regime until the end of the study.

4. A graded exercise treadmill test was done to screen for cardiovascular disease

LIMITATIONS

1. The classification of subjects as sedentary prior to the start of exercise training relied on self-report.

2. Sample sizes (depending on allele frequencies) for the APO E genotypes may not have been sufficient to detect differences in the dependent variable.

3. Measuring only the levels of FVIII:Ag might not represent the chronic change in coagulation profile with training.

4. Screening for cardiovascular disease might have affected the gene frequency of the APO E genotypes in the study population, as the E4 allele has been associated with coronary artery disease and stroke in comparison to E2 and E3.

5. There is always a possibility that differences between genotype groups might be due to other variables not measured in this study or to the possibility that APO E genotype may be in linkage disequilibrium with another gene that is responsible for the observed results.

APPENDIX A: GLOSSARY

<u>DEFINITIONS</u>

1. <u>Allele</u>- Alternative form of a gene at a given locus.

2. <u>Apolipoprotein E</u>- Protein, which is a structural part of the triglyceride rich lipoproteins (chylomicrons and VLDL). Plays an important role in lipid transport and metabolism.

3. <u>Atherogenesis</u>- Formation of an atheroma, which can later evolve into a thrombus; an important step in the pathophysiology of most ischemic disorders.

4. <u>Atherosclerosis</u> – Pathological state characterized by irregular deposition of lipid plaques in the intima of arteries.

5. <u>Coagulation</u> – A series of enzyme catalyzed events that ultimately lead to the formation of a stabilized fibrin clot.

6. <u>Factor VIIIa</u> – The active form of Factor VIII, which takes part in the conversion of Factor X $\rightarrow$ Xa in the coagulation cascade.

7. <u>Factor VIII:Ag/ Factor VIIIC:Ag</u> – Inactive protein form of plasma coagulation Factor VIII, synthesized by liver.

8. <u>Factor VIII:C</u>– Factor VIII coagulant activity.

9. <u>Gene</u> – A unit of inheritance; a sequence of chromosomal DNA that is translated into a functional product (protein).

10. <u>Hemostasis</u> – Arrest of blood flow within blood vessels.

11. <u>Homozygous</u>- Individual having the same allele of the gene in both the loci.

12. <u>Lipoprotein</u> – A lipid-protein aggregate that serves to carry lipids in the body.

13. <u>Locus</u> – The specific location of a gene on a chromosome.

14. <u>Polymorphism</u>- a common genetic variation in a gene at the DNA level.

15. <u>Thrombosis</u> – Formation or presence of a clot within a blood vessel which may cause ischemia and infarction of the tissues supplied by the vessel.

16. <u>Thrombus</u> – A clot in the circulatory system, formed from constituents of blood.

17. <u>VO$_2$ max</u> – Volume of oxygen consumed at maximum effort.

18. <u>Von-Willebrand Factor / Factor VIII R:Ag</u> - Large multimeric glycoprotein that is synthesized in endothelial cells and megakaryocytes; acts as a carrier for Factor VIII in circulation; also prevents premature Factor VIII degradation.

APPENDIX B: CONSENT FORM

CONSENT TO ACT AS A SUBJECT IN AN EXPERIMENTAL STUDY

Project Title: **APO E genotype and HDL changes with Exercise Training**

      I state that I am over 18 years of age and wish to participate in a program of research being conducted by Dr. James Hagberg in the Department of Kinesiology, University of Maryland.

      The purpose of this study is to determine the role that genetics may play in determining how my blood cholesterol levels change with exercise training.

      I already completed a telephone interview that determined that I am not physically active, am 50-75 years of age, not a diabetic or have controlled diabetes, not taking cholesterol-lowering medications, have normal blood pressure or high blood pressure controlled on medications not affecting my cholesterol levels, have no evidence of lung disease, have an appropriate body weight for my height, and have no other medical problems that would keep me from exercising vigorously. Furthermore, if I am a woman, I must be postmenopausal, defined as no menstrual cycles for at least the last 2 years. I understand that if I am a woman and change my hormone replacement therapy regimen during the study, my participation in the study will be terminated. I also understand that if I have a prior history of ulcers or bleeding disorders, I will be excluded from one test that is part of the study. I also understand that I must have somewhat abnormal levels of cholesterol to enter the study.

      I understand that I will complete one orientation and two Screening Visits. The orientation session will present all aspects of the study and my written informed consent will be provided after all of my questions have been answered. For my first Screening Visit, I will report to the laboratory after an overnight fast and a blood sample will be drawn for blood chemistries and blood cholesterol levels. I understand that I may be excluded from the study if this initial blood sample shows elevated levels of glucose in my blood. I understand that a part of the blood sample will be used to obtain my DNA. A blood sample will also be drawn 2 hours after I drink a sugar solution. I understand that a total of 3 tablespoons of blood will be drawn during this visit. I understand that I will be excluded from the study at this point if I have low cholesterol levels, high triglyceride levels, a low red blood cell count, evidence of kidney or liver disease, or evidence of diabetes. I understand that if I remain qualified to this point, on my second Screening Visit I will undergo a treadmill exercise test to determine if I have heart disease. A physical examination will precede the exercise test. I will then complete a test on an exercise treadmill where the treadmill speed and grade will increase every 3 minutes until I cannot continue or symptoms of heart disease develop. Blood pressure, heart rate, and ECG will be recorded before, during and after the test.

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I understand that I will be excluded from the study at this point if I have evidence of heart disease. I understand that if I meet all of these requirements to enter the study, I will undergo 6-8 weeks of instruction in the principles of an American Heart Association low-fat diet and must follow this diet for the remainder of this study. After this I will undergo Baseline

Testing that includes the following tests that will be completed in 5 testing sessions. I will have blood drawn on 2 or 3 occasions from a vein in my arm in the morning after an overnight fast to measure my cholesterol levels and to assess my immune system. I understand that a maximum of 2 tablespoons of blood will be drawn during these visits. I understand that I will also undergo a second exercise test on a treadmill to measure my cardiovascular fitness. The test will start at 70% of the highest heart rate achieved on my first exercise test and the treadmill grade will increase by 2% every 2 minutes. Blood pressure, heart rate, and ECG will be monitored before, during and after the test. The test will be stopped when I no longer can continue. During this test, I will have a noseclip on my nose and I will breathe through a mouthpiece so that the air that I breathe out can be analyzed. I also understand that my dietary habits will be measured by having me record for 7 days all of the food items that I eat. I understand that on another morning after an overnight fast I will have blood samples drawn before and after 3 hours after I drink a glucose solution to assess my risk of developing diabetes; I will also have additional blood drawn prior to this test that will be frozen for future studies that relate directly to the goals of the present study. I understand that 5 tablespoons of blood will be drawn during this visit. I understand that on another occasion after an overnight fast, I will have blood samples drawn from a line (catheter) in my arm before and for 4 hours after drinking 1-2 cups of a high-fat liquid meal. The high-fat meal is made of heavy whipping cream with small amounts of chocolate, sugar, and powdered milk that tastes similar to a rich chocolate shake. I understand that 10 tablespoons of blood will be drawn during this test and will be used to measure how my body absorbs and uses fat from a meal and how my blood clotting, and substances that affect hunger are affected by a fat meal. Before and after I drink the high-fat meal, I understand that I will breath through a mouthpiece while my nose is closed-off with a nose clip and the air that I breath out will be collected and used to determine how much fat I use for energy while sitting at rest. I understand that these tests will be done at the University of Maryland College Park.

I understand that in the morning after an overnight fast I will have blood samples drawn to assess my cholesterol levels and my blood clotting system. I will then have a substance that temporarily stops blood from clotting injected into my arm vein. Blood samples are drawn 10 min later for measurement of chemicals that affect blood cholesterol levels. I understand that if I have a prior history of ulcers or bleeding disorders I will not undergo this test.

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I understand that I will remain in the laboratory for 2-3 hours after this test with pressure on the site where blood samples were drawn to make sure that all bleeding is stopped. I understand that 4 tablespoons of blood will be drawn during this visit. I understand that how much fat and muscle I have will be measured while I lie quietly on a table for 15 to 30 minutes. I understand that the amount of fat I have around my waist will be measured with a CAT scan while I lie quietly on a table. I also understand that these last 3 tests will be done at the VA Medical Center in Baltimore. I understand that during this baseline testing a total of 21 tablespoons of blood will be drawn, which is about two-thirds of the amount of blood given when donating blood. I understand that after completing this testing, for 6 months I will complete 3 exercise sessions each week supervised by study personnel. I understand that I will be instructed on the appropriate warm up and stretching exercises to perform prior to each exercise training session. I will be taught to measure my heart rate and to use heart rate monitors to control how hard I am exercising. The first training sessions will consist of 20 minutes of light exercise. The amount of exercise and how hard I exercise will increase gradually until I am completing 40 minutes of moderate intensity exercise every session. Exercise models include walk/jogging, stair stepping, and cycle, cross-country ski and rowing ergometry. I will be asked to add a 45-60 minute walk to my exercise program on weekends after the first 10 weeks of exercise program. I understand that some of the supervised exercise sessions may be done outside of the exercise facility, but still under the direct supervision of study personnel. I understand that if I lose more weight that expected from my exercise, I will be counseled by a dietician against restricting how much food I will eat. I will also be asked to complete food records during the exercise training program and if major dietary changes have occurred, I will also be counseled by a dietician to resume my original dietary habits.

I understand that after completing 6 months of exercise training, I will have everything reevaluated that was measured before I began the exercise program. I understand that during this testing, a maximum of 21 tablespoons of blood will be drawn, which is about two-thirds of the amount of blood given when donating blood.

I understand that if I qualify for this study that my DNA will be isolated from my blood and analyzed at a number of sites for differences in DNA that may affect how my cholesterol levels change with exercise training. I understand that some of my DNA will also be frozen for future studies. However, these studies can only my DNA at sites that might affect how my cholesterol levels, glucose and insulin levels, bone density, body composition, immunology (disease-fighting), and cardiovascular and blood clotting systems change with exercise training.

All information collected in this study is confidential, and my name will not be identified at any time. I understand that my DNA (genetic material) will be sent to collaborating genetics laboratories that are part of this study and that a sample of my DNA will be kept in the University of Maryland Department of Kinesiology laboratories.

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I understand that my coded blood samples will also be sent to other collaborating laboratories to measure compounds in my blood that relate to blood cholesterol levels and cardiovascular risk. I understand, however, that all my blood samples sent to collaborating laboratories will be identified only by a numeric code. I understand that only investigators at the University of Maryland College Park will know whose name is associated with each coded number. I further understand that the list of names and codes will be retained at the University of Maryland College Park for up to 25 years. I understand the following risks are associated with my participation in this study. (1) The risk of maximal exercise testing is approximately 1 nonfatal event in 10,000 tests and 1 fatal cardiac event in 70,000 tests. Risks will be minimized by having the test administered by a physician and personnel trained in such tests and emergency procedures. I will be screened with a resting ECG and a physical examination prior to this test. An emergency cart with the necessary drugs and a cardiac defibrillator will be present at all testing sessions. (2) There is minimal risk of bruising and infection associated with blood drawing. These risks will be minimized by using sterile techniques and by having experienced personnel draw all blood samples. (3) The risk of the body composition testing is the exposure to X-rays. The amount of X-ray exposure for this test is the same as that occurring during 30 minutes if any activity outside in the sun. (4) The risk associated with the test requiring the injection into an arm vein of a substance that temporarily stops blood clotting is bleeding. This risk will be minimized by excluding persons with bleeding disorders, peptic ulcers, or other blood disorders from the study. The risk is further minimized by placing a pressure bandage on the intravenous access site after the blood sampling and observing the subject for 2-3 hours after the injection. (5) The risk associated with the CAT scan to measure abdominal fat is exposure to X-ray. The X-ray exposure is less than the maximum radiation dose individuals are permitted to be exposed to each year in their occupation. (6) The risks associated with the blood clotting and immune system studies are those related to blood drawing as listed above. (7) The risks associated with the oral glucose tolerance test and the high-fat meal test are those associated with blood drawing, the possibility of having low blood sugar levels at the end of the test, the possibility of having an upset stomach, primarily a stomach ache, after drinking the glucose and/or high fat meals. The risk of low blood sugar levels at the end of the test will be minimized by providing you with a drink and a small snack. (8) The risk of exercise training is the possibility of a heart attack or cardiovascular event. A large physical activity center reported that 1 nonfatal cardiovascular event occurred in 1.7 million walk/jogging miles. These risks will be minimized because I will undergo a cardiovascular evaluation before beginning exercise training. Exercise sessions will be supervised by experienced personnel trained in emergency procedures. An emergency cart with the necessary drugs and a cardiac defibrillator will be present at all supervised exercise training sessions.

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Two study personnel will supervise the outside exercise sessions done at the University of Maryland, College Park though no emergency will be directly available during these sessions. (9) There are no risks associated with the genetic testing because no results of these tests will be given to the participants. This has to be the case because the genetic results are not from clinically approved laboratories

I understand that this study is not designed to help me personally, but may help the investigators to determine who exercise might benefit the most. I understand that I will be provided with my study results and they can be sent to my physician if I request this in writing. I understand that these results are not to be used for clinical diagnostic purposes and that I will not receive the results of my genetic testing. I understand that I am free to ask questions or to withdraw from participation without penalty. I understand that I will be paid $50 for completing baseline testing and the dietary stabilization period. I also understand that I will be paid another $50 for completing 3 months of exercise training and another $100, for a total of $200, for completing the 6 months of exercise training and all final testing, if I complete at least 90% of my exercise training and testing sessions. I understand that if my participation in the study has to be terminated because I change my hormone replacement therapy regimen, I will only be paid for the portion of the study that I have already completed, that is, which of the stages above that I have completed.

In the event of a physical injury resulting from participation in this study, I understand that immediate medical attention is available at the Washington Adventist Hospital or the Baltimore VA Medical Center. However, I understand that the University of Maryland does not provide any medical or hospitalization coverage for participants in this research study nor will the University of Maryland provide any compensation for any injury sustained as a result of participation in this research except as require by law.

Principal Investigator: James Hagberg, PhD, Department of Kinesiology, HLHP Building, University of Maryland, College Park, MD 20742-2611, telephone 301-405-2487.

_____   _____
Subject's signature   Date

_____   _____
Witness   Date

_____   _____
Investigator   Date

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