Association of Apolipoprotein E Polymorphism With Blood Lipids and Maximal Oxygen Uptake in the Sedentary State and After Exercise Training in the HERITAGE Family Study

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The relationship of apolipoprotein E (apo E) genotypes to plasma lipid and maximal oxygen uptake (Vo_{2max}) was studied in the sedentary state and after a supervised exercise training program in black and white men and women. At baseline, the apo E 2/3 genotype was associated with the lowest, and apo E 3/4 and E4/4 with the highest low-density liporpotein (LDL) cholesterol and apo B levels in men and women of both races, while female (not male) carriers of apo E3 had higher high-density lipoprotein (HDL) cholesterol levels than carriers of other genotypes. Very-low-density lipoprotein (VLDL) cholesterol and triglyceride levels were significantly higher in carriers of both apo E2 and apo E4 in white men only. Racial and sex differences were noted in lipid responses to exercise training across genotypes with a significantly greater increase in HDL cholesterol observed only in white female carriers of apo E 2/3 and E3/3, as compared to apo E4/4. Apo E polymorphism was not found to be associated with Vo_{2max} levels either in the sedentary state nor the Vo_{2max} response to exercise training, contrary to previous reports.

APOLIPOPROTEIN E (apo E), a 299–amino acid, arginine-rich glycoprotein, is an integral surface component of chylomicrons, very-low-density lipoproteins (VLDL), and some subclasses of high-density lipoproteins (HDL). Its primary function is as a ligand for receptor-mediated uptake off triglyceride-rich lipoproteins. It also modulates the activity of several lipid-metabolizing enzymes, including lipoprotein lipase (LPL), lecithin cholesterol acyl transferase (LCAT), and cholesterol ester transferase protein. In addition, it appears to be involved with reverse cholesterol transport by HDL.[1,2] The apo E gene, encoded on chromosome 19, is polymorphic with three common alleles coding for 3 isoforms of the apo E proteins, ie, E2, E3, and E4.[1,3] Individuals inherit 1 copy of the apo E gene from each parent in simple Mendelian fashion. This results in 3 homozygous genotypes (E2/2, E3/3, E4/4) and 3 heterozygous genotypes (apo E2/3, E3/4, or E2/4) with apo E3/3. the most prevalent genotype in all populations studied to date.

Apo E polymorphism appears to affect risk of atherosclerotic cardiovascular disease with a strong association of apo E4 to increased risk of coronary heart disease (CHD) and associated mortality, as compared to apo E3, while apo E2 generally is associated with reduced risk of CHD.[1,2,4-8] However, people with type III hyperlipoproteinemia and the apo E2/2 phenotype have an elevated risk of premature CHD. Numerous studies have shown that blood lipid levels associated with apo E polymorphism probably contribute to these differences in CHD rates.[1,2,4-7] Generally carriers of the apo E4 allele have higher, and those with the E2 allele lower, levels of total and low-density lipoprotein (LDL) cholesterol than those with the E3 allele. Apo E polymorphism also appears to play a role in responsiveness of blood lipids to dietary and lipid-lowering drug interventions.[1,9-16] Thus, the apo E gene-environmental interactions contribute to population variance in blood lipid-lipoprotein levels.[14]

Among the multiple mechanisms postulated for reduced risk of CHD with regular endurance exercise is the blood lipid response to training, particularly an increase in plasma HDL cholesterol.[17,18] However, there is a great deal of variability between individuals in response of blood lipids, including HDL cholesterol, to even a standardized exercise training program, as previously reported from the HERITAGE Family Study (HERITAGE).[18,19] Observational and small-scale intervention studies, recently reviewed by Hagberg et al,[14] suggest that apo E polymorphism is a genetic factor contributing to variability in the blood lipid response to exercise training. The principal purpose of this study was to determine in HERITAGE the association of apo E genotype to the blood lipid profile in the baseline sedentary state, and to its contribution to lipid changes following 20 weeks of supervised exercise training in a large biracial group of young and middle-aged men and women. A secondary aim was to compare black and white differences in these variables by apo E genotypes.

In addition, we assessed the possible influence of apo E polymorphism on aerobic fitness at baseline and its change with exercise training. Potential contributions of apo E genotypes to

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cardiorespiratory fitness and its response to training are differences in rate of cell lipid uptake and/or blunting of endothelial-dependent vascular dilatation by elevated LDL cholesterol in carriers of apo E4.[20] It is hypothesized that apo E polymorphism is a genetic contributor to variability in levels of blood lipids and possibly aerobic fitness and in their responses to exercise training.

MATERIALS AND METHODS

Protocol

The HERITAGE study design and procedures have previously been described in detail.[21] In brief, sedentary members of about 200 two-generation black and white families were recruited and exercise-trained under supervision at the participating clinical centers (Laval University, Quebec, Canada; University of Minnesota, Minneapolis; University of Texas, Austin; Arizona State, Tempe and Indiana University, Indianapolis campus). In this investigation, blood lipid levels obtained from subjects in the sedentary state prior to the initiation of exercise training and their responses to training were compared with the subjects' apo E genotypes. The contribution of apo E polymorphism to the training-induced increase in cardiorespiratory endurance also was assessed. The HERITAGE protocol was approved by the institutional review boards protecting human subjects participating in research projects at each of the collaborating centers, and written informed consent was obtained from each participant. Each subject received an incremental honorarium for successful completion of the study.

Subjects

The white participants were members of 2-generation families consisting of both natural parents under 65 years of age and a least 2 offspring aged 17 to 40 years. The black participants were of similar age, but were from family units often as small as 2 first-degree relatives, ie, a natural parent and 1 offspring or 2 full siblings. Race was derived by the self-classification of the participants. Eligibility criteria included requirements for participants to be in good health, sedentary for at least the previous 6 months, to pass a physician-administered physical examination, and to have no significant electrocardiographic abnormalities during a cycle ergometer maximal exercise test.[21] Exclusion criteria included drug treated or untreated hyperlipidemia, drug-treated hypertension or untreated hypertension of greater than stage 1 severity, diabetes mellitus requiring medication, or a body mass index (BMI) exceeding 40 $kg \cdot m^{-2}$. However, in a few instances, the latter criterion was waived by the examining physician, because of the absence of other exclusionary criteria, and a demonstrated ability to perform the prescribed exercise during baseline evaluations.

Clinical Procedures

Following health screening, participants completed health-habit questionnaires assessing smoking and alcohol consumption habits, medication use, and menstrual history; the Atherosclerosis Risk in the Community (ARIC)-Baecke Physical Activity Questionnaire[22]; the Willett Food Frequency Questionnaire[23]; and the Minnesota Eating Pattern Assessment Tool (EPAT), which measures high and low dietary fat sources.[24]

Participants were counseled at baseline and at the midpoint of the training program (10 weeks) not to alter their health habits and to continue their usual eating pattern (as repeatedly assessed by the EPAT), usual physical activity outside of the study (as assessed by the ARIC-Baecke Questionnaire), alcohol and tobacco use, and previously prescribed oral contraceptive or hormonal replacement therapy. Good adherence was generally observed.

Maximal cycle ergometer exercise tests were performed twice on 2 separate days (at least 48 hours apart), before training and twice after completion of training for the determination of Vo_{2max}, as previously described.[21,25] In brief, exercise tests were performed on SensorMedics 800S cycle ergometers (Yorba Linda, CA) connected to a SensorMedics 2900 metabolic measurement cart. Criteria for attainment of Vo_{2max} included a respiratory exchange ratio greater than 1.1, a plateau in Vo_2 (change of <100 $mL \cdot min^{-1}$ during the last 20 seconds of the test), and a heart rate within 10 bpm of the predicted maximal heart rate for the participant's age. All HERITAGE subjects achieved Vo_{2max} by one or more of these criteria in at least one of the two exercise tests both at baseline and following training. A subject's average Vo_{2max} value for the 2 tests at each time period was designated as the Vo_{2max} for the subject if the values were within 5% of each other. If they differed by more than 5%, the higher of the 2 values was designated as the subject's Vo_{2max} for that time period.

Blood Lipid Determinations

Blood lipids were determined as previously described.[19,26] In brief, blood samples were obtained with participants in a semirecumbent position from an antecubital vein into vacutainer tubes containing EDTA in the morning following a 12-hour fast, twice at baseline and 24 and 72 hours after the last exercise training session. For eumenorrheic women, all samples were obtained in the early follicular phase of the menstrual cycle at baseline and post-training, at which time blood cholesterol alterations are reportedly minimal.[27] Plasma samples from the HERITAGE clinical centers in the United States were refrigerated with ice packs and shipped to the Central HERITAGE Lipid Core Laboratory at the Lipid Research Center at Laval University Medical Center for determination of plasma lipids, lipoproteins, apolipoproteins A-I and B, and postheparin lipase activity. This laboratory is a participant in several lipid laboratory certification programs. Cholesterol and triglyceride levels were determined in plasma and lipoproteins by enzymatic methods using a Technicon RA-500 analyzer (Bater, Tarrytown, NY). Plasma VLDL were isolated by ultracentrifugation.[28] The HDL fraction was obtained after precipitation of LDL in the infranatant by the heparin-manganese chloride method.[29] Apo A-1 level was measured in the infranatant and apo B in the plasma and infranatant fraction by the rocket-immunoelectrophoretic method of Laurell.[30] The apolipoprotein measurements were calibrated with reference standards from the Centers for Disease Control and Prevention (Atlanta, GA).

Plasma postheparin LPL and hepatic lipase activities were measured once per subject, before and after exercise training, following a 12-hour overnight fast, and 10 minutes after intravenous administration of heparin (60 IU per kg of body weight) as previously described.[31] Postheparin activities of the 2 lipases were assayed by a modification of the method of Nilsson-Ehle and Ekman,[32] and expressed as millimoles of oleic acid released per milliliter of plasma per minute.

Extensive quality-control procedures were implemented to ensure high-quality lipid and other study data.[33] These included repeat lipid assays in 5% of all samples and analyses of split specimens prepared at each clinical center. Results from plasma specimens containing chylomicrons were discarded for the analyses reported in this study as being suggestive of the nonfasting status of the subject.

To adjust for possible acute or chronic plasma volume changes associated with exercise, plasma total proteins were analyzed using the biuret method (Roche Molecular Biochemicals, Dallas, TX) on the initial pretraining plasma specimen and both post-training specimens. Post-training plasma lipid parameters then were adjusted based on the correlation of pretraining to post-training plasma total proteins levels. In this report, the 2 baseline lipid values and the 2 corrected lipid values following exercise training were averaged, and the differences between these 2 values were considered the responses to training.

Table 1. Baseline Characteristics of the Study Population by Race and Sex (mean ± SEM)

Variable	Men	Women
Black subjects (n)	89	177
Age (yr)	34.6 (12.4)	33.2 (11.4)
Body mass (kg)	84.9 (17.7)	74.10 (16.9)
BMI ($kg \cdot m^{-2}$)	27.2 (5.1)	28.1 (6.3)
Vo_{2max} ($mL \cdot min^{-1}$)	2727 (511)	1744 (356)
Vo_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	33.1 (6.6)	24.3 (5.8)
White subjects	241	252
Age (yr)	36.6 (15.0)	35.1 (14.1)
Body mass (kg)	84.6 (16.3)	67.2 (13.7)
BMI ($kg \cdot m^{-2}$)	26.7 (4.9)	25.0 (4.9)
Vo_{2max} ($mL \cdot min^{-1}$)	3025 (582)	1912 (350)
Vo_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	36.9 (9.0)	29.5 (6.9)

Apo E Genotyping

Venous blood samples were collected in EDTA-containing tubes from each participant during weeks 8 and 12 of training, and were promptly shipped at room temperature to a genetic core laboratory at Laval University. Permanent lymphoplastoid lines were established on each participant by transformation of B lymphocytes with the Epstein-Barr virus. Such cell lines grow well in culture media, and have an infinite life span with chromosomal stability over the years.[21,34] Transformed B lymphocytes were then isolated and cultured until cryopreserved in liquid nitrogen. Apo E genotyping is based on the determination of nucleotide variation in genomic DNA isolated from the permanent lymphoblastoid cell lines.[35] This procedure consists of preparation of genomic DNA by the proteinase K and phenol/chloroform technique. Isolated DNA was dialyzed 4 times against 10 mmol/L Tris-1 mmol/L EDTA (pH 8.0) buffer for 6 hours at 4°C and ethanol precipitated. Apo E polymorphism was typed with the polymerase chain reaction (PCR), using previously described primers,[36] followed by digestion with *Hha*1 restriction enzyme. The PCR was performed in standard buffer (Qiagen, Valencia, CA), and each 15-μl PCR reaction contained 100 ng genomic DNA, 0.2 μmol/L each of dNTPs, and 0.5 U Tag polymerase (Qiagen). The reactions were incubated at 94°C for 5 minutes, 60°C for 45 seconds, and 72°C for 1 minute, followed by 35 cycles of 94°C for 1 minute, annealing at 60°C for 45 seconds, extension at 72°C for another minute, and finally 1 cycle at 72°C for 10 minutes, using a Model 9600 Perkin Elmer thermal cycler (Norwalk, CT).

The amplified PCR products were then digested with 2 U of *Hha*1 (New England BioLabs, Missisauga, ON, Canada) at 37°C for 3 hours. The resulting DNA fragments were electrophoresed on 3.5% agarose gel and visualized under UV light after ethidum staining.

Exercise Training Program

Participants trained on cycle ergometers (Universal Aerocycle, Cedar Rapids, IA) under supervision at each clinical centers, using the same standardized exercise protocol.[21,37] In brief, participants exercised 3 times per week for 20 weeks, progressing from a duration of 30 minutes to 50 minutes per session for the last 6 weeks of training. Similarly, exercise intensity was progressively increased from the heart rate associated with 55% Vo_{2max} at baseline to that associated with 75% Vo_{2max} at baseline for the last 8 weeks of training. The power output of the cycle ergometer was automatically adjusted to the heart rate response during exercise via computerized controls built-in to the cycle ergometers.

Data Analysis

All data were analyzed using a Statistical Analyses System (SAS) package (version 8.01; SAS Institute, Cary, NC). A chi-square test was used to confirm that the observed genotype frequencies were in Hardy-Weinberg equilibrium and to test genotype frequency differences between blacks and whites by sex. We also tested for genotype frequency differences across the previously reported[19] percent change with training in HDL cholesterol by quartiles. Associations between the apo E marker and lipid and lipoprotein phenotypes were analyzed using a MIXED procedure in the SAS software package.[38] Non-independence among family members was adjusted for using a sandwich estimator, which asymptotically yields the same parameter estimates as ordinary least squares or regression methods, but the standard errors and consequently hypothesis tests are adjusted for the dependencies. This method assumes the same degree of dependency among all members within a family. Baseline lipids for each genotype were adjusted for age, and BMI, and blood lipid responses to training by genotype were adjusted for age, baseline BMI, and the baseline value of the lipid.

Values are given as the mean and standard error of the mean (SEM).

RESULTS

Baseline Characteristics

A total of 766 (501 white and 265 black) sedentary men and women participants had suitable plasma lipid batteries and apo E genotyping to be included in the baseline analysis.

Table 1 shows the baseline characteristics of the study population by race and sex. The subjects as a group were overweight based on their mean BMI levels,[39] and their mean Vo_{2max} levels were in the average range for their ages.[40] As previously reported,[26] baseline dietary lipid intake, as assessed by the Willett Food Frequency Questionnaire, was close to current national guidelines,[41] and dietary lipid intake remained relatively stable on repeated EPAT assessments.

Table 2 shows the prevalence of the 3 alleles and 6 genotypes for the HERITAGE population by race. Apo E3 was the most common allele in both races and apo E 3/3 was the most frequent genotype, being present in 49% of the black and 63% of the white subjects ($P < .01$). Black subjects had a significantly higher prevalence of genotypes containing apo E4 ($P < .02$) than the white subjects (21% *v* 12%), and a lower prevalence of apo E3 than the white subjects (70% *v* 79%; $P < .02$), but both races had the same prevalence of the apo E2 allele (9%).

Tables 3 and 4 show the mean ± SEM for baseline plasma lipid levels by apo E genotype for HERITAGE participants by race and sex.. Because of the small numbers of white individuals with the apo E 2/2 genotype (n = 3), the lipid values for

Table 2. Frequency of apo E Alleles and Genotypes by Race

Race	apo E Alleles		
	E2	E3	E4
Black	0.09	0.70	0.21
Whites	0.09	0.79	0.12

	apo E Genotypes					
	E2/2	E2/3	E2/4	E3/3	E3/4	E4/4
Black	0	0.12	0.04	0.49	0.31	0.04
Whites	0.01	0.13	0.02	0.63	0.20	0.01

Table 3. Baseline Plasma Lipids, Lipoproteins, and Apolipoproteins (mean ± SEM in mg · dL^{-1}) Adjusted for Age and BMI in Black Subjects by Sex

	apo E Genotype					*P*
	2/3	2/4	3/3	3/4	4/4	Value
Women (n)	15	6	92	57	6	
Total chol.	146.5 (5.5)^{A}	149.4 (8.6)^{AC}	165.1 (3.7)^{BC}	167.8 (4.1)^{B}	170.3 (12.1)^{AB}	.019
LDL-chol.	92.5 (4.6)^{A}	95.5 (9.2)^{AB}	109.6 (3.6)^{B}	113.4 (3.6)^{B}	119.9 (11.6)^{B}	.007
HDL-chol.	42.8 (2.3)^{AB}	40.1 (2.2)^{B}	45.4 (1.4)^{A}	45.0 (1.7)^{AB}	40.7 (1.6)^{B}	.048
HDL_2-chol	13.8 (2.0)^{AB}	10.0 (1.1)^{B}	14.6 (1.2)^{A}	15.4 (1.4)^{A}	10.6 (1.3)^{B}	.004
HDL_3-chol.	26.9 (1.5)^{A}	28.9 (1.9)^{A}	29.1 (0.9)^{A}	28.2 (0.9)^{A}	29.2 (1.4)^{A}	.667
VLDL chol.	8.5 (1.4)^{A}	9.2 (1.9)^{A}	6.7 (0.6)^{A}	6.4 (0.7)^{A}	7.2 (1.9)^{A}	.397
Triglycerides	74.1 (7.0)^{A}	84.8 (10.5)^{A}	70.5 (3.3)^{A}	68.5 (3.3)^{A}	78.6 (17.5)^{A}	.501
apo A-I	116.1 (3.5)^{A}	125.5 (4.6)^{A}	120.8 (2.6)^{A}	118.0 (2.7)^{A}	115.0 (6.1)^{A}	.283
apo B	68.6 (3.3)^{A}	70.4 (4.1)^{AB}	78.9 (2.7)^{BC}	78.7 (2.4)^{BC}	85.9 (6.2)^{C}	.033
Men (n)	18	4	39	24	4	
Total chol.	161 (7.6)^{AC}	145.7 (9.6)^{A}	174.9 (6.7)^{BC}	194.9 (8.0)^{B}	178.6 (10.3)^{BC}	.013
LDL-chol.	100.1 (4.3)^{A}	97.9 (5.8))^{A}	122.8 (6.3)^{B}	138.7 (6.1)^{B}	135.53 (8.4)^{B}	<.001
HDL-chol.	36.1 (1.9)^{A}	37.2 (2.0)^{A}	39.8 (1.9)^{A}	37.9 (2.5)^{A}	38.7 (3.3)^{A}	.691
HDL_2-chol.	10.1 (1.5)^{A}	10.4 (1.1)^{A}	11.2 (1.3)^{A}	9.4 (1.4)^{A}	11.0 (2.9)^{A}	.827
HDL_3-chol.	24.9 (0.7)^{A}	26.2 (1.9)^{A}	28.0 (1.0)^{A}	27.4 (1.3)^{A}	26.9 (1.9)^{A}	.157
VLDL chol.	14.5 (2.7)^{A}	9.0 (2.7)^{A}	10.3 (1.4)^{A}	11.9 (1.7)^{A}	9.0 (1.6)^{A}	.456
Triglycerides	108.1 (14.2)^{A}	73.0 (13.1)^{A}	85.6 (8.1)^{A}	103.1 (9.6)^{A}	78.2 (10.5)^{A}	.252
apo A-I	109.7 (3.0)^{A}	111.0 (3.3)^{A}	119.3 (3.3)^{A}	116.7 (3.8)^{A}	115.5 (7.0)^{A}	.151
apo B	77.5 (4.2)^{B}	70.1 (4.1)^{A}	87.9 (4.3)^{B}	87.9 (4.3)^{B}	100.3 (4.1)^{C}	.002

NOTE. Means with the same letter superscript are not significantly different.
Abbreviation: chol., cholesterol.

this subgroup was combined with apo E 2/3 for the purpose of data analyses. None of the black subjects were homozygous for apo E2. As previously reported,[28] because of selection criteria HERITAGE participants as a group were normolipidemic and had below average levels of plasma total and LDL cholesterol for North Americans. They also had below average HDL cholesterol levels, and their plasma triglyceride levels were in the 50th to 75th percentile for race, age, and sex.

Significant differences were noted across genotypes for most of the baseline lipid parameters in men and women of both races. As compared to apo E3/3, the most common genotype, apo E 2/3 was associated with lower mean levels of total and

Table 4. Baseline Plasma Lipids, Lipoproteins, and Apolipoproteins (mean ± SEM in mg · dL^{-1}) Adjusted for Age and Baseline BMI in White Subjects by Sex

	apo E Genotype					
	2/2 & 2/3	2/4	3/3	3/4	4/4	*P* Value
Women (n)	38	5	152	60	3	
Total chol.	149.6 (5.0)^{A}	172.1 (18.8)^{AB}	166.4 (2.7)^{B}	169.0 (3.8)^{B}	175.4 (16.6)^{AB}	.034
LDL-chol.	85.8 (4.9)^{A}	105.5 (15.3)^{AB}	108.9 (2.2)^{B}	112.5 (3.2)^{B}	116.0 (16.4)^{B}	<.001
HDL-chol.	42.8 (2.3)^{AB}	40.1 (2.2)^{B}	45.4 (1.4)^{A}	45.0 (1.7)^{AB}	40.7 (1.6)^{B}	.048
HDL_2-chol	21.5 (1.4)^{A}	19.8 (1.1)^{A}	19.1 (1.1)^{A}	16.6 (1.3)^{B}	18.7 (4.2)^{AB}	.049
HDL_3-chol.	30.3 (0.8)^{B}	28.8 (3.4)^{ABC}	27.7 (0.5)^{C}	25.5 (0.7)^{A}	24.4 (0.7)^{A}	<.0001
VLDL chol.	10.6 (0.8)^{A}	12.6 (2.2)^{A}	9.2 (0.8)^{A}	11.6 (1.1)^{A}	12.7 (4.2)^{A}	.061
Triglycerides	84.7 (5.1)^{A}	90.9 (10.1)^{A}	81.1 (5.1)^{A}	91.5 (6.7)^{A}	96.6 (23.2)^{A}	.366
apo A-I	125.3 (2.6)^{A}	120.0 (6.3)^{AB}	117.4 (1.8)^{B}	12.0 (2.7)^{B}	116.4 (1.9)^{B}	.004
apo B	61.2 (4.0)^{A}	80.4 (11.7)^{AB}	77.4 (1.7)^{B}	81.6 (2.3)^{B}	90.5 (14.8)	.002
Men (n)	28	6	164	41	4	
Total chol.	158.6 (8.2)^{AB}	160.2 (9.6)^{AB}	161.2 (5.9)^{A}	172.8 (5.2)^{B}	190.5 (4.5)^{C}	.0001
LDL-chol.	82.4 (6.4)A	100.7 (9.4)^{AB}	107.8 (5.0)^{B}	117.2 (4.3)^{B}	112.2 (21.0)^{AB}	<.005
HDL-chol.	36.9 (2.1)^{ABC}	36.1 (2.0)^{AB}	36.9 (1.5)^{A}	33.7 (1.2)^{BC}	32.6 (2.0)^{C}	.040
HDL_2-chol.	10.7 (1.0)^{BC}	9.2 (0.9)^{AC}	10.9 (0.8)^{AB}	9.3 (0.7)^{BC}	7.8 (0.6)^{A}	<.001
HDL_3-chol.	26.1 (1.5)^{A}	27.2 (2.0)^{A}	25.8 (0.9)^{A}	24.4 (0.8)^{A}	24.6 (1.7)^{A}	.269
VLDL chol.	19.7 (2.9)^{B}	18.7 (2.8)^{B}	13.3 (1.3)^{A}	17.0 (1.6)^{B}	22.7 (10.3)^{AB}	<.004
Triglycerides	124.8 (12.8)^{B}	120.4 (13.9)^{AB}	96.7 (7.2)^{A}	13.9 (7.9)^{B}	152.4 (67.8)^{AB}	.0043
apo A-I	115.8 (5.0)^{A}	114.5 (6.1)^{A}	112.1 (3.4)^{A}	108.6 (2.9)^{A}	105.9 (4.2)^{A}	.242
apo B	74.2 (4.3)^{A}	6.5 (6.8)^{AB}	79.7 (3.6)^{A}	89.6 (3.2)^{B}	97.1 (3.0)^{C}	<.0001

NOTE. Means with the same letter superscript are not significant

Table 5. Training Responses* of Plasma Lipids, Lipoproteins, and Apolipoproteins (mean ± SEM in mg · dL^{-1}) Adjusted for Age and Baseline BMI and Lipid Levels in White Subjects by Sex

	apo E Genotype					
	2/2 & 2/3	2/4	3/3	3/4	4/4	*P* Value
Women (n)	37	5	144	59	3	
Total chol.	3.8 (3.2)^{A}	2.1 (4.7)^{A}	9.6 (2.5)^{A}	7.8 (3.0)^{A}	5.0 (4.6)^{A}	.076
LDL-chol.	−1.6 (3.6)^{AC}	−3.0 (3.6)^{A}	4.9 (2.6)^{B}	3.8 (2.9)^{BC}	6.9 (5.3)^{BC}	.022
HDL-chol.	3.8 (0.8)^{B}	1.7 (1.7)^{AB}	4.1 (0.6)^{B}	3.0 (0.7)^{AB}	1.6 (0.6)^{A}	.0062
HDL_2-chol.	2.3 (0.9)^{AC}	2.5 (1.5)^{ABC}	3.1 (0.6)^{A}	1.2 (0.7)^{C}	−0.6 (1.0)^{BC}	.013
HDL_3-chol.	3.2 (0.6)^{A}	−0.0 (0.8)^{B}	1.2 (0.5)^{B}	0.9 (0.5)^{B}	1.5 (0.78)^{AB}	.013
VLDL chol.	−0.6 (0.9)^{B}	0.1 (1.8)^{B}	0.3 (0.7)^{B}	0.6 (1.1)^{B}	4.4 (0.6)^{A}	−<.0001
Triglycerides	−7.2 (4.9)^{B}	12.0 (9.4)^{B}	0.7 (3.6)^{B}	4.7 (6.0)^{B}	−24.2 (6.8)^{A}	.0024
apo A-I	9.0 (1.9)^{B}	7.3 (3.4)^{B}	7.4 (1.6)^{B}	4.1 (1.7)^{B}	−2.4 (1.6)^{A}	<.0001
apo B	1.8 (3.4)^{A}	−0.9 (3.2)^{A}	4.9 (2.2)^{A}	4.5 (2.3)^{A}	1.4 (4.1)^{A}	.116
Men (n)	28	6	152	41	4	
Total chol.	−2.8 (3.6)^{A}	−11.5 (3.4)^{B}	4.3 (2.8)^{C}	4.0 (2.6)^{AC}	10.6 (2.7)^{C}	<.0001
LDL-chol.	−1.2 (3.1)^{B}	−13.5 (3.5)^{A}	2.9 (2.5)	3.2 (2.5)^{B}	1.8 (6.0)^{B}	<.0001
HDL-chol.	3.5 (0.8)^{A}	0.2 (1.2)^{B}	3.0 (0.6)^{A}	2.5 (0.7)^{AB}	0.2 (1.3)^{B}	.050
HDL_2-chol.	0.6 (0.7)^{A}	0.1 (1.2)^{A}	1.1 (0.6)^{A}	0.4 (0.6)^{A}	−1.5 (0.9)^{A}	.086
HDL_3-chol.	2.6 (0.7)^{A}	0.3 (0.7)^{A}	1.9 (0.5)^{A}	1.8 (0.6)^{A}	1.2 (0.4)^{A}	.113
VLDL chol.	4.3 (1.4)^{A}	3.0 (3.2)^{A}	2.1 (1.2)^{A}	0.8 (1.5)^{A}	14.6 (7.9)^{A}	.082
Triglycerides	−20.7 (6.8)^{A}	5.1 (9.7)^{BC}	−10.2 (5.9)^{AB}	−3.0 (7.8)^{B}	70.2 (35.6)^{C}	.011
apo A-I	8.8 (2.3)^{A}	3.7 (2.8)^{A}	8.1 (1.8)^{A}	7.3 (1.9)^{A}	6.6 (4.0)^{A}	0.572
apo B	0.8 (2.1)^{A}	−1.8 (2.7)^{A}	3.9 (1.6)^{B}	4.1 (1.6)^{B}	12.5 (3.1)^{C}	0.005

*Training responses are defined as the baseline value (mean of 2 assays on different days) minus the post-training value (mean of 2 different days). Means with the same letter are not significantly different.

LDL cholesterol and apo B in both black and white women (but not men). The highest levels of total and LDL cholesterol and apo B were associated with the apo E 3/4 and 4/4 genotypes. Plasma HDL cholesterol levels were similar across genotypes for both groups of men, but not for the women. Both white and black women carriers of apo E3 genotypes had significantly higher HDL cholesterol levels than carriers of the other genotypes. White women with genotypes apo E2/2 or E2/3 also had significantly higher levels of apo A-1 (the principal apolipoprotein associated with HDL particles). Plasma VLDL cholesterol and triglyceride levels were significantly higher in white male (but not female) subjects with genotypes containing either the apo E2 or apo E4 allele, as compared to those with the apo E 3/3 genotype. In contrast, Black subjects showed no significant differences in VLDL cholesterol and triglycerides levels across genotypes. The only significant difference observed in baseline postheparin LPL and hepatic lipase activities across genotypes was a lower mean LPL activity ($P = .006$) in white subjects with the apo E3/4 and E4/4 genotypes, as compared to those with other genotypes (data not shown). Baseline $V_{O_{2max}}$ levels were not statistically different across genotypes in men and women of either race (data not shown).

Exercise Training-Induced Changes

As previously reported, 20 weeks of exercise-training resulted in only a small reduction in body weight and a 15.1% and 18.6% increase in $V_{O_{2max}}$ for men and women, respectively.[26,37] No significant differences were observed across genotypes in the $V_{O_{2max}}$ response to exercise in either black or white women or men (data not shown).

A total of 724 subjects (479 whites and 245 blacks) who completed the study had satisfactory apo E genotyping and lipid data before and after training. Their changes in blood lipids with training by genotypes were used in the analyses reported below. The only significant change in the blood lipid profile with exercise training for the combined group was the previously reported 3.6% mean increase in HDL cholesterol and an associated increase in apo A-1 ($P < .01$).[26] There were no significant differences in the HDL cholesterol response by race, sex, or age. However, there was a great deal of variability in the percent change in HDL cholesterol, with training, ranging from a minus 9.3% in quartile 1 to a plus 18.0% in quartile 4 with only about 15% of the variance explained by multivariate regression analysis using nongenetic variables,[19] and about 30% of the variability attributed to maximal heritability.[42] However, HDL cholesterol changes across quartiles were unrelated to apo E genotypes (data not shown).

Tables 5 and 6 show the adjusted mean changes (and SEM) in plasma lipids following exercise training across the 6 genotypes for the white and black men and women, respectively. In the white subjects (Table 5), significant differences in training-related changes were observed across genotypes for most lipid parameters in both sexes. Genotypes containing the apo E2 allele were associated with significant reductions in LDL cholesterol ($P = .022$) in both sexes, while males with this allele also experienced a significant reduction in apo B ($P < .005$). This was in contrast to significant increases with training in LDL cholesterol and apo B in white subjects with the other genotypes. Significantly greater increases in HDL cholesterol and its apo A-I component associated with genotypes apo E 2/3 and E 3/3, as compared to apo E4/4 ($P = .0062$), were noted only in white women. Apo E 2/3 and E 3/3 genotypes also were associated with the greatest reductions in triglycerides in white men with E3/4 associated with the next largest reduction. In

Table 6. Training Responses of Plasma Lipids, Lipoproteins, and Apolipoproteins (mean ± SEM in mg · dL^{-1}) Adjusted for Age and Baseline BMI and Lipid Levels in Black Subjects by Sex

	Apo E Genotype					
	2/3	2/4	3/3	3/4	4/4	*P* Value
Women (n)	14	6	54	79	6	
Total chol.	1.0 (3.3)^{A}	−7.0 (7.0)^{A}	3.8 (1.8)^{A}	0.5 (2.1)^{A}	2.3 (7.0)^{A}	.484
LDL-chol.	0.6 (2.7)^{A}	−4.8 (5.4)^{A}	3.6 (1.7)^{A}	−0.6 (1.8)^{A}	−2.0 (5.9)^{A}	.181
HDL-chol.	3.1 (1.2)^{A}	2.2 (1.2)^{A}	2.8 (0.7)^{A}	2.7 (1.0)^{A}	3.4 (1.3)^{A}	.946
HDL_2-chol.	0.8 (0.9)^{A}	2.0 (1.5)^{A}	3.3 (0.8)^{A}	2.7 (0.9)^{A}	4.7 (2.0)^{A}	.150
HDL_3-chol.	1.9 (0.8)^{A}	0.7 (1.1)^{A}	0.1 (0.5)^{A}	0.2 (0.7)^{A}	−0.6 (1.6)^{A}	.357
VLDL chol.	−1.4 (1.0)^{A}	−3.0 (2.4)^{A}	−1.1 (0.5)^{A}	−0.1 (0.6)^{A}	2.3 (1.3)^{A}	.175
Triglycerides	−5.3 (4.7)^{A}	−15.2 (.8.8)^{A}	−6.6 (2.9)^{A}	−3.4 (3.1)^{A}	6.3 (6.8)^{A}	.342
apo A-I	6.2 (2.2)^{A}	−9.2 (4.4)^{B}	3.6 (1.1)^{A}	2.3 (1.7)^{A}	4.1 (2.0)^{A}	.043
apo B	−2.9 (1.9)^{A}	−2.4 (3.7)^{A}	0.5 (2.0)^{A}	1.6 (2.4)^{A}	2.3 (5.1)^{A}	.305
Men (n)	18	4	39	24	4	
Total chol.	5.6 (4.5)^{A}	7.2 (7.9)^{A}	4.1 (4.2)^{A}	6.6 (4.5)^{A}	−4.0 (7.7)^{A}	.106
LDL-chol.	−4.0 (3.5)^{AB}	−9.8 (6.1)^{AB}	2.8 (3.6)^{AC}	9.8 (4.3)^{C}	8.0 (4.7)^{B}	−.017
HDL-chol.	0.3 (0.9)^{A}	−2.5 (1.9)^{A}	0.6 (1.2)^{A}	1.5 (1.3)^{A}	−0.5 (1.0)^{A}	.151
HDL_2-chol.	0.2 (0.6)^{A}	−1.6 (1.1)^{A}	1.3 (0.8)^{A}	1.5 (10)^{A}	1.7 (1.1)^{A}	.182
HDL_3-chol.	−0.0 (0.7)^{A}	−0.3 (16)^{A}	0.2 (0.8)^{A}	0.4 (0.8)^{A}	−2.3 (0.9)^{A}	.192
VLDL chol.	−2.1 (1.8)^{A}	−0.5 (2.0)^{A}	0.5 (1.7)^{A}	−2.2 (2.2)^{A}	5.9 (5.4)^{A}	.498
Triglycerides	−20.8 (4.7)^{A}	11.3 (8.3)^{A}	16.45 (10.1)^{A}	−2.7 (7.6)^{A}	36.7 (30.4)^{A}	.114
apo A-I	−0.5 (2.1)^{A}	−5.7 (4.8)^{A}	2.8 (27)^{A}	3.8 (3.1)^{A}	1.2 (3.8)^{A}	.382
apo B	−4.5 (2.7)^{A}	−6.0 (6.6)^{A}	0.4 (2.6)^{A}	7.0 (3.0)^{A}	−0.1 (4.3)^{A}	.101

NOTE. Means with the same letter superscript are not significantly different.

contrast, increased levels of triglycerides were demonstrated in men with other genotypes. In white women, significant mean reductions in VLDL cholesterol and triglycerides were observed only in the small number with the apo E 4/4 genotype (n = 3).

Black subjects showed minimal differences across genotypes in their plasma lipid responses to training (Table 6). In black women, the only significant difference in blood lipid responses to training across genotypes was a significant reduction in apo A-I in those with apo E2/4, as compared with the changes in this variable in those with the other genotypes. For the black men, the only significant difference in lipid response to training across genotypes was a greater reduction in LDL cholesterol in those with apo E 2/4 and E 4/4 than in those with apo E 2/3.In contrast, subjects with the apo E3/3 genotype showed a mean increase in LDL cholesterol ($P = .017$). There were no significant differences in training response in the activities of the 2 postheparin lipases across genotypes in either black or white men and women (data not shown).

DISCUSSION

In this study, apo E3 was the most prevalent allele, and E 3/3 the most common genotype in both white and black subjects. These findings are essentially in agreement with those from Caucasian and non-Caucasian populations from all over the world[1,2,43]; however there is a great deal of variability in prevalence rates for the 3 alleles and 6 genotypes between populations. The only significant racial difference in distribution of the common polymorphisms of apo E noted in this study was a higher prevalence of the apo E4 allele (21%) and the apo E3/4 genotype in black as compared to white subjects. Previous studies in blacks in North America and Africa revealed prevalences of apo E4 of 19% to 26%.[1]

Apo E polymorphism was found in this study to influence plasma lipid levels both in the sedentary state and in their responses to a systematic, moderate-intensity, exercise training program. Further, there were both racial and sex differences in plasma lipid levels between genotypes at baseline in the sedentary state and in the exercise-induced lipid changes. Our baseline data confirmed previous observations from many populations of significantly higher levels of plasma LDL cholesterol and apo B in individuals with the apo E 4/4 and E3/4 genotypes, as compared to those with apo E3/3 or an apo E2–containing genotype.[1,2,43,44] Our finding that those carrying the apo E2 allele had the lowest levels of LDL cholesterol and apo B also is in agreement with previous findings from other populations. Differences in plasma LDL cholesterol associated with apo E polymorphism most likely contribute significantly to the differential risk of CHD associated with apo E polymorphisms in observational epidemiologic studies, ie, an increased risk of CHD associated with apo E4, a reduced risk of CHD associated with apo E2, and an intermediate risk associated with the more common apo E3 allele.[1,2,4-7] It is postulated that the reason for these differences by genotypes in plasma LDL cholesterol levels is related to differences between alleles in their impact on cell receptor clearance rates of triglyceride-rich lipoproteins.[1,2]

Apo E4 is associated with an accelerated rate of both gastrointestinal absorption of cholesterol and uptake of cholesterol by the liver of apo E4 containing lipoproteins. The resulting increase in the hepatic cholesterol pool is postulated to result in a downregulation of LDL receptors, thereby increasing plasma LDL cholesterol levels. In contrast, a limited uptake of apo E2–containing lipoproteins, due to defective receptor binding of this allele, is believed to decrease the liver's cholesterol

pool, resulting in an up regulation of LDL receptors, thereby decreasing plasma LDL cholesterol levels.[1,2]

No consistent relationship was found in this study between the apo E genotype and plasma HDL cholesterol concentration in men, but women with genotypes apo E2/2 or E3/3 had significantly higher levels than those with other genotypes. In contrast, Dallongeville et al,[44] in a meta-analysis of 27 studies involving a total of about 15,000 men and women, reported that HDL cholesterol levels were significantly lower in subjects with the apo E 4/3 phenotype than in those with apo E 3/3. We observed a difference between black and white subjects in the association of apo E polymorphism to plasma levels of triglycerides, as well as to levels of the associated VLDL cholesterol. In white subjects, genotypes containing either the apo E2 or E4 allele had significantly higher levels of these lipids than those with the apo E3 allele, while black subjects showed no significant differences in these variables across genotypes. In the meta-analysis by Dallongeville et al,[44] plasma triglycerides were significantly higher in subjects carrying either the apo E2 allele or the E 4/3 genotype, as compared to those with the E 3/3 genotype, essentially in agreement with the findings in our white subjects. The higher levels of fasting plasma triglycerides observed in white subjects carrying the apo E2 allele again is consistent with the slower plasma clearance of VLDL remnants in carriers of this alleles.[1,2,44] Homozygosity for apo E2 also is reported in about 95% of patients with type III hyperlipoproteinemia (dysbetalipoproteinemia) in which there is delayed removal of VLDL remnants and impaired conversion of intermediate density lipoprotein to LDL. However, only about 5% of people homozygous for apo E2 develop this dyslipidemia.[1,2]

As previously reported, the HERITAGE exercise training program resulted in a significant increase in $V_{O_{2max}}$ in both men and women participants.[26,37] However, apo E polymorphism was unrelated to either baseline $V_{O_{2max}}$ or the $V_{O_{2max}}$ response to exercise training in any of our race and sex subgroups. This is in contrast to the recent findings of Meckes et al.[45] In their study, involving 121 subjects, 24 weeks of endurance exercise testing resulted in a 12.4% mean increase in $V_{O_{2max}}$ in men and a 9.5% mean increase in women; however, women subjects with the apo E 3/3 genotype failed to increase $V_{O_{2max}}$ with training, and men with this genotype had a reduced response, as compared to those with the apo E 2/3 and E 3/4 genotypes. In contrast, in a study involving sedentary, overweight men, after 9 months of training, those carryingthe apo E4 allele (N = 12) experienced the largest mean increase in $V_{O_{2max}}$ (25.9%), as compared to the poorest response (11.1%) in those with the apo E2 allele (n = 6), and an intermediate increase (17.9%) in those with an apo E3 allele (n = 33).[46] Further research is required to clarify the discrepancies in findings among these 3 studies; however, differences in sample size and compliance with the exercise training program are likely to be important confounding variables contributing to the differences between the results of previous studies as compared to HERITAGE.

Controversy also exists as to relationship of apo E polymorphism to the blood lipid response to exercise. The hypothesis that apo E polymorphism affects the blood lipid response to exercise was first tested in the Cardiovascular Risk in Young Finns Study.[47] In this observational study, physical activity was assessed by questionnaire in about 1,500 children and young adults. A moderate inverse association was found between reported physical activity and total and LDL cholesterol in subjects with apo E 4/3, E 3/3, and E 3/2. The apo E3/2 phenotype showed the strongest inverse association, while no association was found with the apo E4/4 phenotype. In another observational study, which involved 129 young and middle-aged men and women in Quebec, Canada (48), an inverse association was found between $V_{O_{2max}}$ level (used as a surrogate for physical activity status) and plasma triglycerides in both apo E2 homozygotes and heterozygotes ($r = -0.55$, $P < .05$), but not in carriers of the apo E4 allele or apo E 3/3. In addition, $V_{O_{2max}}$ was negatively correlated with LDL cholesterol ($r = -0.39$, $P < .05$) in women, but not men, carriers of apo E3/3, and it was positively correlated with plasma HDL cholesterol only in carriers of the apo E3/3 phenotype ($r = 0.51$ for men and $r = 0.65$ for women; $P < .05$).

More recently, Bernstein et al[49] in another observational study, compared plasma lipid levels across apo E allele by physical activity status assessed by questionnaire in a representative population sample of 1708 randomly selected Swiss men and women. For both men and women, apo E4 was associated with higher levels of total and LDL cholesterol, as compared with apo E3, while apo E2 was associated with the lowest levels; moreover apo E2 was associated with a significantly higher HDL cholesterol level than the other alleles. A significant relationship was found between blood lipid levels and the volume of physical activities requiring an energy expenditure of 4 or more times above the resting level, which differed by apo E alleles. Among both men and women, an increasing volume of reported exercise was associated with higher HDL cholesterol and lower triglyceride levels in carriers of apo E4, as compared in carriers of the apo E3 allele, with the least favorable response in carriers of the apo E2 allele. From these data the authors concluded that exercise could counteract some of the potentially deleterious effects of the apo E4 genotype on the blood lipid profile. In contrast, in the present study there was a more favorable lipid response to exercise training in those with genotypes containing apo E2 at least in the white subjects, as compared with other genotypes. These differences between studies may be related to the higher intensity and volume of supervised exercise in HERITAGE.

The first reported experimental study to examine the possible differential effects of exercise training across apo E genotypes was performed by Hagberg et al.[14,46] This study involved 51 overweight, initially sedentary men, 45 to 80 years of age, who were endurance exercise-trained for 9 months. Those carrying at least 1 apo E2 allele (n = 6) had a 2- to 3-fold greater increase in plasma HDL cholesterol, as compared to those carrying apo E3 and E4 alleles. No other significant differences in plasma lipids were observed across alleles. More recently, Meckes et al[45] genetically screened 120 men and women to create groups with an equal number of the 3 most common apo E genotypes (ie, apo E 3/3, E 3./2, and E 3/4), and exercise-trained them for 24 weeks. No significant differences were noted between genotypes in responses of LDL and HDL cholesterol and triglycerides to exercise training.

In the present study, racial and sex differences were noted in lipid response to exercise across apo E genotypes. In white

subjects, the most favorable lipid response to exercise, in terms of reducing risk of CHD, was a significant reduction in LDL cholesterol only in genotypes containing apo E2. White women possessing the apo E 4/4 genotype also experienced a greater reduction in triglycerides, as compared to those with other genotypes. However, in white men significant reductions in triglycerides were observed in those with apo E 2/3 and E3/3 genotypes, as compared to a significant increase with training in triglycerides in those carrying apo E4/4. In addition, a significantly greater increase in HDL cholesterol and apo A-I was observed in white women carrying the apo E3 allele, as compared to those with carriers of apo E 2/4 or E 4/4. Black men (but not women) carriers of apo E2, and those with the apo E4/4 genotypes ($n = 4$) had significantly greater reductions in LDL cholesterol with training, as compared to those with other genotypes. There were no significant differences in changes in triglycerides or HDL cholesterol in the black men and women across genotypes, although in the black women there was some variability across genotypes in apo A-I response to training. Differences between the findings in our study as compared to previous exercise training studies in blood lipid responses across apo E genotypes may be related to differences in study populations, sample size, the training program, and/or unexplained behavioral and environmental factors affecting blood lipid levels. Strengths of our study include the large sample size, supervision of exercise training using a tightly controlled exercise prescription, excellent subject compliance, 2 lipid assays before and after exercise training, control in the younger women for the phase of the menstrual cycle at which blood lipids were assessed, and adjustment for plasma volume changes following exercise.

In summary, this study involving a large biracial population confirms previous observations of significant differences across apo E genotypes in plasma lipids in the sedentary state and in their responses to a systematic endurance exercise training program Geno-types containing apo E4 generally were associated with the least favorable, and those with apo E2 allele, the most favorable, lipid profiles in terms of risk of CHD. Sex and racial differences were observed in the association of apo E genotypes to the response of lipids to exercise training. Similar baseline levels of Vo_{2max} and increases in Vo_{2max} with training were seen across genotypes, indicating in our study population that apo E polymorphism did not contribute to cardiorespiratory endurance.

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