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APOE genotype affects black-white responses of high-density lipoprotein cholesterol subspecies to aerobic exercise training

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Abstract

The objective of the study was to determine whether ethnicity interacts with the *APOE* genotype to influence conventionally measured high-density lipoprotein cholesterol (HDL-C) subfraction levels and nuclear magnetic resonance–measured (HDL_{NMR}-C) particle size at baseline and after training, and the changes with training. After a 6-week dietary stabilization period, men and postmenopausal women 50 to 75 years old underwent baseline testing (NMR lipid, maximum oxygen consumption, body composition, and genotyping assessments). Tests were repeated after completing 24 weeks of endurance exercise training. At baseline, *APOE2/3* blacks had significantly larger particle size ($P < .001$) and higher total HDL_{NMR}-C particle concentration ($P = .006$) than whites. After 6 months of endurance exercise training, *APOE2/3* blacks maintained a significantly larger HDL_{NMR}-C particle size ($P < .001$) and particle concentration of the large HDL_{NMR}-C than *APOE2/3* whites ($P < .001$). In multivariate analyses of variance adjusted for demographic and environmental confounding factors and for training-induced changes in lean body mass and intraabdominal fat, the model explained approximately 33% of the observed variability in training-induced improvements in HDL_{NMR}-C particle size ($P = .002$), with *APOE2/3* blacks having a greater increase in training-induced changes in HDL_{NMR}-C particle size. In a separate but similarly adjusted model for conventionally measured HDL_2-C, the model explained approximately 49% of the observed variability in training-induced changes in HDL_2-C. Ethnicity interacted with the E2/3 genotype at the *APOE* gene locus to influence higher baseline and after-training levels, and greater exercise training–induced improvements in the advantageous HDL-C subfractions in blacks than in whites. *APOE2/3* blacks may benefit more from aerobic fitness to reduce cardiovascular risk.

1. Introduction

Concentrations of high-density lipoprotein cholesterol (HDL-C) and its protective subfractions are inversely related to coronary artery disease and cardiovascular disease (CVD) mortality [1,2]. This inverse relationship of HDL-C to CVD risk is strong, graded, and independent of other plasma lipoprotein lipid levels and nonlipid risk factors [3]. Despite these strong relationships, there are substantial differences in plasma HDL-C levels across different ethnic groups. In fact, higher HDL-C levels have been consistently demonstrated in blacks compared with whites in most population as well as intervention studies [4,5]. Subsequent studies that focused on potential environmental explanations for these differences were unable to readily account for the differences in HDL-C in whites vs blacks [6].

High-density lipoprotein cholesterol particles are heterogeneous with respect to particle diameter, density, composition, and functional properties [7-9]. A more complete assessment of HDL-C particle sizes and concentrations is now possible using nuclear magnetic resonance spectroscopy (NMR). Substantial evidence indicates that these newer NMR measures of plasma

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HDL-C particle size and concentration provide a more precise estimate of CVD risk [10,11].

Exercise training can reduce CVD risk by improving plasma lipoprotein lipid profiles [12-15]. Some recent evidence indicates that the training benefits also extend to NMR measures of HDL-C [16,17]. However, numerous studies have shown highly variable responses to standardized exercise training in terms of plasma lipoprotein lipid levels, suggesting that genetic heterogeneity may influence these training adaptations [18,19]. Independent evidence indicates that ethnicity contributes significantly to interindividual differences in baseline HDL-C values and in exercise training–induced changes in HDL-C [12,18,20]. This observation is supported by the presence of higher HDL-C in blacks despite lower levels of habitual physical activity than whites [4,21]. It is, however, unknown whether such ethnicity-related differences operate through, or interact with, genetic factors to amplify the effects of physical activity on plasma lipoprotein lipids.

One of the most important gene loci known to influence HDL-C metabolism is the *APOE* gene. *APOE* affects the hepatic binding, uptake, and catabolism of several classes of lipoproteins associated with HDL-C and its subfractions [22,23]. Mature HDL-C particles are partly cleared from plasma by binding to specific *APOE* receptors on hepatocytes and by exchanging apoproteins and lipids with other lipoproteins in the tissue [24]. Whereas the E4 allele of the *APOE* gene associates with lower HDL-C levels, the E2 and E3 alleles result in higher plasma HDL-C subfractions, making the *APOE* gene variants an important modifier of HDL-C subfractions [20]. However, it is not known whether ethnicity-specific *APOE* gene–exercise training interactions result in differences between whites and blacks in these newer NMR-measured HDL-C (HDL_{NMR}-C) subfractions.

Given a generally higher HDL-C in blacks than in whites, the graded relationship of *APOE* with HDL-C, and the known influence of exercise training on lipoprotein lipid levels, we hypothesized that *APOE* genotype would have a significant ethnicity-related influence on the levels of HDL-C subfractions and the HDL_{NMR}-C particle size and concentration at baseline and after training, and on training-induced changes.

2. Methods

Sedentary white and black men and women aged 50 to 75 years were screened via telephone to ascertain interest, suitability, and ability to participate in an exercise training intervention. The institutional review boards at the University of Maryland College Park and Howard University approved the study protocol, and written informed consent was obtained during each participant's first laboratory visit. Eligible volunteers were nondiabetic, normotensive or hypertensive with blood pressure (BP) controlled with medication (systolic BP <160 mm Hg, diastolic BP <90 mm Hg), and nonsmokers; had a body mass index less than 37 kg/m^2; were not undergoing regular aerobic exercise; and had no prior history of CVD. All women were postmenopausal and maintained the same hormone replacement therapy (HRT), either on or not on HRT, throughout the study.

During the participant's first laboratory visit, medical histories were reviewed to ensure that subjects met the inclusion criteria; and body mass index less than 37 kg/m^2 was ascertained. Participants had blood chemistries and fasting plasma glucose levels determined and underwent a standard oral glucose tolerance test. Those with fasting glucose greater than 126 mg/dL or 2-hour glucose greater than 200 mg/dL were excluded. Participants had to have at least 1 National Cholesterol Education Program lipid abnormality [25] because this study was part of a larger trial assessing training-induced plasma lipoprotein lipid changes. Maximal treadmill exercise tests were performed; participants whose exercise test was terminated because of CVD signs or symptoms and those who showed other evidence of CVD were excluded.

Participants then completed 6 weeks of instruction on the principles of an American Heart Association (AHA) step 1 diet (<30% calories from fat, ~55% from carbohydrates, ~15% from protein, cholesterol intake <300 mg/d) [26]. Participants completed 7-day food records, adhered to the diet for more than 3 weeks before baseline testing, and maintained adherence to this diet throughout the study. A registered dietician analyzed all food records (Computrition, Chatsworth, CA). Participants completed food records during the exercise training intervention and met with the dietician every 2 to 3 weeks to ensure adherence to the diet.

In the morning after a 12-hour fast, venous blood samples were drawn for analyses of major plasma lipid concentrations and lipoprotein particle size. All baseline blood samples were drawn at the end of the 6-week dietary stabilization period and at least 4 days after any exercise test. Plasma was isolated from blood samples by centrifugation at 3000*g* for 15 minutes at 4°C in the presence of 0.01% EDTA and frozen at −70°C until analyzed. To determine conventional plasma lipoprotein lipids levels, the averages of fasted samples drawn on 2 separate occasions were used. High-density lipoprotein cholesterol was measured after precipitation with dextran sulfate [27]. HDL_2-C and HDL_3-C were separated using a second high–molecular weight dextran sulfate precipitation with HDL_3-C measured and HDL_2-C calculated [28]. Because each lipoprotein in plasma within a given diameter range emits a distinctive lipid NMR signal, the intensity of which is proportional to its bulk lipid mass concentration, these signals were used to determine HDL_{NMR}-C particle size and subfraction concentrations using the second of the 2 samples pooled for conventionally measured HDL-C [29,30]. The HDL-C subfractions were determined as follows: large HDL_{NMR}-C (10-13 nm, similar to HDL-C_{2b}); intermediate HDL_{NMR}-C (8.2-10 nm, similar to HDL-C_{2a} and HDL-C_{3a}); and small HDL_{NMR}-C (7.3-8.2 nm, similar to HDL-C_{3b} and HDL-C_{3c}). A "particle size index,"

describing the mass-weighted average size of particles within each lipoprotein class, was calculated by weighing each subclass concentration by a numerical size designation (1-3), with larger values representing larger particle subclasses. Close agreement has previously been demonstrated between NMR and chemically determined HDL-C ($r = 0.93$) [31]. High-density lipoprotein cholesterol subclass distributions determined by NMR have also been shown to be closely related to conventionally measured HDL-C subfractions [31]. Body composition was assessed by dual-energy x-ray absorptiometry (DPX-L; Lunar, Madison, WI), and subcutaneous fat and intraabdominal fat were quantified at L4 through L5 using a standardized computed tomography scan protocol [32]. Maximum oxygen consumption (Vo_2max) was measured using a graded treadmill protocol [16]. Genomic DNA was extracted from peripheral lymphocytes using standard methods [33]. Subjects were typed at the *APOE* locus as previously described [34].

Participants then underwent 3 supervised exercise training sessions per week for 6 months. Initial sessions consisted of 20 minutes of exercise at 50% Vo_2max and progressed until 40 minutes of exercise at 70% Vo_2max was completed during each session [16,35]. Exercise consisted of treadmill walking/jogging, stair stepping, and cycle and rowing ergometry. Participants added a lower-intensity, unsupervised, 45- to 60-minute walk on the weekend after 12 weeks of training. After exercise training, body composition, Vo_2max, and plasma lipoprotein lipid assessments were repeated as before training. The blood samples for plasma lipoprotein lipid levels were drawn 24 to 36 hours after each subject's last exercise training session. Participants' dietary compliance was confirmed before final testing.

Statistical analyses were performed using the SAS statistical software system (SAS, Cary, NC) [25]. Given the general biological gradient of the *APOE* genotypes (E2/2, E2/3, E3/3, E2/4, E3/4, and E4/4) [20,36] and the relatively small sample size, E2/2, E2/3, and E3/3 were combined and designated as the *E2/E3 group*, whereas E4 hetero- or homozygotes were designated as the *E4 genotype group*. Because there is insufficient information on the biological gradient of E2/4, 2 volunteers having this genotype were excluded from these analyses. In the initial bivariate analysis, we used *t* tests to compare E2/3 vs E4 in the entire sample and to determine ethnicity-related differences in baseline and after-training HDL-C measurements within each genotype group. Multivariate analysis of variance (general linear model approach) was used to evaluate the relationship of ethnicity and *APOE* with training-induced changes in HDL-C. At baseline, complete data were available on 170 subjects (whites = 133, blacks = 37). For the after-training bivariate models, 149 subjects (whites = 120, blacks = 29) had complete data. Because of the confirmed significant relationship of ethnicity with training-induced changes in HDL subfraction ($P < .05$) in the entire group and the interaction of *APOE* gene with ethnicity ($P = .045$) in our preliminary analyses, separate analyses comparing levels of HDL-C and HDL_{NMR}-C particle size and subfraction concentrations in whites vs blacks were conducted for each genotype group. Initial models testing the influence of ethnicity on HDL-C within each *APOE* genotype group were first adjusted for the baseline values of the dependent variable, whereas the second models were further adjusted for age and sex. Because of the significant differences in intraabdominal fat between blacks and whites at baseline, the final models included adjustment for training-related changes in lean body mass (LBM) and intraabdominal fat. Separate analytic models for training-induced changes in HDL-C and HDL_{NMR}-C subfractions were constructed. For ethnicity comparisons, all full models testing differences in training-induced changes in HDL_{NMR}-C and conventional HDL-C subfractions were adjusted for corresponding baseline values of the dependent variables age, sex, and training-induced changes in LBM and intraabdominal fat. Statistical significance was accepted at *P* less than .05.

3. Results

Whites and blacks in the *APOE2/3* genotype group had similar baseline characteristics (Table 1) with respect to body composition and CV fitness. However, *APOE2/3* blacks had significantly less intraabdominal fat than whites. No differences in body composition or CV fitness were evident between *APOE*4 whites and blacks. In the initial *t* test analysis, women on HRT weighed slightly more and had higher LBM at baseline, but were similar in all other characteristics, compared with those not on HRT.

Ethnicity was associated with differences in baseline HDL_{NMR}-C particle size and subfraction levels in the *APOE2/3* group, with blacks having a significantly larger particle size than whites ($P < .001$) (Table 2). The E2/3 blacks also had a significantly higher total HDL_{NMR}-C particle concentration than whites ($P = .006$). This higher total HDL_{NMR}-C particle concentration in E2/3 blacks resulted from a higher large HDL_{NMR}-C particle concentration, which averaged approximately twice that of whites, whereas both groups had similar medium and small HDL_{NMR}-C particle concentrations. The ethnic differences in HDL_{NMR}-C were also evident in the conventional measures of HDL-C, as demonstrated by a significantly higher HDL-C, HDL_2-C, and HDL_3-C levels in E2/3 blacks than E2/3 whites ($P = .002$, $P = .012$, and $P = .009$, respectively), although HDL_3-C was also higher in E2/3 blacks compared with E2/3 whites (Table 3). *APOE*4 blacks and whites had comparable NMR and conventionally measured HDL-C subfractions.

The slightly higher body weight and LBM observed at baseline in women taking HRT vs those not on HRT remained unchanged after training. Six months of aerobic exercise training resulted in favorable responses in both ethnic groups. Although whites and blacks in the E2/3 genotype group and E4 blacks had decreases in intraabdominal fat, E2/3 whites

Table 1
Baseline and after-training characteristics of the sample within each ethnicity and *APOE* genotype groups

Parameter	Baseline		After training	
	Whites	Blacks	Whites	Blacks
APOE2/E3	(n = 91-94)	(n = 22-24)	(n = 79-81)	(n = 18)
Age (y)	58.1 ± 0.6	58.7 ± 1.0	58.1 ± 0.6	59.6 ± 1.3
Sex				
Men	47%	36%	45%	38%
Women	53%	64%	54%	62%
Body weight (kg)	83.2 ± 1.6	81.1 ± 3.0	81.2 ± 1.7†	77.3 ± 3.1†
BMI (kg/m^2)	28.7 ± 0.5	28.8 ± 0.9	27.8 ± 0.4†	27.5 ± 1.0†
LBM (kg)	49.0 ± 12.6	47.7 ± 23.4	49.8 ± 14.0†	47.2 ± 24.3
Total body fat (%)	36.4 ± 0.9	36.2 ± 2.1	34.9 ± 0.9†	34.8 ± 2.8†
Subcutaneous fat (cm^2)	312.9 ± 12.5	334.0 ± 31	306.0 ± 13	323.6 ± 36†
Intraabdominal fat (cm^2)	143.9 ± 5.7	106.7 ± 11*	128.1 ± 5†	91.6 ± 9*
Vo_2max (mL/[kg min])	25.3 ± 0.46	23.6 ± 0.87	29.3 ± 0.6†	27.3 ± 1.7†
APOE 4	(n = 37-39)	(n = 11-13)	(n = 34-39)	(n = 9-11)
Age (y)	58.1 ± 1.0	57.0 ± 1.5	57.9 ± 1.0	57.9 ± 1.7
Sex (%)				
Men	41%	31%	36%	30%
Women	59%	69%	64%	70%
Body weight (kg)	81.0 ± 2.6	86.9 ± 2.9	78.1 ± 2.6	84.4 ± 3.1†
BMI (kg/m^2)	28.5 ± 0.8	30.3 ± 0.9	27.5 ± 0.8	29.7 ± 1.0†
LBM (kg)	46.7 ± 1.7	49.4 ± 3.2	47.1 ± 1.7†	49.5 ± 4.5
Total body fat (%)	37.0 ± 1.6	39.6 ± 2.7	35.0 ± 1.7†	37.8 ± 3.8
Subcutaneous fat (cm^2)	334.4 ± 2.3	364.4 ± 3.0	295.7 ± 1.8	365.6 ± 3.8
Intraabdominal fat (cm^2)	120.2 ± 0.9	120.7 ± 1.3	120.7 ± 1.1	107.1 ± 1.2†
Vo_2max (mL/[kg min])	24.9 ± 0.9	21.7 ± 1.0	28.4 ± 1.1†	24.2 ± 1.6†

Data are mean ± SE. Ranges of sample size for each ethnicity and genotype groups are presented because not all participants completed all measurements.
* Statistically significant difference between ethnic groups with the same genotype at $P < .05$.
† Statistically significant within–ethnic and genotype groups change with training at $P < .05$.

still had a significantly higher intraabdominal fat compared with E2/3 blacks ($P = .003$) (Table 1). E2/3 and E4 blacks and whites had comparable improvements in Vo_2max with CV fitness training, whereas E2/3 blacks maintained the significantly better HDL_{NMR}-C profile, with larger particle size ($P < .001$) and higher particle concentration of large HDL_{NMR}-C than E2/3 whites ($P < .001$) (Table 2). Conversely, although whites and blacks in the E2/E3 genotype group were similar at baseline and had statistically nonsignificant within-group decreases in training-related changes in medium HDL_{NMR}-C particle concentration, after-training levels were significantly lower in E2/3 blacks than whites ($P = .022$) (Table 2). When we examined the relationship between training-induced changes in HDL-C and *APOE* genotype, training-induced improvements in HDL_{NMR}-C particle size were greater in *APOE2/E3* blacks,

Table 2
HDL_{NMR}-C and its subfractions within each ethnicity and genotype group at baseline and after training

	Baseline			After training		
	Whites	Blacks	*P* value	Whites	Blacks	*P* value
APOE2/3 group	(n = 68)	(n = 15)		(n = 68)	(n = 15)	
HDL-C mean particle size (nm)	8.7 ± 0.1	9.2 ± 0.1	<.001	8.8 ± 0.1	9.4 ± 0.1	<.001
HDL-C particle concentration (μmol/L)						
Total (μ/L)	33.6 ± 0.6	34.1 ± 1.2	.006	34.5 ± 0.6	33.7 ± 1.3	.003
Large size (μmol/L)	4.6 ± 0.4	8.7 ± 1.3	<.001	5.6 ± 0.4	10.3 ± 1.0	<.001
Medium size (μmol/L)	6.3 ± 0.7	4.7 ± 1.8	.37	5.3 ± 0.6	2.4 ± 1.0	.022
Small size (μ/L)	22.7 ± 0.7	20.7 ± 1.8	.80	23.7 ± 0.7	21.0 ± 1.5	.251
*APOE*4 group	(n = 21)	(n = 7)		(n = 21)	(n = 7)	
HDL-C mean particle size (nm)	8.8 ± 0.1	8.9 ± 0.2	.60	8.8 ± 0.1	9.1 ± 0.2	.33
HDL-C particle concentration (μmol/L)						
Total (μ/L)	35.1 ± 1.3	32.1 ± 1.5	.20	36.0 ± 1.5	33.5 ± 3.6	.45
Large size (μ/L)	5.8 ± 0.9	5.9 ± 1.1	.94	6.6 ± 0.9	7.4 ± 1.1	.76
Medium size (μ/L)	7.3 ± 1.3	2.7 ± 1.2	.06	7.5 ± 1.1	3.9 ± 1.3	.08
Small size (μ/L)	22.0 ± 1.4	23.5 ± 1.9	.52	22.0 ± 1.2	22.2 ± 2.4	.78

Data are means ± SE. *P* values are for the comparison of white vs black.

Table 3
Conventionally measured HDL-C and its subfraction within each genotype group at baseline and after training

	Baseline			After training		
	Whites	Blacks	*P* value	Whites	Blacks	*P* value
APOE2/3 group	(n = 94)	(n = 24-25)		(n = 81)	(n = 16)	
HDL-C (mg/dL)	47.1 ± 1.6	59.0 ± 3.8	.002	49.2 ± 1.6	58.2 ± 3.8	.023
HDL_2-C (mg/dL)	5.8 ± 1.2	12.4 ± 2.5	.012	6.2 ± 0.9	13.9 ± 3.5	.046
HDL_3-C (mg/dL)	41.4 ± 0.9	46.5 ± 1.8	.009	43.1 ± 0.9	44.5 ± 2.7	.522
*APOE*4 group	(n = 39)	(n = 13)		(n = 33)	(n = 10)	
HDL-C (mg/dL)	49.0 ± 2.4	47.9 ± 4.1	.814	52.2 ± 2.6	48.9 ± 3.7	.524
HDL_2-C (mg/dL)	6.0 ± 1.2	5.2 ± 2.1	.760	7.3 ± 1.5	4.8 ± 2.3	.419
HDL_3-C (mg/dL)	43.1 ± 1.6	42.5 ± 2.2	.839	45.2 ± 1.8	43.8 ± 1.9	.685

Data are means ± SE. *P* values are for the comparison of whites vs blacks.

with the increase being approximately 2.5 times that in the E2/3 whites (0.26 ± 0.06 nm vs 0.10 ± 0.03 nm, $P = .015$) (Fig. 1). However, E4 blacks had the same training-induced increases in HDL_{NMR}-C particle size as E4 whites. Blacks had slight decreases, whereas whites had slight increases, with training in levels of conventionally measured HDL-C compared with baseline; yet HDL levels remained significantly higher in blacks than whites (Table 3). Black vs white differences in after-training levels of cardioprotective HDL_2-C followed the same general trends observed for large HDL_{NMR}-C particle size and concentration, with greater improvement with training in E2/3 blacks than E2/3 whites ($P = .046$). Although whites had slight increases and blacks had slight decreases in HDL_3-C levels, ethnicity did not affect training-related levels of the smaller and less protective HDL_3-C between the groups (Table 3). Within the *APOE*4 genotype group, there was no significant white vs black differences in the levels or the training-induced changes in either NMR or conventionally measured HDL-C subfractions after 6 months of aerobic exercise training.

Initial multivariate analysis for the entire group confirmed our hypothesis of ethnicity-related differences in training-induced changes in HDL subfractions ($P < .05$). Significant interaction of ethnicity with *APOE* in a separate model ($P = .045$) indicates that this ethnicity-related difference differed between E2/E3 and E4. Subsequent multivariate analysis to determine the association of ethnicity with changes in HDL_{NMR}-C particle size within the *APOE2/3* genotype group included ethnicity and baseline HDL_{NMR}-C levels. This model accounted for approximately 20 % of the observed variability in training-induced improvements in HDL particle size ($P < .001$). After adding age and sex to the model, the observed variability increased to approximately 27% ($P < .001$). To control for additional environmental confounding factors, we also adjusted for the effect of training on LBM and intraabdominal fat; this model explained approximately 33% of the observed variability in training-induced improvements in particle size ($P = .002$), with E2/E3 blacks having a disproportionately greater increase in training-induced changes in particle size in the complete model. The models for training-induced changes in HDL_2-C were generally similar to those observed for particle size, with the initial limited model contributing approximately 46% ($P = .011$) to the observed variability. Further adjustment for age and sex did not change the explained variability (~47%, $P = .014$). With additional adjustment for differences in body composition, the model contribution increased to approximately 49% ($P = .005$) of the observed variability in training-induced changes in HDL_2-C. However, the gene and ethnicity effects of aerobic exercise training appear to be limited to HDL_2-C and large particle size, as black vs white differences in training-induced HDL-C changes were generally not significant in the limited model or in the fully adjusted model.

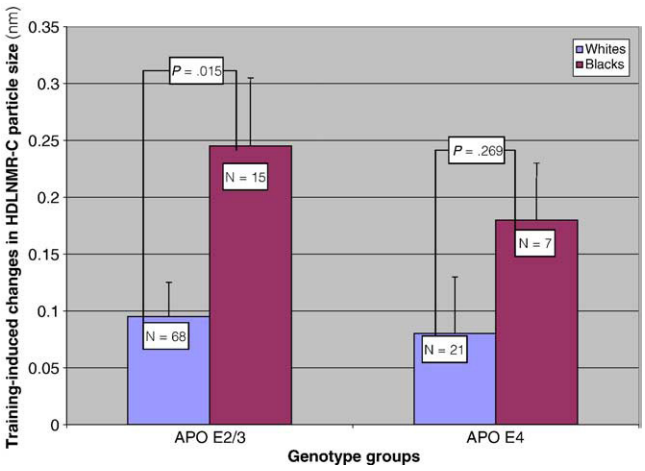

Fig. 1. White and black comparison of training-induced changes in HDL_{NMR}-C particle size within *APOE* genotype.

4. Discussion

In the present study, we showed that ethnicity interacted with the E2/3 genotype at the *APOE* gene locus to influence HDL-C indices at baseline and after training. Compared with whites, black *APOE2/3* carriers had higher baseline levels and greater exercise training–induced improvements in the advantageous HDL-C subfractions, whereas E4 allele carriers did not demonstrate any

evidence of black-white differences in HDL-C at baseline or after 6 months of aerobic exercise training, or in the changes resulting from exercise training.

It is now recognized that HDL-C particles are heterogeneous with respect to particle diameter, density, composition, and functional properties [7-9]. Mature HDL-C particles are partly cleared from plasma by binding to specific apolipoprotein (apo) E receptors on hepatocytes and by exchanging apoproteins and lipids with other lipoproteins in the tissue [24]. Through the activation of lipoprotein lipase, HDL-C transfers apo E and apo C-II to both chylomicrons and to very low-density lipoprotein [37]. Mature HDL-C particles are designated as *HDL$_3$ particles*, which, after losing their cholesterol and gaining triacylglycerol, become the larger cardioprotective HDL$_2$-C particles [38]. Whereas small HDL-C particles are positively related to risk of coronary artery disease [9,39-41], the protective effect of HDL-C has been most consistently observed for large HDL-C particles [9,42]. Although the influence of the *APOE* gene on baseline HDL$_2$-C levels in relatively sedentary populations was previously reported [43], ethnicity-related differences in the effect of *APOE* gene variants on HDL$_{NMR}$-C particle size and concentration, which are newer, more accurate, and a direct measure of HDL-C, have not been described.

Heller and colleagues [44] previously reported that more than 50% of the variation in HDL-C levels in humans is genetically determined and that gene products that influence the amount and nature of lipids contained within HDL-C particles have important effects on the metabolism of HDL-C particles. *APOE* gene is one of the most widely studied polymorphic variants known to affect lipid metabolism. Its 3 common alleles code for 3 isoforms of the apo E protein, that is, ε2, ε3, and ε4 [36]. Whereas apo ε4 preferentially associates with very low-density lipoprotein, apo ε2 and ε3 preferentially associate with HDL-C [45]. In large epidemiologic studies, the hierarchy of the *APOE* phenotypes from the lowest to the highest cholesterol levels was E2/2, E2/3, E3/3, E2/4, E3/4, and E4/4 [20,36,46]. This biological gradient of the *APOE* gene effect on lipid metabolism has important implications for CVD.

Although the exact mechanism by which exercise interacts with *APOE* genotype to increase large particle size HDL-C remains to be fully elucidated, studies in endurance athletes showed that aerobic fitness can increase HDL-C half-life by several days [47]. This fitness-induced prolongation of HDL-C half-life may allow for the modification of HDL-C particle distribution in the circulation, and *APOE2/3* alleles may interact with the increased HDL-C survival caused by exercise to favorably alter HDL-C particle distribution. It is therefore relevant that *APOE* allele frequencies differ between blacks and whites, with *APOE*2 frequency being approximately 27% to 33% higher and E3 approximately 8% to 21% lower in blacks compared with whites [48-50]. Trends for baseline and after-training *APOE* genotypes' frequency distribution between blacks and whites approximate generally reported estimates: 12.8% vs 15.8% for E2/3, 57.9% vs 50% for E3/3, and 29.3% vs 34.2% for E4 heterozygotes. Notably, these differences may underscore some of the ethnicity-related variations in HDL-C levels. Although women on HRT weighed slightly more and had higher LBM at baseline and after training compared with those not on HRT in the initial *t* test, these differences did not explain our findings because the final multiple regression analysis adjusted for body composition.

In addition to possible ethnicity-specific gene variants in HDL-C metabolic pathways, other yet unknown ethnicity-related factors may interact with the *APOE* genotype to influence baseline levels of HDL$_{NMR}$-C particle size and concentration in a relatively sedentary population. Such factors may also have selective interaction with specific alleles of the *APOE* gene to effect higher large HDL$_{NMR}$-C particle size and concentration in blacks than whites. Together, our observations support many epidemiologic studies showing higher HDL-C subfraction levels in blacks than whites, suggesting that higher levels of HDL$_{NMR}$-C and subfraction in blacks may be partly related to the differential *APOE2/3* genotype effects in blacks compared with whites. Future studies must focus on the identification of biological or environmental factors and the evaluation of the interaction of such factors with *APOE* gene.

Training-induced levels of conventionally measured HDL$_2$-C support the presence of higher levels of the large HDL$_{NMR}$-C particles in E2/3 blacks compared with whites. Although we are unaware of previous studies that examined the combined influence of *APOE* gene and ethnicity on training-induced changes in HDL$_{NMR}$-C subfractions, our observation of higher HDL$_2$-C in the *APOE2/3* genotype group is in concordance with previous reports from Wood and Haskell [51] who showed that exercise training–induced increases in plasma HDL-C levels appear to result largely from an increase in the less dense HDL$_2$ subfraction. The greater training-induced changes in HDL$_2$-C levels in our study correlated with our NMR data in *APOE2/3* genotype group and are consistent with a previous report from Hagberg et al [34] who showed that E2-carrier overweight men were more responsive to training-induced changes in HDL$_2$-C than E3 and E4 allele carriers.

The HERITAGE Family Study has reported the strongest supportive evidence for the interactive effects of ethnicity with genetics on plasma lipid lipoprotein responses to aerobic exercise training [20]. For example, Leon et al [20] examined the effect of *APOE* genotype on lipid responses to a 20-week, 3-d/wk exercise training intervention in a family-based cohort of whites and blacks from the HERITAGE study. Distinct race-based differences in exercise training lipid response by *APOE* genotype were observed. Of all races and sexes in the study, white women had the greatest *APOE*-related training-induced changes in conventionally measured HDL-C and subfraction, whereas black women showed significant differences in APO A-I levels by *APOE* genotype. Although our study lacks the power to make sex-based comparisons between blacks and whites, we provide

an important addition to the literature by using a newer and more accurate NMR method to quantify HDL-C particle size and concentration. Given the white-black differences in baseline and after-training HDL_{NMR}-C and subfractions within the *APOE2/3* genotype group in our study, we concur with Rice and colleagues [12] that the discrepancy in black vs white differences in training-induced changes in plasma lipoprotein lipid may indicate genetic heterogeneity in possible underlying pleiotropic genes affecting baseline phenotypic expression and exercise training adaptation. Furthermore, a significantly higher training-induced improvement in HDL_{NMR}-C particle size and HDL_2-C in blacks than whites even after accounting for the contribution of baseline values suggests that our results were independent of baseline differences. This observation supports the likelihood that the *APOE* gene may interact with specific ethnicity-based environmental and/or biological factors to differentially affect plasma lipoprotein lipid responses to standardized aerobic exercise training. Importantly, given the cardioprotective effect of HDL_{NMR}-C and its subfractions, the identification of additional ethnicity-related factors interacting with environmental and/or biological factors to affect HDL_{NMR}-C responses to standardized aerobic exercise training needs further investigation.

One of the important limitations of this study is that it lacks the power to make sex comparisons within *APOE* genotype groups. Second, we are unable to draw hard conclusions on black-white comparison in the E4 group, given the relatively small sample size of this group. Despite this limitation, a major strength of this study was the use of a newer and more accurate NMR technique to show the *APOE* race differences in response to aerobic exercise training. This is especially important, given the heterogeneity in HDL-C particle size response to aerobic exercise training among subjects, suggesting that genotyping may be important to identify those who might benefit more from fitness adaptation to reduce CVD risk. In addition, our study has the advantage of a 6-month standardized aerobic exercise training program, dietary stabilization on the AHA step I diet before training, monitoring of adherence to this diet, and an individually tailored exercise intervention while preserving uniformity in training attendance and exercise volume. In conclusion, we provide new evidence that *APOE2/3* blacks have greater improvement in HDL-C particle size and concentration with exercise training than whites while maintained on a constant AHA step 1 low-fat diet. Given the relatively low level of physical activity in blacks, this study provides new and clinically relevant information that can be useful in encouraging and targeting aerobic exercise training to high–CVD risk subjects with specific genotypes in populations at high CVD risk.

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