High-Density Lipoprotein-Cholesterol, Its Subfractions, and Responses to Exercise Training Are Dependent on Endothelial Lipase Genotype

Amy Halverstadt, Dana A. Phares, Robert E. Ferrell, Kenneth R. Wilund, Andrew P. Goldberg, and James M. Hagberg

Plasma high-density lipoprotein cholesterol (HDL-C) levels are an important independent risk factor for cardiovascular disease (CVD) that can be modified through exercise training. However, levels of HDL-C and its subfractions and their response to standardized exercise training are highly variable among individuals. Such variability suggests that levels of HDL-C, its subfractions, and their response to exercise training may be influenced by genetic variation and the interaction of that genetic variation with physical activity. The endothelial lipase gene (LIPG) may influence HDL-C metabolism and has several recently identified genetic variants. We hypothesized that the LIPG Thr111Ile polymorphism would be associated with variation in HDL-C levels and its subfractions and their response to exercise training. Eighty-three sedentary, healthy 50- to 75-year-old subjects were weight-maintained on an American Heart Association Step 1 Diet and then studied before and after aerobic exercise training. Sample size varied according to outcome measure as complete data was not available for all subjects. Initial age, body composition, and maximum oxygen consumption (Vo_{2max}) did not differ between LIPG genotype groups (CC, n = 41 to 44; CT/TT, n = 37 to 39). Initial total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels were not significantly different between groups. The CT/TT group had lower initial HDL_{2NMR}-C (12 ± 1.0 *v* 17 ± 1.1 mg/dL; *P* = .002) and integrated $HDL_{1,2NMR}$-C (13 ± 1.0 *v* 18 ± 1.1 mg/dL; *P* = .002) levels and somewhat higher initial levels of integrated $HDL_{3,4,5}$-C (31 ± 2.2 *v* 25 ± 2.3 mg/dL; *P* = .06). With exercise training, Vo_{2max} increased, and body weight, total body fat, and visceral adipose tissue decreased similarly in both groups. With training, HDL-C levels increased twice as much (4.4 ± 0.8 *v* 1.9 ± 0.9 mg/dL; *P* = .04), HDL_3-C levels increased almost 2-fold greater (3.8 ± 0.7 *v* 2.2 ± 0.6 mg/dL; *P* = .07), and HDL_{5NMR}-C levels increased more than 4 times as much (2.2 ± 0.8 *v* 0.5 ± 0.6 mg/dL; *P* = .08) in the CC compared to the CT/TT group. We conclude that the LIPG genotype is associated with interindividual variability in HDL-C and its subfractions and their response to exercise training.

THE ENDOTHELIAL lipase gene (LIPG) is a newly identified member of the triglyceride (TG) lipase gene family.[1-9] Unique in its synthesis by endothelial cells, the endothelial lipase (EL) enzyme demonstrates substantial homology to other TG lipase family members (45% identity to lipoprotein lipase [LPL], 40% to hepatic lipase [HL], 27% to pancreatic lipase [PL]).[1-3,6,7,9] The EL enzyme also bears functional similarity to LPL and HL as it is involved in high-density lipoprotein cholesterol (HDL-C) metabolism.[1-11] Previous studies have shown that genetic variations at the LPL and HL loci influence HDL-C levels, suggesting that genetic variation at the LIPG locus may also affect HDL-C levels.[3,10,11]

Of several potentially functional genetic variants identified at the LIPG locus, the Thr111Ile polymorphism is the most common.[6] To our knowledge, the only study assessing the impact of LIPG polymorphisms on HDL-C levels found no difference in genotype or allele distributions between whites with normal or high HDL-C.[6] However, the effect of this variant has not been examined in individuals with HDL-C levels within the normal range or on HDL-C subfractions.

Plasma HDL-C levels are an important independent risk factor for cardiovascular disease (CVD) that can be modified through exercise training.[12-18] For example, Durstine et al summarized current cross-sectional and longitudinal studies and established that 15 to 20 miles of brisk walking or jogging (expenditure of 1,200 to 2,200 kcal per week) was associated with increases in HDL-C of 2 to 8 mg/dL.[12] Kraus et al recently found that a relatively high amount of regular exercise significantly improved overall lipoprotein profiles, including an increase in total HDL concentration, large HDL particle concentration, and average size of HDL particles, even without clinically significant weight loss.[13] Leon et al also found that 20 weeks of endurance exercise training significantly increased HDL-C levels on average by 1.4 mg/dL.[15] With evidence showing that each 1 mg/dL increase in plasma HDL-C levels lowers CVD risk by 4.4%, exercise training may reduce CVD risk by increasing plasma HDL-C levels.[19] Furthermore, recent evidence suggests that HDL-C subfractions, which are also influenced by exercise training, may be of even greater importance to determining CVD risk than conventional HDL-C measures.[20-23] However, the response of HDL-C and its subfractions to standardized exercise training is highly variable among individuals.[12-15,19,20] Therefore, we hypothesized that the LIPG Thr111Ile gene polymorphism would be associated with HDL-C and its subfractions at baseline and with the response of HDL-C and its subfractions to exercise training.

From the Department of Kinesiology, University of Maryland, College Park, MD; Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; and Division of Gerontology, University of Maryland School of Medicine, and Geriatric Research, Education, and Clinical Center, VA Medical Center, Baltimore, MD.

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Address reprint requests to James Hagberg, PhD, Department of Kinesiology, University of Maryland, College Park, MD 20742-2611.

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MATERIALS AND METHODS

Eighty-three healthy sedentary men and women volunteered for this study. Subjects underwent a telephone screening to determine their initial eligibility. The University of Maryland, College Park Institutional Review Board approved the study. Written informed consent was obtained on all subjects, and all procedures were followed in accordance with institutional guidelines. Eligibility for the study required subjects to be healthy, sedentary, 50 to 75 years of age, normotensive or with blood pressure (BP) controlled with medications not affecting plasma lipoprotein lipid levels, nondiabetic, nonsmoking, no history of CVD, and body mass index (BMI) less than 37 kg/m^2. All female participants were more than 2 years past menopause and maintained their hormone replacement therapy (HRT) status (on or not on HRT) constant for the duration of the study.

Screening

Screening visit 1. Medical histories were reviewed and BMI was confirmed to be less than 37 kg/m^2. A fasted blood sample was drawn for genotyping and plasma lipoprotein-lipid profile analysis. Subjects had to have 1 or more National Cholesterol Education Program lipid abnormalities (cholesterol $>$ 200 mg/dL, HDL-C $<$ 40 mg/dL, TG $>$ 200 mg/dL); total cholesterol and low density lipoprotein cholesterol (LDL-C) had to be less than 90th and HDL-C greater than 20th percentile for their age and gender to exclude familial hypercholesterolemia.

Screening visit 2. Each subject had fasting blood samples drawn and underwent a 2-hour 75-g oral glucose tolerance test to ensure fasting glucose levels less than 126 mg/dL and 2-hour glucose levels less than 200 mg/dL.

Screening visit 3. Subjects qualified to this point underwent a physical and cardiovascular examination by a physician to detect cardiovascular (CV), pulmonary, or other chronic diseases that would preclude exercise testing or training.[24] They underwent a Bruce maximal treadmill exercise test to ensure they had no evidence of overt CVD.[25] BP, heart rate, and electrocardiogram (ECG) results were recorded before the test, at the end of every exercise stage, and every other minute for 6 minutes after exercise. The test was terminated when the subject could no longer continue or cardiovascular signs or symptoms occurred.[24] Subjects had to have less than 2 mV ST-segment depression and no cardiovascular signs or symptoms during this test to be included in the study.[24]

Dietary Stabilization

All subjects underwent 6 weeks of dietary instruction (twice a week, 1 hour per session, for a total of 12 hours) with a registered dietician on the American Heart Association Step 1 Diet.[26] Subjects had to follow this prescribed diet and be weight-stable for more than 3 weeks prior to undergoing baseline testing.

Baseline Testing

To analyze plasma lipoprotein lipid levels, subjects had fasted samples drawn on 2 separate occasions, with the values averaged. If these measures differed by greater than 10%, a third separate measurement was included in the average. Plasma TG and cholesterol levels were analyzed in a Center for Disease Control (CDC)-certified lab using a CDC standardized Hitachi 717 autoanalyzer.[27,28] HDL-C was measured after precipitation with dextran sulfate,[29] and LDL-C was calculated using the Friedewald equation.[30] 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.[31] Nuclear magnetic resonance (NMR) techniques (LipoScience, Raleigh, NC) also were used to determine plasma lipoprotein lipids, using techniques previously standardized and validated against conventional methods of separation and analysis.[21,32] NMR measures were made on a single blood sample that had been frozen at −80°C and analyzed at the same time as final test samples. Rough equivalence of NMR and conventional HDL-C measures are as follows: HDL_{1NMR}-C $\sim$ HDL_{3c}-C; HDL_{2NMR}-C $\sim$ HDL_{3b}-C; HDL_{3NMR}-C $\sim$ HDL_{3a}-C; HDL_{4NMR}-C $\sim$ HDL_{2a}-C; and HDL_{5NMR}-C $\sim$ HDL_{2b}-C.[28,29] Additionally, 2 integrated HDL_{NMR}-C subfractions were calculated: $HDL_{3,4,5NMR}$-C, which is more cardioprotective, and $HDL_{1,2NMR}$-C, which is more atherogenic.[21,22,32-34] Body composition was analyzed using dual-energy x-ray absorptiometry (DPX-L; Lunar Corp, Madison, WI) and L4-L5 visceral adipose tissue and subcutaneous adipose tissue were quantified using a standardized computerized tomography (CT) protocol.[35] All subjects underwent a second maximal treadmill exercise test to assess maximum oxygen consumption ($V_{O_{2max}}$) as an index of CV fitness.[36] This test started at 70% of the peak heart rate achieved on the subject's screening exercise test and treadmill grade increased by 2% every 2 minutes.[36] Blood pressure, heart rate, and ECG were monitored and the test was terminated when the subject could no longer continue. V_{O_2} was measured continuously and directly using a customized metabolic system (Marquette Respiratory Mass Spectrometer, Rayfield Mixing Chamber, VMM Ventilatory Turbine) throughout the test and standard criteria were used to determine if a true $V_{O_{2max}}$ had been achieved (no further increase in oxygen uptake with increased work rate [$<$150 mL/min], exceeding age-predicted maximal heart rate, or achieving a respiratory exchange ratio of greater than 1.15).[37] $V_{O_{2max}}$ was measured to derive valid exercise prescriptions for the exercise training intervention and to quantify generalized CV training adaptations.

Genotyping

High-molecular-weight DNA was isolated from peripheral monocytes[38] and genotyped for a C to T transition leading to a threonine to isoleucine substitution at codon 111 of the LIPG protein (T111I) by the fluorescence polarization protocol of Chen et al.[39]

Exercise Training

Subjects underwent 24 weeks of supervised endurance exercise training consisting of 3 sessions per week. Heart rate monitors were used to assess training intensity and ensure that subjects trained at a heart rate corresponding to the appropriate intensity (% of $V_{O_{2max}}$). Training began with 20 minutes at 50% $V_{O_{2max}}$ and progressed to 70% $V_{O_{2max}}$ for 40 minutes where it remained for the final 14 weeks. Subjects added a lower-intensity 45- to 60-minute exercise session during weeks 12 to 24. Inclusion in the final analyses required subjects to have completed $\geq$75% of training sessions. To ensure dietary compliance, subjects completed diet records periodically.

Final Testing

After completing exercise training, all subjects completed the same tests as at baseline. All blood samples were drawn 24 to 36 hours after a usual exercise training session.

Statistics

Data are presented as mean $\pm$ SE. Initial subject characteristics were compared between the LIPG CC and CT/TT genotype groups using independent samples *t* tests. Gender, HRT status, and ethnicity frequency differences and Hardy-Weinberg equilibrium were analyzed using chi-square tests. Analysis of covariance (ANCOVA) was used to compare baseline plasma lipoprotein-lipids between genotype groups with gender, HRT status, and ethnicity as covariates. ANCOVA was used to compare the changes with exercise training between genotype groups with gender, HRT status, ethnicity, and baseline value of the outcome variable as covariates. Paired *t* tests analyzed whole group and within genotype group changes in outcome measures with exercise

Table 1. LIPG Gene Polymorphism Allele and Genotype Frequencies

	Allele Frequency		Genotype Frequency		
Thr111Ile in Exon 3	C (Thr)	T (Ile)	CC	CT	TT
Total study population	0.74	0.26	0.53 (44)	0.42 (35)	0.05 (4)

NOTE. Numbers in parentheses are actual frequencies.

training. Sample size varied according to outcome measure as complete data was not available for all subjects. The sample size for the entire group was 80 to 83 for conventional plasma lipoprotein-lipid measures and 71 for NMR plasma lipoprotein-lipid assessments. LIPG CC genotype group sample size was 42 to 44 for conventional lipids and 35 for NMR lipids; LIPG CT/TT genotype group sample size was 38 to 39 for conventional lipids and 36 for NMR lipids. Transformations were performed on baseline Vo_{2max} (L/min), HDL_3-C, and HDL_{3NMR}-C, and HDL_2-C change with training values due to variance inequalities. Statistical significance was set at $P \leq .05$. Statistical procedures were analyzed on SPSS 10.0 software (SPSS, Inc, Chicago, IL).

With recent evidence suggesting that the current methods available for adjusting family-wise alpha rate in gene association studies involving multiple phenotypes do not take into account the correlation among variables or allows only conclusions about multivariate composites of variables rather than specific variables, no adjustment of the *P* value per test was made.[40] Some protection against the relevance of a family-wise alpha rate error issue is provided by the fact that the study hypothesis was based a priori on specific functional relationships suggested between LIPG and HDL-C metabolism.[1-9,41]

RESULTS

LIPG allele and genotype frequencies did not differ significantly from Hardy-Weinberg expectations (Table 1) and were similar to those reported recently.[6] Due to the small number of rare allele homozygotes (TT, n = 4), the CT and TT genotype groups were combined as T allele carriers for statistical analyses. There were more women than men in the CT/TT genotype group (Table 2). The percentage of women on HRT and the proportions of white/non-white participants were similar in the LIPG genotype groups.

Initial age, body weight, total body fat, visceral adipose tissue and subcutaneous adipose tissue, and Vo_{2max} did not differ between LIPG genotype groups. Initial total cholesterol, LDL-C, and TG levels also were not different between LIPG genotype groups (Table 3). However, the CT/TT genotype group had 30% and 27% lower levels of HDL_{2NMR}-C ($P = .002$) and integrated $HDL_{1,2NMR}$-C ($P = .002$) subfractions, respectively, and 24% higher levels of integrated $HDL_{3,4,5NMR}$-C ($P = .06$) than the CC genotype group. HDL_{3NMR}-C levels were higher in the CT/TT group than in the CC genotype group, but this difference did not reach statistical significance. The CT/TT group also had slightly higher levels than the CC group for a number of other cardioprotective lipoprotein-lipid levels, including HDL-C, HDL_2-C, and HDL_{4NMR}-C, though the differences were not statistically significant.

Table 2. Subject Characteristics at Baseline and Their Changes With Exercise Training

		LIPG Genotype Group	
Characteristics	Total (n = 78-83)	CC (n = 41-44)	CT/TT (n = 37-39)
Age (yr)	57.9 ± 0.6	57.5 ± 0.8	58.4 ± 0.9
Female/male	49/34	21/23	28/11†
% females on HRT	49% (n = 24)	43% (n = 9)	54% (n = 15)
White/non-white	66/17	35/9	31/8
Weight (kg)			
Initial	80.6 ± 1.6	83.1 ± 2.1	77.9 ± 2.5
Change with training	−1.1 ± 0.3*	−1.1 ± 0.4*	−1.2 ± 0.3*
Body fat (%)			
Initial	36.0 ± 1.1	34.5 ± 1.3	37.6 ± 1.6
Change with training	−1.2 ± 0.2*	−1.2 ± 0.3*	−1.2 ± 0.3*
VAT (cm^2)			
Initial	127.8 ± 4.5	130.1 ± 5.8	125.4 ± 7.1
Change with training	−14.4 ± 2.4*	−13.2 ± 3.9*	−15.6 ± 2.8*
SAT (cm^2)			
Initial	308.2 ± 13.6	297.8 ± 17.8	320.0 ± 21.1
Change with training	−0.1 ± 4.1	0.4 ± 5.4	0.3 ± 6.4
Vo_{2max} (L/min)			
Initial	2.0 ± 0.1	2.1 ± 0.1	1.9 ± 0.1
Change with training	0.3 ± 0.02*	0.3 ± 0.03*	0.3 ± 0.02*
Vo_{2max} (mL/kg/min)			
Initial	25.2 ± 0.5	25.7 ± 0.7	24.6 ± 0.8
Change with training	3.8 ± 0.3*	3.6 ± 0.5*	4.1 ± 0.4*

NOTE. Values are expressed as means ± SE except for frequency data.

*Indicates significant change within group after training, $P < .01$.

†$P = .03$ for χ^2 testing differences between genotype groups.

Abbreviations: VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

Table 3. Plasma Lipoprotein-Lipid Levels at Baseline and Their Changes With Exercise Training

Characteristics	Total	Genotype Group		
		CC	CT/TT	P†
Total cholesterol				
Initial	207.7 ± 3.7	207.6 ± 5.2	207.8 ± 5.5	.97
Change with training	−0.2 ± 2.4	−1.3 ± 3.2	0.9 ± 3.6	.99
LDL-C				
Initial	128.9 ± 3.2	128.0 ± 4.5	129.9 ± 4.8	.78
Change with training	1.0 ± 2.3	2.3 ± 3.1	−0.4 ± 3.4	.43
Triglycerides				
Initial	160.2 ± 9.2	166.7 ± 12.8	152.6 ± 13.8	.47
Change with training	−18.4 ± 5.9*	−27.8 ± 9.7*	−7.8 ± 5.9	.16
HDL-C				
Initial	46.2 ± 1.4	45.3 ± 1.8	47.1 ± 1.9	.52
Change with training	3.2 ± 0.6*	4.4 ± 0.8*	1.9 ± 0.9*	.04
HDL_2				
Initial	4.3 ± 0.7	3.4 ± 0.9	5.1 ± 0.9	.18
Change with training	0.4 ± 0.4	1.0 ± 0.3*	−0.3 ± 0.8	.18
HDL_3				
Initial	42.0 ± 0.9	41.0 ± 1.1	42.2 ± 1.2	.66
Change with training	3.0 ± 0.5*	3.8 ± 0.7*	2.2 ± 0.6*	.07
HDL_{5NMR}				
Initial	5.9 ± 0.9	5.8 ± 1.3	6.0 ± 1.2	.93
Change with training	1.3 ± 0.5*	2.2 ± 0.8*	0.5 ± 0.6	.08
HDL_{4NMR}				
Initial	11.0 ± 0.8	10.2 ± 1.1	11.8 ± 1.1	.33
Change with training	2.4 ± 0.5*	2.7 ± 0.7*	2.1 ± 0.8*	.47
HDL_{3NMR}				
Initial	11.6 ± 1.2	9.2 ± 1.5	14.0 ± 1.4	.30
Change with training	−1.7 ± 1.0	−0.3 ± 1.2	−3.1 ± 1.6	.45
HDL_{2NMR}				
Initial	14.0 ± 0.8	16.5 ± 1.1	11.6 ± 1.0	.002
Change with training	0.7 ± 0.7	−0.4 ± 0.9	1.8 ± 1.0	.78
HDL_{1NMR}				
Initial	1.6 ± 0.3	1.6 ± 0.4	1.5 ± 0.4	1.0
Change with training	−0.1 ± 0.3	0.4 ± 0.5	−.04 ± 0.2	.39
$HDL_{3,4,5NMR}$				
Initial	28.4 ± 1.9	25.3 ± 2.3	31.4 ± 2.2	.06
Change with training	2.2 ± 1.1*	4.6 ± 1.4*	−0.1 ± 1.7	.19
$HDL_{1,2NMR}$				
Initial	15.6 ± 0.9	18.1 ± 1.1	13.2 ± 1.0	.002
Change with training	0.8 ± 0.6	−0.2 ± 0.8	1.8 ± 0.9	.78
HDL size				
Initial	8.8 ± .04	8.7 ± 0.7	8.8 ± 0.06	.24
Change with training	0.09 ± .03*	0.3 ± .05*	.03 ± .03	.16

NOTE. Values are expressed as means ± SE in units of mg/dL.

*Indicates significant change within group after training, $P < .05$.

†Probability for difference between the LIPG genotype groups using gender, HRT status, and ethnicity as covariates for initial values and gender, HRT status, ethnicity, and baseline value as covariates for change with training. Total group sample size ranged from 80 to 83 for conventional lipids and is 71 for NMR lipids. CC genotype group sample size ranged from 42 to 44 for conventional lipids and is 35 for all NMR lipids. CT/TT genotype group sample size ranged from 38 to 39 for conventional lipids and is 36 for all NMR lipids.

With exercise training, body weight, total body fat, and visceral adipose tissue decreased similarly in both LIPG genotype groups (Table 2). Vo_{2max} increased similarly in both genotype groups (14% increase in the CC, 16% in the CT/TT genotype groups), which was a statistically significant change within each group; however, the change in Vo_{2max} with training did not differ significantly between the LIPG genotype groups (Table 2). There were no significant changes in plasma total cholesterol or LDL-C levels with training. TG levels decreased by 11% ($P < .05$) with training, but no significant differences existed in responses between genotype groups. Plasma HDL-C levels increased with training in both genotype groups ($P < .05$), but the increase in the CC group was twice that in the CT/TT group ($P = .04$). HDL_3-C levels increased significantly with training in both genotype groups ($P < .05$), with the change being almost 2 times greater in the CC group than CT/TT group, though this difference only approached statistical significance ($P = .07$). HDL_{5NMR}-C levels increased

significantly with training in the CC group ($P < .05$), but did not change significantly in the CT/TT group; however, this difference in change with training between the groups only approached statistical significance ($P = .08$). HDL_{2NMR}-C changes with training were not significantly different within or between groups. The training-induced changes in integrated $HDL_{3,4,5NMR}$-C levels were substantial in the CC genotype group ($P < .05$), while they did not change whatsoever in the CT/TT group; however, the difference between groups did not achieve statistical significance. Changes in integrated $HDL_{1,2NMR}$-C with training were not significantly different between groups. Results were similar for NMR determined plasma lipoprotein-lipid changes with exercise training when using ANCOVA with only gender, HRT status, and ethnicity as covariates. However, the integrated $HDL_{3,4,5NMR}$-C change with training approached significance in this analysis ($P = .06$ in the 3-covariate model *v* $P = .19$ in the 4-covariate model).

DISCUSSION

Plasma HDL-C levels are an independent risk factor for CVD.[16-18] However, plasma HDL-C levels are highly variable, modifiable, and influenced strongly by genetics, environmental factors, and the interaction of genetic and environmental factors.[12-17,19,20,23,42-45] While total plasma HDL-C levels are inversely related to CVD risk, HDL-C subfractions may provide an even better assessment of CVD risk.[20-22] Thus, understanding the genetic and environmental factors involved in determining HDL-C subfraction levels is critical to reducing CVD risk and improving public health.[16,17] Contributing to this understanding, we found an association of the LIPG gene Thr111Ile polymorphism with initial levels of HDL_{2NMR}-C and $HDL_{1,2NMR}$-C and a trend toward association with initial levels of $HDL_{3,4,5NMR}$-C. With exercise training, we found associations of this polymorphism with changes in HDL-C and trends toward associations with changes in HDL_3-C and HDL_{5NMR}-C. All of these data suggest that the LIPG gene may affect HDL-C subfraction levels.

The lipase gene family has several members that affect HDL-C levels through the metabolism of TG and phospholipids, including the enzyme products HL and LPL.[1-3,7-11,18] The LIPG gene is a recently discovered member of this gene family that is thought to function mainly as a phospholipase influencing HDL-C metabolism.[1-3,6,8,9] While the enzymatic mechanism of action and impact on composition and level of HDL particles is not yet understood, EL is unique in that it is synthesized by endothelial cells.[1,3,6,8,9] EL has structural and functional similarity to LPL and HL, providing strong preliminary evidence for a role in HDL-C metabolism.[1-3,6,8,9] Substantiating its effect on HDL-C metabolism, Jaye et al found that overexpression of EL in mice reduced plasma HDL-C levels.[1] Choi et al found that mice overexpressing EL reduced HDL-C levels while mice deficient in EL increased HDL-C levels.[7]

Common polymorphisms at the LPL and HL gene loci have previously been shown to influence plasma HDL-C levels.[10,11] Recently, deLemos et al[6] identified 17 variants at the LIPG locus, of which 6 were thought to be functional. Of those, the most common was the exon 3 Thr111Ile polymorphism.[6] While deLomas[6] did not find an association between this variant and HDL-C levels in individuals with high HDL-C levels, they did not assess the influence of this polymorphism on HDL-C subfractions or in individuals with HDL-C levels within the normal range.

After controlling for a number of covariates, we found important differences in initial levels of HDL-C subfractions between LIPG genotype groups. The CT/TT group had 30% and 27% lower initial levels of HDL_{2NMR}-C and integrated $HDL_{1,2NMR}$-C than the CC group, respectively. The CT/TT group also had 24% higher integrated $HDL_{3,4,5NMR}$-C levels than the CC group. Since the 2 smallest HDL subclasses (HDL_{1NMR}-C and HDL_{2NMR}-C) show a positive association and the 3 largest subclasses (HDL_{3NMR}-C, HDL_{4NMR}-C, and HDL_{5NMR}-C) show an inverse correlation with CHD incidence and severity, the association of the Thr111Ile polymorphism with differences in these subfractions may confer a more cardioprotective lipoprotein profile on the CT/TT compared to the CC group.[21,33,34]

Prolonged endurance exercise training is known to raise HDL-C and HDL_2-C levels.[12-17,19,20,45] However, this response is highly variable among individuals.[15,20,23,45] We found that the LIPG CC genotype group generally increased levels of the more cardioprotective HDL-C subfractions and showed no change in the more atherogenic subfractions with training.[21,33,34] In contrast, the CC/TT group tended to show less improvement with exercise training in cardioprotective subfractions than the CC group and actually showed no change in levels of the cardioprotective $HDL_{3,4,5NMR}$-C. The CT/TT group also tended to increase the more atherogenic HDL_{2NMR}-C with training. Thus, in contrast to having a generally more cardioprotective initial lipoprotein lipid profile in the sedentary state, the CT/TT group appeared to have less positive changes in lipoprotein lipid profiles than the CC group with exercise training. Thus, it would appear that the rare allele generally has a cardioprotective effect in the sedentary state but its effect is ameliorated when individuals become exercise-trained. Alternatively, it is possible that the people with the worse lipoprotein profile at baseline did better with training, and those with more normal levels do not change. However, some studies have shown that this regression to the mean may not apply in this situation, as those with the lowest HDL-C levels have been found to have the greatest increase in HDL-C with exercise training.[20]

The changes in plasma HDL-C and its subfractions with training were also analyzed by ANCOVA using only gender, HRT status, and ethnicity as covariates. The results of these analyses were very similar except in the case of the integrated $HDL_{3,4,5NMR}$-C change with exercise training which approached significance in this analysis. This suggests that HDL-C and HDL-C subfraction changes with exercise training were independent of baseline values and that LIPG genotype was associated with HDL-C subfraction changes with training even after accounting for its effect on baseline lipids.

Major strengths of this study include dietary control, a highly standardized prolonged exercise training, and NMR analysis of HDL-C subfractions. Removing the influence of different diets allowed for improved detection of associations between genotype differences and initial plasma li-

poprotein lipid levels. The standardized exercise training and more precise HDL-C measures, along with the control of diet and initial physical activity level, also allowed for better detection of associations without existing diet and exercise confounding the results.

In summary, the present findings provide further evidence for the association of LIPG genotypes with HDL metabolism and for a significant effect of a common LIPG allelic variant on initial levels and degree of change in HDL-C and HDL-C subfractions with exercise training.

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