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Interactive effects of *APOE* haplotype, sex, and exercise on postheparin plasma lipase activities

Richard L. Seip,[1] Robert F. Zoeller,[2] Theodore J. Angelopoulos,[3] James Salonia,[1] Cherie Bilbie,[1] Niall M. Moyna,[4] Mary P. Miles,[5] Paul S. Visich,[6] Linda S. Pescatello,[7] Paul M. Gordon,[8] Gregory J. Tsongalis,[9] Linda Bausserman,[10] and Paul D. Thompson[1]

[1]*Preventive Cardiology Program, Hartford Hospital, Hartford, Connecticut;* [2]*Florida Atlantic University, Boca Raton;* [3]*University of Central Florida, Orlando, Florida;* [4]*Dublin City University, Dublin, Ireland;* [5]*Montana State University, Bozeman, Montana;* [6]*Central Michigan University, Mt. Pleasant, Michigan;* [7]*University of Connecticut, Storrs, Connecticut;* [8]*University of Michigan, Ann Arbor, Michigan;* [9]*Dartmouth Medical School, Dartmouth Hitchcock Medical Center, Lebanon, New Hampshire; and* [10]*Lifespan Health System, Brown University, Providence, Rhode Island*

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Seip RL, Zoeller RF, Angelopoulos TJ, Salonia J, Bilbie C, Moyna NM, Miles MP, Visich PS, Pescatello LS, Gordon PM, Tsongalis GJ, Bausserman L, Thompson PD. Interactive effects of APOE haplotype, sex, and exercise on postheparin plasma lipase activities. *J Appl Physiol* 110: 1021–1028, 2011. First published February 3, 2011; doi:10.1152/japplphysiol.00287.2010.—Hepatic lipase (HL) and lipoprotein lipase (LPL) activities (HLA, LPLA) modify lipoproteins and facilitate their binding to hepatic receptors. Apolipoprotein E (APOE) physically interacts with the lipases, and the three common haplotypes of the *APOE* gene (ε2, ε3, and ε4) yield protein isoforms (E2, E3, and E4, respectively) that are functionally different. Lipase activities themselves differ by sex and exercise training status. The interaction of *APOE* genotype, exercise training, and sex effects on lipase activities has not been studied. We measured postheparin plasma lipase activities in normolipidemic men and women with the three most common *APOE* genotypes, which are the haplotype combinations ε2/ε3 ($n = 53$), ε3/ε3 ($n = 62$), and ε4/ε3 ($n = 52$), enrolled in 6 mo of aerobic exercise training. These haplotype combinations comprise an estimated 11.6, 62.3, and 21.3% of the population, respectively. Baseline HLA was 35% lower in women than in men ($P < 0.0001$). In men but not women, HLA was higher in ε2/ε3 group compared with ε4/ε3 ($P = 0.01$) and ε3/ε3 ($P = 0.05$). Neither sex nor APOE genotype affected baseline LPLA. Training decreased HLA by 5.2% ($P = 0.018$) with no *APOE* effect. The apparent increase in LPLA following exercise was significant and *APOE* dependent only when corrected for baseline insulin ($P < 0.05$). Exercise decreased LPLA by 0.8 μmol free fatty acid (FFA)·ml^{-1}·h^{-1} (−6%) in ε3/ε3 compared with the combined increases of 6.6% in ε2/ε3 and 12% in ε4/ε3 ($P = 0.018$ vs. ε3/ε3). However, these differences were statistically significant only after correcting for baseline insulin. We conclude that common *APOE* genotypes interact with *1*) sex to modulate HLA regardless of training status, with ε2/ε3 men demonstrating higher HLA than ε3/ε3 or ε4/ε3 men, and *2*) aerobic training to modulate LPLA, regardless of sex, with ε3/ε3 subjects showing a significant decrease compared with an increase in ε2/ε3 and ε3/ε4 after controlling for baseline insulin.

lipoprotein lipase; hepatic lipase; lipids; aerobic capacity; insulin

HEPATIC LIPASE (HL), lipoprotein lipase (LPL), and apolipoprotein E (APOE) participate in the transport, modification, and clearance of lipoproteins, and each has been studied in connection with atherosclerosis. Elevated LPL activity (LPLA) suggests a lower risk for atherosclerotic disease (56), whereas the relationship of HL activity (HLA) to cardiovascular disease risk is less clear (46, 59) and may depend on preexisting lipoprotein concentrations (59). *APOE* genotypes (which are combinations of 2 of the 3 haplotypes) alter the plasma lipid profile (2) and the risk for cardiovascular disease (2, 17, 30, 40). In the present study, we examined the relationships among the lipase activities, *APOE* genotype variants, and exercise in an attempt to further understand how *APOE* genotype might influence the response in lipase activities to chronic exercise.

Circulating APOE is found in the chylomicron, very-low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) fractions of the plasma (35). It serves as a ligand to direct triglyceride-rich lipoprotein lipids to the liver (35) and affects LPLA (38) and HLA (55). The lipolysis of VLDL remnants by HLA converts them to low-density lipoproteins (LDL) (4, 16) and facilitates the transfer of APOE from VLDL to HDL particles (18, 45). APOE also binds to the lipases and proteoglycans found on vessel walls and hepatic sinusoidal spaces (59). In the sinusoidal spaces, it enhances the "docking" of HDL particles for delivery of lipids to the liver (46). In the circulation, APOE enhances the enlargement of HDL particles (30).

Three common haplotype variants of *APOE*, ε2, ε3, and ε4, produce structurally different protein products (3), each with characteristic effects on lipid transport. Postprandial lipemia is greater in carriers of the ε2 haplotype (3) due to the decreased affinity of its product, apolipoprotein E2, for the B(E) receptor (58). The ε4 haplotype product has greater affinity for hepatic LDL receptors, which increases hepatic cholesterol, causing downregulation of the receptors and increased LDL cholesterol(35). Apolipoprotein E2 impedes the in vivo conversion of VLDL to LDL (16), due at least in part to slower hydrolysis of VLDL caused by less stimulation of HLA (55). The metabolic fates of VLDL subpopulations and the origins and rates of formation of large, medium, and small LDL subpopulations differ according to *APOE* genotype (4, 11), effects likely mediated mostly through the apolipoprotein E's effect on HL function and activity. We have shown that *APOE* genotype affects exercise-associated changes in LDL subclasses (47) and have argued that structural differences in apolipoproteins E2, E3, and E4 can account for the different training responses in LDL subclasses (47).

Hepatic lipase activity is lower in women compared with men due to differences in testosterone and estrogen (37). When HLA is low, HDL cholesterol is increased (19, 57). Low HLA

Address for reprint requests and other correspondence: R. L. Seip, Preventive Cardiology-JB704, PO Box 5037, Hartford Hospital, 80 Seymour St., Hartford, CT 06102-5037 (e-mail: rseip@harthosp.org).

and high LPLA, as measured in postheparin plasma (PHP), generally associate with low triglycerides, high HDL cholesterol, and decreased small LDL, a lipid profile that predicts low risk for cardiovascular disease.

Given that *1*) chronic aerobic exercise of vigorous intensity and long duration/high frequency may potentially improve lipid profiles, increase PHP LPLA (48, 54), and decrease PHP HLA (15, 54); *2*) APOE interacts with both lipases (16, 38, 55); and *3*) sex markedly affects HLA (37), we tested the effect of common *APOE* genotype variants on lipase activities at baseline and in response to 6 mo of moderate to vigorous intensity exercise training performed for 40 min, 4 days per week.

METHODS

Study overview. Seven geographic sites identified 566 individuals for *APOE* genotyping to identify ~60 subjects, each with the three most common genotypes ε2/ε3, ε3/ε3, and ε4/ε3. These haplotype combinations comprise an estimated 11.6, 62.3, and 21.3% of the population, respectively (2). *APOE* genotype analysis (described below) was performed by the Hartford Hospital clinical laboratory. One hundred seventy-four subjects ($n = 57$ ε2/ε3, $n = 60$ ε3/ε3, and $n = 57$ ε4/ε3) initiated and 120 completed 6 mo of aerobic exercise training. *APOE* genotypes were evenly distributed among the sites by the coordinating center (Hartford Hospital) to prevent subtle differences in the training regimens among sites from affecting the results.

Subjects. The subjects were healthy nonsmokers without orthopedic problems who were physically inactive for 6 mo before the study (i.e., vigorous activity $< 4\times$/mo). They ranged in age from 18 to 70 yr. Subjects were excluded if they consumed more than two alcoholic beverages daily or if their body mass index (BMI) exceeded 30. Before the study was initiated, participants were informed of all procedures and associated risks and signed an informed consent in accordance with each site's institutional review board for human subject experimentation.

APOE genotype determination. DNA was extracted from leukocytes and *APOE* variants determined using standard techniques (43).

Serum lipid, lipoprotein, and postheparin LPLA and HLA measurements. Collection of blood samples for the determination of serum lipoprotein lipids and postheparin lipase activities were performed after an overnight (12 h) fast in 167 of 174 subjects before training and in 115 of 120 subjects who completed exercise training. Of the 174 recruited, a few simply were missed, whereas others elected to not undergo this test. One subject in the ε4/ε3 group was eliminated because baseline LPLA was >5 SD above the overall mean, leaving 166 subjects.

The 24-h posttraining blood samples were collected the morning after the penultimate exercise session. In the posttraining condition, exercise was performed either the morning or afternoon of the previous day. For both pre- and posttraining lipase activity determinations, and after collection of blood samples for determination of serum lipids, heparin sodium (75 IU/kg body wt) was injected intravenously and a blood sample was obtained 10 min later. Serum and plasma were separated by centrifugation and frozen at −70°C until analyzed. Lipid levels in women before and after training were obtained within 10 days of the onset of menses to avoid variations in lipoprotein values (9).

Total cholesterol, triglycerides (TGs), HDL-cholesterol (HDL-C), the HDL_2 and HDL_3 subfractions, apolipoproteins A1 and B, and PHP LPLA and HLA were determined by the Lipid Research Laboratory (Lifespan Health System, Brown University, Providence, RI) using standard methods (54). All samples from an individual subject were analyzed in the same analysis run at the end of the study to minimize the effect of laboratory variation. Coefficients of variation for lipid measurements in this laboratory were as follows: total cholesterol, 0.8%; TGs, 1.5%; HDL-C, 2.0%; HDL_3 subfraction, 6.3%; apolipoprotein A1, 2.6%; apolipoprotein B, 1.8%; PHP LPLA, 14%; and PHP HLA, 12%.

Insulin and glucose measurements. Glucose was analyzed using an automated glucose analyzer (YSI, Yellow Springs, OH). Insulin was analyzed via radioimmunoassay (Cognoscenti Health Institute, Orlando FL).

Anthropometric measurements. Body weight and height were measured using balance beam scales and wall-mounted tape measures, respectively. Skinfold thicknesses were measured on the right side of the body with the use of calipers to estimate %body fat in men (27) and women (28).

Maximal exercise capacity. Maximal oxygen uptake was measured as the average of the two highest consecutive 30-s values for oxygen uptake at peak exercise during a modified Astrand protocol (42). Blood pressure, 12-lead ECG, oxygen consumption ($\dot{V}O_2$), CO_2, and minute ventilation were also measured.

Aerobic exercise program. Exercise consisted of 6 mo of progressive, supervised exercise. Subjects performed 3 bouts/wk during *month 1* and 4 bouts/wk for *months 2–6*. Bout duration increased from 15 to 40 min during the first 4 wk and was maintained at 40 min/session for *months 2–6*. Intensity was 60–85% of maximal heart rate (HR_{max}) based on pretraining HR maximum.

Exercise energy expenditure. Exercise energy expenditure was estimated from the HR recorded during exercise sessions of that week using individual plots of $\dot{V}O_2$ vs. HR created from pretraining maximal exercise test data. We estimated the $\dot{V}O_2$ corresponding to the training exercise HR intensity and multiplied that $\dot{V}O_2$ by bout duration to obtain total oxygen consumption for each bout. Each liter of oxygen was assumed to represent 5 kcal of energy expenditure.

Statistical analysis. Analysis of variance (ANOVA) or analysis of covariance (ANCOVA) (SPSS version 11.5 GLM procedure) were used to compare *APOE* groups for differences at baseline and responses to exercise training. Covariates used in ANCOVA were selected on the basis of significant correlations and partial correlations ($P < 0.05$) with the dependent variables. Sidak post hoc tests were used to test for differences between *APOE* genotype groups when *F* ratios were significant. In the case of the PHP LPLA changes with exercise, the Helmert contrast post hoc test was used to compare the wild-type (ε3/ε3) group with the combined nonhomozygous subjects.

RESULTS

Baseline measurements. Numbers of men ($n = 82$) and women ($n = 84$) at baseline were practically equal, and *APOE* genotype distribution was equivalent across sex with overall differences in height, weight, waist-to-hip ratio, %body fat, and maximal aerobic capacity in men vs. women (Table 1). By self-report, 151/177 (85%) of the subjects assessed at baseline were of European, 9/177 (5.1%) African, and 9/177 (5.1%)

Table 1. *Subject characteristics at baseline*

	Men	Women	*P* Value
No. of subjects with APOE ε2/ε3, ε3/ε3, ε4/ε3 genotypes	24/32/26	29/27/28	
Age, yr	35.3 ± 10.7	38.2 ± 11.3	0.09
Height, cm	177.2 ± 7.5	164.3 ± 6.5*	<0.01
Weight, kg	88.8 ± 15.5	72.8 ± 13.9*	<0.01
BMI	28.3 ± 4.6	27.0 ± 4.7	0.07
WHR	0.89 ± 6.9	0.77 ± 0.05*	<0.01
%BF	20.8 ± 6.2	24.3 ± 6.3*	<0.01
$\dot{V}O_{2max}$, ml $O_2 \cdot kg^{-1} \cdot min^{-1}$	36.2 ± 7.1	27.8 ± 6.4*	<0.01

Data are means ± SD for $n = 82$ men and 84 women. APOE, apolipoprotein E; BMI, body mass index; WHR, waist-to-hip ratio; %BF, %body fat; $\dot{V}O_{2max}$, maximum oxygen consumption. *$P < 0.01$ indicates a significant difference between sexes.

Table 2. *Lipids, glucose, and insulin at baseline*

	Men	Women	*P* Value
TC, mg/dl	200.5 ± 39.5	200.0 ± 36.5	0.92
TG, mg/dl	151.6 ± 107.9	111.7 ± 59.2*	<0.01
LDL-C, mg/dl	128.2 ± 34.6	125.4 ± 33.3	0.59
HDL-C, mg/dl	42.0 ± 8.7	52.6 ± 14.1*	<0.01
HDL_2-C, mg/dl	8.2 ± 5.5	14.9 ± 9.7*	<0.01
HDL_3-C, mg/dl	34.9 ± 6.9	37.5 ± 7.6	0.02
Glucose, mg/dl	82.6 ± 6.5	80.0 ± 7.9*	0.04
Insulin, μU/ml	7.5 ± 9.9	5.7 ± 3.1	0.12
ApoB, mg/dl	122.7 ± 30.8	113.2 ± 28.3*	0.04
ApoA1, mg/dl	142.4 ± 22.1	162.4 ± 34.0*	<0.01

Data are means ± SD for 82 men and 84 women. TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HDL_2-C and HDL_3-C, HDL_2 and HDL_3 cholesterol subfractions; ApoB and ApoA1, apolipoproteins B and A1. *$P < 0.01$ indicates significant difference between sexes.

Asian ethnogeographic origin. The remainder were of other or unknown ethnogeographic origin.

Fasting serum lipid concentrations reflected normolipidemic profiles for men and women that differed in HDL-C concentrations (Table 2). *APOE* genotype groups were otherwise not different in age, BMI, %body fat, waist-to-hip ratio, and maximal aerobic capacity as indicated by $\dot{V}O_{2max}$ (Table 3). We previously reported an *APOE* genotype effect on changes in LDL-C and HDL-C in a subset of 120 subjects (53). In the present cohort of 166 subjects at baseline, there were trends toward decreased LDL-C in *APOE* ε2/ε3 subjects and decreased HDL-C in ε4/ε3 subjects (see Table 4). When statistically corrected for effects of age and BMI, or for age, BMI, and insulin level, the adjusted mean LDL-C was significantly lower in ε2/ε3 compared with ε3/ε3 and ε4/ε3 subjects (data not shown).

Posttraining measurements. The numbers of men ($n = 53$) and women ($n = 62$) who finished the study with complete lipid and lipase data were approximately equal. *APOE* genotype and sex distribution were also essentially equal [*APOE* ε2/ε3, $n = 38$ (17 male/21 female); *APOE* ε3/ε3, $n = 42$ (21 male/21 female); *APOE* ε3/ε4: $n = 35$ (15 male/20 female)]. Baseline and posttraining values for selected variables are presented by genotype in Table 5.

Training protocol. After the initial progression phase, which lasted ~4 wk, subjects exercised at 75.8 ± 6.1% HR_{max} and expended an estimated 1,250.0 ± 553.9 kcal/wk over the remaining 20 wk of the program. Adherence in those subjects who finished the study, expressed as a percentage of scheduled exercise sessions completed, was 92%.

The effects of *APOE* genotype on PHP lipase activities at baseline and on changes in lipase activities appear in Figs. 1 and 2. ANOVA revealed a significant *APOE* × sex interactive effect on baseline HLA ($P = 0.007$). The highest PHP HLA at baseline was observed in men with ε2/ε3 (26.86 ± 8.52 μmol $FFA \cdot ml^{-1} \cdot h^{-1}$), which was significantly different from that in men with ε3/ε3 (20.78 ± 7.94 μmol $FFA \cdot ml^{-1} \cdot h^{-1}$, $P = 0.007$) and ε4/ε3 (19.74 ± 7.67 μmol $FFA \cdot ml^{-1} \cdot h^{-1}$, $P = 0.002$) and from women with ε2/ε3 (13.60 ± 5.25 μmol $FFA \cdot ml^{-1} \cdot h^{-1}$, $P = 0.0001$). Men and women with ε3/ε3 genotypes also differed (20.78 ± 7.94 vs. 13.08 ± 5.68 μmol $FFA \cdot ml^{-1} \cdot h^{-1}$, respectively, $P = 0.0001$). There were no differences in baseline PHP LPLA due to *APOE* genotype (Fig. 1).

Table 3. *Lack of APOE genotype effect on baseline BMI, %BF, WHR, and $\dot{V}O_{2max}$*

Variable	*APOE* Genotype ε2/ε3	ε3/ε3	ε4/ε3	*APOE* Genotype Effect, *P* Value
n	53	59	54	
Age, yr	37.7 ± 10.8	37.7 ± 12.5	34.8 ± 9.6	0.28
BMI	28.0 ± 4.2	27.8 ± 4.8	27.6 ± 5.2	0.72
%BF	23.0 ± 5.6	22.5 ± 7.0	22.2 ± 6.7	0.82
WHR	0.827 ± 0.093	0.881 ± 0.069	0.827 ± 0.089	0.99
$\dot{V}O_{2max}$, ml $O_2 \cdot kg^{-1} \cdot min^{-1}$	30.9 ± 7.9	32.7 ± 8.1	32.2 ± 7.8	0.64

Data are means ± SD; *n* = no. of subjects with each genotype.

APOE genotype did not affect the exercise training induced decrease in PHP HLA (*F* ratio = 0.34, $P = 0.72$; see Fig. 2). Decreases were −0.47 ± 5.05, −0.94 ± 3.91, and −1.43 ± 3.54 μmol $FFA \cdot ml^{-1} \cdot h^{-1}$ for ε2/ε3, ε3/ε3, and ε4/ε3, respectively (Fig. 2). HLA in men decreased by 1.1–1.9 μmol $FFA \cdot ml^{-1} \cdot h^{-1}$ more than in women, but ANOVA revealed no sex effect (*F* ratio = 3.25, $P = 0.071$) or *APOE* × sex interaction [*F* ratio = 0.06, $P = 0.94$, not significant (NS)]. The covariates baseline HLA and baseline insulin did not alter these responses.

Exercise training changes in PHP LPLA were directionally different between ε3/ε3 (−0.53 ± 3.77) and ε2/ε3 (0.85 ± 3.54) or ε4/ε3 subjects (1.20 ± 5.29) but were not statistically different (ANOVA: *F* ratio = 1.84, $P = 0.163$, NS). Baseline insulin concentration was inversely correlated with the change in LPLA ($r = -0.40$, $P = 0.00002$) and with insulin as a covariate, ANCOVA revealed a significant *APOE* effect on PHP LPLA (*F* ratio = 3.08, $P < 0.05$). Adjusted for baseline insulin, mean changes in LPLA were −0.79 μmol $FFA \cdot ml^{-1} \cdot h^{-1}$ (−6.6%) in ε3/ε3, 0.74 μmol $FFA \cdot ml^{-1} \cdot h^{-1}$ (+6.0%) in ε2/ε3 ($P = 0.23$ vs. ε3/ε3, NS), and 1.34 μmol $FFA \cdot ml^{-1} \cdot h^{-1}$ (+12.0%) in ε4/ε3 ($P = 0.058$ vs. ε3/ε3, NS). With insulin in the model, the change in the wild-type ε3/ε3 group was significantly less than the combined mean of the non-wild-type groups ($P = 0.018$).

DISCUSSION

The present study examined the effect of *APOE* genotype on PHP lipase activities before and after exercise training in men

Table 4. *Lack of APOE genotype effect on baseline lipids, insulin, and glucose*

Variable	*APOE* Genotype ε2/ε3	ε3/ε3	ε4/ε3	*APOE* Genotype Effect, *P* Value
n	53	59	45	
TC, mg/dl	194.8 ± 38.2	207.2 ± 37.8	198.0 ± 37.4	0.19
TG, mg/dl	142.2 ± 103.3	128.8 ± 81.7	123.7 ± 81.1	0.53
LDL-C, mg/dl	118.3 ± 31.0	133.9 ± 32.7	129.9 ± 36.4	0.07
HDL-C, mg/dl	48.5 ± 13.0	48.5 ± 14.0	44.8 ± 11.3	0.11
HDL_2-C, mg/dl	12.2 ± 7.5	10.6 ± 7.8	10.2 ± 7.1	0.28
HDL_3-C, mg/dl	36.0 ± 6.7	36.3 ± 6.0	35.0 ± 7.5	0.58
Glucose, mg/dl	81.4 ± 7.3	81.6 ± 8.2	80.6 ± 6.5	0.80
Insulin, μU/ml	6.1 ± 3.1	5.8 ± 3.1	7.9 ± 11.9	0.87
HOMA	1.23 ± 0.71	1.22 ± 0.69	1.33 ± 0.99	0.68

Data are means ± SD; *n* = no. of subjects with each genotype. HOMA, homeostatic model assessment of insulin resistance.

Table 5. *APOE genotype effect on training response in selected variables for subjects who completed study*

Variable	*APOE* Genotype ε2/ε3 Baseline	ε2/ε3 Posttraining	ε3/ε3 Baseline	ε3/ε3 Posttraining	ε4/ε3 Baseline	ε4/ε3 Posttraining	*APOE* Genotype Effect, *P* Value
Weight, kg	81.7 ± 17.2	80.8 ± 16.2	80.6 ± 16.9	79.3 ± 16.3	81.3 ± 21.0	79.9 ± 19.9	0.89
BMI	28.0 ± 5.0	27.7 ± 4.5	27.2 ± 4.7	26.8 ± 4.4	27.7 ± 5.5	27.3 ± 5.3	0.87
WHR	0.83 ± 0.010	0.82 ± 0.07	0.84 ± 0.09	0.84 ± 0.09	0.84 ± 0.08	0.83 ± 0.08	0.42
%BF	23.3 ± 4.9	22.4 ± 5.0	22.2 ± 6.5	21.3 ± 5.8	22.8 ± 6.8	21.7 ± 6.6	0.83
$\dot{V}O_{2max}$, l/min	2.49 ± 0.86	2.79 ± 0.96	2.62 ± 0.75	2.70 ± 0.80	2.57 ± 0.76	2.91 ± 0.84	0.001*
TC, mg/dl	199.1 ± 38.6	196.2 ± 38.2	208.2 ± 37.3	206.3 ± 36.3	204.2 ± 37.0	205.0 ± 36.8	0.74
TG, mg/dl	142.1 ± 95.0	127.2 ± 79.5	144.1 ± 108.7	123.5 ± 78.3	128.1 ± 75.8	109.6 ± 59.4	0.86
LDL-C, mg/dl	120.7 ± 31.2	122.3 ± 30.0	129.9 ± 35.0	130.8 ± 29.0	132.6 ± 36.1	136.9 ± 35.3	0.70
HDL-C, mg/dl	49.9 ± 12.8	49.7 ± 11.6	49.4 ± 15.1	50.8 ± 14.3	46.0 ± 11.5	46.2 ± 10.3	0.45
Glucose, mg/dl	81.6 ± 6.2	80.9 ± 5.8	82.8 ± 7.1	80.2 ± 5.7	86.4 ± 10.3	83.1 ± 7.5	0.69
Insulin, μU/ml	6.1 ± 3.1	6.0 ± 3.6	5.9 ± 3.2	6.0 ± 3.2	9.0 ± 14.1	6.5 ± 4.7	0.18
HLA, μmol FFA·ml^{-1}·h^{-1}	19.6 ± 9.6	19.0 ± 8.8	16.4 ± 8.3	15.5 ± 6.8	18.7 ± 7.4	17.7 ± 8.2	0.66
LPLA, μmol FFA·ml^{-1}·h^{-1}	12.3 ± 3.1	13.1 ± 4.0	11.9 ± 3.9	11.4 ± 3.5	11.1 ± 3.8	12.4 ± 4.5	0.16

Data are means ± SD for $n = 38$ ε2/ε3, 42 ε3/ε3, and 35 ε4/ε3 subjects. APOE genotype effect is the change from pre- to posttraining. HLA, hepatic lipase activity; LPLA, lipoprotein lipase activity. *$P < 0.001$ indicates significant difference between ε3/ε3 and both ε4/ε3 and ε2/ε3.

and women. Among the more important findings was a physiologically small (5%) yet significant decrease in HLA with training, independent of *APOE*, and there was no significant change in LPLA in any of the three *APOE* genotypes. There was a significant interaction with a trend for a decrease in LPLA in the most common haplotype and an opposite trend for an increase in subjects with the other two less common haplotypes. We believe these findings to be particularly robust given the relatively large sample size and the prolonged and vigorous training protocol.

At baseline there was a sex-specific *APOE* genotype effect on HLA but no effect on LPLA. HLA was elevated by 30% in men with the ε2/ε3 genotype compared with the ε3/ε3 or ε4/ε3 genotype, but not in women. *APOE* genotype did not significantly affect the change in HLA or LPLA with exercise.

Training protocol and the changes in lipase activities. The exercise regime used for this study was of moderate to vigorous intensity but of relatively low caloric expenditure. Although the average kilocalories expended per week was at the low end of the range thought necessary to produce changes in

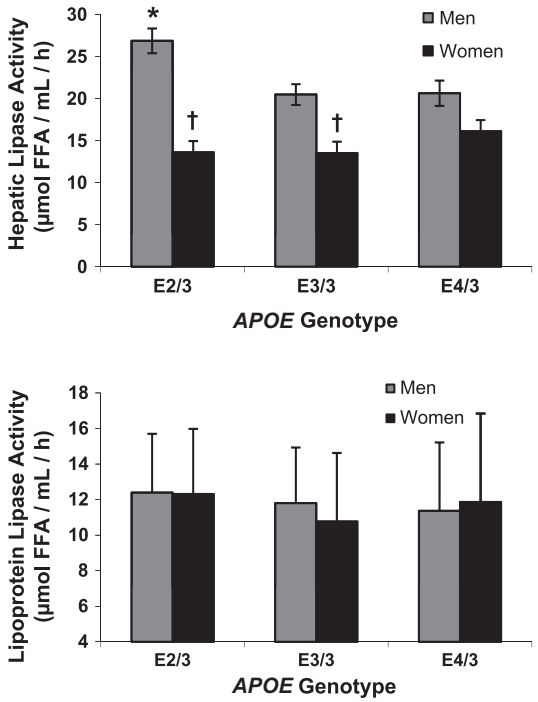

Fig. 1. Lipase activities before exercise training in apolipoprotein E (*APOE*) genotype groups. *Top*: baseline hepatic lipase activity (HLA) in men ($n = 82$) and women ($n = 84$). *$P < 0.05$, ε2/ε3 vs. ε3/ε3 or ε4/ε3 in men. †$P < 0.0001$, men vs. women. *Bottom*: baseline lipoprotein lipase activity (LPLA) in men and women. All data are means ± SD.

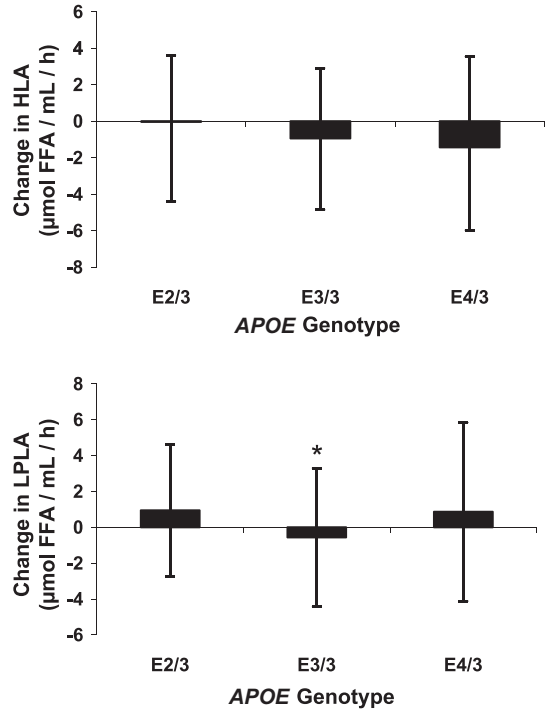

Fig. 2. Changes in lipase activities in response to exercise training. *Top*: change in HLA with exercise training in all subjects ($n = 53$ men and 62 women) who completed exercise training with complete lipase data pre- and posttraining. *Bottom*: change in LPLA with exercise training when controlling for baseline insulin. *$P = 0.018$ vs. ε2/ε3 and ε4/ε3 combined. All data are means ± SD.

lipid profiles and lipase activities (54), the high variability clearly indicates that a significant proportion of subjects were below the proposed threshold of 1,200 kcal/wk. This can be explained, at least in part, by the relatively low aerobic capacity of most of the participants. In other words, although most participants were exercising vigorously in relative terms, the absolute energy expenditure was in proportion to their lower aerobic fitness. Although this could be perceived as a limitation, it also represents a 6-mo exercise program of moderate to vigorous intensity that was well tolerated by the majority of subjects with a dropout rate of <30% and adherence of >90%.

APOE genotype and HLA. The main finding of the present study was a 30% elevation in baseline HLA in men carrying the *APOE* ε2 haplotype, compared with other men. Others have found sex-specific *APOE* genotype effects on lipid parameters. In the Turkish Heart Study ($n > 9{,}000$) (36), TGs were higher only in men with the ε2 haplotype. TGs in the present study were 30% higher (although not significantly) in men with ε2/ε3 compared with men lacking the ε2 haplotype. In *APOE* ε2 carriers, TGs are elevated due in part to poor recognition and uptake of TG-rich particles bearing the ε2 product (32). Hepatic lipase also participates in the uptake of TG-rich particles (21). HLA may be elevated in men with ε2 haplotype to compensate for decreased receptor-mediated uptake of apolipoprotein E2-containing lipoproteins.

In the Turkish study, HDL-C was elevated only in women with the ε2 haplotype (37). In contrast, women with the ε2 haplotype in the present study showed no elevation in HDL-C. In fact, HLA was generally higher in our group of women compared with the Turkish women (6). It is feasible that higher overall HLA in our women favored HDL-C uptake, preventing higher HDL-C levels in those with the ε2 haplotype.

We did not measure sex hormones and did not track use of oral contraceptives, which in hindsight is a limitation. However, 13 of the 62 women who completed the study reported that they were postmenopausal with nine reporting that they were on hormone-replacement therapy (HRT). Menopausal status did not demonstrate an association with baseline or change in HLA ($P > 0.05$). However, among postmenopausal women, those who were on HRT demonstrated significantly lower HLA at baseline (10.29 ± 3.93 vs. 19.11 ± 3.64 μmol FFA·ml^{-1}·h^{-1}, $P = 0.003$). There was no influence of HRT on the change in HLA. The small sample size did not permit for an examination of the influence of *APOE* genotype.

The mechanisms by which the *APOE* ε2/ε3 genotype leads to elevated HLA only in men might be posttranslational, linked to differences in physical behavior of the E2 and E3 proteins (37); genetic, attributable to other gene variants linked to the ε2 allele; or some combination of both. Although HLA was 35% lower overall in women compared with men in our study, other sex-specific parameters differed, including baseline TGs, apolipoproteins A1 and B, HDL-C and its subfractions, and glucose; as well as %body fat and $\dot{V}O_{2max}$ (see Tables 1 and 2). However, none demonstrated a sex × *APOE* genotype interactive pattern in parallel to the sex-specific elevation in HLA in men with ε2/ε3. Baseline LDL-C, HDL-C, and/or total cholesterol, already shown to differ according to *APOE* haplotype (53), also did not alter the *APOE* genotype effect when employed alone or in combination as statistical covariates. Thus the variability in these physiological parameters did not explain the increased HLA in ε2/ε3 men.

Although we did not measure sex hormones in the present study, differences in sex hormones may have a role in the high HLA in ε2 men. Testosterone is 30-fold higher in men compared with women, and estrogen is an order of magnitude higher in women. Testosterone inhibits LDL receptor expression (8) and has no effect on HL expression (41). Estrogen increases LDL receptor expression (1, 25) and decreases HL expression (41). When the hepatic cholesterol pool size is decreased (37) due to slow hepatic uptake of large TG-rich lipoproteins (37) as it is in *APOE* ε2 carriers, HLA (41) and LDL receptor expression increase (41). We hypothesize that men and women *APOE* ε2 carriers utilize different mechanisms to maintain hepatic cholesterol levels: testosterone-induced inhibition of the LDL receptor in ε2 men slows restoration of the hepatic cholesterol pool via that pathway, elevating HLA above its secondary role in lipoprotein uptake (12) to capture lipoprotein lipids. In contrast to men, women ε2 carriers would increase LDL receptor activity (25) to maintain hepatic cholesterol levels through B(E) receptor-mediated uptake.

APOE genotype modulation of the exercise effect on LPLA. Exercise training of sufficient volume and intensity increases LPLA (48, 54) and decreases HLA (14, 54). Increased muscle LPLA can be an important mechanism through which exercise decreases blood TGs (48) and increases HDL-C (10). In the present study, aerobic training estimated to require a mean exercise energy expenditure of 14 kcal·kg^{-1} body wt·wk^{-1} (53) increased mean LPLA by 3%, which was not statistically nor likely clinically significant. The lack of response may be explained, at least in part, by the exercise protocol, which, although of vigorous intensity, was of lower volume compared with those in the literature showing an increase in LPLA and decrease in HLA. The present cohort also had lower baseline values for fasting lipids and was composed largely of normolipidemic persons.

The changes for LPLA, by ε2/ε3, ε3/ε3, and ε3/ε4 genotype, were 0.850, −0.527, and 1.200 μmol FFA·ml^{-1}·h^{-1}, respectively. The distribution by genotype was 38/115 (33.1%), 42/115 (36.5%), and 35/115 (30.4%), respectively. The ε2/ε3, ε3/ε3, the ε3/ε4 genotypes comprised 33, 36.5, and 30.4% of the sample after training, respectively. These proportions differ from the proportions of ε2/ε3, ε3/ε3, and ε3/ε4 genotypes of 11.6, 62.3, and 21.3% that are seen in the general population. Thus 95.3% of the population is defined by the ε2/ε3, ε3/ε3, or ε3/ε4 genotype. The remaining 4.7% are ε2/ε2, ε2/ε4, ε4/ε4, or other genotype. Assuming all other factors that might affect lipase activities and training response (other genetic, environmental) are randomly and equally distributed across *APOE* genotypes, we could extrapolate our findings to the 95.3% of the population whose *APOE* genotypes are ε2/ε3, ε3/ε3, or ε3/ε4. We "weighted" the lipase activity changes as follows: for the ε2/ε3 genotype, multiply by $0.116/0.953 = 0.121$; for the ε3/ε3 genotype, $0.623/0.953 = 0.654$; and for the ε3/ε4 genotype, $0.216/0.953 = 0.227$. Therefore, the weighted average change in LPLA was 0.07 μmol FFA·ml^{-1}·h^{-1}, or 0.6%.

We also found that *APOE* genotype modulates the LPLA response to exercise. Subjects with ε3/ε3, the most frequent genotype in the population but in the present study equally represented with ε2/ε3 and ε4/ε3, showed an unexpected 6% decrease in LPLA adjusted for baseline insulin levels. In contrast, the non-wild-type subjects with ε2/ε3 and ε4/ε3

genotypes increased LPLA significantly compared with the wild-type subjects. Various factors can affect LPLA, including physical interaction with the protein apolipoprotein E, the plasma concentration and distribution of apolipoprotein E, and concentrations of the apolipoprotein C (APOC) family of proteins. The receptor domain of apolipoprotein E binds to LPL (38), and, at least in the case of apolipoprotein E2, LPLA appears impaired (25). However, in the present study the ε2 and ε4 haplotypes tended to show a greater exercise increase in LPLA compared with ε3 carriers. Apolipoprotein E4 preferentially binds to VLDL particles (13). Whether apolipoprotein E4 affects LPL activity differently is not known. It is difficult to explain the training-specific increases associated with the ε4 and ε2 haplotype based on the known physical behaviors of their products. The present findings seem to generate hypotheses rather than align with the existing understanding of lipid metabolism. Measurement of the concentrations of apolipoprotein C1, C2, and C3 and apolipoprotein E and the plasma distribution of apolipoproteins E2, E3, and E4 among VLDL and HDL particles before and after training may provide more insight.

A genetic explanation may be possible, although highly speculative. The *APOC* gene products are crucial regulators of LPL activity. Apolipoprotein C2 is a required cofactor for LPL activation (34, 40), and apolipoproteins C1 and C3 inhibit LPLA (23, 31). That the *APOC* and *APOE* genes lie within 50 kb of one another in the *APOE-APOC1-APOC4* gene cluster on chromosome 19q13.2 makes it possible to hypothesize linkages between the ε3 and *APOC* alleles that might attenuate the exercise increase in LPL activity. At least one example has been reported, an almost complete linkage disequilibrium of an insertion allele of *APOC1* with the *APOE* haplotypes ε2 and ε4 in an eastern European population, suggesting that the deletion in the *APOC1* gene may follow the derivation of all three *APOE* haplotypes on the ε3 allele background (26). LPLA in these subjects has not been measured, and no linkages between *APOE* and *APOC1-APOC4* single-nucleotide polymorphisms (SNPs) were found using the HapMap website (http://www.hapmap.org/).

Implications with respect to exercise. The effect that *APOE* genotype exerts on HLA may have implications for cardiovascular disease risk. Others have identified interactive effects among *APOE* genotype, sex, and exercise training on lipids in cross-sectional studies (5, 49, 52). In the Cardiovascular Disease Risk in Young Finns survey (52), physical activity habits affected the lipid profile across the spectrum of *APOE* genotypes differently in boys and girls. In males only, the highest physical activity level altered LDLC by +14, −6, −11, and −30% in ε4/ε4, ε4/ε3, ε3/ε3, and ε3/ε2 subjects, respectively. In highly active males, the ratio of HDL-C to total cholesterol increased by 6–9% in ε4/ε4, ε4/ε3, and ε3/ε3 subjects, compared with a 6% decrease in ε2/ε3 subjects (52). Of all the common *APOE* genotypes, only in those homozygous for ε3 did activity level effect changes in LDLC and HDLC in the direction of lower cardiovascular disease risk. In contrast, another study suggested that increased physical activity lowers the risk for cardiovascular disease only in those at greatest risk, i.e., sedentary *APOE*4 individuals (5). In a survey of >1,700 subjects (5), high-intensity physical activity levels i*n ε4* imparted greater beneficial effects on HDL-C and TG levels compared with ε2 or ε3 groups. Finally, in 129 adults in Quebec (49), $\dot{V}O_{2max}$ and HDL-C_2 were correlated only in the ε3/ε3 subjects ($r = 0.51$), implying that exercise training to raise $\dot{V}O_{2max}$ selectively imparts a benefit to those with the *APOE* ε3/ε3 genotype. Obesity and insulin resistance varied in that study, and those effects outweighed the effects of *APOE* variants on lipids (49). The present study included sedentary adults with BMI ranging from 20 to 30 and relatively low insulin levels. It would be useful to study the *APOE* effects in subjects with greater variability in BMI, obesity, and insulin resistance to further understand the interaction of *APOE* with these factors.

The HERITAGE study of exercise training reported no *APOE* haplotype-specific effect on the change in PHP HLA and PHP LPLA (33). There was significantly lower LPLA at baseline in white men with ε3/ε4 and ε4/ε4 compared with other genotypes ($P = 0.006$). Despite robust statistical significance, the authors did not indicate whether the LPLA difference affected the lipid profile (33). In another small training study in men (20), relatively high-intensity and -volume exercise training significantly decreased TGs in subjects with the apolipoprotein ε2/ε3 (−23%) and ε3/ε3 (−16%) genotypes, but not in ε4/ε3. Also, HDL-C increased significantly more in the ε2/ε3 (+22%) than in the ε3/ε3 (+9%) and ε4 (+6%) subjects (20). Lipase activities were not measured.

Our group has previously reported in this cohort an effect of *APOE* genotype on the training response of LDL-C, HDL-C, and LDL particle subclasses (47). HLA, through delipidation of VLDL, influences conversion of VLDL to LDL and IDL and the formation of small dense LDL (22). Future exercise studies might examine relationships between baseline HLA levels, changes in HLA, and the changes in LDL subclasses.

Limitations. The present study is unique for its a priori approach to compare equal numbers of subjects with the three most common *APOE* genotypes: ε2/ε3, ε3/ε3, and ε4/ε3. The population frequencies for the ε2/ε3 and ε4/ε3 genotypes are 0.10–0.15, and that of the wild type ε3/ε3 is 0.6 or greater. We obtained similar numbers of subjects with the ε2/ε3, ε3/ε3, and ε4/ε3 genotypes by continuing to recruit and screen volunteers until ~120 carriers of the ε2 and ε4 alleles were identified. Thus the findings carry those limitations inherent to a recruiting process that was not purely random.

With respect to the *APOE* gene itself, our grouping of subjects by ε2, ε3, and ε4 haplotype does not necessarily guarantee homogeneity within the *APOE* gene or nearby genes. Within the classic *APOE* ε2, ε3, and ε4 haplotypes, 18 additional sites of variation at other exons or introns exist (50). It is possible that their effects may confound the metabolic tendencies associated with the functionality of the E2, E3, and E4 proteins. Also, multigenic haplotypes ("physiotypes") are likely to be more informative than individual gene haplotypes with respect to understanding biological traits (51), and this is true of the *APOE* haplotype influence on lipoprotein metabolism (7, 24, 29). More study is needed to identify gene variants and simultaneously define their contributions in response to lifestyle interventions intended to prevent or delay cardiovascular disease. This effort will advance via the application of physiogenomics (44), the study of physiological effects of many genetic variations, simultaneously.

Other limitations include the inclusion of patients with a relatively low entry total cholesterol, the administration of one exercise dosage, and the exclusion of subjects with BMI > 30.

Finally, circulating levels of apolipoprotein E were not measured. Variation in apolipoprotein levels may further clarify the effects of *APOE* haplotype variants on the lipases.

To summarize, this study prospectively investigated whether the three most common *APOE* haplotypes influenced PHP lipase activities before and after exercise training. The experimental groups were balanced for *APOE* genotype and sex. Lipase activities were affected by *APOE* genotype as follows. The *APOE* ε2/ε3 genotype exerted a strong effect that increased HLA in men, not women, regardless of aerobic training status. We have argued that the effect is the result of the different mechanisms by which testosterone and estradiol help regulate hepatic cholesterol economy. In addition, the presence of *APOE* ε3/ε3 attenuated the capacity of aerobic training to increase LPLA, regardless of sex. The mechanism is not known. Altogether, the study demonstrates both a gene × sex interactive effect and an exercise training × gene interactive effect involving PHP lipase activities and *APOE* genotype.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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