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Endurance Exercise Training Effects on Body Fatness, VO2max HDL-C Subfractions, and Glucose Tolerance are Influenced by a PLIN Haplotype in Older Caucasians

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Endurance exercise training effects on body fatness, $\dot{V}O_{2max}$, HDL-C subfractions, and glucose tolerance are influenced by a *PLIN* haplotype in older Caucasians

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Jenkins NT, McKenzie JA, Damcott CM, Witkowski S, Hagberg JM. Endurance exercise training effects on body fatness, $\dot{V}O_{2max}$, HDL-C subfractions, and glucose tolerance are influenced by a *PLIN* haplotype in older Caucasians. *J Appl Physiol* 108: 498–506, 2010. First published October 22, 2009; doi:10.1152/japplphysiol.01018.2009.— Perilipins are lipid droplet-coating proteins that regulate intracellular lipolysis in adipocytes. A haplotype of two perilipin gene (*PLIN*) single nucleotide polymorphisms, 13041A>G and 14995A>T, has been previously associated with obesity risk. Furthermore, the available data indicate that this association may be modified by sex. We hypothesized that this haplotype would associate with body fatness, aerobic fitness, and a number of cardiovascular (CV) risk factor phenotypes before and after a 6-mo endurance exercise training program in sedentary older Caucasians. The major haplotype group (13041A/14995A; $n = 57$) had significantly lower body mass index (BMI) and body fatness compared with noncarriers of the AA haplotype ($n = 44$) before the training intervention. Training improved body composition in both groups, but fatness remained higher in noncarriers than AA carriers after training. This fat retention in noncarriers blunted their maximal oxygen uptake ($\dot{V}O_{2max}$) adaptation to training. Female noncarriers had substantially higher concentrations of several conventionally and NMR-measured HDL-C subfractions than male noncarriers before and after training, but only minimal differences were found between the sexes in the AA haplotype group. Haplotype group differences in baseline and after-training responses to an oral glucose tolerance test (OGTT) also differed by sex, as noncarrier men had the highest baseline area under the insulin curve (insulin AUC), but were the only group to significantly improve insulin AUC with training. The insulin sensitivity index and plasma glucose responses to the OGTT were more favorable in AA carriers than noncarriers before and after training. Overall, our findings suggest that *PLIN* variation explains some of the interindividual differences in the response of obesity and CV phenotypes to exercise training. Furthermore, these data contribute to the growing understanding of *PLIN* as a candidate gene for human obesity and the cardiometabolic consequences of excess adiposity.

genetics; physical activity; lipids; metabolism

THE CARDIOVASCULAR (CV) and metabolic consequences of sedentary behavior are severe and the prevalence of age-related cardiometabolic diseases has reached epidemic proportions. It is increasingly understood that genetic factors contribute to interindividual differences in CV and metabolic disease risk factors. The perilipin gene (*PLIN*) has recently emerged as a candidate gene explaining a portion of these interindividual differences.

The three perilipin protein isoforms encoded by *PLIN* are lipid droplet-coating proteins that regulate intracellular lipolysis (3). In mammalian adipocytes, the phosphorylation state of perilipin regulates access of lipases [e.g., hormone sensitive lipase and adipocyte triglyceride (TG) lipase] to TG in the droplet core, as lipolysis is prevented in the hypophosphorylated state and TG breakdown is elevated on perilipin phosphorylation (6). Experiments in animals have shown that deletion of *PLIN* causes resistance to diet-induced obesity (25), whereas *PLIN* overexpression in cultured adipocytes blunts the actions of lipolytic drugs (22). Moreover, elevated adipocyte *PLIN* mRNA and protein levels were correlated with adiposity in human patients (24). Thus there is support for *PLIN* as a candidate gene contributing to the highly complex, polygenic disease phenotype of human obesity.

Single nucleotide polymorphisms (SNPs) and haplotypes in *PLIN* have been associated with numerous cardiometabolic risk factor phenotypes, including obesity (7, 20, 31–33), insulin resistance (8, 37), and altered TG metabolism during postprandial lipemia (30). *PLIN* variants were also associated with mild weight loss and the accompanying changes in serum free fatty acid concentrations following dietary interventions (7, 20). However, whether variation at the *PLIN* locus modifies exercise training-induced changes in these variables is not known.

Therefore, the purpose of this study was to investigate potential associations of common *PLIN* sequence variants with body fatness and related phenotypes before and after a 6-mo standardized exercise training program in sedentary, older Caucasian men and women. We focused our analyses specifically on a haplotype of two *PLIN* SNPs, 13041A>G (rs2304795) and 14995A>T (rs1052700), that was previously associated with obesity risk, with carriers of haplotype AA being at reduced risk relative to noncarriers (33). We hypothesized that the leaner AA carrier haplotype group would exhibit, in general, more favorable CV-related phenotypes (i.e., higher aerobic capacity, high density lipoprotein lipid-cholesterol levels, and insulin sensitivity; lower circulating TG levels) relative to noncarriers of the AA haplotype before and after endurance exercise training.

MATERIALS AND METHODS

The methods and design of the University of Maryland Gene Exercise Research Study have been described in detail previously (39). Volunteers were enrolled to assess the genetic determinants of

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individual variation in endurance exercise training-induced changes in CV risk factor phenotypes, and data are reported here from 101 Caucasian subjects (46 men, 55 women) who completed all baseline and final testing procedures. The Institutional Review Board at the University of Maryland College Park approved the study and participants provided written informed consent.

Inclusion and exclusion criteria. Subjects were sedentary (aerobic exercise ≤2 times/wk and <20 min/session, sedentary occupation), normotensive or hypertension-controlled (BP <160/99), nondiabetic, nonsmoking men and women aged 50–75 yr who had no prior history of CV disease, were not on lipid- or glucose-lowering medications, and had body mass index (BMI) of <37 kg/m^2. Women were postmenopausal and maintained their hormone replacement therapy status for the duration of the study. Subjects had at least one National Cholesterol Education Program lipid abnormality (total cholesterol >200 mg/dl, HDL-C <40 mg/dl, TG >200 but <400 mg/dl), and total cholesterol and LDL-C must have been <90th and HDL-C >20th percentile for their age and sex to exclude subjects with the possibility of a familial dyslipidemia. To ensure absence of diabetes, plasma glucose concentration had to be <126 mg/dl in the fasted state and <200 mg/dl 2 h following a 75-g oral glucose challenge. A physician-supervised, symptom-limited maximal treadmill exercise test was conducted with blood pressure and ECG monitoring to ensure participants were free of CV disease (1).

Dietary stabilization. Qualified subjects completed a 6-wk American Heart Association Step 1 dietary program (2). Adherence to the prescription of <30% caloric intake from fat, 55% from carbohydrates, and 15% from protein and <300 mg/day cholesterol intake was monitored with 7-day food records by the study dietician. Subjects were admitted to the baseline testing and training phases of the study when they had been weight stable for 3 wk.

Baseline testing. Weight-stable subjects were assessed for body composition by dual-energy x-ray absorptiometry (DXA; Lunar, Madison, WI) and intra-abdominal and subcutaneous fat were quantified at L4-L5 by computed tomography (27). Maximal oxygen uptake ($\dot{V}O_{2max}$) was assessed using a modified Bruce treadmill protocol as previously described (10). $\dot{V}O_2$ was considered maximum using the plateau criteria (i.e., increase in $\dot{V}O_2$ of <150 ml O_2/min with increased work rate). In the absence of a clear plateau in $\dot{V}O_2$, all subjects were verified to reach a respiratory exchange ratio of >1.10 and a peak heart rate within 10 beats of the age-predicted maximum. $\dot{V}O_{2max}$ values were corrected for total body mass (BM) as well as fat-free mass (FFM). A 75 g oral glucose tolerance test (OGTT) was conducted in the morning after an overnight fast and plasma samples were analyzed for glucose and insulin concentrations, and the insulin sensitivity index (ISI) was calculated as previously described (26). Conventional and nuclear magnetic resonance (NMR) measurements of plasma lipoprotein lipids were performed as described by our laboratory previously (15, 16, 39). HDL_{NMR}-C data were obtained on a subset of subjects (n = 33; 12 men and 21 women). HDL_{NMR}-C measurements have been previously validated against conventional measurement techniques (29). Rough equivalence of conventional HDL-C measures and the NMR are as follows: HDL_{1NMR}-C ≈ HDL_{3c}-C; HDL_{2NMR}-C ≈ HDL_{3b}-C; HDL_{3NMR}-C ≈ HDL_{3a}-C; HDL_{4NMR}-C ≈ HDL_{2a}-C; and HDL_{5NMR}-C ≈ HDL_{2b}-C (21, 28, 29). Additionally, three integrated HDL_{NMR}-C subfractions were calculated (15, 16): $HDL_{4,5NMR}$-C and $HDL_{3,4,5NMR}$-C, which are more cardioprotective, and $HDL_{1,2NMR}$-C, which is more atherogenic (15).

Genotyping and haplotype estimation. In addition to the *PLIN* 13041A>G and 14995A>T variants, we also explored whether the 6209T>C and 11482G>A variants were associated with body fatness or CV risk factor phenotypes, as a number of body composition and related phenotypes have been previously associated with these loci (7, 32). All SNPs were genotyped using TaqMan SNP Genotyping Assays (Applied Biosystems) according to the manufacturer's protocol. Pairwise linkage disequilibrium was tested among SNPs using R^2. Haplotypes were inferred using the computational inference program PHASE v2.1 (36). A dataset containing the genotypes for the study participants was input into the PHASE program as described in the program documentation. Resulting haplotypes with a frequency ≥5% were analyzed for association with body composition and cardiometabolic phenotypes.

Exercise training. Participants performed multi-modal (treadmill, cycle, and rowing ergometers) endurance exercise training at an initial volume of 3 day/wk for 20 min/day at a heart rate corresponding to 50% $\dot{V}O_{2max}$. Duration increased by 5 min/session per week for 4 wk until reaching 40 min/session. Intensity was then increased by 5% $\dot{V}O_{2max}$/wk for 4 wk, until reaching 70% $\dot{V}O_{2max}$. A 45- to 60-min walk at home was incorporated into the program after 12 wk. Only subjects who completed >75% of the prescribed training sessions were included in the final analyses.

Final testing. All baseline assessments were repeated after the 24-wk training program. Blood sampling for plasma lipoprotein-lipid profiles and OGTTs were conducted 24–36 h after the final exercise bout.

Statistics. Data were analyzed using SPSS software (Chicago, IL). Assumptions of normality and homoscedasticity were verified for all analyses. Data are presented as means ± SE. Hardy-Weinberg equilibrium was tested using Chi-square tests for each *PLIN* SNP. An omnibus repeated-measures ANOVA was used to test for interactive and main effects of independent variables on outcome phenotypes, with training (baseline vs. after training) as the within-subjects factor and genotype as a between-subjects factor. Between-group differences in training-induced changes were examined with the repeated-measures ANOVA; however, because not all change values differed uniformly among the different phenotypes studied, results are presented as baseline and final values rather than change scores alone. Potential confounding variables including age, sex, and hormone replacement therapy status were assessed using analysis of covariance, but were only included in the final analyses when a significant ($P < 0.05$) proportion of the total variance in the outcome phenotype was explained by the covariate. Sex differences were examined when training × genotype × sex or genotype × sex interaction effects were found to significantly contribute to total phenotype variance. Eta squared (η^2) statistics were calculated to examine the contribution of genotype to the overall variation in a given phenotype, independent from other explanatory variables and covariates. The $\alpha = 0.05$ criterion was used for statistical significance. All post hoc tests were performed with the Bonferroni correction to protect against type I error.

RESULTS

Genotype group sample sizes according to the four *PLIN* SNPs and Hardy-Weinberg equilibrium results are presented in Table 1. The 14995A>T variant was in significant ($P < 0.001$) pairwise linkage disequilibrium with all SNPs: 13041A>G (R^2 = 0.12), 6209T>C (R^2 = 0.15), and 11482G>A (R^2 = 0.15). The 6209T>C and 11482G>A variants were also in significant linkage disequilibrium (R^2 = 0.60, $P < 0.001$). The strongest and most consistent genotype-phenotype associations were observed when subjects who completed all study procedures were examined as 13041A/14995A carriers (n = 57; 34 women and 23 men) vs. noncarriers (n = 44; 21 women and 23 men) haplotype groups (Table 2). Therefore, group comparisons are referred to as AA carriers and noncarriers in all figures. The individual *PLIN* SNPs were also associated with selected phenotypes before and after exercise training (data not shown); however, we report here only the most important associations between the *PLIN* 13041/14995 haplotype and various phenotypes. In addition, HDL_{NMR}-C lipid subfractions were only analyzed from a subset of subjects (n = 22 AA

Table 1. *Genotype frequency information for the four PLIN single nucleotide polymorphisms*

	6209T>C			11482G>A			13041A>G*			14995A>T		
	TT	TC	CC	GG	GA	AA	AA	AG	GG	AA	AT	TT
n	42	52	16	56	39	13	32	62	11	52	43	15
Frequency	0.38	0.47	0.15	0.52	0.36	0.12	0.31	0.59	0.10	0.47	0.39	0.14

Allele frequencies statistically deviate from Hardy-Weinberg equilibrium ($P < 0.05$).

carriers, 7 men and 15 women; $n = 11$ noncarriers, 5 men and 6 women).

Noncarriers of the AA haplotype have higher body fat levels than carriers before and after training. At baseline, AA carriers had significantly lower age- and sex-adjusted total body, trunk, intra-abdominal, and subcutaneous fat and BMI relative to noncarriers of the AA haplotype ($P < 0.05$, Fig. 1). These differences between AA carriers and noncarriers were also observed after training for BMI, total body fat, and trunk fat ($P < 0.05$) despite statistically significant training-induced reductions in these phenotypes for both groups ($P < 0.05$). Intra-abdominal and subcutaneous fat decreased significantly with training in noncarriers ($P < 0.05$) and did not differ between groups after training, as noncarriers experienced significantly larger training-induced reductions in these phenotypes relative to AA carriers ($P < 0.05$). Importantly, these results remained statistically significant when sex and the sex × genotype interaction were included as covariates in the analysis. However, we still chose to examine potential differences in intra-abdominal fatness between men and women because *1*) central obesity is more prevalent in men and is strongly related to hypertriglyceridemia and low circulating HDL-C and *2*) we also found a number of sex × genotype interaction effects on circulating lipids (described below). However, the sex × genotype × training interaction effect on intra-abdominal fat was not statistically significant ($P > 0.05$). There was a significant sex × training effect independent of genotype, as men of both genotype groups had higher intra-abdominal fat than women at baseline ($P < 0.05$), and only men had significant training-induced reductions in intra-abdominal fat ($P < 0.05$). These interaction effects were, however, similar between haplotype groups, and the body composition data stratified by sex displayed identical trends to the genotype × training data shown in Fig. 1.

Training-induced increases in whole body aerobic capacity are blunted in noncarriers of the AA haplotype. AA carriers and noncarriers had similar $\dot{V}O_{2max}$ values at baseline (adjusted for age and sex; $P > 0.05$, Fig. 2). While both groups had significantly higher $\dot{V}O_{2max}$ values after training, noncarriers had significantly lower values than AA carriers after training when $\dot{V}O_{2max}$ was expressed relative to body weight (ml·kg $BM^{-1}\cdot min^{-1}$; $P < 0.05$, Fig. 2*A*). However, after correcting $\dot{V}O_{2max}$ for FFM (ml·kg $FFM^{-1}\cdot min^{-1}$), AA carriers and noncarriers did not differ before or after training ($P > 0.05$; Fig. 2*B*).

Table 2. *Frequencies of PLIN haplotypes and group sample sizes*

PLIN SNP				Carrier	Noncarrier
6209T>C	11482G>A	13041A>G	14995A>T	*n* (freq)	*n* (freq)
X	**X**	**A**	**A**	**57 (0.56)**	**44 (0.44)**
X	X	A	T	12 (0.12)	89 (0.88)
X	X	G	A	30 (0.30)	71 (0.70)
X	X	G	T	2 (0.02)	99 (0.98)

Bold text indicates haplotype groups used for all analyses in the present study.

Sex differences in HDL-C subfractions and TG levels before and after endurance exercise training exist in noncarriers but not carriers of the AA haplotype. There were significant training × genotype × sex interaction effects on a number of conventional (Fig. 3) and NMR-based HDL-C (Fig. 4) measurements after adjusting for age ($P < 0.05$). Bonferroni-corrected post hoc tests revealed three key findings. First, there were differences between sexes within the noncarriers before and after exercise training for all HDL-C subfraction data presented in Figs. 3 and 4, with noncarrier women having higher values than noncarrier men ($P < 0.05$). These sex differences were not observed within AA haplotype carriers, except for HDL_{3NMR}-C before and after training ($P < 0.05$). Second, genotype differences were observed among women, with noncarriers having significantly higher HDL-C, HDL_2-C, HDL_3-C, HDL_{4NMR}-C, $HDL_{3,4,5NMR}$-C, $HDL_{4,5NMR}$-C both before and after training ($P < 0.05$) and HDL-$C_{sizeNMR}$ after training ($P < 0.05$). These between-genotype differences were not observed in men ($P > 0.05$). Third, only noncarrier women experienced training-induced increases in HDL-C, HDL_{4NMR}-C, $HDL_{4,5NMR}$-C, and HDL-$C_{sizeNMR}$ ($P < 0.05$). There were no effects of genotype, sex, training, or their interaction on HDL_{1NMR}-C, HDL_{2NMR}-C, HDL_{5NMR}-C, or $HDL_{1,2NMR}$-C ($P > 0.05$). There was a statistically significant training × genotype × sex interaction effect on plasma TG levels ($P < 0.05$), with noncarrier men having significantly greater TG concentrations than carrier men and noncarrier women after training (Fig. 5, $P < 0.05$). Only noncarrier women had a significant training-induced reduction in TG levels ($P < 0.05$).

Sex and the AA haplotype interactively affect plasma glucose and insulin OGTT responses before and after training. Noncarriers had greater OGTT insulin concentrations at 90 and 180 min than carriers before training ($P < 0.05$; Fig. 6*A*), and the insulin AUC was higher in noncarriers than carriers before training ($P < 0.05$, Fig. 6*B*). Both carriers and noncarriers experienced a reduction in insulin AUC with training ($P < 0.05$), and the 90- and 180-min insulin concentrations were significantly reduced after training in noncarriers such that the two groups did not differ in insulin concentrations at any time point or in insulin AUC after training. Baseline glucose concentrations were higher in noncarriers than carriers at 0 and 90 min of the OGTT ($P < 0.05$; Fig. 6*A*). The 0-min values remained higher in noncarriers than carriers after training ($P < 0.05$), but their 90-min values were reduced after training ($P < 0.05$) such that groups did not differ after training at that time point. Glucose AUC were higher in noncarriers than AA

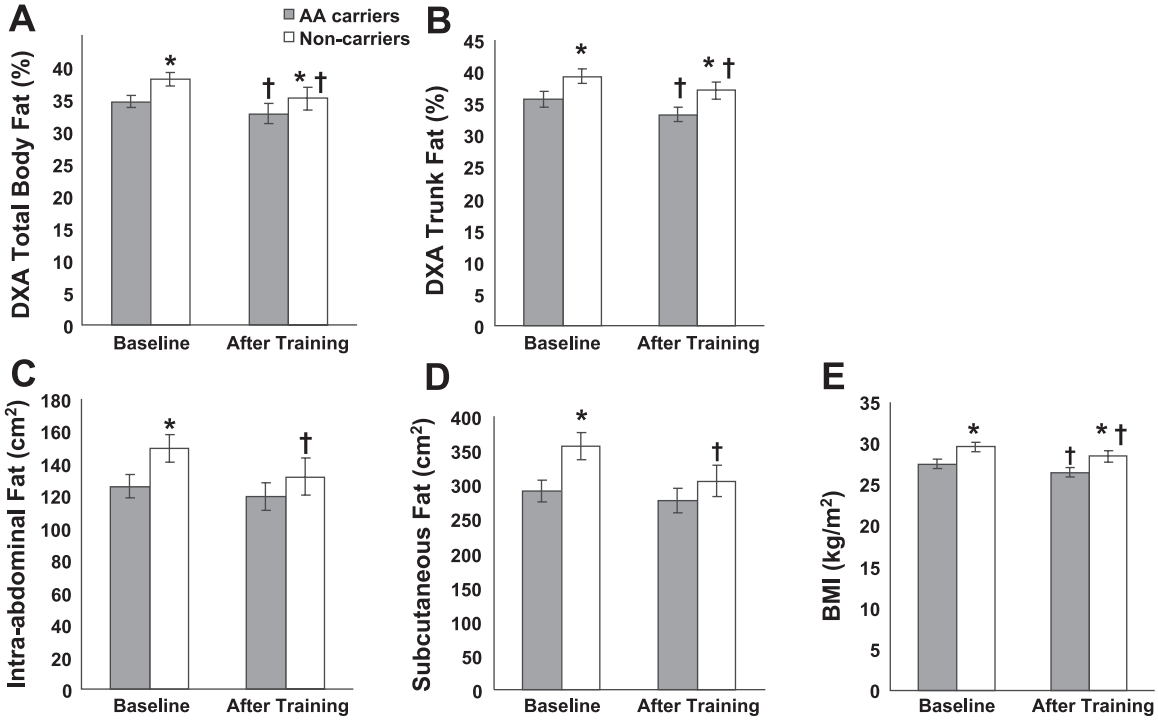

Fig. 1. Influence of the AA haplotype on age- and sex-adjusted body composition phenotypes. *A*, total body; *B*, trunk; *C*, intra-abdominal; *D*, subcutaneous fat; *E*, BMI. *Significant difference between genotypes ($P < 0.05$). †Significant within-genotype change with training ($P < 0.05$).

haplotype carriers both before and after training ($P < 0.05$, Fig. 6*C*). ISI was also lower in noncarriers than carriers both before and after training ($P < 0.05$; Fig. 6*D*). There was a significant training × genotype × sex interaction for insulin AUC ($P < 0.001$; Fig. 7*A*) as noncarrier men had higher insulin AUC compared with all other genotype × sex subgroups at baseline ($P < 0.01$) and a significant reduction in insulin AUC after training ($P < 0.05$). AA men and AA women did not differ in insulin AUC before or after training ($P > 0.05$). There were no sex-specific differences observed for glucose AUC ($P > 0.05$; Fig. 7*B*).

Examination of the η^2 statistics indicated that the *PLIN* AA haplotype accounted for, on average, 2.5% (range 1–6%) of the variation in the different phenotypes where statistical significance was noted.

DISCUSSION

The major finding of this study is that the 13041A/14995A *PLIN* haplotype, which occurs in ~50–55% of white populations (present data and Ref. 33), is associated with generally better cardiorespiratory, body composition, and metabolic phenotypes before and after a 6-mo endurance exercise training program in previously sedentary older Caucasian men and women. In contrast, the noncarrier haplotype is associated with substantially higher conventional and NMR-based HDL-C measures in women. The associations of the haplotype with OGTT variables are also modified by sex, with noncarriers having pronounced differences in these phenotypes between the sexes, but AA carriers showing few differences between men and women. These data are consistent with our hypothesis that carriers of the AA haplotype would have generally more favorable CV-related phenotypes relative to noncarriers of the AA haplotype before and after endurance exercise training. Together with previous findings, our data extend the growing body of evidence implicating *PLIN* as an important genetic contributor to obesity-related risk for CV disease. Furthermore, this is the first documentation of *PLIN* variants modifying the endurance exercise training-induced changes in a number of clinically important CV disease risk factors.

PLIN and PLIN-training interactive effects on body composition and $\dot{V}O_{2max}$. Variation at the *PLIN* locus has been previously associated with numerous obesity- and CV risk-related phenotypes (7, 8, 20, 30–33, 37). The 13041A/14995A haplotype, specifically, has been associated with lower obesity risk, with noncarriers of the AA haplotype having significantly higher obesity rates than AA carriers (33). A haplotype that included these same variants was similarly associated with obesity risk in Malays and Indians (32). The baseline comparisons for body composition in the present study are consistent with these previous findings, as AA individuals had lower BMI, %total and trunk fat, as well as subcutaneous and intra-abdominal fat. Although both AA carriers and noncarriers had significantly lower %total and trunk fat after training relative to baseline, these variables remained significantly elevated in noncarriers compared with carriers following the training program. These body composition data also complement the earlier finding of an association between the 13041/14995 variants and obesity even when statistically controlling for lifestyle factors (e.g., physical activity, smoking, alcohol consumption, diabetes status) (32). Therefore, it appears that relative to carriers, noncarriers of the AA haplotype have higher initial body fat levels that remain elevated after an exercise intervention. These data support earlier indications that the minor *PLIN* alleles confer a propensity to retain higher

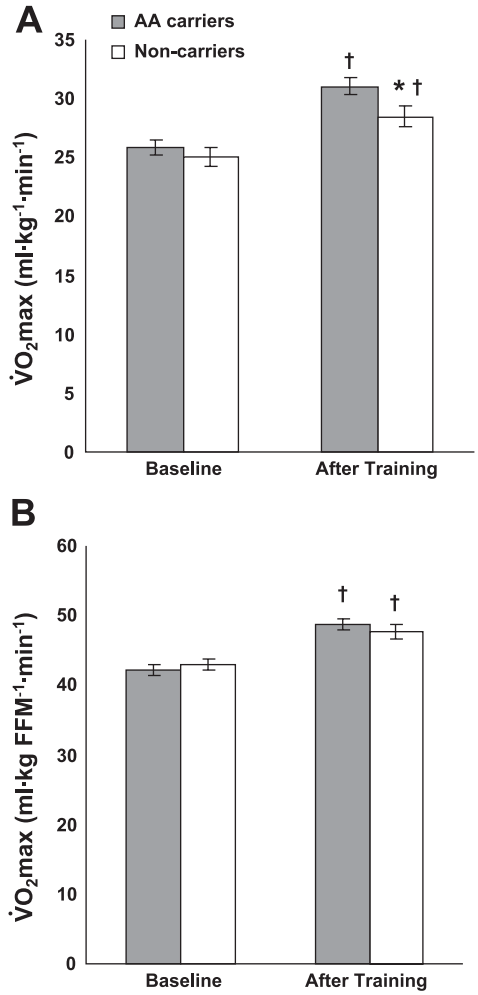

Fig. 2. Influence of the AA haplotype on age- and sex-adjusted maximal oxygen uptake ($\dot{V}O_{2max}$) values. *A*, $\dot{V}O_{2max}$ relative to total body mass (ml·kg BM^{-1}·min^{-1}); *B*, $\dot{V}O_{2max}$ relative to fat free mass (ml·kg FFM^{-1}·min^{-1}). *Significant difference between genotypes ($P < 0.05$). †Significant within-genotype change with training ($P < 0.05$).

body fat levels with changes in dietary and physical activity habits (7, 32).

The tendency for noncarriers of the AA haplotype to carry more total fat blunted their whole body cardiorespiratory adaptations to the training program. Groups had similar $\dot{V}O_{2max}$ (ml·kg BM^{-1}·min^{-1}) at baseline, but after training AA carriers had significantly higher aerobic capacity than noncarriers. However, groups did not differ in $\dot{V}O_{2max}$ when expressed relative to FFM (ml·kg FFM^{-1}·min^{-1}) either before or after training. Together, these data indicate that the lower training-induced increase in whole body $\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1}) of noncarriers after training was entirely a consequence of their higher adiposity than AA carriers and not a result of genotype-dependent differences in CV or muscle function. This may have important implications for noncarriers of the AA haplotype in the population if genomic information is to be used in individualized exercise training prescriptions for improving $\dot{V}O_{2max}$ and body composition. As aerobic fitness expressed in units relative to body weight is strongly linked to CV and all-cause mortality (13), it will be important for future studies to determine whether noncarriers of the AA haplotype require more aggressive, higher-volume exercise training prescriptions and/or dietary modifications to elicit the adaptive responses in body composition and $\dot{V}O_{2max}$ experienced by their AA carrier counterparts.

Sex-specific PLIN and PLIN-training interactive effects on plasma HDL-C subfraction and TG levels. Plasma HDL-C levels independently predict CV disease risk (14), and its subfractions may provide an even better indication of these risks (21, 28, 34). HDL-C levels are highly variable and influenced strongly by sex, genetic factors, environmental factors, and their interactions (11, 12, 23, 38). The present data indicate that the *PLIN* 13041A/14995A haplotype accounts for a portion of this variability. In particular, the well-documented sex dimorphism in HDL-C levels (4, 18, 35) is exacerbated in noncarriers of the AA haplotype. Furthermore, noncarrier women had a significant improvement in the plasma HDL-C profile with training despite having the highest HDL-C and HDL_{NMR}-C values at baseline. This finding is somewhat opposite of what could be expected, as subjects with the lowest baseline HDL-C values typically have the greatest response to exercise interventions (4, 19). On the other hand, the inverse has been shown previously by others (38), and there is evidence that some women can increase their HDL-C levels with exercise training regardless of initial values (35). Our data

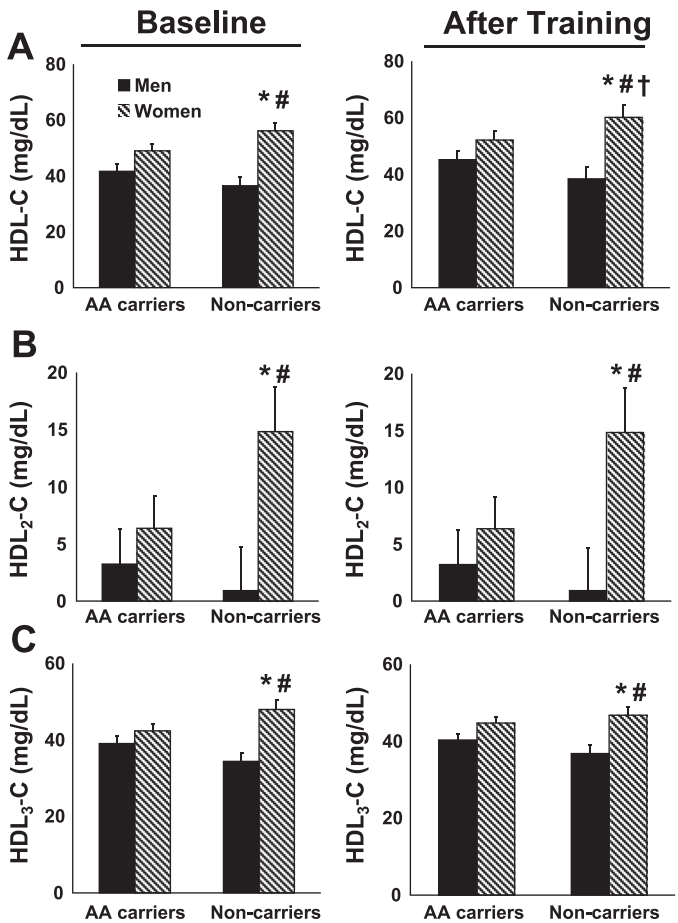

Fig. 3. Influence of the AA haplotype on age-adjusted conventional plasma HDL-C subfractions. *A*, HDL-C; *B*, HDL2-C; *C*, HDL3-C. *Sex-specific difference between genotypes ($P < 0.05$); #Difference between sexes within genotype ($P < 0.05$). †Significant within-genotype change with training ($P < 0.05$).

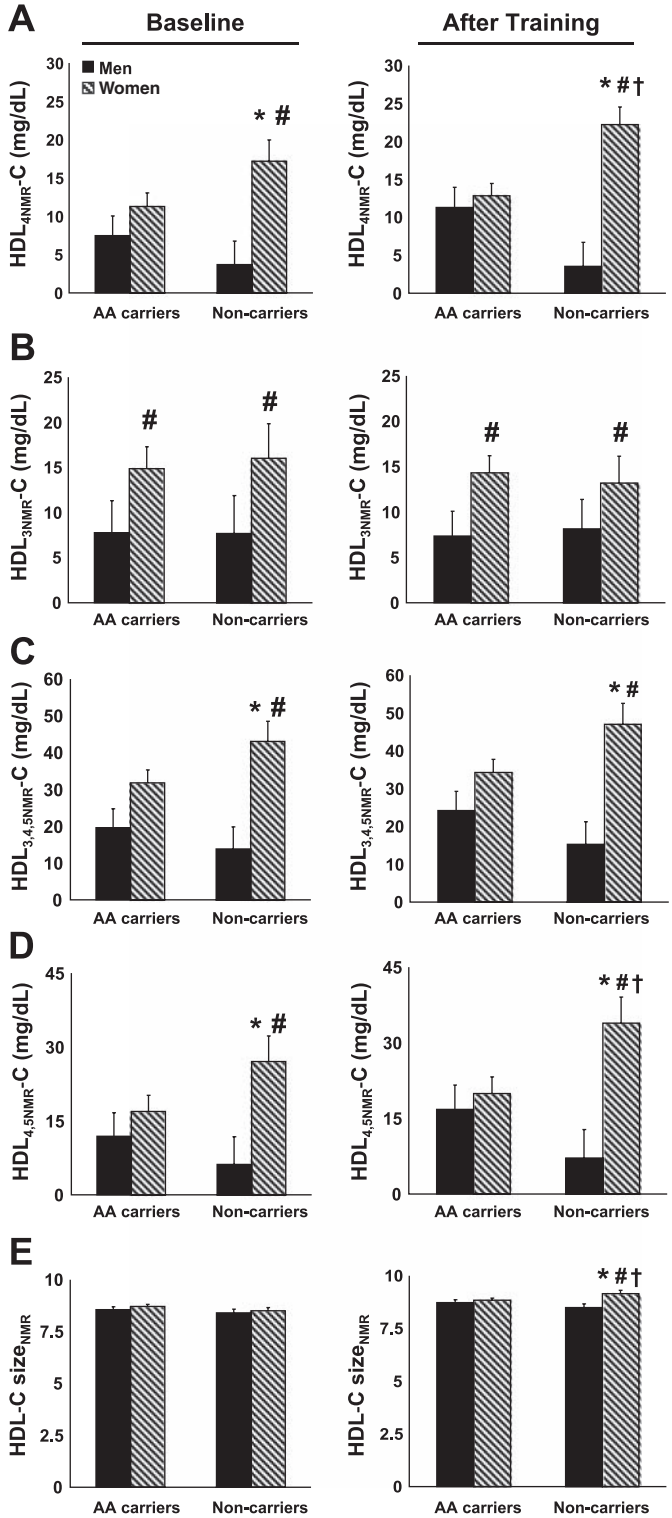

Fig. 4. Influence of the AA haplotype on age-adjusted NMR-based plasma HDL-C subfractions. *A*, HDL_{4NMR}-C; *B*, HDL_{3NMR}-C; *C*, $HDL_{3,4,5NMR}$-C; *D*, $HDL_{4,5NMR}$-C; *E*, HDL-C_{size}. *Sex-specific difference between genotypes ($P < 0.05$). #Difference between sexes within genotype. †Significant within-genotype change with training ($P < 0.05$).

suggest that not possessing the *PLIN* 13041A/14995A haplotype is associated with higher baseline and training-induced changes in HDL-C subfractions in women. It is especially interesting that in these women the cardioprotective subfraction $HDL_{4,5NMR}$-C increased with training, whereas the more atherogenic $HDL_{1,2NMR}$-C subfraction neither changed with training nor differed between genotypes. In parallel, noncarrier women were also the only group to reduce plasma TG concentrations with exercise training, in agreement with evidence that increased catabolism of TG is required to improve HDL-C levels (9, 18).

In addition, our findings may indicate a protective effect of the AA haplotype for men with respect to the atherogenic lipid profile associated with physical inactivity. In contrast to noncarriers, we did not find statistically significant differences in HDL-C subfractions between the sexes among AA carriers in the sedentary or trained states (except for HDL_{3NMR}-C), and noncarrier men had higher TG levels than carrier men after training. These differences persisted after training in noncarrier men, indicating that the noncarrier genotype may confer a resistance to exercise training-induced changes in HDL-C metabolism in these men. Furthermore, this occurred despite similar levels of intra-abdominal body fat between carrier and noncarrier men, which were significantly higher than in women in both haplotype groups. These data were somewhat contrary to our hypothesis, as TG and HDL-C changes with training are generally linked to changes in intra-abdominal fatness (9), although we have previously shown that HDL_{NMR}-C lipids improve with endurance exercise training independent of body composition changes (17).

The *PLIN* haplotype investigated here has been previously associated with obesity risk in white women (33), and a haplotype of the 14995A>T variant with the 11482G>A variant has been associated with the changes in the blood lipid profile in response to a 12-wk caloric restriction intervention (20). Interestingly, *PLIN* variants 6209C>T and 11842A>G were associated with postprandial TG levels in white subjects (30), with a substantially elevated lipemia during the postprandial period evident in carriers of the minor alleles. Although our haplotype approach differed from this previous study, the results are similar in that carriers of minor *PLIN* alleles across a number of SNPs/haplotypes appear to be at elevated risk for proatherogenic lipid profiles. Our data suggest that this is, however, somewhat modifiable in noncarriers of the 13041A/14995A haplotype through regular endurance exercise.

PLIN and PLIN-training interactive effects on insulin sensitivity and glucose tolerance. Exercise training-induced changes in glucose homeostasis vary widely among individuals and may

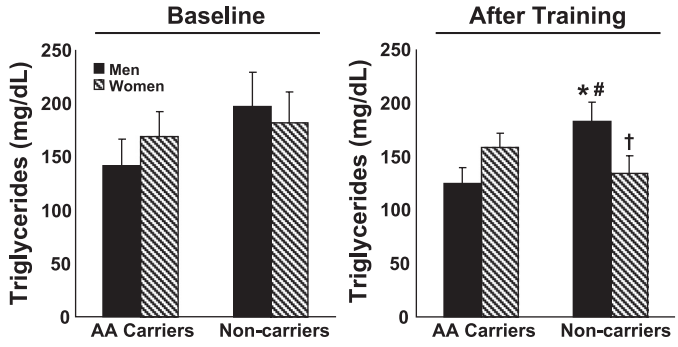

Fig. 5. Influence of the AA haplotype and sex on plasma TG concentrations. *Sex-specific difference between genotypes ($P < 0.05$). #Difference between sexes within genotype. †Significant within-genotype change with training ($P < 0.05$).

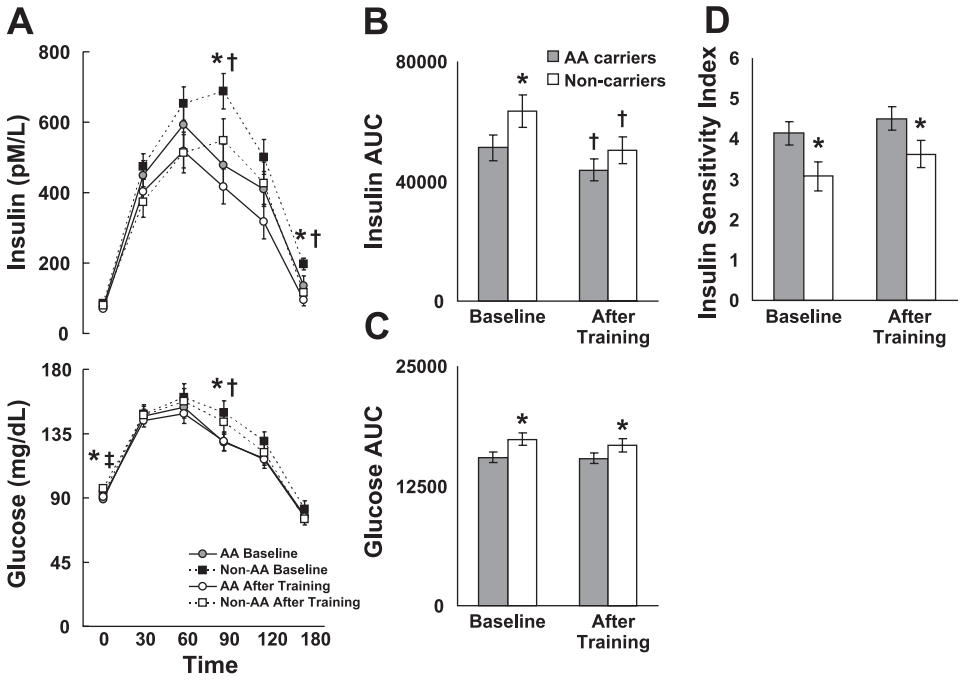

Fig. 6. Influence of the AA haplotype on plasma insulin and glucose responses to an oral glucose tolerance test (OGTT) before and after exercise training. *A*: insulin (*top*) and glucose (*bottom*) data during the-3 h sampling period. *Significant baseline differences between genotype groups ($P < 0.05$). ‡Significant difference between groups after training. †Significant within-genotype change with training in noncarriers ($P < 0.05$). Symbols for insulin AUC, glucose AUC, and insulin sensitivity index (ISI) (*B–D*) are as follows: *significant difference between genotypes ($P < 0.05$); †significant within-genotype change with training ($P < 0.05$).

differ between women and men (5). Our data indicate that carriers of the *PLIN* 13041A/14995A haplotype have higher insulin sensitivity and more favorable insulin and glucose responses to an OGTT compared with noncarriers in both the untrained state and after 6 mo of regular endurance exercise. Consistent with earlier data indicating that previously sedentary insulin-resistant men benefited more from endurance exercise training than women (5), noncarrier men had the highest baseline and greatest training-induced improvement in insulin AUC. On the other hand, there were no significant differences between AA carrier men and women before or after training. Thus the 13041/14995 haplotype variant appears to be an important genetic contributor to differences in baseline and training-induced changes in glucose metabolism between the sexes. Moreover, this association of the haplotype with metabolic phenotypes in parallel with body composition phenotypes extends the earlier finding that the 13041/14995 variant is associated with obesity risk (33).

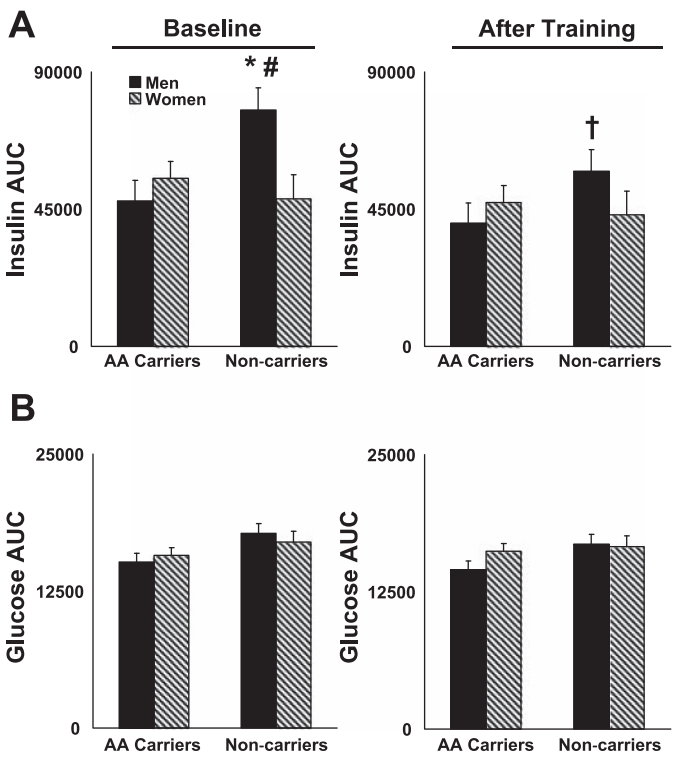

Fig. 7. Genotype × sex interaction effects on insulin and glucose AUC before and after exercise training. *A*, insulin AUC; *B*, glucose AUC. *Sex-specific difference between genotypes ($P < 0.05$). #Difference between sexes within genotype. †Significant within-genotype change with training ($P < 0.05$).

Limitations. There are several limitations of our study. First, we had a relatively small sample size of 101 men and women, and the HDL_{NMR}-C data came from an even smaller subset of these subjects. This precluded thorough, appropriately powered analyses of haplotypes with lower allele frequencies than the 13041/14995 haplotype. Therefore it is possible that we did not detect clinically important effects of other variations at the *PLIN* locus. However, we did detect the large effects associated with the 13041A/14995A haplotype and had excellent statistical power for all reported genotype differences. Second, we can generalize our results and conclusions only to older sedentary Caucasian men and women who are at risk for CV disease, but not to populations of different age, race/ethnicity, or risk factor status. Additionally, future work should determine whether body composition, aerobic fitness, and cardiometabolic risk factors are associated with risk alleles in the *PLIN* gene in other well-characterized populations. Third, our design did not include a control group that remained sedentary throughout the 6-mo study. Therefore, we cannot be certain that differences between groups were not due to confounding variables that we did not measure during the course of the intervention. Finally, the 13041A>G variant deviated from Hardy-Weinberg expectations in our study. However, the haplotype group frequencies in our study (56% carriers, 44%

noncarriers) are somewhat similar to the previously reported data of Qi et al. (49% carriers, 51% noncarriers; Ref. 33). In addition, deviation from Hardy-Weinberg may have occurred because our study population was small and homogenous (i.e., all participants were older sedentary Caucasians with at least one lipid abnormality).

Additionally, it is important to note that the subjects in the present study were instructed to adhere to a regulated diet during the training program, and a research dietician closely monitored dietary records throughout the intervention. Therefore, we believe that the *PLIN* 13041A/14995A effects observed here are specific to exercise training and independent of dietary changes.

Conclusions. In summary, *PLIN* variation appears to influence body composition and CV risk factor phenotypes before and after a 6-mo endurance exercise training program in previously sedentary older Caucasian men and women. The tendency for noncarriers of the 13041A/14995A haplotype to have more body fat than carriers is detrimental to their training-induced adaptations in whole body $\dot{V}O_{2max}$. Sex differences in the lipid profile and glucose/insulin metabolism are more pronounced in noncarriers than in carriers, and while both groups benefit from endurance training, these differences do not appear to be completely normalized following a period of regular endurance exercise. These data support the growing body of evidence supporting *PLIN* as a genetic contributor to the adaptive response of CV and metabolic risk in older individuals to endurance exercise training. Whether carriers of *PLIN* "risk" alleles require more aggressive strategies of primary prevention through regular exercise needs further attention.

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DISCLOSURES

No conflicts of interest were disclosed by the authors.

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