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Peroxisome proliferator-activated receptor-δ polymorphisms are associated with physical performance and plasma lipids: the HERITAGE Family Study

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Hautala AJ, Leon AS, Skinner JS, Rao DC, Bouchard C, Rankinen T. Peroxisome proliferator-activated receptor-δ polymorphisms are associated with physical performance and plasma lipids: the HERITAGE Family Study. *Am J Physiol Heart Circ Physiol* 292: H2498–H2505, 2007. First published January 26, 2007; doi:10.1152/ajpheart.01092.2006.—We tested the hypothesis that peroxisome proliferator-activated receptor-δ (PPARδ) gene polymorphisms are associated with cardiorespiratory fitness and plasma lipid responses to endurance training. Associations between the PPARδ exon 4 +15 C/T and exon 7 +65 A/G polymorphisms and maximal exercise capacity and plasma lipid responses to 20 wk of endurance training were investigated in healthy white (n = 477) and black (n = 264) subjects. In black subjects, the exon 4 +15 C/C homozygotes showed a smaller training-induced increase in maximal oxygen consumption (P = 0.028) than the C/T and T/T genotypes. Similarly, a lower training response in maximal power output was observed in the exon 4 +15 C/C homozygotes (P = 0.005) compared with the heterozygotes and the T/T homozygotes in black subjects, and a similar trend was evident in white subjects (P = 0.087). In white subjects, baseline apolipoprotein A-1 (Apo A-1)levels were higher in the exon 4 +15 C/C (P = 0.011) and exon 7 +65 G/G (P = 0.05) genotypes compared with those in the other genotypes. In white subjects, exon 4 +15 C/C (P = 0.0025) and exon 7 +65 G/G (P = 0.011) genotypes showed significantly greater increases in plasma high-density lipoprotein-cholesterol (HDL-C) levels with endurance training than in the other genotypes, whereas in black subjects the exon 4 +15 CC homozygotes tended to increase (P = 0.057) their Apo A-1 levels more than the T allele carriers. DNA sequence variation in the PPARδ locus is a potential modifier of changes in cardiorespiratory fitness and plasma HDL-C in healthy individuals in response to regular exercise.

cardiorespiratory fitness; high-density lipoprotein cholesterol; exercise training

PEROXISOME PROLIFERATOR-ACTIVATED receptors (PPARs) are nuclear receptors that modulate the function of many target genes, including those involved in lipid metabolism. Three different PPARs—PPARα, PPARγ, and PPARδ—have been characterized, each having a distinct tissue distribution pattern and cellular roles. The role of PPARα on lipid metabolism and fatty acid oxidation is well established, whereas PPARγ is involved in adipocyte differentiation, lipid storage, and insulin sensitivity (4). Activation of PPARδ has been shown to promote fatty acid oxidation in adipocytes and skeletal muscle cells (24).

Endurance training promotes an accumulation of PPARδ protein in skeletal muscle of mice (16). Targeted expression of an activated form of PPARδ in skeletal muscles increased the formation of type 1 muscle fibers and improved running capacity in mice. These mice were able to run as much as twice the distance compared with wild-type littermates. Furthermore, they were resistant to high-fat diet-induced obesity and glucose intolerance (25). Taken together, these results suggest that PPARδ is potentially a molecular regulator of muscle fiber type and a modifier of endurance training-induced changes in physical fitness. However, there are no data available in humans to confirm these findings.

PPARδ has been suggested to play a central role in blood lipid metabolism. Treatment with a PPARδ agonist has increased plasma high-density lipoprotein-cholesterol (HDL-C) concentrations in mice (14) and monkeys (18, 23). Skogsberg and colleagues (20) screened the 5′-untranslated region of the human PPARδ gene for DNA sequence variants and identified four polymorphisms. One of them, a T/C transition in nucleotide 15 of exon 4 (located 87 nucleotides upstream of the start codon), was associated with plasma LDL-cholesterol levels in two cohorts of healthy men (20). The rare C allele that was associated with higher LDL levels was shown to be associated with higher transcriptional activity in vitro and to affect binding of Sp-1 transcription factor. Furthermore, homozygotes for the C allele showed a tendency toward a higher risk of coronary heart disease compared with T/T homozygotes (21).

Regular physical activity and reasonable cardiorespiratory fitness are widely accepted as factors that can potentially reduce all-cause mortality and improve a number of cardiovascular and diabetes risk factors (13). However, there are marked interindividual differences in responsiveness to exercise training, and genetic factors have been shown to contribute to this variability (7). Therefore, we hypothesized that the functional PPARδ exon 4 +15 C/T and a synonymous exon 7 +65 A/G polymorphisms are associated with maximal exercise capacity and blood lipids in the sedentary state and in response to 20 wk of endurance training in sedentary healthy subjects of the HERITAGE Family Study.

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SUBJECTS AND METHODS

Subjects. The study cohort with complete baseline data consists of 478 white subjects (231 males and 247 females) from 99 nuclear families and 272 black subjects (89 males and 183 females) from 114 family units. Training response data were available for 462 white subjects (223 males and 239 females) and 256 black subjects (87 males and 169 females). The study design and inclusion criteria have been described previously (6). Subjects ranged in age from 17 to 65 yr and were healthy, sedentary, and met a number of inclusion and exclusion criteria. Briefly, subjects who had cardiovascular disease, were diabetics, or were being treated with lipid lowering, antihypertensive or hypoglycemic drugs were excluded. The study protocol had been approved by each of the Institutional Review Boards of the HERITAGE Family Study research consortium, and all the subjects gave written, informed consent (6).

Exercise training program. The exercise intensity of the 20-wk training program was standardized for each participant based on the heart rate (HR)/oxygen consumption ($\dot{V}O_2$) relationship measured at baseline (19). During the first 2 wk, the subjects trained at a HR corresponding to 55% of the baseline maximal oxygen consumption ($\dot{V}O_{2\,max}$) for 30 min per session. Duration and intensity of the training sessions were gradually increased to 50 min and the HR associated with 75% of baseline $\dot{V}O_{2\,max}$, which were then sustained for the last 6 wk. Training frequency was 3 times/wk, and all training was performed on cycle ergometers in the laboratory. Trained exercise specialists supervised all exercise sessions.

$\dot{V}O_{2\,max}$ tests. Two maximal exercise tests on separate days both before and after a 20-wk endurance training program were performed using a cycle ergometer (model 800S, SensorMedics, Yorba Linda, CA) for the determination of $\dot{V}O_{2\,max}$ and maximal work output ($\dot{W}_{max}$) as previously described (19).

Body composition. Stature and body mass were measured using a standardized protocol, and body mass index (BMI) was calculated by dividing body mass (in kg) by stature squared (in m^2) (26). Body density was assessed by underwater weighing to determine fat mass, fat-free mass, and relative body fat (%Fat) using race- and sex-specific equations (26).

Plasma lipid determinations. Plasma lipids were determined as previously described (15). In brief, in the morning following a 12-h fast, blood samples were obtained with participants in a semirecumbent position from an antecubital vein into vacutainer tubes containing EDTA. Samples were taken twice both at baseline (2 separate days) and after the last exercise training session (24 and 72 h). Cholesterol and triglyceride levels were determined by enzymatic methods using a Technicon RA-500 analyzer (Bater, Tarrytown, NY). Extensive quality control procedures were implemented to ensure high-quality lipid assays and other study data (10).

Determination of genotypes. The PPARδ single nucleotide polymorphisms (SNPs) were selected from the National Center for Biotechnology Information dbSNP database: a C/T transition of nucleotide 15 in the exon 4 (exon 4 +15 C/T; dbSNP rs2016520) and an A/G transition of nucleotide 65 in the exon 7 (exon 7 +65 A/G; dbSNP rs2076167). The exon 4 +15 C/T SNP is located 87 nucleotides upstream of the start codon and has been shown to affect transcriptional activity of the PPARδ promoter by inducing a binding site for Sp-1 transcription factor (20). The exon 7 +65 A/G SNP is a silent variant in codon 163 (encodes asparagine residue). The SNPs were genotyped using a primer extension method with template-directed incorporation with fluorescence polarization detection. Changes in fluorescence polarization after excitation of the samples by plane-polarized light were measured using a Victor^2 FP Plate Reader (PerkinElmer Life Sciences). The allele calling was done using the SNPscorer genotyping software (PerkinElmer Life Sciences). Haplotypes were constructed using the MERLIN software package (2).

Statistical analyses. A χ^2 test was used to verify whether the observed genotype frequencies were in Hardy-Weinberg equilibrium, and the pairwise linkage disequilibrium between the SNPs was assessed by using the ldmax program available in the GOLD software package (3). Associations between the PPARδ SNPs and physical performance and plasma lipid phenotypes were analyzed by using two complementary methods: a variance components and likelihood ratio test-based procedure in the QTDT software package (1), and a MIXED model-based procedure in the SAS software package. Associations with the haplotypes were analyzed by using the QTDT total association model. Baseline $\dot{V}O_{2\,max}$ and $\dot{W}_{max}$ phenotypes were adjusted for age, sex, and body weight, and baseline plasma lipid phenotypes were adjusted for age, sex, and BMI. The training response phenotypes were adjusted for age, sex, and body weight ($\dot{V}O_{2\,max}$ and $\dot{W}_{max}$) or BMI (plasma lipids) and the baseline value of the phenotype.

The total association model of the QTDT software utilizes a variance-components framework to combine a phenotypic means model and the estimates of additive genetic, residual genetic, and residual environmental variances from a variance-covariance matrix

Table 1. *Baseline characteristics of the subjects*

	Black		White	
	Men	Women	Men	Women
Number of subjects	89	183	231	247
Age, yr	33 (SD 12)	33 (SD 11)	36 (SD 15)	35 (SD 15)
$\dot{V}o_{2max}$, ml/min	2,751 (SD 492)	1,752 (SD 372)	3,032 (SD 583)	1,916 (SD 349)
$\dot{V}o_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	34 (SD 7)	24 (SD 6)	37 (SD 9)	30 (SD 7)
W_{max}, W	200 (SD 37)	124 (SD 29)	226 (SD 51)	143 (SD 32)
W_{max}, W/kg	2.46 (SD 0.61)	1.72 (SD 0.49)	2.77 (SD 0.78)	2.22 (SD 0.64)
BMI, kg/m^2	27.4 (SD 5.6)	28.3 (SD 6.5)	26.6 (SD 4.9)	25.1 (SD 5.0)
FM, kg	20.8 (SD 11.8)	28.1 (SD 12.8)	20.0 (SD 10.9)	21.0 (SD 10.8)
%Fat	23.0 (SD 8.4)	36.1 (SD 8.8)	22.7 (SD 9.0)	30.0 (SD 9.8)
FFM, kg	64.2 (SD 9.1)	46.3 (SD 6.29)	63.5 (SD 7.9)	45.5 (SD 5.2)
HDL-C, mmol/l	0.99 (SD 0.32)	1.13 (SD 0.28)	0.93 (SD 0.20)	1.14 (SD 0.26)
HDL_2-C, mmol/l	0.28 (SD 0.22)	0.40 (SD 0.21)	0.27 (SD 0.12)	0.43 (SD 0.19)
HDL_3-C, mmol/l	0.71 (SD 0.14)	0.73 (SD 0.15)	0.66 (SD 0.12)	0.72 (SD 0.13)
Apo A-1, g/l	1.13 (SD 0.15)	1.16 (SD 0.17)	1.14 (SD 0.16)	1.21 (SD 0.17)
ApoB, g/l	0.83 (SD 0.25)	0.78 (SD 0.21)	0.90 (SD 0.25)	0.82 (SD 0.22)
LDL-C, mmol/l	2.94 (SD 0.83)	2.79 (SD 0.73)	3.06 (SD 0.85)	2.92 (SD 0.77)
TG, mmol/l	1.24 (SD 0.77)	0.91 (SD 0.43)	1.55 (SD 0.90)	1.20 (SD 0.60)

Values are means (SD). $\dot{V}o_{2max}$, maximal oxygen consumption; $\dot{W}_{max}$, maximal work output; BMI, body mass index; FM, fat mass; FFM, fat-free mass; C, cholesterol; Apo, apolipoprotein; TG, triglyceride.

into a single likelihood model (1). The evidence of association is evaluated by maximizing the likelihoods under two conditions: the null hypothesis (L_0) restricts the additive genetic effect of the marker locus to zero ($\beta_a = 0$), whereas the alternative hypothesis does not impose any restrictions to β_a. The quantity of twice the difference of the log likelihoods between the null and the alternative hypotheses $\{2[\ln(L_1) - \ln(L_0)]\}$ is distributed as χ^2 with 1 difference in number of parameters estimated (df). A dominance effect can be tested in a similar manner, but the alternative hypothesis model includes estimates for both additive (β_a) and dominance ($\beta_a \times \beta_a$) genetic effects, and the likelihood-ratio test is based on χ^2 distribution with 2 df (1). In the MIXED model, nonindependence among family members was adjusted for using a "sandwich estimator," which asymptotically yields the same parameter estimates as ordinary least squares or regression methods, but the standard errors and consequently hypothesis tests are adjusted for the dependencies. The method is general, assuming the same degree of dependency among all members within a family.

Since multiple SNPs were used for the association studies, we applied a multiple testing correction proposed by Nyholt (17). Briefly, the method utilizes spectral decomposition of matrixes of pairwise linkage disequilibriums (r) to estimate variance of eigenvalues. The effective number of independent SNPs can be calculated based on the ratio of observed eigenvalue variance and its maximum. The effective number of SNPs can then be used to adjust the standard alpha level (e.g., 5%). Since LD between the PPARδ SNPs differed between black and white subjects, the effective number of SNPs was also different. Consequently, $P < 0.029$ in black subjects and $P < 0.035$ in white subjects were used to reflect statistical significance.

RESULTS

The baseline characteristics of the subjects are presented in Table 1. The frequencies of the exon 4 +15 C and exon 7 +65 G alleles were 0.209 and 0.267 in white subjects and 0.280 and 0.515 in black subjects, respectively. There were no differences in allele and genotype frequencies between men and women. Linkage disequilibrium between the markers was $r^2 = 0.578$ ($D' = 0.894$) in white subjects and $r^2 = 0.278$ ($D' = 0.855$) in black subjects. The genotype frequencies were in Hardy-Weinberg equilibrium in both ethnic groups.

The PPARδ SNPs were not associated with baseline $\dot{V}O_{2\,max}$ or $\dot{W}_{max}$ in black or white subjects (data not shown). In black subjects, the exon 4 +15 C/C homozygotes had smaller training-induced increases in $\dot{V}O_{2\,max}$ and $\dot{W}_{max}$ than the C/T heterozygotes and the T/T homozygotes (Fig. 1, *A* and *B*). A similar pattern in the $\dot{W}_{max}$ training response was also observed in white subjects: both the exon 4 +15 C/C and the exon 7 +65 G/G homozygotes showed a tendency for a smaller increase in $\dot{W}_{max}$ than the other genotypes (Fig. 1, *B* and *D*). The baseline or training response phenotypes of body composition were not associated with the PPARδ SNPs in black or white subjects.

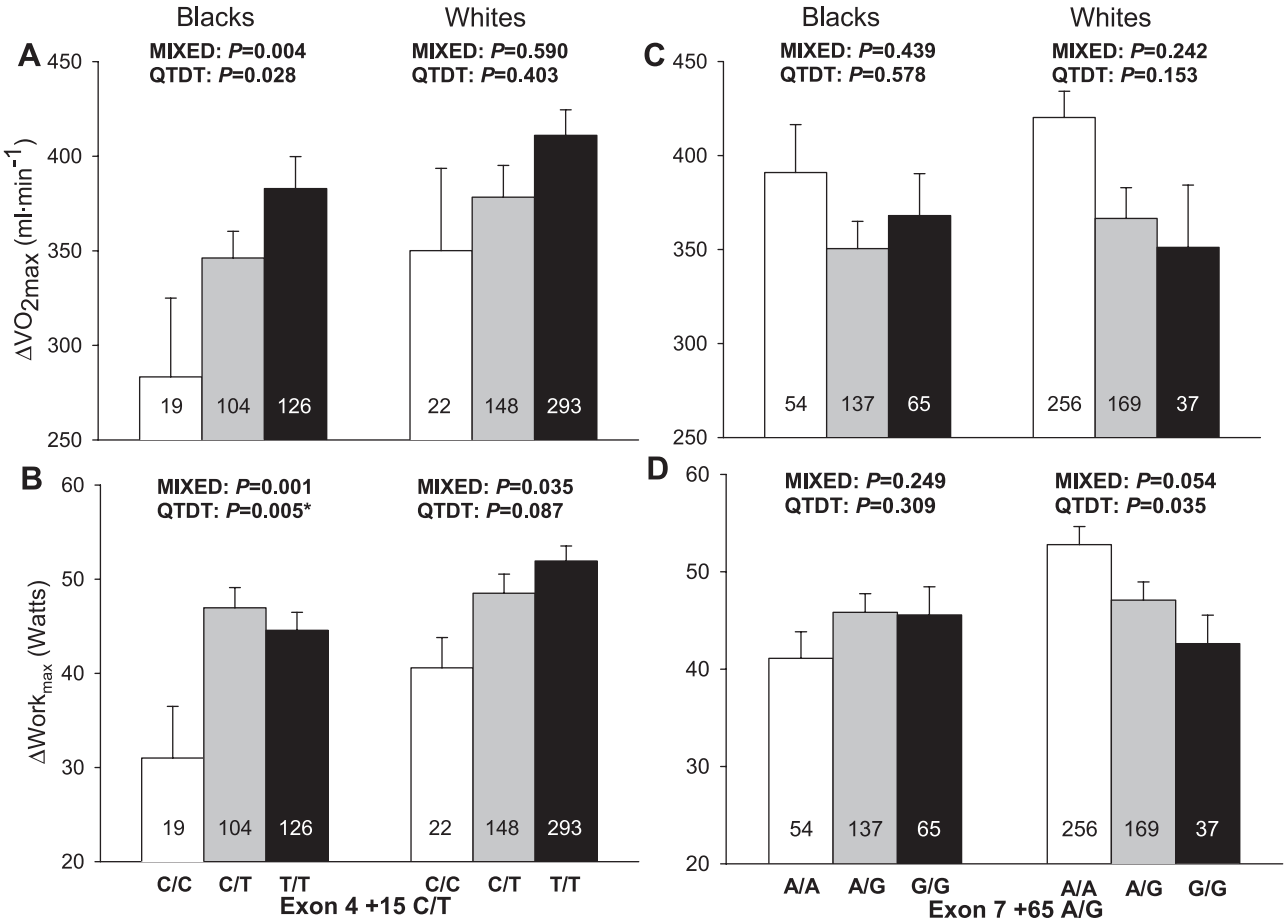

Fig. 1. Exercise training-induced changes in maximal oxygen consumption ($\Delta\dot{V}O_{2\,max}$, ml/min) and maximal power output ($\Delta\dot{W}_{max}$, W) according to the peroxisome proliferator-activated receptor-δ (PPARδ) exon 4 +15 C/T (*A* and *B*) and exon 7 +65 A/G (*C* and *D*) polymorphisms in black and white subjects after adjustment for age, sex, body weight, and the baseline value of the response phenotype. *P* values are derived from the MIXED and QTDT models. *Dominance model. Number of subjects for each genotype is shown within bars.

Table 2. *Plasma lipid phenotypes in the sedentary state and their responses to a 20-wk endurance training program according to the PPARδ exon 4 +15 C/T genotypes in white subjects*

	PPARδ Exon 4 +15 C/T Genotype			*P* Value	
	C/C	C/T	T/T	QTDT	MIXED
n	22/22	155/148	300/293		
HDL-C, mmol/l					
Baseline	1.10 (SD 0.28)	1.06 (SD 0.27)	1.02 (SD 0.25)	0.427	0.423
Response	0.09 (SD 0.14)	0.06 (SD 0.13)	0.03 (SD 0.10)	0.0025	0.0068
HDL_2-C, mmol/l					
Baseline	0.38 (SD 0.21)	0.36 (SD 0.19)	0.34 (SD 0.17)	0.624	0.631
Response	0.05 (SD 0.10)	0.04 (SD 0.11)	0.02 (SD 0.11)	0.049	0.116
HDL_3-C, mmol/l					
Baseline	0.72 (SD 0.15)	0.71 (SD 0.13)	0.68 (SD 0.13)	0.155	0.252
Response	0.04 (SD 0.10)	0.02 (SD 0.10)	0.01 (SD 0.10)	0.049	0.031
Apo A-1, g/l					
Baseline	1.22 (SD 0.20)	1.21 (SD 0.17)	1.16 (SD 0.17)	0.012	0.011
Response	0.04 (SD 0.11)	0.04 (SD 0.12)	0.04 (SD 0.11)	0.170	0.331
LDL-C, mmol/l					
Baseline	3.02 (SD 0.79)	3.01 (SD 0.85)	3.01 (SD 0.81)	0.435	0.527
Response	0.04 (SD 0.43)	0.04 (SD 0.38)	−0.02 (SD 0.37)	0.070	0.128
ApoB, g/l					
Baseline	0.85 (SD 0.20)	0.88 (SD 0.26)	0.86 (SD 0.23)	0.199	0.266
Response	0.03 (SD 0.11)	0.01 (SD 0.10)	0.01 (SD 0.11)	0.108	0.240
TG, mmol/l					
Baseline	1.28 (SD 0.71)	1.46 (SD 0.93)	1.35 (SD 0.72)	0.183	0.334
Response	−0.09 (SD 0.43)	−0.02 (SD 0.44)	−0.01 (SD 0.42)	0.330	0.643

Values are means (SD); *n,* number of subjects (baseline/training response). Baseline phenotypes are adjusted for age, sex, and BMI. Training response phenotypes are adjusted for age, sex, baseline value of phenotype, and BMI. PPARδ, peroxisome proliferator-activated receptor-δ.

In white subjects, baseline Apo A-1 levels were higher in the exon 4 +15 C/C homozygotes than in the other exon 4 +15 genotypes ($P = 0.012$), whereas in black subjects the same genotype tended to have higher baseline HDL-C levels (Tables 2, 3, 4, and 5). The other baseline blood lipid phenotypes did not differ among the PPARδ genotypes. In white subjects, both PPARδ SNPs were associated with the HDL-C training responses ($P = 0.0025$ and $P = 0.011$, Tables 2 and 4). The exon 4 +15 C/C homozygotes increased their HDL-C levels three times more compared with the T/T homozygotes, whereas the exon 7 +65 G/G homozygotes showed 2.3 times greater HDL-C response than the A/A homozygotes. Also, the

Table 3. *Plasma lipid phenotypes in the sedentary state and their responses to a 20-wk endurance training program according to the PPARδ exon 4 +15 C/T genotypes in black subjects*

	PPARδ Exon 4 +15 C/T Genotype			*P* Value	
	C/C	C/T	T/T	QTDT	MIXED
n	21/19	106/104	137/126		
HDL-C, mmol/l					
Baseline	1.14 (SD 0.24)	1.12 (SD 0.35)	1.05 (SD 0.29)	0.039	0.057
Response	0.05 (SD 0.15)	0.03 (SD 0.13)	0.03 (SD 0.13)	0.442	0.960
HDL_2-C, mmol/l					
Baseline	0.42 (SD 0.21)	0.38 (SD 0.26)	0.35 (SD 0.20)	0.101	0.204
Response	0.06 (SD 0.13)	0.04 (SD 0.12)	0.04 (SD 0.11)	0.543	0.907
HDL_3-C, mmol/l					
Baseline	0.73 (SD 0.13)	0.74 (SD 0.14)	0.70 (SD 0.16)	0.091	0.053
Response	−0.02 (SD 0.11)	−0.01 (SD 0.11)	−0.01 (SD 0.11)	0.345	0.612
Apo A-1, g/l					
Baseline	1.16 (SD 0.16)	1.17 (SD 0.16)	1.13 (SD 0.17)	0.310	0.370
Response	0.06 (SD 0.10)	0.01 (SD 0.11)	0.01 (SD 0.13)	0.057	0.034
LDL-C, mmol/l					
Baseline	2.87 (SD 0.67)	2.78 (SD 0.73)	2.89 (SD 0.79)	0.286	0.282
Response	−0.01 (SD 0.41)	0.03 (SD 0.30)	0.01 (SD 0.41)	1.000	0.939
ApoB, g/l					
Baseline	0.79 (SD 0.19)	0.78 (SD 0.21)	0.82 (SD 0.24)	0.118	0.210
Response	0.01 (SD 0.11)	0.02 (SD 0.10)	0.01 (SD 0.11)	0.320	0.661
TG, mmol/l					
Baseline	0.91 (SD 0.38)	1.03 (SD 0.58)	1.06 (SD 0.67)	0.532	0.345
Response	0.02 (SD 0.33)	−0.04 (SD 0.29)	−0.02 (SD 0.50)	0.889	0.838

Values are means (SD); *n,* number of subjects (baseline/training response). Baseline phenotypes are adjusted for age, sex, and BMI. Training response phenotypes are adjusted for age, sex, baseline value of phenotype, and BMI.

Table 4. *Plasma lipid phenotypes in the sedentary state and their responses to a 20-wk endurance training program according to the PPARδ exon 7 +t65 A/G genotypes in white subjects*

	PPARδ Exon 7 +65 A/G Genotype			*P* Value	
	A/A	A/G	G/G	QTDT	MIXED
n	261/256	179/169	38/37		
HDL-C, mmol/l					
Baseline	1.03 (SD 0.25)	1.06 (SD 0.26)	1.06 (SD 0.25)	0.303	0.313
Response	0.03 (SD 0.10)	0.05 (SD 0.13)	0.07 (SD 0.12)	0.011	0.032
HDL_2-C, mmol/l					
Baseline	0.34 (SD 0.18)	0.35 (SD 0.18)	0.35 (SD 0.19)	0.920	0.938
Response	0.01 (SD 0.11)	0.04 (SD 0.12)	0.03 (SD 0.08)	0.015	0.058
HDL_3-C, mmol/l					
Baseline	0.68 (SD 0.13)	0.70 (SD 0.13)	0.71 (SD 0.13)	0.120	0.213
Response	0.02 (SD 0.10)	0.01 (SD 0.10)	0.04 (SD 0.10)	0.279	0.064
Apo A-1, g/l					
Baseline	1.17 (SD 0.16)	1.19 (SD 0.18)	1.22 (SD 0.17)	0.050	0.043
Response	0.04 (SD 0.11)	0.04 (SD 0.12)	0.02 (SD 0.09)	0.442	0.640
LDL-C, mmol/l					
Baseline	3.05 (SD 0.82)	2.91 (SD 0.84)	3.12 (SD 0.78)	1.000	0.340
Response	−0.03 (SD 0.38)	0.04 (SD 0.37)	0.03 (SD 0.38)	0.121	0.243
ApoB, g/l					
Baseline	0.87 (SD 0.24)	0.84 (SD 0.25)	0.90 (SD 0.25)	0.729	0.355
Response	0.01 (SD 0.11)	0.01 (SD 0.11)	0.01 (SD 0.11)	0.192	0.267
TG, mmol/l					
Baseline	1.38 (SD 0.73)	1.34 (SD 0.77)	1.45 (SD 0.92)	0.806	0.775
Response	−0.01 (SD 0.40)	−0.04 (SD 0.43)	−0.07 (SD 0.42)	0.348	0.801

Values are means (SD); *n,* number of subjects (baseline/training response). Baseline phenotypes are adjusted for age, sex, and BMI. Training response phenotypes are adjusted for age, sex, baseline value of phenotype, and BMI.

HDL_3-C tended to increased more ($P = 0.049$) among the exon 4 +15 C/C homozygotes compared with the other genotypes. In black subjects, the exon 4 +15 C/C homozygotes tended to have a greater increase in Apo A-1 levels ($P = 0.057$), whereas the training-induced changes in all other blood lipid phenotypes were similar across genotypes at both markers.

The PPARδ haplotypes were constructed using the "best" option of the Merlin software haplotyping function. We were able to determine the haplotype phase in 228 black and 461 white subjects. The haplotype analyses confirmed the associations detected with individual SNPs (Table 6). In white subjects, homozygotes for the haplotype consisting of the exon 4

Table 5. *Plasma lipid phenotypes in the sedentary state and their responses to a 20-wk endurance training program according to the PPARδ exon 7 +65 A/G genotypes in black subjects*

	PPARδ Exon 7 +65 A/G Genotype			*P* Value	
	A/A	A/G	G/G	QTDT	MIXED
n	61/54	142/137	69/65		
HDL-C, mmol/l					
Baseline	1.03 (SD 0.29)	1.09 (SD 0.26)	1.15 (SD 0.40)	0.041	0.169
Response	0.03 (SD 0.11)	0.03 (SD 0.14)	0.03 (SD 0.13)	0.920	0.928
HDL_2-C, mmol/l					
Baseline	0.34 (SD 0.21)	0.36 (SD 0.19)	0.42 (SD 0.30)	0.254	0.649
Response	0.04 (SD 0.09)	0.04 (SD 0.12)	0.05 (SD 0.12)	0.603	0.606
HDL_3-C, mmol/l					
Baseline	0.69 (SD 0.16)	0.73 (SD 0.14)	0.73 (SD 0.16)	0.066	0.118
Response	−0.01 (SD 0.12)	−0.01 (SD 0.11)	−0.02 (SD 0.12)	0.729	0.639
Apo A-1, g/l					
Baseline	1.12 (SD 0.15)	1.16 (SD 0.17)	1.16 (SD 0.17)	0.209	0.227
Response	0.02 (SD 0.11)	0.01 (SD 0.12)	0.03 (SD 0.12)	0.325	0.157
LDL-C, mmol/l					
Baseline	2.88 (SD 0.81)	2.83 (SD 0.75)	2.88 (SD 0.73)	0.888	0.783
Response	0.02 (SD 0.45)	0.01 (SD 0.35)	0.01 (SD 0.33)	0.841	0.938
ApoB, g/l					
Baseline	0.81 (SD 0.24)	0.80 (SD 0.22)	0.80 (SD 0.22)	1.000	0.892
Response	0.01 (SD 0.13)	0.01 (SD 0.10)	0.02 (SD 0.10)	0.689	0.246
TG, mmol/l					
Baseline	1.06 (SD 0.55)	1.03 (SD 0.65)	0.99 (SD 0.54)	0.888	0.776
Response	−0.01 (SD 0.44)	−0.03 (SD 0.44)	−0.06 (SD 0.28)	0.454	0.572

Values are means (SD); *n,* number of subjects (baseline/training response). Baseline phenotypes are adjusted for age, sex, and BMI. Training response phenotypes are adjusted for age, sex, baseline value of phenotype, and BMI.

Table 6. *Summary of the associations between PPARδ haplotypes and fitness and lipid training responses in the HERITAGE Family Study*

	Haplotype			
Trait	CA	CG	TA	TG
White				
$\dot{V}o_{2max}$	0.507	0.377	0.362	0.59
$\dot{W}_{max}$	0.527	0.146	**0.028**	0.187
HDL-C	1.000	**0.003**	**0.012**	0.862
HDL_2-C	0.233	**0.019**	0.077	1.000
HDL_3-C	0.216	0.139	0.114	0.92
Apo A-1	0.862	0.24	0.406	0.777
TG	0.204	0.128	0.195	0.484
Black				
$\dot{V}o_{2max}$	0.698	0.041	0.442	0.171
$\dot{W}_{max}$	0.92	0.216	0.337	**0.011**
HDL-C	0.386	0.689	0.764	0.777
HDL_2-C	1.000	0.393	0.603	0.777
HDL_3-C	0.159	0.920	0.532	0.764
Apo A-1	0.940	0.267	0.620	0.240
TG	0.362	0.729	0.467	0.458

P values are from total association model of the QTDT software (boldface values indicate statistical significance). See Fig. 2 for details regarding HDL-C and $\dot{W}_{max}$ results in white and black subjects, respectively. Haplotype symbols refer to alleles at the exon 4 +15 and exon 7 +65 loci, respectively.

and exon 7 minor alleles (CG/CG; i.e., the exon 4 +15 C/C homozygotes) showed the greatest increase in plasma HDL-C levels, whereas the exon 4 +15 T/T homozygotes, regardless of alleles in the exon 7 +65 locus (TA/TA and TA/TG haplotypes), had the smallest HDL-C training response ($P = 0.0034$; Fig. 2). Among the exon 4 +15 C/T heterozygotes, the HDL-C training response varied to some extent depending on the exon 7 locus genotype: exon 7 G/G homozygotes (CG/TG) and G/A heterozygotes (CG/TA) showed similar increases in HDL-C as the CG/CG homozygotes, whereas the exon 7 A/A homozygotes (haplotype CA/TA) had HDL response similar to the TA/TA and TA/TG haplotypes. In black subjects, the $\dot{W}_{max}$ training response was also associated with the haplotype ($P = 0.011$; Fig. 2). The CG/CG homozygotes had the smallest improvement in $\dot{W}_{max}$ levels, whereas the homozygotes for the TG haplotype showed the greatest training-induced increases in $\dot{W}_{max}$. Changes in $\dot{W}_{max}$ among the remaining haplotype groups were intermediate to those seen in the CG and TG homozygotes.

DISCUSSION

The main finding of the present study is that DNA sequence variation in the PPARδ gene locus is associated with endurance training-induced changes in cardiorespiratory fitness and plasma HDL-C levels in healthy, but previously sedentary, individuals. The previous studies have shown that considerable heterogeneity occurs in the responsiveness of cardiorespiratory fitness and HDL-C levels to regular endurance training (5, 11). The novel feature of the present study is the contribution of the PPARδ gene locus to endurance training-induced changes in maximal physical performance and plasma lipid profile. These results are interesting from a health point of view because even though the exon 4 +15 C/C homozygotes had quite small training-induced improvements in cardiorespiratory fitness, they showed the greatest increases in HDL-C (white subjects) and Apo A-1 (black subjects) levels. We have previously shown that the changes in cardiorespiratory fitness are not related to changes in HDL-C or other blood lipids (12, 15). The results of the present study highlight the possibility that genetic variation may contribute to the "uncoupling" of the fitness and plasma lipid training responses and that health benefits can accrue from regular endurance training independently of the changes in maximal exercise capacity.

Overexpression of an activated form of PPARδ has been shown to coordinate the responses of oxidative enzymes, mitochondrial biogenesis, and type 1 muscle fiber contractile proteins in mice (16, 22, 24, 25). Skeletal muscle-specific expression of active PPARδ in transgenic mice and PPARδ agonist treatment in wild-type mice increased numbers of type 1 muscle fibers (25). Cheng et al. (8) has reported that PPARδ is a crucial determinant of cardiac fatty acid oxidation in mice and is necessary to maintain energy balance and normal cardiac function. These results indicate that PPARδ and its ligands may play a central role in the regulation of muscle fiber type distribution, endurance performance, and normal cardiac function. Furthermore, it has been suggested that exercise training

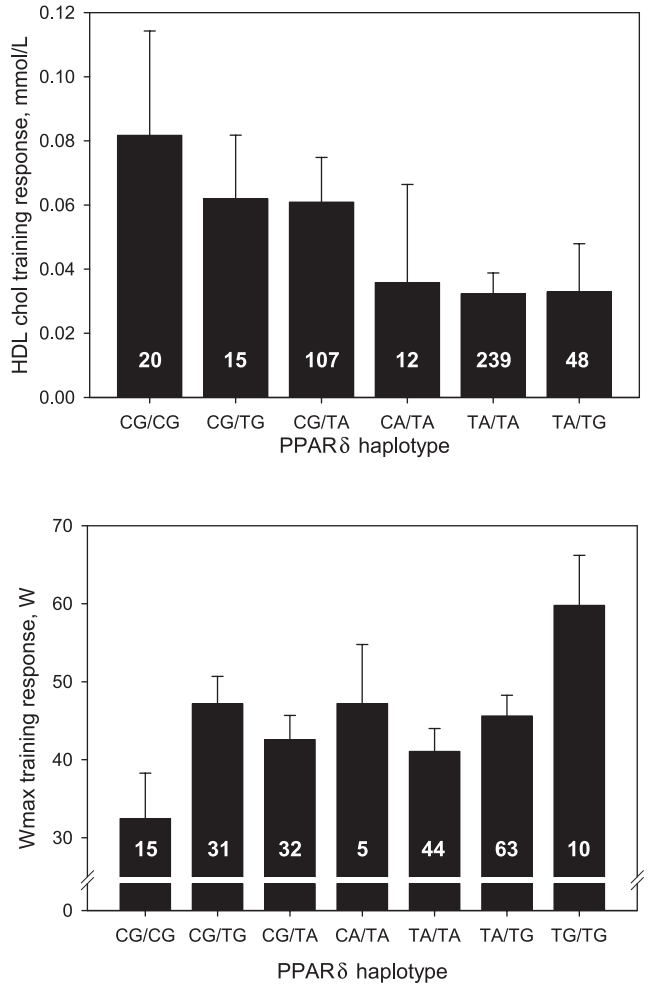

Fig. 2. Endurance training-induced changes in plasma HDL cholesterol (Chol) in white (*top*) and $\dot{W}_{max}$ in black (*bottom*) subjects according to the PPARδ haplotype in the HERITAGE Family Study.

itself activates endogenous ligands for PPARδ as tissues undergo marked increases in fatty acid oxidation (25). In addition, exercise may activate PPARδ by increasing the expression of a transcriptional coactivator PGC-1α (24). Therefore, the results of the present study suggest that PPARδ gene sequence variation could explain some of the interindividual variations in exercise training-induced changes in cardiorespiratory endurance.

A novel finding of the present study is that the PPARδ exon 4 +15 C/T polymorphism, which is located 87 base pairs before the start codon, is associated with training-induced changes in plasma HDL-C. Given that the PPARδ is a transcription factor, the mechanistic model that would explain our findings would require *1*) an exercise-induced increase in PPARδ ligand(s), *2*) an effect of the DNA sequence variants on PPARδ gene function, and *3*) a contribution of PPARδ to the expression of genes involved in HDL-C metabolism. Fatty acids are well-known endogenous ligands of PPARδ, and since exercise increases lipolysis and the availability of free fatty acids (especially in skeletal muscle), this could explain the first condition of the model. It has been documented that the exon 4 +15 C/T polymorphism affects the promoter activity of the PPARδ gene. Constructs containing the C allele had significantly greater promoter activity than those with the T allele (20). The EMSA experiments showed that the nuclear extract proteins bound specifically to DNA probes containing the C allele. Supershift analyses further confirmed that the C allele creates a binding site for transcription factor Sp-1 (20).

Treatment with a PPARδ-specific agonist GW-501516 has been reported to increase plasma HDL-C levels in animal models (18, 23). The same agonist also increased the expression of the ABCA1 gene, which is a key regulator of reverse cholesterol transport, as well as a Apo A-1-specific cholesterol efflux in human macrophages, fibroblasts, and intestinal cell lines (18). These observations were confirmed in the skeletal muscle cell line (9). Thus it is possible that the greater promoter activity of the PPARδ exon 4 +15 C/C homozygotes could result in higher PPARδ levels, which, in the presence of exercise-induced elevated ligand availability, would increase ABCA1 expression and, consequently, activate reverse cholesterol transport. Naturally, this hypothesis must be tested in future studies.

In conclusion, these data from the HERITAGE Family Study suggest that DNA sequence variation in the PPARδ locus is a potential modifier of endurance training-induced changes in cardiorespiratory fitness and plasma HDL-C in healthy, but previously sedentary, subjects.

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