Evidence of LPL gene-exercise interaction for body fat and LPL activity: the HERITAGE Family Study

CHRISTOPHE GARENC,1 LOUIS PÉRUSSE,1 JEAN BERGERON,2 JACQUES GAGNON,1,3 YVON C. CHAGNON,1 INGRID B. BORECKI,4 ARTHUR S. LEON,5 JAMES S. SKINNER,6 JACK H. WILMORE,7 D. C. RAO,4,8 AND CLAUDE BOUCHARD9

1Division of Kinesiology, Department of Preventive Medicine, Laval University, 2Lipid Research Center and 3Laboratory of Molecular Endocrinology, Laval University Medical Research Center, St-Foy, Quebec, Canada G1K 7P4; 4Division of Biostatistics and 5Departments of Genetics and Psychiatry, Washington University Medical School, St. Louis, Missouri 63110; 5School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis, Minnesota 55454; 6Department of Kinesiology, Indiana University, Bloomington, Indiana 47405; 7Department of Health and Kinesiology, Texas A & M University, College Station, Texas 77843; and 9Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana 70803

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IT IS WELL DOCUMENTED that endurance training can reduce adiposity (9, 41, 42). Also, in response to endurance training, there is often a reduction of abdominal visceral fat (AVF), which is associated with improvement of risk factors for coronary heart disease (CHD) such as insulin resistance and dyslipidemia (10). In the HERITAGE Family Study, 20 wk of endurance training resulted, on average, in significant reduction in body mass and body adiposity characterized by gender and race differences (42). However, there were considerable interindividual differences in the changes induced by exercise training. A few twin and family studies (3, 27–30) have shown that these interindividual differences can be attributed, in part, to genetic factors. Thus a study performed with monozygotic twins has shown a significant within-pair similarity in body fat and AVF changes in response to regular exercise (3). Moreover, HERITAGE Family Study reports have concluded that body composition and AVF and abdominal subcutaneous fat (ASF), as well as their responses to endurance training, tend to aggregate in families (27–30).

Lipoprotein lipase (LPL) is the enzyme responsible for the hydrolysis of triglyceride (TG)-rich lipoproteins and, therefore, plays an important role in directing free fatty acids toward adipose and muscle tissues. In the HERITAGE Family Study, the generally more cardio-protective plasma lipoprotein profile observed in black than in white subjects could be attributed, at least in part, to the higher post-heparin LPL (PH-LPL) activity measured in black individuals (7). The studies on the effect of endurance training on PH-LPL activity have yielded inconsistent results. In 13 premenopausal obese women, PH-LPL activity did not change after a 14-mo exercise-training program (8); in another study (20), an increase was observed after only 6 mo of exercise training.

Results from a few studies suggest that LPL activity could be under the influence of genetic factors. In a recent report, PH-LPL activity was significantly influenced by genetic factors, with heritability estimates of
76% in women and 30% in men (26). Large interindividual variation was observed in the adipose tissue LPL activity measured in men in response to exercise (34). Results from a twin study suggested that the changes in adipose tissue LPL activity in response to acute exercise were more similar in monzygotic than in dizygotic twins, suggesting that the response is influenced by genetic factors (33).

A large number of polymorphisms have been described in the human LPL gene (23). One of these polymorphisms is the S447X (Ser447Ter) gene variant located in exon 9 of the gene, which produces an LPL that lacks the COOH-terminal Ser-Gly dipeptide (15, 16). This polymorphism has been shown, at least in some populations, to be associated with a reduced risk of premature CHD (11, 12, 17). Recently, we reported that the S447X polymorphism was associated with reduced levels of TGs and very-low-density lipoprotein TGs, but only in obese, and not in normal-weight, subjects (13). Despite evidence of association between this polymorphism and CHD risk factors, its interaction with exercise training has not been investigated. Because endurance training contributes to a decrease in CHD risk factors (for review see Ref. 10), we hypothesized that the LPL S447X polymorphism could be associated with changes in body fatness and PH-LPL activity in response to 20 wk of endurance training. Finally, we examined whether the correlations between the changes in body fatness and PH-LPL activity were different among carriers and noncarriers of the X447 allele.

MATERIALS AND METHODS

Subjects

Subjects were participants in the HERITAGE Family Study, a multicenter study designed to investigate the role of genetic factors in the cardiovascular and metabolic adaptations to 20 wk of endurance training in white and black families. A total of 259 biologically unrelated sedentary white (93 men and 91 women) and black (25 men and 50 women) subjects from the parental generation and 297 white (140 men and 91 women) and black (25 men and 50 women) sons and 157 daughters) sedentary adult offspring completed the study. They had been subjected to a battery of measurements before and after an endurance-training program. All subjects were required to be in good health to participate in the HERITAGE Family Study protocol and to meet a set of inclusion criteria as summarized elsewhere (2). The institutional review board of each university in the HERITAGE Family Study protocol had approved the protocol. Written informed consent was obtained from each participant.

Endurance-Training Program

Briefly, subjects exercised under supervision on a cycle ergometer (Aerobicycle IV, Universal, Cedar Rapids, IA) three times per week for 20 wk following a standardized protocol. The cycle ergometer was connected to a computer system (Mednet, Universal) that adjusted the power output to ensure that the target heart rates were maintained. For the first 2 wk, subjects trained at a heart rate associated with 55% of their baseline (pretraining) maximal oxygen consumption for 30 min per session. This was gradually increased to 50 min at a heart rate associated with 75% of their baseline maximal oxygen consumption by the end of 14 wk. These conditions were maintained through the remaining 6 wk of the program (2).

Phenotype Measurements

Anthropometric and body density measurements. These measurements have been described in detail previously (42). Body mass index (BMI) was calculated as weight (kg)/height² (m²). The sum of eight skinfolds (subscapular, suprailiac, abdominal, midaxillary, biceps, triceps, medial calf, and thigh) was used to assess the level of subcutaneous fat. Hydrostatic weighing was used to assess body density. Percent body fat was estimated from body density as described elsewhere (43), and fat mass and fat-free mass were derived. AVF, ASF, and total abdominal fat areas. Abdominal fat was assessed by computed tomography, as previously described (29). Scans were obtained between L₄ and L₅. AVF area was defined by drawing a line within the inner portion of the muscle walls surrounding the abdominal cavity. ASF area was obtained by calculating the difference between total abdominal fat (ATF) and AVF areas.

LPL activity. Blood samples were collected after a 12-h overnight fast and 10 min after an intravenous injection of heparin (60 IU/kg body wt). The PH-LPL activity was measured using a modification of the method of Nilsson-Ehle and Ekman (25), as previously described (38). Activities were expressed as nanomoles of oleic acid released per milliliter of plasma per minute.

Genotype Determination

The genotyping of the LPL S447X polymorphism has been described in detail previously (13). Briefly, the primers used for PCR amplification generated a PCR DNA fragment of 137 bp that was cut into two fragments of 117 and 20 bp in the presence of the HinfI cutting site (39). After the amplification, the PCR product was digested overnight at 37°C after addition of 10 U of the restriction enzyme HinfI to the PCR mixture. Resulting fragments were separated by electrophoresis using 10% acrylamide gels, stained with ethidium bromide, and photographed under ultraviolet transmitted light. Here, the allele without the HinfI restriction site is designated the S447 allele (137 bp), whereas the allele with the HinfI restriction site is the X447 allele (117 + 20 bp).

Statistical Analysis

All statistical analyses were performed using SAS (32) software. A χ² test was performed to determine whether parental genotype frequencies for the S447X polymorphism were in Hardy-Weinberg equilibrium and to test for potential race differences in allelic frequencies.

For each phenotype, the response to training was computed as the difference between the pre- and posttraining values. The distribution of each resulting delta (Δ) score was tested for normality using the Shapiro-Wilk test, and all were found to be normally distributed. Association between the S447X polymorphism and each response phenotype (Δ score) was then investigated using an analysis of covariance (General Linear Model procedure of SAS) performed separately in the four race × gender groups. The covariates included in the model were the pretraining value, age, age², and age³, in addition to changes in fat mass for ΔPH-LPL, ΔATF, ΔASF, and ΔAVF. Two genotype groups, i.e., carriers (genotypes S447X and X447X) and noncarriers (genotype S447S) of the X447 allele, were considered in the analyses.
Pearson product-moment coefficients were calculated between change in fat mass and ΔPH-LPL residualized for age and pretraining values to determine whether the relationships between training-induced changes in PH-LPL activity and body fatness variables were dependent on the S447X polymorphism. The Fisher z-test was used to test for differences between correlations in carriers and noncarriers.

RESULTS

The wide range of BMI in white (17–47.5 kg/m²) and black (17.5–44.9 kg/m²) subjects indicates that normal-weight (BMI < 25 kg/m²), overweight (25 ≤ BMI < 30 kg/m²), and obese (BMI ≥ 30 kg/m²) subjects were included in the study. Endurance training resulted in a significant reduction of body fatness in men and women of both races (Table 1). However, BMI did not change significantly after 20 wk of endurance training in black and white women. Men lost more AVF than women in both races (Table 1). The increase in PH-LPL activity with endurance training was significant in white men and women (greater in men than in women) and in black women but not in black men (Table 1).

Allele and genotype frequencies of the S447X polymorphism have been reported in white subjects (13). The frequencies of the X447 allele reach 0.10 and 0.07 in white and black subjects, respectively, and were not significantly different between races [χ² = 1.15, degrees of freedom (df) = 1, P > 0.05]. The frequencies of the S447S (0.82 and 0.85 in white and black subjects, respectively), S447X (0.16 and 0.15 in white and black subjects, respectively), and X447X (0.02 and 0.0 in white and black subjects, respectively), S447X (0.16 and 0.15 in white and black subjects, respectively), and X447X (0.02 and 0.0 in white and black subjects, respectively) genotypes were in Hardy-Weinberg equilibrium in white (χ² = 0.36, df = 1, P > 0.05) and black subjects (χ² = 0.49, df = 1, P > 0.05).

The results of association analysis are presented in Tables 2 and 3 for women and men, respectively. White women carrying the X447 allele exhibited a greater reduction of BMI (P = 0.01), fat mass (P = 0.01), and percent body fat (P = 0.03) in response to training than noncarriers (Table 2). This polymorphism explained 2.6, 2.4, and 1.9% of the total variance in changes in BMI, fat mass, and percent body fat, respectively.

Table 1. Descriptive statistics of body fatness and PH-LPL activity at baseline and in response to 20 wk of endurance training by race and gender

<table>
<thead>
<tr>
<th>White Subjects</th>
<th>Black Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>Men</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.8±0.3</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>20.2±0.7</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>63.6±0.5</td>
</tr>
<tr>
<td>% Body fat</td>
<td>22.9±0.6</td>
</tr>
<tr>
<td>SF8, mm</td>
<td>132±4</td>
</tr>
<tr>
<td>AVF, cm²</td>
<td>110±4</td>
</tr>
<tr>
<td>ASF, cm²</td>
<td>232±9</td>
</tr>
<tr>
<td>ΔTF, cm²</td>
<td>342±12</td>
</tr>
<tr>
<td>PH-LPL, nmol(\text{min}^{-1}\cdot\text{ml}^{-1})</td>
<td>49.3±1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of subjects. LPL, lipoprotein lipase; BMI, body mass index; SF8, sum of 8 skinfolds (subscapular, suprailiac, abdominal, midaxillary, biceps, triceps, medial calf, thigh); AVF, abdominal visceral fat; ASF, abdominal subcutaneous fat; ΔTF, total abdominal fat; PH-LPL, post-heparin plasma LPL activity. *Significant gender difference in white subjects. **Significant gender difference in black subjects. †Significant race difference in men. ‡Significant race difference in women. Significantly different from zero: *P < 0.05, †P < 0.01, ‡P < 0.001.

Table 2. Association of the LPL S447X polymorphism with changes in body fat and PH-LPL activity in white and black women in response to endurance training

<table>
<thead>
<tr>
<th>White Women</th>
<th>Black Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncarriers of the X447</td>
<td>Carriers of the X447</td>
</tr>
<tr>
<td>ΔBMI, kg/m²</td>
<td>0.16±0.056 (305)</td>
</tr>
<tr>
<td>ΔFat mass, kg</td>
<td>-0.47±0.12 (187)</td>
</tr>
<tr>
<td>ΔFat-free mass, kg</td>
<td>0.41±0.08 (197)</td>
</tr>
<tr>
<td>Δ% Body fat</td>
<td>-0.65±0.13 (197)</td>
</tr>
<tr>
<td>ΔSF8, mm</td>
<td>-5.8±2.1 (187)</td>
</tr>
<tr>
<td>ΔAVF, cm²</td>
<td>-3.3±0.8 (193)</td>
</tr>
<tr>
<td>ΔASF, cm²</td>
<td>-8.7±5.1 (193)</td>
</tr>
<tr>
<td>ΔΔTF, cm²</td>
<td>-11.8±1.7 (193)</td>
</tr>
<tr>
<td>ΔPH-LPL, nmol(\text{min}^{-1}\cdot\text{ml}^{-1})</td>
<td>3.3±2.0 (168)</td>
</tr>
</tbody>
</table>

Values are least-squares means ± SE of number of subjects in parentheses.
Black women carrying the X447 allele exhibited a sixfold greater increase in ΔPH-LPL activity (P = 0.02) in response to training than noncarriers (Table 2). The S447X polymorphism accounted for 3.5% of the variance in ΔPH-LPL. Borderline significant association was observed for ΔAVF (P = 0.05) in black women. No significant evidence of association was observed in men (Table 3).

No significant correlations between training-induced changes in PH-LPL and body fat variables were observed in white women and black men. However, in white men, the ΔPH-LPL activity was significantly and negatively correlated with ΔAVF (r = −0.36, P = 0.02 for carriers; r = −0.19, P = 0.009 for noncarriers), ΔASF (r = −0.23, P = 0.002 for noncarriers only), and ΔATF areas (r = −0.33, P = 0.03 for carriers; r = −0.26, P = 0.0006 for noncarriers). As shown in Fig. 1, in black women, ΔPH-LPL activity was positively correlated with the change in fat mass, but only in carriers of the X447 allele (r = 0.76, P = 0.004) and not in noncarriers (r = 0.005, P = 0.96; Fisher z-test: P = 0.005). Similar results (not shown) were observed for change in percent body fat, with a significant correlation in carriers (r = 0.67, P = 0.02) and not in noncarriers (r = 0.05, P = 0.66; Fisher z-test: P = 0.03).

**DISCUSSION**

Many studies have demonstrated that moderate endurance training is sufficient to induce substantial weight loss (for review see Ref. 24). Wilmore et al. (42) reported an average decrease of 3.5 and 2.4% in percent body fat and 6.2 and 3.4% in ATF area in white and black subjects, respectively, in response to 20 wk of endurance training. Although exercise training results in a reduction of body fat on average (6, 42), interindividual variation is partly attributed to genetic factors (3). Results from recent studies suggest that body fat and fat distribution, as well as their changes in response to exercise training, are characterized by significant heritability (27–30). A recessive locus accounting for 18% of the variance in the training changes of AVF area and a dominant locus accounting for 31% of the variance in training changes of fat mass were
recently reported (30). To investigate the interaction between the LPL gene and exercise on body fat and PH-LPL, the response of these phenotypes to 20 wk of endurance training was compared between carriers and noncarriers of the X447 allele. Our results suggest that the LPL S447X polymorphism plays a role in determining the training-induced changes in body fat and PH-LPL activity, but only in women and not in men. The effect of this polymorphism also appeared to be different between races, with a greater reduction of total body fat for carriers in white women, on one hand, and a greater reduction of AVF (adjusted for changes in fat mass) with a greater increase of PH-LPL activity for carriers in black women, on the other hand. Thus, because associations were observed only in women, we can speculate that hormonal factors could be involved in the gender differences observed in the effect of the LPL S447X polymorphism on body fatness.

Studies on the effects of acute exercise on skeletal muscle LPL reveal that muscle LPL mRNA and mass increase and peak between 4 and 8 h after exercise and then begin to fall to reach the preexercise value within 20 h (36, 37). Skeletal muscle LPL activity has also been reported to increase in endurance-trained men with type 1 diabetes (5) and in healthy sedentary men trained for 5–13 consecutive days (35) or 8 wk (19). However, exercise did not affect LPL expression in adipose tissue (35), but training decreased LPL activity (20). Maurie `ge et al. (21) showed that the smaller LPL activity in the ASF of trained than nontrained women was attributed to the smaller adipose cell size, rather than the endurance-training effect per se, since abdominal subcutaneous LPL activity was positively correlated with the fat cell weight. In obese women, training significantly increased the PH-LPL activity (20). Our data suggest that 20 wk of endurance training significantly increased the PH-LPL activity in white subjects. A greater increase in PH-LPL activity was observed in white men than women. In black subjects, only women exhibited a modest but significant increase. In the present study, posttraining PH-LPL activity was measured in the morning after the last exercise test and reflected the acute effect of endurance training. In addition, PH-LPL activity measured 10 min after heparin infusion should reflect skeletal muscle LPL activity, since adipose tissue heparin-released LPL activity appears at a latter stage (4). Thus the increase of PH-LPL activity observed in the present study should mainly reflect the increase in muscle LPL expression. However, this does not exclude the possibility that a decrease in the volume of fat after endurance training could also contribute to the increase in PH-LPL activity.

It has been shown that the X447 allele was associated with higher postprandial clearance level of plasma TGs (18), low TG levels in patients with coronary artery disease (14), and low very-low-density lipoprotein TG levels in some obese subjects (13). We recently reported that the Ser447Ter polymorphism of the LPL gene was not associated with body composition or PH-LPL activity measured at baseline in white subjects (13). The results presented in this study suggest a larger increase in PH-LPL activity in black women carrying the X447 allele than in noncarriers after endurance training. We can speculate that endurance training had a different impact on the mechanisms regulating the expression of the LPL protein depending on the allele carried by the black women. Correlations between changes in PH-LPL activity and fat mass in black women suggested that the degree of fat loss influenced PH-LPL activity and that this relationship is dependent on the S447X LPL polymorphism. Thus, in black women, a greater reduction in fat mass was associated with a greater reduction in PH-LPL in carriers of the X447 allele, while no such relationship was observed in noncarriers.

The issue of gene-exercise interaction has received little attention in the field of exercise science. Recently, it has been suggested that the human genome requires an environment of physical activity to promote normal function and a state of health and that physical inactivity induced abnormal phenotypic expression of our genes (1). Very few intervention studies involving exercise have documented evidence of gene-exercise interaction. For phenotypes related to body composition, significant evidence of interaction was reported with the β3-adrenergic receptor (ADRB3) gene (31), the angiotensin-converting enzyme gene (22), and the insulin-like growth factor I gene (40). In the present study, we showed that the LPL gene could be added to the list of the few genes showing evidence of gene-exercise interaction for phenotypes related to body composition.

The fact that multiple tests were conducted and that the evidence of association is moderate raises the possibility that some of the significant results reported in this study might be due to chance. Although multiple tests were conducted, it is important to keep in mind that the phenotypes investigated are highly correlated and that we are not dealing with such a large number of independent tests. Moreover, the consistency of the results among the correlated phenotypes (e.g., BMI, fat mass, and percent body fat) and the variance accounted for by the LPL polymorphism, which ranged from 2 to 3.5%, indicate that our results are biologically meaningful.

In conclusion, the results presented in this study suggest that the LPL S447X polymorphism is associated with training-induced changes in total body fatness in white women and with changes in AVF and PH-LPL activity in black women. Further studies are needed to confirm these findings in other populations and to test whether other polymorphisms in the LPL gene could be involved in determining the changes in body fat and PH-LPL activity in response to training.

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