Brief Genetics Report

Leptin and Leptin Receptor Gene Polymorphisms and Changes in Glucose Homeostasis in Response to Regular Exercise in Nondiabetic Individuals

The HERITAGE Family Study

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We recently reported that a genomic region close to the leptin locus was linked to fasting insulin response to exercise training in nondiabetic white subjects. We tested the hypothesis that common exonic variants in the leptin (LEP) and leptin receptor (LEPR) genes modify the effects of regular physical activity on glucose homeostasis in nondiabetic whites (n = 397) and blacks (n = 143). In whites, exercise increased insulin sensitivity index (P = 0.041) and disposition index (P = 0.046) in the LEPR 109R allele carriers but not in the K109K homozygotes, increased glucose disappearance index more in the R109R homozygotes than in the K109 allele carriers (P = 0.039), and decreased fasting glucose only in the 109R allele carriers (P = 0.018). We also found an interaction between the LEP A19G and LEPR K109R polymorphisms on the change in fasting insulin in whites (P = 0.010). The association between the LEP A19G polymorphism and the change in insulin was evident only in the LEPR 109R carriers (P = 0.019). The decrease in insulin was strongest in the LEP A19A homozygotes who carried the LEPR 109R allele. Similar interaction was observed in blacks (P = 0.046). Variations in the LEP and LEPR genes are associated with the magnitude of the effects of regular exercise on glucose homeostasis in nondiabetic individuals. *Diabetes* 53: 1603–1608, 2004

Type 2 diabetes is a multifactorial disease that results from a genetic predisposition and various behavioral and environmental risk factors (1). Regular physical activity has been associated with a reduced risk of developing type 2 diabetes (2–4). Physical activity may prevent type 2 diabetes by reducing body adiposity (5,6), increasing insulin sensitivity (5,6), and improving glucose tolerance (6), whereas the effect of exercise on glucose effectiveness and pancreatic β-cell function is unknown. The HERITAGE Family Study indicates (7) that physiological responses to exercise training vary considerably among individuals and that these differences are influenced by genetic factors. However, few studies (8,9) in small numbers of subjects have investigated whether polymorphisms in the candidate genes of type 2 diabetes modify the effects of regular exercise on glucose homeostasis.

Leptin is an adipocyte-secreted hormone that regulates energy homeostasis through central and peripheral mechanisms (10,11). Leptin receptors are present in the hypothalamus but also in tissues regulating glucose homeostasis, including skeletal muscle, liver, pancreas, and adipose tissue (10,11). Leptin has been shown to stimulate glucose uptake and fatty acid oxidation in skeletal muscle (11,12), to prevent lipid accumulation in nonadipose tissues such as skeletal muscle, liver, and pancreatic β-cells (13), and to inhibit insulin secretion through leptin receptors on pancreatic β-cells (14). Mutations in the leptin gene resulting in leptin deficiency cause obesity, insulin resistance, and diabetes in animals (15) and, in rare cases, morbid obesity and hyperinsulinemia in humans (16). Leptin therapy resulted in a sustained weight

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AIR_g, acute insulin response to intravenous glucose; FSIVGT, frequently sampled intravenous glucose tolerance test.

TABLE 1
Baseline characteristics of the subjects

	Whites (n = 397)		Blacks (n = 143)	
	Men	Women	Men	Women
Age (years)	36.3 ± 15.1	34.2 ± 13.8	32.1 ± 10.5	32.2 ± 11.0
BMI (kg/m^2)	26.3 ± 4.6	24.8 ± 5.0	26.9 ± 4.9	28.2 ± 5.9
Body fat mass (%)	22.5 ± 9.1	29.5 ± 9.7	22.9 ± 7.2	36.3 ± 8.5
S_i ($\times 10^{-4}$ min^{-1} per μU/ml)	4.05 ± 2.73	4.99 ± 3.55	2.86 ± 2.50	2.79 ± 2.01
AIR_g (μU/ml)	71.8 ± 60.5	56.9 ± 38.2	158.6 ± 147.1	181.0 ± 180.5
D_i ($\times 10^{-4}$ min^{-1})	227.4 ± 176.9	236.1 ± 169.6	330.8 ± 253.6	375.1 ± 260.2
K_g (%/min)	1.52 ± 0.56	1.73 ± 0.55	1.70 ± 0.64	1.97 ± 0.69
S_g ($\times$ min^{-1})	0.015 ± 0.009	0.018 ± 0.011	0.020 ± 0.015	0.022 ± 0.013
Fasting plasma glucose (mmol/l)	5.2 ± 0.6	4.9 ± 0.5	5.2 ± 0.5	5.0 ± 0.6
Fasting plasma insulin (pmol/l)	69.0 ± 51.6	55.2 ± 30.0	70.2 ± 57.0	85.2 ± 81.0

Data are means ± SD.

reduction and improvement in insulin sensitivity in leptin-deficient individuals (17). Common polymorphisms in the human leptin (LEP) gene have been associated with obesity and leptin levels (18,19). Mutations resulting in a deficient leptin receptor cause obesity and diabetes in animals (20,21) and obesity in humans (22). Common variants in the human leptin receptor (LEPR) gene have been associated with hyperinsulinemia (23,24), type 2 diabetes (23), obesity, and leptin levels (25–28).

We recently reported (29) that a genomic region close to the LEP locus on chromosome 7q31 was linked to the fasting insulin response to exercise training in nondiabetic whites. Here, we tested the hypothesis that common exonic variants in the LEP and LEPR genes, previously associated with hyperinsulinemia, adiposity, or leptin levels (18,19,24–28), modify the effects of regular physical activity on glucose homeostasis in nondiabetic whites and blacks.

RESEARCH DESIGN AND METHODS

The HERITAGE Family Study is a multicenter exercise training study, the main objective of which is to assess the role of genetic factors in cardiovascular, metabolic, and hormonal responses to aerobic exercise training in sedentary families (30). The study design, sampling, and inclusion and exclusion criteria have been described in detail previously (30). In brief, the offspring were required to be aged ≥17 years and the parents aged ≤65 years. The subjects were also required to be sedentary, defined as not having engaged in regular physical activity over the previous 6 months, and free of diabetes, cardiovascular diseases, or other chronic diseases that would prevent their participation in an exercise training program. The study protocol was approved by each of the institutional review boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant. Data on measures of insulin and glucose metabolism were available for 596 individuals. The present analyses included 397 whites (200 men and 197 women) and 143 blacks (61 men and 82 women) for whom complete data were available on all variables.

Exercise training program. The 20-week exercise training program has been described in detail previously (29,30). Briefly, the subjects trained three times per week on cycle ergometers in the laboratory. Exercise intensity was customized for each subject based on the relationship between heart rate and oxygen uptake measured at baseline. During the first 2 weeks, the subjects trained for 30 min per session at a heart rate corresponding to 55% of the baseline $V\text{O}_{2max}$. Duration was gradually increased to 50 min per session, and heart rate was increased to the level corresponding to 75% of the baseline $V\text{O}_{2max}$. This level was sustained for the last 6 weeks. Heart rate was monitored during all training sessions by a computerized cycle ergometer system, which adjusted ergometer resistance to maintain the target heart rate. The subjects were instructed not to change their diet during the intervention.

Phenotype measurements. All phenotypes were measured at baseline and after the 20-week exercise training program. A frequently sampled intravenous glucose tolerance test (FSIVGT) was performed as described (31) in the morning after a 12-h overnight fast and no less than 24 h after the last exercise session. Insulin sensitivity index (S_i) and glucose effectiveness (S_g) were computed by a least-squares fitting of the temporal pattern of glucose and insulin throughout the FSIVGT using the MINMOD program (32). S_i measures the ability of an increment in plasma insulin to enhance the net disappearance of glucose from plasma. S_g measures the ability of glucose per se, independent of changes in plasma insulin, to increase glucose disposal and to suppress endogenous glucose output. Acute insulin response to intravenous glucose (AIR_g), computed as the incremental integrated area under the insulin curve for the first 10 min of the FSIVGT, was used as a measure of insulin secretion. Disposition index (D_i), the product of S_i and AIR_g, is a measure of the ability of the pancreatic β-cells to compensate for insulin resistance (33). Glucose disappearance index (K_g) estimates glucose disappearance (percent per minute) based on the slope of the line derived from least-squares regression of the natural logarithm of plasma glucose from 10 to 60 min during the FSIVGT and was used as a measure of overall glucose tolerance. We did not give a secretagogue at 20 min to provide an accurate measure of K_g. Measurement of fasting plasma insulin, glucose, and C-peptide has been described previously (29). BMI was computed as the ratio of body weight divided by body height squared (kilograms per meter squared). Body fat mass was measured using underwater weighing (34).

Genetic analyses. Genetic analyses have been described previously in detail (26). In brief, genomic DNA was isolated from lymphoblastoid cells. A single nucleotide polymorphism in the LEP gene, an alanine-to-glycine substitution in untranslated exon 1 (A19G), and three single nucleotide polymorphisms in the LEPR gene, a lysine-to-alanine substitution at codon 109 in exon 4 (K109R), a glutamine-to-arginine substitution at codon 223 in exon 6 (Q223R), and a lysine-to-asparagine substitution at codon 656 in exon 16 (K656N), were typed using PCR technology.

Statistical analyses. A χ^2 test was used to compare allele frequencies between whites and blacks and to test whether the genotype distributions were in Hardy-Weinberg equilibrium. Student's t tests were used to compare means of measures of glucose homeostasis at baseline and after exercise training. The associations of the LEP and LEPR gene polymorphisms with the exercise-induced changes in glucose homeostasis, and the interactions among the variants, were analyzed using a MIXED model that takes into account nonindependence among family members. Data were adjusted for age, sex, baseline fat mass, and baseline measure of glucose homeostasis. Further adjustment for the change in fat mass did not affect the associations (data not shown). All analyses were performed with the SAS Statistical Software Package, separately in whites and blacks. Haplotype analyses were performed with the Quantitative Transmission Disequilibrium Test (35).

RESULTS

Baseline characteristics are shown in Table 1. The frequency of the LEP A19 allele was 0.42 in whites and 0.41 in blacks. The frequencies of the LEPR 109R, 223R, and 656N alleles were 0.26, 0.45, and 0.19 in whites and 0.13, 0.54, and 0.15 in blacks, respectively. The frequency of the LEPR 109R allele was significantly higher in whites than in blacks ($P < 0.001$). All genotype frequencies in both races were in Hardy-Weinberg equilibrium.

Physical activity increased S_i by 8.5% ($P = 0.005$), D_i by

9.5% ($P = 0.004$), S_g by 13.0% (<0.001), K_g by 3.2% ($P = 0.030$), and fasting glucose by 1.0% ($P = 0.004$) and decreased AIR_g by 6.6% ($P < 0.001$) and fasting insulin by 10.3% ($P < 0.001$). In blacks, exercise increased S_i by 19.0% ($P = 0.008$), D_i by 18.4% ($P = 0.004$), S_g by 20.7% ($P = 0.004$), and fasting glucose by 2.6% ($P = 0.001$) and decreased AIR_g by 6.7% ($P = 0.016$) and fasting insulin by 14.0% ($P = 0.006$). In whites, physical activity increased S_g by 9.6% ($P = 0.010$) and decreased AIR_g by 6.4% ($P = 0.005$) and fasting insulin by 8.7% ($P < 0.001$). Other changes in glucose homeostasis were not significant.

The LEPR K109R polymorphism was associated with exercise-induced changes in various measures of glucose homeostasis in whites (Fig. 1). Physical activity increased S_i ($P = 0.041$) and D_i ($P = 0.046$) in the 109R allele carriers but not in the K109K homozygotes. Exercise increased K_g more in the R109R homozygotes than in the K109 allele carriers ($P = 0.039$). Physical activity decreased fasting glucose only in the 109R allele carriers ($P = 0.018$). Exercise also reduced fasting insulin more in the R109R homozygotes (-12.7 ± 4.9 pmol/l [mean ± SE]) than in the K109 allele carriers (-8.4 ± 1.8 pmol/l), but the difference was not significant ($P = 0.38$). The LEPR Q223R polymorphism was not significantly associated with changes in glucose homeostasis in whites ($P > 0.05$). The haplotype consisting of the LEPR 109R allele and the LEPR 223R allele was associated with a greater exercise-induced increase in S_i ($P = 0.041$) and D_i ($P = 0.016$) than the other haplotypes in whites. However, the associations of the haplotype mainly reflected the effect of the 109R allele. The K109R and Q223R polymorphisms were not significantly related to changes in glucose homeostasis in blacks ($P > 0.05$).

In whites, exercise increased AIR_g and S_g and decreased fasting glucose in the LEPR N656N homozygotes ($n = 11$), but did not influence glucose homeostasis in the K656N heterozygotes ($n = 129$) or the K656K homozygotes ($n = 256$). The means (±SE) of the AIR_g changes in the N656N homozygotes, the K656N heterozygotes, and the K656K homozygotes were 11.3 ± 5.1, 2.4 ± 2.3, and -1.8 ± 1.4 μU/ml, respectively ($P = 0.030$). The means (±SE) of the S_g changes were 0.015 ± 0.005, 0.002 ± 0.001, and 0.004 ± 0.001%/min ($P = 0.025$), and the means (±SE) of the glucose changes were -2.8 ± 1.1, -0.4 ± 0.6, and 0.2 ± 0.5 mg/dl ($P = 0.052$). There were no N656N homozygotes in blacks, and data on the K656N polymorphism are not presented.

Physical activity decreased fasting insulin more in the LEP A19A homozygotes ($n = 61$, -12.6 ± 3.0 pmol/l [mean ± SE]) than in the A19G heterozygotes ($n = 212$, -7.8 ± 1.8 pmol/l) or the G19G homozygotes ($n = 123$, -7.2 ± 3.0 pmol/l) in whites, but the difference was not significant ($P = 0.19$). As shown in Fig. 2, however, there was a significant interaction between the LEP A19G and LEPR K109R polymorphisms on the exercise-induced change in fasting insulin in whites ($P = 0.010$), and the association between the LEP A19G polymorphism and insulin change was evident only in the LEPR 109R carriers ($P = 0.019$). Consistent with the results in whites, in blacks the LEP A19G polymorphism was not significantly associated with insulin change, but similar interaction between the LEP A19G and LEPR K109R polymorphisms was found ($P = 0.046$).

DISCUSSION

The present study shows that regular physical activity increases insulin sensitivity, but also suggests that regular exercise improves the ability of the pancreatic β-cells to compensate for insulin resistance and increases glucose effectiveness in nondiabetic individuals. A novel finding of the study is that exonic variants in the LEP and LEPR genes modified the effects of regular physical activity on glucose homeostasis in whites. These data support our recent observation (29) in the same nondiabetic population that a genomic region close to the LEP locus was linked to fasting insulin response to exercise training in whites.

The principal finding of this study is that regular physical activity improved insulin sensitivity, pancreatic β-cell compensation for insulin resistance, and glucose tolerance in the LEPR 109R carriers but not in the K109K homozygotes in whites. These results are potentially important from a public health point of view because the LEPR K109K homozygotes, who did not respond to regular exercise, represent >50% of the Caucasian populations (26). Regular physical activity had the strongest fasting insulin decreasing effect in the LEP A19A homozygotes who carried the LEPR 109R allele in whites. We observed similar interaction in blacks, but only three blacks had these genotypes, which complicates the interpretation of the results. These data suggest that both LEP and LEPR genes may contribute to improvements in glucose homeostasis in response to exercise training.

A possible explanation for our findings is that DNA sequence variations in the LEP and LEPR genes induce a mild dysfunction in the leptin-mediated signaling pathway and impair the peripheral effects of leptin (11–14), and thus attenuate the favorable effects of regular physical activity on glucose homeostasis. Although all nucleotide alterations in the LEP and LEPR genes of our study produce amino acid changes and may thus have functional consequences, no true evidence of their functionality is available. Yet, it is possible that the DNA sequence variants of our interest serve as genetic markers for nearby functional variants, which are in linkage disequilibrium with our markers and eventually explain the observed associations.

The differences in the exercise-induced changes among the genotypes were clear, but there was also considerable individual variation in responses even within genotypes. This is likely explained by the multifactorial and multigenic nature of the regulation of glucose homeostasis. The fact that most of the associations were evident only in whites may reflect a limited statistical power in blacks due to a small number of subjects in some genotype groups. Another reason for the lack of agreement may be that the frequency of the LEPR 109R allele was significantly higher in whites than in blacks.

The present study suggests that DNA sequence variations in the LEP and LEPR genes are associated with the magnitude of the effects of regular physical activity on glucose homeostasis in nondiabetic individuals. The

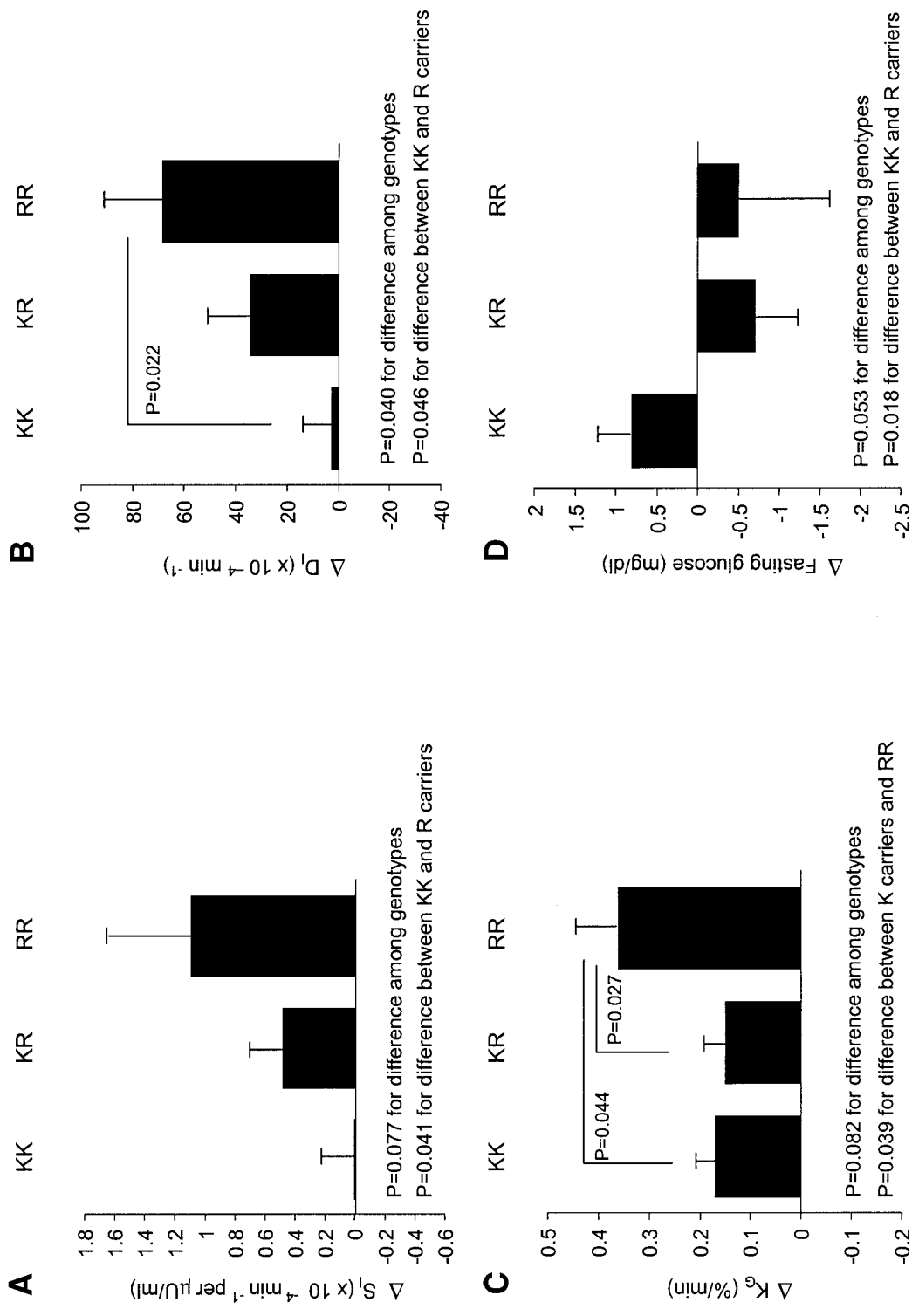

FIG. 1. Exercise-induced changes in insulin sensitivity index (*A*), disposition index (*B*), glucose disappearance index (*C*), and fasting glucose (*D*) according to the LEPR K109R polymorphism in whites after adjustment for age, sex, baseline fat mass, and baseline measure of glucose homeostasis. The numbers of whites with the KK, KR, and RR genotypes were 216 (54%), 152 (38%), and 30 (8%), respectively.

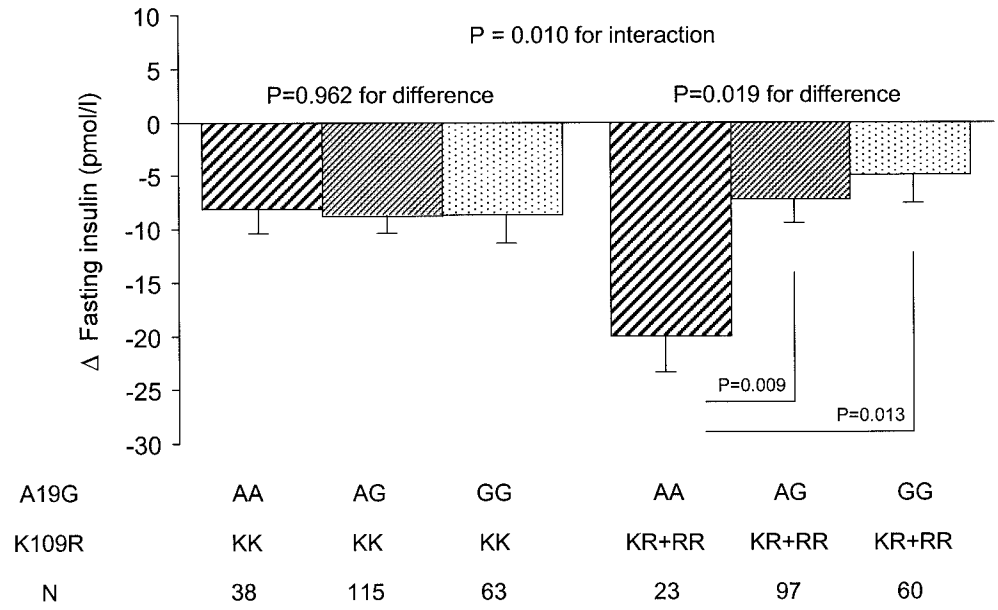

FIG. 2. Exercise-induced changes in fasting insulin according to the LEP A19G polymorphism and the LEPR K109R polymorphism in whites after adjustment for age, sex, baseline fat mass, and baseline fasting insulin.

screening of polymorphisms in the LEP and LEPR genes and other genes may be useful to identify individuals who are most likely to benefit from regular physical activity in terms of the prevention of type 2 diabetes and also those who do not respond to exercise training, for whom other interventions such as dietary changes or drug treatment might be preferable.

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