Influence of the interleukin-6 $-174$ G/C gene polymorphism on exercise training-induced changes in glucose tolerance indexes

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McKenzie, Jennifer A., Edward P. Weiss, Ioana A. Ghiu, Onanong Kulaputana, Dana A. Phares, Robert E. Ferrell, and James M. Hagberg. Influence of the interleukin-6 $-174$ G/C gene polymorphism on exercise training-induced changes in glucose tolerance indexes. J Appl Physiol 97: 1338–1342, 2004. First published June 4, 2004; 10.1152/japplphysiol.00199.2004.—A polymorphism in the IL-6 gene, a G-to-C substitution 176 bp upstream of the ATG translation initiation site, has been associated with diabetes prevalence and insulin resistance. Interventions including exercise training are frequently used to modify cardiovascular disease risk factors. Consequently, this project examined associations between the IL-6 $-174$ genotype and oral glucose tolerance test outcomes in 50- to 75-yr-old sedentary men and postmenopausal women before and after aerobic exercise training. Among the 87 individuals who started the study, 56 were retested after 6 mo of aerobic exercise training. Subject characteristics at baseline did not differ between the IL-6 genotype groups with the exception of fasting glucose, which was higher ($P = 0.02$, covariates age, gender, and ethnicity) in the CC genotype group. The training-induced change in glucose area under the curve during the oral glucose tolerance test varied between the IL-6 $-174$ genotype groups ($P = 0.05$, covariates age, gender, ethnicity, baseline glucose area under the curve, and percent body fat change) with a significant decrease occurring only in the GG genotype group. Insulin outcomes did not differ among the groups at baseline or after training. Training-induced changes in weight, percent body fat, maximal oxygen consumption, fasting glucose, and an insulin sensitivity index also changed similarly among the genotype groups. In conclusion, fasting glucose and the extent to which glucose tolerance changes with exercise training may be influenced by the IL-6 $-174$ gene polymorphism.

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CARDIOVASCULAR DISEASE (CVD) is a health crisis, implicated in over one-third of all deaths in the United States (2). Numerous factors influence an individual’s risk for CVD, including diabetes, obesity, and physical inactivity. Endurance exercise training has been shown to decrease CVD risk, in part by improving glucose tolerance, insulin sensitivity, and body composition (15, 16, 25).

Acute exercise has also been shown to increase levels of circulating interleukin (IL)-6, a cytokine produced from monocytes, endothelial cells, adipocytes, and skeletal muscle tissue. Elevated levels of IL-6 have also been associated with CVD mortality (24, 29) and metabolic conditions including obesity (3, 12, 14, 28), diabetes (23), and insulin resistance (3, 10, 14). In humans, a polymorphism has been reported in the promoter region of the IL-6 gene, a G-to-C substitution, which may influence IL-6 transcription rates (11, 27). This polymorphism has also been associated with reduced plasma IL-6 levels (11) and differences in plasma lipoprotein-lipid levels (9), CVD risk (13), body composition (4), diabetes (18, 30), and insulin sensitivity indexes (ISIs) (4).

Because exercise and the IL-6 $-174$ G/C polymorphism may independently affect glucose and insulin indexes and exercise has been shown to influence IL-6 levels, exercise training and the IL-6 $-174$ G/C gene polymorphism also may interact to affect glucose and insulin levels. Consequently, we hypothesized that common outcomes from oral glucose tolerance tests (OGTTs) would be associated with the IL-6 $-174$ G/C gene polymorphism both before and after 6 mo of aerobic exercise training in individuals at risk for CVD.

MATERIALS AND METHODS

Healthy, 50- to 75-yr-old men and women were screened via telephone to meet the following criteria: sedentary, nondiabetic, nonsmoking, no history of CVD or lung disease, normotensive or blood pressure controlled with non-lipid and non-glucose altering medication, no history of liver or kidney disease, a body mass index of $\leq 37$ kg/m$^2$, and no physical or orthopedic conditions that would preclude exercise. In addition, all women were postmenopausal and agreed to keep their hormone replacement status constant, either receiving or not receiving hormone-replacement therapy (HRT).

Written, informed consent was obtained from all individuals interested in participating in this study, which was approved by the Institutional Review Board at the University of Maryland, College Park. During the first laboratory visit, medical history was reviewed, and height and weight were measured to verify body mass index. After an overnight fast, a blood sample was drawn for genotype and glucose measurements. Standard blood chemistry tests were used to assess liver, kidney, and blood disorders, and a 2-h, 75-g OGTT was performed to assess diabetes status. To be included in the study, subjects had to have a fasting glucose of $<126$ mg/dl, a 2-h glucose of $<200$ mg/dl, and at least one National Cholesterol Education Program lipid abnormality (7). In a second screening visit, subjects underwent a general physical examination and a maximal graded treadmill exercise test (Bruce protocol) to screen for CVD. Subjects were excluded from the study if they exhibited ECG or symptomatic evidence of CVD during or after the exercise test (1).

A registered dietitian then instructed subjects twice a week for 6 wk to consume a diet consistent with the American Heart Association Dietary Guidelines for the General Population (17). Subjects maintained this diet for the duration of the study, with periodic dietary recalls and food frequency questionnaires to ensure adherence. Body weight was stabilized before baseline testing.

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occurred and was maintained ±5% throughout the exercise-training intervention.

After completing the dietary stabilization program, all subjects underwent baseline testing. Body composition was measured using dual-energy X-ray absorptiometry (DPX-L, Lunar, Madison, WI), and intra-abdominal (IAAT) and subcutaneous adipose tissues (SCAT) were assessed using computed tomography (22). A modified graded treadmill exercise test was used to determine maximal \( \dot{V}O_2 \) uptake (\( \dot{V}O_{2\text{max}} \)) (6). A 3-h OGTT was performed in the morning after a 12-h overnight fast. Subjects were asked not to use anti-inflammatory medications during the 24 h preceding the blood-sampling procedures. In addition, subjects were asked to consume at least 250 g of carbohydrate per day for 3 days before the OGTT and recorded all food consumed. Participants were questioned to confirm that they complied with all preparation instructions, and the diet records were collected and examined to ensure adequate carbohydrate intake. A catheter was placed in an antecubital vein, and blood samples were drawn before and at 30, 60, 90, 120, and 180 min after the ingestion of a 75-g glucose solution. The blood samples were centrifuged, and plasma samples were separated and stored at −80°C until assayed for glucose and insulin concentrations. Plasma glucose levels were analyzed with a glucose analyzer (2300 STAT Plus, Yellow Springs Instruments, Yellow Springs, OH) using the glucose oxidase method. Plasma insulin levels were determined by radioimmunoassay (HI-14K kit, Linco Research, St. Charles, MO). Glucose and insulin total areas under the curve (AUC) were calculated using the trapezoidal method.

DNA was isolated using standard techniques and genotyped for the G/C polymorphism in the promoter region of the IL-6 gene using fluorescence polarization (5). Direct DNA sequencing was used to confirm genotypes for a random sample of subjects.

Subjects underwent supervised aerobic exercise training three times per week for 24 wk as described previously (32). Training began at an intensity of 50% \( \dot{V}O_{2\text{max}} \) for 20 min and, by week 9, progressed to 70% \( \dot{V}O_{2\text{max}} \) for 40 min, where it remained for the duration of the study. During weeks 10–24, a lower intensity, 45- to 60-min unsupervised weekend exercise session was added to the training regimen. Only subjects completing ≥80% of the training were included in the data analyses. Overall, subject attendance averaged ≥93%, with 41 of 56 subjects completing ≥90% of the training. Although not significant because of reduced statistical power, the same general genotype-dependent trends persisted when even more stringent attendance criteria were used (>90% adherence).

Final testing occurred after the completion of 24 wk of exercise training. Subjects completed the same assessments as at baseline, except all metabolic measurements were made 24–36 h after an exercise bout.

Statistical procedures were analyzed using SPSS 11.0 software (SPSS, Chicago, IL). Data are presented as means ± SE. Before statistical analyses were conducted, variables deviating from homogeneity of variance and normal distribution assumptions were log transformed. Initial subject characteristics were compared among the IL-6 genotype groups using ANOVA and analysis of covariance covarying for age, gender, and ethnicity. Gender, HRT use, ethnicity, and Hardy-Weinberg equilibrium frequency differences were assessed using \( \chi^2 \) tests. Analysis of covariance was used to compare the exercise training-induced change in OGTT variables among genotype groups. Besides age, gender, and ethnicity, adjustment was also made for percent body fat since adipose tissue can produce IL-6. Analyses were also conducted with and without adjustment for HRT because it has been suggested that IL-6 production may be influenced by HRT use (26). However, HRT use was not found to have a significant effect in the present study; thus it was not included in the final data analyses. Paired t-tests were used to analyze within-genotype group changes with exercise training. Significance was set at \( P \leq 0.05 \).

RESULTS

IL-6 allele and genotype frequencies for the 87 participants at baseline and for the 56 exercise-training intervention participants (Table 1) differed slightly from previous reports (4, 8, 13, 19), with this study having more GG homozygotes and fewer CG heterozygotes. Allele and genotype frequencies for the baseline study population differed from Hardy-Weinberg expectancies (\( \chi^2 = 15.6; P \leq 0.001 \)); however, allele and genotype frequencies for the exercise-trained population were in Hardy-Weinberg equilibrium (\( \chi^2 = 4.1; P = 0.13 \)).

Demographics, body composition, \( \dot{V}O_{2\text{max}} \), fasting insulin, glucose AUC, insulin AUC, and ISI values were similar among the three IL-6 –174 G/C genotype groups at baseline (Table 2). However, fasting glucose differed significantly (\( P = 0.02 \), covariates age, gender, and ethnicity) between the genotype groups, with CC subjects having a higher fasting glucose concentration than both the CG and GG groups (\( P = 0.05 \) and 0.01, respectively). This difference still tended to be significant after also covarying for percent body fat (\( P = 0.06 \)). In addition, although not significant because of reduced statistical power, the same trends were present in both white and nonwhite subject groups.

For the subjects undergoing exercise training, age, gender, the proportion of white to non-white participants, and HRT use did not differ between the IL-6 genotype groups (Table 3). Initial values for weight, percent body fat, SCAT, IAAT, and \( \dot{V}O_{2\text{max}} \) were also comparable among the groups (Table 3), as were initial fasting glucose, fasting insulin, glucose AUC, insulin AUC, and ISI (Table 4).

With exercise training, body weight and SCAT decreased significantly in the GG group (\( P \leq 0.001 \) and 0.04, respectively) but did not change significantly in either the CC or CG genotype groups. Body fat, IAAT, and fasting insulin decreased and ISI increased significantly with training in the CG and GG (all \( P \leq 0.03 \)) but not the CC genotype group. \( \dot{V}O_{2\text{max}} \) increased and insulin AUC decreased similarly and significantly within all IL-6 genotype groups (all \( P \leq 0.02 \)). Fasting glucose did not change significantly with training within the genotype groups. Finally, glucose AUC decreased significantly in the GG group with training (\( P = 0.02 \)) but did not change significantly in the CC or CG groups. The training-induced change in glucose AUC values varied significantly among the genotype groups (\( P = 0.02 \), covariates age, gender, ethnicity, and baseline glucose AUC value) and remained significantly different after also accounting for the change in percent body fat (\( P = 0.05 \)). Although not significant because of reduced

Table 1. IL-6 –174 G/C gene polymorphism allele and genotype frequencies

<table>
<thead>
<tr>
<th>Allele Frequency</th>
<th>Study Sample</th>
<th>Exercise-Trained Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele frequency</td>
<td>C 0.48 (83)</td>
<td>0.43 (48)</td>
</tr>
<tr>
<td></td>
<td>G 0.52 (91)</td>
<td>0.57 (64)</td>
</tr>
<tr>
<td>Genotype frequency</td>
<td>CC 0.33 (29)</td>
<td>0.25 (14)</td>
</tr>
<tr>
<td></td>
<td>CG 0.29 (25)</td>
<td>0.36 (20)</td>
</tr>
<tr>
<td></td>
<td>GG 0.38 (33)</td>
<td>0.39 (22)</td>
</tr>
</tbody>
</table>

Data are frequencies (n value in parentheses).
differed between the genotype groups. The CG and GG groups decreased glucose AUC with training by 13.4 ± 1.9% and 1.9 ± 2.3%, respectively. None of the other allele homozygotes had significant changes within genotype groups with training (P ≤ 0.05). Significant difference among genotype groups, covaried for age, gender, ethnicity, baseline glucose AUC, and change in percent body fat (P ≤ 0.05). Fasting glucose measured in mg/dl; fasting insulin measured in pM/l; glucose AUC measured in mg/dl × min; insulin AUC measured in pM/l × min; ISI calculated according to the method of Matsuda and DeFronzo (20).

**DISCUSSION**

Plasma levels of IL-6 have been associated with CVD and CVD risk factors, including obesity and diabetes. Furthermore, a polymorphism in the IL-6 gene, a G-to-C substitution in the promoter region, has been associated with differences in IL-6 levels and in the prevalence of diabetes and insulin resistance. Fortunately, many CVD risk factors can be modified via environmental interventions, including exercise training. In accordance with this, we have found the IL-6 −174 G/C gene polymorphism to be associated with differences in baseline fasting glucose levels in individuals at risk for CVD. Additionally, we have found that the extent of glucose AUC improvement after aerobic exercise training in these subjects was also associated with the IL-6 gene polymorphism.

Fernandez-Real and colleagues (8) found healthy men and women homozygous for the IL-6 −174 C allele to have increased ISI and lower glucose AUC values after an OGTT compared with G allele carriers. In contrast, Berthier et al. (4) reported trends for higher fasting insulin and OGTT insulin responses in men with the C allele, and Kubaszek and colleagues (19) reported that healthy men and women homozygous for the C allele had lower whole body glucose uptake and insulin sensitivity measured via hyperinsulinemic-euglycemic clamps compared with G allele carriers. Our study in individuals at risk for CVD found differences in fasting glucose at baseline between the genotype groups, with the CC group having a higher fasting glucose concentration than the CG and GG groups. Furthermore, after aerobic exercise training, C allele homozygotes had significantly higher glucose AUC values than the G allele carriers. These differences remained statistically significant after taking into account percent body fat change with training. In fact, the CC group tended to
increase their glucose AUC with training, whereas both the CG and GG groups tended to decrease their glucose AUC with exercise training.

Adipose tissue cells, including SCAT, express and secrete IL-6 (12, 14, 21). In mice lacking the IL-6 gene, both glucose intolerance and obesity develop, with the greatest fat increases in the subcutaneous region (31). Although not statistically significant, SCAT changes with exercise training varied between the genotype groups in the present study, with the C homozygotes experiencing virtually no change in SCAT, the G homozygotes decreasing SCAT, and the CG heterozygotes increasing SCAT. Moreover, these results warrant further research, because substantial variability existed within the groups as to the extent of SCAT change with training.

A limitation in the present study is that the genotype frequencies for the subjects in our baseline comparisons were not in Hardy-Weinberg equilibrium. Consequently, the results from these data may not be representative of the general population because they may reflect some unknown sampling bias in the selection of our study population. Several single-nucleotide polymorphisms have been identified in the promoter region of the IL-6 gene, which could contribute to the observed association. However, due to the low frequency of the minor allele at these other sites, the −174 G/C genotype captures >94% of the single-nucleotide polymorphism haplotype information at this locus (27). Last, due to sample-size constraints, we combined ethnic groups for the analyses and covaried for ethnicity. The same general genotype-dependent trends existed for the glucose indexes that were significantly different (fasting glucose and training-induced change in glucose AUC) when whites and non-whites were analyzed separately, although these results were not significant because of reduced statistical power.

To summarize, although no differences were found in this study with regard to insulin sensitivity, variation marked by the IL-6 −174 G/C polymorphism appears to influence glucose levels at baseline and the extent of change in glucose indexes with aerobic exercise training. This provides further support for the interactive influence of genetics and exercise training on health and fitness outcomes.

REFERENCES


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