**Abstract**

**Objective**: Exercise training has been shown to have anti-inflammatory effects in patients with type 2 diabetes. Changes in interleukin-6 (IL-6) serum concentrations in response to training could contribute to these beneficial effects. However, there are heterogeneous data on whether circulating IL-6 is altered by exercise training. We therefore hypothesize that genetic factors modify the individual changes in IL-6 levels after long-term training.

**Research design and methods**: The −174G/C variant in the IL-6 gene was genotyped in 60 subjects with impaired glucose tolerance. For a 12-month interventional study, patients were randomized into three groups: a control group (n = 16) was compared with one group, which underwent a standardized training program (n = 24) and another group, which was treated with 4 mg rosiglitazone once daily (n = 20).

At baseline, after 1, 6, and 12 months, we measured anthropometric parameters and serum concentration of IL-6 and, at baseline and after 12 months, we determined glucose tolerance and fitness level.

**Results**: Only in subjects carrying the SNP −174C allele did long-term exercise training result in significantly reduced IL-6 serum concentrations. Multivariate linear regression analysis identified the IL-6 genotype as a significant predictor of changes in IL-6 serum concentrations independent of age, gender and improvement in body mass index, hemoglobin (Hb)A1c, and fitness level in response to training.

**Conclusions**: Genetic variants in the IL-6 gene significantly modify changes in IL-6 serum concentrations in response to long-term exercise training programs. Our data suggest that genetic factors are important determinants for the individual response to anti-inflammatory effects of exercise training.
However, there are heterogeneous data on whether and in which direction circulating IL-6 is altered by exercise training. A marked increase in circulating levels of IL-6 after exercise without muscle damage has been a remarkably consistent finding (16). Plasma IL-6 concentration increases in an exponential fashion with exercise and is related to exercise intensity, duration, the mass of muscle recruited, and endurance capacity (16). However, whether these acute effects of exercise on IL-6 concentrations lead to chronically changed IL-6 levels is unclear. In resting state, IL-6 plasma concentrations were not significantly different between elite rowers and non-athletic controls (17), suggesting that body fitness is no major determinant of resting IL-6 plasma concentration. Kadoglou et al. have recently demonstrated that a 16-week aerobic exercise training program significantly reduces IL-6 serum concentrations in patients with T2D (18). Moreover, combined exercise training with rosiglitazone treatment resulted in a greater decrease in circulating IL-6 than exercise alone (19). It was further demonstrated that rosiglitazone treatment reduces both IL-6 serum concentrations in patients with T2D (20) and IL-6 mRNA expression in human s.c. adipose tissue (21). In contrast to these studies, we recently demonstrated that an intensive 4-week exercise training program does not have any effect on IL-6 plasma concentrations (22). Because of these contradictory results, we hypothesize that genetic factors modify the individual changes in IL-6 levels after long-term training. Therefore, we studied the effects of an exercise training program on IL-6 levels in individuals with impaired glucose tolerance (IGT) with different genotypes of the −174 G/C IL-6 polymorphism. Furthermore, to investigate whether improvement of insulin sensitivity might be the major mechanism of reduced IL-6 serum concentrations in response to long-term training, we also examined the effects of a 4 mg rosiglitazone treatment on IL-6 levels in individuals with IGT.

### Research design and methods

#### Subjects

Sixty subjects with IGT had been selected from more than 500 volunteers who were screened by a 75 g oral glucose tolerance test (OGTT). IGT was defined according to American Diabetes Association (ADA) criteria (23). All subjects fulfilled the following baseline inclusion criteria: i) absence of any acute or chronic inflammatory disease; ii) undetected glutamic acid decarboxylase; iii) no medical history of hypertension, i.e., their systolic blood pressure was ≤140 mmHg and diastolic blood pressure was ≤90 mmHg; iv) no clinical evidence of cardiovascular disease; v) no thyroid dysfunction; vi) no concomitant medication, vii) no alcohol or drug abuse; and viii) no pregnancy. During the 1-year training period, daily caloric intake (over a 1-week period) was reported by each subject using dietary diaries once a month. Seventy five patients were initially randomized by a standardized computer method into three different groups. From these 75 individuals, 60 patients completed the entire course of the study in an exercise training group (n = 24), a rosiglitazone treatment group (n = 20), which received 4 mg rosiglitazone once a day as monotherapy and a control group (n = 16), which received usual care. Fifteen patients (one in the training group, five in the rosiglitazone group, and nine in the control group) did not complete the 1-year study due to a lack of compliance. No serious adverse events have been reported during the study. Before randomization, after 4 weeks, 6, and 12 months, blood samples were drawn. Maximal exercise capacity tests were conducted before randomization and after 12 months.

#### Exercise training group

Each subject underwent a twice weekly standardized aerobic 60-min training program, which was monitored and documented by a certified trainer. Each training session consisted of 20-min warming and cool-down periods, 20 min of running or biking, and 20 min of power training. In addition, the participants performed 60 min of swimming on a separate day. The compliance rate was 88%. The study was approved by the ethics committee of the University of Leipzig. All subjects gave written informed consent before taking part in the study.

#### Maximal exercise test

At baseline and after 12 months, all subjects completed graded bicycle ergometer test to volitional exhaustion. After a resting state of at least 3 min to measure steady-state conditions, we used a symptom-limited ramp exercise test with an increase in work load of 10 W/min, starting with unloaded cycling plus the ergometer-related permanent load. Respiratory gas exchange variables were measured continuously throughout the exercise test with an automated open-circuit gas analysis system (Viasys Healthcare, Oxycron Pro, Jaeger, Höchberg, Germany). On maximal exercise, we assessed the maximal power in watt per body weight (Table 1). Peak oxygen uptake (peak VO$_2$) was defined as the highest tens average of oxygen uptake in the last minute of exercise. Exercise tests were applied according to current guidelines for exercise testing (24) with continuous monitoring of electrocardiogram (ECG), blood pressure, and oxygen saturation.

#### Measures of body metabolic parameters

At baseline, after 1, 6, and 12 months, blood samples were taken for the measurement of IL-6, C-reactive protein (CRP), and standard laboratory parameters using the previously described methods (25). In addition, dual-energy X-ray absorptiometry (DEXA) analyses and measurements of anthropometric parameters were
Table 1  Clinical characteristics of study subjects.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=16)</th>
<th>Rosiglitazone (n=20)</th>
<th>Exercise training (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (range, years)</strong></td>
<td>59.1 (49–63)</td>
<td>56.7 (48–61)</td>
<td>58.1 (50–63)</td>
</tr>
<tr>
<td><strong>Gender (M/F)</strong></td>
<td>9/7</td>
<td>10/10</td>
<td>10/14</td>
</tr>
<tr>
<td><strong>Time (months)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>29.8 ± 0.8</td>
<td>29.9 ± 0.8</td>
<td>29.8 ± 0.8</td>
</tr>
<tr>
<td><strong>WHR</strong></td>
<td>1.07 ± 0.03</td>
<td>1.08 ± 0.03</td>
<td>1.09 ± 0.03</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>5.99 ± 0.04</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Fasting plasma insulin</strong></td>
<td>91.2 ± 8.56</td>
<td>86.2 ± 9.19</td>
<td>10.20 ± 0.22</td>
</tr>
<tr>
<td><strong>Fasting plasma glucose</strong></td>
<td>5.63 ± 0.08</td>
<td>5.92 ± 0.07</td>
<td>9.20 ± 0.22</td>
</tr>
<tr>
<td><strong>Maximal power</strong> (W/kg per bw)</td>
<td>1.13 ± 0.06</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>VO₂max (ml/kg per min)</strong></td>
<td>23.5 ± 0.93</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Serum IL-6 (pg/ml)</strong></td>
<td>3.47 ± 0.56</td>
<td>3.19 ± 0.48</td>
<td>3.20 ± 0.54</td>
</tr>
<tr>
<td><strong>Serum CRP (pg/ml)</strong></td>
<td>2.81 ± 0.49</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.E.M.; *P < 0.05 using ANOVA (repeated measures with Bonferroni corrections); †P < 0.05 using paired t-test statistics. Maximal power per body weight was assessed as shown by the bicycle ergometer at maximal exercise.

*1 vs 12 months.
*0 vs 6 months.
*0 vs 12 months.
*1 vs 6 months.
*Comparisons between 0 vs 1 month.
*6 vs 12 months.
performed. BMI was calculated as weight (kg) divided by squared height (m). Waist and hip circumferences were measured and a WHR was calculated. Percentage body fat was measured by DEXA. At baseline and after 12 months, an OGTT was performed according to the ADA criteria (23). Three days prior to the OGTT, the subjects underwent a high carbohydrate diet. The OGTT was performed after an overnight fast with a 75 g standardized glucose solution (Glucodex Solution 75 g; Merieux, Montreal, Canada). Venous blood samples were taken at 0, 60, and 120 min for measurements of plasma glucose concentrations.

Assays
Basal fasting blood samples were taken after an overnight fast to determine glucose, insulin, and standard laboratory parameters. Plasma concentrations of insulin and C-peptide were measured as previously described (25). Serum samples for hsCRP, and IL-6 were stored at −80°C and assayed in triplicate. A high-sensitivity ELISA was used for IL-6 measurement (Quantikine IL-6, R&D Systems, Oxford, UK) as previously described (26), and the high-sensitivity CRP assay was determined by immunonephelometry (Dade-Behring, Milan, Italy). Both interassay and intraassay coefficients of variation were ≤5%.

Genotyping of the −174G/C IL-6 variant
Genotyping of the −174G/C (rs1800795) SNPs was conducted by TaqMan genotyping assay (Applied Biosystems, Inc, Foster City, CA, USA). The genotyping reaction was run on GeneAmp PCR system 9700 (50°C for 2 min, 95°C for 10 min, 95°C for 15 sec, and 62°C for 1 min, for 38 cycles) and was read on an ABI Prism 7500 sequence detector (Applied Biosystems, Inc.).

Statistical analyses
Statistical procedures were analyzed using the SPSS statistical software version 14.0 (SPSS, Inc, Chicago, IL, USA). All data are presented as means ± S.E.M. Prior to statistical analyses, variables deviating from homogeneity of variance and normal distribution assumptions were log transformed. Subject characteristics were compared using two-way ANOVA statistics. Paired t-tests were used to analyze within-genotype group changes with exercise training or rosiglitazone treatment. Linear relationships were assessed by least-square regression analysis. Univariate regression analyses were performed to assess correlates of changes in IL-6 serum concentrations. Multivariate linear relationships were assessed by a general linear model. Hardy–Weinberg equilibrium tests for genotype distributions were performed by the \( \chi^2 \) test. \( P < 0.05 \) was considered to be of statistical significance.

Results

Clinical characteristics of study subjects
In total, 60 subjects with IGT have been prospectively studied over a period of 12 months. At baseline study, the participants have been randomized into a supervised exercise training group (n = 24), a 4 mg rosiglitazone once a day treatment group (n = 20), and a control group (n = 16), which did not receive any intervention. All participants took part in a nutrition education program and had to complete diet diaries. Anthropometric parameters (BMI and WHR), measures of glucose metabolism (fasting plasma glucose and fasting plasma insulin), and IL-6 serum concentrations were determined at baseline and after 1, 6, and 12 months (Table 1). CRP serum concentrations, 2-h plasma glucose after OGTT as well as physical fitness level (peak VO\(_2\)), were determined at baseline and after 12 months (Table 1). There was a significant improvement in physical fitness (peak VO\(_2\)) in subjects who underwent 12 months of aerobic exercise training (Table 1), which was independent of gender (data not shown). Furthermore, exercise training resulted in significantly decreased BMI, WHR, as well as HbA\(_{1c}\) plasma levels of glucose and insulin, and 2-h OGTT plasma glucose levels (Table 1). The exercise intervention resulted in significantly decreased IL-6 and hsCRP serum concentrations (Table 1). In the rosiglitazone treatment group, we found significantly decreased HbA\(_{1c}\) levels, fasting and 2-h OGTT plasma glucose and insulin concentrations (Table 1). In the control group, no significant differences were observed for any of the parameters in the course of the study, except for increased fasting plasma insulin and plasma glucose after 1 year (Table 1).

Correlates of changes in IL-6 serum concentrations
Univariate regression analyses revealed significant positive correlations between changes in IL-6 serum concentrations and exercise-induced changes in HbA\(_{1c}\) and hsCRP after 12 months (Table 2). These correlations remained significant (\( P < 0.05 \)) upon adjusting for age, gender, and changes in BMI (data not shown). There were no significant correlations between changes in IL-6 and changes in BMI, fasting and 2-h plasma glucose, fasting plasma insulin or peak VO\(_2\) (Table 2).

There was a tendency for reduced IL-6 serum concentrations in the rosiglitazone group after 12 months of treatment; however, the decrease did not reach statistical significance (Table 1).

Effect of IL-6 −174G/C genotype on exercise-induced decrease in circulating IL-6
At baseline, the −174G/C variant was associated with 2-h plasma glucose levels in the entire study cohort.
Table 2 Relationships between exercise-induced changes in circulating interleukin-6 levels (ΔIL-6) and changes in anthropometric and biochemical parameters under univariate linear regression analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ΔIL-6 (0–12 months) long-term exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
</tr>
<tr>
<td>Δ BMI (kg/m²)</td>
<td>−0.05</td>
</tr>
<tr>
<td>Δ WHR</td>
<td>−0.01</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td></td>
</tr>
<tr>
<td>Δ Fasting plasma glucose (mmol/l)</td>
<td>−0.04</td>
</tr>
<tr>
<td>Δ 2-h Plasma glucose (mmol/l)</td>
<td>−0.02</td>
</tr>
<tr>
<td>Δ Fasting plasma insulin (pmol/l)</td>
<td>−0.008</td>
</tr>
<tr>
<td>Δ HbA1c (%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
</tr>
<tr>
<td>Δ CRP (pg/ml)</td>
<td>0.38</td>
</tr>
<tr>
<td>Physical fitness</td>
<td></td>
</tr>
<tr>
<td>Δ Peak VO₂ (ml/kg per min)</td>
<td>−0.04</td>
</tr>
<tr>
<td>Δ Maximal power (W/kg per body weight)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Significant correlations (P<0.05) are shown in bold. Statistical significance when P<0.05 is shown in italics.

Discussion

The −174G/C variant in the IL-6 promoter gene has been shown to influence IL-6 transcription in vitro (12). Our study demonstrates for the first time that the −174G/C genotype is a significant and independent predictor of reduced IL-6 serum concentrations after a long-term exercise training program. This suggests that previously reported beneficial anti-inflammatory effects of physical training in patients with T2D (18, 19) are at least modified by genetic factors. The strong genotype, exercise response association for circulating IL-6, might also provide an explanation for the heterogeneous results on whether and in which direction IL-6 serum concentrations are changed by long-term physical exercise (16, 18, 19, 22). Our results further emphasize the fact that genetic factors play an important role in the individual response to exercise training. Obviously, the number of subjects was relatively small and the association between the −174G/C genotype and IL-6 serum concentrations will require confirmation in larger cohorts. However, the fact that the association between the −174G/C genotype and reduced IL-6 serum concentrations in response to long-term exercise without any conceivable adjustment demonstrates the robustness of our finding. Genotype effects might explain the absence of an exercise effect after an intensive 4-week training program in a previous study (22).

It is noteworthy, however, that this decrease was only seen after 12 months but not after 1 month of exercise, which is in accordance with our previous findings (22). Also, the changes in parameters of obesity and glucose homeostasis did not predict changes in IL-6 levels after 4 weeks of training, while a positive correlation between changes in IL-6 concentrations and changes in WHR as well as HbA1c levels was observed after 12 months of exercise. Our data clearly indicate the relevance of exercise duration on levels of this inflammatory marker.
Because exercise and IL-6 polymorphisms may independently affect IL-6 levels, we also investigated whether the effects of exercise on inflammatory markers (IL-6 and CRP levels) as well as on measures of glucose metabolism in subjects with IGT might interact with effects of the −174G/C polymorphism. Improvement of metabolic parameters after long-term exercise training was comparable between the −174G/C genotypic groups. Although McKenzie et al. (27) reported an increase in glucose AUC with training in −174 CC homozygous subjects and a decrease in the GG group, in our study changes in glucose metabolism parameters did not vary between −174G/C genotypic groups. Therefore, our data suggest that the −174G/C genotype does not affect the response to exercise for some key parameters of metabolic assessment including BMI, plasma glucose, and insulin serum concentrations and HbA1c values. However, in our study, long-term exercise induced a significant decrease in levels of inflammatory markers, which was only observed in the group of subjects with the −174C allele (C/C homozygotes + G/C heterozygotes), whereas no significant decrease was seen in the group of subjects homozygous for the −174G allele. The effect of −174G/C on changes in circulating IL-6 levels was independent of obesity and glucose metabolism, as demonstrated by multivariate regression analyses. This further suggests that IL-6 serum concentrations do not significantly contribute to improvement of metabolic parameters and support our previous finding that IL-6 serum concentrations are not related to improved metabolic parameters after a 4-week exercise program (22).

Interactive effects of −174G/C have previously been shown for other phenotypes as well. Möhlig et al. (28) reported that −174G/C interacts with obesity in predicting T2D. Obese individuals with BMI > 28 kg/m²

<table>
<thead>
<tr>
<th>Genotype</th>
<th>G/G (n=6)</th>
<th>G/C + C/C (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>0.40</td>
<td>0.06</td>
</tr>
<tr>
<td>Age (year)</td>
<td>−0.001</td>
<td>0.99</td>
</tr>
<tr>
<td>Δ BMI (kg/m²)</td>
<td>0.07</td>
<td>0.69</td>
</tr>
<tr>
<td>Δ HbA1c (%)</td>
<td>0.42</td>
<td>0.06</td>
</tr>
<tr>
<td>(−174G/C)</td>
<td>0.44</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 4 Analyses of the influence of −174G/C variant on changes in circulating interleukin-6 (ΔIL-6) after 12 months of exercise training in a multiple regression analysis.

*Statistical significance when P<0.05 is shown in italics.

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carrying the C/C genotype had a more than fivefold increased risk of developing T2D compared with remaining genotypes and hence might profit most from weight reduction. Our findings indicate that subjects carrying the C/C or C/G genotypes may profit more from improved physical fitness compared with the G/G homozygotes, particularly regarding reduction of levels of inflammatory markers such as IL-6. IL-6 expression in adipose tissue can be triggered by tumour necrosis factor α (TNFα) (29). Since we did not measure circulating TNFα or adipose tissue expression of TNFα and IL-6, further studies are necessary to elucidate whether the genotype-associated differences in IL-6 response to exercise are mediated by TNFα.

The major limitation of our study is the relatively small sample size due to which we may have lacked statistical power to detect significant differences between genotypic groups for examined metabolic parameters. However, the genotype distribution for the −174G/C variant was in Hardy–Weinberg equilibrium, and therefore we do not expect any unknown sampling bias in the selection of our study subjects. We are aware that it remains to be determined whether the −174G/C is the causal variant responsible for observed effects on exercise-induced changes in circulating IL-6 levels or if it simply serves as a proxy for the actual causal variant(s), which maps nearby and is in linkage disequilibrium with −174G/C. Although several SNPs have been described for the promoter region of the IL-6 gene, the −174G/C captures about 94% of the SNP haplotype information (12).

We further confirm previous studies that long-term exercise training over a period of 12 months positively affected measures related to obesity or IGT. In addition, we found significant improvement of measures of glucose metabolism in subjects treated with 4 mg rosiglitazone for 12 months, which seems to be independent of the −174G/C genotype in the IL-6. Chronic rosiglitazone therapy has recently been shown to reduce IL-6 mRNA expression in human s.c. adipose tissue (21). Recent clinical studies suggest that thiazolidinediones not only exert their actions by enhancing cellular glucose transport but also possess distinct anti-inflammatory properties (20, 30). In patients with T2D, rosiglitazone was shown to significantly reduce IL-6 serum concentrations already after 4 weeks of treatment (20). However, in the rosiglitazone treatment group, there was only a trend for reduced IL-6 serum concentrations in our study. Since reduced circulating IL-6 levels after rosiglitazone treatment have recently been reported (19), the small number of subjects in our rosiglitazone group might be the reason for an insufficient statistical power to detect significant improved IL-6 serum concentrations upon rosiglitazone treatment in our study. Moreover, subjects included in the present study had only IGT and did not have diabetes and, therefore, we cannot exclude possible effects of rosiglitazone on IL-6 and hsCRP in diabetic patients. In contrast to recent reports (31, 32), we did not observe body weight gain after 1-year rosiglitazone treatment. One explanation for the absence of weight gain in the rosiglitazone treatment group might be the lower dose of 4 mg rosiglitazone daily in our study compared with 8 mg daily in many other studies. Another explanation could be that patients with IGT have a different body weight response to rosiglitazone than patients with T2D. It is noteworthy that the absence of weight gain in the rosiglitazone group in our study is consistent with recent results, which did not find significant weight gain after 8 mg rosiglitazone treatment in previously untreated patients with T2D (33).

We cannot exclude possible effects of rosiglitazone on IL-6 circulating levels.

In conclusion, the −174G/C genotype in the IL-6 gene significantly modifies changes in IL-6 serum concentrations in response to long-term exercise training. Our data suggest that genetic factors are important determinants for the individual response to anti-inflammatory effects of exercise training.

Acknowledgements

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References

Elevated interleukin-6 plasma levels are regulated by the promoter region polymorphism of the IL-6 gene in primary Sjogren’s syndrome and correlate with the clinical manifestations of the disease. *Rheumatology* 2001 40 656–661.


Kasapis C & Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers – a systematic review. *Journal of the American College of Cardiology* 2005 45 1563–1569.


29 Fasshauer M, Klein J, Lossner U & Paschke R. Interleukin (IL)-6 mRNA expression is stimulated by insulin, isoprotanol, tumour necrosis factor alpha, growth hormone, and IL-6 in 3T3-L1 adipocytes. *Hormone and Metabolic Research* 2003 35 147–152.


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