CLINICAL STUDY

Long-term exercise training decreases interleukin-6 (IL-6) serum levels in subjects with impaired glucose tolerance: effect of the –174G/C variant in IL-6 gene

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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)A_{1c}, 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.

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Introduction

Chronic activation of innate immune system may play a crucial role in pathophysiology of type 2 diabetes mellitus (T2D) (1). In support of this, increased serum levels of interleukin 6 (IL-6) are associated with increased risk of T2D and insulin resistance (2–4). Furthermore, the IL-6 levels correlate with measures of obesity, such as body mass index (BMI), waist to hip ratio (WHR), and percent body fat (3, 5, 6). The IL-6 gene is therefore a plausible candidate gene involved in susceptibility to T2D. Indeed, numerous studies examined genetic variants in IL-6 and reported their effects on T2D and related traits (7–13). Especially, the –174G/C variant within the IL-6 promoter has been shown to influence IL-6 transcription *in vitro* (12); however, effects of the variant on the IL-6 levels are controversial. Fishman *et al.* showed lower IL-6 serum concentrations in healthy subjects carrying the C/C

genotype (8), whereas Hulkkonen *et al.* did not report any differences in serum levels in function of the IL-6 genotype (9). Also, C/C patients with Sjögren's syndrome had lower IL-6 levels (9), but in a different study, C/C homozygous subjects with abdominal aneurysms showed increased IL-6 concentrations (14).

It has been shown that exercise produces a short-term inflammatory response, whereas both cross-sectional comparisons and longitudinal exercise training studies demonstrate a long-term anti-inflammatory effect (15). However, it is still unclear whether long-term exercise-associated anti-inflammatory effects might be entirely explained by decreased body weight, improved insulin sensitivity, glucose metabolism, and/or fitness level in individuals with impaired glucose metabolism. Moreover, exercise-induced changes in inflammatory markers could contribute to the improvement of insulin sensitivity after physical 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, Oxycon 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.

	Control (<i>n</i> =16)					Rosiglitazone (<i>n</i> =20)					Exercise training (<i>n</i> =24)				
Age (range, years)	59.1 (49–63)					56.7 (48–61)					58.1 (50–63)				
Gender (M/F)	9/7					10/10					10/14				
Time (months)	0	1	6	12		0	1	6	12		0	1	6	12	
BMI (kg/m ²)	29.8±0.8	29.9±0.8	29.8±0.8	29.8±0.8	NS	30.7±0.59	30.8±0.55	30.6±0.56	30.4±0.55	*a	30.1±0.38	29.7±0.38	29.1±0.38	28.7±0.38	*a, b, c, d, e, f
WHR	1.07±0.03	1.08±0.03	1.07±0.03	1.09±0.03	NS	1.12±0.02	1.12±0.01	1.10±0.01	1.09±0.01	NS	1.08±0.03	1.06±0.03	1.03±0.02	1.00±0.01	*a, b, c, d, f
HbA _{1c} (%)	5.99±0.04	–	6.04±0.05	6.02±0.06	NS	5.86±0.02	–	5.84±0.02	5.71±0.02	*c, f	5.96±0.04	–	5.73±0.02	5.63±0.02	*b, c, f
Fasting plasma insulin (pmol/l)	81.2±8.56	86.2±9.19	91.2±9.43	89.0±9.04	*c	89.2±9.46	82.3±7.57	71.8±5.82	65.0±4.45	*a, b, c	104.6±8.8	86.5±7.47	70.4±6.37	62.7±5.41	*a, b, c, d, e
Fasting plasma glucose (mmol/l)	5.63±0.08	5.92±0.07	6.06±0.06	5.94±0.07	*b, c, e	5.79±0.05	5.55±0.05	5.42±0.06	5.36±0.06	*b, c, f	5.70±0.15	5.45±0.18	5.17±0.16	5.16±0.04	*a, b, c, d, e
2-h plasma glucose (mmol/l)	9.50±0.24	–	–	10.20±0.22	NS	9.85±0.2	–	–	7.73±0.23	†	9.68±0.18	–	–	8.25±0.23	†
Maximal power (W/kg per bw)	1.13±0.06	–	–	1.14±0.06	NS	1.38±0.09	–	–	1.43±0.10	NS	1.30±0.04	–	–	1.83±0.06	†
VO _{2max} (ml/kg per min)	23.5±0.93	–	–	24.0±0.97	NS	22.4±0.63	–	–	22.3±0.62	NS	22.6±0.57	–	–	28.6±0.47	†
Serum IL-6 (pg/ml)	3.47±0.56	3.19±0.48	3.81±0.49	3.20±0.54	NS	3.74±0.58	3.26±0.46	3.27±0.56	2.80±0.40	NS	4.08±0.56	4.19±0.55	3.85±0.51	2.60±0.32	*a, c, f
Serum CRP (pg/ml)	2.81±0.49	–	–	2.98±0.43	NS	3.21±0.58	–	–	2.77±0.43	NS	3.58±0.55	–	–	2.49±0.35	†

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.

^a1 vs 12 months.

^b0 vs 6 months.

^c0 vs 12 months.

^d1 vs 6 months.

^eComparisons between 0 vs 1 month.

^f6 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 documented 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 $\leq 5\%$.

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 $\pm$ 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 χ^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 (Δ IL-6) and changes in anthropometric and biochemical parameters under univariate linear regression analysis.

Parameter	Δ IL-6 (0–12 months) long-term exercise	
	R^2	<i>P</i> value
Obesity		
Δ BMI (kg/m ²)	–0.05	<i>0.99</i>
Δ WHR	–0.01	<i>0.41</i>
Glucose metabolism		
Δ Fasting plasma glucose (mmol/l)	–0.04	<i>0.67</i>
Δ 2-h Plasma glucose (mmol/l)	–0.02	<i>0.45</i>
Δ Fasting plasma insulin (pmol/l)	–0.008	<i>0.38</i>
Δ HbA _{1c} (%)	0.22	0.01
Inflammation		
Δ CRP (pg/ml)	0.38	0.001
Physical fitness		
Δ Peak VO ₂ (ml/kg per min)	–0.04	<i>0.82</i>
Δ Maximal power (W/kg per body weight)	0.03	<i>0.19</i>

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

($n=60$). G/G homozygous subjects had a higher mean 2-h plasma glucose level (10 ± 0.16 mmol/l) when compared with carriers of the C allele (G/C heterozygotes + C/C homozygotes; 9.5 ± 0.15 mmol/l; $P < 0.05$). However, the IL-6 genotype did not correlate with any additional clinical parameter (all $P > 0.05$, data not shown) at baseline. In particular, there was no association between the IL-6 –174G/C genotype and IL-6 serum concentrations at baseline. However, significantly reduced IL-6 serum concentrations have only been found in patients who carried the C allele (group of G/C heterozygotes ($N=13$) as well as in a combined group of G/C heterozygotes + C/C homozygotes ($N=2$)). By contrast, IL-6 serum concentration did not change upon the 12-month training program in patients with the –174 G/G genotype of the IL-6 promoter (Table 3). The beneficial effects of exercise on BMI, WHR, fasting plasma glucose, fasting plasma insulin, 2-h plasma glucose, %HbA_{1c}, physical condition, and peak VO₂ were independent of the IL-6 genotype (Table 3). Multivariate linear regression analysis revealed the IL-6 –174 G/C genotype as a significant predictor of changes in IL-6 serum concentrations, which remained significant even upon adjustment for age, gender, and changes in BMI and HbA_{1c} (Table 4). When analyzing the effect of –174 G/C on changes in circulating CRP, the β coefficient remained similar to that observed in the analysis of changes in circulating IL-6 levels (0.44); however, the P value did not reach statistical significance ($P=0.13$).

In contrast to the exercise group, there was no effect of the –174G/C genotype on any of the rosiglitazone-induced changes in measures of glucose metabolism (HbA_{1c} levels, fasting, and 2-h OGTT plasma glucose), as the beneficiary effect of rosiglitazone treatment was evident for all genotypic groups (Table 3).

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 withstood 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 HbA_{1c} levels was observed after 12 months of exercise. Our data clearly indicate the relevance of exercise duration on levels of this inflammatory marker.

Table 3 Clinical characteristics of study subjects.

(A) After long-term exercise training grouped by –174G/C genotypes						
Genotype	G/G (n=9)			G/C + C/C (n=15)		
Age (range, years)	57 (49–67)			64 (54–72)		
Gender (M/F)	2/7			8/7		
Month	0	12		0	12	
BMI (kg/m ²)	29.5±0.5	28.0±0.5	*	30.5±0.5	29.1±0.5	– [†]
WHR	1.05±0.04	0.98±0.02	*	1.13±0.04	1.01±0.02	– [†]
HbA _{1c} (%)	6.08±0.12	5.61±0.03	*	6.03±0.05	5.64±0.02	– [†]
Fasting plasma insulin (pmol/l)	128.4±17.3	85.6±11.0	*	90.4±8.0	50.8±3.8	– [†]
Fasting plasma glucose (mmol/l)	5.83±0.07	5.19±0.08	*	5.93±0.10	5.29±0.04	– [†]
2 h Plasma glucose (mmol/l)	10.25±0.21	8.82±0.27	*	9.33±0.22	7.90±0.31	– [†]
Maximal power (W)	108±6	151±9	*	116±7	154±6	– [†]
Maximal power (W/kg per bw)	1.30±0.05	1.90±0.11	*	1.30±0.07	1.79±0.07	– [†]
Peak VO ₂ (ml/kg per min)	22.52±1.06	27.87±0.80	*	22.66±0.69	29.05±0.57	– [†]
IL-6 (pg/ml)	3.92±0.80	3.06±0.70	NS	4.17±0.76	2.41±0.46	– [†]
CRP (pg/ml)	3.60±0.72	2.93±0.46	NS	3.64±0.77	2.32±0.49	– [†]
(B) After rosiglitazone treatment grouped by –174G/C genotypes						
Genotype	G/G (n=6)			G/C + C/C (n=14)		
Age (range, years)	66 (58–72)			60 (35–71)		
Gender (M/F)	5/1			5/9		
Month	0	12		0	12	
BMI (kg/m ²)	30.5±1.4	30.0±1.4	NS	30.9±0.6	30.6±0.6	NS
WHR	1.14±0.06	1.07±0.02	NS	1.12±0.02	1.09±0.02	NS
HbA _{1c} (%)	5.85±0.03	5.57±0.04	– [†]	5.86±0.08	5.33±0.06	– [§]
Fasting plasma insulin (pmol/l)	71.6±10.3	60.5±13.8	NS	96.9±12.4	69.5±4.7	NS
Fasting plasma glucose (mmol/l)	5.65±0.05	5.15±0.07	– [†]	5.86±0.08	5.33±0.06	– [§]
2 h plasma glucose (mmol/l)	9.86±0.34	7.72±0.19	– [†]	9.85±0.26	7.54±0.24	– [§]
Maximal power (W)	137±23	138±22	NS	116±8	121±9	NS
Maximal power (W/kg per bw)	1.44±0.22	1.47±0.21	NS	1.36±0.11	1.42±0.13	NS
Peak VO ₂ (ml/kg per min)	24.52±0.98	24.40±1.17	NS	21.62±0.70	21.41±0.63	NS
IL-6 (pg/ml)	3.73±1.03	2.59±0.65	NS	3.74±0.73	3.15±0.51	NS
CRP (pg/ml)	3.13±0.94	2.98±0.86	NS	3.35±0.73	2.75±0.53	NS

*Statistical significance of $P < 0.05$ for exercise-induced changes in examined parameters in the group of GG homozygous subjects. [†]Statistical significance of $P < 0.05$ for exercise-induced changes in examined parameters in the group of GC heterozygous + CC homozygous subjects. NS, not significant at $P < 0.05$.

[‡]Statistical significance of $P < 0.05$ for rosiglitazone-induced changes in examined parameters in the group of GG homozygous subjects. [§]Statistical significance of $P < 0.05$ for rosiglitazone-induced changes in examined parameters in the group of GC heterozygous + CC homozygous subjects. NS, not significant at $P < 0.05$.

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 metabolisms in subjects with IGT might interact with effects of the –174G/C IL-6 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 HbA_{1c} 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 4 Analyses of the influence of –174G/C variant on changes in circulating interleukin-6 (Δ IL-6) after 12 months of exercise training in a multiple regression analysis.

	β Coefficient	P value
Age (year)	–0.001	<i>0.99</i>
Gender	0.40	<i>0.06</i>
Δ BMI (kg/m ²)	0.07	<i>0.69</i>
Δ HbA _{1c} (%)	0.42	<i>0.06</i>
Genotype (–174G/C)	0.44	<i>0.04</i>

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

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 (TNF α) (29). Since we did not measure circulating TNF α or adipose tissue expression of TNF α and IL-6, further studies are necessary to elucidate whether the genotype-associated differences in IL-6 response to exercise are mediated by TNF α .

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).

It needs to be mentioned that exercise and pharmaceutical intervention (rosiglitazone) may have similar but not identical effects on insulin sensitivity and, therefore, this comparison should be considered with caution when investigating the mechanisms of a non-pharmaceutical intervention (e.g., exercise) 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.

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