CLINICAL STUDY

Effect of a 4 week physical training program on plasma concentrations of inflammatory markers in patients with abnormal glucose tolerance

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Abstract

Objective: Subclinical chronic inflammation could be a unifying factor linking type 2 diabetes (T2D) and atherosclerosis. The beneficial effects of physical activity on a reduced risk of coronary heart disease could at least in part be mediated by improved markers of inflammation.

Research design and methods: The aim of this study was to determine the effect of 4 weeks of physical training on plasma concentrations of interleukin (IL)-6, C-reactive protein (CRP), adiponectin and IL-10 in 60 individuals with normal glucose tolerance, impaired glucose tolerance (IGT) or T2D.

Results: In patients with IGT and T2D, significant improvement in body fat, fitness level, glucose metabolism and insulin sensitivity after 4 weeks of physical training was associated with significantly improved plasma concentrations of adiponectin and CRP, but not IL-6. Regression analysis demonstrated only for the anti-inflammatory parameters adiponectin and IL-10 a significant relationship with the decrease in fasting plasma glucose, whereas changes in IL-6 and CRP were not significantly related to changes in fasting plasma glucose, body fat, maximal oxygen uptake, or insulin sensitivity. In a multivariate linear regression analysis, only changes in circulating adiponectin, fasting plasma glucose and percentage body fat were determinants of changes in insulin sensitivity.

Conclusions: Physical training was associated with a near normalization of adiponectin and CRP plasma concentrations in subjects with IGT and T2D. Increased insulin sensitivity after training was most strongly related to changes in adiponectin plasma concentrations, in fasting plasma glucose and percentage body fat, whereas changes in IL-6, IL-10 and CRP plasma concentrations did not significantly contribute to improved insulin sensitivity.

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Introduction

Patients with type 2 diabetes mellitus (T2D) have an increased risk for premature atherosclerosis (1, 2). Chronic systemic inflammation is recognized as a part of both atherosclerosis (3) and T2D (4) and could therefore represent the common pathogenic factor in the development of these diseases. Altered plasma concentrations of inflammatory mediators may be pathogenic by inducing systemic endothelial dysfunction. Several studies have demonstrated that elevated markers of inflammation such as interleukin (IL)-6 (5, 6) and C-reactive protein (CRP) (6–8), as well as decreased anti-inflammatory factors including adiponectin (9–11) and IL-10 (12–14) are predictors of insulin resistance and T2D. However, there are other studies

that could not show changes in IL-6, CRP and adiponectin plasma concentrations after regular physical exercise interventions (15–17). Physical activity is associated with reduced risk of cardiovascular disease (CVD), cardiovascular death and total mortality in men with T2D (18). The beneficial effects of physical activity on a reduced CVD risk could at least in part be mediated by improved markers of inflammation. It has been suggested 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 (19). In nondiabetic, insulin-resistant individuals, it was recently shown that exercise training is not associated with improved CRP or adiponectin levels (20). However, the effects of exercise on inflammatory

parameters in patients with T2D need to be further investigated. It is still unclear whether long-term exercise-associated anti-inflammatory effects are entirely explained by changes in body fat content, insulin sensitivity, fitness level or glucose metabolism in individuals with impaired glucose metabolism. Moreover, exercise-induced changes in inflammatory markers could contribute to the improvement of insulin sensitivity after physical training.

We therefore investigated the effects of a 4 week physical training program on plasma concentrations of IL-6, adiponectin, CRP and IL-10. We further asked whether changes in these parameters go beyond that expected from changes in obesity. We finally analyzed the relationship of changes of these inflammation and endothelial dysfunction markers with insulin sensitivity.

Research design and methods

Subjects

Sixty Caucasian men and women (mean age 45.2 ± 3.9 years) were enrolled in a supervised physical training program. Each subject underwent three times a week a 60 min training program, which was monitored and documented by two trainers. Each training session consisted of 20 min warming and cool-down periods, 20 min of running or biking, and 20 min of power-training three times a week. In addition, participants performed 60 min of swimming on a separate day. The compliance rate was 100%. These subjects had been randomly selected from more than 500 volunteers at the beginning of a training program, who were screened for impaired glucose tolerance (IGT) by a 75 g oral glucose-tolerance test (OGTT) and subsequently divided into three different groups according to American Diabetes Association (ADA) criteria (21), i.e. subjects with (i) normal glucose tolerance (NGT), (ii) IGT or (iii) T2D. Subjects with NGT were defined by a fasting plasma glucose < 6.0 mmol/l and a 120 min plasma glucose < 7.8 mmol/l after a 75 g oral glucose load (21). These subjects exhibited no family history of diabetes or T2D during pregnancy ($n = 20$; 9 males, 11 females). Subjects with IGT were defined by a fasting plasma glucose < 6.0 mmol/l and a 120 min plasma glucose > 7.8 mmol/l and < 11.1 mmol/l after a 75 g oral glucose load (21) ($n = 20$; 9 males, 11 females). Subjects with T2D were defined by a fasting plasma glucose > 7 mmol/l and/or a 120 min OGTT glucose > 11.1 mmol/l (21) ($n = 20$; 11 males, 9 females). All subjects fulfilled the following 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 either CVD or peripheral artery disease; (v) no thyroid dysfunction;

(vi) no concomitant medication intake, except for metformin (500 mg twice daily) in the T2D group; (vii) no alcohol or drug abuse; and (viii) no pregnancy. In addition, there was no evidence of diabetic retinopathy or nephropathy in these patients. During the 4 week training period, constant daily caloric intake was reported by each subject using dietary diaries. 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.

Measures of body fat content and OGTT

At baseline and after 4 weeks of training, blood samples were taken for the measurement of IL-6, CRP, IL-10, adiponectin and standard laboratory parameters using the previously described methods (6). In addition dual-energy X-ray absorptiometry (DEXA) analyses and measurements of anthropometric parameters were performed. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m). Waist and hip circumferences were measured and a waist-to-hip ratio (WHR) was calculated. Percentage body fat was measured by DEXA. The OGTT was performed according to ADA criteria (21). Three days prior to the OGTT the patients 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. Insulin sensitivity was assessed with the euglycemic-hyperinsulinemic clamp method as previously described (22, 23).

Maximal exercise test

All subjects completed a graded bicycle-ergometer test to volitional exhaustion by measuring maximal oxygen uptake ($\text{VO}_{2\text{max}}$) with an automated open circuit gas analysis system at baseline. The highest oxygen uptake/minute reached was defined as the $\text{VO}_{2\text{max}}$. Individuals in the supervised physical training subgroup ($n = 60$) completed an additional maximal exercise test after 4 weeks of physical training.

Assays

Basal, fasting blood samples were taken after an overnight fast to determine glucose, insulin, and standard laboratory parameters. Plasma concentrations of insulin, C-peptide, free fatty acid (FFA) and leptin were measured as previously described (22). Plasma adiponectin was assessed using a commercially available RIA kit (Linco Research, St Charles, MO, USA). Serum samples for CRP, IL-6 and IL-10 were stored at -80°C and assayed in triplicate. High-sensitivity ELISAs were used for IL-6 and IL-10 (Quantikine IL-6, IL-10; R&D Systems, Oxford, UK) as previously described (6), and

the high-sensitivity CRP assay was by immunonephelometry (Dade-Behring, Milan, Italy). Both interassay and intraassay coefficients of variation were < 5%.

Statistical analysis

Data are shown as means $\pm$ S.D. unless stated otherwise. Prior to statistical analysis, non-normally distributed parameters were log transformed to approximate a normal distribution. The following statistical tests were used: paired Student's *t*-test, Chi square test, and Pearson's simple correlation. Linear relationships were assessed by least square regression analysis. Multivariate linear relationships were assessed by a general linear model. Statistically analysis was performed using SPSS version 12.0 (SPSS, Chicago, IL, USA). In order to determine whether changes in inflammatory parameters are proportional or disproportional to training-induced changes in total body fat, increased fitness level and insulin sensitivity, random intercept models have been calculated, which considered changes of the inflammatory markers and their interaction with changes in VO_2max , glucose infusion rate during the steady-state of the clamp and percent body fat. These calculations were performed using the software 'Proc mixed' of the SAS 9.1 package (SAS Institute, Inc., Cary, NC, USA).

Results

Subjects

The 60 Caucasian men and women were studied either together or after being divided into three different groups, i.e. subjects with NGT ($n = 20$), or IGT ($n = 20$) or T2D ($n = 20$) (Table 1). Subjects reported daily caloric intake and macronutrient composition was constant during the study period (Table 1). The training effect was confirmed by a significant improvement in VO_2max in all groups (Table 1). Four weeks of physical training resulted in significant decreases in BMI, WHR and percent body fat in all glucose-tolerance groups (Table 1). Moreover, in the IGT and T2D groups, whole-body glucose uptake during the steady-state of a euglycemic-hyperinsulinemic clamp significantly improved after 4 weeks of physical training (Table 1). In patients with T2D, there was an additional significant decrease in fasting plasma glucose concentrations after 4 weeks of physical training, whereas no such difference was detected in the NGT and IGT groups (Table 1).

Correlation of chronic inflammatory markers with parameters of obesity, glucose metabolism, insulin sensitivity and fitness level

Plasma IL-6 concentration was significantly positively correlated with percent body fat (Fig. 1), BMI

($r^2 = 0.11$; $P < 0.001$), fasting plasma glucose ($r^2 = 0.1$; $P < 0.001$) and insulin concentration ($r^2 = 0.2$; $P < 0.001$). There was a significant negative correlation between IL-6 plasma levels and VO_2max and glucose infusion rate during the steady-state of the euglycemic-hyperinsulinemic clamp (Fig. 1). There was a significant negative correlation between adiponectin plasma concentrations and percent body fat (Fig. 2), BMI ($r^2 = 0.1$; $P < 0.001$), fasting plasma glucose ($r^2 = 0.1$; $P < 0.001$) and insulin concentrations ($r^2 = 0.2$; $P < 0.001$). Adiponectin levels were significantly positively correlated with VO_2max and insulin sensitivity (Fig. 2). IL-10 plasma concentrations negatively correlated with percent body fat (Fig. 3), BMI ($r^2 = 0.05$; $P < 0.001$), fasting plasma glucose ($r^2 = 0.03$; $P < 0.001$) and insulin concentration ($r^2 = 0.07$; $P < 0.001$), whereas a significantly positive correlation was found between IL-10 levels and VO_2max and glucose infusion rate during the steady-state of the euglycemic-hyperinsulinemic clamp (Fig. 3). CRP plasma concentration was significantly positively correlated with percent body fat (Fig. 4), BMI ($r^2 = 0.22$; $P < 0.001$), fasting plasma glucose ($r^2 = 0.14$; $P < 0.001$) and insulin concentration ($r^2 = 0.2$; $P < 0.001$). There was a significantly negative correlation between CRP plasma levels and VO_2max and glucose infusion rate during the steady-state of the euglycemic-hyperinsulinemic clamp (Fig. 4).

Effect of 4 weeks of physical training on markers of chronic inflammation

Plasma adiponectin concentration was significantly increased after 4 weeks of physical training in the IGT and T2D groups (Fig. 5). Interestingly, 4 weeks of exercise did not have any effect on IL-6 plasma concentrations (Fig. 5). Moreover, plasma IL-10 concentrations were not significantly different between baseline and after 4 weeks of training. However, there was a tendency for increased IL-10 plasma concentrations in the IGT and T2D groups (Fig. 5). Plasma concentrations of CRP significantly decreased in all three glucose-tolerance groups (Fig. 5).

Effect of training on changes in chronic inflammatory markers beyond that expected from changes in obesity, glucose metabolism, insulin sensitivity and fitness level

Despite the significant decrease in percent body fat and increases in VO_2max and insulin sensitivity, IL-6 plasma concentrations were unchanged after 4 weeks of physical training (Fig. 1). In contrast, the significant increase in adiponectin levels after 4 weeks of physical training (Fig. 5) is closely associated with improvement of insulin sensitivity and disproportionally exceeds the

Table 1 Anthropometric and biochemical parameters (means $\pm$ s.d.) at baseline and after 4 weeks of intensive physical training in subjects with NGT, IGT or T2D. Dietary record data are shown as means during the study for men (M) and women (F).

	NGT (<i>n</i> = 20)		IGT (<i>n</i> = 20)		T2D (<i>n</i> = 20)	
	Baseline	Post-interventional	Baseline	Post-interventional	Baseline	Post-interventional
BMI (kg/m ²)	24.2 $\pm$ 3.1	23.9 $\pm$ 2.7*	29.8 $\pm$ 3.9	29.0 $\pm$ 3.7*	31.3 $\pm$ 3.1	30.5 $\pm$ 2.7*
Body weight (kg)	69.6 $\pm$ 14.0	68.2 $\pm$ 7.7*	87.6 $\pm$ 16.4	84.4 $\pm$ 6.1*	94.6 $\pm$ 19.6	93.0 $\pm$ 8.1*
WHR	0.84 $\pm$ 0.09	0.81 $\pm$ 0.08*	1.20 $\pm$ 0.16	1.17 $\pm$ 0.15*	1.28 $\pm$ 0.12	1.24 $\pm$ 0.11*
Fat mass (%)	24.5 $\pm$ 3.1	23.2 $\pm$ 2.7*	34.9 $\pm$ 8.2	31.5 $\pm$ 7.4*	38.1 $\pm$ 7.9	35.2 $\pm$ 7.6*
FPG (mmol/l)	5.1 $\pm$ 0.5	5.0 $\pm$ 0.3	5.6 $\pm$ 0.5	5.4 $\pm$ 0.6	6.2 $\pm$ 0.6	5.8 $\pm$ 0.4*
2 h OGTT glucose (mmol/l)	5.9 $\pm$ 0.76	5.5 $\pm$ 0.5	9.4 $\pm$ 0.8	8.1 $\pm$ 1.3*	13.0 $\pm$ 1.5	12.6 $\pm$ 2.4
FPI (pmol/l)	66 $\pm$ 34	57 $\pm$ 27	695 $\pm$ 493	379 $\pm$ 323*	319 $\pm$ 212	234 $\pm$ 119
Glucose infusion rate (μ mol/kg/min)	76 $\pm$ 17	85 $\pm$ 15	19 $\pm$ 9	36 $\pm$ 16*	21 $\pm$ 9	32 $\pm$ 11*
VO ₂ max (ml/kg/min)	34.2 $\pm$ 2.6	36.1 $\pm$ 3.2*	26.4 $\pm$ 1.9	28.1 $\pm$ 1.4*	26.1 $\pm$ 1.8	27.5 $\pm$ 1.9*
Triglyceride (mmol/l)	1.4 $\pm$ 0.4	1.5 $\pm$ 0.6	2.5 $\pm$ 0.1	2.2 $\pm$ 0.4*	3.0 $\pm$ 0.1	2.1 $\pm$ 0.5*
FFA (mmol/l)	0.41 $\pm$ 0.19	0.39 $\pm$ 0.18	0.53 $\pm$ 0.24	0.50 $\pm$ 0.24	0.56 $\pm$ 0.26	0.47 $\pm$ 0.22
Leptin (pmol/l)						
M	2.7 $\pm$ 2.0	2.6 $\pm$ 1.7	20.6 $\pm$ 9.0	21.1 $\pm$ 7.5	3.3 $\pm$ 1.8	3.5 $\pm$ 2.0
F	6.0 $\pm$ 2.7	5.8 $\pm$ 2.7	42.2 $\pm$ 23.8	36.7 $\pm$ 19.5	9.0 $\pm$ 1.7	9.0 $\pm$ 2.4
Total cholesterol (mmol/l)	4.6 $\pm$ 0.4	4.7 $\pm$ 0.4	5.3 $\pm$ 0.5	5.5 $\pm$ 0.5	5.6 $\pm$ 0.7	5.4 $\pm$ 0.4
Total HDL (mmol/l)	1.6 $\pm$ 0.4	1.5 $\pm$ 0.2	1.2 $\pm$ 0.3	1.9 $\pm$ 0.3*	1.2 $\pm$ 0.3	1.7 $\pm$ 0.4*
Total LDL (mmol/l)	2.4 $\pm$ 0.4	2.0 $\pm$ 0.4*	3.3 $\pm$ 0.4	2.7 $\pm$ 0.6*	3.4 $\pm$ 0.8	2.8 $\pm$ 0.7*
Dietary record data M/F						
Energy intake (kcal/day)	3538 $\pm$ 802/2537 $\pm$ 512		3633 $\pm$ 538/2987 $\pm$ 674		3942 $\pm$ 967/2818 $\pm$ 635	
Carbohydrate (% of energy)	45.1 $\pm$ 7.5/49.7 $\pm$ 6.9		38.8 $\pm$ 7.3/46.4 $\pm$ 6.4		41.8 $\pm$ 7.7/43.4 $\pm$ 9.1	
Fat (% of energy)	35.4 $\pm$ 6.7/31.3 $\pm$ 8.5		43.6 $\pm$ 9.1/35.4 $\pm$ 9.3		39.8 $\pm$ 4.3/39.4 $\pm$ 6.4	
Protein (% of energy)	19.5 $\pm$ 5.2/19.0 $\pm$ 4.5		17.6 $\pm$ 5.8/18.2 $\pm$ 3.9		18.4 $\pm$ 5.9/17.2 $\pm$ 3.1	

*P < 0.05 for baseline vs after 4 weeks of intensive physical training within each group.

HDL, high-density lipoprotein; LDL, low-density lipoprotein; FPG, fasting plasma glucose; FPI, fasting plasma insulin.

beneficial effects of reduced percent body fat and increased fitness level (VO_2max) (Fig. 2). Increased IL-10 plasma concentration (Fig. 5) were proportional to the improvement of body fat content, VO_2max and insulin sensitivity after 4 weeks of training (Fig. 3).

The significant decrease in CRP levels after 4 weeks of intensive training (Fig. 5) disproportionately exceeds the expected effects from the improvement of insulin sensitivity, reduced percent body fat and increased fitness level (VO_2max) (Fig. 4).

Figure 1 Correlation of plasma IL-6 concentrations with (upper panel) total body fat, (middle panel) maximal aerobic capacity (VO_2max), and (lower panel) glucose infusion rate during the steady-state of a euglycemic–hyperinsulinemic clamp. The inserts in each panel show changes of mean IL-6 from baseline to after physical exercise concentrations. Closed circles represent baseline levels, open circles represent IL-6 concentrations after 4 weeks of intensive physical training ($n = 60$). P -values (t -test) indicate whether IL-6 after-training concentrations are significantly different from the calculated after-training concentration derived from the regression curve equation. The direction of the changes is indicated by arrows.

Figure 2 Correlation of plasma adiponectin concentrations with (upper panel) total body fat, (middle panel) maximal aerobic capacity (VO_2max), and (lower panel) glucose infusion rate during the steady-state of a euglycemic–hyperinsulinemic clamp. The three inserts show changes of mean adiponectin from baseline to after physical exercise concentrations. Closed circles represent baseline levels, open circles represent adiponectin concentrations after 4 weeks of intensive physical training ($n = 60$). P -values (t -test) indicate whether adiponectin after-training concentrations are significantly different from the calculated after-training concentration derived from the regression curve equation. The direction of the changes is indicated by arrows.

Multivariate regression analyses

The correlations found by simple linear regression analysis were further analyzed in more detail. Multivariate linear regression analysis of relative changes in percent body fat, VO_2max , and fasting plasma glucose as

predictors for the relative changes in adiponectin, CRP, IL-6 and IL-10 plasma concentrations revealed only changes in fasting plasma glucose as significant determinants of changes in adiponectin and IL-10 con-

Figure 3 Correlation of plasma IL-10 concentrations with (upper panel) total body fat, (middle panel) maximal aerobic capacity (VO_2max), and (lower panel) glucose infusion rate during the steady-state of a euglycemic-hyperinsulinemic clamp. The three inserts show changes of mean IL-10 from baseline to after physical exercise concentrations. Closed circles represent baseline levels, open circles represent IL-10 concentrations after 4 weeks of intensive physical training ($n = 60$). P -values (t -test) indicate whether IL-10 after-training concentrations are significantly different from the calculated after-training concentration derived from the regression curve equation. The direction of the changes is indicated by arrows.

Figure 4 Correlation of plasma CRP concentrations with (upper panel) total body fat, (middle panel) maximal aerobic capacity (VO_2max), and (lower panel) glucose infusion rate during the steady-state of a euglycemic-hyperinsulinemic clamp. The three inserts show changes of mean CRP from baseline to after physical exercise concentrations. Closed circles represent baseline levels, open circles represent CRP concentrations after 4 weeks of intensive physical training ($n = 60$). P -values (t -test) indicate whether CRP after-training concentrations are significantly different from the calculated after-training concentration derived from the regression curve equation. The direction of the changes is indicated by arrows.

Figure 5 Baseline and post-interventional plasma concentrations of (mean ± s.d.) adiponectin, IL-6, IL-10 and CRP in subjects with NGT ($n = 20$) and IGT ($n = 20$) and in patients with T2D ($n = 20$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ between baseline and after 4 weeks of intensive physical training.

centrations after 4 weeks of physical training (Table 2). We further assessed the role of changes in percent body fat, $VO_2\max$, fasting plasma glucose and plasma concentrations of inflammatory markers as determinants for changes in insulin sensitivity. Glucose infusion rate during the steady-state of the euglycemic–hyperinsulinemic clamp was chosen as the dependent variable in the stepwise multivariate linear regression analysis (Table 3). Changes in adiponectin plasma concentrations, fasting plasma glucose and percentage of body fat emerged as significant determinants of changes in insulin sensitivity after 4 weeks of physical exercise (Table 3). The following parameters did not reach statistical significance ($P > 0.25$) when included one-by-one into the model: ΔCRP ($P = 0.35$), $\Delta IL-10$ ($P = 0.4$), $\Delta IL-6$ ($P = 0.42$) and $\Delta VO_2\max$ ($P = 0.77$).

Discussion

Epidemiological studies have demonstrated that physical activity is associated with reduced risk of CVD, especially in individuals with T2D (18, 24). The reduced CVD risk associated with exercise in patients

with T2D could be mediated by improved markers of inflammation and endothelial dysfunction. We therefore determined the effect of an intensive 4 week physical training on plasma concentrations of the inflammatory parameters IL-6, adiponectin, CRP and IL-10 in 60 individuals with NGT, IGT or T2D. In all three glucose-tolerance groups, fitness level, as measured by $VO_2\max$, significantly increased after the 4 week training period. This training effect was associated with significant decreases in BMI, WHR, and percent body fat. In patients with IGT and T2D, but not in the NGT group, there was an additional effect of training on improved insulin sensitivity. The absence of improved insulin sensitivity in the NGT group was expected, because of the already high degree of insulin sensitivity in these healthy individuals.

The key finding of this study was that 4 weeks of supervised intensive regular exercise were associated with significantly improved adiponectin and CRP plasma concentrations in subjects with IGT and T2D. However, no effect of 4 weeks of exercise training on

Table 2 Multivariate linear regression analysis of relative changes in percent body fat, $VO_2\max$, and fasting plasma glucose as predictors for the relative changes in adiponectin, IL-6, IL-10 and CRP plasma concentrations.

	$\Delta\%$ Body fat β (P -value)	$\Delta VO_2\max$ β (P -value)	ΔFPG β (P -value)
Δ Adiponectin	−0.04 (0.78)	0.1 (0.42)	−0.26 (0.04)
Δ IL-6	−0.09 (0.5)	0.06 (0.7)	0.09 (0.5)
Δ IL-10	−0.08 (0.53)	0.21 (0.1)	0.26 (0.04)
Δ CRP	0.08 (0.5)	−0.07 (0.63)	0.04 (0.7)

Table 3 Stepwise multivariate regression analysis of changes in anthropometric and biochemical parameters as predictors of relative changes in glucose infusion rate (GIR) during the steady-state of euglycemic–hyperinsulinemic clamps. The following parameters did not reach statistical significance ($P > 0.25$) when included one-by-one into the model: ΔCRP , $\Delta IL-10$, $\Delta IL-6$, $\Delta VO_2\max$.

	ΔGIR ($\mu\text{mol/kg/min}$)		
	Step	β	P -value
Δ Adiponectin ($\mu\text{g/ml}$)	1	0.45	0.004
Δ FPG (mmol/l)	2	0.36	0.026
Δ BF (%)	3	−0.23	0.034

FPG, fasting plasma glucose, BF, body fat.

these markers of inflammation was observed in healthy individuals with NGT. This result suggests that physical training can normalize alterations in these plasma parameters associated with abnormal glucose tolerance and could therefore be due to changes in glucose or FFA levels or other metabolic abnormalities.

The increase in adiponectin plasma concentration we observed in our study after a 4 week training program is in contrast to recent studies, in which no alterations of adiponectin levels were found (15, 16). However, distinct characteristics of study populations and training intervention programs could explain these different results.

Our finding that exercise reduces CRP concentrations is supported by at least two longitudinal studies showing that regular training induces a reduction in CRP level (25, 26). However, in contrast to our results it was recently shown that in nondiabetic, insulin-resistant individuals exercise training is not associated with improved CRP or adiponectin levels (20). Moreover, Nicklas *et al.* (17) recently showed that exercise training did not have a significant effect on CRP and IL-6 plasma concentrations, whereas diet-induced weight loss significantly improved these parameters of chronic inflammation. Differences in the duration and intensity of training and more likely in the study population, i.e. patients with IGT or T2D in our cohort, nondiabetic subjects in one previous report (20), and older, overweight or obese patients with osteoarthritis in the other study (17), could explain these discrepant results. The improvement in CRP (Fig. 4) and adiponectin (Fig. 2) plasma concentrations was disproportionately higher than expected from the improvement in percent body fat, VO_2max and insulin sensitivity after training, suggesting additional accompanying effects of exercise to improve plasma concentrations of these parameters. Interestingly, the changes in CRP are not related to changes in IL-6 plasma concentrations, although CRP levels are driven to a large extent by IL-6 (27). This suggests that other factors which significantly changed with the physical training are stronger determinants of CRP plasma concentration in our intervention study.

A marked increase in circulating levels of IL-6 after exercise without muscle damage has been a remarkably consistent finding (28). 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 (28). However, whether these acute effects of exercise on IL-6 concentrations lead to chronically changed IL-6 levels is unclear. In the resting state, IL-6 plasma concentrations were not significantly different between elite rowers and non-athletic controls (29), suggesting that body fitness is no major determinant of resting IL-6 plasma concentration. This is in accord with our results, that 4 weeks of exercise did not have any effect on IL-6 plasma concentrations. Despite a significant correlation of IL-6 plasma concentration with measures of obesity, fitness level, insulin sensitivity

and glucose metabolism, changes in these parameters did not predict changes in IL-6 levels (Fig. 3).

There was a trend for elevated IL-10 plasma concentrations after 4 weeks of exercise in the IGT and T2D groups, suggesting that IL-10 mediates some of the beneficial chronic effects of exercise. The increase of IL-10 levels after 4 weeks of exercise was proportional to the reduction of total body fat, the increase in fitness level and insulin sensitivity, suggesting that changes in IL-10 can be primarily explained by changes of these parameters. Moreover, multivariate regression analysis identified a decreased fasting plasma glucose concentration as a significant determinant of elevated IL-10 plasma concentration.

Subclinical inflammation is associated with insulin resistance and could thereby precede the development of T2D (30, 31). Interestingly, stepwise multivariate regression analysis revealed changes in adiponectin plasma concentration as the strongest predictor of changes in insulin sensitivity as measured by glucose infusion rate during the steady-state of a euglycemic–hyperinsulinemic clamp. This result further supports the concept of adiponectin as an adipokine with insulin-sensitizing effects (32, 33). In addition to plasma adiponectin concentrations, stepwise multivariate regression analysis identified changes in fasting plasma glucose and percent body fat as significant predictors of relative changes in glucose infusion rate during the steady-state of the euglycemic–hyperinsulinemic clamp, thereby confirming the known relationships between insulin sensitivity, hyperglycemia and adiposity. However, we could not confirm our main hypothesis that exercise-induced changes in inflammatory markers could contribute to the improvement of insulin sensitivity after physical training. In our study, changes in inflammatory parameters did not appear to be related to the improvement of insulin sensitivity, suggesting that chronic subclinical inflammation is not the predominant determinant of insulin sensitivity.

In conclusion, in patients with IGT and T2D, physical training led to a near normalization of adiponectin and CRP plasma concentrations. The increase in CRP and adiponectin was disproportionately higher than expected from the improvement in percent body fat, VO_2max and insulin sensitivity after training, suggesting additional beneficial effects of exercise on plasma concentrations of these parameters. Increased insulin sensitivity after training was most strongly related to changes in adiponectin plasma concentrations, in fasting plasma glucose and percent body fat, whereas changes in IL-6, IL-10 and CRP plasma concentrations did not significantly contribute to improved insulin sensitivity.

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