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Effect of aerobic training on plasma levels and subcutaneous abdominal adipose tissue gene expression of adiponectin, leptin, interleukin 6, and tumor necrosis factor α in obese women

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Abstract

Adipocytokines secreted by adipose tissue are suggested to play a role in the development of obesity-related complications. Regular aerobic exercise has been shown to reduce the risk of metabolic complications in obese subjects. The aim of this study was to investigate the effect of aerobic training on gene expression in subcutaneous abdominal adipose tissue (SCAAT) and on plasma levels of several adipocytokines in obese women. Twenty-five obese sedentary premenopausal women (body mass index, 32.18 ± 3.17 kg/m^2) underwent a 12-week aerobic exercise program, with a frequency of 5 d/wk and intensity corresponding to 50% of individual maximal oxygen consumption ($\dot{V}O_2$max) consisting of 2 sessions per week of supervised aerobic exercise and 3 sessions per week of home-based exercise on a bicycle ergometer. Before and after the aerobic training, $\dot{V}O_2$max and body composition were measured and plasma and SCAAT biopsy samples (in a subgroup of 8 subjects) were obtained for determination of plasma and messenger RNA levels of adipocytokines (leptin, adiponectin, interleukin 6, tumor necrosis factor α). The aerobic training resulted in an increase of subjects' $\dot{V}O_2$max by 12.8% (24.6 ± 3.9 vs 27.7 ± 4.8 mL · min^{-1} · kg^{-1}, $P < .05$). Body weight and fat mass were reduced by 5.9% (88.5 ± 8.2 vs 83.3 ± 7.7 kg, $P < .001$) and 6.4% (38.8 ± 4.2% vs 36.3 ± 4.6%, $P < .001$), respectively, and the revised QUantitative Insulin sensitivity ChecK Index (QUICKI) increased (0.43 ± 0.06 vs 0.48 ± 0.06, $P < .05$) during the aerobic training. No aerobic training-induced changes in messenger RNA levels of the investigated genes in SCAAT were observed. A decrease of plasma leptin (24.3 ± 8.7 vs 18.1 ± 8.3 ng/mL, $P < .05$) was detected, whereas plasma levels of other cytokines remained unchanged. In moderately obese females, 3 months' aerobic training did not promote changes in the adipose tissue gene expression or plasma levels of the adipocytokines (except for leptin) involved in a regulation of lipid and carbohydrate metabolism.

1. Introduction

Obesity is known to be associated with a whole body pro-inflammatory state and a number of metabolic disturbances included in the metabolic syndrome. In the search for the mechanism of association between obesity and metabolic syndrome, a role for the adipocytokines secreted by adipose tissue has been suggested. Plasma levels and the expression of adipocytokines in adipose tissue are modified in obese subjects [1-3]. The adipocytokines bring about a number of metabolic actions that might induce or prevent obesity-related metabolic disturbances. Adiponectin increases glucose uptake and fatty acid oxidation by muscle [4] and reduces hepatic gluconeogenesis [5]. A large part of these effects are mediated by the activation of adenosine monophosphate–activated protein kinase(s). Infusion of adiponectin together with leptin reversed insulin resistance in a lipoatrophic mouse model [6]. Tumor necrosis factor α

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(TNF-α) induced insulin resistance in animals and in an in vitro model [7] by interacting with insulin receptor signaling and through activation of lipolysis and inhibition of lipoprotein lipase, the 2 effects concurring to cause increased plasma free fatty acid (FFA) levels [8,9]. Subcutaneous abdominal adipose tissue may not contribute to plasma TNF-α level as no net release from this area was detected [9]. Recently, TNF-α was proposed to have preferentially paracrine effects and to be a regulator of insulin resistance at the tissue level [10]. Association of interleukin 6 (IL-6) with fasting glucose, fasting insulin, and insulin sensitivity was observed in cross-sectional studies [2,11]. Its role in the development of insulin resistance is not completely clear as infusion of IL-6 at doses not higher than those achieved during exercise with stable concentrations of counterregulatory hormones results in unchanged glycemia and induces lipolysis in adipose tissue [12,13].

Aerobic training is considered to be a key part of the therapy for obesity, and numerous studies have shown improvement in the metabolic and cardiovascular status of obese subjects after an aerobic training period. It might be hypothesized that the effect of aerobic training on adipocytokine production and secretion mediates some of the beneficial effects of aerobic training in obesity. Recently, several studies have investigated the effects of the aerobic training on plasma levels of several adipocytokines in different groups of patients. Variable effects of physical training on plasma levels of adiponectin [12,14,15], tumor necrosis factor [16-18], and IL-6 [16,17,19] were found. Moreover, some of the adipocytokines such as TNF-α or IL-6 are also secreted by other tissues in addition to adipose tissue. Consequently, the plasma levels of adipocytokines are not a straightforward reflection of training-induced effects on their production in adipose tissue.

As recently pointed out [20,21], prospective studies of various types of treatment of obesity are necessary to clarify the effect of aerobic training on chronic, systemic inflammation. The aerobic training program is the predominant mode of regular exercise activity used in obese women. Therefore, the aim of this study was to investigate the effect of an intensive (5 days/wk) aerobic training program on plasma and messenger RNA (mRNA) levels of adipocytokines in subcutaneous adipose tissue (SCAAT) in obese female subjects.

2. Subjects and methods

2.1. Subjects

Twenty-five obese premenopausal women (age, 40.4 ± 6.7 years; weight, 88.5 ± 8.2 kg; body mass index [BMI], 32.2 ± 2.2 kg/m^2) were included in the study. None of women had any chronic disease, and all were free of any medication. Pregnancy was excluded at the beginning of the study. All the subjects had been sedentary before this aerobic training program for at least 1 year. The body weight of the subjects had not changed by more than 2 kg during the 3 months preceding the study. They all gave written informed consent before the experiments began. The study was performed according to the Declaration of Helsinki and approved by the ethical committee of the Third Faculty of Medicine, Charles University (Prague, Czech Republic).

2.2. Training program and maximal oxygen consumption determination

Five days before each investigation, maximum exercise test was performed on a bicycle ergometer (Ergoline 800, Ergoline GmbH, Bitz, Germany) in each subject to determine the maximal oxygen consumption ($\dot{V}O_2max$) (Vmax, Sensor Medics, Yorba Linda, CA). An initial work rate of 60 W was followed by a sequential increase in work rate by 25 W every minute until exhaustion. Verbal encouragement was given to attain maximal performance. Heart rate was monitored continuously. Two criteria assessed that the subjects achieved their true $\dot{V}O_2max$ [22]. The maximal heart rate corresponded to that predicted for the given age (179. 6 ± 6.34 beats per minute) before (180.6 ± 9.20 beats per minute; range, 165-205 beats per minute) as well as after (180.8 ± 10.36 beats per minute; range, 167-207 beats per minute) the training, and the achieved respiratory quotient was equal to 1.089 ± 0.025 (range, 1.03- 1.15) before and to 1.090 ± 0.0271 (range, 1.01-1.16) after the training. Calibration check of the spiroergometry system has been performed every week using the commercially available ethanol combustion testing method.

The aerobic training program lasted for 12 weeks and consisted of sessions of aerobic exercise 5 d/wk: (1) twice a week aerobic exercise performed in gymnasium and supervised by an exercise instructor and (2) 3 times a week home-based exercise on an electrically braked bicycle ergometer. Subjects were instructed to exercise at each session for 45 minutes at the intensity corresponding to the individually recommended target heart rates; the individual target heart rate was determined as that corresponding to 50% of individual $\dot{V}O_2max$ as measured during a maximum exercise test.

Each participant was provided with a cardiometer (Polar Accurex Plus Cardiometer, Polar Electric Oy, Kempele, Finland) to check the actual heart rate during exercise sessions. Throughout the program, the intensity of exercise was progressively increased to target heart rates corresponding to 55%, 60%, and 65% $\dot{V}O_2max$, respectively, every 3 weeks. The compliance to the home-based part of the training was found to be good as checked by exercise instructors using training diaries of each subject.

2.3. Experimental protocol

Subjects were investigated at 08:00 AM after an overnight fast in a semirecumbent position before and 72 hours after the last day (to eliminate possible effects of the last bout of exercise) of a 12-week aerobic training program. After a 30-minute rest in a semirecumbent

position, blood samples were drawn from an indwelling venous catheter. In a subgroup of 8 women, a needle biopsy of abdominal SCAAT was performed 15 to 20 cm laterally from the umbilicus, as described before [23].

2.4. Dietary regimen

Before the initial examination and throughout the aerobic training program, patients were instructed to maintain their habitual diet. The dietary intake was estimated by a 7-day food record before the beginning of the study. The maintenance of the dietary regimen was controlled every 4 weeks of the study using a 3-day food record. Adherence to the habitual diet was confirmed by the analysis of these 3-day food records.

2.5. Body composition assessment

Body composition was assessed in fasting condition using dual-energy x-ray absorptiometry performed with a whole-body scanner (Hologic, Siemens, Waltham, MA, the scanner being calibrated daily with Phantom Model DPA/QDR-1). All the measurements were performed during the morning hours (08:00 to 11:00 am), with a difference of maximum 2 hours between the entry and final examination, by the same technician ascribed specifically to this study.

2.6. Messenger RNA analysis

The biopsies of abdominal SCAAT (about 1 g) were used for mRNA quantification. The samples were washed, homogenized in RLT lysis buffer (Qiagen, Courtaboeuf, France) and stored at −80°C until analysis. Total RNA was extracted using the RNeasy total RNA Mini kit (Qiagen). Integrity of RNA was checked on agarose gel, and RNA concentration was determined using a fluorometric assay (Ribogreen, Fluoroskan Ascent, Thermo Electron Corp, Waltham, MA). Reverse transcription was performed with 250 ng of total RNA using random hexamers as primers and Superscript II reverse transcriptase (Invitrogen, Cergy Pontoise, France). Real-time quantitative polymerase chain reaction (qPCR) was performed on a GeneAmp 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). A set of primers was designed for adiponectin and leptin using the software Primer Express 1.5 (Applied Biosystems). For each primer pair, a standard curve was obtained using serial dilutions of human adipose tissue complementary DNA before mRNA quantification. For verification that genomic DNA was not amplified, qPCR was performed on reverse transcription reactions with no addition of reverse transcriptase. Primers and probes for IL-6 and TNF-α were obtained from Applied Biosystems. Each sample was taken in duplicate and 10 ng of complementary DNA was used as a template for real-time polymerase chain reaction. When the difference between the duplicates was more than 0.5 Ct, qPCR was performed again. We used 18S ribosomal RNA (Ribosomal RNA Control TaqMan Assay kit, Applied Biosystems) as control to normalize gene expression.

2.7. Blood analysis

Plasma glucose was determined with a glucose oxidase technique (Biotrol kit, Merck-Clevenot, Nogent-sur-Marne, France) (coefficient of variation [CV], 1.1%-2.0%) and FFA by an enzymatic procedure (Wako kit, Unipath, Dardilly, France) (CV, 2.7%). Plasma insulin concentrations were measured using radioimmunoassay kits from Sanofi Diagnostics Pasteur (Marnes la Coquette, France) (CV, 2.8%-4%).

Plasma triglycerides were determined by spectrophotometry. Adiponectin and leptin plasma levels were determined using radioimmunoassay kits from Linco Research (St Charles, MO) (CV for adiponectin kit, 9.3%; leptin, 3.4%-8.3%) according to the manufacturer's recommendations. Interleukin 6 and TNF-α plasma levels were determined using Quantikine IL-6 (CV 2%-4.2%) and Quantikine High Sensitivity TNF-α kits (CV, 4.6%-5.2%) (R&D Systems, Minneapolis, MN), respectively. The above-mentioned measurements were done in duplicates. Coefficients of variability in our laboratory were in the range of those indicated by kit producers (2%-9%) according to the analyzed substance.

2.8. Statistical analysis

All values are mean ± SD. All analyses were performed using SPSS 12.0 for Windows (SPSS, Chicago, IL). Response of gene expression data was analyzed by a Wilcoxon signed rank test. Correlations were examined by the Pearson correlation coefficient. $P < .05$ was considered statistically significant. The homeostasis model assessment (HOMA) index was computed following this equation: [fasting glycemia (mmol/L) · fasting insulin (mIU/L)]/22.5. The revised QUantitative Insulin sensitivity ChecK Index (QUICKI) was computed following this equation: 1/log [insulin (μIU/mL)] + log[glucose (mg/dL)] + log[NEFA (mmol/L)] [24].

Fold change in the gene expression analysis was calculated for the increasing changes by dividing relative mRNA level values after the intervention by values before the intervention and, for the decreasing changes, by dividing relative mRNA level values before the intervention by values after the intervention.

3. Results

3.1. Anthropometric and biochemical characteristics

Anthropometric, physical fitness, and related biochemical characteristics before and after the aerobic training program are shown in Table 1. An increase in aerobic fitness by 12.8% (24.59 vs 27.74 mL · min^{-1} · kg^{-1}, $P < .05$) as evaluated by $\dot{V}O_2max$ was achieved during the aerobic training program. Body weight decreased by 5.9%, fat mass by 6.52%, and waist circumference by 3.9%. Indices of lipid metabolism remained unchanged except for plasma FFA that decreased after physical intervention.

Table 1
Anthropometric and biochemical characteristics of subjects before and after a 12-week aerobic training program

	Before training	After training	P
Body weight (kg)	88.5 ± 8.2	83.3 ± 7.7	<.001
BMI (kg/m^2)	32.2 ± 2.2	30.4 ± 2.4	<.001
Fat mass (%)	38.8 ± 4.2	36.3 ± 4.6	<.001
Waist circumference (cm)	92.9 ± 7.0	89.3 ± 6.4	<.05
Hip circumference (cm)	114.5 ± 5.8	111.0 ± 5.9	<.001
Waist-hip ratio	0.81 ± 0.04	0.79 ± 0.04	.09
$\dot{V}O_2$max (mL · kg^{-1} · min^{-1})	24.6 ± 3.9	27.7 ± 4.8	<.05
Fasting glucose (mmol/L)	5.0 ± 0.5	5.1 ± 0.3	NS
Fasting insulin (mIU/L)	6.0 ± 3.9	5.5 ± 2.1	NS
Total cholesterol (mmol/L)	5.14 ± 0.9	5.09 ± 0.9	NS
HDL cholesterol (mmol/L)	1.4 ± 0.3	1.4 ± 0.4	NS
Triglycerides (mmol/L)	1.33 ± 0.7	1.30 ± 0.52	NS
FFAs (μmol/L)	670 ± 413	308 ± 128	<.05
HOMA	1.4 ± 0.9	1.2 ± 0.5	NS
rQUICKI	0.43 ± 0.06	0.48 ± 0.06	<.05

Data are presented as mean ± SD (N = 25). NS indicates not significant; HDL, high-density lipoprotein; rQUICKI, revised QUICKI.

3.2. Insulin sensitivity indices

The aerobic training did not influence fasting insulin (6.0 ± 3.9 vs 5.5 ± 2.1 mIU/L, $P > .05$) or fasting glucose level (5.0 ± 0.5 vs 5.1 ± 0.3 mmol/L, $P > .05$). Congruently with this finding, the HOMA index has not changed. Nonetheless, the revised QUICKI index, which takes into account plasma FFA levels, increased by 11.6% during the physical exercise program (0.43 ± 0.06 vs 0.48 ± 0.06, $P < .05$). Data are summarized in Table 1.

3.3. Adipocytokine plasma levels

Plasma leptin declined after the aerobic training program by 25.7% ($P < .001$). No change in plasma adiponectin, TNF-α, and IL-6 was observed. Data are summarized in Table 2.

When leptin plasma levels before and after the training program were adjusted to BMI or fat mass, the aerobic training-induced changes in leptin remained significant, suggesting independent effects of aerobic training on plasma leptin besides reduction of body fat. Plasma leptin, after adjustment to BMI, was reduced by 22.1% and after adjustment to fat mass by 14.6%.

3.4. Expression of adipocytokines in SCAAT

Gene expression was assessed by determining relative mRNA level for adiponectin, leptin, IL-6, and TNF-α in SCAAT. No changes in mRNA in SCAAT for any of the studied cytokines were detected. Data are summarized in Table 3. It is to be noted that, with the number of biopsies analyzed in this study (n = 8), the power analysis shows the minimum detectable fold decrease is 1.9 for leptin, 2.03 for TNF-α, 1.9 for IL-6, and fold increase 1.58 for adiponectin.

Table 2
Plasma levels of adipocytokines before and after 12-week aerobic training program

	Before training	After training	P
Adiponectin (μg/mL)	10.9 ± 6.1	10.0 ± 4.4	NS
Leptin (ng/mL)	24.3 ± 8.7	18.1 ± 8.3	<.001
TNF-α (pg/mL)	6.1 ± 7.6	4.8 ± 4.5	.08
IL-6 (pg/mL)	3.1 ± 3.7	1.4 ± 1.5	NS

Data are presented as mean ± SD (N = 25).

3.5. Association of gene expression in SCAAT with plasma levels of adipocytokines and anthropometric variables

A marked association between TNF-α expression and IL-6 expression ($r = 0.821$, $P = .023$) as well as between TNF-α expression and plasma IL-6 level ($r = 0.811$, $P = .027$) was apparent at the beginning of the study. Adiponectin gene expression was positively associated with adiponectin plasma levels ($r = 0.811$, $P < .05$) and negatively associated with plasma TNF-α ($r = -0.9$, $P < .05$) at the end of the study. Plasma levels of IL-6, leptin, and TNF-α were not associated with their gene expression in subcutaneous adipose tissue either at the beginning or at the end of the study. No correlation between mRNA level and anthropometric variables (weight, BMI, fat mass, waist circumference) or indices of insulin resistance was observed for any of the adipocytokines.

3.6. Association of plasma levels of adipocytokines with anthropometric variables and indices of insulin resistance or metabolic syndrome

Plasma adiponectin concentration showed a close negative association with adiposity ($r = -0.629$, $P < .05$), FFA ($r = -0.562$, $P < .05$), and glycerol ($r = -0.715$, $P < .05$) at the beginning of the study. These correlations were not present at the end of the study. Plasma TNF-α was closely related to adiposity of subjects before and after aerobic training ($r = 0.527$ and $r = 0.438$, respectively, $P < .05$). No significant associations between plasma levels of adipocytokines (adiponectin, leptin, IL-6, or TNF-α) and any of the indices of metabolic syndrome (HOMA, revised QUICKI, fasting glucose, plasma triglycerides, waist circumference, high-density lipoprotein cholesterol, or blood pressure) were observed before or after aerobic training. However, changes in plasma adiponectin were

Table 3
Relative mRNA levels before and after 12-week aerobic training program (data are expressed as arbitrary units obtained after normalization by the 18S ribosomal RNA subunit)

	Percent change	Fold change	P	Minimum detectable fold change
TNF-α	−53.6	Fold decrease 1.49	NS	2.03
Adiponectin	+18.9	Fold increase 1.18	NS	1.58
IL-6	−3.1	Fold decrease 1.03	NS	1.9
Leptin	−32.1	Fold decrease 1.4	NS	1.9

Fold change in the gene expression analysis was calculated for the increasing changes by dividing relative mRNA level values after the intervention by values before the intervention and, for the decreasing changes, by dividing relative mRNA level values before the intervention by values after the intervention (N = 8).

significantly associated with changes in BMI, body weight, glycerol, and FFA ($r = -0.509$, $r = -0.494$, $r = -0.562$, $r = -0.775$, $P < .05$, for FFA $P = .07$). On the other hand, no association between aerobic training-induced changes in plasma adiponectin, leptin, IL-6, or TNF-α, and indices of insulin resistance (plasma insulin, glucose, HOMA, revised QUICKI), body weight, waist circumference, or adiposity were detectable.

4. Discussion

The main aim of this study was to investigate the effect of aerobic training on adipose tissue gene expression and plasma levels of a number of adipocytokines that might play a role in the pathogenesis of metabolic disturbances and the pro-inflammatory state in obese subjects. We focused our attention on adiponectin, leptin, TNF-α, and IL-6 as modifications of plasma, and SCAAT mRNA levels of these adipocytokines have been described in obese subjects [1,23].

In the present study, the aerobic training program induced a decrease of body weight, BMI, body adiposity, and waist circumference. Fasting insulin and fasting glucose levels were not changed by aerobic training, whereas it did promote a marked decrease of fasting plasma FFAs. Aerobic training induced a change in insulin sensitivity when evaluated by the revised QUICKI index; nevertheless, no changes in other indices of insulin resistance such as HOMA or QUICKI were observed. It has been shown previously that the revised QUICKI index, which takes into account the level of plasma FFAs, has a stronger relationship with parameters measured by euglycemic hyperinsulinemic clamp than QUICKI or HOMA indices alone [24,25].

Our results show that aerobic training with mild weight loss did not change plasma adiponectin levels. Studies on the effect of aerobic training on plasma adiponectin have, to date, provided conflicting data. Six months aerobic training under stable body weight had no effect on plasma adiponectin [26] despite decreased plasma insulin levels and increased insulin sensitivity [12,14]. Opposite effects have been published as well—increased plasma adiponectin and insulin sensitivity without changes in body weight [15] or with moderate weight reduction [27]. Such a controversy might partly be explained by the influence of other exercise-induced substances that might negatively influence the production or release of adiponectin from adipocytes. Among these, catecholamines and TNF-α have been shown to decrease adiponectin gene expression in visceral adipose tissue [28,29]. The plasma noradrenaline concentration was higher at the end of our study (215.3 ± 112.9 vs 296.6 ± 92.2 pg/mL, $P = .016$), which suggests that adiponectin gene expression was inhibited (or remained unchanged) due to catecholamine stimulation, thus preventing significant changes in plasma adiponectin.

A strong negative association between pretraining plasma adiponectin and plasma FFA (and glycerol) levels as well as between changes in plasma adiponectin and changes in FFA during aerobic training was observed. A negative association between adiponectin and FFA was previously reported [30], and similarly to our study, this relationship disappeared after the intervention period with the very low-energy diet. Adiponectin intracellular signaling is connected with activation of adenosine monophosphate kinase [4], which has been previously shown to attenuate β-adrenergic stimulation of lipolysis in fat tissue and muscle [31].

Here, we observed neither a change in plasma IL-6 nor a change in IL-6 gene expression in SCAAT. It has been shown that short-term bouts of exercise increase both plasma IL-6 levels as well as IL-6 gene expression in muscle and adipose tissue of healthy subjects [32]. However, longer-term endurance training might have the opposite effect as shown by diminished exercise-induced elevation of muscle IL-6 gene expression and plasma IL-6 after endurance training [33]. Similar data were observed in a group of patients with chronic heart failure or coronary heart disease where plasma IL-6 decreased after 12 weeks or remained unchanged after 6 months of aerobic exercise [16,17]. No significant associations between plasma IL-6 and anthropometric parameters or indices of insulin sensitivity observed in other studies [2,11,34] were observed here.

Expression of IL-6 in SCAAT as well as plasma IL-6 level were closely associated with expression of TNF-α in SCAAT suggesting that, in the adipose tissue, paracrine interactions play an important role. Similar relationships between IL-6 and TNF-α have already been published by other groups [34,35] as well as an association between TNF-α plasma level and adiponectin gene expression [36]. The ability of TNF-α to activate the transcription of IL-6 gene through activation of nuclear factor κB has been proposed [37].

Contradictory findings exist in literature describing reduction [16] as well as no change [2,17,18] of plasma TNF-α induced by diet or physical activity. Tumor necrosis factor α is predominantly secreted by adipose tissue macrophages [38] and is rather considered to be involved in paracrine regulations without being significantly secreted into the circulation [39].

In conclusion, modifications of anthropometric parameters (waist circumference, BMI, fat mass) and of a revised index of insulin resistance induced by aerobic training were not associated with significant changes in gene expression of adiponectin, leptin, TNF-α, or IL-6 in SCAAT. In plasma, only the leptin concentration decreased. The lack of any training-induced change in other circulating adipocytokines in the present study might be associated with the specific type and duration of the training in this study as well as with the sex and degree of obesity of the subjects. Further studies with different populations and longer training periods are warranted. Moreover, the response of adipocytokines to aerobic training might be influenced by interactions between adipocytokines as demonstrated by associations between TNF-α and IL-6 gene expression and plasma IL-6 and adiponectin concentration.

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