Exercise Improves Insulin Sensitivity in the Absence of Changes in Cytokines

REBECCA J. H. M. VERHEGGEN1, FLEUR POELKENS1, SEAN H. P. P. ROERINK2, ROB E. F. S. RAMAKERS1, MILÈNE CATOIRE1, AD R. M. M. HERMUS2, DICK H. J. THIJSSEN1,3, and MARIA T. E. HOPMAN1

1Department of Physiology, Radboud University Medical Center, Radboud Institute for Health Sciences, Nijmegen, THE NETHERLANDS; 2Division of Endocrinology, Department of Internal Medicine, Radboud University Medical Center, Radboud Institute for Health Sciences, Nijmegen, THE NETHERLANDS; and 3Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UNITED KINGDOM

ABSTRACT

VERHEGGEN, R. J. H. M., F. POELKENS, S. H. P. P. ROERINK, R. E. F. S. RAMAKERS, M. CATOIRE, A. R. M. M. HERMUS, D. H. J. THIJSSEN, and M. T. E. HOPMAN. Exercise Improves Insulin Sensitivity in the Absence of Changes in Cytokines. Med. Sci. Sports Exerc., Vol. 48, No. 12, pp. 2378–2386, 2016. Purpose: The benefits of aerobic exercise training on insulin sensitivity in subjects with metabolic syndrome (MetS) are, at least in part, associated with changes in cytokines. Recent studies identified novel cytokines (e.g., fractalkine, omentin, and osteopontin) that are strongly involved in glucose homeostasis and therefore potentially contribute to the exercise-induced changes in insulin sensitivity. Therefore, we aim to examine changes in skeletal muscle RNA expression and plasma levels of novel cytokines after exercise training and correlate these changes to the exercise-induced changes in insulin sensitivity. Methods: Women with metabolic syndrome (MetS, n = 11) and healthy women (n = 10) participated in a 6-month aerobic exercise training intervention (three times a week, 45 min per session at 65%–85% of individual heart rate reserve). Before and after training, we examined insulin sensitivity (M value during hyperinsulinemic euglycemic clamp) and circulating blood levels of cytokines (venous blood sample; leptin, adiponectin, omentin, fractalkin, and osteopontin). The skeletal muscle RNA expression of these cytokines (muscle biopsy) was examined in two subgroups (MetS, n = 6; healthy women, n = 6). Results: At baseline, plasma levels of omentin (85.8 ± 26.2 ng/mL) and adiponectin (5.0 ± 1.7 µg/mL) levels were significantly higher in controls compared with MetS (51.1 ± 27.1; 3.6 ± 1.1 respectively), and leptin levels were lower in controls (18.7 ± 11.5 vs 53.0 ± 23.5 ng/mL). M value was significantly higher in controls (18.7 ± 11.5 vs 53.0 ± 23.5 ng/mL). Results: At baseline, plasma levels of omentin (85.8 ± 26.2 ng/mL) and adiponectin (5.0 ± 1.7 µg/mL) levels were significantly higher in controls compared with MetS (51.1 ± 27.1; 3.6 ± 1.1 respectively), and leptin levels were lower in controls (18.7 ± 11.5 vs 53.0 ± 23.5 ng/mL). M value was significantly higher in controls (18.7 ± 11.5 vs 53.0 ± 23.5 ng/mL). M value was significantly higher in controls (18.7 ± 11.5 vs 53.0 ± 23.5 ng/mL). Exercise training significantly improved M values in both groups (P < 0.01). Exercise training did not alter plasma and skeletal muscle RNA expression levels of cytokines, but no correlation was observed between changes in cytokine level/RNA expression and M values (P > 0.05). Conclusion: Although exercise training successfully improves insulin sensitivity in MetS and healthy women, we found no change in plasma and mRNA expression levels of novel cytokines. Key Words: METABOLIC SYNDROME, EXERCISE TRAINING, RNA EXPRESSION

The metabolic syndrome (MetS) comprises a set of interrelated risk factors resulting in an increased risk for development of type 2 diabetes mellitus and cardiovascular diseases (13,26). The pathophysiology of MetS is characterized by the presence of peripheral insulin resistance, which is caused by a low physical activity level and excessive central adiposity (11). Several previous intervention studies have demonstrated that exercise training is a powerful nonpharmacological tool to improve insulin sensitivity via several metabolic adaptations (18,32). Part of these adaptations may be related to exercise-induced alterations in cytokines. These pro- and anti-inflammatory factors play a pivotal role in the presence of low-grade inflammation that underlies a decline in insulin sensitivity (19,37). Therefore, the measurement of cytokines before and after exercise training may provide potential insight into the mechanisms underlying the benefits of exercise training on insulin sensitivity. Recent studies have identified novel cytokines, which may play an important role in glucose homeostasis and...
presence of inflammation. Fractalkine is secreted in both adipose tissue and skeletal muscle (5,36) and plays a role in the regulation of pancreatic islet β-cell function. In humans, fractalkine has been proven to modulate monocyte adhesion in adipose tissue, thereby influencing chronic inflammation processes (36), and is independently associated with markers of insulin resistance (HOMA-IR) (10). Omentin is a cytokine mainly secreted by visceral adipose tissue. Circulating omentin processes (36), and is independently associated with markers in adipose tissue, thereby influencing chronic inflammation fractalkine has been proven to modulate monocyte adhesion

**METHODS**

**Subjects**

A total of 11 women with MetS and 10 lean, age-matched, sedentary control women were included in this study. Metabolic syndrome was defined as having at least three of five criteria as defined in the Joint Scientific Statement for Harmonizing the Metabolic Syndrome, including waist circumference >88 cm, triglycerides >1.7 mmol/L⁻¹, high-density lipoprotein cholesterol <1.3 mmol/L⁻¹, blood pressure >130/85 mm Hg and/or the use of antihypertensive medication, and fasting glucose levels >6.1 mmol/L⁻¹ (1). Lean women were defined as having a body mass index (BMI) <25 kg m⁻² and the absence of all MetS criteria. Pre-, peri-, and postmenopausal women were included. Women were considered perimenopausal when they experienced a persistent change in menstrual cycles of at least 7 d, or a period of amenorrhea of 60 d or more. Postmenopause was defined as a period of amenorrhea of 12 months or more (15). We excluded women with a medical history of known diabetes mellitus and/or cardiovascular diseases, with liver or renal diseases, who are smoking, who consume more than two units of alcohol (10 g) a day, or who perform regular physical activity >2 h wk⁻¹. Before participation, written informed consent was obtained. This study was approved by the Medical Ethical Committee of the Radboud University Medical Center and was conducted in accordance with the Declaration of Helsinki (2000).

**Study Design**

All subjects who participated in this study were engaged in a 6-month aerobic exercise training intervention. Before and after the intervention, a venapuncture, hyperinsulenic, euglycemic clamp, vastus lateralis muscle biopsy and a maximal cycling test were conducted in each participant.

**Exercise training.** During this training study, all women trained three times a week under the supervision of an experienced researcher. Training consisted of cycling exercise on an ergometer (Lode, Groningen, the Netherlands) starting with a 10-min warm-up, followed by 30 min of exercise at 65% of the individual heart rate reserve, and ending with a 5-min cooldown. Workload was increased based on improvements in physical fitness level across the 6-month intervention. Exercise intensity was monitored and documented using heart rate monitors (Polar RS800; Polar Electro Oy, Kempele, Finland). Women had to attend at least 90% of the training sessions during this 6-month period to be eligible for inclusion of the statistical analysis.

**Measurements**

**Insulin sensitivity.** Peripheral tissue sensitivity to exogenous insulin was measured using a hyperinsulenic euglycemic clamp as previously described (8). The clamp was performed at least 48 h after cessation of the last exercise bout. After an overnight fast (10 h), the subject was placed in the supine position in a quiet, temperature-controlled (22°C–24°C) room. Insulin (Actrapid, Novo-Nordisk, Copenhagen, Denmark) was infused intravenously in a dose of 430 pmol m⁻² min⁻¹ (60 μU m⁻² min⁻¹) for 120 min. Insulin (50 U mL⁻¹) was diluted in 48 mL NaCl 0.9% with the addition of 2 mL blood from the subject to a concentration of 1 U mL⁻¹. Venous plasma glucose concentrations were clamped at 5.0 mmol L⁻¹ by a variable glucose 20% infusion rate, adjusted depending on venous plasma glucose level measured at 5-min intervals. Venous plasma glucose was measured in duplicate, in samples that were immediately centrifuged during 10 s, with use of the glucose oxidation method (Beckman Glucose Analyzer 2; Beckman Instruments Inc., Fullerton, CA). Insulin was measured in duplicate by an in-house radioimmunoassay to conform with the international standard 83/500, using an antihuman insulin antiserum raised in guinea pig and radioiodinated insulin as a tracer. Bound/free separation was conducted by the addition of sheep anti–guinea pig antiserum and precipitation by means of polyethylene glycol. Between and within-run
coefficients of variation were 4.6% and 5.8%, respectively, at a level of 33 mU·L⁻¹. Whole-body glucose disposal during the last 30 min of the euglycemic clamp was calculated as the M value.

**Plasma cytokine levels.** Fasting venous blood samples collected before the start of the hyperinsulinemic euglycemic clamp were used to determine glucose, cholesterol, and triglycerides via standard laboratory methods. Fasting venous blood was sampled at least 48 h after cessation of the last exercise bout. Adiponectin and leptin were measured in duplicate by using DuoSet ELISA development system kits (R&D systems, Minneapolis, MN), free fatty acids using Cobas Mira Plus (Roche Diagnostics Ltd., Basel, Switzerland), inflammatory marker C-reactive protein by Dako high-sensitivity ELISA (Glostrup, Denmark), and fractalkine, omentin, osteopontin, vaspin, and IL-6 by Luminex assay (Austin, TX).

**Skeletal muscle cytokine RNA gene expression.** Before and after the 6-month training period, biopsies from the vastus lateralis muscle were taken after a standardized 250 kcal breakfast (79% carbohydrates, 11.2% protein, and 9.8% fat). The muscle biopsies after the exercise training period were taken at least 48 h after the last exercise session. From the muscle biopsies of six women of each group, total RNA was isolated and purified. The physiological characteristics of these subgroups were representative for the whole group (see Table, Supplemental Digital Content 1, Characteristics of the entire group versus the subgroups, http://links.lww.com/MSS/A722). RNA concentration and purity were measured with a Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA). RNA integrity was analyzed on an Agilent Bioanalyzer (Santa Clara, CA). RNA gene expression profiling was performed using Affymetrix GeneChip Human Gene 1.0 ST arrays (Affymetrix Inc., Santa Clara, CA), according to the manufacturer’s instruction. The average fluorescence intensity of all genes was calculated using the robust multiaarray analysis algorithm, including a quantile normalization and using a background correction for GC content (20). Microarray analysis was performed using the MADMAX pipeline for the statistical analysis of microarray data (24). Quality control was performed, and all arrays met our criteria, except arrays from three participants. Data of these participants were excluded from further analysis. Microarray data were filtered, and probe sets with expression values higher than 20 on more than five arrays were considered to be expressed and were selected for further statistical analysis. Significant differences in expression levels were assessed using intensity-based moderated t-statistics (35). Gene expression was defined as significantly changed when the P value was <0.01, an alpha that is commonly used in gene expression studies (5,35). The protocol used was compliant with the MIAME guidelines, and data have been submitted to the Gene Expression Omnibus repository under no. GSE43760.

**Anthropometry.** Before and after 6 months of endurance training, we examined height and body weight (Seca 888 Scale; Seca, Hamburg, Germany) to calculate BMI. Waist and abdominal circumferences were measured with a measuring tape (Seca 201, Chino, CA) to calculate waist-to-hip ratio. Plasma cholesterol, triglyceride, and glucose were determined in fasting venous blood samples using standard laboratory methods. Before and after the training period, a total body dual-energy x-ray absorptiometry scan was performed to determine lean body mass, total fat mass, and trunk fat mass (QDR 4500 densitometer; Hologic Inc., Waltham, MA).

**Daily activity levels.** At baseline, daily activity levels were assessed with an accelerometer (SenseWear Pro3 Armband; Body Media Inc., Pittsburgh, PA). From each 24-h interval, data were analyzed from 0700 to 2300 h with a minimum on-body time of 85%. At least 3 d was needed to fulfill these criteria to be used for analysis. Time per day spent in vigorous-intensity activities (>6.0 METs) and time per day in very vigorous-intensity activity (>9.0 METs) were calculated (16).

**Dietary intake.** During the training intervention, women were instructed not to change their caloric intake. To assess potential changes in daily food intake, women were asked to record 3-d dietary intake records before and in the last week of the training intervention. Dietary records were analyzed with Eetmeter Software (Voedingscentrum, the Hague, Netherlands). Only data of women who completed the dietary records on at least 3 d before and at the end of the training period were included for analysis.

**Cardiorespiratory fitness level.** Women performed a maximal exercise test on an electrically braked leg cycling ergometer (Angio 300, Lode, Excalibur Sport, Groningen, the Netherlands) using an incremental protocol to assess their cardiorespiratory fitness level. Workload increased by 10 W·min⁻¹ starting at 10 W, until exhaustion. A gas analyzer was used to measure oxygen consumption continuously (Jaeger Benelux BV, Breda, the Netherlands). Maximal oxygen consumption (V̇O₂max) was analyzed as the mean of the last minute of the exercise bout. During the test, heart rate was measured continuously. Two minutes after cessation of the test, capillary blood lactate level (Roche Diagnostics GmbH, Mannheim, Germany) was measured.

**Statistical analysis.** All statistical analyses were conducted in SPSS 20 (Statistical Package for Social Sciences 20.0; SPSS Inc., Chicago, IL). The sample size calculation to achieve statistical power was based on data from previous research published on the effects of exercise training on circulating leptin levels in obese individuals (23). The estimated sample size was 10 participants to detect a difference of 4.1 ng·mL⁻¹ in leptin levels after training (α = 0.05, β = 0.85). Baseline characteristics of the groups were compared using an unpaired t-test. A two-way repeated-measures ANOVA was used to examine the impact of exercise training on V̇O₂max, insulin sensitivity, and cytokines. Correlations between circulating levels of cytokines and the M value were assessed using Spearman’s correlation coefficient. The level of statistical significance for data except the microarray data was defined at α = 0.05. In the microarray analysis,
**Significantly different from Mets at baseline,**

are presented as mean

to hip ratio 0.91 ± 0.08

Significantly different from Mets at baseline,

are presented as mean

groups, pre-, peri-, and postmenopausal women were included

in both MetS and lean control women, but improvement did not differ between the two groups (Table 1). Waist circumference improved significantly in both groups, whereas other

| TABLE 1. Physiological characteristics before and after exercise training. |
|-----------------|-----------------|-----------------|-----------------|
| **Women with MetS (n = 11)** | **Lean Control Women (n = 10)** | **P** |
| **Preexercise** | **Postexercise** | **Preexercise** | **Postexercise** | **Time** | **Group** | **Interaction** |
| **Age (yr)** | 53.2 ± 7.2 | 48.5 ± 10.2 | — | 0.24 | — |
| **Body composition (anthropometry)** | | | | |
| **Weight (kg)** | 96.4 ± 11.3 | 94.8 ± 9.6 | 67.0 ± 6.6** | 66.9 ± 6.2 | 0.18 | < 0.001 | 0.201 |
| **BMI (kg·m⁻²)** | 34.5 ± 3.2 | 34.0 ± 2.9 | 22.8 ± 1.7** | 22.7 ± 1.5 | 0.18 | < 0.001 | 0.23 |
| **Waist (cm)** | 108.6 ± 9.4 | 105.0 ± 8.3 | 80.4 ± 6.4** | 78.7 ± 4.7 | 0.03 | < 0.001 | 0.97 |
| **Waist-to-hip ratio** | 0.91 ± 0.08 | 0.90 ± 0.07 | 0.80 ± 0.05* | 0.78 ± 0.04 | 0.57 | < 0.001 | 0.22 |
| **Body composition (dual-energy x-ray absorptiometry)** | | | | |
| **Lean body mass (kg)** | 56.6 ± 7.2 | 55.9 ± 6.4 | 47.2 ± 4.3* | 47.4 ± 4.1 | 0.62 | 0.002 | 0.24 |
| **Total fat mass (kg)** | 38.6 ± 6.5 | 37.6 ± 5.8 | 19.2 ± 4.3** | 18.7 ± 3.9 | 0.08 | < 0.001 | 0.61 |
| **Trunk fat mass (kg)** | 20.2 ± 4.0 | 19.5 ± 3.1 | 8.4 ± 2.8** | 8.0 ± 2.4 | 0.09 | < 0.001 | 0.54 |
| **Trunk fat percentage (%)** | 52.4 ± 5.1 | 52.0 ± 4.8 | 42.9 ± 7.1* | 42.6 ± 7.0 | 0.47 | 0.002 | 0.91 |
| **Blood markers** | | | | |
| **Fasting glucose (mmol·L⁻¹)** | 5.5 ± 0.6 | 5.7 ± 0.8 | 4.5 ± 0.3** | 4.7 ± 0.4 | 0.10 | < 0.001 | 0.85 |
| **Insulin (mE·L⁻¹)** | 17.4 ± 10.4 | 15.4 ± 5.2 | 8.9 ± 3.0** | 10.0 ± 3.8 | 0.76 | 0.013 | 0.24 |
| **High-density lipoprotein cholesterol (mmol·L⁻¹)** | 1.21 ± 0.30 | 1.38 ± 0.29 | 1.59 ± 0.29 | 1.58 ± 0.19 | 0.49 | 0.007 | 0.29 |
| **Triglycerides (mmol·L⁻¹)** | 1.98 ± 0.84 | 1.83 ± 0.66 | 0.87 ± 0.27* | 0.92 ± 0.22 | 0.61 | < 0.001 | 0.22 |
| **Free fatty acids (mmol·L⁻¹)** | 0.58 ± 0.15 | 0.45 ± 0.18 | 0.52 ± 0.16 | 0.43 ± 0.23 | 0.01 | 0.88 | 0.69 |
| **Blood pressure and heart rate** | | | | |
| **Systolic blood pressure (mm Hg)** | 138 ± 11 | 132 ± 11 | 120 ± 9* | 114 ± 9 | 0.02 | < 0.001 | 0.99 |
| **Diastolic blood pressure (mm Hg)** | 84 ± 5 | 80 ± 7 | 76 ± 5* | 73 ± 6 | 0.03 | 0.001 | 0.90 |
| **Resting heart rate (bpm)** | 68 ± 5 | 59 ± 7 | 60 ± 7* | 58 ± 5 | 0.003 | 0.03 | 0.06 |
| **Physical fitness** | | | | |
| **VO₂max (mL·min⁻¹·kg⁻¹)** | 22.8 ± 4.5 | 25.3 ± 3.8 | 32.0 ± 4.7** | 35.6 ± 5.5 | < 0.001 | < 0.01 | 0.32 |
| **VO₂max (mL·min⁻¹·kg⁻¹·fat-free mass)** | 38.6 ± 6.5 | 42.6 ± 5.6 | 45.5 ± 7.1* | 50.4 ± 7.8 | < 0.001 | 0.023 | 0.48 |
| **Power (W)** | 158 ± 28 | 185 ± 23 | 180 ± 21 | 205 ± 27 | < 0.001 | 0.05 | 0.83 |
| **Menopausal status** | | | | |
| **Premenopausal (n)** | 2 | 3 | — | — | — | — | — |
| **Perimenopausal (n)** | 4 | 4 | — | — | — | — | — |
| **Postmenopausal (n)** | 5 | 4 | — | — | — | — | — |

*Significantly different from Mets at baseline, P < 0.05.
**Significantly different from Mets at baseline, P < 0.001.

RESULTS

Subject Characteristics

The physical characteristics of the women with MetS and the lean sedentary controls are presented in Table 1. In both groups, pre-, peri-, and postmenopausal women were included (Table 1). None of the included women used hormone replacement therapy because this is known to directly influence circulating cytokine levels (38). As a consequence of the selection procedure, women with MetS demonstrated a significantly higher body weight, BMI, blood pressure, and triglycerides at baseline (Table 1). Analysis of physical activity data at baseline demonstrated that the women with MetS spent 10 min·d⁻¹ in vigorous-intensity activities and 0 min·d⁻¹ in very vigorous-intensity activities. Lean controls spent, on average, 19 min·d⁻¹ in vigorous-intensity activities and 0 min·d⁻¹ in very vigorous intensity.

Exercise Training Intervention

Eleven women with MetS and ten lean control women all successfully completed the exercise intervention, with a training compliance of 92%. Cardiorespiratory fitness, determined as the peak oxygen uptake, improved significantly in both MetS and lean control women, but improvement did not differ between the two groups (Table 1). Waist circumference improved significantly in both groups, whereas other body composition measurements showed no change over time but remained different between the two groups. Analysis of the dietary records in a subgroup of women (MetS, n = 4; lean control women, n = 5) showed no significant change in caloric intake during the training intervention (preexercise, 7.6 ± 1.2 MJ·d⁻¹; postexercise, 7.2 ± 1.2 MJ·d⁻¹; P = 0.12). Circulating markers of glucose homeostasis (i.e., fasting glucose and insulin) and lipids showed no change over time in both groups, whereas blood pressure decreased (Table 1). Insulin sensitivity (M value) improved significantly...
in both groups, with no differences in the magnitude of improvement between both groups (Fig. 1). Three of 11 women with MetS (27%) did not meet the criteria for MetS anymore after 6 months of cycling training.

**Cytokines**

**Plasma cytokine levels.** At baseline, plasma levels of omentin and adiponectin were significantly higher in healthy control women, whereas leptin levels were lower in healthy women compared with women with MetS. No differences between the groups were found for the other cytokines (Fig. 2). Exercise training did not change plasma levels of cytokines in both groups (Fig. 2). At baseline, circulating vaspin did not reach the minimum detecting value of 0.16 ng/mL in n = 8 women with MetS and in n = 6 lean control women. Plasma levels of IL-6 did not meet the minimal detection value of 3 pg/mL at baseline in all lean control women (n = 10) and in most women with MetS (n = 9). After the training period, levels of both vaspin and IL-6 remained below our minimal detection limit in these subjects. Because a large proportion of the individuals included in this study did not show detectable circulating IL-6 and vaspin levels, data on both IL-6 and vaspin were excluded from further analysis.

**Skeletal muscle mRNA expression.** The skeletal muscle gene expression levels of novel and known cytokines were compared before and after the training intervention in...
five women with MetS and four lean control women. In both groups, no differences were found in the gene expression levels of the cytokines before and after exercise training (Table 2).

**Correlation Analysis**

At baseline, we found a significant inverse correlation between insulin sensitivity (i.e., M value) and circulating levels of leptin \( R = 0.65, P = 0.002 \), whereas no correlation was found between the M value and adiponectin \( R = 0.35, P = 0.124 \), fractalkine \( R = -0.22, P = 0.33 \), omentin \( R = 0.38, P = 0.09 \), or osteopontin \( R = 0.14, P = 0.55 \). When exploring the exercise training–induced changes in insulin sensitivity (\( \Delta M \) value) and the changes in cytokines (\( \Delta \)fractalkine, \( R = -0.36, P = 0.18 \); \( \Delta \)Omentin, \( R = 0.22, P = 0.34 \); \( \Delta \)Osteopontin, \( R = -0.13, P = 0.59 \); \( \Delta \)Leptin, \( R = -0.24, P = 0.29 \); \( \Delta \)Adiponectin, \( R = 0.37, P = 0.10 \)), no significant correlations were found. Furthermore, no correlation between weight change (\( \Delta \)weight) and changes in \( \Delta \)fractalkine \( R = -0.20, P = 0.40 \), \( \Delta \)Omentin \( R = -0.25, P = 0.27 \), \( \Delta \)Osteopontin \( R = 0.10, P = 0.68 \), \( \Delta \)Leptin \( R = -0.22, P = 0.34 \), or \( \Delta \)Adiponectin \( R = 0.06, P = 0.79 \) was present.

**DISCUSSION**

Our results confirm that exercise training successfully improves insulin sensitivity, in both women with MetS and lean sedentary controls. The improvement in insulin sensitivity after exercise training was not accompanied by changes in circulating levels of novel (i.e., fractalkine, omentin, osteopontin, and vaspin) and traditional (i.e., leptin, adiponectin, and IL-6) cytokines. Furthermore, the gene expression levels of these cytokines in skeletal muscle did not change after exercise training. Taken together, these results suggest that cytokines are not associated with exercise-induced improvements in insulin sensitivity in women.

**Baseline**

In this study, we confirmed by using M value as a gold standard technique that insulin sensitivity in women with MetS is significantly lower compared with age-matched lean controls. Furthermore, we found that lean controls show lower leptin levels and higher omentin and adiponectin levels than women with MetS. Although previous research showed that omentin is lower in subjects with obesity and type 2 diabetes, this study is the first to show that omentin is also lower in women with MetS compared with controls (34,39). Previous studies found that fractalkine, osteopontin, and vaspin are associated with insulin resistance and obesity (2,10,36). However, no baseline differences in circulating levels of cytokines were found between women with MetS and controls in our study. Furthermore, we found no correlation between insulin sensitivity and levels of these cytokines. One important difference with previous studies is that we used the gold standard to measure insulin sensitivity rather than the less invasive, but also less reliable oral glucose tolerance test. Such measures for insulin sensitivity that use fasting levels of glucose and insulin have clear limitations (30) and should therefore be interpreted with caution to examine the relationship between cytokines and insulin sensitivity. Using M value, our study tempers the results of previous work that suggested an association between serum levels of fractalkine, omentin, osteopontin, and vaspin with insulin sensitivity.

**Training**

Six months of aerobic exercise training significantly improved \( \dot{V}O_2_{\text{max}} \) in both women with MetS and sedentary control women. In both groups, no changes in body mass and waist-to-hip ratio were observed. Furthermore, circulating levels of cytokines and their gene expression in skeletal muscle did not change after training. The effect of aerobic exercise training on leptin and adiponectin is not clear and conflicting results have been reported (4,40). Some studies showed a decrease in leptin levels after aerobic exercise training in obese women (14,23,31), whereas others demonstrated no change (31). Some studies have shown that adiponectin increases after exercise training in obese subjects (3,6), but also an absence of a response has been reported (17,31). In our study, we found no change in leptin and adiponectin both in circulating serum levels and on gene expression level in skeletal muscle, despite the significant improvement in M value. Furthermore, our correlation analysis was unable to demonstrate an association between changes in leptin and adiponectin and changes in M value. Taken together, it is unlikely that leptin or adiponectin plays a pivotal role in the mechanisms underlying a change in M value by exercise training.

The response of omentin, fractalkine, and osteopontin to exercise training in humans has scarcely been studied, and evidence is inconclusive. Omentin has been studied most extensively in relation to the exercise training of nondiabetic individuals and in combination with the parameters of glucose homeostasis. Saremi et al. (34) reported an increase in

---

**TABLE 2. Microarray results for gene expression in skeletal muscle.**

<table>
<thead>
<tr>
<th></th>
<th>Women with MetS (n = 5)</th>
<th>Healthy Control Women (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P Corrected P</td>
<td>Value</td>
</tr>
<tr>
<td>Fractalkine (ng·mL(^{-1}))</td>
<td>0.492289</td>
<td>0.997875</td>
</tr>
<tr>
<td>Omentin (ng·mL(^{-1}))</td>
<td>0.970832</td>
<td>0.999025</td>
</tr>
<tr>
<td>Osteopontin (ng·mL(^{-1}))</td>
<td>0.514872</td>
<td>0.997875</td>
</tr>
<tr>
<td>Vaspin (ng·mL(^{-1}))</td>
<td>0.778275</td>
<td>0.997875</td>
</tr>
<tr>
<td>Leptin (ng·mL(^{-1}))</td>
<td>0.0950706</td>
<td>0.997875</td>
</tr>
<tr>
<td>Adiponectin (μg·mL(^{-1}))</td>
<td>0.145784</td>
<td>0.997875</td>
</tr>
</tbody>
</table>

---

**INSULIN SENSITIVITY AND CYTOKINES IN EXERCISE**

Medicine & Science in Sports & Exercise.
circuitating omentin levels after 12 wk of aerobic exercise (five times a week, 50–60 min), which was accompanied by a significant decrease in HOMA-IR and significant weight loss. Another study reported no effect of 3 months (three times a week, 30 min) of exercise on circulating omentin levels women, whereas BMI and HOMA-IR decreased significantly (34,43). One previous study by Catoire et al. (5) examined the response of fractalkine to combined exercise training (two times a week endurance exercise; once a week resistance exercise; 45 min each) of 12 wk and found no change in circulating levels and circulating levels of fractalkine. Our study confirms these findings after a 6-month endurance training intervention in women. Two previous studies investigated circulating osteopontin levels before and after an aerobic training intervention, without assessing measurements of insulin sensitivity, and both found no change (9) (44). In line with previous work, our study demonstrates that omentin, osteopontin, and fractalkine do not change after training. An important difference between studies that did find an effect on cytokines versus those who did not is the presence of weight loss. It has been suggested in previous reviews that considerable weight loss (of at least 5%) is needed to achieve a change in cytokine expression (22,40) and thereby circulating levels. However, in exercise training studies, the relation between weight loss and change in cytokines seems less clear. In some exercise studies, significant weight loss is present, but no change in cytokines occurred (9,34,43). Furthermore, endurance training does not necessarily result in weight loss. During endurance training, adipose tissue depots may decrease, whereas lean body mass and/or circulating blood volume may increase (7,21,33). These counteracting processes may result in the absence of weight loss during exercise training. Indeed, in our study, no correlation was present between weight loss and change in circulating cytokines. Furthermore, we demonstrated significant and clinically relevant improvements in VO2max and insulin sensitivity in the absence of weight loss and changes in plasma cytokine levels and skeletal muscle RNA expression. This suggests that exercise-induced improvements in glycemic control are not accompanied by a change in cytokines.

Another explanation for the differences between training studies on cytokine responses might be related to training frequency and intensity (42). Although we implemented a training frequency of three times a week, Saremi et al. (34) implemented a frequency of five training sessions per week and found a significant increase in omentin levels. Urbanová et al. (43) implemented a protocol three times a week and found no change in omentin levels. Several studies that investigated leptin and adiponectin applied similar training frequency and found conflicting cytokine responses (3,6,14,23). Possibly, a higher training frequency may be needed to cause a change cytokines.

A more plausible explanation for the absence of changes in circulating cytokines and their expression in skeletal muscle in our study relates to a difference in time-dependent responses of cytokines and M value to exercise training. Each training bout elicits acute changes in energy metabolism and homeostasis, and also in circulating cytokine levels and their expression. Some acute changes in cytokines and their expression will persist for hours, whereas others quickly disappear after cessation of exercise (12,25). Potentially, the cytokine response to an acute bout of exercise can influence metabolic adaptations that contribute to a change in insulin sensitivity. However, with our study design, acute changes in cytokines after exercise were not examined. In the discussed exercise training studies, blood (and tissue) samples were collected at 24 h (9,14), 48 h (34,44), or 72 h (31) after the last exercise session, whereas the majority of the authors did not report the timing of sampling. Considering the responses of cytokines to an acute exercise bout, this heterogeneity among study designs might be an explanation for differences found in the response of cytokines to training. Very little is known about the specific time course of expression levels and circulating levels of well investigated and recently discovered cytokines after an exercise bout. This knowledge is needed to interpret each study on its merits.

Limitations. Microarray analysis was performed in a subgroup of nine women, which limited statistical power. Both circulating levels and mRNA expression levels of cytokines in skeletal muscle were analyzed. Because these subgroups were a good representation of the whole group, we do not expect larger numbers would have altered the main outcomes of our study. In this study, pre-, peri-, and postmenopausal women were included, which provides a well representation of the entire female population in this age category. Although the menopause affects fat distribution, its effects on cytokines is less clear (27,29). We therefore do not believe that the main outcome of this study is influenced by menopausal status. In this study, we explored cytokine expression levels in skeletal muscle, whereas adiponectin, omentin, and vaspin might not be expressed in this tissue. However, gene expression analysis allows us to examine the potential role of skeletal muscle as source for circulating cytokines. Because white adipose tissue is also an important source for circulating cytokines, future work should examine expression changes in this tissue (40).

CONCLUSION

In conclusion, this study shows that exercise training successfully improves insulin sensitivity and physical fitness in both women with MetS and lean controls. The improvement in insulin sensitivity after exercise training was not accompanied by or correlated with changes in circulating levels or gene expression levels in skeletal muscle of novel (i.e., fractalkine, omentin, osteopontin, and vaspin) and traditional (i.e., leptin, adiponectin, and IL-6) cytokines. Our data may suggest that exercise-induced improvements in insulin sensitivity are not accompanied by a change in cytokines.
REFERENCES


The results of the present study do not constitute endorsement by the American College of Sports Medicine.

INSULIN SENSITIVITY AND CYTOKINES IN EXERCISE

BASIC SCIENCES


