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In the absence of weight loss, exercise training does not improve adipokines or oxidative stress in overweight children

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Abstract

The aim of the present study was to examine the effect of exercise training on adipokines, inflammatory markers, and oxidative stress in overweight children. Nineteen overweight children were randomly assigned to an aerobic exercise training or sedentary control group for 8 weeks. Measurements included peak oxygen uptake ($V\dot{O}_2$max), body weight and composition, adipokines (C-reactive protein, interleukin 6, tumor necrosis factor α, adiponectin, leptin, and resistin), and oxidative stress (8-isoprostane). There were no differences between groups for change in body weight or composition over the 8 weeks. Exercise training improved $V\dot{O}_2$max (exercise group, 1.64 ± 0.13 to 1.85 ± 0.17 L/min vs control group, 1.83 ± 0.12 to 1.60 ± 0.13 L/min, $P < .05$) but did not change any of the measured adipokines or the marker of systemic oxidative stress, 8-isoprostane. These data suggest that in the absence of weight loss, exercise training alone does not improve the adipokine profile or levels of oxidative stress in overweight children.

1. Introduction

One potential mechanism whereby obesity may increase the risk for future cardiovascular disease is through the increased production of fat cell–derived hormones called adipokines and through increased levels of oxidative stress. Certain adipokines and inflammatory markers such as adiponectin, leptin, resistin, C-reactive protein (CRP), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α) not only affect metabolism, but also act directly on the vasculature to promote atherosclerosis [1-4]. Elevated levels of systemic and vascular oxidative stress are also thought to be directly involved in the atherosclerotic process. Independently, and especially in combination with various adipokines, oxidative stress directly inactivates nitric oxide (NO) [5], which controls vascular tone and protects the artery wall from atherogenic mediators. The resultant decrease in the bioavailability of NO leads to endothelial dysfunction and a proatherogenic environment.

Exercise improves many components of the cardiovascular risk factor profile, including endothelial function [6-10], a surrogate measure of NO bioavailability. Although not as well known, it has been suggested that exercise may also improve levels of adipokines and oxidative stress. Indeed, a large body of evidence exists suggesting that individuals who are either more physically active or more aerobically fit tend to have more favorable adipokine profiles and lower levels of oxidative stress [11-26]. However, most of these data are cross-sectional, and few studies have assessed the direct effects of exercise training on these variables. Many of the controlled intervention studies addressing this issue have shown that exercise improves adipokine and oxidative stress levels; however, most of these trials have reported concomitant improvements in body weight and/or composition that occurred during the exercise training period [27-30] or did not include measures of body fatness [31,32]. Because adipocytes are the main mediators of these hormones, changes in body weight/composition confound the data concerning the direct effects of exercise on these variables.

Recent studies have challenged the notion that exercise directly stimulates improvements in adipokines and inflammatory

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markers independent of weight loss [33-35]. Although these studies are at odds with a large body of previous work, they provide a rationale for additional studies to be performed to further address this question. The aim of the present study was to examine the effect of exercise training on adipokines, inflammatory markers, and oxidative stress in overweight children. We have previously reported data concerning endothelial function and other cardiovascular risk factors in this study sample [8] but at that time did not have available the biochemical markers reported here.

2. Methods

2.1. Subjects

After baseline testing, 20 overweight children (body mass index [BMI] >85th percentile for age and sex) were randomly assigned to either 8 weeks of supervised exercise training or a sedentary control group. All participants and parents/guardians gave written informed assent and consent and the study protocol was reviewed and approved by the University of Minnesota Institutional Review Board. The procedures followed were in accordance with institutional and Health Insurance Portability and Accountability Act (HIPAA) guidelines.

2.2. Laboratory measurements

Before testing, it was confirmed that none of the subjects had been ill or injured in the previous 2 weeks. Testing was performed in the morning at the University of Minnesota General Clinical Research Center after children had fasted for at least 12 hours. Fasting blood samples were collected from an antecubital vein into chilled tubes containing EDTA for the measurement of CRP, IL-6, TNF-α, adiponectin, leptin, resistin, and 8-isoprostane. Plasma was separated by centrifugation at 4°C. The CRP analysis was conducted at Fairview Diagnostic Laboratories, Fairview-University Medical Center (Minneapolis, MN), a Centers for Disease Control and Prevention–certified laboratory. Ultrasensitive CRP was analyzed via rate nephelometry. IL-6, TNF-α, adiponectin, leptin, resistin, and 8-isoprostane were analyzed in the University of Minnesota Cytokine Reference Laboratory by standard techniques with enzyme-linked immunosorbent assay. Inter- and intra-assay coefficients of variation for the adipokines and 8-isoprostane were as follows (presented as inter- and intra-assay coefficient of variation, respectively): IL-6 (3.3-6.4; 1.6-4.2), TNF-α (10.8-16.7; 5.3-8.8), adiponectin (5.8-6.9; 2.5-4.7), leptin (3.5-5.4; 3.0-3.3), resistin (7.8-9.2; 3.8-5.3), 8-isoprostane (6-22; 4-35).

Body composition was determined by dual-energy x-ray absorptiometry. Tanner stage for pubertal development was determined by a trained pediatrician. Aerobic fitness was quantified as peak oxygen uptake ($V\dot{O}_2$max) and was assessed with a graded-intensity protocol on a stationary cycle ergometer starting at 20 W and increasing 20 W every 2 minutes until exhaustion. Expired oxygen and carbon dioxide concentrations and volumes were collected and analyzed with a MedGraphics CPX-D metabolic cart (MedGraphics, St Paul, MN).

2.3. Exercise protocol

Individuals in the control group did not participate in structured exercise and were instructed to maintain current levels of physical activity. All exercise training was supervised, occurred 4 times per week, and consisted of stationary cycling. The exercise intensity and duration was gradually increased throughout the course of the 8-week training program. Participants began the first week exercising at 50% to 60% of $V\dot{O}_2$max for 30 minutes per session (included 5-min warm-up and cool-down); midway through the program, participants were exercising at 60% to 70% of initial $V\dot{O}_2$max for 40 minutes; and at the end of the program, participants were exercising at 70% to 80% of initial $V\dot{O}_2$max for 50 minutes. On the basis of weekly estimated energy expenditure from exercise training, participants averaged approximately 631 kcal for week 2, 853 kcal for week 4, 1066 kcal for week 6, and 1230 kcal for week 8. The heart rate corresponding to the appropriate percentage of $V\dot{O}_2$max was used to monitor exercise intensity.

2.4. Dietary considerations

Participants (and their parents) were instructed to maintain current eating behaviors for the duration of the 8-week study. Dietary intake was assessed by a food frequency questionnaire designed for children (Block Dietary Data Systems, Berkeley, CA) at baseline and at the follow-up visit by a

Table 1
Descriptive characteristics (n = 19)

Variable	Exercise group (n = 9)		Control group (n = 10)	
	Before	After	Before	After
Age (y)	10.8 ± 0.67		11.0 ± 0.71	
Sex	M = 4, F = 5		M = 4, F = 6	
Tanner stage	1.9 ± 0.37		2.3 ± 0.42	
Height (cm)	150.3 ± 3.2	151.5 ± 3.1	152.7 ± 3.8	153.9 ± 3.9
Weight (kg)	74.7 ± 7.7	75.8 ± 8.0	73.5 ± 8.8	74.3 ± 8.9
BMI (kg/m^2)	32.7 ± 2.6	32.7 ± 2.7	30.5 ± 2.3	30.4 ± 2.2
Body fat (%)	45.9 ± 2.1	46.4 ± 2.1	44.6 ± 2.5	45.3 ± 2.4
Trunk fat (%)	46.7 ± 2.2	48.0 ± 2.4	46.1 ± 3.1	46.7 ± 2.6
Energy consumed per day (kcal)	3121 ± 240	2620 ± 200	3038 ± 329	2974 ± 359
Percent energy from fat	35 ± 2	31 ± 2	31 ± 2	33 ± 1
Percent energy from protein	18 ± 1	19 ± 1	18 ± 1	20 ± 1
Percent energy from carbohydrate	48 ± 2	51 ± 2	52 ± 2	48 ± 1

Descriptive characteristics have been previously reported [8]. Data are presented as mean ± SE. There were no significant differences between the groups before and after the intervention period for any of the measured variables.

registered dietician. Nutrient analysis of the questionnaire was performed by using the Dietary Analysis System (Version 4.01, National Cancer Institute, 1997).

2.5. Statistical Analyses

Results are expressed as mean ± SE. Unpaired *t* tests were used to compare baseline variables between groups. Comparison of variables between groups before and after 8 weeks was analyzed by 2-way repeated-measures analysis of variance with Bonferroni post hoc tests where appropriate. The main analysis of interest was the analysis of variance interaction term, which compares the change over time between the groups. An α value of .05 was used to signify statistical significance. Statistical analyses were performed with GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA).

3. Results

Blood samples could not be obtained in 1 subject; therefore, this subject was excluded from the analysis. As reported in a previous publication [8], complete descriptive characteristics of the remaining 19 subjects before and after the intervention period are displayed in Table 1. There were no baseline differences between the exercise and control groups for any of the measured descriptive variables. As previously reported [8], the exercise group demonstrated a significant increase in $V\dot{O}_2$max (exercise group, 1.64 ± 0.13 to 1.85 ± 0.17 L/min vs control group, 1.83 ± 0.12 to 1.60 ± 0.13 L/min, $P < .05$) (Fig. 1) compared with the control group.

Complete adipokine and oxidative stress data are presented in Table 2. At baseline, there were no differences in levels of adipokines or 8-isoprostane between the exercise and control groups. After the 8-week intervention period, there were no differences between groups for any of the measured adipokines or 8-isoprostane.

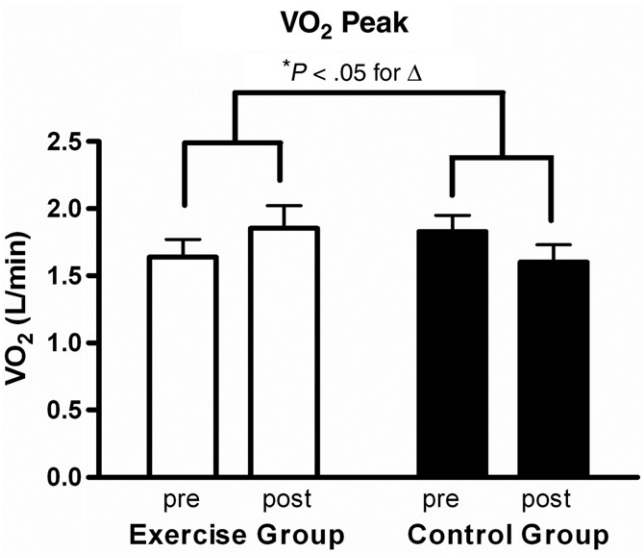

Fig. 1. Changes in $V\dot{O}_2$max over 8 weeks between the exercise and control groups. As previously reported [8], the exercise group significantly improved aerobic fitness level compared with controls.

Table 2
Adipokines and oxidative stress (n = 19)

Variable	Exercise group (n = 9)		Control group (n = 10)	
	Before	After	Before	After
CRP (mg/L)	4.4 ± 1.6	4.8 ± 2.6	5.0 ± 1.2	3.8 ± 0.9
IL-6 (pg/mL)	2.2 ± 0.4	2.3 ± 0.6	3.5 ± 0.7	2.5 ± 0.5
TNF-α (pg/mL)	1.3 ± 0.3	1.4 ± 0.3	1.0 ± 0.1	1.0 ± 0.1
Adiponectin (μg/mL)	5.8 ± 0.6	5.3 ± 0.5	6.0 ± 0.8	6.2 ± 0.8
Leptin (ng/mL)	52.0 ± 14.4	47.3 ± 15.0	41.2 ± 7.2	38.5 ± 7.2
Resistin (ng/mL)	16.4 ± 2.8	16.4 ± 2.7	19.9 ± 2.1	18.7 ± 2.2
8-Isoprostane (pg/mL)	13.7 ± 2.5	11.8 ± 2.4	11.7 ± 1.2	12.0 ± 1.7

Data are presented as mean ± SE. There were no significant differences between the groups before and after the intervention period for any of the measured variables.

4. Discussion

The most important finding in this study is that exercise training, despite improving fitness level, did not improve the adipokine profile or levels of systemic oxidative stress in overweight children. It is important to note that in the current study, exercise training did not change body weight or composition as measured by body mass, BMI, body fat percentage, and percent trunk fat. Without concomitant weight loss or changes in body composition, exercise may have little to no effect on adipokines and oxidative stress.

Although this is a small study, the results contradict suggestions that exercise, by itself, is an anti-inflammatory stimulus and that it improves the adipokine profile. The current findings are not surprising because adipokines are produced and regulated by fat cells, which likely need to be altered in a structural and/or functional way to modify levels of these hormones. Many of the studies supporting a role for exercise in decreasing inflammation are cross-sectional [11-26] and/or fail to report detailed measures of body composition [31,32]. Even the controlled intervention trials that have reported decreases in inflammatory markers with exercise training also report improvements in body weight or composition [27-30], which likely confound the results.

Our results are in agreement with previous exercise training studies showing no change in adipokine levels despite improvements in fitness and insulin sensitivity [34,35]. Marcell et al [34] randomly assigned 51 overweight adults to 16 weeks of either a moderate or intense exercise group or a sedentary control group. Although small but significant improvements were observed for fitness, body composition, and insulin sensitivity, changes in these variables were not associated with improvements in CRP or adiponectin levels. Similarly, Nassis et al [35] reported

that 12 weeks of aerobic exercise training in overweight girls improved fitness and insulin area under the curve during oral glucose tolerance test but failed to improve adiponectin, IL-6, and CRP levels. Similar to the current study, no changes in body weight, BMI, or percent body fat were observed with exercise training, suggesting that exercise alone may not improve the adipokine profile.

Exercise and weight loss likely act via related but distinctly separate mechanisms to improve certain cardiovascular and metabolic risk factors. However, these 2 behavior changes probably act synergistically such that some of the beneficial effects of exercise may actually be mediated through decreases in body fat stores and/or changes in adipocyte function. If a given exercise training regimen does not stimulate decreases in adipocyte number and/or improve adipocyte function, its ability to modify adipokine and oxidative stress levels may be limited or nonexistent. Clearly, the present study does not address this issue in detail because no measures of adipocyte biology and/or function were included, but rather these data provide preliminary evidence that exercise training without concomitant weight loss may have little, if any, effect on these variables.

The current study has some important limitations. The sample size may have been too small to detect statistically significant changes in adipokine and oxidative stress levels. Some of these variables worsened in the control group over the 8 weeks. This study was conducted during the summer months while children were not in school. We speculate, based on conversations with children and parents, that children in the control group engaged in less physical activity during these summer months compared to the school year. This may partially explain why $V\dot{O}_2max$ decreased significantly within the control group. Physiologic changes that occurred from short-term behavior modification in the control group during the summer months (ie, decrease in physical activity) may have been responsible for some of the differences noted between groups. Larger well-controlled trials should be initiated to confirm the findings of the present study with particular focus on the weight loss–independent effects of exercise training on adipokines. Finally, the 8-week duration of exercise training may have been too short and/or the exercise intensity too low to stimulate improvements in adipokines and oxidative stress. Exercise may have a dose- and duration-dependent effect on these variables.

In conclusion, the present study provides evidence that exercise training, in the absence of weight loss or changes in body composition, does not improve the adipokine profile or levels of systemic oxidative stress in overweight children. These findings are contrary to previous reports suggesting that exercise beneficially modifies these variables. It is possible that the positive changes in body composition that often accompanies exercise training is the main mechanism responsible for improving adipokines and oxidative stress and that exercise alone has little to no effect.

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