

# Relationships between adipose tissue and cytokine responses to a randomized controlled exercise training intervention

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## Abstract

Adipose-derived cytokines play a prominent role in mediating the metabolic consequences of obesity and excess body fat. Given this, we hypothesized that alterations in adipose tissue stores incurred with exercise training would be reflected in changes in systemic cytokine concentrations. The Studies of Targeted Risk Reduction Intervention through Defined Exercise, where pronounced changes in adipose tissue stores were observed in the absence of significant changes in dietary intake, provided an ideal setting in which to test this hypothesis. Participants were randomized to 6 months of inactivity or one of 3 types of aerobic exercise training regimens: low-amount-moderate-intensity, low-amount-vigorous-intensity, and high-amount-vigorous-intensity. Plasma samples were collected at baseline and 2 weeks after cessation of 6 months of exercise training or inactivity. In 189 participants, concentrations of 17 cytokines were measured using Bio-Plex Cytokine Assays (Bio-Rad, Hercules, CA); 10 additional cytokines were measured in 60 of these subjects. Of all cytokines tested, the only concentration changes that approached statistical significance were those for granulocyte monocyte-colony stimulating factor and vascular endothelial growth factor, which appeared to increase with training in the low-amount-high-intensity group only ( $P < .05$  for both cytokines). No response to exercise training was noted for any additional cytokine in any of the groups. No relationships were observed between changes in cytokine concentrations and changes in fat mass or other measures of body habitus. In contradiction to our hypothesis, despite significant alterations in body composition, exercise training produced limited cytokine responses.

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## 1. Introduction

The growing epidemic of obesity has promoted increased interest in the role of adipose-derived and systemic inflammatory markers in mediating the metabolic consequences of excess body fat. Adipose tissue and adipocytes have been shown to directly express or secrete a number of inflammatory mediators, often referred to as *adipokines* (reviewed in Trayhurn [1] and highlighted in Table 1). In

addition, observational studies show that obesity is associated with elevated concentrations of a number of inflammatory proteins (reviewed in Nicklas et al [2]).

Given these relationships between inflammatory mediators and adipose tissue stores, a number of investigations have attempted to determine if significant loss of fat mass induces a concomitant change in the systemic concentrations of inflammatory mediators. In the context of loss of fat mass induced via liposuction, such investigations have produced conflicting reports of alterations, or a lack thereof, in systemic concentrations of inflammatory markers [7,8]. In addition, both diet-induced weight loss [9] as well as combined caloric restriction and exercise interventions [10] appear to reduce concentrations of several inflammatory markers.

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However, although exercise training is recognized as a means for significantly improving body composition, there are a limited number of randomized controlled studies performed in the absence of concomitant dietary modifications to lose weight or achieve a “healthier” diet such that

the independent effects of exercise on inflammatory mediators can be assessed. Because multiple inflammatory markers have been implicated in adipose tissue metabolism (as reviewed in Trayhurn [1] and Nicklas et al [2]), we hypothesized that an exercise training intervention that has

Table 1  
Systemic cytokine baseline concentrations and percentage changes with exercise training

	Inactive	Low-amount-moderate-intensity	Low-amount-vigorous-intensity	High-amount-vigorous-intensity
Total (n)	50	42	49	48
Women (n)	27	20	23	18
Men (n)	23	22	26	30
Proinflammatory				
<b>IL-6</b>	17.2 (9.8, 41.7)	27.3 (11.8, 41.7)	26.7 (11.8, 56.8)	31.9 (13.8, 63.0)
	−7.9 (−26.0, 15.3)	18.3 (−10.0, 63.5)	22.4 (−42.3, 84.1)	5.9 (−25.7, 40.8)
<b>IL-8</b>	2.8 (1.7, 4.7)	1.9 (1.1, 2.8)	1.9 (1.4, 3.1)	2.0 (1.2, 3.1)
	4.5 (−20.8, 22.0)	6.0 (−25.9, 52.3)	24.3 (−31.0, 85.6)	−16.3 (−37.4, 45.7)
<b>TNF-<math>\alpha</math></b>	12.3 (3.9, 26.4)	3.1 (1.6, 8.5)	4.6 (2.7, 8.0)	5.0 (3.1, 10.7)
	−13.3 (−39.1, 54.4)	−4.9 (−31.6, 36.6)	−5.1 (−42.3, 63.1)	−15.4 (−42.9, 24.3)
<b>IL-1<math>\beta</math><sup>a</sup></b>	3.4 (1.8, 6.5)	1.7 (0.9, 3.3)	1.8 (1.0, 5.5)	2.4 (1.4, 7.5)
	−7.0 (−31.5, 20.6)	9.2 (−27.2, 31.8)	25.7 (−25.4, 42.4)	−24.6 (−48.3, 28.6)
<b>MCP-1</b>	27.7 (20.5, 37.7)	22.4 (14.9, 28.7)	24.9 (10.9, 33.9)	18.8 (11.2, 33.1)
	−5.6 (−24.2, 9.5)	−9.4 (−28.2, 23.5)	3.5 (−32.5, 50.1)	−6.4 (−30.0, 35.9)
<b>MIP-1<math>\beta</math></b>	30.9 (15.6, 42.1)	18.9 (5.9, 28.1)	23.4 (8.4, 45.5)	16.8 (7.9, 28.0)
	−3.9 (−18.0, 14.3)	1.8 (−37.3, 55.7)	25.1 (−54.7, 100.7)	−4.2 (−41.7, 39.0)
<b>IL-15<sup>b</sup></b>	5.1 (2.2, 8.8)	2.1 (1.2, 2.5)	2.1 (1.8, 2.5)	4.0 (1.0, 30.8)
	−14.5 (−37.9, 29.4)	10.0 (−1.5, 58.7)	14.6 (6.3, 48.7)	25.9 (−1.8, 58.0)
<b>Eotaxin<sup>b</sup></b>	100.3 (72.5, 172.3)	116.5 (82.0, 124.0)	90.8 (60.2, 130.7)	78.0 (71.1, 622.2)
	−6.2 (−19.4, 11.6)	−3.4 (−23.3, 9.1)	−14.0 (−20.0, −6.3)	−2.9 (−20.3, 12.7)
<b>MIP-1<math>\alpha</math><sup>b</sup></b>	5.8 (3.7, 9.07)	4.0 (2.6, 8.1)	2.4 (1.5, 9.7)	5.5 (4.1, 13.8)
	7.0 (−20.6, 40.1)	−11.5 (−42.6, 6.5)	38.2 (7.9, 355.8)	−23.6 (−47.0, 1.3)
<b>RANTES<sup>b</sup></b>	498.6 (349.8, 711.2)	1016.5 (587.4, 1410.3)	939.8 (712.2, 1319.0)	835.9 (720.7, 2558.0)
	−0.9 (−7.7, 4.2)	19.9 (−34.0, 125.5)	53.6 (12.8, 68.1)	7.3 (−46.8, 58.0)
<b>VEGF<sup>b</sup></b>	17.6 (9.8, 25.6)	6.5 (5.0, 10.4)	7.7 (5.7, 8.7)	9.9 (6.0, 14.4)
	4.0 (−21.2, 16.8)	29.4 (−12.0, 97.5)	111.3* <sup>†</sup> (49.4, 175.8)	−17.1 (−29.9, −4.1)
<b>hsCRP</b>	2.1 (1.2, 4.6)	2.1 (1.1, 4.6)	2.1 (1.1, 3.4)	1.5 (0.8, 3.2)
	19.8 (−16.4, 46.0)	12.1 (−41.4, 55.9)	−8.3 (−34.6, 32.8)	3.8 (−23.0, 57.6)
<b>IL-2<sup>a</sup></b>	8.61 (4.1, 22.3)	4.4 (2.1, 6.7)	4.6 (1.5, 6.3)	4.9 (1.8, 14.2)
	−18.5 (−32.2, 9.4)	40.0 (−21.7, 206.3)	0 (−53.8, 162.0)	−21.5 (−40.2, 40.9)
<b>GM-CSF<sup>a</sup></b>	49.2 (26.7, 112.5)	27.7 (9.4, 49.7)	18.4 (9.1, 29.1)	47.7 (9.9, 73.9)
	−19.5 (−59.5, 11.1)	44.3 * (−21.2, 110.0)	67.5* <sup>†</sup> (7.9, 355.6)	−23.2 (54.2, 41.5)
<b>IFN-<math>\gamma</math></b>	52.2 (28.1, 132.6)	15.4 (6.8, 27.7)	12.3 (4.9, 21.4)	17.2 (8.1, 31.0)
	−11.9 (−37.3, 15.4)	−5.3 (−34.0, 45.2)	19.9 (−44.8, 164.9)	−2.6 (−34.9, 59.3)
<b>IL-5</b>	1.7 (1.0, 2.4)	0.9 (0.6, 2.8)	1.2 (0.7, 2.7)	1.4 (0.7, 2.9)
	−4.5 (−27.8, 29.3)	−13.5 (−27.9, 31.4)	9.1 (−36.9, 114.5)	−13.0 (−32.5, 22.4)
<b>IL-7</b>	2.3 (1.4, 5.8)	1.1 (0.8, 2.1)	1.5 (0.9, 2.4)	1.4 (1.1, 3.2)
	0 (−31.4, 28.6)	22.8 (−20.3, 68.2)	22.0 (−20.7, 61.8)	−11.6 (−34.3, 26.4)
<b>IL-12<sup>a</sup></b>	3.9 (2.4, 12.2)	2.4 (1.3, 3.5)	1.9 (0.7, 9.2)	2.5 (1.8, 6.0)
	−21.4 (−63.6, 38.1)	8.5 (−5.4, 22.5)	−8.2 (−88.4, 72.0)	−18.5 (−55.9, 46.2)
<b>IL-13</b>	2.6 (1.6, 4.3)	2.6 (1.1, 6.2)	2.7 (1.4, 9.0)	4.5 (2.1, 8.7)
	−12.1 (−25.6, 38.1)	3.9 (−41.6, 46.8)	26.0 (−31.9, 144.5)	−14.3 (−44.9, 17.0)
<b>IL-17<sup>a</sup></b>	3.8 (1.8, 6.4)	2.4 (0.6, 7.7)	3.6 (1.8, 5.9)	2.6 (1.4, 6.8)
	8.3 (−35.7, 71.2)	15.1 (−34.0, 146.1)	15.3 (−42.9, 173.9)	−23.1 (−44.8, 51.9)
<b>IL-9<sup>ab</sup></b>	12.6 (3.1, 23.7)	71.5 (24.9, 72.6)	2.0 (0.2, 3.8)	8.3 (3.0, 15.9)
	31.7 (−39.3, 54.0)	−42.5 (−67.2, −6.7)	1971.0 (−28.7, 3970.8)	15.9 (−35.3, 53.2)
<b>G-CSF</b>	30.3 (22.0, 44.6)	14.9 (7.4, 26.1)	13.5 (5.4, 21.9)	17.1 (6.9, 25.7)
	−10.6 (−32.3, 14.3)	−1.0 (−32.7, 50.3)	30.1 (−28.5, 150.6)	−8.7 (−38.5, 37.7)
<b>FGF basic<sup>b</sup></b>	27.8 (17.8, 44.8)	14.6 (4.9, 27.6)	19.3 (12.4, 25.5)	17.1 (12.9, 26.5)
	−3.5 (−33.4, 24.1)	−16.5 (−31.9, 28.1)	20.6 (16.2, 40.5)	−23.8 (−36.2, 4.3)
<b>IP-10<sup>b</sup></b>	520.4 (371.8, 703.0)	811.1 (712.6, 1217.5)	1094.2 (716.4, 1299.4)	821.2 (777.0, 1134.4)
	−0.5 (−21.1, 14.2)	−8.4 (−25.6, 15.5)	−23.0 (−43.1, 0.45)	15.6 (−11.4, 41.3)
<b>PDGFbb<sup>b</sup></b>	317.6 (93.2, 927.6)	100.6 (41.7, 194.4)	21.8 (9.0, 75.9)	66.6 (29.0, 312.3)
	−2.8 (−55.2, 80.9)	−28.3 (−78.9, 190.7)	396.5 (9.9, 3673.0)	−10.0 (−50.2, 478.7)

Table 1 (continued)

	Inactive	Low-amount-moderate-intensity	Low-amount-vigorous-intensity	High-amount-vigorous-intensity
Anti-inflammatory				
<b>IL-1ra<sup>b</sup></b>	217.4 (136.3, 388.7) −4.6 (−23.2, 6.5)	97.7 (77.7, 191.1) 3.0 (−27.8, 23.7)	83.0 (70.4, 121.7) 33.5 (−12.1, 121.2)	146.8 (93.4, 807.7) 2.9 (−3.9, 20.4)
<b>IL-10</b>	3.8 (2.1, 7.8) −1.4 (−31.5, 36.9)	2.6 (1.1, 3.7) 21.5 (−22.8, 80.5)	2.7 (1.7, 7.6) 28.8 (−29.6, 99.5)	3.4 (2.2, 10.1) −6.0 (−25.6, 25.0)
IL-4 <sup>a</sup>	1.18 (0.4, 2.6) −5.2 (−43.8, 32.0)	0.5 (0.2, 1.8) 40.0 (−28.0, 177.3)	1.9 (0.2, 4.1) 20.9 (−48.1, 143.8)	1.2 (0.5, 4.8) −13.8 (−54.8, 0.5)

Data are presented as median concentration (picograms per milliliter for cytokines and milligrams per liter for hsCRP) and median percentage change (from baseline). Numbers in parentheses represent lower and upper quartiles for baseline concentration and percentage changes, respectively. Those listed in bold have been shown to be secreted by adipose tissue and/or associated with obesity [1,2,3–6]. MCP-1 indicates monocyte chemoattractant protein 1; MIP-1 $\beta$ , macrophage inflammatory protein 1 $\beta$ ; MIP-1 $\alpha$ , macrophage inflammatory protein 1 $\alpha$ ; RANTES, regulated on activation, normal T-cell expressed and secreted; IFN- $\gamma$ , interferon- $\gamma$ ; G-CSF, granulocyte-colony stimulating factor; FGF basic, fibroblast growth factor basic; IP-10, interferon-inducible protein 10; PDGFbb, platelet-derived growth factor bb; IL-1ra, IL-1 receptor antagonist.

<sup>a</sup> Greater than 25% of subjects had undetectable concentrations of the indicated cytokines.

<sup>b</sup> Measured in 60 subjects only: inactive = 32, low-amount-moderate-intensity = 10, low-amount-vigorous-intensity = 8, high-amount-vigorous-intensity = 10.

\*  $P < .05$  for within-group comparisons.

†  $P < .05$  for between-group comparisons; relative to the inactive group after post hoc testing.

been shown to produce changes in adiposity [11] and anthropomorphic measures [12] in the absence of changes in dietary intake would induce significant modulation in systemic concentrations of these cytokines and inflammatory mediators.

## 2. Methods

### 2.1. Study design

This work is a secondary analysis of the Studies of Targeted Risk Reduction Through Defined Exercise (STRRIDE). Detailed descriptions of the study design including subject recruitment, randomization, exercise training, and outcome variable measurements are published elsewhere [13]. Relevant institutional review boards approved the research protocol, and informed consent was obtained.

### 2.2. Subjects

Inclusion criteria were inactivity (not participating in regular exercise 3 or more times per week and with a peak  $\text{VO}_2$  of less than 40 mL/[kg min]), overweight to mild obesity (body mass index of 25–35 kg/m<sup>2</sup>), dyslipidemia (low-density lipoprotein cholesterol of 130–190 mg/dL or high-density lipoprotein cholesterol <40 mg/dL for men and <45 mg/dL for women), postmenopausal status for women, and age between 40 and 69 years. Exclusion criteria included cigarette smoking, the use of medications known to affect carbohydrate or lipid metabolism (including insulin, oral antidiabetic agents, hydroxymethylglutaryl coenzyme A reductase inhibitors or statins, fibric acid derivatives, bile acid sequestrants, and nicotinic acid), participation in a dietary regimen designed to induce weight loss, presence of diabetes mellitus (fasting glucose >140 mg/dL), hypertension (blood pressure >160/90 mm Hg), known cardiovascular disease, or a musculoskeletal condition that would prohibit exercise training. Persons using hormone replace-

ment therapy or routinely consuming alcoholic beverages were not excluded.

### 2.3. Exercise training

Subjects were randomized to 6 months of continued inactivity or one of 3 aerobic exercise groups: low-amount-moderate-intensity (caloric equivalent of approximately 12 miles/wk or 1200 kcal/wk at 40% to 55% peak  $\text{VO}_2$ ), low-amount-vigorous-intensity (caloric equivalent of approximately 12 miles/wk or 1200 kcal/wk at 65% to 80% peak  $\text{VO}_2$ ), or high-amount-vigorous-intensity (caloric equivalent of approximately 20 miles/wk or 2000 kcal/wk at 65% to 80% peak  $\text{VO}_2$ ). Subjects randomized to exercise completed a 2- to 3-month ramp period to minimize musculoskeletal injuries before the 6 months of prescribed training. The present study is based upon 189 subjects who completed 6 months of prescribed training (or continued inactivity) and had fasting plasma available from both pre- and posttraining time points. Subject flow through STRRIDE has been previously published [14]. Exercise training was supervised; and adherence was calculated as the number of minutes that the individual exercised in the prescribed heart rate range, recorded by a heart rate monitor (Polar Electro, Woodbury, NY), divided by the number of minutes prescribed according to intervention group. Subjects were counseled to maintain dietary intake unchanged throughout the study. Dietary stability was confirmed with 3-day food records and 24-hour dietary recall interviews performed at baseline, at numerous time points during the intervention, and at the conclusion of the study.

### 2.4. Fitness, body composition, lipid, and insulin action measurements

Clinical outcomes were evaluated at baseline (before ramp period) and after 6 months of inactivity or prescribed exercise training. Body mass was measured also after 2 weeks of detraining. Body composition and adiposity

measurements were performed as previously described [12]. Visceral adiposity and subcutaneous adiposity were measured with a single-slice abdominal computed tomographic scan at the L4 vertebra [13,15]. Fat mass was calculated as mass in kilograms  $\times$  percentage of body fat as determined with skin-fold caliper measurements.

Fasting plasma samples were collected at baseline, after 6 months of continued inactivity or exercise training, and again after 2 weeks of training cessation for all subjects. Fasting plasma samples were obtained 2 weeks after cessation of training to assess the sustained effects of exercise-induced reduction in body fat rather than the acute effects of exercise. Because we did not expect any significant changes in body habitus during the 2 weeks after training cessation, using this plasma collection allowed us to obtain the best estimates of the relationships between body habitus and inflammatory mediators at a time point distant from the last bout of exercise, but yet early enough to avoid changes in body composition. This strategy permitted us to assess the sustained effects of long-term exercise training at a time point sufficiently distant from the last bout of exercise to avoid the confounding acute effects of the last bout on inflammatory cytokines. All samples were stored at  $-80^{\circ}\text{C}$  until analysis.

### 2.5. Cytokine measurements

In 189 subjects (88 women, 101 men) who completed 6 months of prescribed training (or continued inactivity) and had fasting plasma available from both pre- and posttraining time points, 17 cytokines concentrations were determined using Bio-Plex Cytokine Assays (171-A11171 and 171-A11127; Bio-Rad, Hercules, CA) according to manufacturer directions. Ten additional cytokines were measured in a smaller subject subset ( $n = 60$ ; 29 women, 31 men).

Samples were diluted 1:2 and incubated overnight at  $4^{\circ}\text{C}$ . A standard range of 0 to 3200 pg/mL was used. Because of concern for plate-to-plate variation in cytokine concentrations, all pre- and postcytokine concentrations were measured on a single plate; and data were analyzed as percentage change as described below. Cytokine concentrations that were below the assay detection limits were treated as missing values. For all cytokines, the manufacturer reported inter- and intraassay coefficients of variation of less than 10% and sensitivities, defined as lower limits of detection, of less than 10 pg/mL. High-sensitivity C-reactive protein (hsCRP) was measured as previously described [14].

### 2.6. Data analysis

Descriptive statistics were calculated for each variable. The percentage change in cytokine concentration and other variables was calculated as follows: [(posttraining cytokine concentration – baseline cytokine concentration)/baseline cytokine concentration] $\times 100$ . Because changes in scores were highly skewed, group differences were assessed with a

nonparametric analysis of variance using a Dunn post hoc test. Pearson product correlations were performed to assess relationships between changes in cytokine concentrations and changes in other variables. For all statistical testing, statistical significance was established at  $P < .05$ . Nonparametric analyses of variance were performed with Prism 4 (GraphPad, San Diego, CA), and all other statistics were performed using SAS Version 8.2 Enterprise Guide (SAS, Cary, NC). Power estimations were derived for each of the 27 cytokines assuming normally distributed data, which provide an approximation of at least 86% of power associated with rank-based data [16].

## 3. Results

The significant improvements in body composition and adiposity we observed in response to exercise training, as well as baseline demographic and metabolic parameters and adherence rates, have been previously reported for the entire STRRIDE subject population [11,12,17,18]. Relative to a slight increase in the fat mass (mean  $\pm$  standard deviation relative change in fat mass) in the inactive control group ( $0.5\% \pm 10.6\%$ ), in each of the exercise groups reported on in this article, we observed a significant reduction in mean fat mass (low-amount-moderate-intensity,  $-5.7\% \pm 10.9\%$ ; low-amount-vigorous-intensity,  $-7.2\% \pm 10.7\%$ ; and high-amount-vigorous-intensity,  $-13.1\% \pm 10.4\%$ ;  $P < .0001$ , post hoc  $P < .05$  for each exercise group vs control). Corresponding to absolute reductions in fat mass of 1.7, 2.1, and 3.7 kg, respectively, these changes in body fat mass occurred in the absence of any clinically or statistically significant changes in reported caloric intake. The mean daily caloric intake change for each group was as follows: inactive,  $-17 \pm 499$  kcal/d; low-amount-moderate-intensity,  $-132 \pm 604$  kcal/d; low-amount-vigorous-intensity,  $189 \pm 627$  kcal/d; and high-amount-vigorous-intensity,  $-36 \pm 525$  kcal/d ( $P < .33$ ). The mean adherence rates for each exercise group were as follows: low-amount-moderate-intensity,  $89.0\% \pm 14.2\%$ ; low-amount-vigorous-intensity,  $92.7\% \pm 10.7\%$ ; and high-amount-vigorous-intensity,  $84.4\% \pm 13.3\%$ . Of the 88 female participants, 46 (52%) reported using hormone replacement therapy.

Changes in median cytokine and hsCRP concentrations are shown in Table 1. Of the 28 molecules analyzed, only changes in granulocyte monocyte-colony stimulating factor (GM-CSF) and vascular endothelial growth factor (VEGF) were found to be significantly different among groups after post hoc testing. Of note, GM-CSF concentrations were undetectable in 44% of the subjects. Relative to the inactive group, the low-amount-vigorous-intensity exercise group showed increases in GM-CSF ( $P < .01$ ) and VEGF ( $P < .05$ ) concentrations. Using within-group analyses, we observed that GM-CSF concentrations increased with exercise training in the low-amount-moderate-intensity group and low-amount-vigorous-intensity group ( $P < .05$ ). An increase in



VEGF concentrations was also detected in the low-amount-vigorous-intensity training group ( $P < .05$ ).

Because adipose tissue is known to produce a number of systemic cytokines with metabolic effects and given the changes in body habitus observed with exercise training, we expected to find the changes in cytokine concentrations to be related to the changes in body composition. However, we did not find a relationship between changes in any cytokine concentration and alterations in body fat mass ( $P > .05$  for all). In addition, cytokine concentration changes were not related to changes in total body mass, subcutaneous adiposity, visceral adiposity, minimal waist circumference, or percentage of body fat as determined with skin-fold caliper measurements ( $P > .05$  for all).

### 3.1. Power of the study

Although criteria regarding the clinical relevance of cytokine concentration changes are lacking, we believe that clinically relevant cytokine concentrations changes are likely to be on the order of several times. Alterations in cytokine concentrations of such magnitudes have been observed in low-grade inflammatory conditions such as obesity [19,20] and dementia [21]. In addition, for many cytokines, differences between healthy controls and recognized inflammatory conditions such as relapsing polychondritis and rheumatoid arthritis have been reported to be several hundred-fold [22]. Based on the smallest group size of 42, at an  $\alpha$  error of .05, we had greater than 90% power to detect a 1 to 3 times difference for most of the cytokines analyzed; the only exceptions to this degree of power were for the 10 cytokines analyzed in only 60 subjects, eotaxin, and interleukin (IL) 12.

## 4. Discussion

We describe the first broad evaluation of cytokine responses in a randomized controlled exercise intervention that excluded concomitant dietary modifications to lose weight. Despite producing significant beneficial changes in fat mass and body habitus, exercise training alone evoked minimal or no responses in systemic cytokine concentrations. These findings support those from a previously reported randomized controlled trial wherein, after 14 weeks of exercise at 65% to 70% peak  $\text{VO}_2$ , no significant changes were observed for adiponectin, CRP, leptin, tumor necrosis factor (TNF)  $\alpha$ , resistin, or IL-6 (measured 48 hours after the last bout of exercise) [23]. Similarly, a randomized controlled trial comparing exercise training and caloric restriction to a healthy lifestyle also demonstrated minimal alterations in TNF- $\alpha$  or adiponectin (measured 48 hours after the last exercise bout) [24]. Our work demonstrates a lack of sustained change in a number of additional classic cytokines, chemokines, and growth factors after exercise training alone. In sum, these results imply that metabolic changes induced by exercise training

occur independent of sustained alterations in the systemic levels of inflammatory mediators, even in the face of a reduction in adiposity.

We observed that despite a mean reduction of adipose tissue mass by  $9\% \pm 11\%$  (or  $2.5 \pm 3.0$  kg), exercise training did not alter cytokine concentrations to any significant degree. In addition, there was no relationship between change in fat mass, or any body composition measure, and change in concentration of any cytokine. Although much larger reductions in adipose tissue mass might produce more measurable and sustained responses in circulating concentrations of adipokines and cytokines, our findings in the setting of moderate, yet significant, reductions of adipose tissue mass prompt us to propose that cytokine changes previously observed with weight loss [7,9,10] are not directly related to changes in adipose tissue mass and are not sustained. One group recently demonstrated that, when induced by diet and exercise, weight loss produced cytokine concentration changes that were transient during the period of weight loss, but lost after a subsequent 2-week period of weight maintenance [25]. Similarly, others have shown that although changes in body mass index were related to changes in TNF- $\alpha$  and changes in soluble TNF- $\alpha$  receptors after 6 months, this relationship did not persist after 18 months of an exercise and/or diet intervention [9]. Thus, because they are not sustained during a maintenance phase of an exercise or dietary weight loss intervention, systemic cytokine concentration changes appear unrelated to changes in the mass of adipose tissue per se; in addition, these and our findings lead us to speculate that cytokine concentration alterations previously reported [7,9,10] may be more directly linked to the rate of change in adipose tissue. Although not directly tested in this investigation, our observations are consistent with this paradigm.

Our observation that there was no change in cytokine concentrations with exercise training confirms and extends those from similar investigations. In addition to those investigations described above [23,24], one other study showed that 4 weeks of combined aerobic and resistance training performed in the setting of constant caloric intake produced changes in percentage of body fat, fitness, and insulin action, but no change was observed in IL-10 or IL-6 concentrations [26]. Similarly, a randomized controlled intervention in older adults with osteoarthritis found that, in the absence of a controlled dietary intervention, 6 months of exercise training reduced body mass by 3.5 kg but did not significantly impact concentrations of IL-6 and TNF- $\alpha$  [9]. In contrast, in the context of uncontrolled observational and cross-sectional interventions, physical activity has been related to reduced inflammatory burden (reviewed in Nicklas et al [2]). Thus, the lack of change in cytokine concentrations with exercise training observed in controlled interventions emphasizes that randomized controlled interventional studies are the best means of determining whether observational associations are causative, correlative, or chance observations.

Nonetheless, the dynamic response of cytokines to acute bouts of exercise and with exercise training appears complex. One limitation of our investigation is that we evaluated cytokine changes based on a single time point after completion of training. A recent nonrandomized, but diet-controlled, 14-week exercise intervention demonstrated reductions in visceral adiposity and IL-6, but not CRP or plasminogen activator inhibitor 1, when plasma was collected 4 days after the last bout of exercise [27]. In the current investigation, we cannot confirm that cytokine concentrations were not similarly reduced earlier in the course of training or the detraining period; but our findings do indicate that any reduction in IL-6 concentrations is not sustained at 2 weeks after the last bout of exercise. Thus, we suggest that the reader be aware of the timing of the last exercise bout relative to sample acquisition when evaluating investigations of exercise training-induced responses in cytokines.

An additional complexity in measuring cytokine responses to exercise is that responses might differ with respect to the underlying level of inflammation in the population studied. Our focus was on individuals at moderate risk for cardiovascular disease as reflected by a median baseline hsCRP of 2.1 mg/L. However, as noted in one investigation where exercise training reduced concentrations of hsCRP only in subjects with baseline concentrations greater than 3 mg/L [28], in populations with higher degrees of baseline inflammation, exercise training might produce observable reductions in inflammatory markers. An additional limitation of our investigation is an inability to account for a number of potential confounders that might influence baseline cytokine concentrations as well as responses of these concentrations to exercise training. These include, but are not limited to, genetic determinants, alcohol use, and hormone replacement therapy use.

In addition, our use of a multiplex assay system should be recognized as a methodological difference when comparing our findings to those that have used individual cytokine assays with lower limits of detection. Specifically, the reported percentage changes, medians, and interquartile ranges might not be directly comparable. As manufacturers develop increasingly sensitive assays over time, particular attention should be paid to assay specifications when comparing results across studies. For example, the manufacturer used in this report now offers a “high sensitivity” cytokine multiplex assay, not available at the time this work was performed, that provides limits of detection of less than 1 pg/mL for all cytokines. Furthermore, we recognize that we did not determine coefficients of variation for the cytokine measurements. As stated above, the manufacturer of this commercially available array system reported that both inter- and intraassay coefficients of variation are less than 10%. Nonetheless, by analyzing these data as percentage change, we limited the impact of interassay variability on our results. In addition, these bead-based assays rely on a minimum of

100 beads per sample, which should produce nominal intraassay coefficients of variation.

Whereas inflammatory cytokines did not decrease with exercise training, we found that concentrations of GM-CSF and VEGF increased with low-amount-vigorous-intensity exercise only. Relative to low-amount-moderate-intensity and high-amount-vigorous-intensity exercise groups, we have found the low-amount-vigorous-intensity exercise group to have a less robust improvement in insulin sensitivity [17] and a trend toward less robust improvements in waist circumference [12] and subcutaneous adipose tissue [11]. Given these findings, the observation that GM-CSF and VEGF increases did not follow a dose response to exercise training, the ability to measure GM-CSF concentrations in only 56% of subjects, and the large number of cytokines evaluated in this investigation, we believe these results require significant validation in other cohorts before they can be accepted as other than chance observations.

In summary, we report that despite robust improvements in adiposity and ectopic (visceral) fat mass, exercise training produced limited changes in systemic concentrations of cytokines. These findings support emerging evidence that systemic cytokine concentrations changes are not directly related to absolute changes in amount of adipose tissue but, rather, may be more likely related to the rate of change in adipose tissue mass. Testing such a hypothesis is worthy of future randomized intervention studies.

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## References

- [1] Trayhurn P. Endocrine and signalling role of adipose tissue: new perspectives on fat. *Acta Physiol Scand* 2005;184:285-93.
- [2] Nicklas BJ, You T, Pahor M. Behavioural treatments for chronic systemic inflammation: effects of dietary weight loss and exercise training. *CMAJ* 2005;172:1199-209.
- [3] Ajuwon KM, Jacobi SK, Kuske JL, Spurlock ME. Interleukin-6 and interleukin-15 are selectively regulated by lipopolysaccharide and interferon-gamma in primary pig adipocytes. *Am J Physiol Regul Integr Comp Physiol* 2004;286:R547-53.
- [4] Vasudevan AR, Wu H, Xydakis AM, Jones PH, Smith EO, Sweeney JF, et al. Eotaxin and obesity. *J Clin Endocrinol Metab* 2006;91:256-61.
- [5] Sell H, Dietze-Schroeder D, Eckardt K, Eckel J. Cytokine secretion by human adipocytes is differentially regulated by adiponectin, AICAR, and troglitazone. *Biochem Biophys Res Commun* 2006;343:700-6.

- [6] Herder C, Haastert B, Muller-Scholz S, Koenig W, Thorand B, Holle R, et al. Association of systemic chemokine concentrations with impaired glucose tolerance and type 2 diabetes: results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). *Diabetes* 2005;54(Suppl 2):S11–S7.
- [7] D'Andrea F, Grella R, Rizzo MR, Grella E, Grella R, Nicoletti G, et al. Changing the metabolic profile by large-volume liposuction: a clinical study conducted with 123 obese women. *Aesthetic Plast Surg* 2005;29:472–8 discussion 9–80, 81.
- [8] Klein S, Fontana L, Young VL, Coggan AR, Kilo C, Patterson BW, et al. Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. *N Engl J Med* 2004;350:2549–57.
- [9] Nicklas BJ, Ambrosius W, Messier SP, Miller GD, Penninx BW, Loeser RF, et al. Diet-induced weight loss, exercise, and chronic inflammation in older, obese adults: a randomized controlled clinical trial. *Am J Clin Nutr* 2004;79:544–51.
- [10] Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, et al. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *Jama* 2004;292:1440–6.
- [11] Slentz CA, Aiken LB, Houmard JA, Bales CW, Johnson JL, Tanner CJ, et al. Inactivity, exercise, and visceral fat. STRRIDE: a randomized, controlled study of exercise intensity and amount. *J Appl Physiol* 2005;99:1613–8.
- [12] Slentz CA, Duscha BD, Johnson JL, Ketchum K, Aiken LB, Samsa GP, et al. Effects of the amount of exercise on body weight, body composition, and measures of central obesity: STRRIDE—a randomized controlled study. *Arch Intern Med* 2004;164:31–9.
- [13] Kraus WE, Torgan CE, Duscha BD, Norris J, Brown SA, Cobb FR, et al. Studies of a targeted risk reduction intervention through defined exercise (STRRIDE). *Med Sci Sports Exerc* 2001;33:1774–84.
- [14] Huffman KM, Samsa GP, Slentz CA, Duscha BD, Johnson JL, Bales CW, et al. Response of high-sensitivity C-reactive protein to exercise training in an at-risk population. *Am Heart J* 2006;152:793–800.
- [15] Ellis KJ. Human body composition: in vivo methods. *Physiol Rev* 2000;80:649–80.
- [16] Conover W. Practical nonparametric statistics. 3rd ed. New York: Wiley and Sons; 1999.
- [17] Houmard JA, Tanner CJ, Slentz CA, Duscha BD, McCartney JS, Kraus WE. Effect of the volume and intensity of exercise training on insulin sensitivity. *J Appl Physiol* 2004;96:101–6.
- [18] Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, McCartney JS, et al. Effects of the amount and intensity of exercise on plasma lipoproteins. *N Engl J Med* 2002;347:1483–92.
- [19] Moon YS, Kim DH, Song DK. Serum tumor necrosis factor- $\alpha$  levels and components of the metabolic syndrome in obese adolescents. *Metabolism* 2004;53:863–7.
- [20] Vendrell J, Broch M, Vilarrasa N, Molina A, Gomez JM, Gutierrez C, et al. Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. *Obes Res* 2004;12:962–71.
- [21] Zuliani G, Ranzini M, Guerra G, Rossi L, Munari MR, Zurlo A, et al. Plasma cytokines profile in older subjects with late onset Alzheimer's disease or vascular dementia. *J Psychiatr Res* 2006.
- [22] Stabler T, Piette JC, Chevalier X, Marini-Portugal A, Kraus VB. Serum cytokine profiles in relapsing polychondritis suggest monocyte/macrophage activation. *Arthritis Rheum* 2004;50:3663–7.
- [23] Giannopoulou I, Fernhall B, Carhart R, Weinstock RS, Baynard T, Figueroa A, et al. Effects of diet and/or exercise on the adipocytokine and inflammatory cytokine levels of postmenopausal women with type 2 diabetes. *Metabolism* 2005;54:866–75.
- [24] Weiss EP, Racette SB, Villareal DT, Fontana L, Steger-May K, Schechtman KB, et al. Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. *Am J Clin Nutr* 2006;84:1033–42.
- [25] Salas-Salvado J, Bullo M, Garcia-Lorda P, Figueredo R, Del Castillo D, Bonada A, et al. Subcutaneous adipose tissue cytokine production is not responsible for the restoration of systemic inflammation markers during weight loss. *Int J Obes (Lond)* 2006;30:1714–20.
- [26] Oberbach A, Tonjes A, Kloting N, Fasshauer M, Kratzsch J, Busse MW, et al. Effect of a 4 week physical training program on plasma concentrations of inflammatory markers in patients with abnormal glucose tolerance. *Eur J Endocrinol* 2006;154:577–85.
- [27] Dekker MJ, Lee S, Hudson R, Kilpatrick K, Graham TE, Ross R, et al. An exercise intervention without weight loss decreases circulating interleukin-6 in lean and obese men with and without type 2 diabetes mellitus. *Metabolism* 2007;56:332–8.
- [28] Lakka TA, Lakka HM, Rankinen T, Leon AS, Rao DC, Skinner JS, et al. Effect of exercise training on plasma levels of C-reactive protein in healthy adults: the HERITAGE Family Study. *Eur Heart J* 2005;26:2018–25.