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Exercise training is not associated with improved levels of C-reactive protein or adiponectin

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Abstract

The purpose of this study was to determine the effect of exercise training on the levels of C-reactive protein (CRP) and adiponectin, and to assess whether exercise-induced changes in insulin resistance could be explained in part by changes in these inflammation markers.

Study participants included 51 middle-aged (45.3 ± 8.3 years; mean ± SD), overweight (33.7 ± 4.8 BMI), insulin-resistant, nondiabetic individuals. Subjects had their insulin sensitivity, body fat, CRP, and adiponectin levels measured, and their predicted maximal fitness calculated before and after 16 weeks of moderate, intense, or no exercise training.

Modest improvements in fitness, body composition, and insulin sensitivity were observed, but these changes were not associated with decreased CRP or increased adiponectin levels, even when subjects were stratified by their change in fitness or obesity. Regression analysis demonstrated that the change in percentage of body fat was significantly related to changes in insulin sensitivity, whereas changes in VO_2 MAX, CRP, and adiponectin were not.

Participation in moderate to intense exercise was not associated with improved measures of chronic inflammation markers, as measured by CRP and adiponectin. Moreover, improvements in insulin sensitivity resulting from exercise or modest weight loss did not appear to be related to changes in these markers.

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1. Introduction

With the recognition that atherosclerosis is in fact an inflammatory condition [1], there has been greater appreciation for the importance of the many sources, consequences, and regulators of pro- and anti-inflammatory factors. An association between plasma C-reactive protein (CRP) concentrations, an excellent marker of inflammation, and cardiovascular disease (CVD) has been noted in both men and women [2,3]. Additional studies have outlined numerous mechanisms by which CRP may directly contribute to vascular inflammation and atherosclerosis [4-6].

Several lines of investigation also suggest the possibility that local and systemic inflammation may be important mediators in the development of insulin resistance and type 2 diabetes in many individuals [7,8]. In support of this notion, epidemiologic studies have demonstrated that several well-accepted markers of inflammation, such as interleukin 6 (IL-6) and CRP, are independent predictors of incident diabetes [7,9]. It is now recognized that adipocytes, particularly those located within the visceral fat, are major secretors of both pro- and anti-inflammatory factors, often referred to as adipokines [10]. Although IL-6 is produced by several tissues, it has been estimated that as much as 30% may be secreted from adipocytes [11]. As IL-6 is the predominant stimulator of hepatic production of CRP, it is clear that fat tissue may be an important direct, as well as indirect, source of cytokines and inflammatory mediators [12].

An association between various adipokines and insulin resistance has been noted in both diabetic and nondiabetic states [13,14]. Of particular interest have been the recent studies demonstrating that adiponectin may play a direct role in mediating insulin-stimulated glucose uptake [15,16].

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Adiponectin has also been demonstrated to have many anti-inflammatory properties, including inhibition of endothelial cell adhesion molecules, down-regulation of macrophage scavenger receptors, inhibition of cytokine signaling, and CRP secretion [17-19]. Animal studies in which the adiponectin gene has been knockedout or overexpressed have clearly demonstrated that adiponectin can also have important effects on the development of atherosclerosis [17,20]. Thus, this unique adipose-derived factor appears to play a central role in the development of both insulin resistance and atherosclerosis, and many of these actions may be through its ability to regulate inflammatory pathways.

Regular exercise has long been associated with reduced cardiovascular risk [21]. This has primarily been attributed to the ability of exercise to improve a variety of metabolic abnormalities and risk factors that are associated with increased atherosclerosis. For example, aerobic exercise has been reported to lower blood pressure, improve dyslipidemia, facilitate weight loss, improve insulin sensitivity, enhance glucose disposal and thereby reduce the incidence of diabetes [22-25]. It has been suggested that many of these improvements may in large part be due to exercise-induced reductions in weight and insulin resistance [26]. Importantly, visceral fat, which is closely linked to insulin sensitivity, appears to be more responsive to exercise training than subcutaneous fat [27]. Given our increased understanding of the above-described relationships between obesity, insulin resistance, inflammation, and atherosclerosis, it is important to assess whether many of the physiologic benefits attributed to exercise could in part be explained by its effect on systemic inflammation.

The goal of this study was therefore twofold: first, to determine the effect of several exercise training regimens on the levels of these important inflammatory mediators, CRP and adiponectin; and, secondly, to assess whether exercise-induced changes in insulin resistance could be explained in part by changes in these inflammation markers.

2. Research design and methods

2.1. Subjects

Fifty-one white men (n = 20) and women (n = 31) aged 45.3 ± 8.3 years (mean ± SD) were selected from a recently completed randomized study comparing the effects of 2 different exercise intensity levels on insulin sensitivity [28] for a further analysis of inflammatory markers. Participants were randomized to 3 groups in blocks of 9 (so that entry could be staggered) after stratification for sex and degree of insulin sensitivity. Subjects had no personal history of diabetes or any major medical condition that would preclude them from participating in the exercise intervention. Two women were on hormone replacement therapy, 1 person was on statin therapy, and 3 individuals reported occasional nonsteroidal anti-inflammatory use. All subjects provided an informed consent (Otago Ethics Committee), had height and

weight recorded, and body composition determined by DEXA (Lunar DPXL, version 1.35, Madison, Wis).

2.2. Insulin sensitivity

Those with fasting glucose of more than 6.1 mmol/L and normal 2-hour oral glucose tolerance test (<200 mg/dL) had their whole-body insulin sensitivity determined using a euglycemic hyperinsulinemic (40 mU/m^2 per minute) clamp, as previously described [28]. A variable-rate glucose infusion was given for 115 minutes and adjusted every 10 minutes. Blood glucose levels were kept as close as possible to 4.5 mmol/L. Plasma insulin levels were measured at 0, 60, 90, and 120 minutes. For this analysis, whole-body insulin sensitivity is estimated by the amount of glucose infused in mg/kg fat-free mass (FFM) per minute. Those with insulin sensitivity in the lowest 25th percentile of that previously determined in a lean (BMI < 27 kg/m^2) population [28] were eligible for this study.

2.3. Submaximal exercise test

All subjects completed a submaximal graded exercise test using a modified Bruce protocol on a motorized treadmill before and after the 16-week exercise intervention, as previously described [28]. Fitness was estimated because the overseeing human subjects review committee was concerned that a maximal exercise test may not be safe or well tolerated in this group of sedentary overweight adults. During the exercise test heart rate was assessed every 30 seconds and the test was terminated when the target heart rate was obtained (75% of the predicted maximum heart rate: 220 − age), or if the participant was unable to continue exercising. Oxygen consumption (VO$_2$) was measured continuously during the exercise test (SensorMedics 2900Z; ViaSys Health Care, Yorba Linda, Calif). Average heart rate and VO$_2$ from the last minute of each stage were plotted and extrapolated to the estimated maximal heart rate and therefore VO$_2$ MAX.

2.4. Exercise intervention

All subjects were inactive at baseline, determined by an assessment of recent participation in physical activity using the Life in New Zealand (LINZ) questionnaire [29]. The aerobic exercise program was planned to incorporate 30 minutes of activity 5 days/wk (at different intensities depending on the group assignment) and took into account preferred activities (mostly walking or jogging outdoors or on a treadmill). An exercise consultant exercised with each participant at least once per week, to ensure appropriate activities were chosen and motivation and compliance remained high. The "moderate" exercise intervention program was based on current health promotion recommendations for activity, which do not specify heart rate targets, only to complete 30 minutes of physical activity most days of the week [30]. The "intense" exercise intervention program aimed to meet the current American College of

Sports Medicine guidelines for developing and maintaining cardiorespiratory and muscular fitness [31,32]. Participants were encouraged to train 5 times per week, consisting of a brief warm-up, followed by approximately 30 minutes per session of aerobic exercise at an intensity of 80% to 90% of age-predicted maximum heart rate (not at an intensity based upon VO_2 MAX), followed by a brief cool-down. The type and duration of physical activity were recorded by participants for both intervention groups on a daily record sheet that was collected and reviewed weekly by the exercise consultant and staff for appropriateness of the exercise intensity and duration. Recommendations for modification of individual exercise regimens were made as needed to ensure exercise goals were obtained. The control group was asked to continue their usual daily routines during the 16-week experimental period.

2.5. Laboratory determinations

Plasma sample aliquots were kept frozen at $-80\ °C$ until analyzed. High sensitivity CRP concentrations were measured using an immunometric assay (DPC Immulite, Los Angeles, Calif). The lower functional sensitivity of the assay is 0.2 mg/L and has been previously demonstrated to perform comparably with the Dade Behring CRP assay [33]. The coefficient of variation (CV) at 1.8 ± 0.2 mg/L (lower limit of the detection) was 12.5%, whereas at 10.7 ± 0.4 mg/L the CV was only 4%. Plasma adiponectin was measured with a radioimmunoassay established by Linco Research, Inc (St Charles, Mo). This assay has a sensitivity of 0.1 μg/mL, intra- and interassay CV of less than 8%. Plasma insulin was determined using the Coat-A-Count iodine 125 radioimmunoassay (DPC, Los Angeles, Calif) which has an interassay CV of less than 10%.

2.6. Statistics

Statistics was carried out via Statistical Package for Social Sciences software (SPSS v 11.5, Chicago, Ill). Of the subjects who reported statin, HRT, or occasional aspirin use, no differences in the primary outcome variables were noted between these individuals and the remaining cohort; therefore, they were not removed from further analysis. As well, these subjects were on these medications before enrolling and remained on them throughout the study; thus any effect of medication would not be different between pre- vs postexercise. As CRP levels were not normally distributed, values were log normalized for analysis; however, nonadjusted values are presented in the results for descriptive purposes only. Group comparisons at baseline were analyzed by analysis of variance (ANOVA). For statistical comparisons between sexes at baseline, the data were analyzed via independent t test. Two statistical strategies were employed when comparing differences among the control and intervention groups: (1) the difference between pre- and posttesting values was calculated for each individual (change score) and the mean change was analyzed by ANOVA, and (2) to control for significant differences present at baseline between sexes in our primary outcome variables, an analysis of covariance (univariate linear model) was used entering baseline values as the covariate to adjust for these differences. Post hoc analyses (LSD) were then performed when warranted. Stepwise regression analyses were performed and variables entered in these regression analyses included age, sex, weight, body fat, BMI, maximum heart rate, VO_2 MAX (ml/kg per minute), fasting glucose, fasting insulin, insulin sensitivity index (mg/kg FFM per minute), and lipid levels when appropriate. Values are expressed as means $\pm$ SEM (unless otherwise noted) and significance was set a priori at .05.

3. Results

3.1. Subject characteristics

Characteristics for the subjects at baseline (before intervention) are described in Table 1. Overall, the group was middle-aged, overweight (with a higher percentage of body fat), and with a pattern of dyslipidemia that was consistent with their reduced insulin sensitivity. Their CRP concentrations were relatively high as a group, with the mean value above the high cardiovascular risk value (3.0 mg/L) recently suggested by the CDC [34].

Certain sex differences were observed at baseline as expected (mean $\pm$ SD), with men having greater total body weight (100.6 ± 16.2 vs 94.0 ± 15.5 kg; $P = .11$) due mostly to a greater amount of lean body mass (61.8 ± 6.5 vs 47.0 ± 5.8 kg; $P = .001$) and less percentage body fat than the women ($32.2\% \pm 7.7\%$ vs $45.5\% \pm 4.7\%$; $P = .001$), resulting in a slightly lower BMI for the men as compared with the women at baseline in this study (32.4 ± 3.9 vs 34.9 ± 5.3; $P = .06$). Predicted maximal aerobic capacity (VO_2 MAX) was also significantly greater in men than in women

Table 1
Group and overall characteristics for the subjects at baseline

	Control (n = 14)	Moderate (n = 17)	Intense (n = 20)	Total (n = 51)
Age (y)	44.1 ± 9.5	47.2 ± 9.2	44.4 ± 6.5	45.3 ± 8.3
Weight (kg)	102.3 ± 18.0	97.5 ± 13.6	92.1 ± 17.3	96.6 ± 16.6
BMI (kg/m²)	35.3 ± 3.7	33.9 ± 4.9	32.5 ± 5.3	33.7 ± 4.8
Body fat (%)	43.7 ± 6.4	39.7 ± 8.3	39.8 ± 7.5	40.8 ± 7.6
VO_2 MAX (mL/kg per min)	29.9 ± 4.5	30.7 ± 6.2	31.0 ± 4.8	30.6 ± 5.1
Insulin sensitivity (mg/kg FFM per min)	8.59 ± 2.08	8.13 ± 2.30	8.97 ± 2.36	8.59 ± 2.25
CRP (mg/L)	5.7 ± 5.1	4.9 ± 3.2	3.4 ± 3.5	4.5 ± 3.9
Adiponectin* (μg/mL)	15.9 ± 7.0	14.8 ± 3.9	12.3 ± 4.8	14.1 ± 5.3

Values are presented as mean $\pm$ SD ($P < .05$); no statistical differences were noted at baseline. Insulin sensitivity is expressed as the amount of glucose infused per kilogram FFM.

* To convert adiponectin to μM/L divide by 30.

(34.4 $\pm$ 3.1 vs 28.2 $\pm$ 4.7 mL/kg per minute; P = .001), although these values represent expected values based on established age and sex norms [35]. Neither fasting glucose (5.1 $\pm$ 0.6 vs 5.1 $\pm$ 0.7 mmol/L; P = NS) nor insulin (17.5 $\pm$ 7.8 vs 17.9 $\pm$ 8.0 mIU/L; P = NS) was significantly different between sexes, whereas insulin sensitivity (8.1 $\pm$ 2.0 vs 9.0 $\pm$ 2.3 mg/kg FFM per minute; P = .13) tended lower in men than in women. Notably, CRP was greater than twofold higher in women than in men (6.9 $\pm$ 1.1 vs 2.8 $\pm$ 0.4 mg/L; P = .004), and adiponectin tended to be higher in women than in men (15.0 $\pm$ 5.5 vs 12.6 $\pm$ 4.9 μg/mL; P = .10).

At baseline, there was a strong positive correlation between logCRP and percentage body fat (r = 0.60; P = .001; Fig. 1A), and a negative correlation between CRP and fitness (r = -0.38; P = 006; Fig. 1C), but no association with insulin sensitivity (r = -0.16; P = NS) in the entire group. When these comparisons were analyzed by sex, a significant relationship (Fig. 1A) was only observed for logCRP and body fat in women (r = 0.73; P = .001) and not in men (r = 0.12; P = NS); and no unique relationship remained between logCRP and fitness or insulin sensitivity in either the women or men. When multivariate stepwise regression analysis was performed to identify which variables predicted baseline logCRP among all the subjects adjusting for sex, body fat (partial r = 0.60; P = .001) was the only significant predictor of logCRP, whereas neither fitness (partial r = -0.03; P = NS) nor insulin sensitivity (partial r = -0.27; P = .06) contributed significantly to the model.

Adiponectin levels at baseline were modestly correlated (Fig. 1B) with body fat (r = 0.27; P = .06) and insulin sensitivity (r = 0.34; P = .02), but not fitness (r = -0.12; P = NS; Fig. 1D) in all subjects. Regression analysis at baseline for adiponectin among all the subjects adjusting for sex demonstrated a significant relationship with insulin sensitivity (partial r = 0.35; P = .01), but not for body fat (partial r = 0.25; P = .09) or fitness (partial r = -0.11; P = NS).

3.2. Exercise intervention

Exercise compliance of participants was assessed by using a score based on attendance to exercise sessions. For the intensive exercise group only, a personal exercise diary was also collected weekly (recording type of exercise, duration, and heart rate achieved). On average, participants attended over 75% of the exercise sessions held 5 times per week. In the intensive group, 85% of the participants were compliant, whereas 71% met the criteria in the moderate intervention group. As stated in the Research design and methods section, exercise intensity was based on average heart rates achieved during the last 10 minutes of exercise and was between 80% and 90% of predicted maximal heart rates in the intensive group; however, no such data were collected in the moderate group. Based on reported average metabolic equivalent (MET) levels for the prescribed activities [36,37], the exercise diaries (in the intense group), and direct observations by the exercise consultant, we estimated that the moderate group exercised at approximately 3.5 METS of intensity per session, whereas the
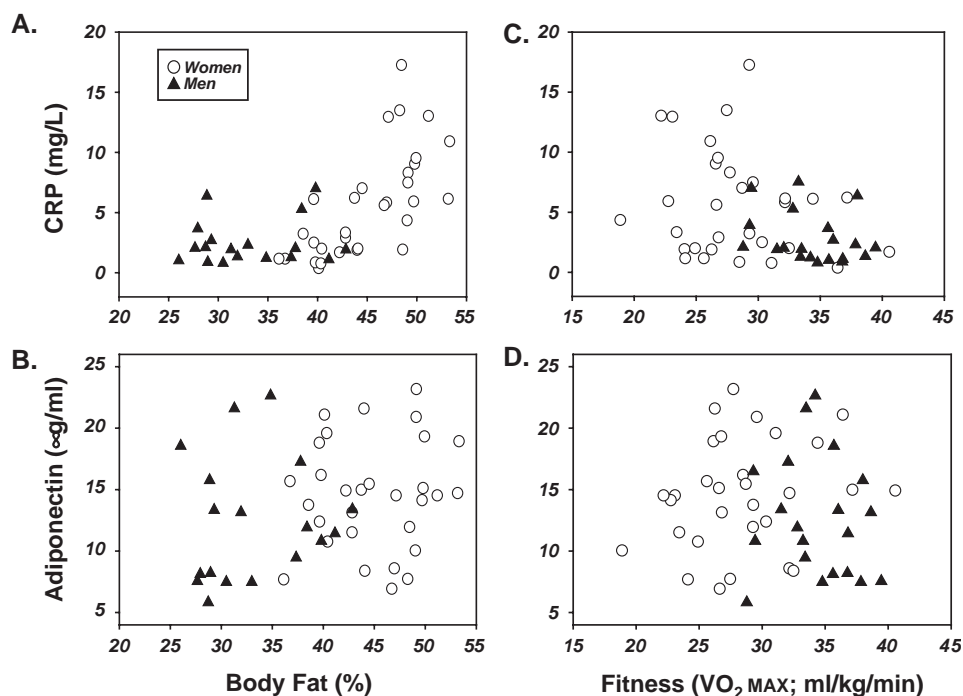

Fig. 1. Baseline levels of CRP and adiponectin in relation to percentage body fat and fitness levels in all subjects. Individual data points for women (open symbol) and men (filled symbol) are shown.

intensive group exercised at an intensity level of approximately 4 to 6 METS. Table 2 describes the mean absolute change in primary outcome variables after 16 weeks of control, moderate, and more intense exercise programs. Both moderate and intense exercise groups demonstrated improved body composition as compared with the control group, with decreased body weight, total body fat, and BMI. Peak heart rate attained during the graded exercise test was reduced in all groups; however, only the intense exercise group demonstrated a significant reduction in heart rate to the peak exercise work rate (control, -3.3 ± 2.2; moderate, -8.0 ± 2.2; intense, -16.1 ± 2.3 beats/min). The changes in body composition and heart rate were accompanied by increased aerobic fitness (ml/kg per minute) in all 3 groups, although only the intense exercise group demonstrated a significant ($P = .001$) increase in predicted VO_2 MAX.

As was previously reported for this group [28], insulin sensitivity (mg/kg FFM per minute) did show a moderate but favorable improvement, although only in the intense exercise group. Surprisingly, no group changes were noted in CRP after training, even in the intense exercise group. Adiponectin increased modestly in both exercise groups as compared with the controls ($P = .09$; Table 2); however, this was due in large part to a decline in the control group, and no statistical improvements were noted in any intervention group ($P = NS$). To ensure that these unimpressive group changes in inflammatory markers were not a result of outliers or differing responses between sexes, we evaluated individual changes in CRP and adiponectin after 16 weeks of exercise and have presented them by sex in Fig. 2. Although large individual variability was noted, with some individuals responding more robustly than others, it is clear that overall there was very little consistent change in CRP or adiponectin in either men or women (Fig. 2).

One possible explanation for the failure to demonstrate major differences in inflammatory markers between exercise groups was that there were insufficient differences by the end of the study in actual exercise activity and fitness levels
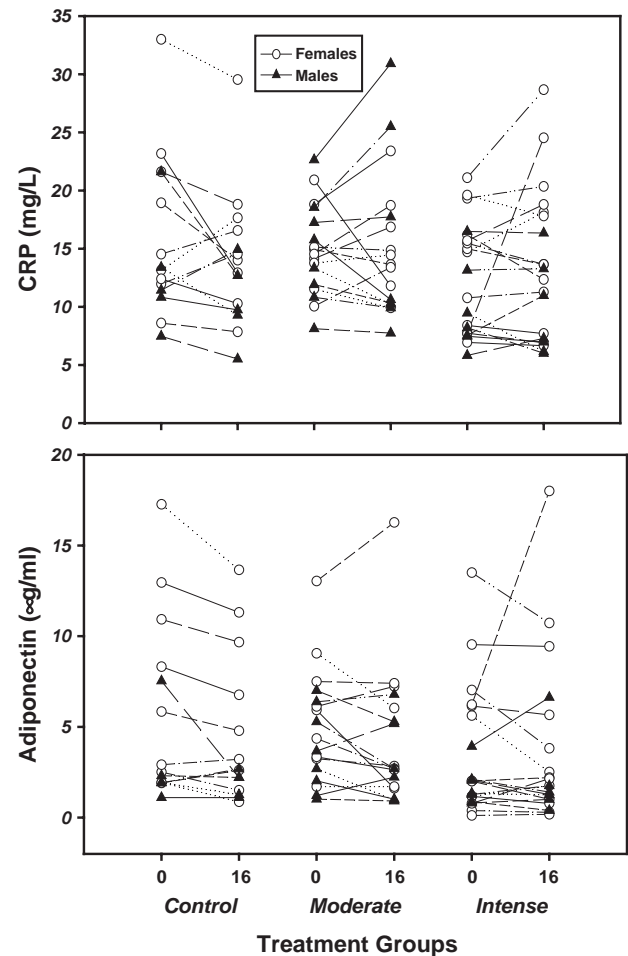

Fig. 2. Paired data points for women (open symbol) and men (filled symbol) are shown for all individuals for levels of inflammatory markers CRP and adiponectin before and after randomization to 16 weeks of control, moderate, or intense exercise programs.

between groups. We therefore reanalyzed the data after separating the subjects into 3 groups based on the absolute change (least, moderate, and greatest) in VO_2 MAX

Table 2
Change (absolute value) in selected characteristics after the 16-week intervention study

	Control (n = 14)	Moderate (n = 17)	Intense (n = 20)
Weight (kg)	-1.1 ± 0.9	$-4.7 \pm 0.8*$	$-5.6 \pm 0.8*$
Body fat (%)	-0.3 ± 0.5	$-1.7 \pm 0.5*$	$-2.4 \pm 0.5*$
VO_2 MAX (mL/kg per min)	2.1 ± 1.1	2.2 ± 1.0	$5.6 \pm 0.9**$
Insulin sensitivity (mg/kg FFM per min)	0.83 ± 0.32	0.96 ± 0.61	$1.65 \pm 0.39**$
CRP (mg/L)	-1.0 ± 0.5	-1.0 ± 0.5	-0.4 ± 0.5
Adiponectin* (μg/mL)	-1.9 ± 1.1	$0.7 \pm 1.0*$	$0.9 \pm 1.0*$

Values are presented as mean $\pm$ SEM ($P < .05$ by analysis of covariance covarying baseline values).

 * Values different than control.

 ** Values different than control + moderate group.

Table 3
Change in (absolute values) of selected characteristics with all subjects separated in tertiles by their VO_2 responses after the 16-week intervention

	Least (n = 19)	Moderate (n = 14)	Greatest (n = 18)
Weight (kg)	-3.0 ± 0.9	-4.6 ± 1.3	-4.3 ± 0.8
Body fat (%)	-1.2 ± 0.5	-0.9 ± 0.5	-2.3 ± 0.6
VO_2 MAX (mL/kg per min)	-0.9 ± 0.3	$2.9 \pm 0.2*$	$8.1 \pm 0.5**$
Insulin sensitivity (mg/kg FFM per min)	0.86 ± 0.20	1.32 ± 0.35	1.46 ± 0.34
CRP (mg/L)	-0.59 ± 0.45	-0.33 ± 0.25	-0.34 ± 0.81
Adiponectin* (μg/mL)	-0.3 ± 1.1	0.2 ± 1.4	0.2 ± 1.0

Values are presented as mean $\pm$ SEM ($P < .05$ by 1-way ANOVA).

 * Values different than the least group.

 ** Values different than least + moderate group.

Table 4
Changes in absolute value of selected characteristics with all subjects separated in tertiles by their body fat loss response after the 16-week intervention

	Least (n = 21)	Moderate (n = 12)	Greatest (n = 16)
Weight (kg)	−1.2 ± 0.7	−4.0 ± 1.3*	−6.9 ± 0.6**
Body fat (%)	0.4 ± 0.2	−1.6 ± 0.1*	−4.0 ± 0.4**
VO$_2$ MAX (mL/kg per min)	2.2 ± 0.8	3.9 ± 1.3	4.8 ± 1.3*
Insulin sensitivity (mg/kg FFM per min)	0.87 ± 0.19	0.55 ± 0.16	2.14 ± 0.53**
CRP (mg/L)	−0.57 ± 0.30	0.54 ± 1.14	−0.87 ± 0.33
Adiponectin* (μg/mL)	−2.0 ± 0.9	1.1 ± 0.9*	1.9 ± 1.3*

Values are presented as mean ± SEM (P < .05 by 1-way ANOVA).
  * Values different than the least group.
  ** Values different than least + moderate group.

(Table 3). Whereas changes in total body weight or body fat across groups were small, there was a significant (P = .001) improvement in insulin sensitivity, particularly in the group with the greatest change in VO$_2$ MAX (Table 3). However, even in this analysis, CRP did not change across the range of improved fitness (Table 3). Adiponectin was higher in both the moderate and greatest-change group in VO$_2$ MAX, but this difference was quite small and not significant. To determine whether changes in weight associated with exercise might be related to changes in inflammatory markers, cohort data were divided into 3 new groups based on their change in body fat (Table 4). Despite a decline of nearly 4% in body fat (≈ 7 kg in weight) in the group with the greatest change, there was no difference in CRP levels among the 3 groups studied. In contrast, both adiponectin and insulin sensitivity were higher in the group with the greatest reduction in body fat. These analyses confirm that participation in moderate to intense exercise in these subjects was not associated with reduced measures of inflammation as measured by CRP, even when subjects were stratified by extent of change in fitness or obesity. Moreover, improvements in insulin sensitivity resulting from exercise or weight loss did not appear to be related to changes in CRP. In contrast, adiponectin did increase modestly across changes in body fat, but did not differ in relation to changes in fitness (Table 4).

To further explore the relationships between exercise and inflammatory markers with measures of insulin sensitivity, several regression analyses were performed. The change in percentage body fat was significantly related (partial r = −0.31; P = .05) to changes in insulin sensitivity, whereas changes in fitness, CRP, and adiponectin were not significant predictors in the model.

4. Discussion

Low aerobic fitness and obesity have both been proposed as independent risk factors for CVD and diabetes [21,38]. One proposed mechanism by which these conditions

contribute to CVD may be related to an increase in circulating inflammatory markers [2,39-41]. Our working hypothesis was that several of the health benefits from exercise training may be related to anti-inflammatory mechanisms. However, a key finding of this study was that 4 months of regular exercise and improved cardiovascular fitness were not associated with significantly improved levels of CRP. Although levels of adiponectin did increase in the intense exercise group, these changes were not consistent across all analyses, were partially related to changes in obesity, and were relatively modest. Furthermore, in multivariate analysis, improvements in fitness were not predictive of changes in adiponectin.

In cross-sectional epidemiologic studies higher aerobic fitness has been associated with lower CRP levels in both women [42] and men [43]. In our study, however, we demonstrated that body fat and not aerobic fitness was significantly related to serum CRP levels in this homogeneous group of overweight and obese individuals. This is consistent with numerous studies that have shown that various measures of obesity are relatively closely associated with measures of inflammation such as CRP [24,44,45]. It is therefore possible that the associations of physical activity patterns with lower levels of inflammation in cross-sectional studies may largely be explained by the more fit individuals having lower body weight, less body fat, and overall better health that often accompanies better fitness in larger more heterogeneous populations.

Interestingly, several weight loss studies of similar duration as the current study have demonstrated that subjects achieving moderate levels of weight loss as in our intense exercise group have impressive and significant declines in CRP [24,44,45]. Moreover, the subjects in these weight-loss studies [44,45] share many characteristics with the subjects participating in our study. On average, they were of similar age, BMI, lipid levels, and when insulin action was assessed; they too were relatively insulin resistant [44]. These data would suggest that weight-loss changes demonstrated in our study would have been expected to induce at least modest reductions in CRP levels. Yet, even when our data were analyzed by tertiles of change in body fat (Table 4), there was little accompanying change in CRP and only modest improvements in adiponectin.

As noted above, the prescribed exercise programs in our current study consisted of relatively frequent exercise sessions, occurring 4.5 times per week, and compliance with this regimen was generally good. This exercise program did result in moderate reductions in body fat (2-4%), increased insulin sensitivity, and improved aerobic fitness levels (≈ 18%), particularly in the intensive group, indicating that the training intensity and duration were sufficient to lead to the expected physiological improvements. In addition, even when all study subjects were stratified by actual changes in fitness, CRP levels still did not decrease across tertiles of VO$_2$ MAX. Thus, the current study results are not readily explained by inadequate

exercise frequency, insufficient weight loss, or a study duration too short to result in physiologic changes.

One potential conclusion is that regular exercise (at this moderate level of intensity) does not improve the levels of inflammatory markers in obese insulin-resistant subjects and may in fact counter the typical effects of moderate weight loss on these risk factors. These results are consistent with those reported in a recent 18-month study of moderate exercise with or without simultaneous caloric restriction in a large group (>50 subjects per intervention arm) of older overweight individuals. Whereas subjects in the weight loss–only group demonstrated substantially more weight loss and a small but significant decline in CRP and other inflammatory markers, the combined weight loss and exercise group and the control group showed no change in markers of inflammation [46]. Interestingly, in a much smaller study in postmenopausal women, these same investigators have found that lesser amounts of diet-induced weight loss were not associated with declines in CRP, but in those both exercising and dieting (and achieving slightly greater weight loss), CRP levels declined significantly [47]. These data taken together seem to imply that moderate regular aerobic exercise alone in sedentary persons is not associated with declines in CRP. On the other hand, if sufficient weight loss occurs with or without concurrent exercise, declines in CRP and changes in other inflammatory markers may occur.

Our results are also consistent with the findings from other prospective studies that evaluated the effects of exercise training on plasma adiponectin [48,49]. Thus, it appears that neither exercise training nor the modest weight loss that frequently accompanies it effectively raises adiponectin. The fact that CRP was not reduced in our intervention groups, when it is known to be responsive to moderate weight loss, is consistent with reports that exercise itself may have some inflammatory consequences [24,50,51]. Exercise can induce striking elevations in IL-6 and other cytokines [24,52,53], which may stimulate increases in CRP. Several of these cytokines (in particular IL-6) are not only inversely related to levels of adiponectin, but have been implicated in the downregulation of adiponectin expression [54]. Thus, exercise-enhanced release of cytokines can be directly implicated in alteration of both CRP and adiponectin levels. The severity of exercise required to elicit these inflammatory responses is not well established, but it appears that the more intense and prolonged the exercise the greater the cytokine elevations [24,51]. Thus, more frequent and more intense exercise is not likely to translate into greater reductions in all measures of inflammation. The time course of these cytokine responses to exercise is not completely established, but the levels of IL-6 and CRP may take up to 2 days to return to baseline levels after intense exercise [55]. As our blood samples were drawn 24 to 48 hours after the last exercise bout, it is conceivable that exercise-induced inflammatory responses had not yet been resolved. Thus, it may be important to conduct more careful time-course assessments of exercise

and inflammation. However, as current recommendations are that individuals exercise at a moderate intensity, for 30 minutes or greater, most days per week [30-32], it is probable that with this frequency of exercise, inflammation markers will remain elevated for much of the time.

There are several other important implications of this study. Changes in insulin resistance resulting from exercise training did not appear to be associated with changes in CRP or adiponectin, even after adjustment for other variables. These data suggest that this important benefit of exercise does not appear to be related to reductions in inflammation or elevations in mediators of glucose disposal such as adiponectin. Although CRP has been used successfully in many studies to provide additional assessment of CVD risk, this study points out several limitations in using a general marker of systemic inflammation, such as CRP, to assess individual CVD risk. Individuals that exercise regularly may indeed reduce their CVD risk by such activity, yet this decrease in risk may not be reflected by ambient CRP levels. In fact, it is possible that strenuous activity may in fact raise CRP levels in the acute and recovery phase, indicating a level of risk that is not appropriate. In addition, as demonstrated in this study, there appears a very strong association of CRP with obesity, with remarkably high values in those with body fat values above 35% (Fig. 1A). It remains to be determined whether this elevation is more a function of fat mass rather than true cardiovascular risk. With obesity increasing dramatically in our population, we may need to at least consider modification of our use of CRP, or its threshold risk levels, in inactive, markedly obese individuals.

Several potential caveats to the findings of this study deserve mention. Although there were both direct evidence (from changes in VO_2 MAX) and indirect support (from changes in anthropomorphic features and laboratory values) that the exercise interventions improved physical fitness, we recognize that the level of exercise intensity conducted by even our intense exercise group may be considered moderate in intensity [56]. We also understand the potential limitations and increased variation of measurement that may occur by extrapolating VO_2 MAX from a submaximal test. Although we measured 2 different factors suggested to be important markers and/or regulators of inflammation, there are many other pro- or anti-inflammatory factors that could be measured. However, CRP in particular has proven to be a relatively useful marker of systemic inflammation and predictor of clinically relevant outcomes [2,3] and is the most commonly measured inflammatory marker. Similarly, there is increasing evidence that adiponectin may be a critical regulator of inflammatory events in a variety of tissues, including the vascular wall. It is also important to note, however, that different forms of adiponectin and its receptors have been identified in animals and humans [57], and it is possible that the biological action of adiponectin may be related more closely to levels of these structural variants rather than to total adiponectin levels [58]. We

cannot exclude the possibility that with more prolonged exercise training, exercise-related inflammation may become blunted, eventually leading to more favorable changes in inflammatory markers. It is also possible that more intense exercise, as occurred in a small observational study of marathon runners [59] or as part of a comprehensive risk-modification program (including changes in diet composition and caloric intake), may contribute to declines in CRP [60]. However, these latter findings as well as a report [61] suggesting that frequent habitual exercise may contribute to reduced levels of CRP in postmenopausal women who were taking estrogen will need to be confirmed in carefully controlled randomized prospective studies. Moreover, as demonstrated in this and our original study, clinically relevant improvements in insulin sensitivity can occur even at these more moderate exercise intensity levels without improvements in markers of inflammation.

In summary, although exercise has many well-recognized beneficial effects [21-23,25,26], this study indicates that (1) moderate-duration exercise training did not have favorable improvements on plasma levels of CRP and adiponectin, and (2) exercise-induced changes in insulin resistance could not be explained by changes in plasma levels of these particular inflammation markers.

Acknowledgment

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