Comparison of Inflammatory Responses After Acute Moderate Aerobic Cycling in Healthy Young Active and Inactive Men

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ABSTRACT

In view of available findings about exercise-induced inflammation, the present study was conducted to investigate the effect of acute moderate cycling on systemic inflammatory responses in healthy young active and inactive men. A quasi-experimental pre-post design was used to study 12 healthy young inactive men (aged 21.01 ± 1.1 years, body fat 16.7 ± 1.2% and VO2max 45.01 ± 5.83 ml/kg/min) and 12 young active men (aged 21.02 ± 1.2 years, body fat 12.04 ± 2.72% and VO2max 59.63 ± 2.15 ml/kg/min). One week after preliminary measurements, all subjects participated in an acute moderate cycling protocol (45 min with 50% VO2max). Blood samples were drawn before and immediately after the exercise. Complete blood cell counts, fasting blood sugar, serum interleukin-6 (IL-6), interleukin-10 (IL-10), C-reactive protein (CRP) and plasma stress hormones (cortisol and epinephrine) were determined. Data were analyzed by the independent samples t-test and Pearson’s correlation test at α < 0.05. Our results demonstrated that total and differential circulating leukocyte counts and serum IL-6, IL-10, and CRP concentrations, along with plasma epinephrine and cortisol levels, were increased immediately after the acute moderate cycling protocol in both active and inactive men (P < 0.01). Furthermore, the positive correlation between the post-exercise total leukocyte counts and serum IL-10 was significant (P = 0.011). However, the correlations between the exercise-induced reduction of fasting blood glucose and total leukocyte counts with serum CRP and IL-6 after exercise were not significant (P > 0.05). The small exercise-induced elevation of inflammatory marker concentrations suggests that the intensity or duration of the acute moderate cycling episode may not have been sufficient to induce a substantial systemic inflammatory response in young active or inactive men. Therefore, it can be concluded that the moderate exercise appears to be safe from an immunological point of view in young active and inactive men, and moderate exercise cycling can be recommended for both groups. Biomed. Int. 2011; 2: 64-71. ©2011 Biomedicine International, Inc.

Key words: Aerobic, exercise, inflammation, physiology

INTRODUCTION

Chronic diseases account for ~60% of total mortality worldwide. Available evidence suggests that engaging in moderate exercise or physical activity should benefit immune function and reduce susceptibility to infection and chronic non-communicable diseases. However, it has been demonstrated that a single bout of exercise leads to an acute
inflammatory response characterized by leukocytosis and an increase in circulating pro-inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-10 (IL-10), or an elevation in systemic C-reactive protein (CRP). This is controversial: some studies have shown that one bout of moderate exercise elicits no detectable change in various markers of inflammation, whereas other studies utilizing moderate-intensity exercise (cycling or running) have demonstrated that acute moderate exercise leads to significant changes in certain inflammatory markers. The immune system components in the resting state are similar in active and inactive individuals, but responses to exercise show differences. These responses depend on many factors such as age, sex, fitness and conditioning, environmental conditions and so on.

Because these findings concerning the role of personal conditioning and the effect of exercise on inflammatory markers are inconsistent, the present study was conducted to investigate changes in inflammatory response markers (leukocytosis, serum IL-6, IL-10 and CRP) after 45 min of acute moderate aerobic cycling in healthy young active and inactive men.

MATERIALS AND METHODS

This study was conducted using a quasi-experimental pre-post design on 12 healthy young inactive men and 12 healthy young active men. All subjects were free of disease and infection. The experimental procedures and protocols were approved by the Ethics Committee of the Tabriz University of Medical Science and all participants were informed about the purpose and risks of the study before written informed consent was obtained. All subjects were living on the Tabriz University campus, so nutritional and other conditions were controlled. They had not participated in any heavy sports program during the previous six months. The exercise was carried out in the department of physiology, Tabriz University of Medical Science during the 2009-2010 academic sessions.

Preliminary measurements

One week before the study, health screening, anthropometric measurements and maximal oxygen uptake measurements were completed in a single session. Height, body weight, BMI and fat percentage for both pre- and post-exercise data were determined by an experienced expert. Body fat percentage (BF) was measured in millimeters using a skin-fold caliper (Eiken MK-60; Meikosha Co., Tokyo, Japan) and a standard protocol (ACSM three-site: triceps, suprailiac and abdomen). All measurements were done on the right side of the body.

The incremental maximal cycle test (Astrand, 1965) was performed on a cycle ergometer (Ergometrics 800S, Sensor Medics, Yorba Linda, USA) for estimation of \( \text{VO}_{2\text{max}} \). During the Astrand maximal test, respiratory gas exchange was continuously measured breath-by-breath using the computerized standard open circuit technique (Sensor medics Vmax 29, Yorba Linda, CA, USA). Heart rate was measured with a heart rate monitor (Polar Electro, Kempele, Finland) and continuously supervised by a physician during all testing periods. Also, perceived exertion (PRE) was subjectively rated on the Borg scale during the final minute of the exercise protocol.

Experimental design and procedures

Subjects were asked to refrain from exercise and any anti-inflammatory medications for 72 h before the bout of moderate aerobic cycling. However, one week after the preliminary measurement, all subjects participated in a moderate cycling protocol (50% \( \text{VO}_{2\text{max}} \) for 45
min). The exercise protocol was conducted at laboratory temperature (22-25 °C) at 9-10 am. A short self-paced warm-up (5 min) was allowed.

Blood samples (7 ml) were collected from an antecubital vein immediately before and after the exercise protocol. Immediately after collection, one ml of blood was sent for CBC-H1 analysis and a three milliliters aliquot was dispensed into tubes and left to clot at room temperature for 10 min in order to collect serum. The remainder of each sample was dispensed into EDTA-coated tubes and centrifuged for 10 min at 3000 g to collect plasma. The plasma and serum samples were stored at −80°C until the day of assay. Plasma fasting blood glucose concentration was measured using an enzymatic reaction with glucose oxidase (Cobas Mira assay; Roche, Basel, Switzerland). Red blood cells (RBC), total and differential leukocyte counts, and hemoglobin (Hgb) and hematocrit (Hct) levels were determined using an automatic blood analyzer (Technicon H1, Technicon, Tarrytown, NY, USA).

Serum IL-6, IL-10, and CRP were analyzed using commercial solid-phase high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (Sanquin, Amsterdam-Netherlands for IL-6 and IL-10; Pars Azmoon, Iran, for CRP). The concentrations of plasma epinephrine and cortisol (ng mL⁻¹) were also determined by high-sensitivity ELISA kits (IBL-Hamburg, Germany). The intra-assay variation for all ELISA measurements was between 3 and 7%. Leukocyte counts were adjusted for percentage changes in blood volume, whereas plasma and serum variables were adjusted according to percentage changes in plasma and blood volume, as calculated from hemoglobin and hematocrit.²¹

Statistical analysis
Data were expressed as means (± SD) and checked for normal distribution using the Kolmogorov–Smirnov test. Differences in parameter changes between the two groups were analyzed by an independent t-test. All statistical analyses were performed using the SPSS statistical software package (SPSS version 15.0 for Windows, SPSS Inc., Chicago, IL, USA). The significance level was set at α < 0.05.

RESULTS
Table 1 shows the anthropometric and physiological characteristics of the two groups of participants. All resting and post-exercise serum inflammatory marker concentrations (acute phase protein) were within the population reference range. The active subjects, who had participated in an aerobic program for four months, were of similar age, height, and BMI to the inactive subjects but had higher maximum oxygen consumption (Table 1).

Table 2 shows that the percentage changes in all inflammatory markers (CRP, IL-6, IL-10 and total leukocyte counts) were greater in the inactive than the active men. Stress hormones (i.e., cortisol and epinephrine) levels also increased in both groups, but the increases were greater in inactive than active men (Figures 1 and 2).

DISCUSSION
In this study, we compared the influence of moderate cycling exercise on inflammatory markers in healthy young active and inactive men. Our results showed that in the resting state (basal condition) the immunological component did not differ significantly between the two study groups (active and inactive) (P > 0.05). Moderate exercise increased the inflammatory markers in both groups, but the increases were greater in the inactive than
the active men. Recent studies have shown that exercise type (running vs. cycling), individual fitness and conditioning, duration, intensity (particularly) and history of exercise (active vs inactive), sex and age are important in determining the inflammatory response to exercise. Some previous studies have shown that moderate exercise (50% VO\textsubscript{max}) does not change systemic markers of inflammation, while others have demonstrated that acute moderate exercise leads to significant changes in certain inflammatory markers.

Table 1: Anthropometric and physiological characteristics of subjects.

<table>
<thead>
<tr>
<th>Character</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Year)</td>
<td>Active</td>
<td>12</td>
<td>21.2</td>
<td>1.2</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>12</td>
<td>21.01</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Active</td>
<td>12</td>
<td>64</td>
<td>4.24</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>12</td>
<td>71.42</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Active</td>
<td>12</td>
<td>175.57</td>
<td>5.45</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>12</td>
<td>177.42</td>
<td>5.94</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg m\textsuperscript{-2})</td>
<td>Active</td>
<td>12</td>
<td>20.79</td>
<td>1.44</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>12</td>
<td>22.56</td>
<td>2.83</td>
<td></td>
</tr>
<tr>
<td>Body fat (%))</td>
<td>Active</td>
<td>12</td>
<td>12.04</td>
<td>2.72</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>12</td>
<td>16.7</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>VO\textsubscript{max} (ml min\textsuperscript{-1} kg\textsuperscript{-1})</td>
<td>Active</td>
<td>12</td>
<td>59.63</td>
<td>2.15</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>12</td>
<td>45</td>
<td>5.83</td>
<td></td>
</tr>
<tr>
<td>AT* (Anaerobic threshold (VO\textsubscript{max} %))</td>
<td>Active</td>
<td>12</td>
<td>0.52</td>
<td>0.08</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>12</td>
<td>0.60</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Comparison of exercise-induced inflammatory responses in healthy young active and inactive men after moderate exercise.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean Difference (pre-exercise minus post-exercise)</th>
<th>Percentage change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Leukocytes (×10\textsuperscript{9} L\textsuperscript{-1})</td>
<td>Active</td>
<td>0.81</td>
<td>14.84</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>1.37</td>
<td>23.03</td>
<td></td>
</tr>
<tr>
<td>Blood Sugar (mg dl\textsuperscript{-1})</td>
<td>Active</td>
<td>-3</td>
<td>-4.54</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>-5.8</td>
<td>-7.63</td>
<td></td>
</tr>
<tr>
<td>CRP (mg L\textsuperscript{-1})</td>
<td>Active</td>
<td>0.24</td>
<td>53.33</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>0.43</td>
<td>84.98</td>
<td></td>
</tr>
<tr>
<td>Interlukin-6 (pg ml\textsuperscript{-1})</td>
<td>Active</td>
<td>0.42</td>
<td>36.38</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>0.75</td>
<td>61.68</td>
<td></td>
</tr>
<tr>
<td>Interlukin-10 (pg ml\textsuperscript{-1})</td>
<td>Active</td>
<td>0.63</td>
<td>26.40</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Inactive</td>
<td>1</td>
<td>40.69</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Comparison of percentage changes in epinephrine level between young active and inactive men.

Figure 2: Comparison of percentage changes in cortisol level between young active and inactive men.
Total leukocyte count

Any stressor such as exercise load (with different intensities and durations) or changes in environmental temperature\textsuperscript{12-25} could lead to an increase in total and differential leukocyte counts resulting from the recruitment of cells from the spleen and bone marrow into the circulation, mediated at least in part by cardiovascular factors and particularly by stress hormones such as cortisol and epinephrine\textsuperscript{26} and depending on age and gender.\textsuperscript{16} In the present study, the leukocyte counts increased by 14.84 % in active and 23.04 % in inactive men after moderate exercise (Table 2). Previous studies have demonstrated that peripheral blood leukocyte counts increase immediately after acute moderate exercise (running and cycling), sometimes being elevated 2-3 fold,\textsuperscript{11,27} and can remain elevated for several hours after prolonged exercise.\textsuperscript{3,8,10} Moyna et al. found a rapid, albeit transient, alteration in the number of circulating leukocytes during and following an acute progressive incremental exercise test that was independent of gender and fitness.\textsuperscript{28} In agreement with this finding, Nieman and colleagues demonstrated a modest increase in total leukocyte count and serum IL-6 concentration 1 h after a 30-min treadmill walk at 60–65% VO\textsubscript{2}max in young women,\textsuperscript{10} whereas Markovitch and coworkers showed that walking at moderate intensity (50% VO\textsubscript{2}max) for 30 min did not change systemic markers of inflammation in middle-aged men.\textsuperscript{9} In accordance to our findings, Blannin and coworkers showed that counts of circulating leukocytes, lymphocytes, and neutrophils increased significantly less in a trained than in a control group.\textsuperscript{29}

Cytokines

The results in the present study demonstrated that the inflammatory cytokines (IL-6, IL-10) increased immediately after the acute moderate intensity cycling (50% VO\textsubscript{2}max for 45 min) in both active and inactive men, but significantly more in the latter group. Consistent with our findings, Giraldo and coworkers found the profile of pro-/anti-inflammatory cytokine release to be better following moderate exercise. However, moderate-intensity exercise had a greater effect on serum concentrations of IL-6 than short-term intense exercise.\textsuperscript{13} Peake and coworkers reported that the percentage change in plasma IL-10 concentrations from pre- to immediately post-exercise correlated significantly with the percentage change in the plasma concentrations of IL-6 and cortisol over the same time period.\textsuperscript{12} Scharhage et al. observed an almost 10-fold increase in IL-6 after 4 h of cycling with moderate intensity, indicating a moderate acute phase response.\textsuperscript{11} The greater increases in IL-6 after moderate exercise reported in this study (up to 1.5-fold) compared with others\textsuperscript{10} can be explained by the higher mechanical muscular strain due to differences in exercise duration (45 min vs 4 h); IL-10 follows the increase in IL-6 level.

CRP

In contrast to the 3.5– to 4-fold increases in CRP concentrations 16-24 h post-exercise reported in some previous studies,\textsuperscript{11,31} we observed only a small though significant increase in serum CRP concentration immediately after moderate exercise in both groups. Other studies have reported a small decrease in CRP concentrations one hour after exercise.\textsuperscript{13} These discrepancies could be due to differences in the sampling periods and lag of CRP appearance in the circulation after different exercise intensities. Thus, Niess and colleagues demonstrated a clear lag period between the exercise stimulus and the appearance of CRP in the circulation, with peak concentrations typically 24 h post-exercise.\textsuperscript{25} In contrast to our findings, Markovitch et al. found that neither pro- nor anti-inflammatory markers changed significantly after moderate exercise, so the long-term anti-inflammatory and
anti-atherogenic effects of regular moderate-intensity physical activity cannot be explained in terms of a net anti-inflammatory effect of any single exercise bout. Nevertheless, some studies have demonstrated that acute moderate exercise leads to significant changes in certain inflammatory markers.10,11

**Stress hormones**

In the present study, we observed increases of cortisol and epinephrine after moderate exercise in both groups, but the increases were greater in inactive than active men (*Figures 1 and 2*). Stress hormones play an important role in modulation of pro-inflammatory cytokines and down-regulation of the immune response. Blasio *et al.* showed that stress hormones such as cortisol and epinephrine mediate leukocytes and other inflammatory factors. In accordance with our finding, some researchers have shown that epinephrine and cortisol increase after different intensities of exercise. In contrast, other researchers in this area have even found a decrease in cortisol level after moderate and intense exercise in sedentary women, and no change in epinephrine after moderate exercise. It should be noted that different methods have been used to measure serum cytokine concentrations such as flow cytometry or a cytometric bead array (CBA), and these methods are not sensitive enough to detect relatively minor changes in levels after various types of exercise. In contrast, enzyme-linked immunosorbent assays (ELISA) are sensitive enough to detect the small changes in the concentrations of human cytokines after different exercise routines.

In view of the controversies in this field, it could be interesting to measure the inflammatory marker levels at different times after the cessation of the exercise bout (e.g. 0, 1, 2, 5 and 24 h) to determine whether the changes observed are transient or sustained. Further investigations looking at the effects of different intensities and durations of exercise on the immediate changes in these parameters would seem worthwhile.

**CONCLUSIONS**

Moderate exercise induced changes in some inflammatory markers in both active and inactive men. The increases after moderate-intensity cycling were greater in the inactive than the active subjects, but this mode, intensity and duration of acute moderate exercise might not have been sufficient to induce a substantial inflammatory response in either group. Although it be concluded that personal conditioning and fitness are important in modulating responses to exercise, this type of moderate exercise is safe for both active and inactive young men.

**ACKNOWLEDGEMENTS**

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