Acute moderate-intensity exercise in middle-aged men has neither an anti-nor proinflammatory effect

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Markovitch D, Tyrrell RM, Thompson D. Acute moderate-intensity exercise in middle-aged men has neither an anti-nor proinflammatory effect. J Appl Physiol 105: 260–265, 2008. First published May 8, 2008; doi:10.1152/japplphysiol.00096.2008.—Strenuous exercise induces an initial pro- and subsequent anti-inflammatory response, and it has been suggested that this may be one of the ways that regular exercise reduces chronic inflammation and therefore the risk of cardiovascular disease. However, public health recommendations emphasize moderate-intensity physical activity, and it is important to understand whether moderate-intensity exercise has a similar anti-inflammatory effect. Twelve sedentary male volunteers (age 54 ± 4 yr) completed two main trials, moderate-intensity exercise and rest (30 min at 50% maximal oxygen uptake vs. sitting, respectively). There were no significant changes in circulating neutrophils, lymphocytes, monocytes, or serum interleukin-6, interleukin-10, and C-reactive protein concentration over the 7 days following exercise. Similarly, lymphocyte adhesion to cultured endothelial cells and heme oxygenase-1 (HO-1) expression in lymphocytes and monocytes were not affected by walking at any time point. These results suggest that the long-term anti-inflammatory and antiatherogenic effects of regular moderate-intensity physical activity must be explained by something other than a profound net anti-inflammatory response to each exercise bout since a single bout of walking did not lead to a change in various markers of inflammation or lymphocyte adherence to cultured endothelial cells.

moderate exercise; inflammation; cytokines; lymphocytes; adhesion

ATHEROSCLEROSIS results from interactions between modified lipoproteins, monocyte-derived macrophages, T cells, and the normal cellular elements of the arterial wall, and there is growing evidence to show that chronic low-grade inflammation participates centrally in all stages of this disease (7, 15). Systemic concentrations of C-reactive protein (CRP) and IL-6 have been identified as particularly useful risk factors for cardiovascular disease (3, 32). Although many different cell types are important in atherogenesis, there is now good evidence that lymphocytes play a pivotal role in all stages of the disease. For example, Song and colleagues (34) demonstrated that atherosclerotic-prone lymphocyte-deficient mice have atherosclerotic lesions approximately half the size of control animals. It also appears that increased expression of anti-inflammatory changes within lymphocytes may make them more resistant to participation in atherogenesis. For example, mice with T cells overexpressing IL-10 had lesions arrested at the fatty streak stage (despite on-going hyperlipidemia) (28). Interestingly, it appears that the anti-inflammatory effect of IL-10 is mediated by the expression of the anti-inflammatory protein heme oxygenase-1 (HO-1) (14). HO-1 is oxidant responsive and anti-inflammatory in a range of conditions, with effects ranging from the desensitization of adhesive responses in leukocytes through to the resolution of inflammation (41, 42), and research confirms that leukocyte HO-1 can play an important role in atheroprotection (23).

Physical inactivity accelerates the development of major noncommunicable disease, with studies showing a particularly marked and consistent effect on cardiovascular disease (40). Several studies have demonstrated a strong and consistent inverse relationship between physical fitness and leukocyte count and markers of inflammation such as serum IL-6 and serum CRP (11–13), whereas serum IL-10 is positively related to fitness (10). Extensive research has shown that during and immediately after acute prolonged and very demanding exercise, there is a dramatic increase in leukocyte cell count and serum IL-6, which, in the hours following exercise, is accompanied by an increase in the anti-inflammatory cytokine IL-10 (4, 24). Furthermore, acute prolonged exercise has been shown to increase the expression of the oxidant-responsive anti-inflammatory gene HO-1 in lymphocytes (21, 38). Consequently, it has been hypothesized that the long-term effect of exercise and protection against diseases associated with chronic low-grade inflammation may be ascribed to the anti-inflammatory response elicited by each bout of acute exercise (27).

Public health physical activity recommendations state that moderate-intensity aerobic physical activity for a minimum of 30 min on at least 5 days each week confers substantial protection against chronic diseases such as cardiovascular disease (5, 9). It is possible that some of the benefits derived from such activity are largely related to acute changes induced by the last bout of activity. For example, the regular upregulation of anti-inflammatory molecules such as HO-1 may contribute to an overall downregulation of proatherogenic inflammatory pathways in the same way that acute regular changes in lipid metabolism may play a role in the long-term benefits of regular exercise (8). While this appears to be an attractive hypothesis in the context of the anti-inflammatory effects of demanding vigorous exercise, there is less evidence that this is relevant to moderate-intensity physical activity as described in most physical activity recommendations.

It is essential that we understand the antiatherogenic mechanisms by which regular moderate exercise exerts its protective effect. One explanation is a regular acute upregulation of anti-inflammatory and antiatherogenic pathways, although there is no evidence to show that this is the case. We hypoth-

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esized that an acute bout of physical activity would lead to a modest upregulation of anti-inflammatory pathways such as lymphocyte HO-1 and serum IL-10 in sedentary middle-aged men and that this may underpin some of the atheroprotective benefits of moderate-intensity exercise.

MATERIALS AND METHODS

Subjects. Twelve sedentary male volunteers took part in this investigation, which was approved by the local ethics committee (mean age, height, body mass, BMI, and body fat percentage were 54 ± 4 yr, 177 ± 3 cm, 87 ± 9 kg, 28 ± 3 kg/m², and 22 ± 2%). Each volunteer provided written informed consent before participation. Volunteers who smoked, had a BMI > 35 kg/m², or took regular medication were excluded from taking part in this study. Mean estimated maximal oxygen uptake (V\textsubscript{O\textsubscript{2max}}) was 34.4 ± 3.9 ml·kg⁻¹·min⁻¹.

Preliminary measurements. Habitual physical activity energy expenditure was estimated using combined accelerometry and heart rate with branched equation modeling using an objective validated instrument (39). This tool was worn for eight continuous days, collecting data measurements every minute during both day and night. The data from the first day was discarded as this was not a full 24-h measurement. The time expended by each subject below threshold values of 4 metabolic equivalents (METs; low intensity), between 4 and 5.9 METs (moderate intensity), and above 6 METs (vigor intensity) over the 7-day testing period was calculated using in-house software (31). This allowed us to classify whether individuals were meeting age-specific physical activity recommendations.

The V\textsubscript{O\textsubscript{2max}} of each subject was determined using a submaximal speed test followed by an incremental incline test on a treadmill (Woodway, ELG 70 Weiss). The submaximal protocol consisted of four steady-state exercise stages, each lasting 4 min, with speed increasing by 0.8 km/h with each stage. One-minute expired air samples were collected at the end of each stage of exercise using Douglas bags. Following adequate recovery, subjects completed the incremental incline test, consisting of 3-min exercise stages with the incline increasing by 3% at the end of each stage. The test was terminated once heart rate (HR) for each subject reached 75–85% of their age-predicted HR maximum. Expired gas samples were collected in the final minute of each stage along with HR and rating of perceived exertion (RPE). V\textsubscript{O2max} was estimated using linear regression, and oxygen uptake was used to calculate relative exercise intensity.

Experimental design and procedures. Subjects completed one exercise trial and one rest trial (30-min quiet sitting) in a randomized order ~10 days apart. During the 72-h period before each main trial, subjects recorded their food and fluid intake, and this was replicated twice with PBS and then resuspended with fresh culture medium (31).

Adherent lymphocytes were imaged by a fluorescent microscope (HB1010-AF, Nikon, Japan) fitted with a digital camera (Coolpix E995RCUK, Nikon, Japan). Both microscope and camera were used at a fixed magnification. Ten images were taken per 3-cm plate, with the investigator blinded to the coding of the pictures to avoid bias. The number of lymphocytes counted for both plates from the same sample was averaged to provide the mean adherence per sample (20 pictures).

Flow cytometry. Lymphocyte and monocyte HO-1 protein was analyzed by indirect immunofluorescence using a monoclonal mouse anti-HO-1 antibody IgG and FITC-conjugated goat antimouse polyclonal immunoglobulin antibody as described previously (16). Briefly, ~1 × 10⁶ mononuclear cells were fixed and permeabilized in 2 ml 70% ice-cold ethanol, and samples were kept at 4°C until subsequent analysis. Cell analysis was performed in a Becton Dickinson FACScan (Cellquest version 3.3 software) using 488-nm excitation and detection in the green fluorescence channel (FL1: 530 ± 30 nm bandpass filter). HO-1 protein was expressed as the fold change in median fluorescence intensity (MFI) relative to the baseline pretest sample.
Table 1. Total leukocyte and subset counts following 30-min walking or sitting

<table>
<thead>
<tr>
<th>Time</th>
<th>Leukocytes</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>5.26±1.47</td>
<td>2.98±1.22</td>
<td>1.61±0.28</td>
<td>0.42±0.11</td>
</tr>
<tr>
<td>Exercise</td>
<td>5.44±1.52</td>
<td>3.17±1.37</td>
<td>1.62±0.31</td>
<td>0.41±0.10</td>
</tr>
<tr>
<td>0 h</td>
<td>5.18±1.49</td>
<td>3.01±1.23</td>
<td>1.53±0.24</td>
<td>0.40±0.12</td>
</tr>
<tr>
<td>2 h</td>
<td>5.61±1.62*</td>
<td>3.37±1.35</td>
<td>1.62±0.26</td>
<td>0.40±0.11</td>
</tr>
<tr>
<td>24 h</td>
<td>5.17±1.55</td>
<td>2.98±1.39</td>
<td>1.53±0.24</td>
<td>0.42±0.09</td>
</tr>
<tr>
<td>48 h</td>
<td>5.08±1.78</td>
<td>2.90±1.62</td>
<td>1.52±0.25</td>
<td>0.43±0.10</td>
</tr>
<tr>
<td>72 h</td>
<td>4.85±1.33</td>
<td>2.75±1.29</td>
<td>1.50±0.23</td>
<td>0.40±0.08</td>
</tr>
<tr>
<td>168 h</td>
<td>5.09±1.15</td>
<td>2.95±0.89</td>
<td>1.51±0.23</td>
<td>0.40±0.08</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 12. *Significant effect of time between 0 h and 2 h (P < 0.05).

Statistics. A two-way ANOVA with repeated measures was used to compare results between trials and over time. Where a main effect was observed, post hoc tests using Bonferroni adjustment were made for multiple comparisons. Normality plots with tests (Shapiro-Wilks) were performed and log transformed before statistical analysis where data were not normally distributed. For single comparisons, a paired Student’s t-test was used to determine whether any differences existed between conditions. Values are presented as means ± SD. Significance was accepted at the 5% level. Data were analyzed using SPSS version 14 (SPSS, Chicago, IL). We have also reported effect size (ES) between trials for the change from baseline as suggested by Thomas and colleagues (37). Because of the large number of repeated measures for multiple parameters we have only reported large effects (ES > 0.8) (37).

RESULTS

Physical activity analysis. The mean daily time spent in moderate activities (>4 METs) accumulated in bouts of >10 min was 11 ± 11 min. None of the participants achieved exercise recommendations of a minimum of 30 min of moderate-intensity exercise (accumulated in bouts of 10 min) at least 5 days/wk. Over the entire 7-day record, 10 volunteers did not perform a single bout of moderate-intensity activity lasting more than 30 min.

Trial data. The mean treadmill speed of the 30-min walk was 5.5 ± 0.6 km/h, which elicited an oxygen uptake of 53.0 ± 2.4% VO2max (18.3 ± 2.6 ml·kg⁻¹·min⁻¹). Energy expenditure during the 30-min exercise trial was 191 ± 27 kcal, and mean HR was 115 ± 7 beats/min. Baseline blood lactate concentration was 0.53 ± 0.19 and 0.58 ± 0.22 mmol/l in the rest and exercise trials, respectively, and did not change in response to sitting or walking. There were no differences between trials in the amount or composition of food consumed over each 3-day period before the main trial day (data not shown).

Leukocyte counts. Total leukocyte count did not differ between exercise and rest trials; however, there was a main effect of time (t6, F = 4.019, P < 0.05) that was observed between the immediately posttrial sample (0 h) and 2 h posttrial (P < 0.05) (Table 1). Although both neutrophil and lymphocyte counts changed over time (t6, F = 3.169, P < 0.05; and t6, F = 3.957, P < 0.05, for neutrophils and lymphocytes, respectively), post hoc comparisons did not show significant differences between time points. Monocyte counts did not differ between exercise and rest trials or over time.

Serum inflammatory markers. IL-6 concentration, IL-10 concentration, and CRP concentration did not change following exercise or rest trials (Table 2).

HO-1 protein. Lymphocyte and monocyte HO-1 protein expression did not change following exercise and rest trials (Table 3).

Adhesion assay. There was no difference in lymphocyte adhesion to cultured endothelial cells between exercise or rest trials, but there was a main effect of time (t6, F = 5.845, P < 0.05) at 2 h (P < 0.05) (Fig. 1). While there were no observed differences at any time point, there was a large effect size statistic (ES = 1.3, 1.1, and 1.3) between trials for the change from baseline after 48, 72, and 168 h, respectively, although this appears to be largely accounted for by a relative increase in baseline in the rest trial (Fig. 1).

Table 2. Serum IL-6, IL-10, and CRP concentrations at 0, 2, 24, 48, 72, and 168 h following each trial

<table>
<thead>
<tr>
<th>Time</th>
<th>IL-6, pg/ml</th>
<th>IL-10, pg/ml</th>
<th>CRP, mg/l</th>
<th>IL-6, pg/ml</th>
<th>IL-10, pg/ml</th>
<th>CRP, mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>1.33±1.23</td>
<td>0.76±0.79</td>
<td>1.72±1.25</td>
<td>1.32±2.10</td>
<td>1.71±2.87</td>
<td>1.58±1.00</td>
</tr>
<tr>
<td>Exercise</td>
<td>1.39±1.11</td>
<td>0.26±0.45</td>
<td>1.50±1.32</td>
<td>1.47±2.27</td>
<td>1.14±2.32</td>
<td>1.57±1.00</td>
</tr>
<tr>
<td>0 h</td>
<td>1.54±1.40</td>
<td>0.45±0.69</td>
<td>1.65±1.20</td>
<td>1.46±2.00</td>
<td>1.97±2.68</td>
<td>1.67±1.07</td>
</tr>
<tr>
<td>2 h</td>
<td>1.21±1.66</td>
<td>1.24±1.76</td>
<td>1.58±0.99</td>
<td>1.17±1.20</td>
<td>2.11±1.96</td>
<td>1.53±0.94</td>
</tr>
<tr>
<td>24 h</td>
<td>1.29±1.78</td>
<td>0.76±1.20</td>
<td>1.57±1.17</td>
<td>1.34±1.78</td>
<td>1.49±1.58</td>
<td>1.44±1.14</td>
</tr>
<tr>
<td>48 h</td>
<td>1.19±1.69</td>
<td>1.32±1.55</td>
<td>1.56±1.54</td>
<td>1.56±1.97</td>
<td>1.89±2.85</td>
<td>1.57±1.51</td>
</tr>
<tr>
<td>72 h</td>
<td>1.63±1.60</td>
<td>1.73±2.29</td>
<td>1.68±1.38</td>
<td>1.32±1.59</td>
<td>2.02±3.89</td>
<td>1.84±1.19</td>
</tr>
<tr>
<td>168 h</td>
<td>1.32±1.59</td>
<td>1.84±1.19</td>
<td>1.32±1.59</td>
<td>1.84±1.19</td>
<td>2.02±3.89</td>
<td>1.84±1.19</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 12. CRP, C-reactive protein.
DISCUSSION

A single bout of treadmill walking at 50% \( \dot{V}O_{2\text{max}} \) did not elicit a detectable change in various markers of inflammation (pro- and anti-inflammatory) or lymphocyte adherence to cultured endothelial cells in middle-aged men. The estimated intensity of the exercise protocol was equivalent to 5.2 ± 0.7 METs. The threshold for physical activity of a moderate intensity in middle aged individuals is between 4 and 5.9 METs (31); therefore the treadmill exercise used in this investigation is typical of the moderate-intensity physical activity that is recommended for public health.

It has been proposed that regular physical activity is anti-inflammatory partly because of the changes induced by each individual bout of exercise (26, 27). Vigorous exercise elevates serum IL-6 concentration, which may be anti-inflammatory through the increased production of IL-1 receptor antagonist (IL-1ra) and IL-10 (27). Furthermore, vigorous exercise increases the lymphocyte expression of the anti-inflammatory enzyme HO-1 at protein and mRNA levels (21, 38). Lymphocytes play a pivotal role in inflammation-induced atherogenesis, and the increased transcription of the anti-inflammatory gene HO-1 in these cells following acute strenuous exercise would point toward a potentially important atheroprotective mechanism initiated by exercise. The overall impact of such changes could potentially affect proatherosclerotic aspects of cell function (adherence to stimulated endothelial cells and leukocyte cytokine secretion). For example, a single bout of exercise has been shown to reduce lymphocyte adhesion to cultured endothelial cells (17). However, in the present study, we did not observe changes in any of these measures (leukocyte cell count, CRP, IL-6, IL-10, lymphocyte HO-1, or lymphocyte adhesion) over a 7-day period in response to a single bout of moderate-intensity exercise.

Some previous studies utilizing moderate-intensity exercise have shown that exercise elicits changes in certain inflammatory markers, and others have found no effect (18, 20, 30). In contrast to our findings, Nieman and coworkers (20) demonstrated a modest increase in total leukocyte count and serum IL-6 concentration 1 h following a 30-min treadmill walk at 60–65% \( \dot{V}O_{2\text{max}} \) in young women who were accustomed to regular walking. It is not clear whether differences in subject characteristics (such as sex, age, or fitness) or exercise intensity (50% vs. 60–65% \( \dot{V}O_{2\text{max}} \)) explain this apparent discrepancy. To date, only one published study has investigated the effect of moderate-intensity exercise on serum concentrations of the anti-inflammatory cytokine IL-10, in which the systemic concentration of IL-10 in young well-trained male runners did not change following 60 min of running at 60% \( \dot{V}O_{2\text{max}} \), and this would support our findings (25). In summary, it appears that moderate-intensity exercise does not increase circulating pro- and anti-inflammatory markers in middle-aged men who are unaccustomed to exercise and that some kind of intensity or duration threshold must be achieved to elicit an acute change in measures such as IL-6 and IL-10.

We also found no effect on the expression of the anti-inflammatory protein HO-1 in lymphocytes or in lymphocyte adhesion to cultured endothelial cells. Lymphocyte HO-1 expression is upregulated in response to prolonged demanding exercise (21, 38), but eccentric contractions and a short exhaustive run have been shown to have no effect on HO-1 protein expression in leukocytes (6). In vitro adhesion assays have been used to demonstrate a reduction in lymphocyte adhesion to human umbilical venous endothelial cells in healthy individuals (mean age 36 yr) following a 25-min cycle ergometer exercise at 75% \( \dot{V}O_{2\text{max}} \), which was accompanied by a significant increase in serum IL-6 concentrations immediately postexercise (18). However, leukocyte-endothelial adhesion following 20 min of steady-state exercise at 65–70% \( \dot{V}O_{2\text{max}} \) seems to be dependent on fitness or absolute work rate because young physically fit individuals (age, 32 ± 11 yr; \( \dot{V}O_{2\text{max}} \), 43.9 ± 5.1 ml·kg\(^{-1}\)·min\(^{-1}\)) demonstrate an attenuation in leukocyte-endothelial adhesion, whereas less fit older individuals (age, 45 ± 9 yr; \( \dot{V}O_{2\text{max}} \), 27.2 ± 5.8 ml·kg\(^{-1}\)·min\(^{-1}\)) do not seem
to show the same response (17). The subjects in the present investigation were similar in characteristics to these unfit individuals, and it appears that an acute bout of moderate-intensity exercise in middle-aged men has no effect on either the expression of a key anti-inflammatory gene in lymphocytes (HO-1) or lymphocyte-endothelial adhesion ex vivo.

It seems that exercise intensity and duration are both important in determining the inflammatory response to exercise, with intensity being particularly important. In the present investigation we did not observe an inflammatory response following a 30-min walking protocol at 50% \(V_{O2max}\). Other studies have shown that exercise of a shorter or similar duration can elicit an inflammatory response, but only if the intensity is higher (36, 43). Similarly, low-intensity protocols that are very prolonged in duration (1-legged dynamic knee extensor exercise for 5 h at 40% \(V_{O2max}\)) also induce an inflammatory response (35). It is also noteworthy that studies that employ more intense exercise protocols tend to use younger and fitter individuals, and this means that not only is there a difference in terms of relative work rate (e.g., 50% \(V_{O2max}\) vs. 60% \(V_{O2max}\)), but there will be an even more pronounced difference in absolute work rate because of an age-related decline in capacity (e.g., in absolute running speeds or METs). The individuals in the present investigation did not meet physical activity recommendations although they were of average fitness for their age (2). Therefore, it appears that moderate-intensity exercise of a duration that would meet public health recommendations in middle-aged men is insufficient to induce a pro- or anti-inflammatory response.

An alternative explanation for the lack of change could be that the subjects were so accustomed to this kind of exercise that it did not represent a challenge or perturbation to the system. However, on the basis of the observed habitual physical activity data, this does not appear to be the case as all the subjects were inactive and the majority did not complete a single 30-min bout of exercise (of at least moderate intensity) during a typical week. Therefore, the volunteers were unaccustomed to the exercise challenge used in the present investigation.

The results from the present investigation indicate that even though regular moderate-intensity exercise appears to be associated with reduced markers of inflammation in cross-sectional comparisons (11, 13, 29), this does not appear to be explained by acute inflammatory changes induced by each exercise bout. As a result, there must be other explanations that account for the anti-inflammatory and antiatherogenic effect of long-term, moderate-intensity exercise. It is possible that the acute bout of exercise elicited some very subtle changes in other tissues that were not assessed in the present investigation. This notwithstanding, we feel that it is more likely that other changes associated with regular moderate-intensity exercise ultimately explain the anti-inflammatory effect. For example, a reduction in body mass following exercise training (22) or fat mass through dietary intervention (19) can be anti-inflammatory and reduce chronic inflammation. Furthermore, positive changes in lipid and glycemic profiles could also explain the anti-inflammatory effect of regular moderate-intensity exercise (33). Carefully constructed randomized controlled trials are required to better understand the anti-inflammatory effect of regular moderate-intensity exercise.

In summary, walking at a moderate intensity for 30 min did not change systemic markers of inflammation (total leukocyte cell counts, serum IL-6, IL-10, or CRP concentrations), lymphocyte or monocyte expression of the anti-inflammatory enzyme HO-1, or lymphocyte adhesion to endothelial cells. These findings suggest that the long-term anti-inflammatory and antiatherogenic effects of regular moderate-intensity physical activity must be explained by something other than a transient net anti-inflammatory response to each exercise bout and are instead perhaps explained by other changes that accumulate when exercise is performed over weeks and months.

GRANTS

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REFERENCES


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