Physical fitness attenuates leukocyte-endothelial adhesion in response to acute exercise

Paul J. Mills,1 Suzi Hong,1 Laura Redwine,2 Steven M. Carter,2 Albert Chiu,2 Michael G. Ziegler,2 Joel E. Dimsdale,1 and Alan S. Maisel2

Departments of 1Psychiatry and 2Medicine, University of California, San Diego, La Jolla, California

Submitted 2 February 2006; accepted in final form 8 May 2006

Mills, Paul J., Suzi Hong, Laura Redwine, Steven M. Carter, Albert Chiu, Michael G. Ziegler, Joel E. Dimsdale, and Alan S. Maisel. Physical fitness attenuates leukocyte-endothelial adhesion in response to acute exercise. J Appl Physiol 101: 785–788, 2006. First published May 25, 2006; doi:10.1152/japplphysiol.00135.2006.—Studies suggest that physical fitness promotes cardiovascular health, including improved endothelial function and possibly reduced inflammatory responses to stressors. This study examined the effects of fitness on leukocyte-endothelial adhesion in response to an acute exercise challenge. Peripheral blood mononuclear cell (PBMC) adhesion to human umbilical venous endothelial cells (HUVEC) was examined in 18 more-fit and 19 less-fit individuals [mean age 39 yr (SD 11)] before and after a 20-min treadmill exercise at 65–70% peak oxygen consumption. PBMC were isolated from whole blood (Ficoll-Paque) at rest and immediately after exercise. HUVEC were incubated for 4 h in the presence of cytokines IL-1 and IL-8 to activate endothelial adhesion molecule expression. Fit subjects showed a significant reduction in PBMC-HUVEC adhesion after exercise (P < 0.01) compared with less-fit subjects, who showed no significant change. Regardless of fitness levels, both at rest and in response to exercise, soluble ICAM-1 in the incubation media attenuated PBMC-HUVEC adhesion by ~81% (P < 0.001). The findings indicate that immune cells that demarginate in response to exercise have reduced ability to adhere in individuals who are physically fit, an effect apparently independent of ICAM-1 binding. The findings provide evidence of how physical fitness might protect individuals from inflammatory responses to exercise.

endothelium; adhesion; soluble intercellular adhesion molecule-1; peripheral blood mononuclear cell

CARDIORESPIRATORY FITNESS and regular physical activity are associated with a reduction in cardiovascular disease risk factors and mortality (2, 20, 27). One of the mechanisms through which physical fitness might promote cardiovascular health is supporting anti-inflammatory processes (14, 37).

An important step in the inflammatory process of relevance to cardiovascular disease is the cell adhesion molecule-mediated adhesion of leukocytes to the vascular endothelium (28). Sites of intimal thickening and atheromatous plaques show marked increases in subendothelial infiltrates of T lymphocytes and macrophages expressing high densities of CD11a (34). The expression of CD11a on circulating monocytes is greater in patients with atherosclerosis (15). CD11a is the α-subunit of the β2-integrin lymphocyte function-associated antigen-1 (CD11a/CD18), which binds to the ligand intercellular adhesion molecule-1 (ICAM-1) mediating firm leukocyte adhesion onto the endothelium (19). Mature cells typically express higher densities of integrins such as CD11a and lower densities of the selectin CD62L (19). The expression of β2-integrins such as CD11a are important for the adhesion of lymphocytes to endothelial cells (4, 5). Many of the mechanisms of leukocyte-endothelial adhesion were elucidated using in vitro human umbilical venous endothelial cells (HUVEC) models. Such studies, representing a broad array of basic and clinical sciences, have demonstrated the effects of cytokine stimulation on upregulating endothelial cell adhesion molecule expression and the mechanisms of leukocyte endothelial cell adhesion (18, 32, 39).

Acute exercise typically produces a dramatic redistribution in the number and phenotypic profiles of leukocytes in the peripheral circulation (9, 14, 26, 38). Studies consistently show that a characteristic of these redistributed leukocytes is an increased expression of CD11a on lymphocytes and CD11b on monocytes and a decreased expression of CD62L (16, 17, 31). The decrease in CD62L in response to stressors is related to the increase in integrin expression (29). Our laboratory has previously shown that the acute exercise-induced increase in CD11a expressing lymphocytes is attenuated in fit individuals (11).

In light of observations that physical fitness promotes cardiovascular health through anti-inflammatory mechanisms, as well as a relative reduction of leukocyte CD11a expression after exercise in fit individuals, this study examined the effects of physical fitness on PBMC-HUVEC adhesion after an acute exercise challenge in a group of fit and not fit individuals.

METHODS

Subjects. Thirty-seven volunteers participated after providing written, informed consent. Other than mild hypertension in 10 of the subjects (blood pressure >140/90 mmHg but <180/110 mmHg), all subjects were identified as healthy after a history and physical by a physician. All potential subjects underwent an ECG to ensure that there were no cardiac abnormalities before participation, and only those with normal ECGs were studied. Individuals who had a history of heart disease, liver or renal disease, diabetes, psychosis, severe asthma, pregnancy, morbid obesity (%ideal body weight >150), or current use of prescription medication were excluded. Volunteers were recruited from the local community and compensated financially for their participation. The protocol was approved by the University of California, San Diego (UCSD), Institutional Review Board.

Protocol. Subjects completed the Leisure Time Exercise Questionnaire (LTEQ) to assess subjects’ habitual exercise (8, 12). LTEQ is a simple self-administered questionnaire designed to measure an individual’s regular physical activity level. A total score was calculated by multiplying frequencies of weekly exercise of different intensity by the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Fit</th>
<th>Nonfit</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Age,† yr</td>
<td>32.3 (10.8)</td>
<td>45.1 (8.8)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>8/10</td>
<td>13/6</td>
</tr>
<tr>
<td>Hypertension status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(hypertensive/normotensive)</td>
<td>3/15</td>
<td>7/12</td>
</tr>
<tr>
<td>VO2 peak, ml·kg⁻¹·min⁻¹</td>
<td>43.87 (5.05)</td>
<td>27.13 (5.79)</td>
</tr>
<tr>
<td>Body mass index,† kg/m²</td>
<td>23.67 (3.3)</td>
<td>26.36 (4.7)</td>
</tr>
<tr>
<td>Exercise,‡ amount of physical activity/week</td>
<td>74.0 (41.3)</td>
<td>39.9 (23.1)</td>
</tr>
<tr>
<td>Screening systolic blood pressure, mmHg</td>
<td>121.4 (17.5)</td>
<td>128.0 (15.9)</td>
</tr>
<tr>
<td>Screening diastolic blood pressure,§ mmHg</td>
<td>72.7 (12.7)</td>
<td>81.4 (10.3)</td>
</tr>
</tbody>
</table>

Values are means (SD); n, no. of subjects. VO2 peak, peak oxygen consumption. *Leisure Time Exercise Questionnaire [Godin and Shephard (8)] †P < 0.01; ‡P ≤ 0.05.

Table 2. Metabolic and cardiorespiratory responses during a 20-min steady-state exercise at 65–70% VO2 peak in fit and nonfit subjects

<table>
<thead>
<tr>
<th></th>
<th>Fit</th>
<th>Nonfit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average respiratory exchange ratio</td>
<td>0.952 (0.092)</td>
<td>0.937 (0.05)</td>
</tr>
<tr>
<td>Average metabolic equivalents,*</td>
<td>8.45 (1.22)</td>
<td>5.48 (0.71)</td>
</tr>
<tr>
<td>Average systolic blood pressure,† mmHg</td>
<td>152.7 (26.5)</td>
<td>155.9 (22.2)</td>
</tr>
<tr>
<td>Average diastolic blood pressure,§ mmHg</td>
<td>71.8 (10.7)</td>
<td>81.8 (10.2)</td>
</tr>
<tr>
<td>Average ratings of perceived exertion</td>
<td>15.34 (1.51)</td>
<td>13.05 (1.89)</td>
</tr>
</tbody>
</table>

Values are means (SD); *P < 0.001; †P < 0.01.
Fig. 1. Peripheral blood mononuclear cell (PBMC) adhesion to human umbilical venous endothelial cells (HUVEC) at rest and after 20 min of steady-state exercise at 65–70% peak oxygen consumption in 18 fit and 19 nonfit individuals. Top: percentage of cytokine-stimulated over nonstimulated condition. Fit subjects showed a significant reduction in PBMC-HUVEC adhesion after exercise compared with less fit subjects (P < 0.01). Bottom: percentage of cytokine-stimulated plus 350 ng/ml soluble ICAM (sICAM-1) over nonstimulated condition. Regardless of fitness levels, both at rest and in response to exercise, the presence of sICAM-1 in media attenuated PBMC-HUVEC adhesion ∼80% (P < 0.001).

We also ran separate repeated-measures ANCOVAs to examine the potential effects of hypertension and gender on PBMC-HUVEC adhesion. The 10 subjects with mild hypertension were not on antihypertensive medication at the time of testing. There were no significant effects of hypertension or gender on adhesion in this sample. There were not enough subjects to examine possible fitness by hypertension or fitness by gender interactions.

DISCUSSION

In attempts to understand how physical fitness promotes cardiovascular health, prior studies have examined vascular endothelial function. These studies suggest that being physically fit is associated with enhanced endothelium-dependent vasodilation (24, 36), although such findings have not been universal (22). Whereas loss of endothelial vasodilatory function is thought to contribute to the increased risk of atherosclerosis and thrombosis, fewer studies have examined the effects of physical fitness on leukocyte function as a mediator of atherogenic-associated changes in endothelial tissue. In humans, in vitro lymphocyte adherence to a nylon matrix was reported to be reduced in athletes compared with healthy sedentary individuals (30). In BALB/cJ mice, a moderate-intensity aerobic exercise training program attenuated leukocyte infiltration to the lung (25).

To expand on previous findings, this study examined in vitro adhesion of PBMC to a standardized endothelial cell line prestimulated with cytokines to upregulate endothelial cellular adhesion molecules. Although we did not observe a significant effect of fitness on PBMC adhesion at rest, group differences were observed in response to the exercise challenge. The reduced in vitro adhesion of PBMCs after exercise in the more-fit subjects suggests that exercise leads to a circulatory environment of reduced leukocyte adhesion to endothelial cells in more-fit individuals. The acute response to exercise is a redistribution in the number and phenotypic profiles of leukocytes in the peripheral circulation, including a decreased expression of CD62L and an increased expression of CD11a on lymphocytes and CD11b on monocytes (16, 17, 31). Based on such observations, one might expect an increase in their adhesion to endothelial cells. Our laboratory has previously reported that more fit individuals have significantly fewer CD11a expressing leukocytes after exercise compared with less fit individuals (11), which led us to expect a relative reduction in adhesion in the more-fit subjects. However, a conformational change to the activated form of CD11a/CD18 is necessary for effective high avidity binding for firm attachment of leukocytes to endothelium (3). Exercise might not be lead to conditions necessary for sufficient CD11a/CD18 activation (3, 35).

Whereas endothelial ICAM-1 activation supports leukocyte-endothelial adhesion, sICAM-1 acts to inhibit adhesion because it binds to CD11a/CD18 leaving less available CD11a/CD18 for endothelial ICAM-1 binding (1, 23). We found that sICAM-1 in the incubation media had the same inhibitory effect on PBMC adhesion in fit as in less-fit subjects. Given our laboratory’s earlier observations in fit subjects of decreased CD11a expression on peripheral leukocytes after exercise (11), we expected a smaller inhibitory effect of sICAM-1 in our more-fit subjects. Although prior studies indicate that the number of CD11a-expressing cells increase anywhere from 35 to 110% with acute exercise (11, 16, 17), the inhibition of adhesion in our study was similar regardless of whether the cells were taken before or after exercise. Thus, regardless of an anticipated higher PBMC CD11a expression postexercise in less-fit subjects, sICAM-1 led to a similar degree of inhibition of PBMC adhesion to HUVECs in all subjects. We chose to focus on ICAM-1 as a potential mechanism because of our laboratory’s prior observations (11, 16). In pilot studies, we had examined 350 and 700 ng/ml concentrations of sICAM-1 for their ability to inhibit PBMC-HUVEC binding. These concentrations were chosen from the literature to represent medium and high circulating levels, respectively (33). The 700 ng/ml concentration nearly completely eliminated adhesion, whereas the 350 ng/ml concentration decreased it by our target of ∼70%. We thus used this concentration in the study. In addition to ICAM-1, cytokine-stimulated HUVEC express other adhesion molecules that are relevant to leukocyte adhesion, including VCAM-1 and E-selectin (18, 32, 39). It could
certainly be the case that an effect of fitness on endothelial adhesion is mediated by other adhesion molecules.

Our fit subjects were younger and had lower blood pressure and a marginally lower BMI. This would be expected given that this group of individuals was much more physically active than the less-fit subjects. We controlled for these differences statistically. In addition, we ran a repeated-measures ANOVA without these covariates, and the same significant finding of reduced adhesion in the more-fit subjects was evident, suggesting that the difference in adhesion was likely related to differences in fitness rather than these other factors.

In summary, findings from this study suggest that immune cells that demarginate in response to acute exercise in more physically fit subjects have a reduced ability to adhere to endothelial cells. The mechanisms of this effect require elucidation, but the findings suggest how physical fitness might protect individuals from excessive inflammatory responses to acute stressors.

GRANTS
This work was supported by National Heart, Lung, and Blood Institute Grants HL-57265 and HL-073355 and UCSD General Clinical Research Center Grant M01 RR-00827.

REFERENCES