Aerobic exercise attenuates inducible TNF production in humans

Richard P. Sloan,1,2 Peter A. Shapiro,3 Ronald E. DeMeersman,4 Paula S. McKinley,1,2 Kevin J. Tracey,5 Iordan Slavov,1,2 Yixin Fang,1,2 and Pamela D. Flood6

1Division of Behavioral Medicine, Department of Psychiatry, Columbia University, 2New York State Psychiatric Institute, 3Division of Consultation Liaison Psychiatry, Department of Psychiatry, Columbia University Medical Center, 4Department of Rehabilitation Medicine, Columbia University Medical Center, 5The Feinstein Institute for Medical Research at North Shore-LIJ Health System, and 6Department of Anesthesiology, Columbia University Medical Center, New York, New York

Submitted 5 February 2007; accepted in final form 9 July 2007

Sloan RP, Shapiro PA, DeMeersman RE, McKinley PS, Tracey KJ, Slavov I, Fang Y, Flood PD. Aerobic exercise attenuates inducible TNF production in humans. J Appl Physiol 103: 1007–1011, 2007.—Aerobic exercise reduces coronary heart disease risk, but the mechanisms of this protection are not fully understood. Atherosclerosis is an inflammatory disease mediated by monocyte-derived macrophages, which accumulate in arterial plaques and become activated to release factors, including cytokines, that cause damage. Here we studied the effects of aerobic training on monocyte production of tumor necrosis factor (TNF) in whole blood ex vivo. Healthy young sedentary adults (n = 61, age 20–45 yr) were randomized to a moderate- (M) or a high- (H) intensity 12-wk training program. Whole blood was extracted before and after training, and then it was stimulated by addition of lipopolysaccharide (LPS); inducible TNF was measured in the plasma. Data were analyzed according to intention to treat principles using a random-effect model to determine the impact of training group on maximal aerobic capacity and LPS-stimulated TNF after correcting for covariates. Analyses revealed improvement in aerobic capacity in both the H (9%) and the M (7%) groups. However, aerobic training led to significant (P < 0.001) decreases in TNF release only in the H group. These data suggest that in healthy young adults, a 12-wk high-intensity aerobic training program downregulates blood monocyte production of stimulated cytokine release.

AEROBIC EXERCISE IS WIDELY RECOGNIZED TO REDUCE THE RISK OF CORONARY HEART DISEASE, SO MUCH SO THAT CONSSENSUS PANELS ROUTINELY RECOMMEND PHYSICAL ACTIVITY AS PART OF A CARDIOPROTECTIVE REGIMEN FOR HEALTHY PEOPLE (14, 15). SURPRISINGLY, DESPITE THE WEALTH OF CLINICAL AND EPIDEMIOLOGICAL DATA INDICATING BENEFIT, THE PHYSIOLOGICAL OR MECHANISTIC BASIS OF THIS PROTECTION IS UNKNOWN. SOME RECENT STUDIES HAVE SUGGESTED THAT EXERCISE MAY PROMOTE CARDIOPROTECTION THROUGH ANTI-INFLAMMATORY EFFECTS (5, 7, 17) AND THAT THESE EFFECTS MAY BE DOSE DEPENDENT (5, 9).

Monocytes and macrophages participate in the pathogenesis of atherosclerotic plaque formation and in the development of cardiovascular complications (18). When activated in the arterial wall, macrophages produce cytokines and other factors that mediate damage. For example, overexpression of tumor necrosis factor (TNF) has been implicated in plaque stability, endothelial dysfunction, and inflammatory damage in the arterial wall. TNF-mediated activation of endothelial cells modulates extracellular matrix degradation by inducing the expression of matrix metalloproteinases (16, 21). In human umbilical vein endothelial cells, TNF increases the level of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase (6), and in rat mesenteric microvessels, TNF induces significant leukocyte adhesion (23).

Although intraplaque TNF synthesis contributes to pathogenesis, it is not possible to measure these tissue levels in healthy volunteers. A number of recent studies, however, have utilized surrogate measures of synthesis by measuring TNF production in whole blood stimulated with endotoxin ex vivo. Here, we report that in a study examining the impact of two levels of aerobic training in sedentary young adults, high- but not moderate-intensity exercise led to decreased production of TNF by monocytes in whole blood ex vivo.

METHODS AND MATERIALS

Subjects

Subjects enrolled in this study were 61 healthy, sedentary nonsmoking women (n = 51) and men (n = 10), recruited from Columbia University Medical Center (CUMC). The mean age was 32.5 yr (range 21–45; SD = 6.9). Subjects were eligible if they were not exercising regularly and did not exceed American Heart Association standards for average fitness [maximum aerobic fitness (VO2max) ≤43 and 37 ml·kg⁻¹·min⁻¹ for men and women, respectively] (1). Subjects were excluded if screening indicated symptoms of affective disorder, psychosis, or substance abuse, or if they currently used psychotropic medication, as established by telephone screen. Subjects also were excluded if they had any medical condition that affected the autonomic nervous system or cardiovascular system. All subjects provided written informed consent. The CUMC Institutional Review Board approved this study.

Experimental Protocol

Subjects were recruited by flyers posted throughout the institution. After telephone screening to determine eligibility, they performed an incremental exercise test on a cycle ergometer during the initial laboratory session.

VO2max was assessed by a graded exercise test on an Ergoline 800S electronically braked cycle ergometer (SensorMedics, Anaheim, CA). Each subject began exercising at 30 W for 2 min, and the work rate was continually increased by 30 W every 2 min until VO2max criteria (respiratory quotient ≥1.1, increases in ventilation without concomitant increases in O2 uptake maximum age-predicted heart rate (HR) was reached and/or volitional fatigue) were reached. Minute ventilation was measured by a pneumotachometer connected to a FLO-1.
volume transducer module (PHYSIO-DYNE Instrument, Quogue, NY). Percentage of expired O2 and CO2 were measured using paramagnetic O2 and infrared CO2 analyzers connected to a computerized system (MAX-1, PHYSIO-DYNE Instrument) and calibrated against known medical-grade gases. The highest O2 uptake value attained during the graded exercise test was considered \( V_O_{2\text{max}} \) (3).

The exercise training protocol comprised self-directed exercise; however, to increase adherence qualifying subjects were assigned to one of the study’s research assistants as a “coach” who followed and encouraged their progress throughout the 12-wk training period. Subjects first completed a 2-wk stretching run-in period in the same facility used for the aerobic training phase of the study. Those who completed a minimum of seven 30-min stretching sessions were randomized to either the high- or moderate-intensity aerobic training program. Coaches contacted each subject on a weekly basis to monitor progress and to provide training guidance where needed.

After completion of training, subjects returned for postraining \( V_O_{2\text{max}} \) and laboratory testing (session 2). Data collection staff were blind to group assignment. Incentives for participation included a 4-mo membership in a fitness facility used for the study’s exercise protocol and $300 for completing the two laboratory sessions. In addition, after completing all study activities, subjects with exercise adherence rates of \( \geq 90\% \) received one session with a personal trainer and an additional 2-mo membership at the fitness facility to use however they chose.

**Conditioning Programs**

All exercise sessions in both conditions consisted of 10–15 min of warm-up and cooldown stretching and 30–40 min of aerobic exercise. These sessions were carried out 4 days/wk in the employee fitness center (PlusOne Health Management) on the CUMC campus.

**Stretching.** For both training conditions, subjects were instructed to stretch before and following exercise. Stretching consisted of arm circles, neck rotations, toe reach, gluteal stretches, lateral leg swings, Achilles stretch, and ankle rolls.

**High-intensity aerobic training.** This comprised the experimental condition in the study. Subjects in this group were permitted to do any or all of several aerobic activities, including cycling on a stationary bicycle, running on a treadmill, climbing on a step machine, or using an elliptical step machine. Subjects gradually progressed to a high-intensity work level. For weeks 1 and 2 of the 12-wk program, they trained at 55–60% of their maximum HR as determined during the initial cardopulmonary stress test. In weeks 3 and 4, they increased to 65–75% of maximum HR, and in weeks 5–12, they trained at 75–80% of maximum HR.

**Moderate-intensity aerobic training.** This comprised an active control condition in the study. As in the high-intensity program, subjects were permitted to select from the same aerobic equipment and activities. Subjects in this group trained throughout the 12-wk program at 55–60% of maximum HR.

All training sessions in both conditions consisted of 10–15 min of warm-up and cooldown and 30–40 min of intense workout. These sessions were carried out 4 days/wk.

**Quality Control/Adherence**

Throughout all training sessions, subjects wore Polars 610i model heart rate monitors that recorded HR continuously throughout the session. After each session, subjects downloaded their HR data directly from the Polar monitor to a personal computer via infrared port transmission. Study staff reviewed the HR data on a weekly basis to document adherence to training intensity levels. At each training session, subjects also completed logs containing the date, duration, and type of exercise activities performed at that session.

**TNF**

During morning laboratory visits, venous whole blood samples were obtained from volunteers before (session 1) and after (session 2) exercise training. Postexercise training samples were collected at least 1 day following the last training session.

Whole blood was stimulated by addition of lipopolysaccharide ([LPS] 0, 0.1, 1, 10 and 100 ng/ml). Plasma was prepared and TNF levels measured using DuoSet Elisa Development kit (no. DY210, R&D Systems) according to the manufacturer’s instructions.

Approximately 7 ml of blood were collected into a heparinized blood collection tube. Heparinized blood was immediately incubated at 37°C with low CO2 (2%) until aliquoted in experimental setup (incubation did not exceed 1 h). Endotoxin (LPS, *Escherichia coli* 0111:B4, Sigma catalog no. L4130) was resuspended to 5 mg/ml, sonicated for 30 min, vortexed well, and diluted with 1× PBS to create a working 1 mg/ml stock. The LPS stock was serially diluted with 1× PBS to final concentrations of 100, 10, 1, 0.1, and 0 ng/ml in blood aliquots. Microfuge tubes aliquoted with blood and endotoxin were gently mixed on a vortexer and incubated in a test tube rack on a rocking platform at 37°C, low CO2 for 4 h. Microfuge tubes were removed from incubation, centrifuged in a tabletop microfuge [5 min, 2,040 relative centrifugal force (5,000 revolution/min in Microfuge 5415C)], and the plasma was harvested and frozen at −20°C for future analysis.

**Statistical Analysis**

Data on aerobic capacity and TNF were analyzed using a mixed-effect model to determine the effect of group assignment and testing session after correcting for age, sex, and body mass index (BMI). TNF data were log transformed before analysis to stabilize their variance and achieve homoscedasticity.

**RESULTS**

**Descriptive Data**

Sixty-one subjects were randomized and tested before training. Fifty-one (83.6%) returned for testing following training (26 of 30 in the moderate-intensity group and 25 of 31 in the high-intensity group). Overall, 49.2% attended at least 42 training sessions (50.0% in the moderate-intensity group and 48.4% in the high intensity group).

In 5 of the 51 subjects, the assay was performed incorrectly. Data from one additional subject were excluded from analysis after it was determined that she had exercised within 24 h before the blood draw. Thus 45 subjects had acceptable TNF measures both before and after training.

Characteristics of the two groups of subjects before training are presented in Table 1. At study entry, as the table indicates, the high- and moderate-intensity training groups did not differ significantly from each other in potential covariates.

**Effect of Training on Aerobic Capacity**

\( V_O_{2\text{max}} \) was aerobic capacity was measured before and after completion of exercise training. As shown in Table 2, training
Table 1. Subject characteristics at study entry

<table>
<thead>
<tr>
<th>Effect</th>
<th>Session Pretraining</th>
<th>Session Posttraining</th>
<th>F (df)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>32.1±7.0</td>
<td>33.0±7.0</td>
<td>8.83</td>
<td>0.01</td>
</tr>
<tr>
<td>Weight, lb.</td>
<td>154.9±31.5</td>
<td>149.2±18.5</td>
<td>6.38</td>
<td>0.001</td>
</tr>
<tr>
<td>Height, in.</td>
<td>65.0±3.4</td>
<td>64.9±2.9</td>
<td>0.62</td>
<td>0.414</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.6±3.5</td>
<td>25.0±3.2</td>
<td>0.70</td>
<td>0.4849</td>
</tr>
<tr>
<td>V˙O₂max, ml·kg⁻¹·min⁻¹</td>
<td>26.5±5.8</td>
<td>26.3±5.2</td>
<td>0.09</td>
<td>0.9282</td>
</tr>
<tr>
<td>Log V˙O₂max</td>
<td>4.3±0.8</td>
<td>4.1±0.7</td>
<td>1.37</td>
<td>0.1765</td>
</tr>
<tr>
<td>Log TFN0</td>
<td>4.4±0.8</td>
<td>4.2±0.6</td>
<td>1.29</td>
<td>0.2020</td>
</tr>
<tr>
<td>Log TFN1</td>
<td>4.7±0.7</td>
<td>4.6±0.6</td>
<td>0.56</td>
<td>0.5761</td>
</tr>
<tr>
<td>Log TFN10</td>
<td>5.1±0.6</td>
<td>5.0±0.6</td>
<td>0.59</td>
<td>0.5555</td>
</tr>
<tr>
<td>Log TFN100</td>
<td>5.4±0.6</td>
<td>5.2±0.6</td>
<td>1.26</td>
<td>0.2135</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects. *P < 0.001, †P < 0.01. BMI, body mass index; V˙O₂max, maximal O₂ uptake; TFN0, TFN0.1, TFN1, TFN10, and TFN100, 0, 0.1, 1, 10, and 100 pg/ml tumor necrosis factor.

significantly increased V˙O₂max in each training condition. Analysis revealed significant effects of session [F(1,97) = 6.38, P = 0.01], sex [F(1,97) = 20.95, P < 0.001], age [F(1,97) = 9.53, P < 0.01], and BMI [F(1,97) = 52.42, P < 0.001]. Mean V˙O₂max increased by 9% in the high-intensity group and by 7% in the moderate-intensity group. However, the two groups did not differ in the increase in V˙O₂max, as indicated by the lack of a significant group × session interaction.

Inducible TNF Release in Whole Blood

Previous studies have shown that addition of LPS to human whole blood stimulates the production of TNF by monocytes in the sample (2). Table 3 shows that the effect of testing session was highly significant [F(1,363) = 15.42, P < 0.001], indicating that aerobic training significantly attenuated inducible TNF production.

However, the significant group × session interaction [F(1,363) = 8.83, P < 0.01] indicates a group difference in this effect of training. As Fig. 1 indicates, only the high-intensity exercise training regimen led to a decrease in inducible TNF release. Post hoc tests confirmed that the difference in pre- to posttraining inducible TNF release was significant in the high-intensity group [t (363) = −0.31, P < 0.001] but not in the moderate-intensity group [t (363) = −0.04, not significant]. Age, BMI, and sex did not significantly influence inducible TNF release in these subjects.

DISCUSSION

Our data demonstrate that a 12-wk aerobic training program significantly decreases inducible TNF production in whole blood in sedentary young adults but that this effect was seen only in the high-intensity training group. Previous studies on the anti-inflammatory effects of exercise training have been equivocal, reflecting differences in subject selection, inflammatory markers, and training activities. Most commonly, C-reactive protein (CRP) has been the inflammatory index of choice. In the largest and best-documented study, 20 wk of aerobic training administered to 35-yr-old healthy subjects led to a reduction in CRP only among those with elevated levels at study entry (11). Other studies have yielded reductions in CRP (8, 13) or shown no impact (17). Recently, Devaraj et al. (4) have reported that LPS-stimulated whole...
blood TNF synthesis was associated with increased CRP levels.

We studied the production of TNF by monocytes stimulated with endotoxin because it is a validated measure of immunological responsive to innate stimuli (2). Endotoxin activates TNF production via a mechanism of signal transduction that is dependent on the Toll-like receptor 4 (TLR4). Activation of TLR4 has been implicated in mediating the onset and development of atherosclerosis (12). The advantage of this approach is that it allows direct measurement of monocyte responses via a TLR4-dependent pathway in the extracellular milieu as occurs in vivo. The present study design does not allow us to conclude that TLR4 is the only TLR activated, but these data suggest the value of studying the effects of exercise on TLR signaling.

The progression and development of atherosclerosis depends in part on monocytes migrating to blood vessels, where they become activated to secrete cytokines. Exercise training increases parasympathetic outflow, which in turn may downregulate cytokine release through a cholinergic receptor-dependent mechanism. This hypothesis is based on the recent description of the cholinergic anti-inflammatory pathway, a mechanism whereby acetylcholine released from the vagus nerve specifically inhibits the synthesis of TNF and other cytokines in monocytes (22). Stimulation of vagus nerve activity in animals decreases levels of TNF during endotoxemia, sepsis, and ischemia (19, 20). Taken together with the present results, it is plausible to consider that increased vagus nerve activity resulting from exercise downregulates TNF production by endotoxin activated whole blood ex vivo.

These results have important implications for understanding the effects of exercise on the innate immune response to bacterial endotoxin, and they provide a testable hypothesis for understanding the beneficial effects of exercise that have been reported for atherosclerosis and other inflammatory diseases, i.e., by increasing the activity of the cholinergic anti-inflammatory pathway.

**Limitations**

The principal limitation of the study is the absence of a non-aerobic-training control group to rule out the possibility that the decline in TNF was the product either of repeated testing or engaging in any kind of nonspecific exercise. Data on the temporal stability of inflammatory markers are limited, but in the setting of percutaneous coronary revascularization, TNF was stable in 234 patients with coronary artery disease over a period of 18 mo (10). The only longitudinal study to employ a nonaerobic training control group found that in participants in a flexibility training condition had significantly smaller reductions in CRP and IL-6 compared with an aerobic training condition (8). Finally, the absence of an effect on TNF in the moderate-intensity training group argues against an effect of repeated testing.

In the future, it will be important to determine whether other TLR4 monocyte responses are downregulated in these subjects. Moreover, our participants achieved only modest improvements in aerobic capacity, and we did not observe a difference in the magnitude of the improvement moderate- and high-intensity groups, despite the difference in the TNF response. Future studies should examine further the effects of higher and lower exercise intensity levels on the suppression of TNF.

It also is theoretically possible that exercise decreased white blood cell counts in the whole blood samples, which in turn decreased TNF synthesis (9). Moreover, we cannot assume that the monocyte response in the blood is identical to the response in plaque. Finally, most of the subjects in this study were women, and future studies should include equal numbers of men and women.

These limitations notwithstanding, the study has some notable strengths. First, the study was a randomized controlled trial of two different aerobic training intensities. Second, monitoring of adherence to the training regimens was thorough. Subjects were followed closely to attend training sessions as prescribed and to maintain the appropriate level of HR during training. Finally, because subjects were young and, correspondingly, at very low risk for heart disease, the attenuation of the TNF response to LPS suggests a potentially clinically significant effect even in low-risk subjects.

**Conclusions**

In this study, we demonstrated that a 12-wk program of high- but not moderate-intensity aerobic conditioning resulted in a significant reduction in inducible TNF synthesis in whole blood in a sample of young, healthy, and sedentary adults. These findings suggest that one cardioprotective mechanism of exercise is by decreasing proinflammatory cytokine synthesis.

**ACKNOWLEDGMENTS**

The authors are grateful to the expert assistance of Claire Golden, Sushma Rambaran, and Eugene Festa and the staff of the PlusOne Fitness Center.

**GRANTS**

This study was supported by a grant from the American Heart Association, Heritage Affiliate (to R. P. Sloan) and by the Nathaniel Wharton Fund and the Vidalda Foundation. Work in K. J. Tracey’s laboratory was supported in part by the National Institute of General Medical Sciences at the National Institutes of Health (Grant MO1 RR-018535).

**REFERENCES**


