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Aerobic Exercise Training and Inducible Inflammation: Results of a Randomized Controlled Trial in Healthy, Young Adults

Richard P. Sloan, PhD; Peter A. Shapiro, MD; Paula S. McKinley, PhD;* Matthew Bartels, MD;[†] Daichi Shimbo, MD; Vincenzo Lauriola, MS; Wahida Karmally, RD; Martina Pavlicova, PhD; C. Jean Choi, MS; Tse-Hwei Choo, MS; Jennifer M. Scodes, MS; Pamela Flood, MD;[‡] Kevin J. Tracey, MD

Background—Consensus panels regularly recommend aerobic exercise for its health-promoting properties, due in part to presumed anti-inflammatory effects, but many studies show no such effect, possibly related to study differences in participants, interventions, inflammatory markers, and statistical approaches. This variability makes an unequivocal determination of the anti-inflammatory effects of aerobic training elusive.

Methods and Results—We conducted a randomized controlled trial of 12 weeks of aerobic exercise training or a wait list control condition followed by 4 weeks of sedentary deconditioning on lipopolysaccharide (0, 0.1, and 1.0 ng/mL)-inducible tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and on toll-like receptor 4 in 119 healthy, sedentary young adults. Aerobic capacity by cardiopulmonary exercise testing was measured at study entry (T1) and after training (T2) and deconditioning (T3). Despite a 15% increase in maximal oxygen consumption, there were no changes in inflammatory markers. Additional analyses revealed a differential longitudinal aerobic exercise training effect by lipopolysaccharide level in inducible TNF-α (P=0.08) and IL-6 (P=0.011), showing T1 to T2 increases rather than decreases in inducible (lipopolysaccharide 0.1, 1.0 versus 0.0 ng/mL) TNF-α (51% increase, P=0.041) and IL-6 (42% increase, P=0.11), and significant T2 to T3 decreases in inducible TNF-α (54% decrease, P=0.007) and IL-6 (55% decrease, P<0.001). There were no significant changes in either group at the 0.0 ng/mL lipopolysaccharide level for TNF-α or IL-6.

Conclusions—The failure to support the primary hypotheses and the unexpected post hoc findings of an exercise-training–induced proinflammatory response raise questions about whether and under what conditions exercise training has anti-inflammatory effects.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01335737. **(*J Am Heart Assoc.* 2018;7: e010201. DOI: 10.1161/JAHA.118.010201.)**

Key Words: clinical trial • exercise training • inflammation

One mechanism by which exercise contributes to cardioprotection may be via its anti-inflammatory effects.[1–3] Observational studies generally support this hypothesis. For example, the 13 748 participants in the NHANES (National Health and Nutrition Examination Survey) III study who engaged in regular aerobic dancing or jogging were less likely to have elevated levels of C-reactive protein (CRP), white blood cell count, and fibrinogen,[1,2] and men in the British

From the Divisions of Behavioral Medicine (R.P.S., P.S.M., V.L.) and Consultation/Liaison Psychiatry (P.A.S.), Department of Psychiatry, and Departments of Rehabilitation Medicine (M.B.), Medicine (D.S.), Anesthesiology (P.F.) and Biostatistics, Mailman School of Public Health (M.P.) and Irving Institute for Clinical and Translational Research (W.K.), Columbia University Medical Center, New York, NY; New York State Psychiatric Institute, New York, NY (R.P.S., C.J.C., T.-H.C., J.M.S.); The Feinstein Institute for Medical Research, Northwell Health, Manhassett, NY (K.J.T.).

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*Dr Paula S. McKinley is currently located at the Division of General Internal Medicine, Department of Medicine, Montefiore Medical Center, Bronx, NY.

[†]Dr Matthew Bartels is currently located at the Department of Rehabilitation Medicine, Montefiore Medical Center, Bronx, NY.

[‡]Dr Pamela Flood is currently located at the Anesthesia Department, Stanford, CA.

Correspondence to: Richard P. Sloan, PhD, Division of Behavioral Medicine, Department of Psychiatry, Columbia University Medical Center, 622 West 168th St, New York, NY 10032. E-mail: rps7@columbia.edu

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Clinical Perspective

What Is New?

- The cardioprotective effects of aerobic exercise training are well-established in patients and healthy subjects.
- Hypothesized mechanisms for the protective effect included training-induced anti-inflammatory effects.
- Twelve weeks of training yielded the expected significant increase in aerobic capacity in the training but not the wait-list control condition, but contrary to expectation, there were no treatment effects on ex vivo lipopolysaccharide-induced levels of tumor necrosis factor-α or interleukin-6 (IL-6).
- Post hoc analyses revealed that training led to an increase in tumor necrosis factor-α and IL-6 induced by 0.1 and 1.0 ng/mL lipopolysaccharide compared with 0.0 ng/mL lipopolysaccharide.

What Are the Clinical Implications?

- In young, healthy participants, aerobic exercise training may not elicit anti-inflammatory effects.
- In fact, training may elicit a proinflammatory effect, consistent with an immune system that evolved to combat sterile injury and infectious challenge.

Regional Heart Study who either maintained a minimum of light physical activity or became physically active over a 20-year period had lower levels of CRP and white blood cell count than less active groups.[4] Although some studies have failed to find such cross-sectional relationships,[5,6] a literature review identified at least 9 large cohort studies, with almost all showing the predicted inverse dose response effect between physical activity and measures of inflammation.[7]

However, intervention studies to date have had less consistent findings. Among negative studies, Lakka et al reported no overall effect of a 20-week aerobic training program on CRP in 652 sedentary, healthy 35-year-old participants, but a positive effect among those subjects in the top tertile of CRP (>3.0 mg/L) at study entry.[8] Aerobic training programs for 140 middle-aged men[3] and 162 men and women did not lead to reductions in CRP.[9] Aerobic training with or without dietary intervention led to improvements in aerobic capacity, but did not change interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), or CRP, in young, moderately overweight men.[10] In 25 overweight men (aged 52.8±7.2 years), a 16-week aerobic training program had no effect on IL-6, TNF-α, or CRP.[11] Libardi et al reported similar findings.[12] In 102 sedentary men and women, aerobic training led to improvements in fitness but did not change IL-6 or CRP.[13]

Other studies, in contrast, do show the hypothesized anti-inflammatory effects of exercise training, with reductions in CRP[14,15] and IL-6[15,16] and white blood cell count.[17] In a small randomized controlled trial of 49 sedentary men aged 45 to 64 years, a 24-week aerobic training program followed by 2 weeks of deconditioning led to a significant increase in maximal oxygen consumption (VO_2max) that was retained after deconditioning by a per-protocol analysis of the 41 participants who completed the study.[18] Serum IL-6 fell significantly in the exercise group after training and rose after deconditioning.

These inconsistencies are likely to be the product of many factors that vary across studies: (1) observational versus interventional studies; (2) study design (eg, exercise alone versus exercise plus weight loss); (3) differences in participants (eg, old versus middle aged; men, women, or both; healthy versus unhealthy patients; overweight/obese versus normal weight); (4) differences in training protocols (short-term versus long-term regimens); (5) differences in inflammatory markers assessed; and (6) differences in data analytic approaches. In view of these many differences, it is not surprising that the literature is inconsistent.

A feature shared by all of the studies discussed above is measurement of in vivo circulating levels of inflammatory markers. A disadvantage of this design is failure to account for the complex role of tissues other than monocytes—adipocytes, endothelial cells, muscle—in generating circulating inflammatory cytokines. A more precise estimation of the effect of exercise on monocyte-related inflammation may be achieved with ex vivo stimulation of white cells by lipopolysaccharide. While influenced by differences in stimulation concentrations, incubation time, and cell preparation,[19] ex vivo stimulation by lipopolysaccharide nonetheless may more accurately reflect immune regulation.[20] Many human[21,22] and animal[23–25] studies (though not all—see below) report the predicted anti-inflammatory effects of exercise training on lipopolysaccharide-inducible cytokine release.

With this report, we try to bring some clarity to this matter. Because even results of studies on CRP have yielded inconsistent findings, we focused on upstream inflammatory markers (TNF-α and IL-6, and toll-like receptor 4 [TLR4], whose activation initiates an NFκB-mediated cascade culminating in the production of TNF-α and IL-6). We focused on the effects of aerobic exercise training on ex vivo lipopolysaccharide-stimulated generation of inflammatory markers by peripheral whole blood monocytes. We conducted a randomized controlled trial and conducted our primary analysis on the basis of intention-to-treat group assignment. Additionally, we conducted per-protocol analyses to determine the effects of changes in inflammatory markers, specifically among those subjects who adhered to the exercise protocol. Furthermore, because we measured cardiorespiratory fitness throughout the study, we were able to confirm the fitness

effect of the exercise intervention. Finally, we included a 4-week sedentary deconditioning period as a further test of the effect of reversal of improvements in fitness on inflammatory markers.

The primary aim of the present study was to test the hypothesis that lipopolysaccharide-inducible monocyte production of TNF-α in whole blood ex vivo would decrease more from study entry (T1) to 12-week follow-up (T2) in the aerobic exercise group, compared with the wait-list control group, and that this effect would be reversed by sedentary deconditioning. The secondary aims were to examine whether a similar decrease occurs in the lipopolysaccharide-inducible production of IL-6 and in TLR4.

Methods

Research Materials Transparency

Upon receiving a request to the corresponding author from a member of the research community, we will make data, research and analytic methods, and materials used to conduct this study available for purposes of reproducing the results or replicating the procedure.

Participants

A total of 119 healthy young adults (age 20–45 years) were recruited from the Columbia University Medical Center/New York Presbyterian Hospital community. Recruitment was by flyers posted throughout the Medical Center and electronic bulletin boards. Subjects were nonsmoking, sedentary, habitual nonexercisers. The study was approved by the Institutional Review Board of the New York State Psychiatric Institute and was registered at ClinicalTrials.gov: NCT01335737.

Study Protocol

The study protocol is depicted in the Consort Diagram (see Figure 1). To determine eligibility, potential participants completed a phone screening including the Baecke Physical Activity assessment.[26] Those who were regular exercisers, defined as a score of ≥10 on this scale (range, 3–15 points), were excluded from further participation. Qualifying participants provided informed consent and were further screened for medical conditions that contraindicated exercise training, use of hormonal birth control, and body mass index >33 or <18 kg/m^2. Those still eligible completed a maximal cardiopulmonary exercise test (CPET). Participants who qualified as no better than average fitness by American Heart Association standards (VO_2max <43 mL/kg per minute for men, <37 mL/kg per minute for women) were eligible to continue in the study. Following the CPET, all participants went through a 2-week run-in period described below. They then were scheduled for the time 1 (T1) measurement session, during which they provided a fasting blood sample. After the blood draw, they were provided with a light breakfast, then completed several mood inventories and participated in a psychophysiology stress challenge in which high-frequency heart rate variability was measured. These data will be reported elsewhere. Women were scheduled for testing during their midluteal menstrual phase to control for the effects of menstrual cycle variation on cardiac autonomic control, as described below.[27] Those who completed this measurement session were randomized to either the 12-week aerobic training program or a wait-list control condition using random block assignment stratified by sex. Identical measurement sessions were conducted after the intervention (time 2 [T2]) and again after 4 weeks of sedentary deconditioning (time 3 [T3]). Participants received up to $210 compensation for the various testing sessions in the study. To encourage adherence, those who completed 85% of their training sessions received 2 months of bonus gym membership. Wait-list participants received 5 months of gym membership after their final measurement session.

Physical activity outside of the training sessions was monitored using a pedometer (Omron HJ-710ITTFFP) to assess potential changes in unsupervised physical activity. All participants in both the training and wait-list groups were required to wear the step counter throughout the 16 weeks of training and deconditioning to assess adherence to a sedentary lifestyle.

Run-in Stretching Period

During this 2-week period, participants came to the Fitness Center 4 times/week for 30 minutes of stretching. Each participant was assigned a research assistant "coach" who provided detailed instruction on stretches, consisting of arm circles, neck rotations, toe reach, gluteal stretches, lateral leg swings, Achilles stretch, and ankle rolls. All participants wore Polar heart rate monitors during the stretching sessions. Coaches monitored participants' adherence to the stretching protocol via gym attendance records and heart rate (HR) monitor data. Only those participants who attended at least 7 sessions were permitted to continue in the study.

Aerobic Training Program

For 12 weeks, participants randomized to aerobic training group came to the Fitness Center for 4 sessions/week according to a schedule they determined. Their coaches provided them with guidelines specifying their training goals to assure that they exercised at the appropriate level of intensity.

All training sessions consisted of 10 to 15 minutes of warm-up and cool-down and 30 to 40 minutes of workout.

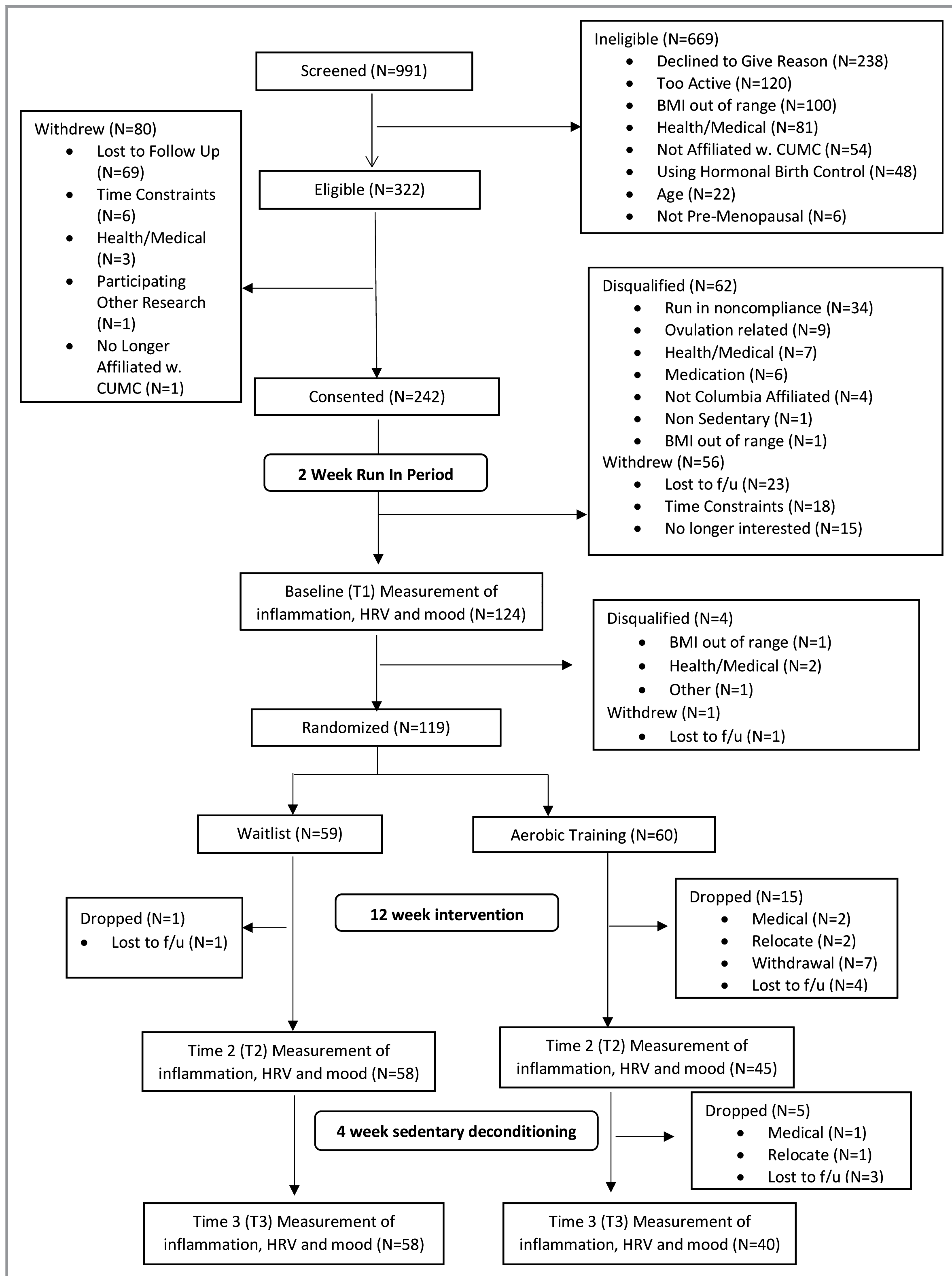

Figure 1. CONSORT diagram.

Subjects were permitted to select from a series of aerobic activities and for weeks 1 and 2 of the program, they trained at 55% to 65% of maximum HR as established during their qualifying CPET. In weeks 3 and 4, they increased their intensity to 65% to 75% of maximum HR, and in weeks 5 to 12, they trained at 75% of maximum HR.

To exercise at their target HR, participants wore a Polar Electro model s610i HR monitor during each training session. This monitor provided a digital display of HR and recorded HR throughout the training session. At the end of each session, participants uploaded the data from the monitor into a computer located in the Fitness Center. Coaches instructed participants on the use of the monitors and on how to upload data. These data were used to verify that they trained as prescribed.

Adherence to the training programs were documented by weekly logs, computerized attendance records at the facility, and data from HR monitors used during each training session. Subjects were contacted on a weekly basis by their coaches to monitor their progress. If the performance of participants in the aerobic training condition fell out of range (described below), they were contacted more frequently until they returned to prescribed training levels.

Sedentary Deconditioning

After completion of training and posttraining testing, aerobic training group participants refrained from any type of exercise for a 4-week period. During this time, they were contacted by their coaches on a weekly basis to encourage adherence to this deconditioning phase of the study.

Wait-List Control Condition

Subjects randomized to the wait-list group maintained their sedentary lifestyle after their qualifying testing session for 12 weeks plus the 4-week period corresponding to the deconditioning period, for a total of 16 weeks.

Laboratory Testing Sessions

After the run-in period, qualifying participants arrived at the Behavioral Medicine Laboratory at 8 AM after an overnight fast. Forty-five milliliters of venous whole blood was drawn for cytokine and hormone analysis. After the blood draw, they received a light breakfast. Waist circumference was measured with a Gulick anthropometric tape. Height was measured using the Seca 214 Portable Height Rod (Seca Corporation, Hamburg, Germany). Weight and body composition were measured using a Tanita BF-350 monitor (Tanita UK Limited, Middlesex, UK).

Measurement of Aerobic Capacity

Maximum aerobic fitness was measured by a graded exercise test on an Ergoline 800S electronic-braked cycle ergometer (SensorMedics Corporation, Anaheim, CA). All subjects had their peak ventilatory capacity (maximum voluntary ventilation) determined before the exercise test via a Vmax Encore System (Sensormedics, Yorba Linda, CA). Peak exercise capacity was determined by subjects having all subjects achieve at least 2 of the following: >85% peak predicted heart rate (220 − age), maximal exertion with limitation due to dyspnea, maximum exertion with muscle fatigue, respiratory exchange ratio >1.10, or increasing wattage with plateau of VO_2. Each subject had a 5-minute resting phase followed by a 3-minute warm-up and then a progressive ramped exercise test at 15 W/min until achieving VO_2max criteria (respiratory quotient ≥1.1, increases in ventilation without concomitant increases in VO_2, maximum age-predicted HR was reached, and/or volitional fatigue). Minute ventilation, expired oxygen, and carbon dioxide were measured using Vmax Encore Metabolic system (Sensormedics, Inc, Loma Linda, CA). The flow sensor and the gas analyzer were calibrated against known medical-grade gases before each test. VO_2max was determined from the peak 20-second average of the breath-by-breath measurement of the VO_2. Identical test procedures were carried out at the end of the training and deconditioning phases of the trial. Anaerobic threshold was determined for each subject using the V-slope technique.[28] Subjects qualifying as below average fitness by American Heart Association standards (VO_2max <43 mL/kg per minute for men, <37 mg/kg per minute for women) proceeded to the 2-week run-in stretching period.

Treatment Assessments

Inflammation Measures

We used a standardized method to activate cytokine release in whole blood ex vivo by addition of lipopolysaccharide (0, 0.1 and 1 ng/mL). Blood was collected into a heparinized blood collection tube. Endotoxin (lipopolysaccharide, e coli 0111:B4, Sigma cat. No. L4130) was sonicated for 30 minutes, vortexed well, and diluted with 1X phosphate-buffered saline (PBS) to create a working 1 mg/mL stock. The lipopolysaccharide stock was serially diluted with 1X PBS to final concentrations of 1, 0.1, and 0 ng/mL in blood aliquots. Microfuge tubes aliquoted with blood and endotoxin were gently pulsed on a vortexer and incubated in a test tube rack on a rocking platform at 37°C, low CO_2 for 4 hours. The Microfuge tubes were removed from incubation, centrifuged in a tabletop microfuge (5 minutes, 2040 g), after which plasma was collected and stored at −20°C for future analysis of cytokine levels.

Multiplex Analysis of Cytokines

We quantified stimulated and study entry circulating TNF-α and IL-6 using a Discovery Assay called the Human Cytokine Array Focused 11-Plex (Eve Technologies Corporation, Calgary, AB, Canada). The multiplex assay was performed at Eve Technologies by using the Bio-Plex 200 system (Bio-Rad

Laboratories, Inc, Hercules, CA, USA), and a Milliplex human cytokine kit (Millipore, St Charles, MO, USA) according to their protocol. The assay sensitivities of TNF-α and IL-6 were 0.1 and 0.3 pg/mL, respectively. Individual analyte values and other assay details are available on the Eve Technologies website (www.evetechnologies.com) or in the Milliplex protocol (http://www.emdmillipore.com/US/en/life-science-research/protein-detection-quantification/milliplex-multiplex-assays-using-luminex/UjGb.qB.8WQAAAE_rn8RHeN.,nav). All assays were done in duplicate.

Western Blot Analysis of TLR4

Denatured samples (20 μg/lane) with β-mercaptoethanol were separated on a 10% sodium dodecyl sulfate polyacrylamide gel, and the proteins in the gel were then electrotransferred to nitrocellulose membranes (Biorad) in PBS +20% methanol. Following the transfer, the membrane was blocked with 5% defat milk in PBS with 0.1% Tween (PBS-T), then incubated with rabbit antihuman TLR4 primary antibody (1:500, Santa Cruz Biotechnology). The membrane was washed 3 times with PBS-T and incubated with horseradish peroxidase–conjugated antirabbit secondary antibody (Abcam). After washing the membrane 3 more times, TLR4 bands were visualized with the use of the ECL kit (Amersham). Afterwards, the same membrane was stripped of the TRL4 antibodies in 2% sodium dodecyl sulfate, 0.8% β-mercaptoethanol PBS Striping buffer and blotted with mouse antihuman β-actin primary antibody and horseradish peroxidase–conjugated antimouse secondary antibody (Abcam) and visualized as above. The optical density of TLR4 and β-actin bands was measured with the use of the GelDoc system (Biorad), and TLR4 was expressed as a ratio of TLR4 density to that of β-actin (in arbitrary units; see Figure 2).

Dietary Assessment

We used the Block Brief 2000 FFQ (NutritionQuest, Berkeley, CA) to assess diet. This questionnaire was designed to provide estimates of usual and customary dietary intake. The food list for this questionnaire was developed from the NHANES III dietary recall data. The nutrient database was developed from the US Department of Agriculture's Nutrient Database for Standard Reference. Individual portion size is asked, and pictures are provided. Using data from this FFQ, we derived the alternative Mediterranean diet index.[29]

Statistical Analyses

The effect of aerobic training on all outcomes was analyzed using longitudinal mixed effect models with either log-link functions (for outcomes TNF-α, IL-6, and TLR4) or identity-link functions (for outcomes VO_2max and fat-free mass) to match

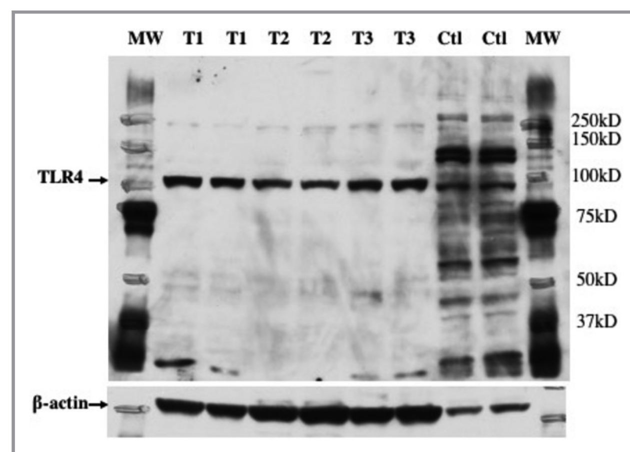

Figure 2. Western blots of TLR4 from a participant with T1, T2, and T3 blood draws. A representative Western blot of peripheral blood mononuclear cell lysates probed with monoclonal antibodies against TLR4 and ß-actin. 20 μg of cell lysates were loaded on the gel in duplicates (columns 2, 3, for T1; columns 4, 5 for T2, and columns 6, 7 for T3). Ctl indicates HEL293 cell lysate with TLR4 serving as positive control; MW, molecular weight marker. The arrows in the left margin indicate the TLR4 and ß-actin proteins detected. MWs are listed on the right.

their variables' distributions. A random intercept was used to account for within-subject correlations over time and the models adjusted for unequal variance among treatment groups. All unadjusted models included the effect of time, treatment group, the 2-way interaction between time and treatment group, and the corresponding T1 measure. Adjusted models included the additional covariates of age, sex, T1 body fat percentage, and T1 alternative Mediterranean diet index that have previously shown associations with the outcome measures.[30,31] Models of TNF-α and IL-6 were fit separately for each stimulation level (lipopolysaccharide 0.0, 0.1, and 1.0 ng/mL).

To estimate the within-group changes from T1 to T2 for each outcome, the observed values at all sessions were centered by subtracting the grand mean of the corresponding T1 values, and the same model described above was performed. Subtracting a constant from the outcome and baseline values does not affect the relationship but provides a way to estimate the changes from T1 to T2 within group and assess their significance (Data S1).[32] For each outcome, a total of 3 prespecified contrasts were obtained: (1) the treatment group effect at T2, (2) the difference from T1 to T2 for the aerobic exercise training condition, and (3) the difference from T1 to T2 for the wait-list condition.

All primary outcome analyses were conducted using an intent-to-treat (ITT) sample. In addition, all primary outcome models were analyzed using a per-protocol sample. The per-protocol sample was defined as completing at least 50% of scheduled exercise training sessions, having a blood draw

within 18 hours to 10 days after the last exercise training session, and completing the CPET within 14 days of the last exercise training session.

The original sample size of 182 participants (or 128 completers) was chosen to ensure sufficient power (at least 90%) of a 2-sided test with level of significance of 5% to detect true effect sizes of 0.67 or greater between the aerobic exercise training and the wait-list groups with respect to the primary outcome of TNF-α.[33]

To assess the moderation effect by baseline inflammation, that is, the effect of having high versus low levels of inflammation at study entry on the longitudinal effect of treatment assignment, each inflammatory measure at study entry was dichotomized into high and low groups using a median split. A 3-way interaction among treatment, time, and the corresponding dichotomized study entry inflammatory marker was analyzed using models similar to those described above.

To explore potential longitudinal treatment differences between lipopolysaccharide-inducible levels of TNF-α and IL-6, the lipopolysaccharide-inducible level was dichotomized into lipopolysaccharide 0.0 ng/mL versus lipopolysaccharide 0.1 and 1.0 ng/mL. A 3-way interaction among lipopolysaccharide-inducible level, treatment, and time and subsequent 2-way interactions and corresponding main effects were analyzed. These models were adjusted by the same covariates as primary outcome models.

TNF-α was missing for 5 (4.2%) participants at T1, 20 (16.8%) at T2, and 26 (21.8%) at T3. IL-6 was missing for 7 (5.9%) participants at T1, 23 (19.3%) at T2, and 25 (21.0%) at T3. TLR4 was missing for 10 (8.4%) participants at T1, 26 (21.9%) at T2, and 30 (25.2%) at T3. The logistic regressions for the missing values failed to identify any significant predictors of missingness, so the unobserved values were assumed to be missing at random. Additionally, a sensitivity analysis using multiple imputation via the Markov chain Monte Carlo method was performed on TNF-α, IL-6, and TLR4, and the results were consistent with the primary analyses on the basis of the ITT sample.[34] Because no violations of missing-at-random assumptions were identified, and because the imputation analyses results did not deviate from the results based on ITT sample, study dropouts are not expected to lead to any meaningful biases.

All analyses were performed using SAS software (version 9.4, SAS Institute, Cary, NC), and all statistical tests were 2-sided at a significance level of 5%.

Results

Sample Recruitment and Adherence

We screened 991 potential participants, of whom 119 (63 women, 56 men) were recruited and randomized. Of the 60 participants randomized to the aerobic training condition, 15 dropped out during the training and before T2 testing (see CONSORT diagram in Figure 1). An additional 5 participants dropped out during the sedentary deconditioning phase. Only 1 participant in the wait-list condition dropped out, between T1 and T2 testing. In the aerobic exercise group, an average of 33.5 (SD=17.4) of the 48 training sessions (70%) were completed, and among T2 completers, 45 participants attended a mean of 39.8 (SD=13.6) of the scheduled 48 training sessions (83%). Among the 40 who completed training and all 3 testing sessions, the attendance rate was 90%. There were no protocol-related adverse events.

In the aerobic exercise group for the ITT sample (n=60), the median number of days between the last completed exercise training session and posttraining (T2) blood draw was 3 days (interquartile range [IQR], 2–8 days). The median number of days between the last completed exercise training session and posttraining (T2) CPET was 4 days (IQR, 2–6 days).

A total of 90 subjects (aerobic exercise group, n=31; wait-list group, n=59) were included in the per-protocol sample. In this sample, participants in the aerobic exercise group completed an average of 44.2 exercise training sessions (SD=10.2). The median number of days for the time between the last completed exercise training session and posttraining (T2) blood draw as well as posttraining (T2) CPET was 3 days (IQR, 2–5 days).

Demographics

Time 1 demographic characteristics as well as measures of aerobic fitness and inflammation are presented for each group in Table 1. There were no significant differences between the aerobic training and wait-list groups in any of the T1 measures. The average age of participants in both groups was 31 (SD=6) and the mean body mass index was 25 kg/m^2 (SD=4). Participants were 33.6% white, 27.7% Asian, 17.6% black or African American, 0.8% Native Hawaiian or Pacific Islander, and 20.2% other races. Overall, study entry circulating inflammatory markers were low (median [IQR] of CRP, 0.68 mg/L [0.31–2.32]; IL-6, 1.33 pg/mL [0.98–2.62]; TNF-α, 8.45 pg/mL [6.88–10.00]).

Aerobic Capacity

In the unadjusted model on the ITT sample, the aerobic training group achieved a significantly greater improvement in aerobic capacity on average by 5.00 mL/kg per minute ($P<0.001$) compared with the wait-list group at T2. Within the aerobic training group, VO_2max significantly increased by 4.13 mL/kg per minute at T2, relative to T1 ($P<0.001$), but did not change within the wait-list group ($P=0.08$). The 2-way interaction between treatment group and time was not

Table 1. Demographic and T1 Characteristics of the Participants Randomized to Exercise and Wait-List Group (N=119)

	Wait List (n=59)		Aerobic Training (n=60)	
	N	Mean (SD) or n (%)	N	Mean (SD) or n (%)
Age, y	59	31.4 (6.2)	60	31.2 (5.7)
Sex	59		60	
Male		28 (47.5%)		28 (46.7%)
Female		31 (52.5%)		32 (53.3%)
BMI, kg/m^2	59	24.9 (3.8)	59	24.9 (3.8)
Body fat %	56	25.0 (9.5)	58	25.9 (9.7)
AMED index	57	4.4 (1.7)	60	4.3 (1.8)
Race	59		60	
Asian		18 (30.5%)		15 (25.0%)
Native Hawaiian or Pacific Islander		0 (0.0%)		1 (1.7%)
Black or African American		11 (18.6%)		10 (16.7%)
White		20 (33.9%)		20 (33.3%)
Other		10 (16.9%)		14 (23.3%)
Ethnicity	59		60	
Hispanic		12 (20.3%)		19 (31.7%)
Not Hispanic		47 (79.7%)		41 (68.3%)

AMED indicates alternative Mediterranean diet; BMI, body mass index.

significant, indicating that treatment differences at T2 and T3 are not significantly different (P=0.19). Similar results were found when using the per-protocol sample.

Observed descriptive summaries and adjusted model results using the ITT sample are presented in Table 2. After controlling for additional covariates of age, sex, T1 body fat percentage, and T1 alternative Mediterranean diet index, adjusted model ITT results were similar to the unadjusted model ITT results except for a reduction in VO_2max by 1.00 mL/kg per minute from T1 to T2 in the wait-list group that reached statistical significance (P=0.045; see Figure 3). However, in the adjusted model on the per-protocol sample, the reduction in VO_2max from T1 to T2 (by 0.77 mL/kg per minute) in the wait-list group was no longer statistically significant (P=0.11).

Physical Activity Independent of the Training Sessions

During the 12-week training program, the mean (SD) number of steps/day for the exercise and wait-list groups were 6842 (2294) and 6017 (1983), respectively (P=0.051). During the 4-week deconditioning period, the mean (SD) number of steps/day was also not significantly different between the exercise (6051 [2207]) and wait-list (5566 [2265]) groups (P=0.34). Pearson correlations showed no significant correlations between the average number of steps/day and unstimulated or stimulated levels of IL-6 or TNF-α at any point in the study.

Fat-Free Mass

In the unadjusted analysis on the ITT sample, the average amount of fat-free mass in both the aerobic training and wait-list groups was not significantly different at T2 (P=0.37). Fat-free mass did not change for those within the aerobic training group from T1 to T2 (P=0.37), nor did it change for those in the wait-list group (P=0.72). The 2-way interaction between treatment group and time was not significant, indicating that no significant treatment difference at T2 compared to T3 were found (P=0.53). The adjusted analyses on the ITT sample (see Table 2, Figure 4) showed similar results. Results for the per-protocol sample were similar except that within the aerobic training group, fat-free mass significantly increased from T1 to T2 by 3.06 pounds (P=0.011) in the unadjusted analyses and by 2.98 pounds (P=0.009) in the adjusted analyses.

Inducible TNF-α Release in Whole Blood

In unadjusted analyses using the ITT sample, at T2, there was no significant effect of the aerobic training intervention on inducible TNF-α release at T2 (lipopolysaccharide 0.0 ng/mL, P=0.62; lipopolysaccharide 0.1 ng/mL, P=0.12; lipopolysaccharide 1.0 ng/mL: P=0.19). Similarly, there were no significant changes from T1 to T2 within treatment groups. The 2-way interaction between treatment group and time was not significant, indicating no significant treatment differences between T2 and T3 (lipopolysaccharide 0.0 ng/mL, P=0.32; lipopolysaccharide 0.1 ng/mL, P=0.26; lipopolysaccharide 1.0 ng/mL: P=0.10). The unadjusted and adjusted analyses using the ITT sample and per-protocol sample were similar. Observed descriptive summaries and adjusted model results using the ITT sample are presented in Table 2 and Figure 5.

Inducible IL-6 Release in Whole Blood and TLR4

In the unadjusted models using the ITT sample, there was no significant effect of the aerobic training intervention on inducible IL-6 release (lipopolysaccharide 0.0 ng/mL: P=0.69; lipopolysaccharide 0.1 ng/mL: P=0.31; lipopolysaccharide 1.0 ng/mL: P=0.29) or on TLR4 (P=0.49) at T2. There were also no significant changes from T1 to T2 for each treatment group on IL-6 or TLR4. The nonsignificant 2-way interaction between treatment group and time for inducible IL-6 release

Table 2. Summary of Observed Descriptive Summaries of ITT Sample and Adjusted Model Results of Fitness Measures and Inflammation Markers (N=119)

Measure	Wait-List Group Mean (SD) or Median (IQR)			Aerobic Training Group Mean (SD) or Median (IQR)		
	T1	T2 (12 Weeks)	T3 (16 Weeks)	T1	T2 (12 Weeks)	T3 (16 Weeks)
VO_2max	31.00 (6.4)	30.11 (6.2)*[†]	29.38 (6.5)*	30.39 (6.9)	34.74 (8.4)*[‡]	33.28 (8.3)*
Fat-free mass	118.81 (25.3)	115.60 (23.5)	117.16 (24.1)	113.48 (22.1)	117.08 (23.3)[§]	117.84 (24.0)
Inducible TNF-α[‖]						
Lipopolysaccharide 0.0 ng/mL	11.61 (5.4–25.0)	10.68 (5.0–22.5)	11.99 (6.4–19.1)	11.77 (7.7–19.3)	11.20 (6.87–23.1)	14.83 (7.0–33.4)
Lipopolysaccharide 0.1 ng/mL	142.55 (36.7–364.2)	101.79 (33.8–350.0)	85.70 (22.6–323.1)	109.25 (25.7–354.1)	131.86 (33.7–506.3)	113.69 (18.0–289.6)
Lipopolysaccharide 1.0 ng/mL	434.02 (142.8–1105.5)	501.91 (117.6–872.9)	449.9 (80.6–1076.2)	607.13 (84.8–1453.2)	681.54 (247.6–1604.6)	432.09 (141.7–1056.3)
Inducible interleukin-6[‖]						
Lipopolysaccharide 0.0 ng/mL	4.53 (2.6–8.3)	3.96 (2.5–14.0)	4.16 (2.7–6.9)	5.73 (3.5–10.0)	5.49 (4.3–9.5)	6.11 (3.7–20.3)
Lipopolysaccharide 0.1 ng/mL	248.93 (44.7–484.7)	211.90 (63.7–445.2)	219.63 (22.6–605.5)	236.66 (21.6–548.7)	245.21 (101.1–649.9)	151.99 (10.1–408.5)
Lipopolysaccharide 1.0 ng/mL	586.87 (122.8–1456.0)	600.97 (132.6–1485.0)	572.27 (151.1–1351.3)	799.28 (226.1–1498.8)	866.58 (358.5–1466.3)	414.59 (138.5–1281.7)
TLR4[‖]	0.50 (0.3–0.9)	0.46 (0.2–0.7)	0.43 (0.2–0.8)	0.41 (0.2–0.9)	0.38 (0.2–0.6)	0.37 (0.2–0.7)

IQR indicates interquartile range; ITT, intent-to-treat; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-α; VO_2max, maximal oxygen consumption.
*Values significantly different between groups at the equivalent time point in the adjusted model ($P<0.001$).
†Values significantly different between T1 and particular time in the same group in the adjusted model ($P<0.05$). In per-protocol analyses, this is no longer significant for the wait-list group ($P>0.05$).
‡Values significantly different between T1 and particular time in the same group in the adjusted model ($P<0.001$).
§Values significantly different between T1 and particular time in the same group in the per-protocol adjusted and unadjusted models ($P<0.05$).
‖Median and IQR are presented for outcomes that had skewed distribution and were log-transformed in the analyses.
¶Values significantly different between groups at the equivalent time point in the adjusted model ($P<0.05$).

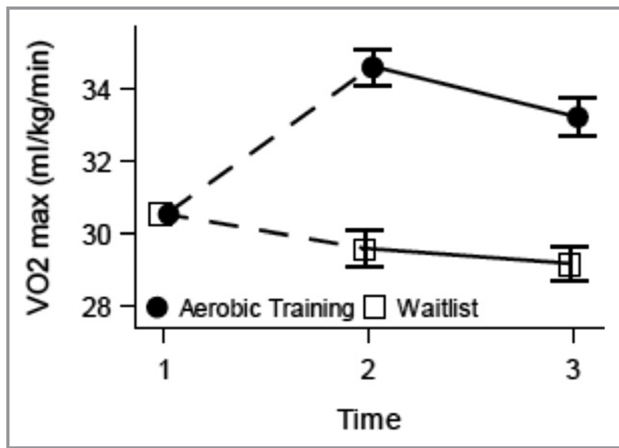

Figure 3. Observed T1 mean plotted with adjusted model estimated T2 and T3 means and standard errors of VO_2max on the intent-to-treat (ITT) sample.

(lipopolysaccharide 0.0 ng/mL, P=0.08; lipopolysaccharide 0.1 ng/mL, P=0.22) and TLR4 (P=0.81) indicates no significant treatment difference between T2 and T3. However, there was a significant 2-way interaction for IL-6 lipopolysaccharide 1.0 ng/mL, suggesting a differential treatment effect at T2 compared with T3 (P=0.019). Adjusted model results for IL-6 and TLR4 using the ITT sample are presented in Table 2. Unadjusted and adjusted model results were similar for the ITT and per-protocol samples.

Moderation of the Treatment Effect by Baseline Level of Inflammation Markers

The medians and IQR for the low TNF-α groups with lipopolysaccharide 0.0, 0.1, and 1.0 ng/mL stimulation were 7.7 (4.6–9.7), 25.1 (13.8–49.9), and 66.1 (18.9–291.8), respectively, and for the high group were 23.4 (17.3–66.5), 321.6 (179.1–494.6), and 1188.3 (526.7–1790.8), respectively. The medians and IQR for the low IL-6 groups with lipopolysaccharide 0.0, 0.1, and 1.0 ng/mL stimulation were 3.9 (2.6–5.2), 13.0 (5.0–77.0), and 69.5 (20.1–312.8), respectively, and for the high groups were 11.6 (6.7–99.2), 388.3 (271.5–830.4), and 1406.0 (828.9–1568.9), respectively. The median and IQR for the low TLR4 group was 0.19 (0.1–0.3) and for the high group was 0.7 (0.5–1.1).

The 3-way interaction between dichotomized (high and low) T1 inflammatory marker levels (TNF-α, IL-6, and TLR4), treatment, and time was not significant for any inflammatory outcomes defined above in either the unadjusted or adjusted models. That is, there was no significant difference in treatment effect over time between participants with high versus low levels of the T1 inflammatory markers.

Figure 4. Observed T1 mean plotted with adjusted model estimated T2 and T3 means and standard errors of fat-free mass (lbs) on ITT sample.

Effect of Inducible Lipopolysaccharide Levels on TNFα and IL-6

Examination of Table 2 and Figure 5 indicates that in the training group but not in the wait-list condition, exposure to 0.1 and 1.0 ng/mL lipopolysaccharide increased levels of TNF-α and IL-6 at T2, but not for the 0.0 ng/mL lipopolysaccharide condition. The adjusted analyses using the ITT sample revealed a differential longitudinal treatment effect by lipopolysaccharide condition in inducible TNF-α (P=0.08) and IL-6 (P=0.011). Post hoc comparisons showed increases from T1 to T2 in inducible (lipopolysaccharide 0.1 ng/mL and lipopolysaccharide 1.0 ng/mL) TNF-α (estimated percent change 51%; P=0.041) and IL-6 (estimated percent change 42%; P=0.11), as well as significant decreases from T2 to T3 in inducible (lipopolysaccharide 0.1 ng/mL and lipopolysaccharide1.0 ng/mL) TNF-α (estimated percent change −54%; P=0.007) and IL-6 (estimated percent change −55%; P<0.001). No significant changes were seen in either the training or wait-list groups at the 0.0 ng/mL lipopolysaccharide condition for either TNF-α and IL-6.

Discussion

We conducted an adequately powered, randomized controlled trial in a cohort of young, healthy, sedentary adults, contrasting the effects of a 12-week aerobic exercise training program and a wait-list control condition on lipopolysaccharide-inducible TNF-α, IL-6, and TLR4, following ITT principles of analysis. Although the training program yielded a 15% increase in aerobic capacity, planned ITT analysis showed that there were no effects on any of these inflammatory markers. Results of the per-protocol analysis were similar. In order to rule out a "floor effect," that is, the possibility that the negative finding was due to our subjects having entered the study with already relatively low levels of inflammatory markers, we conducted a secondary analysis, which

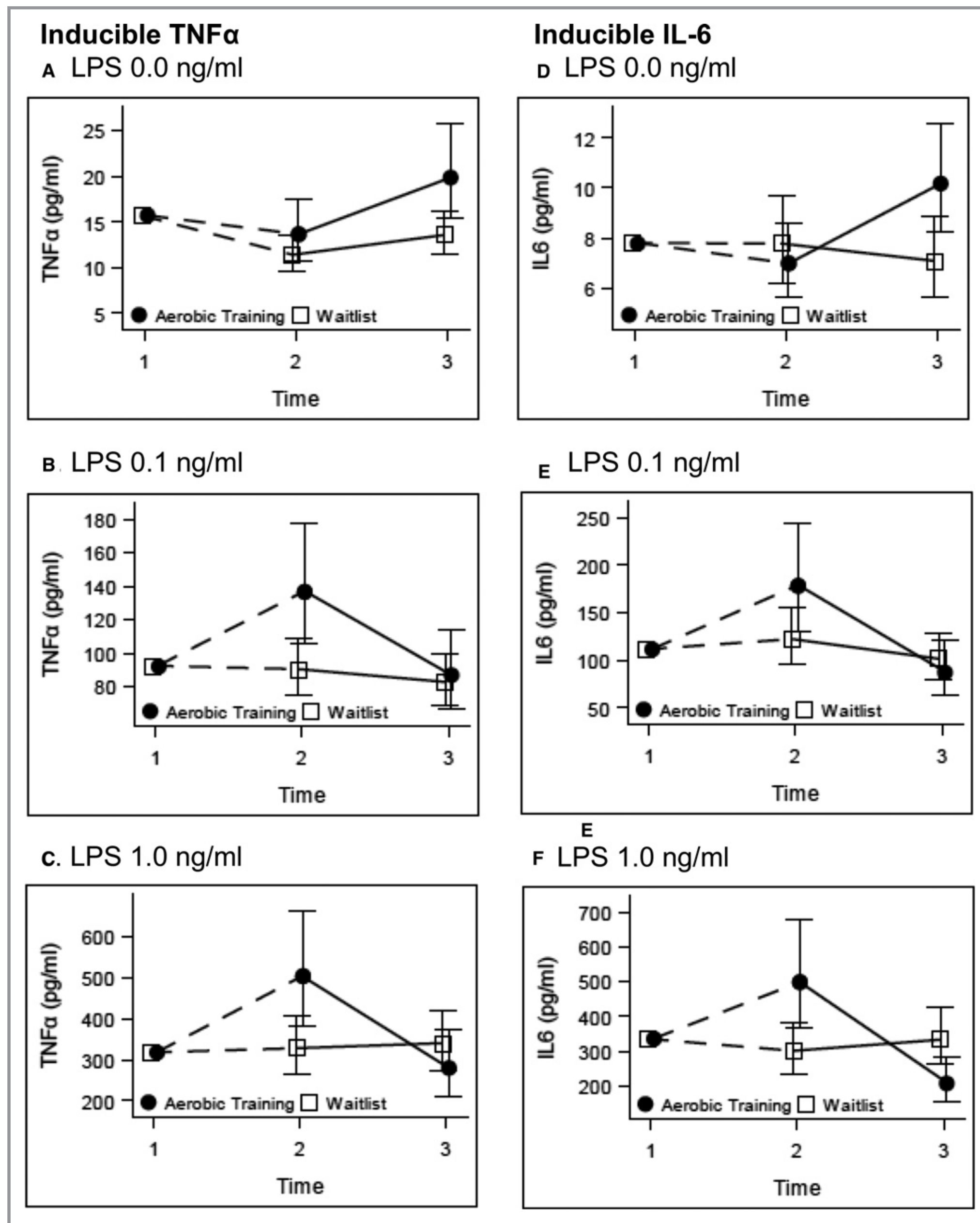

Figure 5. Observed T1 geometric mean plotted with adjusted model estimated T2 and T3 geometric means and standard errors of inducible TNF-α at lipopolysaccharide **(A)** 0.0 ng/mL, **(B)** 0.1 ng/mL, and **(C)** 1.0 ng/mL; and inducible interleukin-6 at lipopolysaccharide **(D)** 0.0 ng/mL, **(E)** 0.1 ng/mL, and **(F)** 1.0 ng/mL. All figures were based on the ITT sample.

confirmed that there were no differences in effects of treatment in participants with higher versus lower levels of inflammatory markers at study entry. The failure of the primary hypothesis, that training would lead to a reduced TNF-α response to lipopolysaccharide challenge, cannot be explained by the lack of a training effect because aerobic capacity increased significantly in the exercise group but did not change in the wait-list condition. Aerobic group participants were adherent to the training protocol, as indicated by exercise logs, gym attendance records, and, most importantly, training HR levels as measured by HR recordings obtained during exercise. These data are consistent with the improvement in aerobic capacity in the training but not the wait-list group.

Intriguingly, post hoc analyses revealed that exercise training *enhanced* rather than attenuated the IL-6 and TNF-α response to the 0.1 and 1.0 ng/mL lipopolysaccharide conditions and that this effect was reversed by sedentary

deconditioning. This finding, although preliminary, is inconsistent with the prevailing view that the health promotional benefits of exercise training derive in part through its anti-inflammatory effects. Nonetheless, some studies have reported similar findings. A small study contrasting endurance-trained and sedentary men found that lipopolysaccharide-induced monocyte production of IL-6 was greater in the trained group.[35] In 79 middle-aged women in the SWAN (Study of Women's Health Across the Nation) study, self-reported levels of physical activity were associated with greater lipopolysaccharide-induced production of IL-6, interleukin-1ß, and TNF-α.[36] In vivo administration of lipopolysaccharide to healthy, young, trained or untrained men revealed tissue-specific induced inflammatory responses: Compared with their untrained counterparts, trained subjects had an enhanced lipopolysaccharide-induced TNF-α and IL-6 mRNA expression in skeletal muscle and slightly reduced responses systemically and in adipose tissue.[37] Some animal studies also report increases in ex vivo lipopolysaccharide-stimulation models, with exercise training leading to increases in TNF-α but not IL-6 in rats[38,39]; in interleukin-1ß, interleukin-12, TNF-α, and interferon-γ in mice,[40–43] and in TNF-α and interferon-ß in horses.[44]

Under ordinary circumstances, inflammatory responses to challenge are under tight feedback control by activation of the hypothalamic-pituitary-adrenal axis, leading to increased production of glucocorticoids, which can inhibit inflammation. Reduced glucocorticoid production or sensitivity disinhibits this suppressive process, resulting in greater cytokine production. It is possible that exercise training functions in this way, eliciting increases in lipopolysaccharide-inducible cytokines by attenuating glucocorticoid sensitivity. Some studies report findings consistent with this hypothesis. For example, dexamethasone inhibition of lipopolysaccharide-induced IL-6 was lower in trained compared with untrained men.[35] In 18 male cadets in the Brazilian Air Force Academy, 6 weeks of intensive exercise training led to a reduction in basal cortisol levels, and in glucocorticoid receptor (GR) mRNA levels and decreased glucocorticoid sensitivity, measured as the response to intravenous low-dose dexamethasone.[45,46] Binding capacity of GRs in peripheral blood monocytes was lower in semiprofessional soccer players than in young and older comparison subjects.[47] Highly trained swimmers had substantially lower GR-α mRNA expression than untrained control subjects.[48] The increased basal cortisol levels[49–51] seen in endurance athletes may function to downregulate GR activity.[52] GR-binding capacity in peripheral leukocytes was lower in trained athletes compared with untrained controls.[53] These studies all are consistent with the hypothesis that aerobic exercise training leads to reduced feedback control of inflammation by diminished GR activity, resulting in an enhanced inflammatory response to challenge.

Recent theoretical and empirical work is consistent with this conclusion, asking in effect why evolution would confer a survival advantage to diminished immunocompetence when the immune system's primary function is to protect the organism from threat, for example, infection or sterile injury,[54,55] by eliminating the cause of cell injury, removing necrotic cells, and initiating tissue repair. Thus, there is a clear survival advantage of having an immune system capable of a robust response to threat so long as the response does not persist beyond resolution of that threat. Viewed in this way, exercise training still is a health-promoting behavior, increasing the capacity to mount an enhanced, rather than diminished, inflammatory response to challenge. This position is consistent with the work of Miller and colleagues[56] and, more generally, of the views of Dhabhar and colleagues[54,57,58] and Dienstbier,[59] who argued for the health benefits of significant responsiveness to challenge.

Curiously, precisely this same finding—increased lipopolysaccharide-induced cytokine production—is reported in studies investigating the effects of risk-enhancing psychosocial characteristics, for example, caregiving for a sick relative or early life adversity or traits like hostility and depression. For example, Miller and Chen showed that young women raised in harsh family settings had elevated IL-6 responses to lipopolysaccharide challenge associated with GR desensitization.[60] Suarez et al found that greater levels of hostility and depressive symptomatology were associated with greater lipopolysaccharide-induced cytokine production in healthy women[61] and men.[62] Parents of children with cancer had diminished dexamethasone-induced suppression of lipopolysaccharide-induced IL-6 but not TNF-α or interleukin-1ß production compared with a control group.[63]

Thus, both aerobic exercise training, a health promoting activity, and psychosocial disadvantage, associated with elevated risk, show the same phenotypic inflammatory responses to infectious challenge. Why exercise training and caregiving stress should share a common mechanism is unclear. However, an enhanced inflammatory response to challenge has survival value only to the extent that it abates after successful resolution of the threat.[54,57,58] Resolution of inflammation, once considered merely the passive dilution of granulocytes and the return to preinflammation levels of mononuclear cells, now is seen as an active process characterized by programmed apoptosis of leukocytes and phagocytic clearance cellular debris[64,65] that may operate independently of proinflammatory processes. It is possible that while exercise training and psychosocial disadvantage promote an enhanced response to ex vivo infectious challenge, they differ in the resolution of challenge. Testing this would require measurement of anti-inflammatory cytokines, for example, interleukin-10 and other proresolution mediators, and measurement of the proinflammatory cytokine

response induced by lipopolysaccharide challenge over time to determine whether it persists or resolves. Neither our study nor those examining psychosocial risk have collected the data required to test this hypothesis.

It is possible that because we conducted an ITT analysis, which includes data from all randomized participants irrespective of whether they completed the trial, our sample is significantly different from other studies, most of which analyzed data from only those participants who completed both waves of data collection, as discussed above. By including participants who dropped out after randomization but before T2 testing, we may be biasing our findings against the possibility of a significant effect of training. However, the per-protocol analysis, in which data only from participants who completed the study were analyzed, still found no anti-inflammatory effects of aerobic training.

Additionally, participants in the aerobic exercise arm of the trial were permitted to choose from among various training modalities (eg, cycling, Stairmaster, treadmill) so long as they achieved their target HRs during training, but the cardiopulmonary exercise tests were conducted on cycle ergometers. Multiple studies have shown that when CPET is performed with a modality different than the one used during training (eg, swimmers tested on a cycle ergometer), the transfer of training effects is incomplete.[66,67] Therefore, our postintervention CPET measurement may have underestimated the training-induced increase in aerobic fitness in some of our subjects. However, underestimation would only strengthen the conclusion that aerobic exercise training does not have a significant effect on inflammation as measured in this study.

Finally, our trial was relatively brief in length—only 12 weeks of training. Many studies reporting longer training programs, some as long as many years, show the predicted anti-inflammatory effects of training. However, this explanation, too, is unlikely to account for the failure of the training program to produce an anti-inflammatory effect because many longer studies show no effect[9–12] and many studies of shorter interventions have the predicted effects.[14,16,33]

Strengths and Limitations

Strengths of this study include a large sample size, prospective design, a training plus deconditioning phase, randomization, fidelity to the intervention with good adherence achieved by coaching, and analysis by ITT principles. Additionally, because participants in this study exercised on their own, these findings have greater generalizability compared with studies in which all training sessions were supervised. Limitations include the relative youth and good health of the subjects and variability in the specific exercise equipment chosen by the subject so long as they were able to train at the target HR. We are unable to determine the effect of this variation. Other potential limitations include the proportion of missing data and variation in the interval from last exercise session to the posttraining (T2) blood draw, as well as the lack of power to explore potential longitudinal treatment differences between lipopolysaccharide-inducible levels of TNF-α and IL-6 using the 3-way interaction between lipopolysaccharide-inducible level, treatment, and time and subsequent contrasts performed.

In this trial, 16.8% and 19.3% of the T2 (posttraining) data were missing for our primary outcomes TNF-α and IL-6, respectively, based on the ITT sample of 119 randomized participants. By comparison, for example, of 855 enrolled participants in the HERITAGE study,[8] 652 had complete data; that is, the rate of missing data was 23.7%. Reports of other trials, many of them smaller, also report equivalent or higher rates of missing data[68–70] or fail to report them at all.[18,71–74] These studies suggest that the rate of missing data in our trial was typical for exercise training trials.

As in most clinical trials, exercise related or otherwise, there was variation in the timing of the postintervention data collection. In the aerobic exercise group, the median interval between the last training session and posttraining (T2) blood draw was 3 days IQR, 2–8 days) for the ITT sample and 3 days (IQR, 2–5 days) for the per-protocol sample. It is conceivable that this variation may have influenced the posttraining levels of TNF-α or IL-6, with any training effect diminishing with an increasing interval. The literature provides little guidance regarding the maximum interval between the last exercise session and the blood draw to control for the loss of an effect of the training regimen. Thompson et al showed that for circulating IL-6 but for no other inflammatory marker, an exercise-training–induced reduction was lost after 2 weeks of deconditioning.[18] In another study, 12 weeks of aerobic training led to a reduction in s-intercellular adhesion molecule-1, which returned to baseline after 4 weeks of deconditioning, but there was no similar effect on CRP or TNF-α. IL-6 did not change after training but unexpectedly rose after deconditioning.[75]

Given the limited evidence on this matter, we examined the change from T1 to T2 in TNF-α and IL-6 from the exercise group as a function of the interval between the last training session and blood draw. As Figure S1 indicates, up to an interval of 10 days, there is considerable variability in the magnitude of change in TNF-α. However, starting at about 10 days, the variability drops substantially, showing that after this time point, T1 and T2 values were similar, consistent with the view that an effect of training persists for about 10 days.

More broadly, however, variations in timing of data collection in human clinical trials are inevitable. Even in the

best-designed protocols with the most motivated participants, the real-life considerations of human subjects with busy and complicated lives may interfere with perfect compliance with all aspects of the trial. However, with sufficiently large samples and random assignment, we can minimize their impact.

Importantly, we elected to focus on inducible cytokine responses to exercise training and not circulating levels because the latter reflect the contribution of adipocytes, endothelial cells, and muscle, in addition to monocytes/macrophages. However, it is clear that these approaches measure different facets of the inflammatory process: Circulating measures examine levels of systemic inflammation, whereas inducible measures reflect the ability of cells to produce cytokines in response to challenge. Many, but as indicated above, by no means all of the studies demonstrating anti-inflammatory effects of exercise training measured circulating levels of inflammation.

Finally, it is difficult to conclusively infer changes in chronic inflammatory status using a single snapshot sample, given most markers can vary considerably depending on a variety of factors including sleep status, diet, time of last exercise bout, and so on.

Conclusions

This 12-week randomized controlled trial of aerobic exercise training in healthy, sedentary, young adults produced a 15% increase in VO_2max and a significant increase for fat-free mass in the per-protocol treatment group and not in the control group but failed to support the hypothesis that training would reduce inducible TNF-α, IL-6, and TLR4. However, post hoc analysis revealed a training-induced enhanced IL-6 and TNF-α response to lipopolysaccharide stimulation, a finding consistent with the possibility that health benefits of aerobic exercise training include a robust but time-limited proinflammatory response to challenge. The failure to support the primary hypotheses and the unexpected post hoc findings raise questions about whether and under what conditions exercise training has anti-inflammatory effects.

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Disclosures

None.

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Supplemental Material

Data S1.

Supplemental Statistical Methods

Analysis of covariance (ANCOVA) with grand mean centering was used to assess treatment effects and within-group changes of aerobic capacity, fat free mass, and inflammation. The model used for estimating these effects was as follows:

$$Y_{ij} - \overline{Y}_0 = \beta_0 + b_{oi} + \beta_1{*}(Y_{0i} - \overline{Y}_0) + \beta_2{*}trt_i + \beta_3{*}time_j + \beta_4{*}trt_i{*}time_j \quad (1)$$

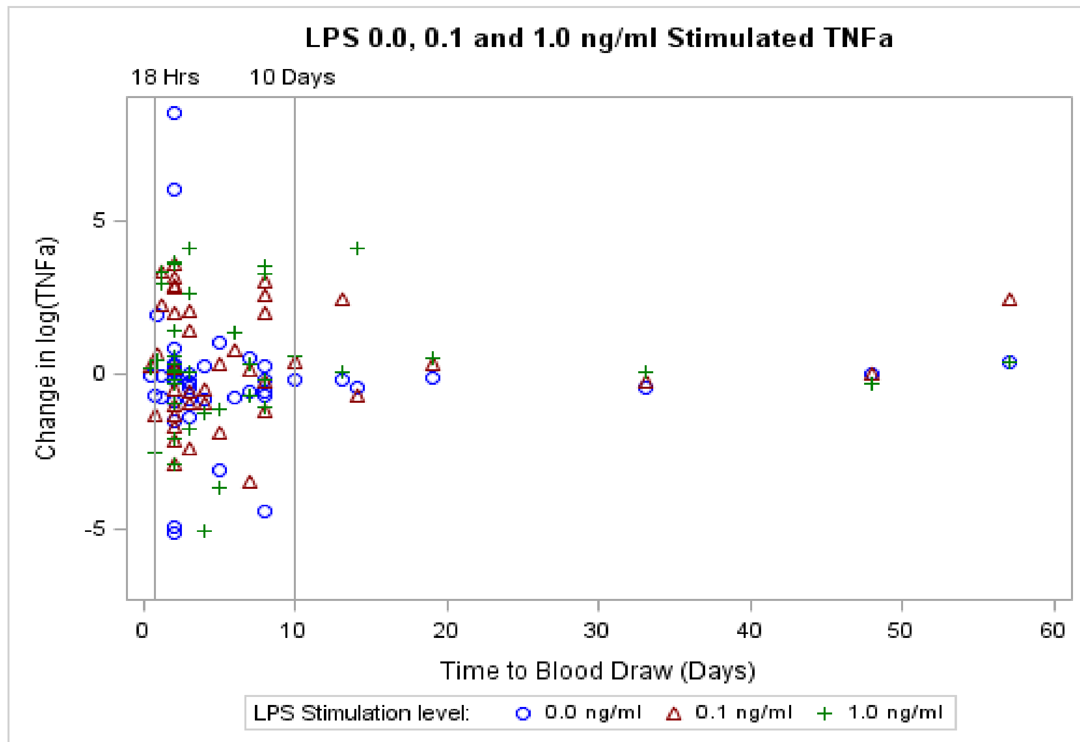
Where i is the index for subject, j is the index for time (Session 2, Session 3), Y is the outcome value, $\overline{Y}_0$ is the grand (across all subjects and both groups) mean of the outcome at baseline, β_0 is the overall model intercept, b_{oi} is the random intercept for subject i, β_1 is slope of the association between the outcome at baseline and follow-up, β_2 is the treatment effect at session 2, β_3 is the change in outcome from Session 2 to Session 3 for the waitlist group, and β_4 is the treatment difference in change from Session 2 to Session 3. "Waitlist" was the reference value for trt. "Session 2" was the reference value for time.

This model is equivalent to modeling the Session 2 and Session 3 outcome values, while adjusting for baseline, as:

$$Y_{ij} = \beta_0 + b_{oi} + \beta_1{*}Y_{0i} + \beta_2{*}trt_i + \beta_3{*}time_j + \beta_4{*}trt_i{*}time_j \quad (2)$$

in that the error variances for the 2 models are identical (Mulligan and Wiesen, 2003). Moreover, the treatment effect at Session 2, β_2 (which is the primary parameter of interest for this study), has the same interpretation in both models, Similar for β_3 and β_4. However, model (1) is preferable, as it is more straightforward to estimate within-group change from baseline in the outcome: Using model (1), the mean change from baseline to Session 2 for the waitlist group is simply equal to β_0; the mean change from baseline to Session 2 for the exercise group is equal to $\beta_0 + \beta_2$; the mean change from baseline to Session 3 for the waitlist group is equal to $\beta_0 + \beta_3$; and, finally, the mean change from baseline to Session 3 for the exercise group is equal to $\beta_0 + \beta_2 + \beta_3 + \beta_4$.

Figure S1. Change in TNFα, stimulated by 0.0, 0.1, and 1.0 ng/mL LPS, from study entry to post-conditioning as a function of the interval between the final exercise training session and the T2 blood draw.



Up to about 10 days, there is substantial variability in the change in TNFα, after which little change is seen.

Supplemental Reference:

1. Mulligan NW, Wiesen C. Using the analysis of covariance to increase the power of priming experiments. *Canadian Journal of Experimental Psychology.* 2003; 57: 152−166.