Effect of exercise on in vitro immune function: a 12-month randomized, controlled trial among postmenopausal women

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Campbell PT, Wener MH, Sorensen B, Wood B, Chen-Levy Z, Potter JD, McTiernan A, Ulrich CM. Effect of exercise on in vitro immune function: a 12-month randomized, controlled trial among postmenopausal women. J Appl Physiol 104: 1648–1655, 2008. First published April 10, 2008; doi:10.1152/japplphysiol.01349.2007.—Cross-sectional studies suggest that moderate physical activity is associated with enhanced resting immune function; however, few randomized controlled trials have investigated this link. We investigated the effect of 12-mo aerobic exercise, relative to stretching control, on in vitro immune function in a randomized, controlled trial of 115 postmenopausal, overweight, or obese sedentary women, aged 50–75 yr. The exercise goal was ≥45 min/day, 5 days/wk. Control women participated in 1 day/wk stretching classes. Immune markers (natural killer cell cytotoxicity, T-lymphocyte proliferation, immune cell counts and phenotypes, and serum immunoglobulins) were assessed at baseline, 3 mo, and 12 mo under strict blood-draw criteria. General estimation equations evaluated intervention effects at 3 and 12 mo, controlling for baseline. Of the 115 women who began the trial, blood samples were available from 109 at 3 mo (95%) and 108 at 12 mo (94%). From baseline to 12 mo, the exercise group participated in 87% of the prescribed physical activity minutes per week and increased maximal \( \Delta \)O\(_2\) uptake by 13.8%; controls experienced no change in fitness. The main outcomes, natural killer cell cytotoxicity and T-lymphocyte proliferation, did not differ between groups at 3 and 12 mo. Secondary outcome and subgroup (e.g., stratification by baseline categories of body mass index, immune status, C-reactive protein, and age) analyses did not show any clear patterns of association. This 12-mo randomized, controlled trial showed no effect of aerobic exercise on in vitro immune function, despite excellent retention, high adherence, and demonstrable efficacy of the exercise intervention.

Observational studies indicate that physical activity is associated with reduced risk of mortality (24) and a broad spectrum of chronic diseases and conditions. Physical activity is also inversely associated with conditions linked to immunity, including upper respiratory tract infection (URTl) (4), which suggests that improved immune surveillance may be one mechanism that connects exercise to reduced disease risk. Because many immune responses decline with age (3, 17, 28, 45), and older persons are especially prone to malignancy and infection, the impact of exercise on immune competence among older persons is of particular interest. Although the effect of acute exercise on immune function has been examined extensively (14, 38, 39), studies on long-term exercise are especially rare.

Cross-sectional studies of in vitro immune function quite consistently suggest enhanced natural killer (NK) cell cytotoxicity (20, 34) and T-lymphocyte proliferation (6, 16, 34, 44) among highly trained athletes vs. untrained subjects. Cross-sectional studies of immune cell counts and phenotypes have been less consistent (14, 38, 39). Some exercise training studies with rodents suggest improvements in natural cytotoxicity and T-lymphocyte proliferation, as well as enhanced counts of T cells, B cells, and immunoglobulins (8, 9, 12, 22, 23, 37), but these results are not universal (13, 21). Exercise training has been also shown to augment recovery of lymphocytes after energy restriction among rodents in vivo (7) and to reduce stress-induced antibody formation (30). Although cross-sectional and rodent studies provide some insight on links between exercise and immune function potentially relevant to human health, causality is best established in randomized, controlled trials of exercise intervention.

Accordingly, several randomized, controlled trials have investigated the effect of moderate-intensity aerobic exercise on NK cell cytotoxicity and T-lymphocyte proliferation. Perhaps the most convincing data come from trials with at least 10 individuals per intervention and control group and ≤15% drop-out. Studies that meet these criteria (10, 29, 34, 35, 41, 47) indicate essentially no effect of exercise intervention on immune function. Similarly, exercise intervention studies on counts and percentages of NK cells, leukocytes, and lymphocytes have largely suggested no effect of exercise (2, 5, 31, 32, 34, 36, 40–42, 47). Although the majority of these studies suggest little to no effect of exercise on immune function, caveats about previous work include limited statistical power because of small sample sizes (generally, between 10 and 25 participants per arm) and relatively short intervention periods (typically, studies were <12 wk in duration).

In a randomized, controlled trial of 115 previously sedentary, postmenopausal, overweight, or obese women, we examined the effect of 12-mo aerobic exercise intervention, compared with stretching control, on several markers of immune status (NK cytotoxicity; T-lymphocyte proliferation; cell counts and phenotypes of T cells, B cells, and NK cells, measured by flow cytometry; and serum concentrations of immunoglobulins). We hypothesized that the exercise intervention would improve in vitro immune function. Previous work from this study reported excellent retention, high adherence, strong efficacy of the exercise intervention, and no measurable contamination from changes in diet (18, 25–27).
METHODS

Design Overview

This study was a 12-mo randomized, controlled trial that compared the effect of a moderate-intensity aerobic exercise intervention to a stretching control program on markers of immune function and phenotype, 3- and 12-mo after randomization. The study was conducted during 1997–2001 at the Fred Hutchinson Cancer Research Center (FHCRC) and at the University of Washington (UW). All study procedures, including written and informed consent, were reviewed and approved by the FHCRC Institutional Review Board.

Setting and Participants

Participants (n = 115) were a subset of women (n = 173) recruited for an exercise intervention trial who met additional criteria for this separately funded study of measurement of immunological outcomes. Eligibility criteria were as follows: postmenopausal; aged 50–75 yr; in good health; nonsmoker; sedentary [<60 min/wk of moderate- and vigorous-intensity recreational activity and maximal O2 uptake (V˙O2 max) < 25.0 ml·kg\(^{-1}\)·min\(^{-1}\)]; not taking postmenopausal hormones in the past 6 mo; alcohol consumption of fewer than two drinks per day; body mass index (BMI) between 25 and 40 kg/m\(^2\) (or BMI 24.0–24.9 if body fat > 33%); no personal history of invasive cancer, diabetes, cardiovascular disease, or asthma; no current serious allergies; no regular (twice per week or more) use of aspirin or other nonsteroidal anti-inflammatory medications; and no use of corticosteroids or other medications known to affect immune function. Women were further excluded if they volunteered to lose weight, had a previous surgery for weight loss, or were currently attempting, or planning to attempt, weight loss by taking diet pills or entering a structured weight-loss program.

Women were recruited through a combination of mass mailings and media placements. Details on recruitment have been published previously (46). In the parent trial, 173 women were randomly assigned to the exercise intervention (n = 87) or control (n = 86) groups. For this study of immune function, a subset of 53 exercisers and 62 controls were eligible (Fig. 1).

Fig. 1. Flow of participants through the study. N, no. of subjects.

Randomization, Exercise Intervention, and Exercise Adherence

Randomization was stratified by BMI (<27.5 or ≥27.5 kg/m\(^2\)) to ensure equal numbers of heavier and lighter women in each study group. Approximately equal numbers of exercisers and controls were enrolled during each month of study recruitment.

The exercise intervention progressed to at least 45 min of moderate-intensity exercise 5 days/wk. During months 1–3, participants were required to attend three supervised exercise sessions per week at a study facility (University of Washington or a commercial gym) and to exercise 2 days/wk at home. For months 4–12, participants were required to attend at least one session per week at a study facility and to perform the remaining exercise sessions either at home or at the facility. Because women recruited into this study were physically inactive and had low aerobic fitness at baseline, the training program started at 40% of observed maximal heart rate (HR) for 16 min per session and increased to 60–75% of maximal HR for 45 min per session by week 8, where it was maintained for the remainder of the study. Participants wore Polar HR monitors during exercise sessions. Facility sessions consisted of treadmill walking and stationary bicycling. Strength training to fatigue was recommended, but not required, to decrease risk of injury and maintain joint stability. Resistance training consisted of two sets of 10 repetitions of leg extension, leg curls, leg press, chest press, and seated dumbbell row. A variety of home exercises were suggested and encouraged, including walking, aerobics, and bicycling. Participants were encouraged to wear their HR monitors when exercising at home. Participant adherence was assessed via daily activity logs, on which exercise intervention participants reported the type and duration of exercise they performed, and the maximal HR and rating of perceived exertion achieved during the session. Activity logs were reviewed weekly by study staff to monitor compliance and intervene when needed.

Control participants attended 60-min stretching and relaxation sessions 1 day/wk for 12 mo and were asked not to change exercise habits. Both exercisers and stretchers were asked not to modify their dietary intake for the duration of the study. After their assessments at the conclusion of the study, control women were offered three training sessions with the exercise physiologist.
Main Outcomes

All women came to the University of Washington Medical Center for blood draws between 7:30 and 8:30 AM after a 12-h fast, with the following stringent blood draw criteria: no infection or symptoms of any infection for ≥7 days; adequate sleep (6–9 h); no exercise or alcohol for 24 h; no topical corticosteroids or aspirin for 48 h; no systemic antihistamines or corticosteroids for 1 wk; and no immunizations during the previous 3 wk. All participants were contacted 1 wk after blood draw to track recent illness; several reported being ill shortly after blood draw and returned for a second blood draw when symptom free. Blood was collected into one 3-ml EDTA tube for enumeration and phenotyping of immune cells and two 7-ml heparinized tubes for the NK cell cytotoxicity assay. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by Ficoll-Hypaque separation and cryopreserved in liquid nitrogen. All assays were conducted at the UW Clinical Immunology Laboratory (M. H. Wener).

Lymphocyte enumeration. Lymphocytes were isolated from whole blood with the use of a whole blood lysis technique, between December 2002 and June 2003. Four-color flow cytometry (XL-MCL, Beckman Coulter, Miami, FL), with antibodies (Beckman Coulter: CD3, 4, 8, 16, 45; Beckton Dickinson, San Jose, CA: CD19, 56) to appropriate clusters of differentiation, was used to identify total numbers of T cells (CD3+, 45+), CD4+ and CD8+ T-cell subsets, B cells (CD19+, 45+), and NK cells (CD3−, 16/56+, 45−).

NK cell cytotoxicity. The flow-cytometry assay for measuring NK cytotoxicity was conducted on fresh tissue immediately after blood draw, as described previously (43). To prepare NK cytotoxicity assay effector cells, peripheral blood lymphocytes were obtained from fresh 14-ml heparin-anticoagulated blood by Ficoll-Hypaque separation followed by two washes with RPMI cell culture medium (without phenol red). To prepare the target cells, K562 cells (in the log phase of growth) were washed twice with phosphate-buffered saline/bovine serum albumin and incubated with 3′,3′-diododecylxocarbocyanine perchlorate (DiO; Live/Dead cytotoxicity kit no. L7010, Molecular Probes, Eugene, OR) at a concentration of 2 × 106 cells/ml for 20 min at 37°C with 5% CO2. The cells were then washed twice with phosphate-buffered saline and serum albumin, resuspended in RPMI to a concentration of 1 × 106 cells/ml, and filtered through a 35-μm strainer. Mononuclear cells were prepared by Ficoll-Hypaque differential centrifugation of blood effector cells; diluted corresponding to final effector-to-target cell ratios (E/T) of 50:1, 25:1, 12.5:1, and 6.25:1; and incubated with the DiO-labeled K562 cell suspension (target cells) for 4 h at 37°C with 5% CO2. Following incubation, propidium iodide (0.03 mg/ml final concentration) was added to each tube to identify dead cells. The percentage of dead target cells (i.e., propidium iodide (0.03 mg/ml final concentration) was added to each viability of thawed lymphocytes for this assay was ~90%. Cells from two quality control samples were included in every experiment; coefficients of variation for the assays with PHA and anti-CD3 were ~23 and 16%, respectively. PBMC were prepared by Ficoll-Hypaque separation, and 1 ml aliquots of 5–10 × 106 cells/ml were frozen in 30% fetal calf serum, 60% RPMI, and 10% DMSO (Gibco, Githersburg, MD).

For the [3H]thymidine incorporation, 200 μl of 5 × 104 cells/well were incubated in microtiter plates with 25 μl PHA of 0.1 and 0.5 μg/ml in five replicates each. After incubation for 72 h at 37°C, cells were pulsed for 24 h with 25 μl [3H]thymidine, harvested, and counted with a β-counter.

For the cell division tracking method, 170 μl of 105 cell/ml in RPMI were used for each sample. Carboxy-fluorescein diacetate, succinimidyl ester (Molecular Probes), a precursor of carboxyfluorescein diacetate succinimidyl ester, was added to the cell suspension at a final concentration of 10 μM. Cells were incubated for 10 min, washed twice, and resuspended in 3.0 ml of Complete Medium, and 180 μl were then pipetted into 16 wells of a microtiter plate (100,000 cells/well). Next, 20 μg of 2 ng/ml anti-CD3 antibody (BD Biosciences) were added to eight of the wells to specifically stimulate T lymphocytes. The remaining eight wells were used as control unstimulated cells. Following incubation for 3 days at 37°C, identical wells were pooled into 5-ml sterile tubes containing 2 ml Complete Medium and incubated for 3 more days. On the 6th day, cells were harvested, and the carboxyfluorescein diacetate succinimidyl esterFITC intensity of viable lymphocytes was measured with a flow cytometer (XL-MCL, Beckman Coulter, Miami, FL).

Immunoglobulin A (IgA), G (IgG), and M (IgM) assays were performed by nephelometry using the Behring Nephelometer II analyzer (Dade-Behring Diagnostics, Deerfield, IL). The intrabatch and interbatch coefficients of variation for IgA were 6.9 and 6.1%; for IgG, 3.8 and 5.4%; and for IgM, 10.8 and 9.2%, respectively.

Body Composition and Distribution

At baseline, 3 mo, and 12 mo, body weight to the nearest 0.1 kg and height to the nearest 0.1 cm were taken in duplicate, and the average was used to compute the BMI (kg/m2). At baseline and 12 mo, total body fat and percent body fat were analyzed by dual-energy X-ray absorptiometry (QDR 1500; Hologic, Waltham, MA); and intra-abdominal and subcutaneous fat were obtained via a one-slice computed tomography at L4–5 (CT; model CT 9800 scanner; General Electric, Waukesha, WI), with coefficients of variation of 1.2%.

Other Study Measures

Data were collected on demographic information, medical history, health habits, medication use, reproductive and body weight history, total energy intake (via 120-item self-administered food-frequency questionnaire), and frequency, duration, and intensity of physical activity (via self-administered Minnesota Physical Activity Questionnaire). VO2 max was assessed at baseline and at 12 mo using a Medgraphics automated metabolic cart (Medgraphics, St. Paul, MN), where participants completed a maximal-graded treadmill test. The test started at 3.0 mph and 0% grade. Speed was raised to 3.5 mph at 2 min, and thereafter grade was increased by 2% every 2 min until the participant reached volitional fatigue. No treadmill tests were terminated for reasons other than volitional fatigue. Energy expenditure was estimated from the daily physical activity logs and the compendium of physical activities (1).

Statistical Methods

Intervention effects were assessed on the immune outcomes at 3 and 12 mo after randomization, based on comparisons between the groups as defined at randomization. Changes in immune outcomes between groups were assessed via generalized estimating equations (GEE), which account for repeated observations on the same subjects over time. Baseline measures of each outcome were included in GEE.
models. In secondary analyses, we examined subgroup effects according to baseline values of BMI (<30 or ≥30 kg/m²), median split of each immune measure, age (≤60 or >60 yr), and C-reactive protein (≤3 or >3 mg/l), and stratified by 12-mo changes in VO₂max, exercise adherence, and percent body fat. Most immune measures were non-normally distributed and were log transformed. Missing data were omitted from the GEE analyses. No adjustments were made for multiple comparisons. All statistical tests were two sided. All analyses were performed with SAS software (version 9.1, SAS Institute, Cary, NC).

RESULTS

Study Population

One hundred fifteen women were randomized for the study: 53 to intervention and 62 to control. At 3 mo, blood samples were available from 50 exercisers and 59 controls. At 12 mo, blood samples were available from 50 exercisers and 59 controls (Fig. 1).

There were no significant differences between exercise and control groups at baseline (Table 1). On average, women were 61 yr old, and 88% were non-Hispanic white. Over 12 mo, the intervention group participated in moderate activity an average of 3.8 ± 1.3 days/wk for 166.4 ± 76.1 min/wk. Women in the exercise arm expended, on average, 3,828 ± 2,053 kJ/wk. At 12 mo, exercisers and controls increased VO₂max by 13.8 and 0.1%, respectively (P value: ≤0.0001). Similarly, the exercise intervention resulted in decreased body weight vs. control (exercise: −1.8 kg; control: +0.3 kg; P value: 0.002), and decreased percent body fat (exercise: −1.5%; control: +0.02%, P value: ≤0.0001). There were no differences between or within groups for total caloric intake throughout the trial (all P values ≥0.05).

Intervention Effects

There were no statistically significant intervention effects on the main study outcomes, NK cytotoxicity (Fig. 2) and T-lymphocyte proliferation (Fig. 3), at 3 and 12 mo; results were consistent when analyzed using various methods for NK cytotoxicity (e.g., at other E/T) and T-lymphocyte proliferation (e.g., using cell division tracking). Similarly, no intervention effects were noted for secondary outcomes of counts and percentages of T cells and B cells, measured by flow cytometry (Table 2, percent data not shown). IgA increased more among stretchers than among exercisers at 3 mo postrandomization (P = 0.04), but no differences were noted at 12 mo (Table 3). No intervention effects were noted for IgG and IgM (Table 3).

Subgroup analyses largely indicated no effect of exercise intervention when stratified by baseline values of BMI median split of each immune measure, age or C-reactive protein. Similarly, no clear patterns of association were noted when stratified by 12-mo changes in VO₂max, exercise adherence, and percent body fat. None of the subgroup analyses is presented. Considering the large number of statistical tests performed, we believe that the few statistically significant results occurred by chance.

DISCUSSION

In this randomized, controlled trial of aerobic exercise vs. stretching control among 115 overweight or obese, but otherwise healthy, postmenopausal women, we found no evidence for an effect of aerobic exercise intervention on primary (in vitro NK cytotoxicity and T-lymphocyte proliferation) and secondary immune end points, 3 and 12 mo postintervention. The study had a drop-out rate of 6% over 12 mo, indicating excellent retention. Women randomized to the exercise arm participated in 84.5% of the prescribed physical activity and increased VO₂max by 13.8%, suggesting good adherence and efficacy of the exercise intervention. Women in the control group experienced no change in aerobic fitness, indicating lack of compliance or study drop-out/drop-in effects.

A previous study from this trial reported on the effect of exercise intervention on the incidence of colds and URTI (4);
cold and URTI data were collected from self-reported questionnaire, conducted quarterly. The results suggested that exercisers relative to stretchers experienced fewer colds over the 12 mo of the study ($P_{H0.02}$). The effect was most pronounced in the final 3 mo of the study, where a greater than threefold difference in the incidence of colds was observed among stretchers relative to exercisers. The combined results from the previous and present studies suggest that acute immune changes may occur with each exercise session to enhance overall immunosurveillance against cold pathogens; however, this effect does not translate to chronic resting changes on in vitro immune function.

These null findings are consistent with two previous shorter and smaller randomized, controlled trials conducted by Nieman et al. (34, 35) among apparently healthy women. In the first study, 32 women (mean age, 73 yr) were randomized to either supervised brisk walking for 30–40 min/session, 5 days/wk, at 60% HR reserve, or to a stretching control group (34). Thirty women completed the 12-wk intervention, with no statistically significant intervention effects on in vitro NK cell cytotoxicity and T-lymphocyte proliferation, despite significant improvement in aerobic fitness in the intervention group. A more recent study among obese women yielded similar results (35); among the 21 exercisers relative to the 22 controls who completed the study, no exercise intervention effects were noted for in vitro NK cell cytotoxicity and T lymphocyte proliferation.

Fig. 2. Effect of exercise vs. control on natural killer (NK) cell cytotoxicity (effector-to-target cell ratio: 12.5:1). Open bars, exercisers; solid bars, controls. Exercisers vs. controls: $P$ value for 3 mo $= 0.83$; $P$ value for 12 mo $= 0.54$.

Fig. 3. Effect of exercise vs. control on lymphocyte proliferation [phytohemagglutinin (PHA) stimulated at 0.1 g/ml]. Open bars, exercisers; solid bars, controls. Exercisers vs. controls: $P$ value for 3 mo $= 0.50$; $P$ value for 12 mo $= 0.59$.

Table 2. Effect of exercise compared to stretching control on immune cells counts as measured by flow cytometry: 3- and 12-month comparisons

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<tr>
<th></th>
<th>3 mo</th>
<th>12 mo</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>Geometric Mean (95% CI)</td>
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<tr>
<td>T-cell counts</td>
<td></td>
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<tr>
<td>T cells (CD3/CD45)</td>
<td>53</td>
<td>1,231 (1,140–1,381)</td>
</tr>
<tr>
<td>Helper T cells (CD3/CD4)</td>
<td>53</td>
<td>854 (785–930)</td>
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<tr>
<td>Cytotoxic T cells (CD3/CD8)</td>
<td>53</td>
<td>326 (289–368)</td>
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<tr>
<td>CD4/CD8 ratio</td>
<td>53</td>
<td>2.31 (2.01–2.68)</td>
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<tr>
<td>B-cell counts</td>
<td>53</td>
<td>246 (219–277)</td>
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<tr>
<td>NK cell counts</td>
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<td></td>
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<tr>
<td>NK cells (CD3−/CD56+ CD16+CD57+)</td>
<td>53</td>
<td>128 (110–148)</td>
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Values are geometric means with 95% confidence interval (CI) in parentheses; N, no. of subjects; NK, natural killer; $P$ values indicate changes from baseline to *3 mo and †12 mo among exercisers vs. controls, controlling for baseline values.
proliferation. Our results on T-cell proliferation may be different from studies that used fresh rather than cryopreserved cells. We chose the latter approach to reduce batch-to-batch variation over the course of this study that extended over several years.

Others have reported enhanced in vitro NK cell cytotoxicity after exercise intervention in smaller studies. Results from a 10-wk study among 25 elderly nuns (mean age, 77 yr), in which the intervention consisted of 20–50 min of brisk walking 3 days/wk, suggested improved NK cell cytotoxicity, but no effect on in vitro T-lymphocyte proliferation (10). Both NK cell cytotoxicity and T-cell function were suggestively enhanced after a 6-mo exercise intervention (10.220.33.6 on July 31, 2017 http://jap.physiology.org/ Downloaded from). Fairey et al. (11) randomized 53 breast cancer survivors to 15 wk of aerobic exercise (3 days/wk) or to a no-exercise control group. NK cell cytotoxicity increased by 5–6% among exercisers relative to controls, and no changes were noted for neutrophils, mononuclear cells, and PHA-stimulated [3H]thymidine incorporation (11). Recently, Yang et al. (48) reported that traditional Chinese exercise improved antibody response to influenza among 50 older adults (mean age, 77 yr), with equivocal results for immune response to influenza A strains H1N1 and H3N2.

The somewhat discrepant findings between randomized, controlled trials may be explained by several factors. First, the average age among participants in randomized, controlled trials has varied considerably. One of the only previous studies to report statistically significant exercise effects on in vitro NK cytotoxicity, despite a relatively brief intervention period and low dose of exercise, was conducted among the oldest participants (10). Second, another study to report an intervention effect was conducted among breast cancer survivors (11). As aging and cancer treatment are both associated with depressed immunity, these results may suggest that the immune-enhancing effects of exercise are restricted to those persons with compromised immunity or those recovering from major illness. In the present study, we found no evidence that participants who, at baseline, were below the median for various immune measures experienced any immune enhancement from exercise, although it is unlikely that the current participants’ immune status is directly comparable to breast cancer survivors or to elderly subjects. An additional potential bias in exercise intervention trials is socialization. In the present study, controls met once per week to maximize retention without adding undue costs. A socialization bias may have been problematic, if we had observed any exercise effects on immune function since exercisers met more often. Given our null results, however, we do not feel that such a bias was a concern.

Women assigned to the intervention lost, on average, only ~2 kg of body weight, so we could not assess the effects of large amounts of weight loss on immune function. In a four-arm study, Nieman et al. (35) demonstrated that 12-wk caloric restriction (average weight loss: 7.9 kg) among overweight or obese women resulted in decreased lymphocyte proliferation. In subgroup analyses, we saw no suggestion of intervention effects by weight-loss strata.

Our study has limitations. First, the exercise intervention was only 1 yr in duration. To our knowledge, this is the longest duration of any randomized, controlled trial of exercise and immune function, but it is plausible that even longer periods of exercise intervention are required to modify immune function.
Cross-sectional studies of older endurance-trained female athletes, relative to sedentary females, support this hypothesis (34). Second, we evaluated in vitro blood immune function at rest; future studies may consider taking tissue-specific biopsy samples and/or measuring training effects on immune function by taking samples during or immediately after exercise to detect potential transient effects of exercise, as has been suggested for exercise effects on leukocyte counts (33).

Future studies should also consider exercise effects on clinically relevant outcomes, such as response to vaccination or delayed type hypersensitivity skin challenge. A previous study among 27 elderly participants reported that 10-mo aerobic exercise resulted in greater antibody titer response to influenza H1N1 and H3N2 strains and Granzyme B activity to A/Panama/2007/99 than did controls (19). Grant et al. (15) assessed the effect of aerobic exercise training on in vivo response to keyhole limpet hemocyanin (KLH). The exercise intervention resulted in higher anti-KLH response to IgG2 and IgM, but not IgG1, relative to control. Additionally, this study highlights one of the challenges faced when working with more invasive immune assays of the 109 participants enrolled in the randomized controlled trial, 54% did not participate in KLH administration (55 refused, 4 had contraindications to KLH administration). We considered implementing more invasive measures of immune function in the present trial, but opted against because they may have reasonably impacted study adherence and long-term retention.

In summary, this randomized, controlled trial identified no effects on in vitro immune function of a year-long aerobic exercise intervention relative to a stretching control group, despite decreased colds among exercisers (4), excellent adherence and retention, and positive efficacy of the exercise intervention.

GRANTS

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REFERENCES


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