Chronic Resistance Exercise Training Improves Natural Killer Cell Activity in Older Women

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Background. Regular exercise has been reported to slow the age-associated declines in natural killer cell activity (NKCA). To evaluate this response, we recruited older, postmenopausal women (65–85 years old) to fill one of two groups: training (10 weeks of resistance exercise; TR) or control.

Methods. Blood samples were collected from an arm vein in the TR group at rest (PRE), immediately following (POST), and 2 hours (2H) following an acute bout of resistance exercise both before (BEFORE) and after (AFTER) training. Leukocytes and NKCA were determined by flow cytometry and a whole blood 51Cr release assay, respectively.

Results. Acute exercise increased total leukocyte (p < .05), CD8 (p < .05), CD4 (p < .05), and CD56 counts (p < .05), but there was no effect of training. NKCA was greater TR-AFTER-PRE (136%), -POST (80%), and -2H (127%) compared to similar values from TR-BEFORE (p < .05).

Conclusion. Increased resting NKCA after chronic resistance training suggests that immunity has been improved.

ACUTE, high-intensity exercise may suppress the immune system during 1–6 hours of recovery. High-intensity training, according to the “j-loop” hypothesis, may increase the risk of upper respiratory tract infection (URT) (1). For example, a single strenuous bout of exercise may suppress the innate immune system for up to 24 hours after exercise (2). During this “open window” there may be an increased risk of illness (2). The “j-loop” and “open window” models were developed to describe how the immune system responds to the stress of exercise, but because most researchers used younger (18- to 35-year-old) participants, their responses may (3) or may not (4) be representative of the responses in older participants (>60).

Advancement of physical age is often associated with a reduction in immune system function, which can lead to an increased risk of infection and disease (1). Others have suggested that immunosenescence is the most likely explanation for age-related disruptions in immunity; however, the exact mechanism by which these cells become senescent is not fully understood (5,6). Exercise training has been suggested as a treatment to slow the apparent age-associated decline in immunity (3,7–11).

Whereas several physical changes associated with chronic training in persons of advanced age are understood (5,7–9,11), the response of the immune system to exercise and exercise training is not. Exercise training has been reported to increase (12), suppress (10), or not change (7–9,11) the activity of the innate immune system, as measured by changes in natural killer (NK) cell number and function. In a previous study from our laboratory, we reported (8) that NK activity (NKCA) was not affected following an acute bout of resistance exercise or by 10 weeks of resistance exercise training (70% of 1 repetition maximum (RM) in older (67- to 84-year-old) women. However, they were tested at the same absolute workload before and after training (different relative intensity). Therefore, it is possible that post-testing resistance exercise-trained older participants at a higher workload (same relative workload as pre training) would result in a post exercise decline in NKCA as previously shown in younger participants (9).

We hypothesize that high-intensity exercise (80% of 1RM) may reduce post exercise NKCA and that 10 weeks of resistance exercise training will not alter resting NKCA. The purpose of this study was to examine the effect of a 10-week resistance exercise training program on NK number and activity when participants were pre- and post-tested at the same relative intensity.

METHODS

Participants

All testing procedures used in the study were approved by the Committee on the Use of Human Subjects in Research at Purdue University. The participants in the present study were originally recruited as part of a larger study examining the effect of hormone status on strength training (8). Preliminary analyses revealed that hormone status did not influence NK response to strength training; therefore, these participants were pooled and reported as a single training group. Before any assessments were completed, all participants read and signed a university-approved informed consent form. Prior to exercise training, participants underwent a thorough medical screening by the study physician including: medical history evaluation, resting electrocardiogram (to screen for cardiac abnormalities at rest), exercise test with electrocardiogram (to screen for cardiac abnormalities caused by exercise), and a physical screening for musculoskeletal abnormalities. After the participant was “cleared” to participate by the study physician, participants were also asked to obtain approval from their personal physician so that they could review the exclusionary criteria for the study. Following screening and clearance, the participants were placed into one of two groups: trained groups (TR) or a control (CON) group (Table 1).

Acclimation

Participants were acclimated to the correct procedure for performing the following exercises: seated leg press, knee
CON sat quietly in the laboratory while TR exercised during the course of the experimental trial. A similar number of stretching. Participants were allowed water intake ad libitum of 1RM). The participants were allowed a brief walking warm-up. Training, the participants performed eight repetitions in the first set resistance exercise training workloads. Participants were reassessed at the end of training (week 10). Resistance Exercise Training After acclimation, TR trained three times per week, on alternate days, performing three sets of the 10 above-mentioned exercises, whereas CON did not train. During the first week of training, the participants performed eight repetitions in the first two sets at a resistance equal to 70% of the 1RM and in the third set performed as many repetitions as possible. At least a 1-minute recovery was allowed between sets. Intensity was increased to 80% of 1RM for the 2nd through 10th weeks of training. When a participant was able to complete more than 12 repetitions in the third set, the resistance was increased for the subsequent session. Each participant’s 8RM and 1RM was estimated from the 8RM and was used to set resistance exercise training workloads. Participants were allowed at least a 24-hour recovery between acclimation sessions. Experimental Trial TR completed a single resistance exercise bout before (BEFORE) and after (AFTER) 10 weeks of training. Participants arrived at the laboratory between 6:30 and 7 AM at least 7 days after their last exercise session and completed a single resistance exercise bout consisting of two sets of eight repetitions and a third set to failure on the above-mentioned resistance exercises (~80% of 1RM). The participants were allowed a brief walking warm-up (10 minutes) followed immediately by a short period of stretching. Participants were allowed water intake ad libitum during the course of the experimental trial. A similar number of CON sat quietly in the laboratory while TR exercised. Blood Sampling Venous blood was collected from a peripheral arm vein by a trained technician before exercise (PRE), immediately after exercise (POST, within 2 minutes of completing last repetition), and 2 hours after exercise (2H). Blood samples were collected into evacuated tubes (Becton Dickinson, St. Louis, MO) containing sodium heparin and were gently inverted until determination of leukocyte counts and NKCA. Flow Cytometry Total leukocyte count was determined using a particle counter (Z2: Beckman Coulter, Miami, FL). Leukocyte counts were completed in duplicate. Leukocyte fractions were prepared by diluting whole blood (1:25) in an ammonia chloride solution (1.5 M; Sigma-Aldrich, St. Louis, MO). After red blood cell lysis, isolated leukocytes were washed twice with magnesium-free phosphate-buffered saline (Sigma-Aldrich). After the final wash, leukocytes were suspended in magnesium-free phosphate-buffered saline (2 × 10^6 leukocytes · mL⁻¹) and aliquoted (100 μl) into three separate 12 mm × 75 mm polystyrene tubes (Sarstedt, St. Louis, MO). The following combinations of monoclonal antibodies (20 μl) were then added to each tube: CD3-fluorecein isothiocyanate (FITC) and CD56-phycoerythrin (PE), CD4-FITC and C8-PE, or a two-color isotype control (Beckman-Coulter). Primary gates were established for lymphocytes based on forward and side scatter, and secondary gates were established to enumerate NK (CD3⁻/56^+), helper T cell (CD4^+), and cytotoxic T cell (CD8^+) count. Gate position was set using the isotype control. Quality control was completed each day using a set of standard sized polystyrene beads (Beckman-Coulter). All analysis was completed using an XL-MCL flow cytometer equipped with an air-cooled 488 nm argon laser (Beckman-Coulter). NKCA NKCA was determined using a whole blood ⁵¹Cr release assay as described previously (4, 7–9). Briefly, sodium heparin-treated whole blood aliquots (150 μl) were incubated in triplicate with four concentrations of ⁵¹Cr-labeled K562 target cells (2, 1, 0.5, and 0.25 × 10^⁶ cells ml⁻¹). Measures of spontaneous and total release were determined by substituting RPMI 1640 or 1% Triton X-100 (Sigma-Aldrich) for blood, respectively. Following a 4-hour incubation, cell-free supernatants were removed and transferred to separate tubes, then counts per minute were determined from a gamma counter (Packard Cobra; Boston, MA). Percent lysis was determined for each target cell concentration, and was expressed on a 1:1 effector-to-target cell basis using kinetic plotting (13–15). Statistics Prior to statistical analysis, assumptions of constant variance and normality of the data were assessed. If an assumption violation was detected, then the data were stabilized by transformation prior to further statistical analysis. Participant characteristics were compared between groups using a Student t test. All participant characteristics were reported as the mean ± standard deviation (SD). Blood measurements were compared using a two (TR, CON) × two (BEFORE, AFTER) × three (PRE, POST, 2H) factor analysis of variance (ANOVA) with repeated measures on the second and third factors. All significant alpha values were adjusted using the Huynh–Feldt method to account for the repeated measures design. When significant effects were detected (p < .05), the location of significance was determined using separate Student t tests with a Bonferroni correction for multiple comparisons. All statistical analysis was completed using SPSS version 11.0.1 (Chicago, IL). Table 1. Participant Characteristics for Women Completing 10 Weeks of Resistance Exercise Training (TR) or Control (CON)  | Characteristics | TR (N = 19) | CON (N = 6) |
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*Indicates greater than before (p < .05).
8RM = 8-repetition maximum.
RESULTS

Training Improvements in Physical Capacity

A significant Group × Training interaction was found for chest press 8RM where TR-AFTER was greater than TR-BEFORE (34%), CON-BEFORE (50%), and CON-AFTER (64%) (Table 1). A Group × Training interaction was also found for leg press 8RM where TR-AFTER was greater than TR-BEFORE (23%), CON-BEFORE (26%), and CON-AFTER (39%) (Table 1). Similar improvements were observed for the other eight resistance exercises.

Leukocyte Count

A significant main effect for exercise time (F = 39.688, p < .001) and a Group × Exercise time (F = 6.515, p < .001) were found for total leukocyte count. CON-PRE (−14%) and CON-POST (−17%) were significantly lower than TR-POST. Also TR-POST was 14% greater than TR-PRE.

Flow Cytometry

Significant Group × Training (F = 3.439, p = .039) and Group × Exercise time (F = 2.750, p = .026) interactions were found for helper T cell (CD4+) count (Figure 1). TR-PRE was greater than CON-PRE (67%), CON-POST (107%), and CON-2H (54%). Also TR-POST was greater than CON-PRE (48%) and CON-POST (84%). Finally, TR-BEFORE was greater than CON-BEFORE (68%) and CON-AFTER (71%); TR-AFTER was greater than CON-BEFORE (53%) and CON-AFTER (56%).

NKCA

Significant Group × Training (F = 4.433, p = .011) and Group × Exercise time (F = 7.757, p < .001) interactions were found for NKCA (Figure 2). TR-AFTER was greater than TR-BEFORE (38%), CON-BEFORE (60%), and CON-AFTER (36%). Also, the response to acute exercise was greater PRE (136%), POST (80%), and 2H (127%) in TR-AFTER than in TR-BEFORE. Finally, TR-AFTER-PRE (30%), POST (74%), and 2H (12%) were greater than corresponding time points for CON-AFTER.

DISCUSSION

Similar to previous reports, we found a significant increase in muscle strength as a result of the training program (8,9). The training stimulus (progressive training program at 80% of 1RM) increased NKCA at rest and in response to an acute bout of exercise. However, the training did not statistically alter total or differential leukocyte counts. We did not observe any evidence to suggest that an "open window" response occurred in the present study. Based on the present and our previous studies (7,8), it is possible that resistance exercise does not result in postexercise suppression of NKCA in individuals of advanced age or that the exercise intensity of the present study was not great enough to elicit such a response.

The increase in NKCA at rest, observed in the present study, is consistent with the "j-loop" hypothesis. It was not the goal of this study to impose a severe training load on the participants, but rather to provide a significant exercise stimulus (i.e., 80% of 1RM). The participants in the present study were tested at a higher intensity than were those in our previous study (7), but we still did not find a training-induced suppression of NKCA. Lack of NKCA suppression is also supported by the findings of others (1,4,16). Therefore, based on the principles of the "j-loop"
hypothesis, it is reasonable to assume that our training stimuli would be in the moderate range.

As stated previously, there are conflicting reports in the literature as to the effect of exercise training on NKCA. Our present findings agree with those of other researchers (17,18) who reported an increase in NKCA following a period of exercise training in older individuals. However, our present findings for NKCA do not agree with our previous study (8) and others (10). The key difference between our previous study (4) and the present study was the intensity of the exercise stimulus chosen (i.e., 70% vs 80% of 1RM). Rincon and colleagues (10) used an exercise stimulus (70% of 1RM) similar to that in our previous study (8), except that their participants were frail elderly women and ours were healthy, ambulatory women. Therefore, the decline in NKCA following resistance training that they reported (19) may not be applicable to our present or previous study. The present study was not designed to evaluate the effect of different training intensities, so we are unable to determine which intensity provides the most dramatic changes in NKCA.

The present study was not designed to evaluate a mechanism by which resistance training alters NKCA, which may be related to T-cell balance or another yet to be determined response (16,20). The relative balance between type 1 (T1) and type 2 (T2) T cells alters an individual’s response to disease and infection (5,6,13). T-cell balance has been reported to shift in favor of a T2 response as a result of aging (5,6,20) and acute exercise (13,21). T2 dominance results in suppression of NKCA. Some authors (6,13,22) have suggested that chronic exercise training may prevent suppression of NKCA by maintaining the relative balance between T1 and T2 cells. In the present study, we reported a significant difference between TR and CON for helper T cell count at several time points. Although we cannot fully explain the difference in helper T cell count, this difference may have influenced changes in NKCA.

Summary

The key findings of the present study was that chronic resistance training did not decrease, but rather increased NKCA at rest and in response to an acute bout of exercise. This finding suggests that the “‘j-loop’ hypothesis can be applied to exercising seniors. Also the present study, along with our previous studies (7,8), provides evidence that postexercise suppression of NKCA may not occur in exercising seniors. We hypothesized that changes in T-cell balance may explain the training-induced increase in NKCA. However, more research is needed to determine the mechanism by which chronic exercise training influences T-cell balance in individuals of advanced age.

Acknowledgment

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References


Figure 4. Natural killer cell cytotoxicity determined at rest (PRE), immediately after (POST), and 2 hours after (2H) an acute bout of 10 resistance exercises before and after 10 weeks of training. *Less than TR-PRE (p < .05). †Less than before training (p < .05).