

Strength, workload, anaerobic intensity and the immune response to resistance exercise in women

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Abstract

Aim: The mechanism linking exercise intensity to the magnitude of the immune response is not completely understood. The purpose of this investigation was to determine whether the immune response to resistance exercise was associated with (1) changes in workload or (2) anaerobic exercise intensity.

Methods: Previously untrained women underwent 6 months of resistance training for lower and upper body (TOTAL, $n = 34$) or for upper body alone (UPPER, $n = 30$). Lymphocyte subsets [T (CD3+), CD4+, CD8+, NK and B], functional markers (CD45RA+ and CD45RO+), and mitogen (phytohemagglutinin-M, concanavalin A and pokeweed mitogen) and superantigen (*staphylococcus a. cowans*)-stimulated proliferation were measured from blood samples collected pre- and post-exercise for a squat resistance exercise consisting of six sets of 10 repetitions at 75% of one repetition maximum. This protocol was performed before (T0) and after 3 (T3) and 6 months (T6) of training.

Results: Lymphocyte recruitment to the circulation and proliferation following resistance exercise did not differ between training groups at any time, although the TOTAL group performed at a higher workload as training progressed. With respect to anaerobic intensity, exercise-induced increases in NK, CD4+, CD8+ and B lymphocyte concentrations were 42 ($P = 0.07$), 76 ($P < 0.05$), 72 ($P < 0.05$) and 242% ($P < 0.01$) greater in women in the highest compared with the lowest post-exercise lactate quartiles. Lymphocyte proliferation did not differ between lactate quartiles.

Conclusions: Anaerobic intensity, rather than increased strength and workload, is associated with the number of lymphocytes recruited to the circulation, but not T and B cell proliferation responses.

Keywords B cells, lymphocyte proliferation, natural killer cells, T cells.

The mechanisms contributing to the variability amongst individuals in the immune response to exercise stress need to be identified so that we can characterize the positive and negative influences of exercise training on the immune system. Exercise-induced increases in lymphocyte trafficking are typically considered to be controlled by catecholamine levels (Kappel *et al.* 1991, Landmann

1992, Benschop *et al.* 1997) and delayed decreases by cortisol (Pedersen *et al.* 1997). A number of influences including adrenaline, cortisol, prostaglandins, β -endorphins and cytokines are suspected to modulate lymphocyte functions (Kappel *et al.* 1991, Pedersen *et al.* 1997).

Anaerobic exercise intensity is typically indicated by the magnitude of the rise in blood lactate concentration.

There is a strong association between adrenaline and lactate concentrations in the blood during and following high intensity exercise (Schneider *et al.* 2000). Thus, one component of anaerobic intensity that influences the magnitude of the immune response is adrenaline. However, lactate is a key factor potentially contributing to the mechanism of the immune response to exercise that has yet to be investigated in this context. Neutrophils produce lactate at sites of inflammation and the corresponding drop in pH modulates a number of immune responses (Lardner 2001). Surface expression of adhesion molecules, natural killer (NK) cell activity and lymphocyte proliferation all respond to decreases in pH (Loeffler *et al.* 1992, Buhner *et al.* 1993, El Habbal *et al.* 1997, Zünd *et al.* 1997, Fischer *et al.* 2000, Drbal *et al.* 2001). Thus, we hypothesized that some of the variability amongst individuals in their responses to exercise may be the result of differences in blood lactate and anaerobic intensity.

Another factor that might induce variability is the absolute workload or work rate during exercise. For example, stronger individuals perform at a higher absolute workload during exercise when the relative workload is equal amongst all individuals. Our previous research indicated that the immune response to resistance exercise was greater in women with higher strength than in women with lower strength (Dohi *et al.* 2001). Thus, we hypothesized that as women gain strength during resistance training the magnitude of the immune response to acute resistance exercise also would increase.

The purpose of this investigation was twofold: (1) to determine whether increased strength, and thus absolute work capacity, influenced the magnitude of the immune response to resistance training; and (2) to determine whether differences in anaerobic exercise intensity account for any of the variability in the lymphocyte response to resistance exercise. The magnitude of the response elicited by exercise is the focus herein because we have previously reported that resting immune cell concentrations and T and B cell proliferation are not influenced by 6 months of resistance training in younger women (Miles *et al.* 2002).

Materials and methods

Subjects

College-aged (18–30 years) women recruited to participate in this investigation were informed of the potential risks associated with this investigation and signed an informed consent document approved by the university Institutional Review Board and by the Human Use Review Office of the Army Surgeon General, Washington, DC. Prior to the study, a physician medically screened subjects for inclusion. None of

the subjects had any confounding orthopaedic, endocrine or other disorders that would contraindicate participation in a heavy resistance or endurance training programme. None of the women reported using prescription medications or being current smokers.

Experimental design

Subjects recruited for exercise training were assigned to one of two resistance-training groups, matched for initial body size and strength. As body size and strength influence training capacity, it was more important to match the groups for these variables than to risk differences between groups because of random assignment. Resistance training was designed for the total body (TOTAL, $n = 34$) or upper body only (UPPER, $n = 30$). Squat 1 repetition maximum (1 RM) strength was determined and at least 48 h later a six set by 10 RM resistance exercise test was performed at 75% of 1 RM. Venous blood samples were collected pre- and immediately post-exercise to assess the magnitude of the exercise-induced immune response. Immune assessments included lymphocyte subsets [T (CD3+), CD4+, CD8+, NK and B], functional markers (CD45RA+ and CD45RO+), and mitogen (phytohemagglutinin-M, concanavalin A and pokeweed mitogen) and superantigen (*staphylococcus a. cowans*) stimulated proliferation pre- and post-exercise. These assessments were made before the training began (T0, September/October), after 3 months of training (T3, November/December) and after 6 months of training (T6, April/May).

Strength

One-repetition maximum (1 RM) tests were used to determine squat strength. The testing protocol has been described previously (Kraemer *et al.* 1991). Testing consisted of two warm-up sets using three to five repetitions at 60 and 80% of estimated 1 RM followed by three to five subsequent attempts to determine 1 RM load. The highest mass (kg) lifted with proper form was used as the 1 RM test score.

Resistance exercise test

The resistance exercise test was performed between 6:30 and 10:00 AM after an overnight fast. For each subject, the times for T3 and T6 tests were the time for the T0 blood collection ± 1 h, with a goal of exactly the same time as the T0 collection. Peripheral blood from a forearm vein was collected using a standard venipuncture technique. All samples were collected with subjects lying down.

Each subject rested quietly in the laboratory prior to the pre-exercise blood collection. Fifteen minutes later a light warm-up on a cycle ergometer was performed for

2–3 min. The resistance exercise test consisted of six sets of 10 RM squats with 2 min rest between sets. Initially, the workload was 75% of 1 RM. If the subject failed to perform the 10 repetitions because of fatigue on any give set, the load was immediately adjusted to permit completion of the remaining repetitions. The resistance exercise test took 12–15 min to complete on average. Post-exercise blood samples were collected immediately post-exercise.

Training programmes

Details of each training programme have been described in detail previously (Kraemer *et al.* 2001). A trainer supervised all subjects during all workouts to insure adherence to proper techniques and exercise protocols. All exercise-training groups completed two 12-week meso-cycles separated by 3 weeks of active rest (period of low intensity recreational activities). Training occurred 3 days week⁻¹. Within each 12-week meso-cycle were three 4-week micro-cycles. The number of repetitions decreased and the proportion of the 1 RM for resistance increased from micro-cycle 1 to micro-cycle 3. For the UPPER group, the first and third workouts of the week consisted of three sets each of bench press, seated rows, dumb-bell presses, latissimus dorsi pull downs, biceps curls, triceps push-downs, rotational abdominal crunches and back extensions. The second training session of the week consisted of dumb-bell inclined presses, front pull downs, upright rows, dumb-bell rows, dumb-bell curls, dumb-bell triceps extension and sit-ups. For the TOTAL group, the first and third workouts of the week consisted of three sets each of squats, leg curls, calf raises, narrow bench presses, dumb-bell rows, dumb-bell triceps extensions, dumb-bell curls and abdominal crunches. The second workout of the week consisted of leg extensions, leg curls, calf raises, bench presses, seated rows, triceps push-downs, biceps curls and sit-ups.

Complete blood count

Complete blood counts were performed using an automated haematology analyser (Coulter Corporation, Miami, FL, USA). Leucocyte differentials to indicate proportion of the white blood cell population that were neutrophils, lymphocytes or monocytes were performed manually for the first 32 subjects (first year of the study) and taken from the automated analysis for the remaining 32 subjects.

Leucocyte labelling

Within 6 h of collection, leucocytes were labelled for two-colour analysis using fluorescein isothiocyanate (FITC) and phycoerythrin (PE) conjugated mono-

clonal antibodies (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) using a whole blood staining method. An FITC and PE conjugated isotype control was used to determine background fluorescence. Bit map gates based on cell size and granularity were used to distinguish lymphocytes and neutrophils for fluorescence analysis, and a CD45-FITC/CD14-PE stain was used to determine the purity of the lymphocyte population. The surface antibodies used for subset identification were CD3-CD16+ 56+ for NK cells, CD3-CD19+ for B cells, CD3+ CD4+ for CD4+ T cells (generally T helper cells), CD3+CD8+ for CD8+ T cells (generally T cytotoxic cells), CD45RA (naïve lymphocytes) and CD45RO (memory lymphocytes). Briefly, 10 µL of an FITC conjugated and 10 µL PE conjugated antibody, or 10 µL of a Simultest™ FITC/PE antibody pair, was pipetted into a tube and 100 µL of whole blood was added, vortexed gently, and incubated for 20 min in the dark at 4 °C. After incubation, 2 mL of FACSLyse (Becton Dickinson Immunocytometry systems) solution was added to each of the tubes before vortexing and incubating for 10 min in the dark at room temperature for erythrocyte lysing. Tubes were centrifuged for 4 min at 250 × g, the supernatant was vacuum aspirated, the cell pellet was washed with 1 mL of phosphate-buffered saline (PBS) without magnesium or calcium, centrifuged and vacuum aspirated again. The pellet was suspended in a fixative solution of 1% formaldehyde in PBS without magnesium or calcium.

Samples were stored refrigerated until analysis by flow cytometry usually within 1 day, but always within 3 days of labelling. For 32 of the subjects, 10 000 events were collected using a Coulter EPICS 753 flow cytometer (Coulter Corporation, Hiialeah, FL, USA). Raw flow data were analysed using EPICS software (version 4.0, Coulter Corporation) to determine proportions of fluorescent-labelled lymphocytes. For the remaining 32 subjects, 5000 events within the lymphocyte scatter gate were collected using a Coulter XL flow cytometer. Raw data were analysed using Coulter System II (version 1.0). Concentrations of cells with each surface marker were calculated by multiplying lymphocyte concentration by the proportion with positive fluorescence for cell surface marker. Lymphocyte proportions were corrected for contamination by platelets and debris by dividing the raw proportion by the proportion purity calculated from the CD45+ proportion of the lymphocyte scatter gate.

Lymphocyte proliferation

Peripheral blood mononuclear cells for the mitogen assay were isolated from acid citrate dextrose (ACD) whole blood using density gradient centrifugation. After centrifugation and removal of plasma, the leucocyte

buffy coat was collected, diluted with an equal volume of PBS, and a volume of Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) equal to two-thirds the volume of the diluted buffy coat was underlaid. This gradient was centrifuged at $2400 \times g$ for 30 min at 23 °C. The mononuclear cell layer was removed and washed twice with PBS and suspended in RPMI 1640 culture medium (supplemented with 10% foetal calf serum, 2 mM L-glutamine, 50 mM 2-mercaptoethanol, 100 U mL⁻¹ Penicillin and 100 g mL⁻¹ streptomycin sulphate) at a concentration of 2×10^6 cells mL⁻¹. Lymphocyte proliferative response to mitogen stimulation was measured for optimized doses of phytohemagglutinin-M (PHA, 4.0 and 10.0 µg mL⁻¹), concanavalin A (ConA, 4.0 and 10.0 µg mL⁻¹), pokeweed mitogen (PWM, 0.25 and 2.5 µg mL⁻¹), and *staphylococcus a. cowans* (Sac, 1 : 100 000 and 1 : 10 000 dilution with RPMI). Six replicates of 100 µL of isolated cells and 100 µL of medium with each mitogen dilution, plus a media control, were incubated for 70–74 h at 37 °C and 5% CO₂ in 96-well round bottom plates. During the last 6 h of incubation, 1.0 µCi of ³H-thymidine was added to each well. The cells from each well were collected on a glass fibre filtermat (Wallac Inc., Gaithersburg, MD, USA) using an automated cell harvester (7025 Combi Cellharvester, Skatron, Inc., Sterling, VA, USA). After drying, the filtermats were bagged, 10 mL of scintillation fluid was added, and the incorporation of ³H-thymidine by proliferating cells was quantitated using a betaplate liquid scintillation counter (model 1250, Wallac Inc.).

Serum lactate concentrations

Serum lactate concentration was determined in duplicate using a 1500 Sport L-Lactate Analyser (Yellow Springs Instruments, Yellow Springs, OH, USA).

Statistical analysis

The magnitude of the immune response to acute exercise was calculated by subtracting the pre-exercise value from the post-exercise value for all variables. A two-way analysis of variance (ANOVA) with repeated measures (3×2) was used to detect changes over time (T0, T3 and T6) and amongst groups (TOTAL vs. UPPER). A separate ANOVA (3×2) comparing the magnitude of the immune response between the upper (HI-LAC) and lower (LO-LAC) quartiles for subjects with the highest and lowest post-exercise blood lactate concentrations was performed to determine whether anaerobic intensity influenced the magnitude of the immune response to resistance exercise. The Tukey HSD *post hoc* test was used to determine the location of differences when significant main effects were detected. Significance was set at $\alpha = 0.05$.

Results

TOTAL vs. UPPER groups

Squat 1 RM increased (group \times time $P < 0.001$) to a greater degree in the TOTAL group (+22.4 and +36.4% TOTAL compared with +5.8 and +11.1% UPPER at 3 and 6 months, respectively) (Fig. 1a). Post-exercise lactate concentrations increased (group \times time $P = 0.02$) slightly over time for the TOTAL group, but not for the UPPER group (Fig. 1b).

While granulocytes, monocytes and lymphocytes all increased ($P < 0.001$) from pre- to post-exercise, there were no differences in the magnitude of these increases between groups or over time (Table 1).

The exercise-induced rise in blood-borne lymphocyte concentrations was not influenced by increased strength. However, influx of some subsets was transiently enhanced after 3 months of training (Table 1). Concentrations of all lymphocyte subsets increased ($P < 0.001$) from pre- to post-exercise. The magnitude



Figure 1 Mean data (\pm SD) for squat 1 RM (a) and post-exercise serum lactate concentration (b) pre- to post-exercise for women in response to the 6×10 RM squat resistance exercise test for women who trained both the upper and lower body (TOTAL) or upper body only (UPPER). Measurements were made before resistance training began (T0), and after 3 (T3) and 6 months (T6) of resistance training, respectively. * $P < 0.05$ between time points; # $P < 0.05$ between groups.

Table 1 Changes in leucocyte and lymphocyte subset concentrations ($\times 10^9 \text{ L}^{-1}$) for TOTAL and UPPER groups from pre- to post-exercise

	TOTAL			UPPER		
	T0	T3	T6	T0	T3	T6
Leucocyte differential						
Granulocytes	1.39 \pm 1.42	1.74 \pm 1.49	1.59 \pm 1.01	1.84 \pm 1.31	1.31 \pm 0.65	1.39 \pm 0.83
Monocytes	0.29 \pm 0.35	0.25 \pm 0.45	0.25 \pm 0.18	0.21 \pm 0.33	0.22 \pm 0.31	0.19 \pm 0.27
Lymphocytes	1.85 \pm 0.84	1.81 \pm 0.99	1.89 \pm 0.81	1.57 \pm 0.85	1.71 \pm 0.92	1.56 \pm 0.75
Lymphocyte subsets						
NK	0.67 \pm 0.39	0.65 \pm 0.42	0.71 \pm 0.35	0.73 \pm 0.38	0.67 \pm 0.42	0.68 \pm 0.38
CD3- CD8+	0.16 \pm 0.25	0.33 \pm 0.22†	0.24 \pm 0.13†‡	0.25 \pm 0.16	0.46 \pm 0.24†	0.33 \pm 0.14†‡
T	0.97 \pm 0.60	1.06 \pm 0.78	1.01 \pm 0.54	0.70 \pm 0.54	0.91 \pm 0.75	0.69 \pm 0.50
CD4 + T*	0.34 \pm 0.36	0.48 \pm 0.59†	0.37 \pm 0.29	0.18 \pm 0.19	0.40 \pm 0.43†	0.17 \pm 0.24
CD8 + T	0.47 \pm 0.33	0.50 \pm 0.36	0.51 \pm 0.36	0.37 \pm 0.38	0.43 \pm 0.34	0.34 \pm 0.29
CD45RA+	1.37 \pm 0.65	1.27 \pm 0.83	1.14 \pm 0.53	1.08 \pm 0.35	1.39 \pm 0.76	1.12 \pm 0.60
CD45RO+	0.49 \pm 0.40	0.54 \pm 0.51†	0.36 \pm 0.29	0.36 \pm 0.22	0.42 \pm 0.32†	0.26 \pm 0.23
B	0.08 \pm 0.08	0.11 \pm 0.12†	0.06 \pm 0.07	0.07 \pm 0.08	0.13 \pm 0.18†	0.08 \pm 0.10

Values = mean \pm SD. † $P < 0.05$ compared with T0; ‡ $P < 0.05$ compared with T3. * $P < 0.05$ between TOTAL and UPPER groups.

Table 2 Changes in lymphocyte proliferation (uptake of ^3H -thymidine, corrected counts per minute) for TOTAL and UPPER groups from pre- to post-exercise

	TOTAL			UPPER		
	T0	T3	T6	T0	T3	T6
Con A	-1413 (12178)	-5866 (12621)	-3380 (14021)	-4118 (13570)	-5867 (12598)	-4027 (10401)
PHA	727 (17791)	-4342 (15154)	406 (19361)	4422 (17873)	-7386 (20151)	-2324 (15689)
PWM	-4804 (11142)	-11264† (11226)	-4910‡ (11579)	-7620 (11355)	-11159† (7082)	229‡ (9302)
Sac	-1330 (4701)	-407 (6079)	-642 (3038)	-1470 (4634)	-2266 (2665)	-1505 (2755)

Values = mean (SD). † $P < 0.05$ compared with T0; ‡ $P < 0.05$ compared with T3.

of the increase was similar between TOTAL and UPPER groups and over time for NK, T, CD8+ T and CD45RA+ lymphocytes. The magnitude of the increase was similar between groups but larger at T3 compared with T0 and or T6 for CD4+ T cells ($P = 0.01$), non-T (CD3-) CD8+ ($P < 0.001$), CD45RO+ ($P = 0.01$) and B lymphocytes ($P = 0.02$).

Lymphocyte proliferation decreased ($P < 0.001$) from pre- to post-exercise across all measurements. The magnitude of the decrease was similar over time and between groups for the majority of the stimulation conditions (Table 2). The magnitude of the decrease from pre- to post-exercise for PWM stimulation was greater after 3 months of training compared with the other time points.

Anaerobic intensity

To determine whether anaerobic intensity reflected by lactate production is associated with immune responses

to resistance exercise, separate repeated measures ANOVAS were run comparing the highest T0 lactate quartile (HI-LAC, $n = 17$) with the lowest quartile (LO-LAC, $n = 15$). Women in the HI-LAC group had post-exercise responses $\geq 11.92 \text{ mmol L}^{-1}$, LO-LAC $\leq 7.62 \text{ mmol L}^{-1}$. Participants in the both the HI-LAC and LO-LAC groups were spread across both training groups. These groups did not differ significantly in their squat 1-RM strength, and thus the intensity of exercise for the $6 \times 10 \text{ RM}$ exercise test (Fig. 2a). Further, the relative magnitude of the lactate response was consistent for each group across the training period (Fig. 2b). Thus, the women in each group were consistently high or low in their lactate responses to the $6 \times 10 \text{ RM}$ exercise test.

The HI-LAC group had a trend ($P = 0.07$) towards greater pre- to post-exercise increase in NK cells (Fig. 3a) and significantly greater increases ($P < 0.05$) for total lymphocytes, CD3-CD8+ lymphocytes (Fig. 3b), B cells (Fig. 3c), T cells (Fig. 4a), CD4+ T

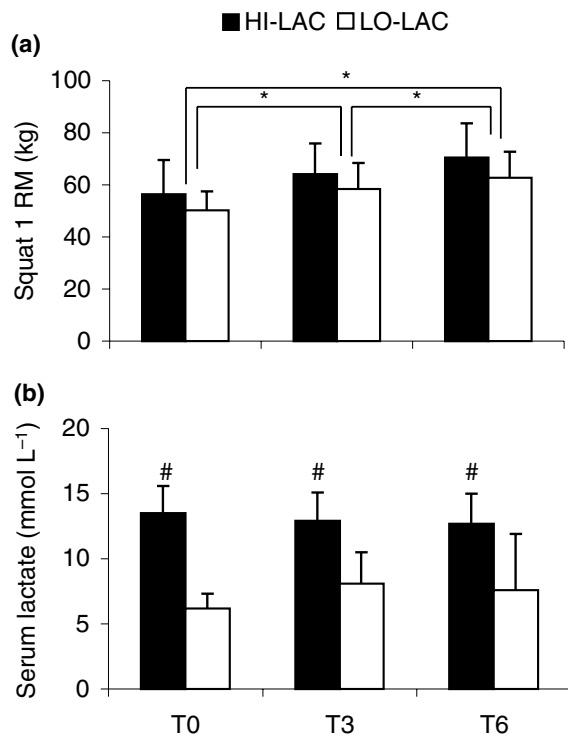


Figure 2 Mean data (\pm SD) for squat 1 RM and post-exercise serum lactate concentration pre- to post-exercise for women in response to the 6×10 RM squat resistance exercise test for women in the high lactate (HI-LAC) and low lactate (LO-LAC) response groups. Measurements were made before resistance training began (T0), and after 3 (T3) and 6 months (T6) of resistance training, respectively. * $P < 0.05$ between time points; # $P < 0.05$ between groups.

cells (Fig. 4b) and CD8+ T cells (Fig. 4c). There were no differences between HI-LAC and LO-LAC groups for the change in granulocytes, monocytes, CDRA+ lymphocytes or CDRO+ lymphocytes. Interestingly, the magnitude of the change in lymphocyte proliferation was not different between groups (for the sake of brevity, data not shown).

Discussion

The primary finding of this investigation is that the number of leucocytes that can be mobilized during resistance exercise is associated with the anaerobic exercise intensity as reflected by lactate production. The magnitude of the immune cell redistribution to the circulation was not associated with training status or strength levels and workload. We also found that lymphocyte recruitment to the circulation and the suppression of lymphocyte proliferation post-exercise was greater after 3 months of training compared with pre-training and after 6 months of training.

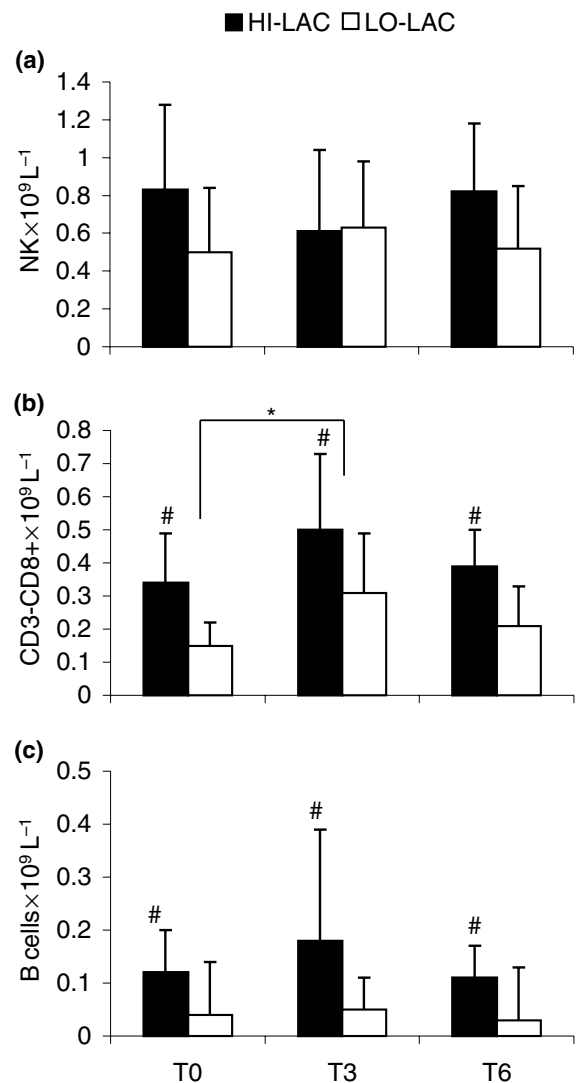


Figure 3 Mean data (\pm SD) for the change in NK cell (a), CD3-CD8+ cell (b) and B cell (c) concentration pre- to post-exercise for women in response to the 6×10 RM squat resistance exercise test for women in the high lactate (HI-LAC) and low lactate (LO-LAC) response groups. Measurements were made before resistance training began (T0), and after 3 (T3) and 6 months (T6) of resistance training, respectively. * $P < 0.05$ between time points; # $P < 0.05$ between groups.

Women from the highest (HI-LAC) and lowest (LO-LAC) post-exercise lactate concentration quartiles were compared and our data indicate that lactate production during the exercise was related to the magnitude of the immune response to resistance exercise. The positive relationship between adrenaline and lactate levels (Schneider *et al.* 2000) is likely to account for much of the immune differences between the HI-LAC and LO-LAC groups. However, the differences between the two groups are not entirely consistent with the known effects of adrenaline on the

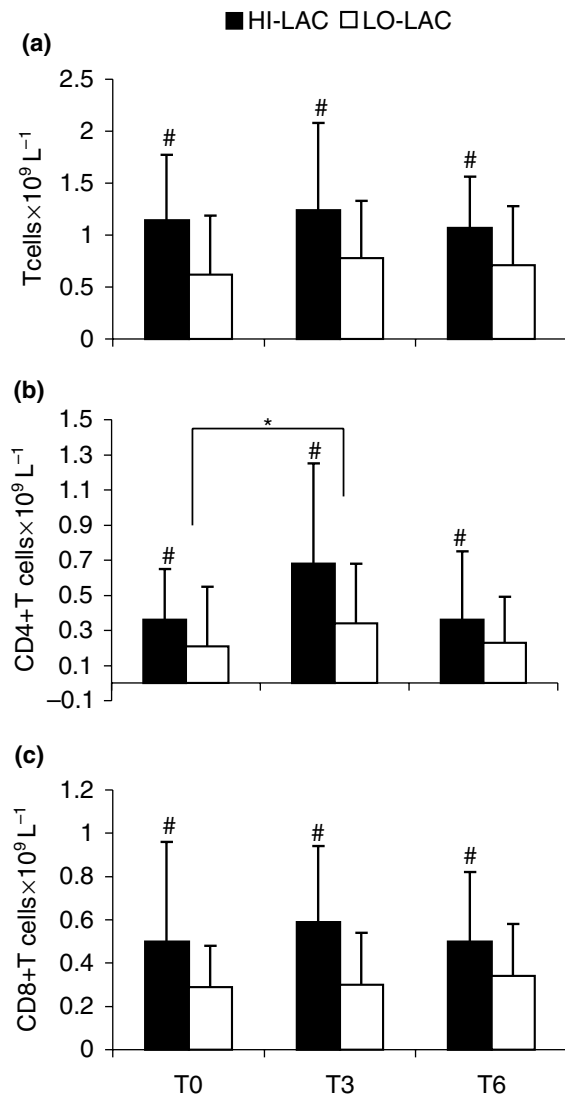


Figure 4 Mean data (\pm SD) for the change in T cell (a), CD4+ T cell (b) and CD8+ T cell (c) concentration pre- to post-exercise for women in response to the 6×10 RM squat exercise test for women in the high lactate (HI-LAC) and low lactate (LO-LAC) response groups. Measurements were made before resistance training began (T0), and after 3 (T3) and 6 months (T6) of resistance training, respectively. * $P < 0.05$ between time points; # $P < 0.05$ between groups.

immune system. For example, the cell population most dramatically recruited to the circulation by adrenaline is NK cells (Kappel *et al.* 1991, Landmann 1992, Benschop *et al.* 1997). In our study, the difference between groups in the relative rise in NK cells during the exercise was less for NK cells than for T and B lymphocytes. Were the differences between groups simply the effect of adrenaline, the reverse would have been measured. Thus, these data provoke the consideration of lactate itself as a modulator of immune cell trafficking and perhaps function.

Lactate does have an influence on immune cells and appears to be a component of some immune responses. Neutrophils produce lactate at sites of inflammation as a means of lowering pH and luring additional cells to the site of inflammation (Ratner 1992, Lardner 2001). Lactate-induced changes in adhesion molecule expression may influence NK cell activity and lymphocyte proliferation. For example, Mac-1, LFA-1 and ICAM-1 are up-regulated and L-selectin is down-regulated by acidic pH (Buhrer *et al.* 1993, El Habbal *et al.* 1997, Zünd *et al.* 1997, Drbal *et al.* 2001). Stimulation of the Na⁺-K⁺-ATPase in skeletal muscle during endotoxemia elevates blood lactate (Bundgaard *et al.* 2003). This is further evidence that lactate may be a logical molecule to modulate immune function. This teleological hypothesis of lactate as one of the links between exercise and the associated immune responses merits additional research.

There are many influences of lymphocyte recruitment to the circulation that need to be considered. It should be noted that if lower exercise-induced blood lactate levels occur because of low carbohydrate availability, then higher lactate levels are not associated with greater mobilization of lymphocytes to the circulation (Mitchell *et al.* 1998). Similarly, carbohydrate supplementation during endurance exercise can result in lower post-exercise lymphocyte concentrations but no difference in lactate (Nieman *et al.* 1998). Adrenaline-induced mobilization of lymphocytes to the circulation, particularly NK cells, is well documented (Kappel *et al.* 1991, Landmann 1992, Benschop *et al.* 1997). Natural killer cells were the only population of lymphocytes that did not differ between HI-LAC and LO-LAC groups. Thus, we suspect that β -adrenergic stimulation prevails as the dominant stimulus for NK cell mobilization, but that other populations of lymphocytes additionally may be influenced by lactate and or blood pH.

Lymphocyte proliferation did not differ between groups with high and low post-exercise blood lactate levels. Enhanced lymphocyte proliferation in response to a decrease in pH has been demonstrated in a single study (Loeffler *et al.* 1992). No other investigations of the effect of lactate or pH on lymphocyte proliferation could be found. In the present investigation, the proliferation assay was performed on isolated lymphocytes and the influence of lactate or low pH in whole blood at the time of collection was not likely to be discernible with the used methodology. Further research to identify the influence of exercise-induced acidosis on immune cell function may reveal a new mechanism of interaction between exercise and the immune system.

It is tempting to suggest that individuals in the HI-LAC group worked harder during the resistance exercise bout. However, the relative intensity was the same for all subjects and the HI-LAC group had

post-exercise lactate concentrations roughly double that of the LO-LAC group. There may be inherent differences amongst the subjects in their ability to activate glycolytic motor units or to motivate themselves to produce a true maximum effort. Additionally, the difference may be that the HI-LAC group had a greater mass of glycolytic muscle fibres because lactate production during strenuous exercise has been correlated to the proportion of type II muscle (Bishop *et al.* 2000). Individuals in the HI-LAC and LO-LAC groups were consistent in their lactate responses across all three testing sessions. Resistance training does not increase the concentration of glycolytic enzymes per unit of muscle, e.g. phosphofructokinase (Tesch *et al.* 1987); thus it is not surprising that the lactate responses were consistent across 6 months of training.

Neither the initial strength level nor the gain in strength during training affected the lymphocyte response. All subjects performed the resistance exercise 6×10 RM squat tests at the same relative workload throughout the investigation (75% of 1 RM). The TOTAL group trained their legs, increased their squat 1 RM much more than the UPPER group, and performed the greatest amount of absolute work during the 6×10 RM squat test at the end of 6 months of training. The resistance exercise-induced increase in lymphocytes was the same after 6 months of training for both the TOTAL and UPPER groups, as it was pre-training. This finding is consistent with studies of longitudinal resistance training in older women (Flynn *et al.* 1999), cross-sectional comparisons of resistance or interval exercise between weight-trained and non-weight-trained individuals (Nieman *et al.* 1994, Potteiger *et al.* 2001) and high and low fitness individuals (Fry *et al.* 1992). This is also consistent with our previous finding that the number of lymphocytes recruited to the circulation during resistance exercise was similar in magnitude for both high and low strength women (Dohi *et al.* 2001). Thus, factors associated with a greater absolute workload during resistance exercise generally do not influence the magnitude of the lymphocyte response to resistance exercise if the relative workload is similar.

The lymphocyte proliferation response to mitogen stimulation is variable following resistance exercise. Nieman *et al.* (1995) measured a decrease in proliferation that was attributable to decreased proportions of T cells rather than a decrease on a per cell basis. Our previous research indicated that high strength women decreased and low strength women increased the T and/or B cell proliferation on a per cell basis immediately following resistance exercise (Dohi *et al.* 2001). Potteiger *et al.* (2001) measured decreased proliferation 3 h post-resistance exercise in untrained but not trained

women. Thus, the lymphocyte proliferation response to resistance exercise is inconsistent.

The enhancement of the acute immune response to resistance exercise after 3 months of training is intriguing. There was a greater exercise-induced increase in several of the lymphocyte subsets (CD4+ T, CD3-CD8+, CD45RO+ and B) and a greater decrease in some lymphocyte proliferation conditions (PHA at optimal, and PWM at suboptimal and optimal) from pre- to post-exercise after 3, but not 6 months of resistance training. The magnitude of the decrease in lymphocyte proliferation after 3 months was roughly equal amongst groups. Thus, the larger decrease from pre- to post-exercise at this time point was not a function of greater absolute work because the workload did not increase significantly for the UPPER group. Exercise training typically does not increase the magnitude of the immune response to acute exercise (Woods *et al.* 1999). Increasing training intensity has been shown to modulate the immune response to acute exercise in some studies (Verde *et al.* 1992). Perhaps, a systemic adaptation occurring after 3 months of training mimicked an alteration in training intensity and homeostatic balance was re-established by 6 months of training. Alternatively, there may have been a seasonal influence on the immune response to acute exercise as the 3-month time point fell in either November or December for all our subjects. This possibility cannot be ruled out without a non-training control group for comparison. Instead of a non-training control group, we chose a group of women who were training muscles other than those involved in the squat exercise test for comparison so that we could isolate the influence of increased strength, independent of the effects of resistance training.

In conclusion, high levels of blood lactate were associated with increased recruitment of T and B, and perhaps NK, lymphocytes to the circulation, but did not influence the proliferation response of T and B lymphocytes. There is evidence to support the hypothesis that blood lactate increases may be part of the mechanism to increase lymphocyte concentration in the circulation. The magnitude of exercise-induced immune cell responses to resistance exercise occurred after 3 but not 6 months of resistance training. This may have been due to temporary systemic adaptations to training or possibly seasonal variation in immune responsiveness to stress.

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