Leukocyte adhesion molecule expression during intense resistance exercise

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Leukocyte adhesion molecule expression during intense resistance exercise. J. Appl. Physiol. 84(5): 1604–1609, 1998.—We hypothesized that expression of L-selectin and VLA-4 integrin adhesion molecules would influence cell type-specific redistribution during exercise. Women subjects performed six sets of 10-repetition maximum squats. L-selectin and VLA-4 integrin were measured by using flow cytometry pre- and postexercise on peripheral blood neutrophils and lymphocytes (n = 29 subjects) and lymphocyte subsets (n = 70 subjects), respectively. Neutrophil concentration increased 41.8% (P < 0.001), whereas the percent expressing L-selectin was unchanged (79%). Lymphocyte concentration increased 61.8% (P < 0.001). The percent of T cells expressing L-selectin decreased from 73.5 ± 8.9 to 68.2 ± 11.4% (P < 0.001); the combined population of natural killer and B cells expressing L-selectin decreased from 80.4 ± 22.5 to 62.7 ± 25.8% (P < 0.001). VLA-4 integrin was expressed by nearly all lymphocytes both pre- and postexercise. The proportional decrease in L-selectin positive cells could have resulted from 1) shedding of L-selectin, 2) selective entry of L-selectin-negative subsets, or 3) selective removal of L-selectin-positive subsets.

very late antigen-4 integrin; L-selectin; neutrophils; lymphocytes; cortisol

EXERCISE CAUSES a redistribution of leukocytes that varies by cell type. Cell type-specific redistribution of leukocytes hinges on surface adhesion molecules. Expression and activation of several surface adhesion molecules by distinct leukocyte populations dictates cell type-specific adhesion in different tissues (7, 22, 24). The tissues to which cells will adhere, the stimuli that cause release into the circulation, and the stimuli that elicit extravasation are determined by the adhesion molecule profile of a given leukocyte type. Furthermore, the profile of adhesion molecules expressed on different leukocyte populations is influenced by systemic factors. For example, some adhesion molecules are upregulated, whereas others are downregulated, by cortisol (5, 6).

Adhesion is mediated by two general classes of molecules: selectins and integrins. Selectins are responsible for the slowing down and rolling of circulating leukocytes near sites of adhesion (7, 15). This slowing gives local chemotactic factors a chance to activate integrins that provide firm attachment to various ligands generally found on the endothelium (7). In this model for leukocyte migration to sites of inflammation, the two cellular adhesion molecules work in concert to achieve leukocyte adherence. Uksila et al. (24) demonstrated that leukocyte adhesion in different tissues for leukocyte storage was dependent on variable expression of many different adhesion molecules for cell type-specific adhesion. From this, we infer that release of leukocytes from sites of storage into the circulation also occurs in a specific manner according to the profile of adhesion molecules.

L-selectin and very late antigen-4 (VLA-4) integrin are among the many leukocyte adhesion molecules that influence the trafficking of leukocytes. L-selectin is expressed on many leukocytes and binds to carbohydrate structures on endothelial cells that are either in peripheral lymph nodes or activated by inflammation (15). The VLA-4 integrin is a β2/α4-subunit complex (CD49d) involved in directing the migration of leukocytes to inflammation and lymphoid tissues (11). Vascular cell adhesion molecule-1 and fibronectin are VLA-4 ligands. Reduced localization of leukocytes at sites of inflammation has been demonstrated in mice lacking L-selectin (7). Reduced lymphocyte adhesion to activated endothelial cells has been measured after VLA-4 blockade with antibodies (11). Thus, L-selectin and VLA-4 are important in leukocyte adhesion and may influence leukocyte redistribution during exercise.

Therefore, investigation of L-selectin and VLA-4 may help elucidate the mechanism of differential recruitment of various leukocyte populations to the circulation during exercise. Exercise disproportionately influences trafficking of natural killer (NK) cells and granulocytes (see Ref. 17 for review). Adhesion by NK cells is particularly dependent on VLA-4 (10), and adhesion by neutrophils is particularly dependent on L-selectin (5, 6). Thus, VLA-4 integrin has particular relevance to the investigation of exercise and leukocyte adhesion molecule expression. We hypothesized that cell types entering the circulation preferentially during exercise would have a distinct profile of adhesion molecules compared with those cell types less affected by exercise. Consequently, the L-selectin and VLA-4 expression on circulating leukocytes would change to favor cell types entering the circulation during exercise. The purpose of this investigation was to determine whether the influx of neutrophils and lymphocytes during brief, heavy-resistance exercise changed the expression of L-selectin and VLA-4 on these two leukocyte populations (experiment 1) and on specific lymphocyte subsets (experiment 2). Experiment 2 was carried out to follow up on the findings of experiment 1.
METHODS

Subjects. This investigation was approved by the Institutional Review Board for the Use of Human Subjects at Pennsylvania State University. Healthy women between the ages of 18 and 35 yr were recruited as subjects, and they gave written informed consent before participating. Two separate experiments were performed by using the same subject-testing protocol; 29 and 70 women participated in experiments 1 and 2, respectively. Descriptive data for both groups are given in Table 1.

Exercise. A brief, heavy, squat-resistance exercise was chosen for this investigation because it elicits quick and dramatic increases in exercise-related stimuli and provides a similar stress among subjects relative to their strength and body mass. At least 3 days before the squat-performance test, the one-repetition maximum (1 RM) for the squat exercise was determined for each subject. Subjects were coached in proper squat technique at this time. On the day of the squat-performance test, subjects reported to the laboratory between 630 and 1300 after at least 4 h of fasting. After the resting-condition blood sample was collected, subjects performed 2–3 min of low-tension stationary cycling as a warm-up before beginning the squat exercise. The squat mass during the performance test was equal to 75% of the 1 RM. Subjects began in the fully upright position and lowered the weight until the femur reached a position parallel to the floor. Six sets of 10 repetitions were performed, with 2 min of active rest between sets. If subjects were unable to complete 10 repetitions within a set at the starting weight, a small amount of weight was removed for subsequent sets. The squat exercise and determination of 1 RM were performed by using a computerized Smith-like machine (Plyometric Power System, Lismore, New South Wales, Australia) previously described in detail (27). Completion of the squat-performance test took ~15 min.

Blood collection. Peripheral blood was collected from a forearm vein by using a 20-gauge needle and a standard venipuncture technique. Samples were collected immediately pre- and within 5 min postexercise into 5-ml vacuum tubes containing 0.5% EDTA anticoagulant (Becton Dickinson, Franklin Lakes, NJ). Two tubes were collected for the assays in this investigation: one for labeling leukocytes and one for complete blood count (CBC). Subjects were lying down during collection of blood pre- and postexercise.

Table 1. Subject characteristics

<table>
<thead>
<tr>
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<th>Experiment 1</th>
<th>Experiment 2</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>29</td>
<td>70</td>
</tr>
<tr>
<td>Age, yr</td>
<td>22.9 ± 3.6</td>
<td>23.4 ± 4.2</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.65 ± 0.07</td>
<td>1.66 ± 0.07</td>
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<tr>
<td>Mass, kg</td>
<td>61.3 ± 8.7</td>
<td>64.9 ± 10.1</td>
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<tr>
<td>Squat 1 RM, kg</td>
<td>52.3 ± 13.9</td>
<td>51.6 ± 11.2</td>
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</tbody>
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Values are means ± SD; n, no. of women; 1 RM, 1 repetition maximum.

Leukocyte labeling. Within 6 h of collection, leukocytes were labeled for two-color analysis by using FITC- and phycoerythrin (PE)-conjugated monoclonal antibodies (Becton Dickinson Immunocytometry Systems, San Jose, CA) and a whole blood staining method. In experiment 1, CD62L-FITC and CD49d-PE were used to determine the percentages of lymphocytes and neutrophils bearing L-selectin and VLA-4, respectively. Additionally, CD3-FITC, CD19-PE, and CD16 + 56-PE were used to determine percentages of T, B, and NK (CD3−) lymphocytes, respectively. In experiment 2, additional stains, including CD3-FITC/CD49d-PE and CD3-PE/CD62L-FITC, were used to elucidate which lymphocyte subsets had VLA-4 and L-selectin adhesion molecule surface expression. A 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 lymphocyte and neutrophil populations.

With a minor modification for the use of a new flow cytometer in experiment 2, the same double-labeled staining procedure was used for all samples. Briefly, 10 µl of a FITC-labeled and 10 µl of PE-labeled antibody, or 10 µl of a Simultest FITC and PE antibody pair, were pipetted into a tube, and 100 µl of whole blood were 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 were 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, and the cells were washed with 1 ml PBS without magnesium or calcium, centrifuged, and vacuum aspirated again. The pellet was resuspended in a fixative solution of 1% formaldehyde in PBS without magnesium or calcium. In experiments 1 and 2, the samples were resuspended in 100 and 500 µl of 1% formaldehyde, respectively. Samples were refrigerated and stored until they were analyzed by flow cytometry, usually within 1 day, but always within 3 days of labeling.

From each stained sample in experiment 1, 10,000 events were collected by using an EPICS 753 flow cytometer (Coulter). Raw flow data were analyzed by using EPICS software (version 4.0, Coulter) to determine proportions of fluorescently labeled lymphocytes. In experiment 2, neutrophils were not analyzed, and 5,000 events within the lymphocyte scatter gate were collected by using a model XL flow cytometer (Coulter). Raw data were analyzed by using Coulter System II (version 1.0, Coulter). Concentrations of cells with each surface marker were calculated by multiplying the neutrophil or lymphocyte concentration by the percentage with positive fluorescence for cell surface marker. To account for contamination by platelets and debris, we corrected lymphocyte percent by dividing the raw percent by the CD45+ proportion of the lymphocyte scatter gate. Neutrophil purity was generally >99%; therefore, no correction was necessary.

Flow cytometric analyses were reliable from test to test. To determine the reliability of the assay, two separate blood samples were collected from eight women in the resting condition. For the proportions of CD3+ /CD62L+, CD3−/CD62L+, CD3+/CD49d+, and CD3−/CD49d+ lymphocytes, no differences were found between the first and second samples (paired t-test), and Pearson product-moment correlation coefficients were all greater than r = 0.9. To determine the test-retest reliability of the exercise response, we performed the same analyses for a sample of 10 of the subjects in...
experiment 2 who repeated the exercise 3 mo later. No differences were found between the first and second blood samples from the resting condition or between the first and second postexercise samples, except for CD3+/CD62L+/lymphocytes (means were within 5% of each other). Pearson product-moment correlations for paired data from the two separate testing sessions ranged from r = 0.67 to r = 0.77. These data indicate that the measures used in this investigation were stable and consistent for test-retest within the same day for resting condition samples and on separate days for the exercise response.

Statistical analysis. Means ± SD were calculated. Differences between pre- and postexercise measures were detected by using Bonferroni alpha-corrected paired t-tests. Significance was set at alpha = 0.05.

RESULTS

Exercise increased (P < 0.001) the concentration of lymphocytes and neutrophils in both experiments 1 and 2 (Table 2). In experiment 1, neutrophil concentration increased 41.8% with a net increase of 1.71 ± 1.13 cells × 10⁹/liter. Most neutrophils expressed L-selectin (CD62L+), and this percent did not change during the exercise (Table 3). Very few neutrophils expressed VLA-4 (CD49d−) pre- or postexercise. A small percent (21%) did not express either adhesion molecule. The concentration of CD62L+ neutrophils increased from pre- to postexercise (P < 0.001; Fig. 1).

In experiment 2, percentages of T, B, and NK lymphocytes in the resting condition were 74.7 ± 6.3, 8.8 ± 2.8, and 13.9 ± 7.2%, respectively. Although the majority of lymphocytes in the circulation at rest were T cells, the lymphocyte increase during exercise was attributable to roughly equal influxes of additional T and NK cells (Table 2).

Lymphocytes expressing either or both L-selectin and VLA-4 increased in concentration (P < 0.001) in experiments 1 and 2. To avoid duplication, only the data from experiment 2, in which additional lymphocyte parameters were measured, are presented. There was an increase (P < 0.001) in the concentration of lymphocytes expressing VLA-4 (Fig. 2). The percentage of lymphocytes expressing VLA-4 was ~95% pre- and postexercise (Table 3). The percentage of lymphocytes coexpressing VLA-4 and L-selectin decreased, as did the percentage of lymphocytes expressing only L-selectin (P < 0.001 for both). Thus, in contrast to neutrophils, there was a decrease in the percentage of lymphocytes expressing L-selectin. The decreased percentage of CD62L+ lymphocytes was a function of a decreased proportion of T cells and an increased proportion of NK cells postexercise (Fig. 3). The CD62L+ proportion of T cells (CD3+) and the combined B and NK cell population (CD3−) both decreased (P < 0.001). However, the concentration of all subsets expressing CD49d and/or CD62L increased (P < 0.001) (Fig. 4). The change in concentration for subset populations indicates that the decrease in percentage of CD62L+ lymphocytes occurred because ~50% of the incoming cells were CD62L+ positive compared with 73.5 and 85.2% of CD3+ and CD3− cells at rest, respectively (Fig. 3).

The surface density of L-selectin was altered slightly on neutrophils but not on lymphocytes. In experiment 1,

| Table 3. Percentages of leukocytes expressing L-selectin and VLA-4 integrin |
|-----------------|-----------------|-----------------|-----------------|
|                  | L-Selectin       | L-Selectin      | VLA-4           |
|                  | Only, %          | and VLA-4, %    | Only, %         |
|                  | (Neutrophils = 29) | (Lymphocytes = 70) | (Lymphocytes = 70) |
| Preexercise      | 7.86 ± 14.3      | 7.0 ± 5.9       | 0.8 ± 1.1       |
| Postexercise     | 7.90 ± 16.8      | 6.7 ± 4.9       | 0.6 ± 0.7       |
| Neutrophils (n=29) | 5.8 ± 4.9        | 62.3 ± 8.1      | 32.4 ± 8.4      |
| Lymphocytes (n=70) | 4.9 ± 2.2        | 50.1 ± 8.9*     | 44.5 ± 9.0*     |

Values are means ± SD; n, no. of women. VLA-4, very late antigen 4. *P < 0.001 compared to preexercise.
the mean log fluorescence intensity of CD62L on neutrophils increased (P < 0.05) from 3.8 ± 1.1 to 4.0 ± 1.2 relative units on a three-decade log scale. In experiments 1 and 2, the mean log fluorescence intensity of CD49d or CD62L on lymphocytes did not change from pre- to postexercise.

Plasma cortisol concentrations were 984.9 ± 444.2 and 963.9 ± 433.9 µg/dl pre- and postexercise, respectively. The postexercise decrease was not significant.

DISCUSSION

Brief heavy-resistance exercise caused large increases in neutrophils and lymphocytes in the circulation. The same proportion of neutrophils, but a decreased proportion of lymphocytes, expressed L-selectin after the exercise. Neutrophils do not express VLA-4 (1), but the majority of lymphocytes measured in the circulation did express this molecule. The observed differences between neutrophils and lymphocytes are consistent with the model proposed by Spertini et al. (22), in which activation and deactivation of adhesion molecules occurs in a leukocyte lineage-specific manner.

The neutrophilia and lymphocytosis measured in this investigation are consistent with the findings of other investigations. Kraemer et al. (12) measured similar neutrophil responses to a comparable resistance-exercise protocol and found that these changes were not linked to elevations in cortisol. The concentration changes in lymphocyte subsets measured in this investigation are slightly lower than, but comparable with, those measured by Nieman et al. (16) in response to exhaustive resistance exercise in men.

The fluorescence intensity of L-selectin positive neutrophils increased, indicating that the surface density of this molecule was greater on cells in the circulation during exercise compared with those at rest. It is
thought that the immediate rise in neutrophils during brief exercise is a result of catecholamine-induced demargination, whereas delayed neutrophilia is stimulated by elevations in cortisol (19). Additionally, decreased neutrophil adhesion to endothelial cells in vitro has been linked to cAMP-induced changes in the endothelium (4). Although modest delayed neutrophilia has been measured in the absence of elevated cortisol (21), neutrophils appear to enter the circulation from the bone marrow by shedding L-selectin in response to increased cortisol (5, 6). The exercise in this investigation was brief, cortisol was not elevated, and banded neutrophils (indicative of bone marrow release) did not increase. These findings suggest that the neutrophil increase represents demargination. Thus, our data suggest that the density of L-selectin on marginated neutrophils was greater than on circulating neutrophils during resting conditions.

The percentage of T and combined NK and B cell populations expressing L-selectin decreased. L-selectin-negative NK cells entering the circulation could account for nearly all of the decrease in L-selectin expression within the NK and B cell population. Kurokawa et al. (13) found that the CD8+ T cells that increased in the circulation during exercise were L-selectin negative. The same occurrence was likely in the present investigation, because there was a strong positive correlation between L-selectin-negative T cells and CD8+ T cells (r = 0.89, P < 0.001, data not shown). Decreased percentages of lymphocytes expressing L-selectin could occur because L-selectin expression on these cells was less in the noncirculating pool, or because L-selectin was shed before entering the circulation or while in the circulation.

The possibility that L-selectin was shed could have been further investigated by measuring soluble L-selectin in the plasma. However, our data provide evidence that the interpretation of increases in soluble L-selectin should be made carefully. For example, while the surface density of L-selectin on neutrophils increased, the proportion of lymphocytes expressing L-selectin decreased, and the concentration of both neutrophils and lymphocytes expressing L-selectin increased. Neutrophils leaving the bone marrow shed L-selectin before entering the circulation (25), and neutrophils leaving the circulation shed L-selectin before extravasation (26). Thus soluble L-selectin elevations could result either from neutrophils or lymphocytes entering or leaving the circulation or from L-selectin shedding by either population while in the circulation. As a result, it would be extremely difficult to determine which of these events contributed to the increase in soluble L-selectin.

Our finding that 88% of T cells and at least 97% of the combined B and NK cell population expressed VLA-4 both pre- and postexercise cannot be used to suggest that lymphocyte trafficking during exercise is not influenced by this molecule. Adherence by VLA-4 (7) and other integrins (23) is altered by induced changes between low- and high-affinity conformations. In our investigation, discrimination between conformations of VLA-4 was not possible by using the CD49d antibody. Decreased NK cell adhesion (3) and increased NK cell concentration in the circulation (20) have been measured in response to β2-adrenergic stimulation. β2-Adrenergic stimulation can alter the density of several different adhesion molecules (20). However, in addition to finding the same surface density of VLA-4 before and after exercise, we also found that in vitro addition of L-epinephrine to whole blood did not change the density of VLA-4 (data not shown).

In conclusion, we found that the proportion of neutrophils expressing L-selectin did not change during brief, heavy-resistance exercise. However, the surface density of L-selectin was greater for the neutrophils in the circulation after the exercise. These findings suggest that same proportion of expression, but a greater surface density of L-selectin on neutrophils, may be in the marginated pool. L-selectin expression decreased on T cells and on the combined B and NK cell population in the circulation. Potential mechanisms for enrichment of L-selectin negative lymphocytes in the circulation during exercise include the following possibilities: 1) selective recruitment of cells not expressing L-selectin; 2) selective removal of L-selectin positive lymphocytes from the circulation; or 3) lymphocyte...
specific shedding of L-selectin. The expression of VLA-4 was high on nearly all lymphocytes, suggesting either that this molecule has no role in trafficking during exercise or that its role in trafficking is a function of a conformational change or of epithelial ligand activation.

We thank all the students who assisted in the collection of these data, especially Brad Nindl and Linc Gotshalk, as well as the subjects who gave their time and energy to participate. This project was supported by Department of Defense US Army Grant DAMD 17-95-C-5069 (to W. J. Kraemer). The assistance of the Pennsylvania State General Clinical Research Center at the Noll Physiology Laboratory, supported by National Institutes of Health Grant M01-RR-10732, in providing complete blood counts in experiment 2, is appreciated.

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Received 14 August 1997; accepted in final form 20 January 1998.

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