The Acute Response of Apoptosis and Migration to Resistance Exercise Is Protocol-Dependent

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Abstract

The aim of the present study was to compare the acute effects of resistance exercise (RE) designed for hypertrophy or local muscle endurance (LME) on CD4+ and CD8+ T cell apoptosis and migration. 14 untrained subjects (age 20.5 ± 0.8 years, body mass 70.0 ± 12.8 kg, body mass index 24.0 ± 3.2 kg/m²), women (N = 11) and men (N = 3) completed 2 RE sessions (3 sets of 9 exercises) designed for hypertrophy at 10 repetitions maximum (RM) and LME at 60% of 10RM with 1-min rest-intervals between sets and exercises. The investigated lymphocytes were: CD4+, CD4+/CD69RA+, CD8+ and CD8+/CD69RA+ with cell surface markers annexin V and CX3CR1 analyzed by flow cytometry. Percentage of CD4+ positive for annexin V+ were higher immediately following and 24h after the hypertrophy protocol as compared with LME, while CD4+ positive for CX3CR1 were higher immediately after and lower at the 24h time point after LME as compared with the hypertrophy session. CD8+ lymphocytes responded similarly to the hypertrophy and LME protocols with elevations in both cellular migration and cell death immediately following and 24h after the bouts (p ≤ 0.05). Considering that the acute response of CD4+ lymphocytes to RE is protocol-dependent, a gradual adaptation to a hypertrophy program could minimize the effect on CD4+ lymphocytes and reduce the potential susceptibility to antigens during this timeframe. This would also be interesting for a RE program designed for LME based on the observed CD8+ lymphocyte response.

Introduction

Resistance exercise (RE) stimulates adaptational processes that are expressed through increases in strength, power, hypertrophy, and local muscular endurance [1], while the achievement of specific goals and physiological responses are emphasized through manipulation of prescriptive variables such as the modality, load, volume, exercise order, rest interval and types of strength to be trained [1]. Although the positive effects of RE on muscle strength, muscle mass, functional capacity, blood pressure, body composition and health-related parameters have been demonstrated [1, 12, 17, 18], less is known on the modulating effects of this exercise modality on immune cell markers of migration and apoptosis. Previous studies that focused on immune cell function were designed to induce muscle damage or were performed with training methodologies that may not reflect practical conditions [5, 9]. Thus, Pereira et al. [11], highlighted the importance of characterizing and describing the immune response to acute RE, while it may aid the future design and dose-response prescription. In this sense, the blood lymphocyte count is known to fall below resting values (lymphocytopenia) within 30 min to 2 h after exercise cessation and return naturally to normal values within 6–24 h [10]. Part of this decrease in lymphocytes may be associated with programmed cell death (apoptosis), movement of cells from the circulation (migration) or a combination of both [6, 8]. Annexin V is a member of the annexin family of intracellular proteins that binds to phosphatidylserine and can be used to identify apoptotic cells [13]. Moreover, lymphocytes naturally migrate to and from the lymphoid pools to maintain the immuno pool. This migration process may be identified by the expression of CX3CR1 (known as fractalkine, neurotactin), since the interaction of CX3CR1 to its ligand mediates cell adhesion and migration [4].

References

A recent study from Pereira et al. [11], found significant apoptosis and migration of CD4+ and CD8+ lymphocytes up to 24 h after RE, with minimal effects of rest interval length. Nevertheless, it remains to be determined how the different RE protocols would affect apoptosis and migration in CD8+ and CD4+ T cells subset. Interestingly, when comparing 3 different RE sessions, designed for hypertrophy, local muscle endurance or maximal strength, Asano et al. [2], revealed that the hypertrophy protocol induced a higher increase of creatine kinase (indicator of muscle damage), lactate concentration and hypoglycemia in untrained subjects. Thus, the aim of the present study was to compare the acute effects of RE designed for hypertrophy or LME on CD4+ and CD8+ T cell apoptosis and migration in untrained individuals. We hypothesized that the hypertrophy protocol would induce a higher modulation on cell surface markers for apoptosis and migration in CD8+ and CD4+ T lymphocytes up to 24 h of recovery.

Methods
Subjects
14 healthy untrained young subjects (age 20.5 ± 0.8 years, body mass 70.0 ± 12.8 kg, body mass index 24.0 ± 3.2 kg/m², body fat percentage 15.5 ± 6.2, fat-free mass 58.6 ± 9.4 kg), volunteered to participate in the study. The power of sample is mentioned in the statistical methods. Men and women were included in the present study as it has been previously shown that neither gender nor menstrual cycle phase influence exercise-induced lymphocyte apoptosis in untrained subjects [8]. Following the classifications from the ACSM [1], subjects were considered untrained, as they had no previous experience with regular RE. Subjects using medical drugs, dietary supplements, anabolic steroids, or women taking any oral contraceptives for at least 6 months before the study were excluded. Additionally, the recruited subjects had to be free of immune, muscular or cardiovascular diseases. Voluntarily written informed consent was obtained, and the study was approved by the Human Subject Review Board of Western Kentucky University. Additionally, the study met the ethical standards proposed by of the International Journal of Sports Medicine [3].

Study design
Subjects underwent 8 testing sessions and were advised to refrain from strenuous exercise during the study. A summary of the experimental design is presented in Fig. 1. Before each RE session, subjects rested for 10 min, and a blood sample was obtained (PRE) by finger-prick procedures [6]. Subjects also had their blood collected immediately post-exercise (POST), 2 h and 24 h after exercise. Lymphocytes subsets investigated were CD4+, CD4+/CD69RA+, CD8+ and CD8+/CD69RA+ with cell surface markers for apoptosis (annexin V) and migration (CX3CR1).

Familiarization to RE and testing
Subjects received standardized instructions on RE technique and performed one exercise for each main muscle group with 3 sets of 10–12 submaximal repetitions. 72 h after the second familiarization session, subjects underwent the 10RM tests for all exercises using a counterbalanced order on 4 separated days with a minimum of 3 days between them (Fig. 1). The heaviest load achieved on the test days was considered as the initial 10RM. No exercise was allowed in the 72 h between tests to avoid interference in the test-retest reliability. Additionally, to minimize the error during tests, the following strategies were adopted according to Tibana et al. [16]: a) standardized instructions concerning the testing procedure were given to the participants before the test; and b) the mass of all weights and bars used were determined using a precision scale. The 10RM were determined in fewer than 5 attempts with a rest interval of 5 min between them, and 10 min was allowed before the beginning of the next testing exercise. On each test and re-test trial, 4 or 5 exercises were tested using a counterbalanced order, alternating lower and upper limbs. All test-retest and training sessions were performed at the same time of the day. The intraclass correlation coefficient (model 1,1 one-way random single measures) revealed an optimal reliability for all exercises (R = 0.99).
Experimental sessions
5 days after the familiarization and testing sessions, subjects were submitted to a second baseline blood sample at the same time of the day. As expected, this guaranteed that the familiarization period did not modify the immune parameters to be evaluated. Following a warm-up set of 10 repetitions of chest press at 40% of 10RM, subjects randomly (simple randomization) completed the hypertrophy or LME RE sessions according to the recommendations of the ACSM [1] with one week between each.
Experimental sessions consisted of 9 exercises in the following order: chest press, leg press, front lat pull-down, seated leg extension, upright row, seated leg curl, triceps extension, calf press and biceps curl (Cybex International, Medway, MA, USA). For all exercises, 3 sets were performed at 10RM in the hypertrophy session and at 60% of 10RM in the LME session with a rest interval of 1 min between sets and exercises. The mean duration of the sessions was 50–60 min. Table 1 summarizes the total repetitions performed in the 3 sets and the training volume (total repetitions × load) in each exercise for the hypertrophy and LME sessions. In addition, the subjects were encouraged to avoid smoking, alcohol, caffeine, unusual physical activity and to maintain their usual daily activities, including sleeping hours.

Blood analysis
Flow cytometry antibodies and buffers were obtained from e-Bioscience (San Diego, Calif., USA). The investigated lymphocytes subsets were: CD4+, CD4+/CD69RA+, CD8+ and CD8+/CD69RA+ with cell surface markers annexin V and CX3CR1. Finger-prick blood samples were obtained before familiarization, PRE, POST, 2 h and 24 h after the experimental sessions. The protocol of blood analysis for lymphocyte subsets has been described previously [6]. Whole blood (20μL) was added to an appropriate antibody panel (250μL) and incubated in a dark room for 30 min. After incubation, samples were centrifuged for 5–10 min, decanted and thoroughly vortexed before the addition of the red blood cell lysis buffer. After the 10 min lysis period, phosphate buffered saline was added and samples were centrifuged, decanted, and vortexed before analysis by flow cytometry (C6, Accuri, Ann Arbor, Mich., USA). Cell apoptosis and migration were determined through the use of annexin V (BioLegend, San Diego, Calif., USA) and CX3CR1 (BioLegend, San Diego, Calif., USA), respectively. At least 10000 events were counted in the lymphocyte gate, initially determined from front and side-scatter characteristics. Further gating to distinguish CD4+ and CD8+ populations was determined in the fluorescence channel (FL-2) via the phycoerythrin (PE) fluorochrome.

Statistical analyses
In all calculations, the alpha level was set to p ≤ 0.05. The Shapiro-Wilk normality test and the homoscedasticity test were used. To control for sphericity, the correction of Greenhouse-Geisser was applied. Absolute changes from rest (Δ baseline) values were calculated according to the following formula: \[(\text{measure-baseline}) \cdot \text{baseline-1} \] · 100. As we expected the absolute change from rest with regard to apoptotic and migratory markers to be similar to the change in cell volume, the Chi squared test (χ²) was utilized. As previously described, based on our previous investigation [7], a large effect size was anticipated. However, to employ a conservative approach, sample size was calculated using a medium effect size corresponding to a 25% relative increase in post-exercise apoptosis. To detect this increase in lymphocyte apoptosis, it was determined that a minimum of at least 8 subjects was necessary. The statistical analyses were performed using SPSS 20.0 (SPSS, Chicago, IL).

Results
The number of repetitions and total volume in each exercise are presented in Table 1. The total number of repetitions in chest press (p < 0.001), leg press (p < 0.001), front lat pull-down (p ≤ 0.001), knee extension (p < 0.001), upright row (p < 0.001), leg curl (p ≤ 0.001), calf press (p ≤ 0.001), triceps extension (p ≤ 0.001) and biceps curls (p ≤ 0.001) were significantly higher in the LME protocol compared to the hypertrophy protocol. The total volume for chest press (p = 0.002), leg press (p = 0.05), front lat pull down (p = 0.009), upright row (p = 0.001), leg curl (p < 0.001), triceps extension (p = 0.004) and biceps curl (p = 0.05) exercise was significantly higher in LME as compared with hypertrophy, with no differences for knee extension (p = 0.25) and calf press (p = 0.13) exercises.

CD4+ cell counts displayed no statistical changes after hypertrophy (p = 0.26) and LME protocols (p = 0.326) (Fig. 2a, b). Nonetheless, there was an increase in the percentage of CD4+ positive for annexin V and CX3CR1 cells immediately after and 24 h post hypertrophy, with no differences in the 2 h post time point (Fig. 2a, b).

Percentage of CD4+ for annexin V decreased immediately after and increased 24 h post LME session (Fig. 2b), while CD4+ cells for CXCR1 only increased immediately after (Fig. 2b). There was no difference in CD4+ marked with CX3CR1 2 h and 24 h post LME (Fig. 2b).

CD8+ total cell count presented no statistical change after hypertrophy (p = 0.09), but was significantly increased following the LME protocol (p = 0.018) (Fig. 2c, d). Additionally, there was a significant increase in CD8+ for annexin V and CX3CR1 immediately after, 2 h and 24 h post hypertrophy (Fig. 2c, d). Similar results were found after the LME protocol, except for the

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Table 1: Total number of repetitions and volume for Hypertrophy and Local Muscle Endurance (LME) protocols.

<table>
<thead>
<tr>
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<th>Hypertrophy</th>
<th>LME</th>
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<tr>
<td><strong>Total number of repetitions</strong></td>
<td></td>
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<tr>
<td>chest press</td>
<td>26.4 ± 1.0</td>
<td>43.6 ± 1.7</td>
<td>&lt;0.001</td>
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<tr>
<td>leg press</td>
<td>36.7 ± 1.7</td>
<td>63.2 ± 2.8</td>
<td>&lt;0.001</td>
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<td>front lat pull-down</td>
<td>28.0 ± 1.0</td>
<td>51.5 ± 3.1</td>
<td>&lt;0.001</td>
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<tr>
<td>knee extension</td>
<td>29.0 ± 1.4</td>
<td>43.2 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>upright row</td>
<td>26.5 ± 1.1</td>
<td>46.5 ± 2.1</td>
<td>&lt;0.001</td>
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<tr>
<td>leg curl</td>
<td>28.3 ± 1.0</td>
<td>48.5 ± 1.8</td>
<td>&lt;0.001</td>
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<tr>
<td>triceps extension</td>
<td>26.0 ± 1.8</td>
<td>50.7 ± 3.2</td>
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<td>calf press</td>
<td>38.2 ± 2.4</td>
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<td>biceps curl</td>
<td>27.0 ± 1.5</td>
<td>43.9 ± 1.6</td>
<td>&lt;0.001</td>
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<td><strong>Total volume (kg)</strong></td>
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<td>1009.6 ± 87.8</td>
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<td>1901.9 ± 206.5</td>
<td>0.25</td>
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<tr>
<td>upright row</td>
<td>5085.8 ± 27.4</td>
<td>6319.9 ± 37.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>leg curl</td>
<td>1442.8 ± 72.9</td>
<td>1761.4 ± 115.4</td>
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<td>31740.0 ± 238.2</td>
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<td>biceps curl</td>
<td>542.0 ± 75.6</td>
<td>616.8 ± 36.6</td>
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Data are means ± SEM. *Significantly different from Hyp-1, P < 0.05.
Discussion

The purpose of this investigation was to observe the T lymphocyte immune response induced by RE protocols designed toward hypertrophy compared to LME. We hypothesized that the hypertrophy protocol would influence cell surface markers for apoptosis (annexin V) and migration (Cx3CR1) to a greater extent than the LME protocol. The main findings are as follows: 1) the LME protocol induced an increase in cellular migration of CD4+ lymphocytes immediately following the RE bout, whereas the hypertrophy protocol induced increases in both migration and cell death immediately after; 2) at 24 h following the LME protocol both cell migration and apoptotic CD4+ lymphocytes were significantly elevated, while no differences were noted with either marker 24 h following the hypertrophy protocol; 3) CD8+ lymphocytes responded similarly to the hypertrophy and LME protocols with elevations in both cellular migration and cell death noted immediately post RE as well as 24 h after the bouts. Thus, our initial hypotheses were partially correct.

Regarding CD4+ lymphocytes, we found that the hypertrophy RE protocol induced significant changes in both biomarkers of interest (annexin V and Cx3CR1) immediately post-exercise, whereas the LME protocol displayed changes in cell migration only. We have begun describing the relationship between cell volume, cell death and cell migration in terms of the expected change (i.e., the change from baseline) [7, 11], as others have proposed that the post-exercise lymphocytopenia observed following a bout is due only to extravasation [14]. Within this framework, it would be reasonable to conclude that the change...
in apoptotic cells would match the change in cell volume. However, our previous findings indicate an elevation in the apoptotic cell count at various exercise modalities and intensities. For example, during incremental treadmill running the change in CD4+ apoptotic cells were significantly increased at 76% and 87% VO\textsubscript{2}max, but not evident immediately following exercise [7]. The reason for this is that a much greater cellular migration response is evident at higher intensities (87% VO\textsubscript{2}max) and serves to remove the apoptotic CD4+ cells from the vasculature. This is similar to the pattern found in the LME protocol design of the present investigation, where only changes in cellular migration in CD4+ cells were noted immediately following the bout. A similar observation was recently reported in CD4+ lymphocytes with regard to RE programs designed for hypertrophy comparing 1-min and 3-min rest periods between sets [11]. The protocol that employed a greater rest period (3-min) displayed no evidence of CD4+ apoptotic cell changes immediately post-exercise, whereas increased apoptotic and migratory markers were reported utilizing the protocol with shorter rest [11].

Resistance training protocols which focus on hypertrophy vs. LME have been compared in the literature [2]. Asano et al. [2], utilized healthy men who had no history of strength training, and subjected them to hypertrophy protocols (1–3 sets of 10–12 repetitions at 70% of the 1RM with 1.5-min rest intervals) and muscular endurance protocols (15 repetitions at 60% of 1RM with 1-min rest intervals). They found that the hypertrophy protocol elicited higher responses from the cardiovascular system (heart rate and systolic blood pressure) and the metabolic measures of blood lactate and creatine kinase when compared to the LME protocol [2]. While these factors were not measured in the present investigation, it is tempting to speculate that a resistance training program designed for hypertrophy represents higher mechanical and metabolic stress which affected the immune measurements obtained in the current study.

Krüger et al. [5], suggest a major role of cortisol in mediating apoptosis after intensive resistance training (75% of 1RM). On the other hand, attenuation of apoptosis by adding mifepristone into media demonstrated that glucocorticoids receptors are involved in apoptosis signaling. It was often speculated that an

Fig. 3 Comparison data between hypertrophy and local muscle endurance protocols (average ± SEM) is for apoptosis (annexin V), and cellular migration (CX3CR1) in CD4+ (panels a and b) and CD8+ lymphocytes (panels c and d) immediately following the resistance exercise and 2 h and 24 h following bouts. Significance was determined using the Chi squared test (χ\textsuperscript{2}) comparing the observed changes in annexin V and CX3CR1 to the expected changes in cell count. *Indicates significant difference between protocols at p = 0.01.

increase in cortisol contributes to post-exercise lymphopenia by affecting lymphocyte migration patterns or by increasing lymphocyte apoptosis. Regarding the hormone response, Smilios et al. [15], examined the effects of the number of sets on testosterone, cortisol, and growth hormone (hGH) responses after maximum strength (MS), muscular hypertrophy (MH) and LME protocols. Cortisol and hGH were higher after the hypertrophy trial than the strength and hypertrophy workouts. Furthermore, cortisol and hGH were higher after the LME workout as compared with the strength and hypertrophy workouts. Taken together these results may explain, at least in part, the differences in apoptotic response to hypertrophy and LME protocols.

Regarding CD8+ lymphocytes, we found significant elevations in markers of cell death and cellular migration following both types of RE protocols. This finding is not surprising, as CD8+ lymphocytes have been reported to respond to a greater extent to exercise compared to their CD4+ counterparts, likely due to the effect of epinephrine and the greater volume of B2-adrenergic receptors known to be present on cytotoxic T cells. Previous research utilizing incremental treadmill running to exhaustion has reported increases in CX3CR1, but not annexin V, in CD8+ lymphocytes immediately following exercise [7]. These findings are different compared to the present investigation and likely due to the exercise protocol used (i.e., primarily aerobic treadmill running vs. resistance exercise employing upper and lower body muscle groups). The continuous characteristic of the aerobic protocol (higher time under stress) vs. intermittent during RE may have possibly contributed to these differences. Pereira et al. [11] found a similar CD8+ lymphocyte response with regard to elevated migratory and cell death markers immediately following muscular hypertrophy protocols that utilized 1- and 3-min rest periods. The findings of the present investigation confirm that this immune response in CD8+ cells extends to RE protocols that are designed for LME as well.

In conclusion, this investigation is the first to report that the acute response of CD4+ lymphocytes to resistance exercise is protocol-dependent. While future research should be directed toward the clinical implications of this finding, exercise practitioners should be aware of this immune response when prescribing resistance training protocols to novice individuals at the onset of a program. It is possible that a gradual accommodation period to a hypertrophy program as prescribed by the American College of Sports Medicine [1] could blunt the effect on CD4+ lymphocytes observed in the post-exercise period and reduce the potential susceptibility to antigens during this timeframe. In addition, a similar accommodation period for resistance exercise programs designed for local muscular endurance would be recommended based on the observed CD8+ lymphocyte response.

**Conflict of interest:** The authors have no conflict of interest to declare.

**References**