Adaptation to Eccentric Exercise: Neutrophils and E-selectin During Early Recovery

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Catalog Data

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Mots-clés: myosite, lésion du muscle, adhésion leucocytaire

Abstract/Résumé
The purpose was to determine the responses of blood neutrophils and E-selectin concentrations during early recovery (< 24 hr) from 2 bouts of eccentric exercise. Subjects (N = 9) completed 2 bouts of eccentric arm exercise using their non-dominant arm (Bout 1 and Bout 2) and 1 non-exercise control condition. The exercise bouts were separated by 4 weeks, and the control condition preceded Bout 1. Neutrophil concentrations were significantly higher at 3, 6, and 9 hr post-exercise for Bout 1 relative to Bout 2 and control. No significant changes in blood E-selectin concentrations were observed. Isometric strength deficit was similar for Bout 1 and Bout 2 at 5 min and 3 hr post-exercise and was significantly greater for Bout 1 relative to Bout 2 at 6, 9, and 24 hr post-exercise. The adaptation to eccentric exercise is associated with a lower concentration of blood neutrophils during early recovery. The neutrophilia associated with novel eccentric arm exercise precedes secondary changes in isometric strength and is not associated with changes in the concentration of blood E-selectin.

Le premier objectif de cette étude est d’évaluer l’adaptation des neutrophiles sanguins et la concentration de sélectine-E au cours des 24 h suivant deux séances d’exercices pliométriques. Les neuf sujets participent aux deux séances d’exercices pliométriques du bras non dominant (S1 et S2) et à une séance de contrôle. La séance de contrôle précède S1 espacée de S2 par 4 semaines. Les concentrations de neutrophiles sont significativement

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plus importantes 3, 6 et 9 h après S1 qu’après S2 et la séance de contrôle. Il n’y a pas de variation significative des concentrations de sélectine-E. La diminution de force isométrique après 5 min et après 3 h de récupération est semblable au cours des deux séances mais elle est significativement plus importante 6 h, 9 h et 24 h après S1 qu’après S2. En conclusion, l’adaptation aux exercices pliométriques est associée à une plus faible concentration de neutrophiles dans les 24 h suivant la séance d’exercices. De plus, les neutrophiles observés au cours de la deuxième séance sont présents avant la diminution subséquente de force isométrique et ne sont pas associés aux variations de la concentration de sélectine-E.

Introduction

Skeletal muscle injury, induced by eccentric exercise, is associated with muscle inflammation, soreness, and a loss of joint range of motion and strength (Clarkson et al., 1992). The inflammatory response to novel eccentric exercise consists of neutrophilia, neutrophil activation, and the accumulation of neutrophils in injured muscle during early recovery (<24 hr; Fielding et al., 1993; MacIntyre et al., 2000; MacIntyre et al., 1996; Pizza et al., 1996; Pizza et al., 1995; Smith et al., 1989). Although the biological function of neutrophils in contraction-induced muscle injury is unknown, they may exacerbate the injury and/or initiate muscle regeneration.

An important preliminary step in ascertaining the biological function of neutrophils in contraction-induced muscle injury is the determination of the temporal relationship between blood neutrophil concentrations and isometric force deficit, a measure of the totality of muscle injury (Faulkner and Brooks, 1994). The adaptation to eccentric exercise provides an interesting model to study this relationship because responses to injurious eccentric exercise (Bout 1) can be compared to relatively non-injurious eccentric exercise (Bout 2).

Newham and colleagues (1987) have reported that the adaptation to eccentric exercise does not influence the initial isometric force deficit but rather, the force deficit during prolonged (> 24 hr) recovery. The isometric force deficit during early recovery (<24 hr), however, was not determined by Newham and colleagues (1987). Since blood (Pizza et al., 1996; Pizza et al., 1995, Smith et al., 1989) and muscle (Fielding et al., 1993; MacIntyre et al., 2000) neutrophil concentrations are elevated during early recovery (<24 hr), determining their temporal relationship to the isometric force deficit will provide insight on the role of neutrophils in contraction-induced muscle injury. We hypothesized that elevations in neutrophil concentrations would precede secondary changes in the isometric force deficit and that the adaptation to eccentric exercise would be associated with a lower concentration of neutrophils during early recovery.

Although neutrophils infiltrate skeletal muscle following eccentric exercise (Fielding et al., 1993; MacIntyre et al., 2000), little is known about the kinetics and the cellular events of neutrophil extravasation. Neutrophil extravasation consists of a complex series of events that are regulated and coordinated sequentially by adhesion molecules on neutrophils (e.g., CD11/CD18 and sialyl-Lewisa) and the expression of counter-receptors on endothelium (e.g., ICAM-1 and E-selectin; Kishimoto and Rothlein, 1994).

E-selectin, restricted to the luminal surface of endothelial cells, is rapidly (1–2 hr) induced by inflammatory cytokines (IL-1β and TNF-α; Newman et al.,
The induction of E-selectin promotes the rolling of the leukocyte along the surface of the endothelium and has been correlated with extravasation of neutrophils into inflammatory lesions (Kishimoto and Rothlein, 1994). The extracellular portion of E-selectin is released from endothelial cells, and its appearance in the blood is suggestive of endothelial cell activation (Newman et al., 1993). Since elevations of blood E-selectin concentration have been reported in a variety of systemic inflammatory conditions (Carson et al., 1993; Newman et al., 1993), we hypothesized that blood E-selectin concentrations would be elevated following novel eccentric exercise.

The primary purpose of the study was to determine the temporal relationship between neutrophils and maximal isometric force following 2 bouts of eccentric exercise. A secondary purpose was to determine the responses in blood neutrophils and E-selectin during early recovery (< 24 hr) from 2 bouts of eccentric arm exercise and a control non-exercise condition.

**Methods**

**SUBJECTS**

Nine untrained males participated in this study after signing an institutionally approved informed consent. Subjects were physically active without participating in activity specific to the forearm flexors for the previous 9 months.

**EXPERIMENTAL DESIGN**

Subjects completed 2 bouts of eccentric arm exercise using their non-dominant arm (Bout 1 and Bout 2) and 1 non-exercise control condition (CT). The exercise bouts were separated by 4 weeks, and CT preceded Bout 1. The testing protocol for CT duplicated the testing sequence of Bout 1 and Bout 2.

**eccentric arm exercise**

The exercise consisted of 25 maximal forced-lengthened contractions of the forearm flexors using a computerized arm-curl bench (Nosaka and Clarkson, 1996; Pizza et al., 1996). Subjects began with the forearm flexed, and the forearm was extended in 4 s. The subjects' forearm was then returned to the starting position without resistance. Peak and average force were determined for each eccentric contraction using a calibrated load cell (Omega International Corp., Stamford, CT) that was interfaced with a microcomputer.

**BLOOD SAMPLING AND ANALYZES**

Blood samples were obtained from an arm vein before and 3, 6, 9, and 24 hr post-exercise. Blood samples were taken from each subject at the same time of day for all conditions. Neutrophil concentrations were determined by performing complete and differential blood counts using standard laboratory techniques (Brown, 1973). Serum samples were analyzed for circulating E-selectin using an ELISA technique (R&D Systems, Minneapolis, MN).
ISOMETRIC STRENGTH AND MUSCLE SORENESS

Isometric strength was assessed before and 5 min, 3, 6, 9, and 24 hr post-exercise, whereas muscle soreness was assessed before and 3, 6, 9, and 24 hr post-exercise. Peak isometric strength was determined by having subjects contract the forearm flexors maximally when their arm was positioned at 90° of flexion. Muscle soreness was assessed by having an investigator passively move the subject’s arm 3 times through its range of motion. Subjects were asked to rate their soreness on a visual analog scale (VAS) from 1 (normal) to 10 (very, very sore). Subjects marked a point on the VAS, and the distance in millimeters from the beginning of the scale to the mark was measured and used as an indicator of muscle soreness. Indirect markers of muscle injury were measured twice, and the mean scores were used for statistical analyses. Previous investigators have established the validity and reliability of using the VAS for assessing pain (O’Connor and Cook, 1999), whereas we have demonstrated the reliability of our isometric strength assessment ($r = 0.96; N = 12$).

STATISTICAL ANALYSES

A repeated measures analysis of variance was used to analyze the main effects and the interaction effect for all dependent measures. The Huynh-Feldt Epsilon was applied to degrees of freedom to account for violation of the sphericity assumption. The Tukey post-hoc test was used to locate the differences between means when the observed F ratio was statistically significant ($p < .05$).

Results

ISOMETRIC STRENGTH AND MUSCLE SORENESS

Baseline isometric force was similar between CT and Bout 1; however, isometric strength did not completely recover prior to Bout 2 (Bout 1 = 206.2 ± 9.1 N, Bout 2 = 187.7 ± 11.6 N, CT = 196.4 ± 30 N). Because of differences in baseline isometric force between Bout 1 and Bout 2, relative changes in isometric force post-exercise are presented in Figure 1. The percent change in isometric force during recovery was calculated relative to baseline isometric force. The percent change in isometric force was significantly higher for CT relative to Bout 1 and Bout 2. Isometric strength deficit was similar for Bout 1 and Bout 2 at 5 min and 3 hr post-exercise and was significantly different between the bouts at 6 hr, 9 hr, and 24 hr post-exercise. Muscle soreness (Figure 2) was significantly higher for Bout 1 relative to Bout 2 and CT. Muscle soreness was higher at 24 hr post-exercise for Bout 2 relative to CT.

NEUTROPHILS AND E-SELECTIN

Blood neutrophils were significantly higher at 3, 6, and 9 hr post-exercise for Bout 1 relative to Bout 2 and CT (Figure 3). The concentrations of neutrophils were similar between Bout 2 and CT. Blood E-selectin concentrations were not influenced by eccentric exercise (Table 1).
Figure 1. Maximum voluntary isometric strength expressed as a percent change from baseline values. Mean ± SE. *significantly different from bout 2, #significantly different from bout 1 and bout 2.

Figure 2. Muscle Soreness. Mean ± SE. Subjects rated their soreness on a visual analog scale from 1 (normal) to 10 (very, very sore). The distance in millimeters from the beginning of the scale to their mark was measured and used as a indicator of muscle soreness. *significantly different from bout 2 and control, #significance for bout 2 relative to control.
Figure 3. Blood neutrophil concentrations (x 106 ml⁻¹). Mean ± SE. *significantly different from bout 2 and control.

Table 1 Blood E-selectin Concentrations (ng ml⁻¹; Mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>3 hr</th>
<th>6 hr</th>
<th>9 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>68.0 ± 11.4</td>
<td>65.4 ± 9.8</td>
<td>64.4 ± 9.3</td>
<td>62.6 ± 5.7</td>
<td>61.5 ± 5.5</td>
</tr>
<tr>
<td>Bout 1</td>
<td>72.5 ± 10.0</td>
<td>73.5 ± 9.9</td>
<td>67.4 ± 9.3</td>
<td>74.6 ± 11.5</td>
<td>75.1 ± 8.7</td>
</tr>
<tr>
<td>Bout 2</td>
<td>69.2 ± 8.0</td>
<td>69.0 ± 10.4</td>
<td>62.2 ± 5.5</td>
<td>67.1 ± 10.2</td>
<td>68.4 ± 12.5</td>
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Discussion

We have recently reported that the adaptation to eccentric exercise is associated with a lower concentration of neutrophils at 6 and 12 hr of recovery (Pizza et al., 1996). The present study extends these observations by reporting that differences in neutrophil concentrations between 2 bouts of eccentric arm exercise are apparent within 3 hr of recovery (Figure 3). The major finding of the present study, however, is that the neutrophilia associated with novel eccentric exercise (Bout 1) precedes secondary changes in the isometric force deficit (3–24 hr; Figure 1).

The mechanisms for the mobilization of neutrophils following novel eccentric exercise and their reduced response to a repeated bout of eccentric exercise are not known. Cannon and colleagues (1994) have reported a positive correlation between C3a des Arg and neutrophils and suggested that complement activation contributes to the neutrophilia following novel eccentric exercise. Other established chemoattractants (e.g., leukotrienes, prostaglandins, cytokines), activated endothelium (e.g., superoxide anion), and products released from injured muscle may
also contribute to the neutrophilia following injurious exercise. Further study is needed on factors that regulate blood neutrophil concentrations following novel and repeated eccentric exercise.

Previous investigators have described changes in isometric strength following repeated bouts of eccentric exercise (Clarkson et al., 1992; Newham et al., 1987); however, ours is the first to describe changes during early recovery (< 24 hr post-exercise). The similarity in the initial force deficit (5 min post-exercise) for Bout 1 and Bout 2 was still evident at 3 hr post-exercise when muscle weakness resulting from metabolic fatigue should have been resolved (Figure 1). Faulkner and Brooks (1994) have recommended that the isometric force deficit at 3 hr post-eccentric exercise be interpreted to represent the initial injury. Thus, the similarity in isometric force at 5 min and 3 hr post-exercise may indicate that the adaptation to eccentric exercise does not influence the initial injury. These results are consistent with Newham and colleagues (1987) who reported similar isometric force deficits immediately following 2 bouts of eccentric arm exercise that were separated by 2 weeks. Our results extend those of Newham and colleagues (1987) by demonstrating that differences between repeated bouts of eccentric exercise in isometric force become apparent within 6 hr of recovery and increase in magnitude during 24 hr of recovery (Figure 1). Because changes in isometric force deficits after 3 hr post-exercise represent secondary injury (Faulkner and Brooks 1994), our isometric force data may indicate that the mechanisms responsible for the adaptation to eccentric exercise are those that cause secondary injury and not the initial injury. Further study examining both ultrastructural changes and isometric force deficits to repeated bouts of eccentric exercise, however, is needed before definitive conclusions can be made.

The initial injury following eccentric exercise has been attributed to mechanical factors (Faulkner and Brooks, 1994; Warren et al., 1993) and calpain activation (Belcastro et al., 1998), whereas reactive oxygen and nitrogen species (Pizza et al., 1998; Zerba et al., 1990) and neutrophils have been suggested to contribute to secondary muscle injury. Although no direct evidence exists, neutrophils have been hypothesized to exacerbate the initial muscle injury following novel eccentric exercise. Credence for this hypothesis is provided by Fielding and colleagues (1993) and MacIntyre and colleagues (1996, 2000), who reported a positive correlation between muscle neutrophil concentration and Z-line disruption and a temporal relationship between radiolabeled leukocytes and a decline in eccentric torque, respectively, during early recovery (< 24 hr) from eccentric exercise.

Our results demonstrate that changes in blood neutrophil concentrations precede secondary changes in isometric strength following novel eccentric exercise. If neutrophils do contribute to secondary injury, it is reasonable to assume that elevated blood neutrophils would precede increases in muscle neutrophil concentrations and, hence, the delayed structural and functional changes. Further study is needed to directly determine the role of neutrophils in exercise-induced muscle injury.

The non-significant changes in blood E-selectin concentrations following Bout 1 (Table 1) were contrary to our hypothesis. Our hypothesis was based on the reported increase in IL-1 and TNF-à (Cannon et al., 1991; Cannon et al., 1989) and muscle neutrophil concentrations (Fielding et al., 1993; MacIntyre et al. 2000) during early recovery from eccentric exercise. Based on research that has demon-
strated an increase in muscle neutrophil concentrations (Fielding et al., 1993; MacIntyre et al. 2000), the lack of a significant change in blood E-selectin concentrations may indicate that the induction of E-selectin is not required for the early extravasation of leukocytes following eccentric exercise. An alternative interpretation is that the magnitude of the inflammatory response to the eccentric arm exercise may not have been sufficient enough to increase blood concentrations of E-selectin.

In conclusion, the adaptation to eccentric exercise is associated with a lower concentration of blood neutrophils during early recovery. In addition, the neutrophilia associated with novel eccentric exercise precedes secondary changes in isometric strength and is not associated with changes in the concentration of blood E-selectin. These data provide the foundation for future experimentation on the contribution of neutrophils to muscle injury and regeneration.

References


