Activity and immobilization after eccentric exercise: II. Serum CK

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ABSTRACT

SAYERS, S. P., P. M. CLARKSON, and J. LEE. Activity and immobilization after eccentric exercise: II. Serum CK. Med. Sci. Sports Exerc., Vol. 32, No. 9, pp. 1593–1597, 2000. Purpose: The purpose of the present study was to examine the effect of muscle activity level on serum creatine kinase (CK) activity after high-force eccentric exercise of the elbow flexors. Methods: Twenty-six male volunteers were randomly assigned to one of three groups for a 4-d treatment period after exercise: immobilization (N = 9), control (N = 8), and light exercise (N = 9). During the treatment period, the immobilization group had their arm casted and supported in a sling at 90°. The control group had no restriction of their arm activity. The light exercise group performed a daily exercise regimen of 50 biceps curls with a 5-lb dumbbell. Serum CK activity was obtained by venipuncture for three consecutive days before eccentric exercise and during the 4-d treatment period. To quantify activity of the arm, CSA (Computer Science and Applications, Inc.) activity-monitoring devices were worn. Results: Serum CK measurements revealed that there was a significant group by time interaction in the analysis of variance (P < 0.05). Peak serum CK activity of the immobilized group (668 IU) was lower than either the control (4230 IU) or light exercise (2740 IU) group. During the treatment period, activity level among the three groups was significantly different from each other (P < 0.001): 529 counts·min⁻¹ for the immobilization group, 944 counts·min⁻¹ for the control group, and 1334 counts·min⁻¹ for the light exercise group. Conclusions: These results suggest that immobilization of exercised damaged muscle during recovery significantly blunted serum CK activity, which may be due to attenuated removal of CK from the muscle and/or decrease lymphatic transport. Key Words: CK VARIABILITY, LYMPH TRANSPORT, ACTIVITY MONITORING, MUSCLE DAMAGE

Creatine kinase (CK) is an enzyme found in muscle tissue. After eccentric exercise, serum or plasma CK is elevated, most likely due to fiber damage (3,5,8,19). CK activity in the blood reaches a peak approximately 3–5 d after exercise (6), and this peak reflects both CK release from the damaged muscle and its clearance by the reticuloendothelial system (5). However, there is a large variability in the CK response among similarly exercised individuals. Some individuals show small increases after strenuous exercise, whereas others show increases many thousands of times resting levels. Intersubject variability in serum CK activity ranged from 236 IU to 25,244 IU in one study (19) and from 500 IU to 34,500 IU in another (15).

Several studies have tried to explain the variability in the CK response to exercise. Clarkson and Ebbeling (4) examined whether subjects who showed only a small increase in CK had a specific CK inhibitor in the blood, as had been found in patients with muscular disease who showed little increase in CK. They found no evidence of CK inhibitors, thus this could not explain the large CK variability. Two studies, Nosaka and Clarkson (18) and Norton et al. (16) examined whether differences in the amount of muscle mass or lean body mass (LBM) had any effect on CK variability after strenuous exercise. Neither study observed any influence of muscle mass or LBM on serum CK levels. Nosaka and Clarkson (17) and Apple et al. (1) suggested that different enzyme disappearance rates may play a role in serum CK variability. However, there is no clear explanation for the variability among individuals in the serum CK response after eccentric exercise.

The role of activity has been examined to determine the effects of lymphatic transport on serum CK levels (10,12,13). The lymphatic system is a pumpless system, unlike the vascular system, which contains a muscular layer inside the vessels to assist in transport. The lymphatic system therefore depends on external forces to assist in transport of fluids. Pressure changes during breathing, arterial pulsations, and skeletal muscle activity are essential to propel lymph through the system (14). Larger proteins like CK, which are released from damaged muscle into the interstitial spaces, are prevented from being taken up by the capillaries due to their size, but are easily taken up by the lymphatics.

Because activity facilitates the movement of enzyme such as CK from the interstitium into the blood through the lymphatic system, an increase in muscle activity may result in elevated CK levels in the blood. Jackson et al. (11) observed that young ambulatory Duchenne muscular dystrophy (DMD) patients had higher CK levels than nonambulatory DMD patients, possibly due to activity status. However, results from two studies examining serum CK...
responses to additional exercise bouts in the days after high-force eccentric exercise were equivocal (7,21).

The purpose of the study was to determine whether activity of the muscle would have an impact on serum CK levels after high-force eccentric exercise. The hypothesis to be tested was that an increase in activity would increase the serum CK response to eccentric exercise, while a decrease in activity would reduce the serum CK response to eccentric exercise.

METHODS

Subjects. The 26 subjects used in this study were the same as in the previous study (Part I) that examined the effect of activity on recovery of muscle function (20). After a detailed explanation of the study, subjects signed an informed consent document consistent with the guidelines of the University of Massachusetts Human Subjects Review Committee. Subjects were then randomly assigned to one of three groups: immobilization (N = 9), control (N = 8), or light exercise (N = 9).

Study design. The study design involved a 7-d protocol, 3 d of baseline serum CK measures and activity level of the nondominant arm, an eccentric exercise session, and 4 d of post-exercise CK measures and arm activity level. The exercise regimen has been designed to induce muscle damage through eccentric contractions using a modified preacher curl apparatus and is described in Part I of this study (20). Serum CK measures were obtained for 3 consecutive days before eccentric exercise and for 4 consecutive days after exercise. To quantify activity of the arm during the study, Computer Science and Application, Inc. (CSA) activity-monitoring devices were worn on the wrist of the nondominant arm for 3 consecutive days before eccentric exercise and for 4 days after eccentric exercise. After serum CK measurements on the final day of baseline measurements, a strenuous eccentric exercise session consisting of 50 maximal eccentric contractions of the elbow flexors of the nondominant arm was performed by each subject. Immediately after eccentric exercise, the immobilization group had their arm immobilized in a cast and secured in a sling at 90° for the duration of the study. On days 1 through 4 of the treatment period, the immobilization, control, and light exercise groups visited the laboratory in the morning for venipuncture. Each group also had the activity of their exercised arm monitored with CSA activity monitors. Only the light exercise group was required to perform a daily exercise regimen of 50 bicep curls with a 5-lb dumbbell. Two sets of 25 bicep curls were performed with a 2-min rest between sets. The 5-lb weight was chosen to increase the activity of the arm during the light exercise without promoting further damage to the eccentrically exercised muscle fibers. To assess acute changes in serum CK activity after the bicep curl exercise, the light exercise group had blood drawn both before and immediately after the exercise.

Serum CK. Blood samples were taken from subjects for a determination of serum CK activity. Venipuncture at the antecubital vein was performed by trained laboratory personnel. Samples were allowed to clot at room temperature and were centrifuged to separate the components and were stored at −20°C. Biochemical analyses were performed using a Bausch & Lomb (Rochester, NY) spectronic 1001 spectrophotometer and a commercially available diagnostic kit (Sigma Diagnostics no. 47–50, St. Louis).

Activity monitors. Subjects were required to wear activity-monitoring devices on the wrist of their nondominant arm during all waking hours for 3 d during baseline data collection and for 4 d after eccentric exercise. Pilot testing revealed measurable differences in arm activity level among the three treatment conditions when using the CSA activity monitors attached to the wrist. The activity monitor (CSA, Inc., Shalimar FL, model 7164) is a single plane accelerometer (5.1 × 4.1 × 1.5 cm, 43 g), assessing motion in the vertical plane (9). The activity monitor assessed vertical accelerations ranging from 0.05–2.0 G and is band limited with a frequency response from 0.25 to 2.5 Hz (at 0.75 Hz, one “count” of activity is equal to 0.0167 G). Normal body motion is detected by these parameters and high frequency vibrations are filtered. An analog bandpass filter acts to filter the acceleration signal and this signal is digitized by an 8 bit A/D converter at 10 samples s⁻¹. Each digitized signal was summed over a user-specified time interval and the activity counts were stored internally. At the end of the three-day baseline period and at the end of the 4-d post-exercise period, activity monitors were downloaded into a custom written software program on a Gateway 2000 personal computer (model no. GP6–350, North Sioux City, SD). The activity monitoring devices contained no visual display for subjects to monitor or alter their own activity level. Before being outfitted with the activity monitor, subjects were given oral and written instructions regarding their use. Subjects were also given data sheets to record all time periods that the activity monitor was not attached to the wrist, for instance, during sleeping hours or when taking a shower. The activity monitor recorded the amount of arm activity based on accelerations of the arm, with activity counts being greater per movement for larger amplitude movements. Arm activity in counts was divided by the number of minutes that the monitor was in use throughout the day, resulting in the expression of activity of the arm in counts per minute.

Statistics. To assess reliability of the baseline measures of arm activity level and serum CK activity, an intraclass R and a repeated measures analysis of variance (RANOVA) was used. Changes in arm activity from baseline to immediately post-eccentric exercise were assessed using RANOVA. To assess changes during the treatment period, arm activity level and serum CK activity were evaluated using RANOVA. The level of significance for all tests was set at P < 0.05. A Tukey’s honestly significant difference (HSD) post hoc test was used to assess differences among the groups during the treatment period, and a Bonferroni adjustment was used to account for the number of tests. For the analysis of serum CK activity during the treatment period among the three groups, the Bonferroni test adjusted the level of significance to P = 0.004. Student’s t-tests for
dependent samples were used to assess pre- to post-light exercise differences in serum CK activity. A log transformation was performed on the CK values for analysis.

RESULTS

There were no significant differences among the three groups or over time in baseline arm activity level ($P > 0.05$) or serum CK activity ($P > 0.05$). Intraclass R values for baseline arm activity level and serum CK activity were $R = 0.74$ and $R = 0.81$, respectively. RANOVA for arm activity revealed that there were significant group main effects ($P < 0.001$) and time main effects ($P < 0.001$), but no significant group by time interaction ($P = 0.11$) during the period from baseline to 1 d post-eccentric exercise (see Fig. 1).

The treatment period was then examined to determine whether the three treatments had an effect on arm activity (Fig. 1). There was no significant interaction among groups in arm activity level during the treatment period ($P = 0.75$), indicating that the pattern of response was similar among the groups. However, the group main effects were significant ($P < 0.001$). A Tukey’s HSD test for group main effects revealed that the arm activity of the light exercise group during the treatment period was significantly higher than the immobilization and control groups ($P < 0.001$). The arm activity level of the control group was also significantly higher than the immobilization group during the treatment period ($P < 0.001$).

There was a significant group by time interaction in serum CK activity over the treatment period ($P < 0.05$) indicating that the three groups responded differently during the treatment period. Figure 2 shows changes in serum CK activity among the three groups during the baseline and treatment periods. The immobilization group had the lowest serum CK levels on each of the 4 d (see Fig. 2). A Tukey’s HSD tests revealed serum CK activity in the immobilization group was significantly lower than the serum CK activity in both the control or light exercise group during the second, third, and fourth days of treatment ($P < 0.004$), but not on the first day ($P > 0.004$). At no point during the treatment period was serum CK activity in the control and light exercise groups significantly different from each other ($P > 0.004$).

The light exercise group had venipuncture performed before and immediately after their light exercise session on each of the 4 d of treatment for the measurement of serum CK activity. There was slightly higher serum CK activity after each of the first two light exercise sessions as compared with pre-light exercise values on day 1 and 2 of the treatment period (see Table 1). Much larger differences in pre- to post-light exercise serum CK activity were evident on days 3 and 4 of the treatment period, but the differences were not statistically significant. Differences in pre-light exercise serum CK activity and post-light exercise serum CK activity were statistically significant on the first day of the treatment period ($P = 0.03$), but not during day 2 ($P = 0.08$), day 3 ($P = 0.07$) or day 4 ($P = 0.08$) of the treatment period.

DISCUSSION

The objective of the present study was to examine different levels of physical activity after high-force eccentric exercise and to compare these activity levels with serum CK activity. Two previous studies had examined additional exercise bouts in the days after high-force eccentric exercise. The additional activity caused an increased serum CK response in one study (21) but not in the other (7). Other than the present study, only one study has shown that inactivity after strenuous exercise resulted in lower serum CK activity (10). Havas et al. (10) examined the effect of bed rest for 24 h after an 18-km run and observed a smaller CK response in subjects ($N = 6$) who were confined to bed compared with normal active controls ($N = 5$). Although the

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**Figure 1**—Arm activity level expressed in counts per minute among the three groups during the treatment period. Pre represents the mean arm activity counts during the baseline period, before eccentric exercise. Days 1–4 represent mean arm activity counts during the 4-d treatment period. Data represent means ± SEM.

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**Figure 2**—Serum creatine kinase (CK) activity expressed in international units (IU) among the three groups during the treatment period. Pre represents the mean serum CK activity during the baseline period, before eccentric exercise. Days 1–4 represent the serum CK activity during the 4-d treatment period. Data represent means ± SEM.
difference in the serum CK response was small between groups and the sample size was small, the data tended to suggest that inactivity either reduced CK release from the muscle or reduced lymphatic transport after strenuous exercise.

Arm activity level proved to be adequately assessed using the activity monitoring devices. When the time period from baseline to one day post-eccentric exercise was assessed among the three groups (see Fig. 1), the immobilization group showed a significant reduction in activity, which is what would be expected by immobilizing the arm. However, these devices were also able to detect reductions in activity due to the eccentric exercise alone. Figure 1 shows that the control and light exercise group both exhibited a reduction in arm activity during the 24-h time period after eccentric exercise. This reduction in activity after eccentric exercise could have been due to muscle damage and/or soreness, which could reduce the daily use of the arm resulting in a decrease in measured activity.

It was also of interest to observe whether the three treatments had any effect on arm activity level during the 4-d treatment period (days 1–4). Figure 1 shows that the immobilization group showed a significant reduction in activity compared with the control group. The control group and the control group had significantly greater arm activity than the immobilization group. Although this pattern was what we expected, it appeared that the amount of change for the light exercise group compared with control was greater than expected. Doing 50 biceps curls alone would not be expected to result in such large differences in arm activity between the control and light exercise groups. However, research has shown that exercising a sore muscle may reduce muscle pain during the exercise period (2). Thus, exercising the sore limb during the treatment period most likely resulted in a reduced perception of muscle soreness. This, in turn, may have enabled the subjects to engage their exercised arm in more activity during the 24-h time period after eccentric exercise.

We hypothesized that subjects with the greatest amount of arm activity during the days after high-force eccentric exercise would have the greatest release of CK into the blood. This would be due to “flushing” CK out of the muscle into the interstitial spaces and into the blood through the lymphatic system. Increases in activity did result in increased serum CK activity, however, not in a dose-response manner because there was no significant difference between the control and light exercise groups in the CK response. The large variability in the serum CK response in the control and light exercise group was perhaps too great to detect a significant difference between these groups (see Fig. 2).

Acute bouts of activity appeared to influence serum CK activity. Research has shown that increasing the muscular activity of dogs resulted in an increase in the lymphatic transport of CK and other enzymes compared to anesthetized or resting dogs (12). An examination of the light exercise group in the present study revealed differences in serum CK activity after the light exercise bout on each of the treatment days. Although differences in pre-light exercise and post-light exercise serum CK values were only statistically significant on day 1 (P = 0.03), low P-values on day 2 (P = 0.08), day 3 (P = 0.07), and day 4 (P = 0.08) indicated a trend toward an acute rise in serum CK activity with increased muscular activity. Perhaps during the 50 light biceps curls, activity of the arm forced the CK that had accumulated in the lymph through the lymphatic system and into the blood.

We also hypothesized that subjects with the lowest amount of arm activity would have the lowest serum CK values. This would be due to the reduction in lymphatic transport with inactivity of the muscle. Consequently, significant differences were observed in serum CK activity between the immobilization group and the control group and between the immobilization and light exercise group. Figure 2 shows that immobilization severely blunted the CK response during the days when peak serum CK activity is expected to rise substantially, three to five days after eccentric exercise (6), and even reduced the variability within this group.

In conclusion, muscle activity (either acute activity or activity imposed over several days) appears to play some role in the variability of the serum CK response to eccentric exercise. Due to the large variability in the serum CK response in the control and light exercise groups, possible differences in CK activity between these groups could not be detected. Thus, further research with larger sample sizes will be necessary to determine whether increased muscular activity results in increased serum CK activity after eccentric exercise. Immobilization, however, did result in a significant blunting of the serum CK response, which could be due to a decrease in lymphatic transport with inactivity. Future studies are needed that address whether reductions in serum CK activity are due to an attenuated release of CK from the muscle during immobilization (suggesting accelerated healing), or whether the serum CK response is merely delayed by immobilization (suggesting reduced lymphatic activity).

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### Table 1. Serum CK values of light exercise group pre- and post-light exercise.

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<th>Day 1</th>
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<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>Baseline</td>
<td>112 ± 45</td>
<td>237 ± 79</td>
<td>257 ± 83*</td>
<td>1012 ± 406</td>
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<tr>
<td>Day 1</td>
<td>2397 ± 880</td>
<td>2735 ± 1000</td>
<td>2740 ± 914</td>
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*indicates a significant increase from pre-to-post light exercise.

Serum CK values (mean ± SEM) are expressed in IU.
REFERENCES


