Davies RC, Eston RG, Fulford J, Rowlands AV, Jones AM. Muscle damage alters the metabolic response to dynamic exercise in humans: a \(^{31}\)P-MRS study. J Appl Physiol 111: 782–790, 2011. First published June 30, 2011; doi:10.1152/japplphysiol.01021.2010.—We used \(^{31}\)P-magnetic resonance spectroscopy to test the hypothesis that exercise-induced muscle damage (EIMD) alters the muscle metabolic response to dynamic exercise, and that this contributes to the observed reduction in exercise tolerance following EIMD in humans. Ten healthy, physically active men performed incremental knee extensor exercise inside the bore of a whole body 1.5-T superconducting magnet before (pre) and 48 h after (post) performing 100 squats with a load corresponding to 70% of body mass. There were significant changes in all markers of muscle damage (perceived muscle soreness, creatine kinase activity, nonoxidative metabolism, with this contributing to the decrease in exercise tolerance following EIMD, 13). The mean differences were significantly elevated following EIMD. These changes are associated with an acute inflammatory response and delayed onset muscle soreness (28) and peripheral fatigue factors originating within the damaged muscle tissue may also be involved in the reduced time to exhaustion following eccentric, muscle-damaging exercise. Using muscle biopsy procedures, Asp et al. (2) reported a reduction of muscle glycogen content in human subjects following muscle-damaging exercise, which was associated with a 23% reduction in maximal work capacity during incremental knee extensor exercise. Other changes in metabolic function following eccentric exercise include impaired muscle glycogen resynthesis (2, 3) and an elevated blood lactate response during exercise (2, 18, 19). It is feasible that these changes reflect a shift in the muscle metabolic profile to an increased reliance on nonoxidative metabolism, with this contributing to the decreased endurance capacity following EIMD (2). However, the precise nature of the accelerated fatigue development experienced following EIMD is poorly understood.

Noninvasive evaluation of muscle metabolism can be achieved using \(^{31}\)P-magnetic resonance spectroscopy (\(^{31}\)P-MRS). Studies using this technology have reported an increase in the resting inorganic phosphate (Pi)-to-phosphocreatine (PCr) ratio (Pi/PCr) following EIMD, suggestive of an increase in resting muscle metabolism (26, 27, 33, 41). Several of the candidate mechanisms for the accelerated development of fatigue and reductions in peak power observed following eccentric exercise, including increases in Pi concentration ([Pi]) and reductions in pH, can be measured using \(^{31}\)P-MRS. Rodenburg et al. (41) reported no difference in the Pi/PCr, pH, or peak power during graded knee extensor exercise performed 24 h after a bout of stepping exercise designed to induce EIMD. However, the authors concluded that the lack of change in several markers of muscle damage indicated that the muscle-damaging protocol employed was not sufficient to alter muscle metabolism (41).

Following a period of endurance training, incremental knee extensor exercise tolerance is enhanced with the profiles of [Pi]/[PCr], [PCR], and pH, all evidencing a rightward shift when plotted against work rate (21). It is possible that, following a bout of muscle-damaging exercise, the reduced exercise tolerance could be associated with a leftward shift in these \(^{31}\)P metabolite-time profiles. Alternatively, an unchanged muscle
metabolite response would indicate that other, potentially central, mechanisms may be responsible for any decrements in endurance capacity following EIMD. The purpose of this study was, therefore, to investigate the effect of a well-defined bout of eccentric, muscle-damaging exercise (7, 8, 14, 15) on changes in muscle metabolism during dynamic incremental knee extensor exercise. We used 

\[ \text{MRS} \] to test the hypothesis that EIMD alters the muscle metabolic response to dynamic exercise and thus contributes to reduced exercise tolerance in humans.

**METHODS**

**Subjects**

Ten healthy, physically active male subjects (age, 22 ± 4 yr; mass, 78.2 ± 8.8 kg, height, 1.79 ± 0.08 m) volunteered to participate in this study. All participants were asymptomatic of illness and preexisting injuries and had not performed any resistance training of the lower limbs within the previous 6 mo. Participants provided written, informed consent to participate in the study, which had been approved by the Institutional Ethics Committee.

**Experimental Procedures**

Dynamic incremental knee extensor exercise was performed inside the bore of a whole body MRI system before and 48 h after performing 100 muscle-damaging squats. Markers of muscle damage were measured before and 24 h after the squatting protocol and 2 h before and after the two knee extensor exercise tests. Participants were instructed to report to the laboratory at the same time of day (∼1 h) on four separate occasions (Fig. 1).

**Assessment of muscle damage.** All markers of muscle damage, i.e., perceived muscle soreness [using a 0–10 visual analog scale (VAS)], creatine kinase (CK) activity, and isokinetic peak torque (30°/s), were measured in the order listed, immediately before, and 24 and 48 h after performing the eccentric, muscle-damaging exercise protocol (Fig. 1).

Perceived soreness of the knee extensors was assessed using a blank 0–10 VAS. The VAS consisted of a 10-cm line labeled from left (no soreness) to right (worst soreness ever). Participants squatted to 90° knee flexion with hands on hips and then placed a mark on the VAS to indicate their level of soreness. Perceived pain was then quantified by measuring the distance to the mark on the line to the nearest 0.1 cm.

Plasma CK activity was assessed from fingertip capillary blood samples. The sample was centrifuged at 4,000 rpm (2,000 g) for 5 min, and two 20 µl samples of plasma were then added to 1 ml of reagent (Randox CK-NAC 110, Randox Laboratories, Crumlin, Ant- rinis, UK). The solution was then incubated at 37°C, and absorbance at 340 nm was recorded by spectrophotometry (Jenway 6310 spectro- photometer, Jenway, Essex, UK) at 1, 2, 3, and 4 min. CK values were calculated using the formula CK (U/l) = 8.095 × Absorbance 340 nm/min. The mean CK value of the two samples was calculated and used for subsequent analysis. Normal serum values of 24–195 U/l are reported for men using this method (46).

Isokinetic peak torque was measured using a Biodex B-2000 isokinetic dynamometer (Biodex, Shirley, NY), which was calibrated before each data collection session in accordance with the manufacturer’s guidelines. Following familiarization sessions, participants performed five maximal voluntary contractions at 30°/s, with a rest period of 30 s between contractions. The highest of the five maximal voluntary contractions was recorded. Visual feedback, displaying real-time torque, was used to encourage maximal effort. These procedures have been used previously (7, 8, 14, 15).

**Dynamic incremental knee extensor exercise.** Single-legged, knee-extension exercise tests were conducted at the same time of day (∼1 h) for each participant, before and 48 h after performing eccentric, muscle-damaging exercise (Fig. 1). The dynamic knee-extensor exercise tests were conducted in the prone position, with the subjects positioned inside a whole body MRI system. A 6-cm 

\[ \text{P-MRS} \] transmit-receive surface coil was placed within the subject bed, and the subject was asked to lie on it such that the coil was centered over the quadriceps muscle of the leg to be exercised. Subjects were then secured to the ergometer bed with Velcro straps at the thigh, buttocks, and lower back to minimize extraneous movement during the protocol. The foot of the leg to be exercised was connected to a pulley system that permitted a nonmagnetic weight to be lifted and lowered and work rate to be calculated.

Exercise was performed at a rate of 40 repetitions/min, with the subjects lifting and lowering the mass over a distance of ~0.22 m in accordance with a visual cue projected onto the front wall of the scanner room. The contraction phase of the knee extensors and the 

\[ \text{P-MRS} \] interrogation of the quadriceps occurred in unison. After a 2-min period of rest, the subjects commenced knee-extension exercise against an initial basket load of 1 kg. Thereafter, the basket load was increased by 0.5 kg every 30 s, until the subjects were no longer able to maintain the kicking frequency at 40 repetitions/min. The subjects received strong verbal encouragement to continue for as long as possible.

**Eccentric, muscle-damaging exercise protocol.** Participants completed 100 (Smith) squats, performed as 10 sets of 10 repetitions, with the load on the bar corresponding to ~70% of each participant’s body mass. Before commencing, all participants were instructed in correct and safe lifting technique. The bar was positioned on the participant’s shoulders, and feet were positioned under the bar, with the back straight and legs fully extended (knee = 180°). The descent phase involved eccentric action of the knee extensors to lower the bar to a knee angle of just past 90°. The lifting phase involved concentric action to return the bar to the starting position. This protocol has previously been used to induce EIMD (7, 8, 14, 15).

**Measurements**

**MRS measurements.** MRS was performed in the Peninsula Magnetic Resonance Research Centre using a 1.5-T superconducting magnetic resonance scanner (Philips Gyroscan Clinical Intera, Philips Medical Systems, Best, the Netherlands). Initially, fast-field echo images were acquired to determine whether the muscle was positioned correctly relative to the coil. This was aided by placing cod liver oil capsules, which yield high-intensity signal points within the image, adjacent to the coil, allowing its orientation relative to the muscle...
volume under examination to be assessed. A number of preacquisition steps were carried out to optimize the signal from the muscle under investigation. Tuning and matching of the coil were then performed, followed by an automatic shimming protocol undertaken within a volume that defined the quadriceps muscle. To ensure that the examined muscle was consistently at the same point relative to the coil during exercise, the subject was visually queued via a display consisting of two vertical bars, one that moved at a constant rate with a frequency of 0.67 Hz and one that monitored foot movements via a sensor present within the pulley to which they were connected. Thus the subject endeavored to match the movements of these two bars. The work done by the subjects was recorded via a nonmagnetic strain gauge present within the pulley mechanism. Before exercise, during exercise, and during recovery, data were acquired every 1.5 s, via a pulse/acquire sequence, with a spectral width of 1,500 Hz and 1,000 data points. Phase cycling with eight phase cycles was employed, to compensate for any 90° pulse imperfections, leading to a spectra being acquired every 12 s. The subsequent spectra were quantified via peak fitting, assuming prior knowledge, using the jMRUI (version 2) software package and the AMARES fitting algorithm (36, 50).

Spectra were fitted assuming the presence of the following peaks: P₁, phosphodiester, PCr, α-ATP (2 peaks, amplitude ratio 1:1), γ-ATP (2 peaks, amplitude ratio 1:1), and β-ATP (3 peaks, amplitude ratio 1:2:1). In all cases, relative amplitudes were corrected for partial saturation because of the short repetition time relative to the longitudinal relaxation time constant T₁. Corrections were determined by first acquiring an unsaturated spectra, which was free of T₁ effects, (repetition time, 30 s), and calculating the PCr/Pₐ. This ratio was also determined during exercise and compared with the unsaturated PCr/Pₐ, thereby allowing the appropriate correction for differences in T₁ values for PCr and Pₐ to be made. During exercise, T₁ was assumed to remain unchanged (10), although it is recognized that any differences in T₁ due to pH may lead to inaccuracies in the Pₐ/PCr at higher work rates. Absolute concentrations were estimated by calculating the size of peak areas relative to β-ATP, which was set at 8.2 mM. The Pₐ/PCr was determined from the respective Pₐ and PCr spectral areas, as obtained during the quantification procedure. Intracellular pH was calculated from the chemical shift of the Pₐ, spectral peak relative to the PCr (33). During the course of the exercise, the full width at half-maximum of the Pₐ peak broadened from ~6 Hz at rest to ~25 Hz at end exercise. ADP concentration was calculated as described by Kemp et al. (25).

To determine the intracellular threshold (IT) during incremental exercise, piecewise linear regression was used. Briefly, different two-line combinations were fitted to the [Pi]/[PiCr]-work rate and pH-work rate relationships, until the lowest sum of squared residuals was found (32). The point at which this particular two-line combination intersected was defined as the IT. ADP decay time constants postexercise were determined by fitting a single exponential of the form ADP = ADPend − A[1 − exp (−t/T)], where t is time, T represents the ADP decay time constant, and A is the difference in ADP values between end exercise and rest.

**Statistical Analysis**

Changes in the markers of muscle damage (perceived muscle soreness, CK activity, and isokinetic peak torque) were analyzed using a series of one-way repeated-measures ANOVAs. As the CK activity data were not normally distributed, these values were log-transformed before statistical analysis (47). Paired t-tests were used to determine significant differences in resting and end-exercise values between the two conditions. Where data were not normally distributed, Wilcoxon tests were also run. Changes in the MRS measurements (P, PCr, P/PiCr, and pH) at 0, 2, 4, and 6 min of incremental exercise were examined using separate two-way fully repeated-measure ANOVAs (condition × time). The assumption of sphericity was evaluated using Mauchly’s test. Where sphericity was violated (P < 0.05), the Greenhouse-Geisser (GG) correction factor was applied. All data were analyzed using the statistical software package SPSS for Windows (version 13), with statistical significance set at 0.05. Values are presented as means ± SD.

**RESULTS**

**Muscle Damage**

There were significant changes in all markers of muscle damage following eccentric exercise. Table 1 shows changes in muscle soreness, CK activity, and isokinetic peak torque before and after eccentric exercise. Muscle soreness increased 24 h after eccentric exercise, with the highest values reported at 48 h [F(2,18) = 26.22, P < 0.05]. Plasma CK activity increased after eccentric exercise, with the highest values observed at 24 h [before exercise (pre), 178 ± 61 U/L; 24 h after exercise (post), 798 ± 692 U/L; FG(1.9,9) = 15.02, P < 0.05]. Isokinetic peak torque (30°/s) decreased by 15% at 24 h and remained 11% lower than baseline values at 48 h [F(2,18) = 14.33, P < 0.05].

**MRS Measurements**

Resting [Pi] was significantly elevated following eccentric exercise [T₀ = −3.70, P < 0.01]. This resulted in an elevated [Pi]/[PiCr] [T₀ = −3.68, P < 0.01]. No changes in resting [PiCr] [T₀ = 1.911, P > 0.05] or pH [T₀ = −0.087, P > 0.05] were observed.

Before eccentric exercise, all 10 participants completed a minimum of 7 min of incremental exercise before reaching volitional exhaustion. However, 48 h after eccentric exercise, the minimum time achieved by all participants was reduced to 6 min. Thus the baseline and first 6 min of incremental exercise were analyzed to examine changes in muscle metabolic responses. Muscle metabolic responses during incremental exercise are presented in Table 2, and a representative baseline spectra obtained before and after the muscle damaging protocol is shown in Fig. 2. There was a significant main effect for time for all muscle metabolic responses, with [PiCr] and pH values declining, and [Pi] and [Pi]/[PiCr] values increasing as the incremental exercise progressed (all P < 0.05). There was a significant main effect for condition for [Pi] [F(1,9) = 7.080, P < 0.05], with the [Pi] value being significantly elevated following eccentric exercise. As a direct consequence of the elevated [Pi], there was also a significant main effect for

| Table 1. Changes in markers of muscle damage before and 24 h and 48 h after eccentric exercise |
|----------------------------------|--|--|--|
| Measured Variable | Before | 24 h | 48 h |
| Soreness (0–10 VAS) | 0.5 ± 0.3 (0.1–1.1) | 5.0 ± 2.0* (1.8–8.2) | 5.5 ± 3.2* (1.6–9.7) |
| CK activity, U/L | 178 ± 61 (97–270) | 798 ± 692* (196–2,481) | 409 ± 264* (213–1,088) |
| Peak torque (N·m), 30°/s | 310 ± 47 (227–366) | 265 ± 66* (113–340) | 275 ± 77* (115–361) |

Values are means ± SD (range). VAS, visual analog scale (0–10). CK, creatine kinase. *Significantly different from preexercise value (P < 0.05).
condition for [P]/[PCR], with values being significantly higher following eccentric exercise \( F(1,9) = 5.908, P < 0.05 \). In contrast, [PCR] and pH values were not different between conditions (both \( P > 0.05 \)). There were no significant interactions of condition and time for any of the measured muscle metabolic variables. Changes in [PCR], [P]/[PCR], and pH are illustrated for a typical participant in Figs. 3, 4, and 5, respectively.

Time to exhaustion and the associated peak work rate attained during the incremental knee extensor exercise were significantly reduced following muscle damage \( t(9) = 4.85, P < 0.001 \) and \( t(9) = 5.21, P < 0.001 \), respectively. Values at exhaustion are presented in Table 3. The data for [P]/[PCR] at exhaustion were not normally distributed, and, therefore, Wilcoxon tests were used to analyze these data. However, the results did not differ from the \( t \)-test results, and so, for consistency, the results of the paired \( t \)-tests are presented. pH and [PCR] values at exhaustion were higher \( t(9) = -2.34, P < 0.05 \) and \( t(9) = -4.49, P < 0.01 \), respectively, and [P]/[PCR] values at exhaustion were lower \( t(9) = 2.346, P < 0.05 \) following eccentric exercise. However, [P] at exhaustion was not significantly different following eccentric exercise \( t(9) = -0.496, P > 0.05 \). The IT was not significantly altered by eccentric exercise \( t(9) = 0.51, P > 0.05 \). Values at IT are presented in Table 3. The time constant describing the recovery of ADP concentration postexercise was not significantly different between conditions \[pre: 16.4 \pm 8.6; post: 17.9 \pm 7.2 \text{s}; t(9) = -0.689, P > 0.05\].

DISCUSSION

To our knowledge, this is the first study to investigate changes in muscle metabolism with \(^{31}\)P-MRS following an effective muscle-damaging protocol. The principal original finding of this investigation was that the reduction in time to exhaustion consequent to a bout of eccentric, muscle-damaging exercise was not associated with a greater depletion of [PCR] or fall in pH. Specifically, time to exhaustion was reduced by 12% following the muscle-damaging exercise, but the temporal changes in [PCR] and pH were similar in the two experimental conditions, such that end-exercise [PCR] and pH were significantly higher 48 h after the muscle-damaging exercise (Figs. 3 and 5). In contrast, the end-exercise [P] was not significantly different between the pre- and post-EIMD conditions, with an elevated resting P value in the post-EIMD condition maintained throughout the whole exercise bout. These results indicate that the reduced exercise tolerance following EIMD may be related either to the increased [P] that was observed at rest and throughout incremental exercise, or to other unmeasured peripheral or central factors. Importantly, however, the results allow us to discount a greater rate of nonoxidative energy metabolism (as inferred from the changes in [PCR] and pH) as important mediators of the reduced exercise tolerance following EIMD.

Our findings provide novel insights into changes in muscle metabolite responses to dynamic exercise subsequent to EIMD. Previous research has reported unaltered P/PCR, pH, and peak power during incremental exercise following a prior bout of eccentric exercise (41). However, these authors speculated that the bench-stepping protocol employed to induce damage was not sufficiently rigorous to elicit changes in exercise metabolism. Indeed, the lack of change in several markers of muscle damage, including maximum power output, suggests that their eccentric exercise protocol was ineffective (41). Participants in the present study completed a bout of eccentric, muscle-damaging exercise, comprising 100 squats with the load on the bar set at 70% body mass. This eccentric exercise protocol is known to be effective in inducing damage (7, 8, 14, 15). Indeed, in the present study, there were changes in all measured symptoms and markers of muscle damage. The disruption of sarcomeres and myocyte membranes during unaccustomed eccentric exercise leads to immediate and prolonged reductions in peak torque due to dysfunction of the excitation-contraction mechanism (39). The 11% reduction in isokinetic peak torque observed at 48 h is consistent with previous studies that have employed this same eccentric exercise protocol (7, 8, 14, 15). Furthermore, the significant increase in plasma CK activity is indicative of increased membrane permeability. Impaired maximal force production may, in part, account for the reduction in time to exhaustion and the associated 14% reduction in peak work rate values. The accelerated rate of fatigue experienced by our participants is consistent with observations of impaired endurance performance brought about by prior eccentric exercise (2, 15, 30, 48).

Peripheral fatigue factors originating from within the damaged muscle tissue have been implicated in the reduction in

---

Table 2. Muscle metabolic responses during incremental exercise before and 48 h after eccentric, muscle-damaging exercise

<table>
<thead>
<tr>
<th>Measured Variable</th>
<th>0 Min</th>
<th>2 Min</th>
<th>4 Min</th>
<th>6 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>[PCR]† mM</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td></td>
<td>38.3 ± 1.2 (35.4–39.5)</td>
<td>37.9 ± 1.4 (35.3–40.0)</td>
<td>35.0 ± 2.1 (32.0–38.1)</td>
<td>34.1 ± 2.6 (30.4–37.8)</td>
</tr>
</tbody>
</table>

†Significant main effect for time (0, 2, 4, and 6 min) (\( P < 0.05 \)).
endurance capacity associated with EIMD. Specifically, increased blood lactate (2, 6, 12) and increased utilization of glycogen stores (2) following eccentric exercise have been attributed to a putative shift toward an increased reliance on nonoxidative energy production, leading to impaired endurance performance (2, 6, 12). However, in the present study, the [PCr] and pH values for a given work rate were not significantly altered, although the performance of incremental knee extensor exercise was impaired.

Muscle [PCr] and O2 uptake demonstrate similar kinetic profiles during transitions to higher work rates (4, 29, 31, 42), indicating that the control of oxidative phosphorylation is closely linked to the creatine kinase reaction (29, 34). Thus our observation of an unchanged [PCr] profile during incremental exercise is consistent with reports of unchanged O2 uptake kinetics following eccentric exercise (14, 43). These data indicate that EIMD does not compromise oxidative function during dynamic exercise. When arterial O2 content is reduced, the accelerated development of peripheral muscle fatigue has been associated with greater PCr hydrolysis and increased acidosis (20). However, the reduced exercise tolerance observed in the present study was not accompanied by any increase in the rate of PCr degradation or accelerated fall in pH, suggesting that O2 delivery to the exercising muscle was not compromised following EIMD.

The rate of PCr resynthesis following the cessation of exercise can be used to provide an index of maximal oxidative capacity. However, intracellular pH is known to affect the rate of PCr recovery (49). Given that there was a significant difference between the end-exercise pH values determined pre- and post-EIMD, we, therefore, determined the time constant describing the ADP concentration decay postexercise as an
alternative measure of oxidative capacity. The recovery kinetics of [ADP] have been shown to be either independent of, or to have a low dependency on, pH (49). The time constants for [ADP] were not different before and after muscle damage, supporting the proposition that muscle damage does not compromise muscle oxidative function.

The reduction in time to exhaustion resulted in significant alterations in end-exercise [PCr] values. Before the bout of muscle-damaging exercise, the mean depletion of the PCr pool was >80% at the end of the incremental exercise, with several subjects almost completely depleting their muscle PCr. However, 48 h after the muscle damage was induced, participants reached exhaustion sooner, with a mean depletion of the PCr pool of ~62%. Thus, end-exercise [PCr] was higher when incremental exercise was performed in the muscle-damaged condition. Similarly, the intracellular pH-work rate profile was similar during incremental exercise following EIMD. There was no significant difference in the pH values for a given work rate.

Table 3. Values at the intracellular threshold and at exhaustion before and 48 h after muscle-damaging exercise

<table>
<thead>
<tr>
<th>Measured Variable</th>
<th>Pre</th>
<th>48 h Post</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intracellular threshold</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time, s</td>
<td>326 ± 77 (174–432)</td>
<td>316 ± 65 (198–432)</td>
</tr>
<tr>
<td>[Pi]/[PCr] ratio</td>
<td>0.30 ± 0.14 (0.14–0.59)</td>
<td>0.31 ± 0.10 (0.18–0.54)</td>
</tr>
<tr>
<td>pH</td>
<td>7.07 ± 0.02 (7.05–7.12)</td>
<td>7.08 ± 0.02 (7.06–7.12)</td>
</tr>
<tr>
<td><strong>Exhaustion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time, s</td>
<td>519 ± 56 (427–585)</td>
<td>459 ± 63 (352–565)*</td>
</tr>
<tr>
<td>[PCr], mM</td>
<td>7.2 ± 1.7 (0.9–15.0)</td>
<td>14.5 ± 2.1 (6.0–25.2)*</td>
</tr>
<tr>
<td>[Pi], mM</td>
<td>19.7 ± 1.9 (8.5–28.0)</td>
<td>21.1 ± 2.6 (10.8–37.8)</td>
</tr>
<tr>
<td>[Pi]/[PCr] ratio</td>
<td>2.09 (1.74–14.4)‡</td>
<td>1.46 (0.85–2.29)‡*</td>
</tr>
<tr>
<td>pH</td>
<td>6.75 ± 0.04 (6.5–6.9)</td>
<td>6.83 ± 0.04 (6.6–7.0)‡*</td>
</tr>
</tbody>
</table>

Values are means ± SD (range). *Significantly different from precentric exercise (P < 0.05). ‡End-exercise [Pi]/[PCr] ratio data were not normally distributed; therefore, values are presented as median (interquartile range). The intracellular threshold (see METHODS) was not significantly altered by eccentric exercise (τ0.5 = 0.51, P = 0.620).
before compared with after EIMD, but end-exercise pH was higher due to the shorter time to exhaustion. Furthermore, the unchanged [PCr] and pH responses to incremental exercise resulted in there being no significant difference in the IT following eccentric exercise. Low [PCR] and pH have been implicated in the process of muscle fatigue (51, 52), particularly during high-intensity exercise, where the limit of tolerance appears to coincide with the attainment of a consistent intracellular environment (20, 51, 53). However, on the basis of our observations, low [PCR] and pH values are not associated with the reduction in exercise tolerance that accompanies EIMD.

It should be noted that intracellular pH and the associated muscle-to-blood H⁺ concentration gradient depends on a complex interplay of intracellular and extracellular factors, as well as local perfusion and buffering reactions (40). As a result, changes in local blood flow may have an impact on proton efflux rates and on local pH values (49). Previous work has indicated that there may be some changes in local perfusion following EIMD (14). However, pH during incremental exercise was not altered, and pH at exhaustion was actually higher following EIMD, indicating that any reduction in perfusion did not have a large effect on pH, and also that pH per se does not determine changes in exercise tolerance following EIMD.

At rest, an increased [Pi] resulted in a 50% increase in the resting [Pi]/[PCr] 48 h after eccentric exercise. These findings are consistent with several previous studies using ³¹P-MRS, which also reported increases in resting [Pi]/[PCr] following eccentric exercise (26, 27, 33, 41). This elevation in resting [Pi] may be interpreted as evidence for an increase in the rate of ATP turnover associated with an increased resting metabolic rate. The repair and remodeling of tissue damaged via eccentric exercise, such as that reported by Yu et al. (54) and Yu and Thornell (55), could lead to such an increase in the rate of ATP turnover. Furthermore, the resting muscle glycolytic content of eccentrically exercised muscle is reported to be ~22% lower than in the control condition 48 h after an eccentric exercise bout (2). This difference, which particularly affects the preferentially damaged type II fibers, has been attributed to increased resting muscle glycolgen utilization (2). An alternative explanation for the increase in [Pi]/[PCR], which, importantly, results from increased [Pi], but not decreased [PCR], may be linked to the substantial microvascular dysfunction reported following eccentric exercise (23). Although [PCR] data presented herein suggest that there may be no limitation to O₂ delivery per se, there may be other consequences of the impaired muscle microcirculatory flow observed by Kano and colleagues (23). For example, an increase in nonflowing capillaries, in conjunction with impaired perfusion and diffusion properties, might possibly lead to an accumulation of phosphate in the intramuscular space.

Increases in resting [Pi] and [Pi]/[PCr] following a period of cast immobilization have been implicated in the associated loss of muscular strength (37, 38). Similarly, increases in the resting [Pi]/[PCr] of patients with postpolio residual paralysis, a condition that is characterized by decreased endurance capacity and muscular weakness, is related to the severity of paralysis (44). Increases in intracellular [Pi] can inhibit force production via direct action on cross-bridge formation or on other sites in the excitation-contraction pathway and may play a key role in the development of muscle fatigue (16, 52). Thus the reduction in peak torque and exercise tolerance observed in the present study following muscle damage may be related to the increased [Pi] and [Pi]/[PCr] that was observed not only at rest, but also during incremental exercise. The observation that the significant increases in [Pi] and [Pi]/[PCr] at rest at 48 h post-EIMD were also evident during the dynamic exercise test is of particular interest. The rate of increase in [Pi] was not altered as a result of the muscle damage. However, the earlier termination of the test at 48 h resulted in there being no significant difference in end-exercise [Pi] values. It might, therefore, be speculated that exercise intolerance was mediated by [Pi]. It has been proposed that Pi may enter the sarcoplasmic reticulum and bind to Ca²⁺, reducing free Ca²⁺ and contributing to the reduced Ca²⁺ release observed during fatigue (52). However, it should be cautioned that wide intersubject variability in [Pi] values, along with complications in the measurement of [Pi] using ³¹P-MRS, limit confidence in this interpretation. Specifically, as a result of the small concentration of Pi at rest, there is significant potential error in its assessment and thus also in calculation of the percent increase in [Pi] during exercise relative to this baseline value. This may in some way explain the nonconstant value of the sum of PCR and Pi during the course of the exercise, although there is also some suggestion that Pi may enter either mitochondrial or phosphomonester pools, rendering it temporally undetectable (5).

Central fatigue factors, including the production of inflammatory cytokines, may also be involved in the reduced time to exhaustion following EIMD observed herein. Carmichael et al. (9) have reported that increases in brain interleukin-1β in areas responsible for movement, motivation, and the perception of effort and pain, were associated with decreases in treadmill run times to fatigue in mice. In human subjects, it has been proposed that the increased sense of effort reported during dynamic exercise following eccentric exercise influences time trial performance (30, 48). The duration that an individual can exercise to “volitional exhaustion” is clearly regulated by a complex interaction of central and peripheral factors, with the decision to terminate exercise proposed to be a conscious behavior based on the perception of alterations in subconscious homeostatic control systems (45). It is beyond the scope of this study to determine whether central or peripheral factors make the greater contribution to the accelerated fatigue development experienced with EIMD.

In conclusion, the results of this study suggest that the reduced exercise tolerance following EIMD cannot be attributed to a greater rate of nonoxidative energy metabolism (as inferred from the changes in [PCR] and pH). Although we cannot exclude an important role for centrally mediated fatigue, our results indicate that increases in resting [Pi] that are maintained during exercise may be a contributory factor to the reduced exercise tolerance that is observed following EIMD.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


20. Hogan MC, Richardson RS, Haseler LJ. 
8. Byrne C, Eston R. 
13. Clarkson PM, Nosaka K, Braun B. 
15. Debold EP, Romatowski J, Fitts RH. 


2. Performed 48 h later.


24. Kano Y, Sampei K, Matsudo H. 


1. Braun WA, Dutto DJ.


2. Byrnes C, Eston R. 

43. Schneider DA, Berwick JP, Sabapathy S, Minahan CL.


