Muscle metabolism and acid-base status during exercise in forearm work-related myalgia measured with $^{31}$P-MRS

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1School of Physical and Health Education, Nipissing University, North Bay; and 2Department of Kinesiology, Faculty of Applied Health Science, University of Waterloo, and 3Department of Medical Biophysics, Schulich School of Medicine and Dentistry, University of Western Ontario, 4Imaging Division, Lawson Health Research Institute, St. Joseph’s Health Care, and 5School of Kinesiology, Faculty of Health Sciences, University of Western Ontario, London, Ontario Canada

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Raymer GH, Green HJ, Ranney DA, Marsh GD, Thompson RT. Muscle metabolism and acid-base status during exercise in forearm work-related myalgia measured with $^{31}$P-MRS. J Appl Physiol 106: 1198–1206, 2009. First published December 26, 2008; doi:10.1152/japplphysiol.90925.2008.—In this study, we examined muscle metabolic and acid-base status during incremental wrist extension exercise in the forearm of individuals with work-related myalgia (WRM). Eighteen women employed in full-time occupations involving repetitive forearm labor were recruited in this cross-sectional study. Nine of these women were diagnosed with WRM, while the other nine had no previous WRM history and were used as age-matched controls (Con). Phosphorus-31 magnetic resonance spectroscopy ($^{31}$P-MRS) was used to noninvasively monitor the intracellular concentrations of phosphocreatine ($[\text{PCr}]$) and inorganic phosphate ($[\text{Pi}]$) as well as intracellular pH ($[\text{pHi}]$) status during exercise in WRM and Con. We observed a 38% decreased work capacity in WRM compared with Con [0.18 W (SD 0.03) vs. 0.28 W (SD 0.10); $P = 0.007$]. Piecewise linear regression of the incremental exercise data revealed that the onset of a faster decrease in $[\text{Pi}]$ ($i.e.$, the pH threshold, $[\text{pHT}]$) and the onset of a faster increase in $[\text{Pi}]/[\text{PCr}]$ (i.e., the phosphorylation threshold, PT) occurred at a 14% relatively lower power output in WRM [pHT: 45.2% (SD 5.3) vs. 59.0% (SD 4.6), $P < 0.001$; PT: 44.8% (SD 4.3) vs. 57.8% (SD 3.1), $P < 0.001$; % of peak power output, Con vs. WRM, respectively]. Monoeponential modeling of the kinetics of $[\text{PCr}]$ and $[\text{pHi}]$ recovery following exercise demonstrated a slower ($P = 0.005$) time constant ($\tau$) for $[\text{PCr}]$ in WRM [113 s (SD 25)] vs. Con [77 s (SD 23)] and a slower ($P = 0.007$) $\tau$ for $[\text{pHi}]$, in WRM [370 s (SD 178)] vs. Con [179 s (SD 52)]. In conclusion, our results suggest that WRM is associated with an increased reliance on nonoxidative metabolism. Possible mechanisms include a reduction in local muscle blood flow and perfusion, an increased ATP cost of force production, or both.

phosphorus magnetic resonance spectroscopy; extensor carpi radialis brevis; wrist extension; intracellular pH; phosphocreatine; intracellular threshold; recovery; repetitive strain injury

THE MUSCLES PRODUCING WRIST EXTENSION MOVEMENTS, including the extensor carpi radialis brevis muscle (ECRB), are commonly used in daily living and occupational tasks. Individuals performing repeated manual tasks involving wrist extension are at risk of developing a repetitive strain injury also referred to as work-related myalgia (WRM),1 a general term used to describe pain and discomfort that result from repetitive movements. In 2000–2001, 1 in 10 Canadians aged 20 or older, or an estimated 2.3 million people, reported WRM that was serious enough to limit their normal activities (49). WRM also represents a major component of worker disability and discomfort: ~44% of workers involved in repetitive upper limb tasks have reported muscle pain and tenderness (44).

While the pathophysiology of WRM is not well understood, it is clear that this disorder is commonly manifested in occupational settings that demand prolonged static and/or highly repetitive loads, often at low amplitude (19). Anatomic factors, such as biomechanical stresses on soft tissue and nerves, have been identified as important considerations (18); however, data suggest that abnormalities in forearm muscle blood flow (4, 33–35, 37) may represent a significant part of the etiology. Regardless of the origin of this disorder, extended muscle usage or contractile activity could result in muscle cellular defects leading to peripheral muscular fatigue and weakness that are characteristics of WRM (4, 15, 43). In this view, assessment of cellular metabolism in the ECRB may be vital to our understanding of WRM.

A reduction in local muscle blood flow has previously been shown to be associated with trapezius WRM (37), as has lower capillary-to-muscle fiber area ratios (27). “Ragged red” fibers, or muscle fiber structure breakdown, have also been observed in WRM (32, 34, 36, 37) and may also be a possible consequence of a localized ischemia (22). Ischemia, particularly when present during contractile activity, may increase the levels of reactive oxygen species (ROS) such as the superoxide radical anion ($O_2^{-}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($\cdot OH$). These ROS are particularly destructive to phospholipid membranes, cation pumps, and mitochondria in the cell (12, 30). Ischemia also has important consequences for cellular metabolic status. The generation of adenosine triphosphate (ATP) becomes more anaerobic dependent with a greater accumulation of metabolic by-products such as adenosine diphosphate (ADP) and inorganic phosphate (Pi). Both ADP and Pi act to stimulate the oxidative and nonoxidative pathways, ensuring close coupling between ATP demand and supply. Stable or slowly increasing values of ADP concentration ([ADP]) indicate a close coupling between ATP demand and oxidative ATP supply, whereas more rapid increases could reflect an inability of oxidative phosphorylation to meet ATP demands (11) and/or reflect metabolic adjustments (i.e., a

1 Forearm work-related myalgia was called repetitive strain injury before a 1987 moratorium banned the use of this term, considering it to be a reversible neurosis (see Ref. 43).
reduction in the intracellular energy state) required to maintain a given level of oxidative phosphorylation (20, 53).

In the present study, we have used phosphorus-31 magnetic resonance spectroscopy (31P-MRS) to measure muscle high-energy phosphate status in WRM. With 31P-MRS, the relative concentrations of ATP ([ATP]) and phosphocreatine ([PCr]) are easily determined, but [ADP] is typically too low to be detected above the background noise in the spectrum. However, because the consumption of ATP is stoichiometrically equivalent to the hydrolysis of PCr to P:\textsubscript{i}, the ratio [P:\textsubscript{i}]/[PCr] may be used as an index of ADP to ATP regeneration, and therefore a reflection of cellular metabolic status (5, 6). Because forearm WRM has been shown to be associated with reduced muscle blood flow (4), we sought to determine whether 31P-MRS could provide insight into the relative balance of oxidative and nonoxidative ATP production in forearm WRM. Specifically, we measured muscle metabolic status (i.e., [P:\textsubscript{i}]/[PCr]) and acid-base status [intracellular pH (pHi)] in the ECRB of individuals with and without WRM before, during, and after an incremental wrist extension exercise protocol to volitional fatigue. The [P:\textsubscript{i}]/[PCr] and pHi data collected during exercise were plotted against the rising power output for each subject and modeled with a piecewise linear regression function (52) to determine inflections in the rate of [P:\textsubscript{i}]/[PCr] increase and pHi decline. The [P:\textsubscript{i}]/[PCr]- and pHi\textsubscript{2}-power output relationships during an incremental exercise test have previously been studied in young and old individuals and with exercise training or pharmacological interventions. In general, an earlier onset of a more rapid phase of [P:\textsubscript{i}]/[PCr] increases suggests a decrease in muscle oxidative potential (41), while a delayed onset suggests an enhanced aerobic ATP production in the face of rising power output demands (40, 45). Similarly, a more favorable pHi\textsubscript{2}-power output relationship can also predispose to increased exercise tolerance (40, 45). Therefore, in this study we hypothesized that forearm WRM would be associated with reduced exercise tolerance, an earlier onset of a more rapid increase in [P:\textsubscript{i}]/[PCr], and an earlier onset of a more rapid phase of intracellular acidification.

METHODS

Participants. Nine women with WRM and nine matched healthy female control subjects (Con) volunteered for the study. Inclusion in the WRM group was based on a diagnosis of WRM involving the ECRB by one of the investigators, an anatomist and orthopedic physician (D. A. Ranney) with an intensive clinical history of diagnosing WRM. Pain due to repetitive work, tenderness of ECRB, and physician (D. A. Ranney) with an intensive clinical history of diagnosing WRM. Pain due to repetitive work, tenderness of ECRB, and occupation (36, 37). Characteristics of WRM and Con study in both groups. Inclusion criteria were therefore similar to those of previous WRM studies (36, 37). Characteristics of WRM and Con participants are shown in Table 1. To determine the reproducibility of the experimental protocol, five women from the Con group and three additional healthy female volunteers (total n = 8) underwent repeated tests with 1 wk separating the two trials. Before the experiment, all procedures and any potential risks were explained to each volunteer, and an informed consent document was signed before participation.

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<thead>
<tr>
<th>Table 1. Participant characteristics</th>
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<td>Mass, kg</td>
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Values are given as means (SD). WRM, work-related myalgia; Con, control.

*Significant difference (P < 0.05) compared with Con.

The study was approved by the University of Waterloo Ethics Review of Research Involving Human Participants.

Experimental protocol. An incremental wrist flexion exercise test was performed to assess metabolic and acid-base status in the ECRB. Before the start of exercise, participants were positioned seated in a chair next to a custom-built nonmagnetic wrist extension ergometer inserted inside the bore of a 1.9-T superconducting magnet. The dominant arm of each participant was placed inside the ergometer by placing the arm in full extension and then abducting to 90\textdegree. In this position, the wrist was in a slightly supinated position, allowing participants to grasp a handle attached to the lever of the ergometer while a padded strap rested across the back of the metacarpophalangeal joints. Exercise was performed by extension of the wrist, which moved the handle through a range of motion (ROM) of ~40\textdegree. The position of the handle and strap was adjusted for each participant so that the pivot of the lever centered on the axis of the wrist joint. Pilot testing in our laboratory confirmed that this task activated primarily the ECRB, because the integrated electromyography (iEMG) signal in the ECRB significantly increased in a progressive manner throughout the incremental exercise protocol, with only a small increase in the iEMG signal in the extensor carpi radialis longus and the extensor carpi ulnaris and no iEMG signal increase in the flexor carpi ulnaris (9).

Exercise consisted of repeated wrist extension at a frequency of 0.167 Hz (3-s concentric contraction/3-s eccentric contraction). The frequency of exercise contraction was selected to simulate low-intensity, near-isometric occupational tasks that may predispose to WRM. The extension of the wrist in the ergometer raised and lowered a water reservoir outside the bore of the magnet through the use of a cable and pulley system. Adjusting the water level in the suspended reservoir changed the resistance; thus the resistance was increased in a ramp-like fashion by pumping water into the reservoir at a constant rate (0.200 l/min) by means of a roller pump (Cole-Parmer Instruments, Chicago, IL.). A metronome set at 0.167 Hz was used to help participants maintain the proper contraction frequency. To ensure that they maintained a consistent ROM, the ergometer was interfaced to a computer data acquisition system allowing a light-emitting diode (LED) to signal and record the start (i.e., 0\textdegree) and end (i.e., 40\textdegree) of wrist extension ROM.

After a 3-min period during which resting measurements were taken, participants began wrist extension exercise while water was continuously added to a 0.20-kg reservoir at a rate of 0.20 kg/min. Exercise was terminated at volitional fatigue, or at the point where participants were unable to maintain the full ROM as determined by their inability to illuminate the LED at full ROM (i.e., 40\textdegree wrist extension) on three successive contractions. The work done by each participant was calculated by using the known repetition rate (0.167
extension exercise, the ECRB \(1H\)-NMR signal intensity is visibly greater than the extensor carpi radialis brevis muscle (ECRB). B: after a bout of wrist extension exercise, the ECRB \(1H\)-NMR signal intensity is visibly greater than the flexor carpi ulnaris muscle, confirming that our exercise protocol primarily activated the ECRB. rf, Radio frequency.

Hz), the arc distance of the lever (0.07 m), and the weight of the reservoir and water [0.20 kg + (flow rate × exercise time)] and standard physical relationships. This produced a ramp slope of −0.025 W/min. The actual flow rate of water into the reservoir was calculated as the total volume of water added during the exercise test divided by the time to fatigue.

\(31P\)-MRS. Data were obtained with a 30-cm-bore, 1.9-T superconducting magnet interfaced with a SMIS/IMRIS console (Surrey Medical Imaging Systems, Guilford, UK; Innovative Magnetic Resonance Imaging Systems, Winnipeg, MB, Canada). When the participant’s arm was placed in the ergometer within the bore of the magnet, the dorsal aspect of the forearm was situated over a 4-cm dual-tuned \(1H\)-\(31P\) surface coil, ~4–7 cm distal to the lateral epicondyle of the humerus. In this position, the nuclear magnetic resonance (NMR) signal was obtained primarily from the ECRB, as confirmed by pilot \(1H\)-NMR imaging (Fig. 1). All spectra were acquired with a 3-ms 90° adiabatic radio frequency pulse, a 3.3-kHz receiver bandwidth, a 8-μs delay time, and 2,048 complex data points with a 300-μs dwell time. Each spectrum was produced from an average of 10 free induction decay (FID) signals with a repetition time (TR) of 6 s (total acquisition time of 60 s). Data were collected continuously throughout rest and exercise and during the recovery period (12 min) immediately following cessation of exercise. An example of the Fourier-transformed FID signals (i.e., \(31P\)-MRS spectra) collected during an incremental exercise protocol in one healthy subject is shown in Fig. 2.

Quantification of the \(31P\)-MRS metabolite data was performed in the time (acquisition) domain by fitting each \(31P\) FID to a sum of damped sinusoids, which could be varied in terms of amplitude, phase, delay time, damping constant, and frequency. This method utilized prior knowledge and a nonlinear least-squares algorithm to iteratively reduce the difference in error between the actual data and the experimental model (2). \([\text{PCr}]\) and \([\text{Pi}]\) were determined from the amplitude of the exponential model function at time \(t = 0\). For each parameter, metabolite concentration was expressed relative to total phosphate signal ([Phos]), and because only ratios of metabolites were used, correction factors were not applied. \(pH\) was determined from the chemical shift of \(P_i\) with respect to PCr.

Data analyses. For the purposes of plotting against time or power output, each spectrum or data point was assumed to represent the midpoint of the acquisition time. To facilitate determination of the biphasic parameters of \([\text{P}_i]/[\text{PCr}]\), a logarithm-linear transformation was used. Thus log([P]i/[PCr]) and \(pH\) were plotted as a function of power output for each subject. Piecewise linear regression analysis was then applied to these plots by use of an algorithm that estimated the slope and intercept parameters of two regression functions and determined an inflection at which the slope of the two lines diverged. An \(F\) test \((P < 0.05)\) was used to evaluate whether a single or multiple regression provided the optimal fit of the data. The location of the

<table>
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<th>Table 2. Time to fatigue and peak power output during incremental wrist extension exercise</th>
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<td>-----------------------------------------------</td>
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<tr>
<td>Time to fatigue, min</td>
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<td>Peak power output, W</td>
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Values are given as means (SD). *Significant difference \((P < 0.05)\) compared with Con.

Fig. 1. A: pilot \(1H\)-nuclear magnetic resonance (NMR) image from 1 subject, showing the approximate placement of the 4-cm \(31P\)-NMR surface coil above the extensor carpi radialis brevis muscle (ECRB). B: a bout of wrist extension exercise, the ECRB \(1H\)-NMR signal intensity is visibly greater than the flexor carpi ulnaris muscle, confirming that our exercise protocol primarily activated the ECRB. rf, Radio frequency.

Fig. 2. Representative \(31P\)-magnetic resonance spectroscopy (MRS) spectra collected from the ECRB from a typical healthy female volunteer. Spectra (time resolution = 60 s) were collected starting at rest (time = 0 min) throughout incremental wrist extension exercise and during 12 min of resting recovery. Labels indicate peaks used for metabolite quantification. \(P_i\), inorganic phosphate; \(\text{PCr}\), phosphocreatine.

Fig. 3. Intracellular pH and log([P]i/[PCr])−power output relationship during incremental wrist extension exercise in a typical healthy female volunteer. Solid lines show piecewise linear regression for detection \((P < 0.05)\) of the onset of intracellular acidosis (pH threshold, \(pHT\)) and the onset of a rapid phase of log([P]i/[PCr]) increase (phosphorylation threshold, PT).
estimated inflection point was confirmed by visual inspection to ensure a fit that coincided with the best visual representation of the data.

**Statistical analysis.** Statistical analyses were performed with the Sigmastat (v3.5) statistical program for the PC (Systat). Comparisons between WRM and Con for the biphasic parameters of pH, and log([Pi]/[PCr]) and the monoexponential recovery parameters of [PCr] and pH

were identified as the onset of intracellular acidosis (i.e., the pH threshold, pHT) and the onset of more rapid changes in these parameters. Inflection points marking the transition between the slow to more rapid phases were identified as the onset of intracellular acidosis (i.e., the pH threshold, pHT), and the end of incremental exercise. At rest, pH
 was not different in WRM compared with Con [WRM: 7.01 (0.04) vs. Con: 7.03 (0.03); P = 0.373]. However, the pHT occurred earlier in WRM, such that the transition from the slow to the rapid phase of pH decline was observed at a 0.09-W lower power output in WRM compared with Con (Table 5). However, to control for the confounding observation of a reduced peak power output (PPO) in the incremental exercise test in WRM, the pHT was also expressed in relative terms, as a percentage of PPO (%PPO). Thus the pHT occurred at a 14% relatively lower (P < 0.001) power output in WRM (Table 5).

Finally, despite the earlier occurrence of acidification in WRM, there was no difference in the total amount of acid accumulation, because the pH
 reached at the point of cessation of exercise was not different (P = 0.696) between groups [WRM 6.55 (0.19) vs. Con 6.52 (0.09)]. The biphasic parameters (i.e., slope and intercept) describing the initial and rapid phases of pH
 decline in both WRM and Con are presented in Table 6, showing no difference between WRM and Con in the slope or rate of acidification either before (P = 0.286) or after (P = 0.198) the pHT.

**Muscle metabolic status.** Figure 5 shows the muscle metabolic status [as estimated by log([Pi]/[PCr])] at rest, at the onset of a more rapid change in log([Pi]/[PCr]) (i.e., the PT), and at the end of incremental exercise. Similar to pH
, rest log([Pi]/[PCr]) was not different in WRM compared with Con [WRM −0.85 (0.20) vs. Con −0.79 (0.22); P = 0.559]. However, the PT occurred earlier in WRM, such that the transition from the slow to the rapid phase of log([Pi]/[PCr]) increase was observed at a 13% relatively lower (P < 0.001) %PPO in WRM compared with Con (Table 5). Despite the earlier occurrence of

**RESULTS**

Compared with Con, WRM had a 38% lower (P = 0.007) peak power output and thus a shorter (P = 0.007) time to fatigue (Table 2). In both WRM and Con, the ECRB metabolic and acid-base response during incremental wrist extension exercise was characterized by an initial phase of slow pH
 decline and log([Pi]/[PCr]) increase, followed by a phase of a more rapid rate of change in these parameters. Inflection points marking the transition between the slow to more rapid phases were identified as the onset of intracellular acidosis (i.e., the pH threshold, pHT) and the onset of more rapid changes in [Pi]/[PCr] (i.e., the phosphorylation potential threshold, PT). A typical example of the modeling of the biphasic pH
 and log([Pi]/[PCr]) response during incremental exercise in a healthy female volunteer is shown in Fig. 3.

The test-retest reproducibility of the incremental wrist extension exercise protocol is summarized in Tables 3 and 4. The power output at the pH
 threshold and PT in a subgroup (n = 8) of healthy female volunteers was strong (r = 0.966 and r = 0.944, test and retest, respectively), showing a significant (P < 0.001) correlation and no statistical differences (P = 0.526 and P = 0.852) between the two tests. The monoexponential modeling of the [PCr] recovery data demonstrated good correlation between test and retest (r = 0.857) for the [PCr] time constant (τ) (P = 0.006); however, the mean [PCr] τ was 13 s lower in the retest (P = 0.016).

**Muscle acid-base status.** Figure 4 shows the ECRB muscle pH status at rest, at the onset of intracellular acidosis (i.e., the pHT), and at the end of incremental exercise. At rest, pH
 was not different in WRM compared with Con [WRM: 7.01 (0.04) vs. Con: 7.03 (0.03); P = 0.373]. However, the pHT occurred earlier in WRM, such that the transition from the slow to the rapid phase of pH decline was observed at a 0.09-W lower power output (P = 0.002) power output in WRM compared with Con (Table 5).

Finally, despite the earlier onset of acidification in WRM, there was no difference in the total amount of acid accumulation, because the pH
 reached at the point of cessation of exercise was not different (P = 0.696) between groups [WRM 6.55 (0.19) vs. Con 6.52 (0.09)]. The biphasic parameters (i.e., slope and intercept) describing the initial and rapid phases of pH
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, rest log([Pi]/[PCr]) was not different in WRM compared with Con [WRM −0.85 (0.20) vs. Con −0.79 (0.22); P = 0.559]. However, the PT occurred earlier in WRM, such that the transition from the slow to the rapid phase of log([Pi]/[PCr]) increase was observed at a 13% relatively lower (P < 0.001) %PPO in WRM compared with Con (Table 5). Despite the earlier occurrence of

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**Table 3. Test-retest reproducibility of pH and PT in healthy women during incremental wrist extension exercise test**

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<th>Test</th>
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<td>pH</td>
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<tr>
<td>Power, W</td>
<td>0.17 (0.06)*†</td>
<td>0.17 (0.05)</td>
<td>−0.194 (P = 0.852)</td>
<td>0.944 (P &lt; 0.001)</td>
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<tr>
<td>Log([Pi]/[PCr])</td>
<td>57.7 (6.9)*†</td>
<td>55.3 (3.8)</td>
<td>1.286 (P = 0.239)</td>
<td>0.836 (P &lt; 0.010)</td>
</tr>
<tr>
<td>pH</td>
<td>7.00 (0.04)</td>
<td>6.98 (0.08)</td>
<td>0.674 (P = 0.522)</td>
<td>0.137 (P = 0.746)</td>
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<tr>
<td>PT</td>
<td></td>
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<tr>
<td>Power, W</td>
<td>−0.24 (0.22)</td>
<td>−0.26 (0.15)</td>
<td>0.573 (P = 0.584)</td>
<td>0.479 (P = 0.230)</td>
</tr>
<tr>
<td>Power, %PPO</td>
<td>57.5 (5.3)*</td>
<td>55.3 (3.8)</td>
<td>1.286 (P = 0.239)</td>
<td>0.836 (P &lt; 0.010)</td>
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<td>Log([Pi]/[PCr])</td>
<td>0.24 (0.22)</td>
<td>0.26 (0.15)</td>
<td>0.573 (P = 0.584)</td>
<td>0.479 (P = 0.230)</td>
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Values are given as means (SD); n = 8 subjects. pH, pH threshold; %PPO, % peak power output; PT, phosphorylation threshold; [Pi], inorganic phosphate concentration; [PCr], phosphocreatine concentration. *Significant correlation with test 2 (P < 0.05); †significant correlation with PT (P < 0.05). No significant differences between test and retest.

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**Table 4. Test-retest reproducibility of [PCr] monoexponential recovery parameters in healthy women during incremental wrist extension exercise test**

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<tbody>
<tr>
<td>Amplitude (a), %</td>
<td>54.2 (12.3)*†</td>
<td>60.6 (7.7)</td>
<td>−2.219 (P = 0.062)</td>
<td>0.762 (P = 0.028)</td>
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<tr>
<td>Time constant (τ), s</td>
<td>50.6 (11.7)*†</td>
<td>44.9 (9.8)</td>
<td>2.095 (P = 0.074)</td>
<td>0.756 (P = 0.030)</td>
</tr>
<tr>
<td>Time constant (τ), s</td>
<td>86 (21)*†</td>
<td>73 (21)</td>
<td>3.147 (P = 0.016)</td>
<td>0.857 (P = 0.006)</td>
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Values are given as means (SD); n = 8 subjects. Monoexponential recovery parameters are given for the function y = y0 + a(1 − e−bτ), where the time constant (τ) = 1/b. [PCr] is expressed as % of resting [PCr]. *Significant difference (P < 0.05) compared with retest; †significant correlation with retest (P < 0.05).
the more rapid phase of log([Pi]/[PCr]) increase, the value reached at the end of exercise was similar (P = 0.273) between groups [WRM 0.82 (0.38) vs. Con 0.60 (0.44)]. The biphasic parameters (i.e., slope and intercept) describing the initial and rapid phases of log([Pi]/[PCr]) increase in both WRM and Con are presented in Table 6, showing no difference between WRM and Con in the slope of log([Pi]/[PCr]) increase either before (P = 0.073) or after (P = 0.102) the PT.

Metabolic and acid-base status during recovery from exercise. The recovery or return to baseline of [PCr] and pH_i during the time period immediately following incremental exercise is shown in Fig. 6. In WRM, [PCr] was lower than in Con (P < 0.001) at 30 s and 90 s after cessation of exercise. No significant differences were detected for any of the time points for pH_i recovery. Monoexponential modeling of the [PCr]-recovery time relationship (Table 7) demonstrated a slower (P = 0.005) time constant (τ) for [PCr] in WRM [113 s (SD 25)] compared with Con [77 s (SD 23)].

**DISCUSSION**

We examined the metabolic and acid-base response to incremental exercise in the ECRB in participants with forearm WRM. Our major findings in this study were 1) a 38% reduced wrist extension exercise tolerance in participants with WRM; 2) an earlier onset of the rapid phase of pH_i decline in WRM such that it occurred at a 14% relatively lower power output in these participants; 3) a 13% relatively earlier onset of a more rapid phase of log([Pi]/[PCr]) increase; and 4) slower restoration of PCr during recovery from exercise.

To the best of our knowledge, this is the first study of metabolic and acid-base status in patients with WRM using 31P-MRS. The noninvasive nature of this technique makes it ideally suited for investigating muscle metabolism in small muscles such as the ECRB as well as in patients with WRM in whom muscle biopsy sampling may not be practical. In healthy female subjects, we demonstrated our incremental wrist extension exercise protocol to have strong test-retest reproducibility for determination of the power outputs associated with the onset of the more rapid phases of pH_i decline and log([Pi]/[PCr]) increase. While the test-retest correlation of the [PCr] recovery τ was also significant, there was a significant 13-s-lower mean value for τ in the retest, suggesting that the reproducibility of the [PCr] recovery kinetics from a single bout of incremental wrist extension exercise should be considered with caution. Thus the pH_T and the PT are the preferred and more reliable indexes of ECRB metabolic and acid-base status in our exercise protocol.

It is our conclusion that the earlier onset of the pH_T and the PT in WRM contributed to the reduced exercise tolerance in WRM, possibly due to a reduction in the rate or contribution of oxidative (i.e., aerobic) ATP production. A decreased rate or contribution of aerobic ATP production in WRM may be interpreted from the relatively earlier onset of the rapid phase in log([Pi]/[PCr]) (indicative of an increased reliance on substrate-level phosphorylation). The proportion of [Pi] to [PCr] reflects the metabolic activity of the muscle and has often been used as an in vivo estimate of [ADP], a key metabolic regulator. Mitochondrial oxidative phosphorylation and, to a lesser extent cellular glycolysis, are regulated by the relative concentrations of ADP and Pi. With increasing power outputs, the breakdown of ATP and PCr results in an accumulation of ADP and Pi, which stimulate mitochondrial respiration and glycolysis, ensuring close coupling between ATP utilization and ATP resynthesis. Because log([Pi]/[PCr]) is a good in vivo estimate of [ADP], the log([Pi]/[PCr])-power output relationship provides an index of oxidative phosphorylation and, to a lesser extent, glycolytic activity. Thus stable values of log([Pi]/[PCr]) early in an incremental exercise protocol indicate an adequate energy status, whereas rapid increases in this ratio later in incremental exercise reflect a more rapid increase in intracellular [ADP]. This implies that the rapid phase of log([Pi]/[PCr]) increase ultimately reflects the inability of oxidative phosphorylation to meet ATP demands (11) and/or the meta-
bolic adjustments (i.e., a reduction in the intracellular energy state) required to maintain oxidative phosphorylation (7, 53). An increased contribution of anaerobic metabolism in WRM would be expected to lead to an increased accumulation of metabolites such as Pi and lactate. This appears to have occurred in WRM, because we observed an earlier onset of intracellular acidosis in this group. This leftward shift in the pHi-power output relationship in WRM suggests the presence of an intracellular acid-base status that likely predisposed to reduced exercise tolerance in these individuals (45). Metabolic acidosis has been shown to inhibit oxidative phosphorylation (25) and may contribute to muscle fatigue through mechanisms related to allosteric inhibition of the rate-limiting enzymes phosphofructokinase and glycogen phosphorylase, decreased release of Ca\textsubscript{2+} from the sarcoplasmic reticulum, and a reduction in the number and force of muscle cross-bridge activations (12).

A consequence of the leftward shift in the log([Pi]/[PCr])-power output relationships in WRM could be an increased [ADP] signal required to stimulate/maintain oxidative phosphorylation at a given power output/ATP demand. This suggests a corresponding decrease in muscle O\textsubscript{2} availability (i.e., lower PO\textsubscript{2}) (53). Indeed, reduced muscle blood flow and O\textsubscript{2} consumption in individuals with forearm WRM have recently been reported (4). If muscle PO\textsubscript{2} was similarly reduced in the ECRB of the WRM group in the present study, our data would support a reduction in the contribution of oxidative metabolism and/or a compensatory increased reliance on anaerobic metabolism. However, it is noted that a reduced metabolic efficiency (i.e., an increased ATP cost of force production) and/or reduced mitochondrial activity in WRM due to cellular or mitochondrial defects (e.g., electron transport chain uncoupling) cannot be ruled out. In persons with WRM, the chronic repeated cycle of low-intensity contraction and relaxation would be expected to systematically challenge the metabolic processes to supply the ATP needed for contractile activity. Should these demands persist over time, it is possible that abnormalities in the type I (slow twitch) muscle fiber population would become manifest. For example, the presence of “moth-eaten” muscle fibers [muscle fibers lacking in activity

Table 5. Onset (or threshold) of rapid decrease in intracellular pH and rapid increase in log([Pi]/[PCr]) during incremental wrist extension exercise

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>PO, W</th>
<th>%PPO</th>
<th>pH</th>
<th>PO, W</th>
<th>%PPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>WRM</td>
<td>6.97 (0.04)</td>
<td>0.08 (0.02)*</td>
<td>45.2 (5.3)*</td>
<td>−0.20 (0.20)</td>
<td>0.08 (0.02)*</td>
<td>44.8 (4.3)*</td>
</tr>
<tr>
<td>Con</td>
<td>6.98 (0.05)</td>
<td>0.17 (0.07)</td>
<td>59.0 (4.7)</td>
<td>−0.15 (0.19)</td>
<td>0.16 (0.06)</td>
<td>57.8 (3.1)</td>
</tr>
<tr>
<td>P</td>
<td>0.600</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.649</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are given as means (SD). PO, power output. *Significant difference (P < 0.05) compared with Con.

Table 6. Biphasic parameters of intracellular pH and log([Pi]/[PCr]) during incremental wrist extension exercise

<table>
<thead>
<tr>
<th></th>
<th>Initial Phase (below threshold)</th>
<th>Rapid Phase (above threshold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Slope (m1)</td>
<td>Intercept (b1)</td>
</tr>
<tr>
<td>WRM</td>
<td>−0.38 (0.34)†</td>
<td>7.00 (0.05)*†</td>
</tr>
<tr>
<td>Con</td>
<td>−0.58 (0.44)†</td>
<td>7.06 (0.04)*†</td>
</tr>
<tr>
<td>log([Pi]/[PCr])</td>
<td>WRM</td>
<td>6.83 (2.05)*</td>
</tr>
<tr>
<td>Con</td>
<td>4.08 (3.76)*</td>
<td>−0.67 (0.23)*</td>
</tr>
</tbody>
</table>

Values are given as means (SD). Slope and intercept parameters are given for the piecewise linear function $y = m_{x}x + b_{1}$ ($x < \text{threshold}$); $y = m_{2}x + b_{2}$ ($x > \text{threshold}$). pH, intracellular pH threshold; PT, log([Pi]/[PCr]) threshold. *Significant difference (P < 0.05) compared with Con; †significant difference (P < 0.05) compared with rapid phase.

Fig. 5. Intracellular log([Pi]/[PCr])-power output relationship during incremental wrist extension exercise in participants with WRM compared with Con. Data are expressed relative to power output (top) and as % of peak power output (bottom). Data are presented as means ± SD. *Significant difference (P < 0.05) compared with Con.
of mitochondrial enzyme nicotinamide adenine dinucleotide trazolium reductase (NADH-TR) and deficits in cytochrome-c oxidase (COX) may be interpreted as evidence of disturbed oxidative metabolism (26, 27, 39). Both NADH-TR and COX are essential for the generation of mitochondrial ATP, and decreased levels of these enzymes may provide an explanation for previous reports showing decreased resting [ATP] in women suffering from trapezius WRM (36, 38). The mechanism responsible for reduced mitochondrial NADH-TR and COX in WRM is uncertain, but one possible culprit may be ischemia in the contracting musculature. In at least one animal study, moth-eaten fibers have been shown to be induced by ischemia (22).

A reduced contribution of oxidative phosphorylation and a greater accumulation of metabolites is typically observed during exercise under ischemic conditions (29) and in participants with peripheral vascular disease (28). If reduced muscle blood flow and muscle O₂ consumption were present in during exercise in WRM, this could suggest that forearm WRM has pathophysiology similar to ischemia-reperfusion. Ischemia-reperfusion injury is most often observed in the heart but is also prevalent in skeletal muscle after a temporary occlusion of blood flow (46). It has been suggested that pathology of forearm WRM is associated with the presence of a local static muscle contraction that restricts blood flow during exercise (51). The pattern of exercise in our incremental wrist extension exercise protocol (3-s concentric contraction/3-s eccentric contraction) was selected to simulate low-intensity, near-isometric occupational tasks that may predispose to WRM (19). Whether our dynamic wrist extension exercise protocol impaired muscle flow in our WRM group cannot be determined from our data; however, one interpretation of our observation of a slower rate (i.e., time constant) of PCr resynthesis following exercise in WRM may be a reduction in muscle O₂ availability (21). Because PCr resynthesis occurs exclusively in the mitochondria, and because it depends entirely on oxidative phosphorylation and O₂ supply, a longer PCr time constant would imply a reduction in cellular Po₂ in WRM. Reduced oxidative phosphorylation is a recognized occurrence in healthy individuals exercising under ischemic conditions (29) and in individuals with peripheral vascular disease (28). Reductions in forearm muscle blood flow and O₂ consumption have also been reported during rhythmic handgrip exercise in participants with WRM (4), and an inverse relationship between pain level and cell capillarity in participants with trapezius WRM has been shown to exist (27).

Ischemia-reperfusion, if present in WRM, may lead to a degenerative process that decreases vascular and metabolic function. For example, ROS are known to increase during metabolic stress such as that induced by increased contractile activity and ischemia-reperfusion (47). ROS are particularly destructive to phospholipid membranes, cation pumps, and mitochondria in the cell (12, 30). Not surprisingly, the existence of “ragged red” fibers (muscle fibers with fiber structure breakdown) (32) and “moth-eaten” fibers (muscle fibers lacking in activity of NADH-TR) (36) as well as larger fiber areas and lower capillary-to-fiber area ratios in the type I fibers (36) have been reported in persons with WRM. ROS may also be disruptive to the excitation-contraction (EC) process. During EC, the excitation signal must be conducted to the interior of the cell via the sarcolemma and T tubule and ultimately to the sarcoplasmic reticulum, where the Ca²⁺-release channels (ryanodine receptors) and Ca²⁺-uptake proteins are located. For

![Graph showing recovery of [PCr] and intracellular pH](image)

**Fig. 6. Recovery of [PCr] (top) and intracellular pH (bottom) during the recovery time period following incremental wrist extension exercise in participants with WRM compared with Con. Data are presented as means ± SD.**

*Significant difference (P < 0.05) compared with Con.

<table>
<thead>
<tr>
<th>[PCr]</th>
<th>Baseline (y₀), %</th>
<th>Amplitude (a), %</th>
<th>Time constant (τ), s</th>
</tr>
</thead>
<tbody>
<tr>
<td>WRM</td>
<td>45.2 (9.7)*</td>
<td>64.2 (11.0)*</td>
<td>113 (25)*</td>
</tr>
<tr>
<td>Con</td>
<td>58.3 (10.0)</td>
<td>49.5 (12.7)</td>
<td>77 (23)</td>
</tr>
</tbody>
</table>

Values are given as mean (SD). Monoexponential recovery parameters are given for the function \( y = y₀ + a \left(1 - e^{-\frac{t}{\tau}}\right) \), where time constant \( \tau = \frac{1}{b} \). [PCr] expressed as % of resting [PCr]. *Significant difference (P < 0.05) compared with Con.
signal transduction to remain viable, resulting in an appropriate muscle contraction, Na\(^+\) and K\(^+\) gradients across the membrane and Ca\(^{2+}\) cycling must be protected. These processes appear to be altered with activity (15). As an example, the Na\(^+\)-K\(^+\)-ATPase, which establishes membrane gradients for Na\(^+\) and K\(^+\) after excitation, becomes inactivated with chronic use (16). Similar effects have also been reported for the Ca\(^{2+}\)-ATPase and the Ca\(^{2+}\)-release channel (12). Although the specific mechanism involved in this inactivation remains to be defined, ROS damage may be likely (30).

A number of limitations are acknowledged in the present study. First, the 60-s time resolution of the \(^{31}\)P-MRS spectra was approximately twice as long as we have used in previous studies examining the wrist flexor muscles (13, 45). The reason we used a longer time resolution in the present study is because the ECRB has a smaller cross-sectional area than the flexor digitorum superficialis we have studied in wrist flexion exercise, contributing to less signal-to-noise ratio (SNR) when using a \(^{31}\)P surface coil and nonlocalized \(^{31}\)P-MRS. Also, SNR is proportional to the thickness of adipose tissue, and we observed in pilot testing with MR imaging that females typically possess greater forearm superficial adipose thickness compared with males. Therefore, as we have previously averaged 5 or 6 \(^{31}\)P-MRS spectra in the wrist flexor muscles in healthy males, in the present study we averaged together 10 sequential \(^{31}\)P-MRS spectra to achieve a comparable level of SNR. Unfortunately, this averaging was performed at the expense of decreased time resolution. The extent to which this could have influenced the accuracy of mathematical modeling of the biphasic parameters is unknown.

It is also noted as a limitation that the logarithm transformation of the [Pi]/[PCr] data may disrupt the Gaussian noise distribution and therefore possibly affect the piecewise least-squares regression results. Nevertheless, modeling the [Pi]/[PCr] and pH\(_{i}\) data as having two distinct linear phases appears to be common practice in the literature (1, 3, 8, 10, 14, 17, 24, 31, 42, 48). In either case, it is possible to argue that the data are more appropriately fit to an exponential function. In this case, a definite breakpoint or “threshold” cannot be identified because of the continuous nature of such a curve (23, 50).

Regardless, it is important to note that while a given algorithm or model used may produce different absolute values, a rightward or leftward shift of any standardized point on such a curve, whether it is termed a “threshold” or not, will reflect a meaningful change in the data.

In summary, for the first time using \(^{31}\)P-MRS we have described the dynamics of ECRB metabolic and acid-base status during exercise in individuals with forearm WRM. The major findings of our study were that 1) compared with individuals without WRM, individuals with WRM had a relatively earlier onset of a phase of more rapid increases in log([Pi]/[PCr]); 2) individuals with WRM had a relatively earlier onset of intracellular acidosis; and 3) individuals with WRM displayed a slower rate of PCr resynthesis after incremental wrist extension exercise. Together, these observations suggest that WRM may have contributed to an increased reliance on nonoxidative metabolism with rising power output/ATP demands. Possible mechanisms include a reduction in local muscle blood flow and perfusion, an increased ATP cost of force production, or both.

The likelihood that the above findings contributed to a greater predisposition to fatigue in WRM may be of significant clinical importance when considering the physical demands associated with activities of daily living and repetitive occupational tasks for those living with WRM. Manual labor tasks in industry or within the home environment are typically an “all-or-none” task (i.e., they require a minimum absolute force requirement to be performed successfully). In this light, the significantly worsened metabolic and acid-base status for a given absolute power output in individuals suffering from WRM could be expected to have a severe impact on work tolerance. For example, the typical repetitive low-intensity task performed by workers in industrial settings generally requires power outputs within the initial stable (i.e., steady state) region of the [Pi]/[PCr]- and pH\(_{i}\)-power output curves. However, in individuals with WRM, a leftward shift of these curves could translate to the same power output requirement existing within the phase of rapid [Pi]/[PCr] increase and intracellular acid accumulation, in which case work capacity would be expected to be reduced, and workers or employers attempting to mitigate symptoms of WRM might seek to lower the absolute work intensity for a given task.

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REFERENCES


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