Prior exercise delays the onset of acidosis during incremental exercise

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Abstract

The effects of prior moderate and prior heavy intensity exercise on the subsequent metabolic response to incremental exercise were examined. Healthy, young adult subjects \( n = 8 \) performed three randomized plantar-flexion exercise tests: 1) an incremental exercise test \( \sim 0.6 \) W/min) to volitional fatigue (RAMP); 2) RAMP preceded by 6 min moderate-intensity, constant-load exercise below the intracellular pH threshold (pHT; MOD-RAMP); and 3) RAMP preceded by 6 min heavy-intensity, constant-load exercise above pHT (HVY-RAMP); the constant-load and incremental exercise periods were separated by 6 min rest. \(^{31}\)Phosphorus magnetic resonance spectroscopy was used to continuously monitor intracellular pH, phosphocreatine ([PCr]), and inorganic phosphate ([P\(_i\)]). No differences in exercise performance or the metabolic response to exercise were observed between RAMP and MOD-RAMP. However, compared to RAMP, a 14 % (SD 10) increase (\( P<0.01 \)) in peak power output (PPO) was observed in HVY-RAMP. The improved exercise performance in HVY-RAMP was accompanied by a delayed (\( P=0.01 \)) onset of intracellular acidosis [HVY-RAMP: 60.4 % PPO (SD 11.7) vs. RAMP: 45.8 % PPO (SD 9.4)], and a delayed (\( P<0.01 \)) onset of rapid increases in [P\(_i\)]/[PCr] [HVY-RAMP: 61.5 % PPO (SD 12.0) vs. RAMP: 45.1 % PPO (SD 9.1)]. In conclusion, prior heavy-intensity delayed the onset of intracellular acidosis and enhanced exercise performance during a subsequent incremental exercise test.
Keywords

Phosphorus magnetic resonance spectroscopy, 31P-MRS, warm-up, phosphocreatine, intracellular pH, plantar-flexion
Introduction

A prior bout of heavy-intensity ‘warm-up’ exercise has been shown to result in acceleration or amplification of the phase II oxygen uptake (VO$_2$) response and an attenuation of the subsequent VO$_2$ slow component in subsequent constant load exercise (2; 4; 6; 10; 20; 23). These altered pulmonary VO$_2$ responses have been associated with decreased phosphocreatine (PCr) utilization (23). However, a recent study demonstrating the elimination of the VO$_2$ slow component following prior heavy exercise found evidence of increased PCr breakdown and reduced efficiency during subsequent exercise (24). Therefore, despite a general consensus in the literature on prior exercise reducing the VO$_2$ slow component and altering the phase II VO$_2$ response, the metabolic effects of prior exercise have yet to be elucidated.

A prior bout of heavy intensity exercise may modulate the metabolic response to a subsequent bout via a number of mechanisms. One commonly proposed candidate is a vasodilation in the active muscle caused by the accumulation of vasoactive metabolites such as H$^+$ (9; 19). This mechanism is proposed to increase blood flow and O$_2$ delivery to exercising muscle as well as reducing or eliminating regional perfusion heterogeneities that otherwise existed following the onset of heavy exercise (14). Accumulation of H$^+$ is also expected to cause decreased binding affinity of hemoglobin for O$_2$ (i.e. an increased Bohr shift of the haemoglobin-oxygen dissociation curve) resulting in increased O$_2$ availability to the working muscle (9). However, evidence that a metabolic acidosis is not a prerequisite for the altered VO$_2$ response following prior exercise can be found in studies demonstrating that prior low- and moderate-intensity exercise that did not cause an elevated blood lactate concentration still resulted in a subsequent reduction of the VO$_2$
slow component (17; 18). These studies suggest that accumulation of H⁺ during prior heavy-intensity exercise does not directly contribute to modulating the VO₂ response.

In the present study, we studied the effects of an altered intracellular metabolic and acid-base status resulting from a prior bout of exercise on the subsequent metabolic response to incremental exercise. While it has previously been shown that prior heavy-intensity exercise causes a significant reduction in the total exercise-induced [PCr] decrement, the metabolic effects of prior exercise on an incremental exercise protocol are unknown. In contrast to constant-load exercise, an incremental exercise protocol begins at a very light intensity and gradually increasing ATP demands. Using whole-body cycling exercise it has been previously shown that prior intense exercise results in a steeper VO₂-power output slope during subsequent incremental exercise only at exercise intensities above the gas exchange threshold (GET) (13). One interpretation of this observation may be that prior exercise does not affect the rate at which oxidative phosphorylation increases during the early stages of subsequent incremental exercise, but that prior exercise may increase muscle O₂ consumption above the GET. This may suggest that exercise economy at heavy work rates is sensitive to the prior activity of the exercising muscles. However, the effects of a prior bout of exercise on the intracellular metabolic and acid-base response during a subsequent incremental exercise have yet to be clearly identified.

Therefore, we used phosphorus-31 magnetic resonance spectroscopy (³¹P-MRS) to monitor the muscle metabolic and acid-base status during an incremental plantarflexion exercise protocol with no prior exercise, prior moderate-intensity exercise, and prior heavy-intensity exercise. Prior moderate-intensity exercise was selected because it
did not produce an accumulation of intracellular H⁺, while prior heavy-intensity exercise allowed comparison to exercise which did generate a significant acidosis. We hypothesized that a rightward shift of the pHᵢ- and [Pᵢ]/[PCr]-power output relationships towards higher power outputs would be evident following prior heavy-intensity exercise because an accumulation of H⁺ would enhance local blood flow and perfusion. In contrast, we hypothesized this effect would not be evident after prior moderate-intensity exercise due to a lack of H⁺ accumulation. Finally, we expected the favourable metabolic response resulting from prior heavy-intensity exercise to be associated with enhanced exercise performance during the subsequent incremental bout.

Methods

Subjects. Eight male volunteers participated in the study [age 24 years (SD 4); mass 76.7 kg (SD 6.4); height 1.82 m (SD 0.04)]. Before the experiment, all procedures and any potential risks were explained to each subject, and an informed consent document was signed prior to participation. The study was approved by The University of Western Ontario Review Board for Health Sciences Research Involving Human Subjects.

Experimental Protocol. Subjects were studied on three occasions: 1) progressive plantar-flexion exercise to fatigue (RAMP); 2) RAMP preceded by a bout of moderate-intensity exercise (MOD-RAMP); and 3) RAMP preceded by a bout of heavy-intensity exercise (HVY-RAMP). The order of these experiments were randomized, and separated by at least 72 hours. In addition, each subject completed at least one familiarization progressive exercise protocol to ensure proper compliance with the exercise protocol. From the familiarization progressive exercise test(s), the onset of intracellular acidosis
(described below) was determined in order to calculate workloads corresponding to MOD and HVY.

Subjects reported to the laboratory at least 2 hours after a light meal and after abstaining from caffeine-containing foods and beverages. Prior to the start of exercise, subjects lay supine on a table with the legs positioned in a custom-built MR-compatible ankle exercise ergometer (21). The dominant leg of each subject was positioned in the ergometer foot securely attached to a lever or footplate on the ergometer. The footplate was aligned such that the pivot of the lever centred on the axis of the ankle joint. The subjects remained supine throughout the entire protocol.

The exercise consisted of repeatedly depressing the footplate at a frequency of 0.5 Hz (1-s contraction/1-s relaxation) through a range of motion of ~35°. This action raised and lowered a water reservoir, in which the resistance was manipulated by adding known volumes of water for the constant load prior exercise bouts, or by adding water in a constant ramp-like fashion by means of a roller pump (Cole-Parmer Instruments, Chicago, IL). A metronome set at 0.5 Hz was used to help subjects maintain the proper contraction frequency. To ensure subjects maintained a consistent range of motion (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°) and end (i.e. 35°) of plantar-flexion ROM. Exercise was terminated at volitional fatigue, or at the point where subjects were unable to maintain the full ROM as determined by their inability to illuminate the LED at full ROM (i.e. 35° plantar-flexion) on 3 successive contractions.

After a 3-min period during which resting measurements were recorded, subjects began exercise. In RAMP, exercise commenced at the same time that water started to
flow continuously into the reservoir at a rate of 1.4 kg/min. Power output was calculated using the known repetition rate (0.5Hz), the displacement of the suspended reservoir (0.08 m), and the weight of the reservoir + water added [1.7 kg + (1.4kg/min * exercise time)] using standard physical relationships. This produced a ramp slope of ~0.6 W/min from an initial load of ~0.7 W. 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.

In MOD-RAMP and HVY-RAMP, a 3-min period of resting data collection was followed by a 6-min bout of constant-load MOD or HVY exercise. The power outputs corresponding to MOD and HVY were calculated for each subject from the familiarization progressive exercise test as follows. Logarithm-transformed intracellular [H+] data (i.e. pH) were plotted as a function of power output for each subject, and a piecewise linear regression algorithm was applied to these plots for detection of a threshold or onset of rapid increases in intracellular [H+]. The power output corresponding to MOD was taken as the power output midway between the initial load and the load corresponding to the onset of a rapid acidification (i.e. pHT). Similarly, the power output corresponding to HVY was taken as the power output midway between pHT and the peak power output achieved. The actual power outputs employed in MOD and HVY are summarized in Table 1. Following the completion of 6-min MOD or HVY constant-load exercise, subjects rested comfortably for 6 min and then performed a RAMP exercise protocol identical to that described above.

\(^{31}\text{P-MRS.}\) Intracellular muscle metabolism was studied using \(^{31}\text{P-MRS}\) with the ankle exercise ergometer positioned within the bore of the magnet. Data were obtained
using a 64 cm bore, 3.0 Tesla superconducting magnet interfaced with a SMIS/IMRIS console (Surrey Medical Imaging Systems, Guilford, U.K.; Innovative Magnetic Resonance Imaging Systems, Winnipeg, Canada). A 4 cm square $^{31}$P surface coil was securely fastened over the belly of the lateral gastrocnemius of the dominant leg used for exercise. An external reference standard containing methylene diphosphate (MDP) was affixed to the opposite side of the surface coil. All spectra were acquired with a 3ms 90° adiabatic radio-frequency (rf) pulse, a 5.0 kHz receiver bandwidth and 2048 complex data points. Prior to commencing the experimental protocol, two baseline spectra were acquired. The first baseline spectrum was an average of 6 acquisitions having a repetition time (TR) of 30 s. The second baseline spectrum was the average of the final 24 acquisitions of a 30-acquisition spectrum with TR = 5 s. Calculation of the longitudinal relaxation ($T_1$) correction factors to account for steady-state precession was therefore obtained from the differences in amplitude of the $^{31}$P metabolites in the first baseline spectrum (no $T_1$ effects) from the second baseline spectrum ($T_1$ saturated). All subsequent spectra obtained during the experimental protocol were collected continuously and each spectrum consisted of the average of 3 spectra (TR = 5 s, total acquisition time = 15 s).

Data analyses. Quantification of the $^{31}$P-MRS metabolite data was performed in the time (acquisition) domain by fitting each $^{31}$P 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 non-linear least squares algorithm to iteratively reduce the difference in error between the actual data and the experimental model. The concentrations of the phosphate compounds phosphocreatine ([PCr]) and
inorganic phosphate ([P_i]) were determined from the amplitude of the exponential model function at time equal zero. Final metabolite concentrations were expressed relative to MDP concentration ([MDP]), an external reference standard of known concentration. Normalizing metabolite concentrations to the external standard allowed comparisons between subjects in absolute units, and corrected for small changes in signal amplitude due to motion artefacts that may have been caused by the exercising muscle. Intracellular pH (pH_i) was calculated from the chemical shift (ppm) of the P_i peak relative to PCr. Adenosine diphosphate concentration [ADP] was calculated by assuming a total creatine concentration of 42 mM and that the creatine kinase reaction was at equilibrium. The equilibrium constant was adjusted for intracellular [H^+], which was calculated from pH_i.

For the purposes of plotting against time or power output, each spectra or data point was assumed to represent the midpoint of the acquisition time. To facilitate determination of the biphasic parameters of intracellular [H^+] and [P_i]/[PCr], a logarithm-linear transformation was used. Thus, for intracellular [H^+] data, pH_i (pH_i = -log[H^+]) was plotted as a function of power output for each subject. Similarly, log([P_i]/[PCr]) facilitated detection of biphasic parameters in the [P_i]/[PCr] ratio. Piecewise linear regression analysis was then applied to these plots by use of an algorithm which 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 estimated inflection point was confirmed by visual inspection, and the estimation algorithm was could be adjusted if necessary to provide a fit which coincided with the best visual representation of the data.
Statistical Analysis. Statistical analyses were performed using Sigmastat v3.1 statistical program for the PC (Systat Inc.). The test-retest reliability of the incremental plantar-flexion exercise protocol was analyzed by comparing the Familiarization protocol to the RAMP protocol. Pearson correlations, as well the coefficient of variability (CV) and the 95% confidence intervals of the CV were calculated for this purpose (12). Differences between RAMP, MOD-RAMP, and HVY-RAMP were examined with a repeated measures analysis of variance. A significant $F$ ratio was further analyzed via Student-Newman-Keuls post hoc analysis. In all cases, a $P$ value of less than 0.05 was used to determine the rejection of the null hypothesis. Data are presented as mean (SD).

Results

Exercise performance. Each of the three RAMP protocols were identical except that in the MOD- and HVY-RAMP protocols the incremental exercise was preceded by a bout of constant-load moderate- and heavy-intensity exercise, respectively. Exercise performance in each of the RAMP protocols is summarized in Table 2. Test-retest reliability of the incremental exercise protocol, assessed by comparison of the familiarization protocol to RAMP, was evident by a strong correlation of $r=0.94$ ($P<0.001$) and coefficient of variation $CV=4.2\%$ (95% CI 2.7 – 8.7%) for peak power output. Similarly, the piecewise regression analysis method for detection of the onset of rapid decreases in pH$_i$ (i.e. pHT) also demonstrated good reliability with a test-retest correlation of $r=0.93$ ($P<0.001$) and $CV=5.7\%$ (95% CI 3.7 – 11.9%). The time-to-fatigue (TTF) and peak power output (PPO) were greater in HVY-RAMP compared to
RAMP ($P=0.04$); no differences were observed between RAMP and MOD-RAMP ($P=0.11$) or between HVY-RAMP and MOD-RAMP ($P=0.31$).

*Intramuscular acid-base status.* At rest, pH$_i$ was similar ($P>0.05$) in each trial (RAMP: 6.99 (0.03); MOD-RAMP: 6.99 (0.02); HVY-RAMP: 6.98 (0.01)). In MOD-RAMP, pH$_i$ remained similar to resting values at the end of the 6-min moderate-intensity constant-load exercise (Rest: 6.99 (0.02) vs. MOD: 6.97 (0.03), $P=0.16$). In contrast, 6-min of prior heavy-intensity exercise (HVY) caused a significant decrease in pH$_i$ (Rest: 6.98 (0.01) vs. HVY: 6.65 (0.13), $P<0.01$). After 6-min of resting recovery from prior exercise in MOD-RAMP and HVY-RAMP (i.e. immediately prior to the start of incremental exercise), pH$_i$ was not different from Rest in either condition (MOD-RAMP: 6.98 (0.03), $P=0.24$; HVY-RAMP: pH$_i$, 6.96 (0.08), $P=0.16$). The value of pH$_i$ at the point of fatigue at the end of incremental exercise was not different in MOD-RAMP (6.64 (0.15), $P=0.33$) or in HVY-RAMP (6.61 (0.16), $P=0.51$) compared to RAMP (6.57 (0.18)).

Data from a representative subject showing pH$_i$ throughout the entire RAMP, MOD-RAMP, and HVY-RAMP protocols are presented in Fig. 1. During incremental exercise, pH$_i$ displayed a biphasic decline, with an initial slow and a later fast component (Fig. 3). Piecewise linear regression analysis determined that in every subject, the data were best described by fitting two linear components compared to a single linear fit ($P<0.05$). An inflection or transition point between the slow and fast linear components is represented by the pHT. The slope of the pH$_i$-power output relationship was less ($P<0.05$) below the pHT than above, with no differences in the rate of rapid pH$_i$ decline (above pHT) between conditions (Table 3). The onset of intracellular acidosis (Table 4)
occurred at 45.8 (9.4) % of peak power output (% PPO) in RAMP, which was not different than MOD-RAMP (47.5 (15.9) % PPO, P=0.70). However, in HVY-RAMP, the pHT occurred at a higher power output during exercise (60.4 (11.7) % PPO, P=0.01).

**Intramuscular metabolite status.** At rest, [P_i]/[PCr] was similar (P>0.05) in each trial (RAMP: 0.09 (0.02); MOD-RAMP: 0.08 (0.03); HVY-RAMP: 0.06 (0.02)). In both MOD-RAMP and HVY-RAMP, [P_i]/[PCr] was elevated (P<0.01) at the end of 6-min constant-load exercise. However, the increase during constant-load exercise in HVY-RAMP was greater (P<0.01) than in MOD-RAMP (MOD-RAMP: Rest, 0.08 (0.03) vs. MOD, 0.24 (0.10); HVY-RAMP: Rest, 0.06 (0.02) vs. HVY, 0.99 (0.38)). After 6-min resting recovery from prior exercise, [P_i]/[PCr] returned to baseline levels in MOD-RAMP (0.07 (0.03), P=0.94) or to below baseline levels in HVY-RAMP, (0.03 (0.02), P<0.01).

Data from a representative subject showing log[P_i]/[PCr] throughout the entire RAMP, MOD-RAMP, and HVY-RAMP protocols is presented in Fig. 2. In each condition and for every subject, [P_i]/[PCr] displayed a biphasic response (P<0.05) which facilitated modeling using a logarithm-linear transformation. Thus, log[P_i]/[PCr] data demonstrated an initial slow and later fast component (Fig. 3). A inflection or region of transition (PT) from the slow to fast components was detected in all subjects. The slope of the log[P_i]/[PCr]-power output relationship was less (P<0.05) below the PT than above. In each condition, the occurrence of the PT was also coincident and correlated with the pHT (RAMP: r=0.97, P<0.001; MOD-RAMP: r=0.97, P<.001; HVY-RAMP: r=0.99, P<0.001). In HVY-RAMP, the rate of log[P_i]/[PCr] increase above the PT was greater compared to RAMP (P=0.02) or MOD-RAMP (P=0.04) (Table 3). The onset of
rapid increases in log[Pᵢ]/[PCr] occurred at 45.1 (9.1) % PPO in RAMP, which was not
different than MOD-RAMP (44.8 (17.8) % PPO, P=0.93). However, in HVY-RAMP the
PT occurred at a significantly higher power output (61.5 (12.0) % PPO) than RAMP
(P<0.01) or MOD-RAMP (P<0.01).

Finally, calculated mean intracellular [ADP] data for all subjects is plotted
relative to %PPO during the incremental exercise protocol in Fig. 4. There was
significantly greater [ADP] at 90, 95, and 100 %PPO in HVY-RAMP compared to
RAMP or MOD-RAMP (P<0.001). There were no differences between RAMP and
MOD-RAMP.

**Discussion**

In this study, we used ³¹P-MRS to examine the effects of prior bouts of moderate-
and heavy-intensity exercise on the intramuscular metabolic and acid-base responses to
incremental plantar-flexion exercise to fatigue. The major findings of this study were that
during an incremental exercise protocol: 1) prior heavy-intensity exercise (HVY-RAMP)
shifted the intracellular pH (pHᵢ) -power output relationship towards higher power
outputs (i.e. delayed onset of acidification); 2) prior heavy-intensity exercise shifted the
[Pᵢ]/[PCr]-power output relationship towards higher power outputs (i.e. attenuated PCr
breakdown); 3) prior heavy-intensity exercise was associated with an increased time-to-
fatigue (TTF) and peak power output (PPO) during this subsequent incremental exercise
protocol; and 4) the metabolic and acid-base response during incremental exercise was
unchanged following prior moderate-intensity exercise (MOD-RAMP) compared to no
prior exercise (RAMP).
One hypothesis of this study was that prior heavy-intensity exercise would cause a rightward shift in the pH\textsubscript{t}- and [P\textsubscript{i}]/[PCr]-power output relationships. The premise for this hypothesis was that an accumulation of intracellular H\textsuperscript{+} during prior HVY exercise would cause an increase in [H\textsuperscript{+}] in the surrounding vasculature, resulting in local vasodilation and a decreased binding affinity of hemoglobin for oxygen. For a given power output, this could provide greater convective and diffusive O\textsubscript{2} delivery, possibly producing or causing a greater aerobic contribution to energy production. In HVY-RAMP, the rightward-shift of the pH\textsubscript{t}- and [P\textsubscript{i}]/[PCr]-power output relationship indicate a more favourable metabolic status and a decreased reliance on substrate level phosphorylation. Stable values of [P\textsubscript{i}]/[PCr] indicate an adequate energy status, whereas rapid increases in this ratio reflect the inability of oxidative phosphorylation to meet ATP demands (7) and/or the metabolic adjustments (i.e. a reduction in the intracellular energy state) required to maintain oxidative phosphorylation (11; 26).

A further possible interpretation of the rightward-shift in the [P\textsubscript{i}]/[PCr] power output relationship is a decreased [ADP] signal required to stimulate or maintain oxidative phosphorylation at a given power output/ATP demand, suggesting a corresponding increase in muscle O\textsubscript{2} availability (i.e. greater pO\textsubscript{2}) (26). This may suggest a greater contribution of oxidative metabolism during moderate to heavy work rates (and/or decreased reliance on anaerobic metabolism). However, in the region of the pH\textsubscript{T} and PT we observed no differences in calculated [ADP] (Fig. 4). This observation may be interpreted as evidence that pO\textsubscript{2} was not different between conditions. If this is true, the delayed onset of acidosis in HVY-RAMP may have directly caused the delayed increase in [P\textsubscript{i}]/[PCr] via the creatine kinase reaction. However, a greater contribution of aerobic
metabolism during exercise intensities corresponding to the rightward shift pH-T in HVY-RAMP can not be ruled out, as it has been shown that a given [ADP] produces a greater oxidative flux in the absence of intracellular acidosis (16).

It is noted that while a significant intracellular acidosis was created during the bout of prior heavy-intensity exercise, it was observed that in HVY-RAMP, the pH_i had returned to baseline during the ensuing 6-min of resting recovery. As local muscle blood flow, perfusion, or vasodilation were not measured in this study, it is unknown whether the recovery of pH_i may have influenced or possibly mitigated the hypothesized response to H^+ in the surrounding vasculature following heavy-intensity exercise. Thus, from our data, enhanced O_2 delivery in HVY-RAMP can not be stated with certainty.

A secondary finding in this study was the observation of a greater rate of decline in [Pi]/[PCr] and greater [ADP] during the later stages of incremental exercise in HVY-RAMP. This may be interpreted to suggest reduced exercise economy in this condition. Similarly, Jones and Carter (13) demonstrated a steeper VO_2/work-rate slope above the gas exchange threshold when incremental exercise was preceded by prior heavy-intensity cycling. Sahlin et al (24) also demonstrated reduced gross efficiency and increased total PCr breakdown during 10 minutes of submaximal exercise preceded by prior HVY. Together, this would imply that exercise economy at heavy work rates is sensitive to the prior activity of the engaged muscles. One explanation may be that fatigue or glycogen depletion of the type-II fibres during the bout of prior HVY exercise resulted in a greater activation of the type-I muscle fibres during the later portions of the subsequent incremental bout (1; 5). An increased O_2 or ATP requirement for the same external work rate might therefore be expected if additional muscle fibres (of any type) were recruited.
when a bout of prior fatiguing or heavy-intensity exercise was present. However, the contribution and pattern of fibre activation and the recruitment of other muscles (e.g. soleus, medial gastrocnemius) can not be determined from our data.

In this study, it was hypothesized that a rightward shift in the pH$i$-power output relationship would maintain a more favourable pH$i$ status that would predispose to increased exercise tolerance (22). Other studies using cycle ergometry have found similar improvements in performance following prior HVY exercise (3; 15; 25). Metabolic acidosis has been shown to inhibit oxidative phosphorylation (16) 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$^{2+}$ from the sarcoplasmic reticulum, and a reduction in the number and force of muscle cross-bridge activations (8). Additionally, the effect of prior heavy-intensity exercise on muscle fibre activation may influence the balance between aerobic and anaerobic contributions to energy metabolism. An increased recruitment of the more aerobic type-I muscle fibres may be responsible for an increased muscle O$_2$ (13). An increased aerobic contribution of energy metabolism might result in reduced accumulation of metabolites such as H$^+$ and P$_i$ and decreased breakdown of PCr as was observed in the present study. However, reduced gross efficiency during heavy work rates may explain the lack of performance enhancement in some studies. Ultimately, whether the performance of prior heavy-intensity exercise will improve performance in a subsequent bout may depend on the balance between improvements in energy metabolism versus reductions in gross efficiency, and the extent to which variables such as duration or intensity of prior exercise will modulate these factors.
In summary, in this study we observed a delayed onset of intracellular acidosis during incremental exercise when preceded by a bout of heavy-intensity exercise 6 minutes prior. This delayed onset of acidification was also associated with a delayed onset of rapid changes in [P_i]/[PCr]. Together, these changes resulted in a greater exercise tolerance in HVY-RAMP, and may have been a result of a greater oxidative flux given the maintenance of a more favourable intracellular pH. We suggest that possible mechanisms may include improved muscle blood flow and perfusion, greater activation of type-I muscle fibres, or both.
### Tables

**Table 1.** Power output at onset of threshold of intracellular acidification (pHT) during a familiarization incremental plantar-flexion exercise protocol, and mean moderate-intensity (MOD) and heavy-intensity (HVY) work rates used during subsequent experimental trials.

<table>
<thead>
<tr>
<th></th>
<th>Power Output at pHT</th>
<th>Moderate (MOD) Work Rate</th>
<th>Heavy (HVY) Work Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power (W)</td>
<td>3.7 (1.4)</td>
<td>2.8 (0.9)</td>
<td>5.6 (1.1)</td>
</tr>
<tr>
<td>% PPO (%)</td>
<td>45.0 (10.6)</td>
<td>33.5 (6.7)</td>
<td>70.3 (13.4)</td>
</tr>
</tbody>
</table>

Values given as mean (SD). %PPO, percentage of peak power output.

**Table 2.** Time to fatigue and peak power output during incremental exercise in the familiarization, RAMP, MOD-RAMP, and HVY-RAMP trials.

<table>
<thead>
<tr>
<th></th>
<th>Time to fatigue, min</th>
<th>Peak Power Output, W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familiarization</td>
<td>14.0 (3.4)</td>
<td>8.1 (1.4)</td>
</tr>
<tr>
<td>RAMP</td>
<td>13.9 (3.7)</td>
<td>8.1 (1.2)</td>
</tr>
<tr>
<td>MOD-RAMP</td>
<td>15.3 (3.7)</td>
<td>8.8 (1.5)</td>
</tr>
<tr>
<td>HVY-RAMP</td>
<td>16.6 (5.2)*</td>
<td>9.1 (0.9)*</td>
</tr>
</tbody>
</table>

Values given as mean (SD). *Significant difference (P<0.05) compared to RAMP. No difference between MOD-RAMP and HVY-RAMP.

**Table 3.** Biphasic parameters of intracellular pH and log [Pi]/[PCr] during incremental exercise in RAMP, MOD-RAMP, and HVY-RAMP.

<table>
<thead>
<tr>
<th></th>
<th>Initial Phase (Below Threshold)</th>
<th>Rapid Phase (Above Threshold)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope ($m_1$)</td>
<td>Intercept ($b_1$)</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAMP</td>
<td>-0.01 (0.01)</td>
<td>7.01 (0.03)</td>
</tr>
<tr>
<td>MOD-RAMP</td>
<td>0.00 (0.00)*</td>
<td>6.98 (0.02)</td>
</tr>
<tr>
<td>HVY-RAMP</td>
<td>-0.01 (0.01)†</td>
<td>6.96 (0.06)</td>
</tr>
<tr>
<td>PT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAMP</td>
<td>0.05 (0.02)</td>
<td>-0.88 (0.21)</td>
</tr>
<tr>
<td>MOD-RAMP</td>
<td>0.06 (0.02)</td>
<td>-1.08 (0.19)</td>
</tr>
<tr>
<td>HVY-RAMP</td>
<td>0.07 (0.02)*</td>
<td>-0.79 (0.57)</td>
</tr>
</tbody>
</table>

Values given as mean (SD). Slope and intercept parameters are given for the piecewise linear function: $y = m_1x + b_1$ (x < threshold); $y = m_2x + b_2$ (x > threshold). pH, intracellular pH threshold; PT, log[P_i]/[PCr] threshold. *Significant difference (P<0.05) compared to RAMP. †Significant difference (P<0.05) compared to MOD-RAMP.
Table 4. Onset (or threshold) of a rapid decrease in intracellular pH (pHT) and the rapid increases in log [Pi]/[PCr] (PT) during incremental exercise in RAMP, MOD-RAMP, and HVY-RAMP.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>PO, W</th>
<th>%PPO</th>
<th>log[Pi]/[PCr]</th>
<th>PO, W</th>
<th>%PPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAMP</td>
<td>6.96 (0.05)</td>
<td>3.8 (1.3)</td>
<td>45.8 (9.4)</td>
<td>-0.52 (0.23)</td>
<td>3.7 (1.2)</td>
<td>45.1 (9.1)</td>
</tr>
<tr>
<td>MOD-RAMP</td>
<td>6.99 (0.02)</td>
<td>4.2 (2.0)</td>
<td>47.5 (15.9)</td>
<td>-0.67 (0.23)</td>
<td>4.0 (2.1)</td>
<td>44.8 (17.8)</td>
</tr>
<tr>
<td>HVY-RAMP</td>
<td>6.91 (0.09)</td>
<td>5.5 (1.2)*†</td>
<td>60.4 (11.7) *†</td>
<td>0.01 (0.55) *†</td>
<td>5.6 (1.3)*†</td>
<td>61.5 (12.0)*†</td>
</tr>
</tbody>
</table>

Values given as mean (SD). PO, power output. *Significant difference (P<0.05) compared to RAMP. †Significant difference (P<0.05) compared to MOD-RAMP.

Figure Captions

Fig. 1. Typical intracellular pH (pH<sub>i</sub>) response to incremental exercise in a representative subject (Subject 3) with no prior exercise (RAMP), prior moderate-intensity exercise (MOD-RAMP), or prior heavy-intensity exercise (HVY-RAMP). Data presented for entire protocol, with time = 0 seconds indicating the start of incremental exercise in each condition. Arrows indicate power output corresponding to the intracellular pH threshold (pHT) for this subject.

Fig. 2. Typical intracellular [Pi]/[PCr] response to incremental exercise in a representative subject (Subject 3) with no prior exercise (RAMP), prior moderate-intensity exercise (MOD-RAMP), or prior heavy-intensity exercise (HVY-RAMP). Data presented for entire protocol, with time = 0 seconds indicating the start of incremental exercise in each condition. Arrows indicate power output corresponding to the log[Pi]/[PCr] threshold (PT) for this subject.

Fig. 3. Intracellular pH (pH<sub>i</sub>) and log[Pi]/[PCr] during incremental exercise in a representative subject (Subject 2) with no prior exercise (RAMP), prior moderate-intensity exercise (MOD-RAMP), or prior heavy-intensity exercise (HVY-RAMP). Solid lines indicate piecewise linear regression ‘best-fit’. Arrows indicate power output corresponding to the intracellular pH threshold (pHT) or log[Pi]/[PCr] threshold (PT) for this subject.

Fig. 4. Calculated intracellular [ADP] during incremental exercise (mean values for all subjects) with no prior exercise (RAMP), prior moderate-intensity exercise (MOD-RAMP), or prior heavy-intensity exercise (HVY-RAMP). Data are plotted as percentage of peak power output. *Significant difference compared to RAMP or MOD-RAMP.
Figures

Figure 1.
Figure 2.
Figure 3.
Figure 4.
REFERENCES


17. **Koppo K and Bouckaert J.** In humans the oxygen uptake slow component is reduced by prior exercise of high as well as low intensity. *Eur J Appl Physiol* 83: 559-565, 2000.


