Influence of intermittent hypoxic training on muscle energetics and exercise tolerance

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Holliss BA, Fulford J, Vanhatalo A, Pedlar CR, Jones AM. Influence of intermittent hypoxic training on muscle energetics and exercise tolerance. J Appl Physiol 114: 611–619, 2013. First published January 10, 2013; doi:10.1152/japplphysiol.01331.2012.—Intermittent hypoxic training (IHT), whereby athletes live at an altitude or near sea level while undertaking a portion of their training under normobaric or hypobaric hypoxia, has been suggested to be a possibly worthwhile strategy to enhance athletic performance (6, 27). However, there is controversy surrounding the mechanisms of physiological adaptations to IHT, and the extent of the potential performance advantages (24).

Under physiological hypoxia, the O2 homeostasis-regulating transcription factor, hypoxia-inducible factor 1α (HIF-1α) is activated, initiating a range of adaptations to preserve O2 delivery (18, 33), of which the best documented is the hepatic and renal release of erythropoietin (EPO). Given sufficient hypoxic dose, this will result in a sustained increase in the circulating EPO concentration and, consequently, increased erythropoiesis (25). The response timeline is still in question but, for example, it has been reported that after a 4-wk phase of resting intermittent hypoxic exposure (3 h/day, 5 days/wk, at a simulated altitude of 4,000–5,500 m), there were no significant changes in total hemoglobin mass, red cell volume, or other red cell indices compared with a placebo group (10). It is therefore not surprising that studies have failed to measure an increased total hemoglobin mass following IHT interventions, which use relatively short-duration total hypoxic exposures (13, 24, 45).

In addition to hematological effects, a sustained high level of HIF-1α is also known to be associated with a range of other adaptations that enhance muscle O2 homeostasis (33). These adaptations include enhanced tissue perfusion linked to angiogenesis (38), improved mitochondrial efficiency and control of mitochondrial respiration (29, 32), and enhanced hydrogen ion (H+) buffering capacity (9). Due to the invasive nature of assessing these variables, most studies have been restricted to small sample sizes or resting measures, such that the influence of IHT on skeletal muscle metabolism during dynamic exercise has not been comprehensively investigated. The noninvasive technique, 31P magnetic resonance spectroscopy (31P-MRS) has been utilized to assess muscle energetics during exercise in response to a range of interventions (1, 4, 5, 12, 15–17, 34, 42).

Greater muscle oxidative capacity is reflected in faster postexercise phosphocreatine (PCr) resynthesis (39); when muscle acidosis is avoided, the speed of [PCr] recovery provides a valid estimate of in vivo oxidative capacity (4, 12, 26, 35).

To our knowledge, only one study has used 31P-MRS to investigate the effects of IHT on the muscle metabolic responses to exercise (21). In that study, four combination skiers trained for 60 min twice a day for 4 consecutive days at a simulated altitude equivalent to 2,000 m (21). The PCr recovery time constant ([PCr]-τ) was significantly faster post-IHT (mean change of −19%) but remained unchanged in the eight control participants who undertook no training (21). The IHT modality (running/cycling) was different from the 31P-MRS test exercise modality (repeated right-knee extensions), and because the control group remained inactive, it was not possible to assess the effect of the hypoxic stimulus, per se, compared with the effects of normobaric normoxic running/cycling. However, the faster [PCr]-τ suggests enhanced muscle oxidative capacity following just 8 h of IHT. If confirmed, this would provide a strong evidence base for the use of IHT by athletes.

The purpose of this study was therefore to investigate the muscle metabolic responses to exercise following a short, intense period of IHT. We used a study design in which
subjects trained one leg in normoxia (as a control; INT) and the other in hypoxia. The same exercise modality (knee extension) was used for all training and \(^{31}\)P-MRS tests. We hypothesized that 1) muscle metabolic perturbation (as assessed by changes in [PCr], pH, and inorganic phosphate concentration, expressed as [Pi], would be attenuated during submaximal exercise); 2) [PCr] recovery kinetics following exercise would be faster; and 3) the time-to-exhaustion during incremental exercise would be extended following both IHT and INT in both normoxia and hypoxia, but that the effects would be greater following IHT.

**METHODS**

**Participants and experimental design.** After institutional ethical approval, nine physically active, healthy men participated in this study (mean ± SD: age, 21.5 ± 3.7 yr; body mass, 75.5 ± 11.7 kg; stature, 1.79 ± 0.03 m). Prior to testing, each participant completed a physical activity readiness questionnaire and provided written informed consent. The participants’ reported habitual exercise ranged from four sessions of 45 min duration per week to five sessions of 90 min duration per week. The participants were engaged in training for a variety of recreational sports (soccer, cycling, running, rowing, and hockey) and could be best described as moderately trained.

Participants’ legs were randomly allocated into the normoxic or hypoxic training group [i.e., one leg was trained while they inhaled normoxic gas (INT); the other was trained while inhaling hypoxic gas (IHT)]. All participants completed 1) one single-leg knee-extension exercise test protocol practice (described below) and, after a 48-h break, one incremental test to volitional exhaustion under hypoxic conditions, for familiarization purposes; 2) pretraining testing, consisting of four \(^{31}\)P-MRS test protocols, two for each leg in each of normoxia and hypoxia, 3) 3 wk of intensive IHT (experimental leg) and INT (control leg); and 4) post-pretraining testing in which the pretraining test protocols were repeated.

**\(^{31}\)P-MRS testing.** All testing took place with the subjects in a prone position inside the bore of a 1.5 T superconducting magnet (Gyroscan Clinical Intera, Philips Medical Systems, Best, Netherlands). Participants had Velcro straps securely fastened around the thighs, hips, and lower back, and the foot of the test leg was fastened to a pulley system via a padded sling. The \(^{31}\)P-MRS test protocol then commenced. Knee-extension exercise was performed using a custom-built nonferromagnetic ergometer over a distance of ~0.22 m in time with a visual queue that coincided with magnetic resonance (MR) pulse acquisition (40 pulses/min). The protocol included a 4-min moderate-intensity exercise bout; 6 min of rest; two 24-s high-intensity bouts separated by 3 min and 36 s of rest; then a 5-min 36-s rest and, finally, an incremental test to the limit of tolerance. The work rates applied were calculated from pilot testing undertaken during the familiarization period. The moderate-intensity exercise rate was performed at a load of 1 kg lower than that eliciting the pH threshold, and the 24-s high-intensity bouts were performed at the peak work rate achieved during the familiarization incremental test. The duration and intensity of this 24-s exercise bout was based on a phase of pilot testing to find the optimal exercise intensity and duration to elicit a drop to 50–60% of baseline [PCr] without a concomitant reduction in intracellular pH. It is known that PCr recovery is not sensitive to differences in end-exercise [PCr] when pH is not altered (37). For the incremental exercise test, the initial resistance was 0.5 kg, and this was increased by 0.5 kg every 30 s until volitional exhaustion. The [Pi]/[PCr] and pH during the incremental tests was plotted against work rate, and a pH threshold was identified, as described by Barker et al. (2). We did not measure pulmonary gas exchange during the \(^{31}\)P-MRS tests due to restrictions related to the magnetic environment and the small VO\(_2\) amplitude (and low signal-to-noise ratio) during single-legged knee-extension exercise performed in the bore of the magnet.

**Training intervention.** The participants completed 3 wk of intensive IHT (experimental leg) and INT (control leg). Participants trained 5 times per week and thus completed 15 sessions in total over the 3-wk training intervention. Each training session consisted of two identical 25-min phases, one in which the IHT leg was trained, and one in which the INT leg was trained (in a randomized, alternating order). In the IHT condition, the training program totaled 316 min of active IHT or 375 min of hypoxia inspiration, including rest intervals. We based our training intervention on previous studies showing that 3 wk of IHT significantly improved peak power output compared with normoxic training during incremental exercise in hypoxia (30); and 384 min of IHT (over 6 wk) improves mitochondrial function, VO\(_{2\text{max}}\), and endurance exercise performance compared with normoxic training (47). The training program particularly emphasized high-intensity interval training because this has been shown to be particularly effective in invoking rapid muscle metabolic adaptations and improvements in endurance fitness (8, 40). Indeed, Forbes et al. (5) have reported that just six sessions of high-intensity training results in significant speeding of [PCr]-\(\gamma\). We therefore anticipated that an intense, well-controlled, 3-wk training intervention would result in significant muscle metabolic adaptations that would underpin an enhanced incremental exercise test performance, and that these adaptations may be greater in IHT compared with INT (30, 47).

After being securely fastened to the exercise apparatus as previously described, the single-leg knee-extension exercise training commenced with 2.5 min at the work rate corresponding to the pH threshold in the IHT leg as measured in hypoxia. This was immediately followed by 2.5 min at a work rate 10% higher than that of the pH threshold, then a further 5 min at a work rate 20% above the pH threshold. After a 30-s rest, high-intensity interval exercise commenced. During Week 1 of training, this consisted of 10 × 60-s exercise bouts (with 30-s passive recovery intervals), while during Week 2 and Week 3 of training this consisted of 10 × 70-s exercise bouts (with 20 s of passive recovery), with the work rate being the mean of the pH threshold and the peak work rate attained during incremental exercise in the IHT leg in hypoxia. Arterial O2 saturation (SaO\(_2\)) and heart rate (HR), which were assessed using pulse-oximetry (Nonin 7500FO, Nonin Medical, Plymouth, MN); and the rating of perceived exertion (RPE), which was assessed with the Borg scale (3), were recorded after the initial 5 and 10 min of continuous exercise, and then after the fifth and tenth bouts of interval exercise. Work rates were identical for each leg regardless of FIO\(_2\), and were increased by 0.5 kg when RPE ≥15 after the fifth interval. The inspirate FIO\(_2\) (14.5 ± 0.1% for IHT; 20.9 ± 0.0% for INT) was kept constant throughout by 10.2 ± 0.3% for IHT; 20.9 ± 0.0% for INT) was checked before, during, and after each testing session using a Servomex 5200 Paramagnetic Analyzer (Servomex, Crowborough, UK) as described below.

**Inspired gases.** The inspirate was generated by a Cloud 9 hypoxic generator (Sporting Edge, Basingstoke, UK), placed in the MR control room, connected to a 10-m extension pipe, which fed into a 150-liter Douglas Bag (Cranlea & Co, Birmingham, UK). This acted as a reservoir and mixing chamber, and had a separate output pipe feeding into a Hans Rudolf one-way valve (Cranlea & Co) connected to a face mask from which the subject could breathe, with an expired air exit. Thus the flow rate was maintained constant, and no rebreathing of expired air occurred. The O\(_2\) and CO\(_2\) concentration of the inspirate was checked by a researcher in the MR control room during every test using the Servomex 5200, taking samples via a 10-m capillary tube. This analyzer was calibrated prior to each use with a 16.0% O\(_2\), 8.0% CO\(_2\), and 76.0% N gas mix (BOC Special Gases, Guildford, UK). For all normoxic tests and training sessions, the O\(_2\) filters were inactivated, yielding an FIO\(_2\) of 20.9 ± 0.0% and an FICO\(_2\) of 0.05 ± 0.00%, whereas during hypoxic tests and training sessions, an FIO\(_2\) of 14.5 ± 0.1% and an FICO\(_2\) of 0.04 ± 0.00% were produced (simulating ~3,000 m altitude). During testing, both the subject and the researcher administering the test were blinded to the FIO\(_2\), with only the researcher in the MR control room being aware of the FIO\(_2\). Moreover, subjects were blinded to the FIO\(_2\) during all training sessions.
$^{31}$P-MRS procedures. Prior to the exercise test beginning, absolute baseline concentrations of metabolites were established via a technique similar to that described by Kemp et al. (19) using a 6-cm $^{31}$P transmit/receive surface coil. Subjects were positioned within the scanner with the coil placed within the scanner bed and positioned such that the subject’s quadriceps muscle was centered directly over it and a phosphoric acid source was directly beneath it. After initially acquiring images to confirm that the m. rectus femoris was positioned correctly relative to the coil, spatially localized spectroscopy was undertaken to determine the relative signal intensities obtained from the phosphoric acid source and P$_i$ from the subject’s quadriceps. On completion of the exercise protocol and after the subject had been removed from the scanner, subsequent scans were obtained to compare the signals obtained from the same phosphoric acid standard and an external P$_i$ solution of known concentration. The localized voxel sampled within the external solution was of the same dimensions and distance from the coil as from the muscle previously, allowing the calculation of muscle [P$_i$] following corrections for relative coil loading. Absolute concentrations of PCr and ATP were subsequently calculated via the ratio of [P$_i$]:[PCr] and [P$_i$]:[ATP], respectively.

Following metabolite concentration determinations, the phosphoric acid source was removed from the scanner bed and the subjects were securely fastened to the exercise apparatus as previously described. Images were acquired to confirm the quadriceps muscle was positioned directly above the 6-cm $^{31}$P coil, and subjects commenced breathing the inspirate, which was continued for 30 min prior to the commencement of $^{31}$P data acquisition. Initially, a number of preacquisition steps were carried out to optimize the signal from the muscle under investigation. Matching and tuning of the coil was performed and an automatic shimming protocol undertaken within a volume that defined the quadriceps muscle. A baseline spectrum before exercise was then acquired with long repetition time (TR = 20 s) in which the relative unsaturated peak amplitudes could be determined. Two minutes of further rest then followed to acquire baseline MR sequences, after which the single-legged knee-extension exercise test protocol commenced, as previously described. During the 2-min resting baseline and the subsequent exercise protocol, $^{31}$P data were acquired every 1.5 s, with a spectral width of 1,500 Hz and 1K data points. Phase cycling with four phase cycles was employed, leading to a line and the subsequent exercise protocol, $^{31}$P data were acquired every 6.0 s.

Data analyses. The acquired spectra were quantified via peak fitting, assuming prior knowledge, using the jMRUI (version 3) software package employing the AMARES fitting algorithm (41). Spectra were fitted assuming the presence of the following peaks: P$_i$, phosphodiester, PCr, α-ATP (two peaks, amplitude ratio 1:1), γ-ATP (two peaks, amplitude ratio 1:1), and β-ATP (three peaks, amplitude ratio 1:2:1). Intracellular pH was calculated using the chemical shift of the P$_i$ spectral peak relative to the PCr peak (35). [ADP] was calculated via knowledge of [P$_i$], [PCr], and pH values as described by Kemp et al. (20), taking into account the dependency of rate constants on pH. The oxidative ATP turnover rate (ATP-Ox) was determined on the basis of the hyperbolic relationship between [ATP] production rate and free cytosolic [ADP], and calculated using the PCr recovery time constant determined from the 24-s bout (22, 23).

The 4-min moderate-intensity exercise bout values of [PCr], [ADP], [P$_i$], [P$_i$]/[PCr], ATP-Ox, and pH were calculated as the mean between 90 s and 210 s of exercise (i.e., 120 s total sample time, omitting the first 90 s and final 30 s). End-exercise values were quantified as the mean of the final three data points (i.e., final 18 s) prior to volitional exhaustion.

For the [PCr] values following the 24-s high-intensity exercise period, [PCr] recovery was fitted with Prism 5 software (GraphPad, La Jolla, CA) by a single exponential of the form:

$$[\text{PCr}]_{\text{end}} = [\text{PCr}]_{\text{end}} + [\text{PCr}]_0(1 - \exp(-t/\tau))$$

where [PCr]$_{\text{end}}$ is the value at the end of exercise, [PCr]$_0$ is the difference between the [PCr] at end-exercise and when fully recovered, t is the time from exercise cessation, and τ is the time constant for the exponential recovery of [PCr]. The [PCr]−τ from each of the two 24-s exercise bouts was determined separately and the mean of the two values was then calculated.

Statistics. Separate repeated-measures ANCOVA with mixed measures were used for each of the two test conditions (normoxic vs. hypoxic) to assess differences in changes of each $^{31}$P-MRS variable ([PCr], [ADP], [P$_i$], [P$_i$]/[PCr], ATP-Ox, and pH) during the moderate-intensity exercise bouts and the incremental exercise tests to exhaustion, between the two training conditions (INT vs. IHT). The pH threshold and the time-to-exhaustion during the incremental tests were compared in the same way. Before calculating the monophasic [PCr]−τ from the 24-s high-intensity exercise bouts, paired-samples t-tests were used to assess any differences between resting baseline and end 24-s pH. Differences between pretraining [PCr]−τ under normoxic and hypoxic conditions were also assessed using paired-samples t-tests, and differences in [PCr]−τ resulting from INT vs. IHT were assessed by ANCOVA. Pretraining values were used as covariates for all ANCOVAs. Results are expressed as mean ± SD. All t-tests and ANCOVAs were performed using PASW Statistics (v18.0, IBM SPSS, Portsmouth, UK), and the probability level of $P < 0.05$ was considered to represent a significant difference.

RESULTS

$\text{SaO}_2$ in normoxia and hypoxia. Prior to training during moderate-intensity exercise in normoxia, the hypoxic inspirate resulted in an $\text{SaO}_2$ of 91 ± 1% compared with the normoxic inspirate, which resulted in an $\text{SaO}_2$ of 98 ± 1%. There were no significant changes in these $\text{SaO}_2$ values after training ($P > 0.05$).

$^{31}$P-MRS variables during moderate-intensity exercise. The moderate-intensity exercise test results are summarized in Tables 1 and 2. Significant overall training effects existed for most end-exercise MR variables when IHT and INT data were combined. Most end-exercise MR variables when IHT and INT data were compared across both training conditions ($P < 0.05$).

### Table 1. $^{31}$P-MRS variables measured during the moderate-intensity exercise bout while breathing the normoxic inspirate

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normoxic trained leg</th>
<th>Hypoxic trained leg</th>
<th>Main effect for time</th>
<th>Interaction effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretraining</td>
<td>Post-training</td>
<td>Pretraining</td>
<td>Post-training</td>
<td>$F$</td>
</tr>
<tr>
<td>Baseline [PCr] (mM)</td>
<td>32.2 ± 3.8</td>
<td>31.2 ± 2.5</td>
<td>33.1 ± 3.8</td>
<td>31.8 ± 3.6</td>
</tr>
<tr>
<td>End-exercise [PCr] (mM)</td>
<td>27.2 ± 5.0</td>
<td>26.5 ± 2.3</td>
<td>27.5 ± 3.8</td>
<td>27.8 ± 3.6</td>
</tr>
<tr>
<td>Δ[PCr] (mM)</td>
<td>5.1 ± 2.0</td>
<td>4.7 ± 2.6</td>
<td>5.5 ± 2.0</td>
<td>4.5 ± 1.8</td>
</tr>
<tr>
<td>End-exercise PCr (%)</td>
<td>83.5 ± 7.3</td>
<td>85.0 ± 7.7</td>
<td>83.2 ± 5.9</td>
<td>86.5 ± 4.8</td>
</tr>
<tr>
<td>End-exercise ATP-Ox (mM/s)</td>
<td>0.38 ± 0.08</td>
<td>0.46 ± 0.15</td>
<td>0.44 ± 0.12</td>
<td>0.50 ± 0.14</td>
</tr>
<tr>
<td>End-exercise [ADP] (μM)</td>
<td>15.3 ± 7.1</td>
<td>15.3 ± 7.1</td>
<td>16.4 ± 5.9</td>
<td>12.4 ± 3.5</td>
</tr>
<tr>
<td>End-exercise [P$_i$] (mM)</td>
<td>7.9 ± 2.3</td>
<td>7.2 ± 2.8</td>
<td>9.0 ± 2.8</td>
<td>6.6 ± 1.7</td>
</tr>
<tr>
<td>End-exercise [P$_i$]/[PCr]</td>
<td>0.31 ± 0.13</td>
<td>0.28 ± 0.13</td>
<td>0.34 ± 0.12</td>
<td>0.25 ± 0.06</td>
</tr>
<tr>
<td>End-exercise pH</td>
<td>7.03 ± 0.04</td>
<td>7.04 ± 0.03</td>
<td>7.03 ± 0.04</td>
<td>7.05 ± 0.03</td>
</tr>
</tbody>
</table>

Δ[PCr] indicates difference in [PCr] between baseline and end-exercise. *Significant training effect across both training conditions ($P < 0.05$).
combined (see Tables 1 and 2 for ANCOVA-derived $F$ and $P$ values). However, there were no significant interactions; that is, changes resulting from IHT were not significantly different from INT. The absolute [PCr] at rest and over the final 30 s of moderate-intensity exercise were slightly but significantly reduced after both IHT and INT training (Fig. 1), but there was no difference between the two training conditions. However, $\Delta$[PCr] (i.e., the magnitude of PCr degradation) during exercise was reduced by both types of training and there was an interaction in hypoxia, such that IHT spared PCr utilization to a greater extent than INT. The end-exercise pH was higher following training when subjects breathed hypoxic gas but not normoxic gas (Tables 1 and 2).

$\textbf{PCr recovery kinetics.}$ The results for PCr recovery kinetics are summarized in Table 3. There were no significant differences between resting pH and the pH measured after the 24-s high-intensity exercise bout, with a mean change of only $-0.01 \pm 0.01$ units. There were no significant [PCr]-$\tau$ differences pretraining between the legs that had been selected for INT and the legs that had been selected for IHT, whether tested in normoxia ($21 \pm 3$ s vs. $20 \pm 4$ s; $t = 0.81, P = 0.44$) or hypoxia ($28 \pm 4$ s vs. $29 \pm 5$ s; $t = -0.53, P = 0.61$). As expected, before training, [PCr]-$\tau$ was significantly faster in normoxia compared with hypoxia in the legs that had been selected for INT ($21 \pm 3$ s vs. $28 \pm 4$ s; $t = -4.74, P = 0.001$) and the legs that had been selected for IHT ($20 \pm 4$ s vs. $29 \pm 5$ s; $t = -5.43, P = 0.001$). The [PCr]-$\tau$ was significantly reduced after both INT and IHT under both normoxic and hypoxic test conditions (Table 3 and Fig. 2). In hypoxia, the [PCr]-$\tau$ reduction was significantly greater after IHT ($-7 \pm 3$ s) compared with after INT ($-4 \pm 2$ s) [$F_{(1,16)} = 14.46, P = 0.002$]. Although [PCr]-$\tau$ in normoxia tended to decrease more

<table>
<thead>
<tr>
<th>$\text{PCr}$ concentration (mM)</th>
<th>$\Delta$[PCr] (mM)</th>
<th>End-exercise [ADP] (µM)</th>
<th>End-exercise [Pi]/[PCr]</th>
<th>End-exercise pH</th>
<th>$\text{PCr}$ recovery kinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Baseline [PCr]}$</td>
<td>$\text{End-exercise [PCr]}$</td>
<td>$\text{End-exercise [Pi]}$</td>
<td>$\text{End-exercise pH}$</td>
<td>$\text{Normoxic trained leg}$</td>
<td>$\text{Hypoxic trained leg}$</td>
</tr>
<tr>
<td>34.1 ± 4.4</td>
<td>28.5 ± 4.4</td>
<td>5.6 ± 3.5</td>
<td>83.7 ± 9.2</td>
<td>16.1 ± 8.0</td>
<td>8.2 ± 2.9</td>
</tr>
<tr>
<td>32.5 ± 3.1</td>
<td>27.4 ± 3.1</td>
<td>5.1 ± 1.9</td>
<td>84.4 ± 5.6</td>
<td>14.1 ± 6.5</td>
<td>6.5 ± 2.8</td>
</tr>
<tr>
<td>34.4 ± 4.2</td>
<td>28.4 ± 5.0</td>
<td>6.0 ± 2.8</td>
<td>82.1 ± 7.9</td>
<td>16.3 ± 5.6</td>
<td>8.7 ± 2.5</td>
</tr>
<tr>
<td>32.0 ± 4.3</td>
<td>27.4 ± 4.6</td>
<td>4.6 ± 1.6</td>
<td>85.2 ± 5.6</td>
<td>16.0 ± 3.4</td>
<td>7.1 ± 1.6</td>
</tr>
<tr>
<td>6.12 ± 0.03</td>
<td>6.54 ± 0.02</td>
<td>14.20 ± 0.02</td>
<td>20.12 ± &lt;0.001*</td>
<td>11.32 ± 0.01*</td>
<td>7.22 ± 0.02*</td>
</tr>
<tr>
<td>0.19 ± 0.672</td>
<td>&lt;0.001*</td>
<td>0.995</td>
<td>36.07 ± &lt;0.001*</td>
<td>1.21 ± 0.29</td>
<td>0.13 ± 0.72</td>
</tr>
</tbody>
</table>

*Significant difference between INT and IHT legs ($P < 0.05$). †Significant training effect across both training conditions ($P < 0.05$). $\Delta$[PCr] indicates difference in [PCr] between baseline and end-exercise.

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**Table 2.** $^{31}$P-MRS variables measured during the moderate-intensity exercise bout while breathing the hypoxic inspirate

**Fig. 1.** Mean combined (see Tables 1 and 2 for ANCOVA-derived $F$ and $P$ values). However, there were no significant interactions; that is, changes resulting from IHT were not significantly different from INT. The absolute [PCr] at rest and over the final 30 s of moderate-intensity exercise were slightly but significantly reduced after both IHT and INT training (Fig. 1), but there was no difference between the two training conditions. However, $\Delta$[PCr] (i.e., the magnitude of PCr degradation) during exercise was reduced by both types of training and there was an interaction in hypoxia, such that IHT spared PCr utilization to a greater extent than INT. The end-exercise pH was higher following training when subjects breathed hypoxic gas but not normoxic gas (Tables 1 and 2).

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**Fig. 1.** Mean ± SD muscle [PCr] during 2-min passive rest (baseline values); the 4-min moderate-intensity exercise bout; and 3 subsequent min of passive rest pretraining (●) and post-training (○). Note that absolute [PCr] at rest and over the final 30 s of exercise were significantly reduced after both IHT and INT [i.e., there was a training effect when tested under normoxia ($P = 0.001$) and under hypoxia ($P = 0.023$) but there were no significant differences between the two training conditions (no interaction effect)].

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after IHT (−3 ± 3 s) compared with INT (−2 ± 2 s), this was not statistically significant (F_{1,15} = 2.98, P = 0.11).

**31P-MRS variables and exercise tolerance during incremental exercise.** The incremental exercise test results are summarized in Tables 4 and 5. There were no significant differences between the improvements in time-to-exhaustion post-IHT compared with post-INT regardless of whether subjects were tested in normoxia (122 ± 41 s vs. 128 ± 67 s) or hypoxia (78 ± 54 s vs. 106 ± 45 s); indeed there was only a significant overall training effect for time-to-exhaustion under normoxic test conditions when INT and IHT data were combined (Tables 4 and 5). There were significant overall training effects when both IHT and INT data were combined for absolute and relative [PCr] at the limit of tolerance or at the pH threshold during the incremental tests between IHT and INT (Tables 4 and 5).

**DISCUSSION**

To our knowledge, this is the first study to investigate the influence of IHT compared with INT on muscle energetics during exercise in normoxia and hypoxia. Overall, IHT had only limited effects on the muscle metabolic responses to exercise. During moderate-intensity exercise, the effects of training were similar between IHT and INT, although in hypoxia, Δ[PCr] was reduced to a slightly greater extent by IHT. Similarly, there were no significant differences in the effects of training on the pH threshold or any of the other 31P-MRS-derived variables between IHT and INT during incremental exercise. Compared with INT, IHT resulted in a slightly but significantly faster [PCr]-τ in hypoxia and there was a tendency for there to be a similar effect in normoxia. However, changes in time-to-exhaustion during incremental exercise were not significantly different between IHT and INT either in normoxia or hypoxia.

**PCr recovery kinetics.** The pretraining differences in [PCr]-τ between normoxic and hypoxic test conditions of 7–9 s (34–42% slower) confirm that, in hypoxia, recovery from high-intensity exercise is substantially impaired. Our results are similar to those of Haseler et al. (12) who reported a 34% difference between [PCr]-τ measured in 21% FIO2 compared with 10% FIO2.

The reduction in [PCr]-τ in hypoxia following IHT was significantly greater than the reduction following INT (26% vs.

---

**Table 3. [PCr]-τ measured following the 24-s high-intensity exercise bout**

<table>
<thead>
<tr>
<th></th>
<th>Normoxic trained leg</th>
<th>Hypoxic trained leg</th>
<th>Main effect for time</th>
<th>Interaction effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretraining</td>
<td>Post-training</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>[PCr]-τ (s) while breathing the normoxic inspirate</td>
<td>20.9 ± 2.9</td>
<td>19.1 ± 3.2</td>
<td>3.90</td>
<td>0.07</td>
</tr>
<tr>
<td>[PCr]-τ (s) while breathing the hypoxic inspirate</td>
<td>27.8 ± 4.3</td>
<td>24.1 ± 3.7</td>
<td>28.6 ± 4.5</td>
<td>21.2 ± 2.7</td>
</tr>
</tbody>
</table>

*Significant difference between INT and IHT legs (P < 0.05). †Significant training effect across both training conditions (P < 0.05).

---

**Fig. 2.** [PCr]-τ after the 24-s severe-intensity exercise bout pre- and post-training. Gray bars illustrate mean values; black lines illustrate individual responses. *Significant difference between INT and IHT (P < 0.05); #significant effect across both training conditions (P < 0.05). Note that under hypoxic conditions, all tests revealed a significant overall training effect (i.e., a faster [PCr]-τ after training, but most importantly, under hypoxic conditions there was a significant interaction (P = 0.002), showing that the IHT group realized a greater [PCr]-τ reduction than the INT group.)
13%), suggesting that adding a hypoxic stimulus to normal training may augment the physiological adaptations that modulate the [PCr]-τ in hypoxia. The [PCr]-τ reduction of 17% post-IHT in normoxia is similar to the significant 19% change reported by Kuno et al. (21) following 4 consecutive days of IHT (~480 min exposure). Unfortunately, that study did not include an INT control condition, such that it is not possible to determine to what extent hypoxia per se influenced the training adaptation above and beyond normal exercise training. However, collectively, these two studies suggest that IHT speeds muscle [PCr]-τ, which has been proposed to reflect muscle oxidative capacity (11).

The high-intensity training stimulus we employed is similar to that used in the study by Vogt et al. (43), in which subjects completed either high- or low-intensity INT or IHT for 6 wk. These authors reported a significant gain in subsarcolemmal mitochondria density in the high- (+130%) and low- (+100%) intensity IHT groups, but not in the high- (+1%) or low- (−13%) intensity INT groups (43). Similarly, Geiser et al. (7) reported that total and subsarcolemmal mitochondrial densities increased by +54% and +105%, respectively, following high-intensity IHT compared with +24% and +13%, respectively, following high-intensity INT. The results of the present study are consistent with these findings: assuming a sufficient muscle O2 supply, an increase in mitochondrial volume would be expected to result in faster [PCr]-τ (11, 12, 26, 35, 39), as was observed post-IHT. It is important to note, however, that the improvement in [PCr]-τ post-IHT was only different from that observed post-INT when subjects were tested in hypoxia. If IHT had promoted mitochondrial biogenesis to a greater extent than INT, a significantly faster [PCr]-τ might also have been expected in normoxia. Therefore, it is likely that the faster [PCr]-τ observed after IHT was not solely due to enhanced mitochondrial biogenesis. It is known that postexercise PCr recovery is heavily influenced by the muscle oxygenation status (11, 12). It may be speculated therefore, that compared with INT, IHT caused physiological adaptations that resulted in a greater enhancement of muscle O2 delivery in hypoxia.

In hypoxia, the mitogen-activated protein kinase pathway is stimulated, enhancing the activity of HIF-1α. A range of molecular and structural changes subsequently take place (14), including enhanced transcriptional activation of vascular endothelial growth factor (VEGF) (28). Geiser et al. (7) reported that capillary density increased significantly (by +12%) following high-intensity IHT, whereas there was no significant change following high-intensity INT. Vogt et al. (43) also reported significant increases in VEGF mRNA and capillary density after high-intensity IHT by +52% and +19%, respectively, with no significant changes after low-intensity IHT or high-intensity or low-intensity INT. Although not consistently found (47), these differences in capillary density changes between high-intensity IHT and INT (7, 43) suggest that IHT might result in enhanced muscle oxygenation. If so, this may contribute to the reduced [PCr]-τ observed post-IHT in hypoxia (12). Faster [PCr]-τ following IHT would be expected to result in less fatigue and to enable better maintenance of performance during intermittent high-intensity exercise (39). It may therefore be speculated that athletes competing in sports requiring repetitive sprints might obtain an advantage from performing IHT prior to competition, especially at

### Table 4. 31P-MRS variables and exercise tolerance measured during the incremental test while breathing the normoxic inspirate

<table>
<thead>
<tr>
<th></th>
<th>Normoxic trained leg</th>
<th>Hypoxic trained leg</th>
<th>Main effect for time</th>
<th>Interaction effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretraining</td>
<td>Post-training</td>
<td>Pretraining</td>
<td>Post-training</td>
</tr>
<tr>
<td>pH threshold (s)</td>
<td>424 ± 74</td>
<td>485 ± 57</td>
<td>441 ± 88</td>
<td>488 ± 76</td>
</tr>
<tr>
<td>[PCr] at T-Lim (mM)</td>
<td>16.4 ± 5.2</td>
<td>11.3 ± 3.4</td>
<td>16.2 ± 5.0</td>
<td>10.8 ± 3.0</td>
</tr>
<tr>
<td>PCr at T-Lim (%)</td>
<td>49.9 ± 11.8</td>
<td>36.0 ± 9.8</td>
<td>48.2 ± 13.2</td>
<td>33.9 ± 8.7</td>
</tr>
<tr>
<td>[ADP] at T-Lim (μM)</td>
<td>42.4 ± 20.7</td>
<td>56.4 ± 30.7</td>
<td>45.7 ± 19.3</td>
<td>59.0 ± 24.7</td>
</tr>
<tr>
<td>[P] at T-Lim (mM)</td>
<td>15.4 ± 5.7</td>
<td>19.6 ± 6.0</td>
<td>18.5 ± 6.6</td>
<td>18.3 ± 6.5</td>
</tr>
<tr>
<td>[PCr]/[ADP] at T-Lim</td>
<td>1.30 ± 0.72</td>
<td>1.95 ± 0.97</td>
<td>1.33 ± 0.81</td>
<td>1.90 ± 0.98</td>
</tr>
<tr>
<td>pH at T-Lim</td>
<td>6.86 ± 0.11</td>
<td>6.71 ± 0.15</td>
<td>6.85 ± 0.19</td>
<td>6.76 ± 0.19</td>
</tr>
<tr>
<td>ATP-Ox at T-Lim (mM/s)</td>
<td>0.67 ± 0.13</td>
<td>0.90 ± 0.20</td>
<td>0.76 ± 0.14</td>
<td>1.10 ± 0.39</td>
</tr>
<tr>
<td>T-Lim (s)</td>
<td>488 ± 63</td>
<td>616 ± 42</td>
<td>505 ± 59</td>
<td>627 ± 57</td>
</tr>
</tbody>
</table>

T-Lim indicates limit of tolerance. *Significant training effect across both training conditions (P < 0.05).

### Table 5. 31P-MRS variables and exercise tolerance measured during the incremental test while breathing the hypoxic inspirate

<table>
<thead>
<tr>
<th></th>
<th>Normoxic trained leg</th>
<th>Hypoxic trained leg</th>
<th>Main effect for time</th>
<th>Interaction effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretraining</td>
<td>Post-training</td>
<td>Pretraining</td>
<td>Post-training</td>
</tr>
<tr>
<td>pH threshold (s)</td>
<td>457 ± 76</td>
<td>486 ± 74</td>
<td>438 ± 79</td>
<td>474 ± 76</td>
</tr>
<tr>
<td>[PCr] at T-Lim (mM)</td>
<td>16.5 ± 4.9</td>
<td>10.2 ± 2.7</td>
<td>17.7 ± 7.7</td>
<td>11.1 ± 3.7</td>
</tr>
<tr>
<td>PCr at T-Lim (%)</td>
<td>48.3 ± 10.7</td>
<td>31.1 ± 7.5</td>
<td>50.1 ± 17.4</td>
<td>34.4 ± 9.1</td>
</tr>
<tr>
<td>[ADP] at T-Lim (μM)</td>
<td>44.9 ± 12.5</td>
<td>65.9 ± 19.5</td>
<td>42.1 ± 16.2</td>
<td>67.0 ± 16.5</td>
</tr>
<tr>
<td>[P] at T-Lim (mM)</td>
<td>15.9 ± 3.8</td>
<td>19.2 ± 7.9</td>
<td>17.9 ± 7.3</td>
<td>19.5 ± 5.9</td>
</tr>
<tr>
<td>[PCr]/[ADP] at T-Lim</td>
<td>1.06 ± 0.45</td>
<td>2.09 ± 1.11</td>
<td>1.23 ± 0.86</td>
<td>1.98 ± 1.02</td>
</tr>
<tr>
<td>pH at T-Lim</td>
<td>6.88 ± 0.10</td>
<td>6.76 ± 0.13</td>
<td>6.85 ± 0.17</td>
<td>6.76 ± 0.21</td>
</tr>
<tr>
<td>ATP-Ox at T-Lim (mM/s)</td>
<td>0.54 ± 0.06</td>
<td>0.81 ± 0.13</td>
<td>0.52 ± 0.19</td>
<td>0.88 ± 0.15</td>
</tr>
<tr>
<td>T-Lim (s)</td>
<td>509 ± 40</td>
<td>615 ± 45</td>
<td>518 ± 41</td>
<td>595 ± 57</td>
</tr>
</tbody>
</table>

T-Lim indicates limit of tolerance. *Significant training effect across both training conditions (P < 0.05).
Both, IHT does not result in a greater improvement in exercise tolerance in indices of mitochondrial function, capillarization, or small, such that it is not certain to have functional relevance (see Muscle metabolic responses to incremental exercise and time-to-exhaustion).

Muscle metabolic responses during moderate-intensity exercise. There were essentially no differences in the muscle metabolic response to moderate-intensity exercise resulting from IHT compared with INT. The only difference between conditions was that Δ[PCr] was reduced to a greater extent following IHT compared with INT when subjects exercised in hypoxia. This sparing of the extent of PCr degradation suggests a lower muscle metabolic perturbation in hypoxia following IHT. The mechanistic basis for this effect is uncertain but might be linked to enhanced local muscle oxygenation (12, 46). However, although there were differences in Δ[PCr] following IHT and INT, the end-exercise [PCr] was not different and thus the functional significance of this change is questionable.

Muscle metabolic responses to incremental exercise and time-to-exhaustion. There were essentially no differences in the muscle metabolic response to incremental exercise following IHT and INT. On average, the subjects were able to sustain exercise longer after training in both normoxia and hypoxia, but in hypoxia, the intersubject variability precluded the attainment of statistical significance. Importantly, there was no additional improvement in time-to-exhaustion following IHT compared with INT.

Our results are consistent with several previous studies (7, 31, 43) in showing that, despite seemingly favorable adaptations in indices of mitochondrial function, capillarization, or both, IHT does not result in a greater improvement in exercise performance in normoxia compared with INT. Similar to the present study, using a double-blind placebo-controlled design, Truijens et al. (40) added high-intensity interval training (three sessions per week for 5 wk) to the programs of trained swimmers and found that IHT was no more effective than INT in improving 100-m or 400-m time trial performance. Our results contrast with those of Terrados et al. (36) and Zoll et al. (47) who reported that exercise tolerance was significantly increased after IHT but not INT in well-trained athletes. It is possible that differences in the reported effectiveness of IHT reflect interstudy differences in subject training status and the total hypoxic dose administered. It should be noted that in the Zoll et al. (47) study, face masks were used to administer the inspirate in the IHT group only, so the lack of blinding may have resulted in a performance-enhancing placebo effect. In the present study, subjects remained blinded to the FIO2 during both training and testing (at the completion of the study, 4 out of 9 subjects correctly guessed which leg had been trained in hypoxia) and the performance tests were conducted in a double blind manner.

Experimental considerations. Our study employed an incremental exercise test to exhaustion to assess maximal aerobic performance, but we recognize that incremental tests are less sensitive than constant-work-rate tests for assessing changes in exercise tolerance following an intervention (44). Also, the training intervention was relatively short (3 wk and 15 sessions), and we cannot exclude the possibility that IHT might have more effectively enhanced performance if it had been practiced for longer. The moderate training status of our subjects is also an important consideration. Previous studies have suggested that differences in performance changes between INT and IHT may be more likely in highly trained (36,
Intermittent Hypoxic Training and Muscle Energetics • Holliss BA et al.

47) compared with less well-trained (7, 43) subjects. It should also be noted that the IHT and INT training sessions were completed at the same absolute intensity such that the IHT leg was trained at a slightly higher relative intensity. It is possible that this contributed to the minor differences (for example, in [PCR]-τ) observed between IHT and INT. The localized muscle mass engaged by our single-legged exercise training and testing modality precluded the measurement of VO2max and did not simulate the oxidative energy demand that would be experienced during whole-body exercise. However, studies that have used separate training groups or cross-over designs are subject to the normal daily variations in subjects’ activities outside of the controlled training and test environments. One of the key strengths of the present investigation is the single-legged study design, which ruled out any placebo effects and allowed noninvasive interrogation and comparison of the muscle metabolic adaptations to IHT compared with INT in the same subjects.

In conclusion, compared with INT, IHT resulted in no meaningful changes in the muscle metabolic response to moderate-intensity constant-rate exercise or exhaustive incremental exercise. However, in hypoxia only, IHT resulted in a small but statistically significant reduction of [PCR]-τ in the recovery from high-intensity exercise. Although the reduced [PCR]-τ in hypoxia may reflect increased muscle oxidative capacity following IHT, the practical importance of this is questionable, given that IHT was no more effective than INT in enhancing incremental exercise performance either in hypoxia or normoxia.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES


