Skeletal muscle ATP turnover by \(^{31}\)P magnetic resonance spectroscopy during moderate and heavy bilateral knee-extension

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Key Points Summary

- Heavy-intensity exercise causes a progressive increase in energy demand that contributes to exercise limitation.
- This inefficiency arises within the locomotor muscles and is thought to be due to an increase in ATP cost of power production, however the responsible mechanism is unresolved.
- We measured these by combined pulmonary gas exchange and muscle magnetic resonance spectroscopy during moderate and heavy exercise in humans.
- Muscle ATP synthesis rate increased throughout constant power heavy exercise, but this increase was unrelated to the progression of whole body inefficiency.
- Our data indicate that increased ATP requirement is not the sole cause of heavy exercise inefficiency and other mechanisms, such as increased $O_2$ cost of ATP resynthesis, may contribute.
Abstract

During constant power high-intensity exercise, the expected increase in oxygen uptake (\(\dot{V}O_2\)) is supplemented by a \(\dot{V}O_2\) slow component (\(\dot{V}O_{2sc}\)), reflecting reduced work efficiency predominantly within the locomotor muscles. The intracellular source of inefficiency is postulated to be an increase in the ATP cost of power production (an increase in P/W). To test this hypothesis, we measured intramuscular ATP turnover with \(^3^1^P\) magnetic resonance spectroscopy (MRS) and whole-body \(\dot{V}O_2\) during moderate (MOD) and heavy (HVY) bilateral knee-extension in healthy participants (n=14). Unlocalised \(^3^1^P\) spectra were collected from the quadriceps throughout using a dual-tuned (\(^1^H\) and \(^3^1^P\)) surface coil with a simple pulse and acquire pulse sequence. Total ATP turnover rate (ATP\(_{tot}\)) was estimated at exercise cessation from direct measurements of the dynamics of phosphocreatine (PCr) and proton handling.

Between 3 and 8 min during MOD there was no discernable \(\dot{V}O_{2sc}\) (mean ± SD: 0.06 ± 0.12 L.min\(^{-1}\)) or change in [PCr] (30 ± 8 vs. 32 ± 7 mM) or ATP\(_{tot}\) (24 ± 14 vs. 17 ± 14 mM.min\(^{-1}\); each \(p =\) n.s.). During HVY the \(\dot{V}O_{2sc}\) was 0.37 ± 0.16 L.min\(^{-1}\) (22 ± 8%), [PCr] decreased (19 ± 7 vs. 18 ± 7 mM, or 12 ± 15%; \(p < 0.05\)) and ATP\(_{tot}\) increased (38 ± 16 vs. 44 ± 14 mM.min\(^{-1}\), or 26 ± 30%; \(p < 0.05\)) between 3 and 8 min. However, the increase in ATP\(_{tot}\) (\(\Delta\)ATP\(_{tot}\)) was not correlated to the \(\dot{V}O_{2sc}\) during HVY (\(r^2 = 0.06; p =\) n.s.). This lack of relationship between \(\Delta\)ATP\(_{tot}\) and the \(\dot{V}O_{2sc}\), together with a steepening of the [PCr]-\(\dot{V}O_2\) relationship in HVY, suggests that reduced work efficiency during heavy exercise arises from both contractile (P/W) and mitochondrial (the \(O_2\) cost of ATP resynthesis; P/O) sources.
Abbreviations

A, amplitude; ATP$_{tot}$, total ATP turnover rate; D, ATP production from PCr breakdown; $k$, rate constant; L, ATP production from glycogenolysis; LT, lactate threshold; MRS, magnetic resonance spectroscopy; PCr, phosphocreatine; PCr$_{sc}$, phosphocreatine slow component; pH$_i$, intramuscular pH; Pi, inorganic phosphate; P/O, ATP yield per O → H$_2$O; P/W, ATP cost per unit work; Q, ATP production from oxidative phosphorylation; RI, ramp incremental exercise; S$_p$O$_2$, arterial oxygenation; $\tau$, time constant; $V_{[\text{PCr}]}$, initial rate of PCr resynthesis; $\dot{V}$O$_2$, rate of whole-body O$_2$ uptake; $\dot{V}$O$_{2peak}$, peak rate of O$_2$ uptake; $\dot{V}$O$_{2sc}$, slow component of O$_2$ uptake
Introduction

During constant power exercise below the lactate threshold (LT; moderate intensity), the rate of pulmonary oxygen uptake ($\dot{V}O_2$) increases exponentially, reaching a steady-state within 2-3 min. A $\dot{V}O_2$ steady-state indicates that the exercise-related energy transfer is accounted for by oxidative phosphorylation. However, above the LT (heavy intensity), the dynamics of $\dot{V}O_2$ become complicated by an additional, slow component ($\dot{V}O_{2sc}$) (Poole et al., 1994). This becomes especially important at power outputs above critical power, where the $\dot{V}O_{2sc}$ will draw $\dot{V}O_2$ inexorably toward its physiologic maximum. In this intensity domain the limit of tolerance is reached rapidly, and the exercise cannot continue unless the power output is reduced below critical power (Coats et al., 2003). Although the $\dot{V}O_{2sc}$ is intimately related to exercise intolerance (Murgatroyd et al., 2011), the aetiology of the $\dot{V}O_{2sc}$ remains poorly understood.

The $\dot{V}O_{2sc}$ represents an impairment of exercise economy, and is predominantly (~85%) due to increased O$_2$ consumption in the muscles engaged in the locomotor work (Poole et al., 1991; Rossiter et al., 2002; Krstrup et al., 2009). However, the intracellular source of this inefficiency is uncertain. It has been postulated that the $\dot{V}O_{2sc}$ is related to an increased phosphate cost of force or power production. That is, an increase in the rate of ATP consumption per unit power output (or P/W) is met instantaneously by phosphocreatine (PCr) (via the Lohmann reaction) whose breakdown signals an increase in the rate of oxidative phosphorylation (Rossiter et al., 2002). However, distinguishing between this and the alternative hypothesis, that supra-LT exercise is associated with reductions in mitochondrial coupling (Krustrup et al., 2003), i.e. the ratio of the ATP resynthesized per oxygen converted to water (P/O), is technically challenging in humans.
To test these two hypotheses requires knowledge of dynamic changes in total ATP turnover rate (ATP\textsubscript{tot}) in concert with power output and VO\textsubscript{2}. Specifically, were the intramuscular source of the VO\textsubscript{2} slow component to be caused by an increase in P/W (in line with current views; (Rossiter, 2011; Poole & Jones, 2012)), then the slow component magnitude during heavy exercise would be strongly related to the magnitude of the change in ATP\textsubscript{tot}. Alternatively, if no proportionality between the VO\textsubscript{2} slow component and the change in ATP\textsubscript{tot} were evident, then changes in P/W could not be the sole source of the VO\textsubscript{2} slow component.

The technical challenge thus becomes, how best to establish ATP\textsubscript{tot} during heavy intensity exercise that elicits a VO\textsubscript{2} slow component? One approach uses \textsuperscript{31}P magnetic resonance spectroscopy (MRS) (Kemp et al., 2001; Layec et al., 2009a) to partition ATP delivery from oxidative phosphorylation, PCr breakdown, and glycogenolysis. \textsuperscript{31}P MRS provides direct measurement of [PCr], and allows glycogenolytic rate (a relatively minor component of ATP\textsubscript{tot} in exercise of this kind) to be estimated using reasonable assumptions about muscle H\textsuperscript{+} buffering (Kemp et al., 2001; Kemp et al., 2014). Several methods have been proposed to calculate oxidative ATP yield using \textsuperscript{31}P MRS, but these show poor agreement (Layec et al., 2011). Previous studies to estimate ATP\textsubscript{tot} during supra-LT exercise have assumed linear VO\textsubscript{2}/[PCr] and a fixed time constant (τ) of PCr breakdown and resynthesis (Meyer, 1988; Walter et al., 1999; Lanza et al., 2005; Faraut et al., 2007) or first-order [ADP]VO\textsubscript{2} in order to transform [PCr] into a rate of oxidative ATP turnover (Layec et al., 2009a). However, it is clear that the VO\textsubscript{2}-[PCr] relationship is not linear through the intensity domains (Kemp, 2008; Wust et al., 2011; Kemp et al., 2014), and accordingly τPCr is not invariant across exercise intensities (Yoshida & Watari, 1993, 1994; Rossiter et al., 2002; Jones et al., 2008), making this an unreliable assumption on which to base estimation of ATP\textsubscript{tot}. Assuming τPCr to be invariant is equivalent to assuming that any change in [PCr] is directly proportional to change in ATP\textsubscript{tot}; when τPCr
changes across exercise intensity and/or duration, this proportionality is lost (Kemp et al., 2014).

These new findings mean that the close coherence between [PCr] and VO₂ during the slow component phase (Rossiter et al., 2002; Layec et al., 2009a) is no longer sufficient evidence to imply that an increase in P/W alone is the responsible mechanism. Consequently, a direct measurement of oxidative ATP yield during supra-LT exercise, that does not rely on these assumptions, is required to distinguish whether change in P/W is the dominant mechanism for the VO₂sc.

Oxidative ATP turnover (the dominant proportion of ATPtot) at exercise cessation may be directly measured from the initial rate of post-exercise PCr resynthesis ($V_{i[PCr]}$), easily measured by ³¹P MRS; the only assumptions required (the evidence for which is reviewed elsewhere (Kemp et al., 2014)) are that PCr recovery is driven overwhelmingly by oxidative ATP synthesis, and that any basal component of ATP turnover (i.e. ATP production not available for use by myosin ATPase, SERCA, or Na⁺/K⁺ ATPase during exercise or PCr resynthesis during recovery) is small and reasonably constant. Therefore, temporal characterisation of oxidative energy yield during dynamic exercise can be made simply by halting the exercise and measuring $V_{i[PCr]}$.

Although this method has inherently poor temporal resolution (it is only valid at the instant of exercise cessation), it provides the accuracy necessary to isolate the intracellular source of inefficiency during high intensity exercise. The other, much smaller, components of ATPtot can be estimated at end-exercise by ³¹P MRS in ways that are relatively robust against uncertainty or changes in the underpinning assumptions.

Therefore, the purpose of this study was to characterise the rate of ATP turnover during sub- and supra-LT exercise in human quadriceps during bilateral, prone, knee-extension exercise using ³¹P MRS. The rate of pulmonary oxygen uptake was measured under the same conditions.
to quantify the $\dot{V}O_2$ slow component. We hypothesised that the close association between the dynamics of the [PCr] and $\dot{V}O_2$ slow components during supra-LT exercise would be reflected in the dynamics of $ATP_{tot}$ (measured independently), thereby confirming the hypothesis that increased P/W during heavy intensity exercise is the predominant mechanism of the $\dot{V}O_2$ slow component.

Materials and Methods

Ethical Approval

The Biological Sciences Faculty Research Ethics Committee, University of Leeds, and the University of Liverpool Committee on Research Ethics approved this study, and all procedures complied with the latest revision of the Declaration of Helsinki. Written informed consent was obtained from all volunteers prior to their participation in the study.

Participants

Fourteen healthy volunteers (1 female, 13 males) agreed to participate in this study (mean ± standard deviation (SD): age 27 ± 8 y; height 177 ± 8 cm; mass 75 ± 12 kg; bilateral knee-extension $\dot{V}O_2$peak 2.0 ± 0.5 L.min$^{-1}$). All participants were undertaking a regular exercise regimen, ranging from recreational fitness to amateur competitive sport. Volunteers were screened for cardiovascular disease risk with a resting ECG and a health history questionnaire.

Exercise protocols

All exercise tests were undertaken on an MR-compatible computer-controlled electromagnetically-braked knee-extension ergometer (MRI Ergometer Up/Down, Lode BV, Groningen, NL) customised for use at 3T by the addition of extended carbon fibre lever arms. The participants lay prone with their feet strapped into moulded plastic stirrups, which were
attached to carbon-fibre/aluminium arms, linking to the ergometer crank arms. The participants’ hips were secured to the patient bed with nylon and Velcro straps in order to isolate power production to the quadriceps and minimise movement from hip flexion/extension. Knee movements were constrained by the scanner bore, allowing for approximately 35° of bilateral knee-extension (Whipp et al., 1999; Cannon et al., 2013). No resistance was applied during knee flexion, other than a constant work required to lift the mass of the lower leg.

The testing protocol began with a rigorous familiarisation phase that took place in a temperature-controlled laboratory with pulmonary gas exchange measurements. Ramp incremental and constant power protocols were completed until reproducible physiologic measurements were obtained across two consecutive visits for each condition. The second phase of the study took place within the bore of an MR scanner for measurement of muscle phosphates. The same MRI ergometer was used for both phases of the protocol.

Initially, participants completed a ramp incremental (RI) exercise test to the limit of tolerance. For this, participants lay at rest for ~3-4 min, followed by a low power exercise (5 W) for ~2-4 min. The power was then increased as a function of time at 2-5 W.min⁻¹ (the rate of increase was dependent on the volunteer’s size and strength) until the limit of tolerance was reached. Ramp rates were adjusted using ‘trial and error’ to determine a ramp rate that resulted in a ramp protocol of approximately 10-12 min. The frequency of knee-extension was constrained at 90 min⁻¹ with the use of a metronome. This cadence was chosen to allow synchronization with the MR scanner acquisitions (1 pulse per 2 knee-extensions), and also acted to ensure that the ergometer flywheel was maintained above its minimum operating speed. The RI was terminated upon the participant being unable to maintain the required cadence, despite strong verbal encouragement. The results of the RI were used to determine the $\dot{V}O_{2peak}$, and to calculate
power for subsequent tests. There is a substantial learning effect with the exercise model (large gains in peak power were achieved with consecutive tests) and therefore typically more than 3 RI tests were completed by each participant until reproducible performances were achieved.

A series of constant power exercise tests were then undertaken. These consisted of an 8 min moderate intensity bout, followed by a 6 min rest, and an 8 min heavy intensity exercise bout. During moderate intensity exercise the target power was 80% of estimated LT (\(\sim 60-70\% \dot{V}O_{2\text{peak}}\)), and during heavy intensity bouts the target power was half way between estimated LT and \(\dot{V}O_{2\text{peak}}\). These intensity domains were confirmed post hoc from the profile of \(\dot{V}O_2\) during constant power bouts (Whipp, 1996). If necessary, power was adjusted in subsequent familiarisation tests to ensure the absence (moderate) or presence (heavy) of the \(\dot{V}O_{2\text{sc}}\). Once familiarised, participants repeated this protocol 3 times on separate days to combine respired gas exchange data and improve signal:noise.

During the second phase of experiments, participants completed constant power bouts within the bore of the superconducting magnet for \(^{31}\)P MRS. Two trials of constant power tests were completed in a random order consisting of: 1) 4 min of rest, followed by 3 min of moderate exercise, 6 min rest, 3 min of heavy exercise; and 2) 4 min of rest, followed by 8 min of moderate exercise, 6 min rest, 8 min heavy exercise. Each protocol was preceded by \(\sim 10\) min of magnet shimming to optimise the MRS signal, and separated by at least 30 min outside of the MR scanner. Therefore approximately 60-90 min elapsed between the two exercise trials.

*Pulmonary gas exchange*

Participants breathed through a low resistance (< 0.1 KPa.L\(^{-1}\).s\(^{-1}\) at 15 L.s\(^{-1}\)), low dead space (90 mL) mouthpiece for the measurement of respired gases. Flow rates and volumes were
measured with an infrared turbine flow sensor (Interface Associates, Laguna Niguel, CA, USA), while a quadrupole mass spectrometer was used to measure respired gas concentrations after sampling air at 0.5 mL.s\(^{-1}\) from the mouthpiece (MSX, nSpire Health Ltd, Hertford, UK). Gas concentration signals were time aligned with the flow sensor signal using proprietary software for the calculation of breath-by-breath gas exchange. These algorithms identified the end of each breath with the flow sensor and time aligned the changes in respired gases.

Prior to each experiment the flow sensor and gas analysers were calibrated according to the manufacturers’ instructions. The turbine volume transducers were calibrated with a 3 L syringe (Hans Rudolph Inc., Shawnee, KS, USA). The calibration was completed with flow rates ranging from 0.2 to 6 L.s\(^{-1}\), mimicking flow rates expected for humans at rest and during exercise. After the completion of the flow sensor calibration, the flow volumes were verified over ten syringe strokes of varying flow rates, and accepted when the means were within ± 0.01 L, with a SD and coefficient of variation of 0.02 L and 1%, respectively. Additionally, the mass spectrometer was calibrated with atmospheric air and precision-verified gases with concentrations of O\(_2\), CO\(_2\) and N\(_2\) spanning the physiologic range. Following each experiment, mass spectrometer calibration factor drift was verified as negligible by sampling the calibration gases.

**Data analyses for pulmonary measures**

Breath-by-breath \(\dot{V}O_2\) was filtered for errant breaths (i.e. values resulting after sighs, swallows, coughs etc., defined as residing outside of 99% prediction limits) (Lamarra et al., 1987). Responses from like transitions were combined to improve signal:noise using an averaging technique that preserves the breath-by-breath density measured during the exercise transition. This method requires time aligning and sorting of all \(\dot{V}O_2\) data from exercise transitions in the time domain. Time and \(\dot{V}O_2\) are then averaged into bins of \(n\) breaths, where \(n\) is the number of
exercise transitions completed (Murgatroyd et al., 2011). The magnitude of the $\dot{V}O_{2\text{sc}}$ was expressed as the difference in $\dot{V}O_2$ between 3 min and 8 min of exercise.

Power output and flywheel speed from the ergometer were sampled continuously and digitised by a data recording system and stored on a PC (PowerLab 8/30 with LabChart Pro, ADInstruments Pty Ltd, Bella Vista, NSW, AU).

$^{31}P$ magnetic resonance spectroscopy

Muscle phosphorus-containing metabolites were measured with a 3T superconducting magnet (Magnetom Trio, Siemens AG, Erlangen, DE). A one-pulse MRS acquisition was employed using a dual tuned ($^1H$ and $^{31}P$) 15 cm and 18 cm diameter surface RF coil (RAPID Biomedical GmbH, Rimpar, DE), which was placed under the knee extensors, half way between the hip and knee. The concave RF coil was stabilised with sandbags and was secured to the table once the participants’ hips were strapped to the scanner table. A series of axial, sagittal and coronal gradient-recalled echo images of the thigh were acquired to confirm the placement of the RF coil relative to the knee extensor muscles and to prescribe the volume over which shimming was achieved. Subsequently, a standard $^1H$ shimming protocol was used to optimise the homogeneity of the magnetic field ($\beta_0$). A fully relaxed spectrum (repetition time (TR) of 10 s; number of scans = 4) was initially obtained to provide a high-resolution unsaturated resting spectrum along with a 32 scan spectrum with a TR of 2 s. Following this, free induction decays for $^{31}P$ spectra were collected every 2 s with a spectral width of 3200 Hz and 1024 data points throughout the rest-to-exercise-to-rest transitions. $^{31}P$ data were averaged over four acquisitions yielding a datum every 8 s.
Kinetic analysis of $^{31}$P MRS data

Signal intensities, frequencies and line widths of inorganic phosphate (Pi), PCr, γ-ATP, α-ATP, and β-ATP, were determined using Java-based Magnetic Resonance User Interface (jMRUI) (Naressi et al., 2001) in order to transform the raw data into a time series for each of the phosphates of interest. Intramuscular pH ($pH_i$) was estimated from the chemical shift of Pi (Moon & Richards, 1973):

$$pH_i = 6.75 + \log(\delta - 3.27/5.69 - \delta)$$

Equation 1

where $\delta$ is the chemical shift of the Pi peak, relative to PCr.

PCr kinetics were modelled using non-linear least squares regression (OriginPro 7.5, OriginLab Corp., Northampton, MA, USA). $^{31}$P MRS data were filtered for errant values resulting from artefact (Rossiter et al., 2000) prior to characterisation with the following function:

$$[PCr] = [PCr]_0 + A \cdot (1 - e^{-t/\tau})$$

Equation 2

where $\tau$ is a time constant, $[PCr]_0$, $[PCr]_0$, and $A$ are the time variant form, baseline, and fundamental amplitude, respectively. The fitting window was determined from an iterative process (Rossiter et al., 2001) to ensure the exclusion of phase III (steady state or slow component, depending on the intensity domain). The magnitude of the PCr slow component ($[PCr]_{sc}$) was expressed as the difference in $[PCr]$ between the 3rd and 8th min of exercise.

ATP$_{tot}$ was estimated from the contributions from oxidative phosphorylation ($Q$), PCr breakdown ($D$), and glycogenolysis ($L$), which were determined from the $^{31}$P MRS data acquired during
exercise and recovery, using methods described elsewhere (Kemp et al., 2001; Kemp et al., 2007; Layec et al., 2011; Kemp et al., 2014), and outlined below.

**ATP production from PCr breakdown (D)**

The rate of PCr breakdown by creatine kinase (D) yields one component of ATP production (in mM.min\(^{-1}\)), and was determined over 32 s (4 spectra) immediately prior to exercise cessation.

\[ D = \frac{d[PCr]}{dt} \]  
Equation 3

In the present experiments, where [PCr] is either close to steady state or changing only slowly by the end of exercise, D is a very small component of end-exercise ATP\(_{tot}\).

**ATP production from oxidative phosphorylation (Q)**

The rate of oxidative ATP yield (Q) is reflected in the rate of [PCr] recovery at the instant of exercise cessation (\(V_{[PCr]}\)), and was calculated (mM.min\(^{-1}\)) as:

\[ V_{[PCr]} = k \cdot A \]  
Equation 4

where A is the amplitude of [PCr] change (in mM). The rate constant (k) was estimated by fitting the PCr recovery kinetics with the following function:

\[ [PCr]_t = [PCr]_{end} \cdot A \cdot (1 - e^{-kt}) \]  
Equation 5

where [PCr]\(_t\) is the time-dependent variant of [PCr], and [PCr]\(_{end}\) is the concentration of PCr measured at the end of exercise. We make the well-evidenced assumption (Kemp et al., 2014) that the rate of suprabasal oxidative synthesis at the start of recovery (\(V_{[PCr]}\) from Equation 4) is a good estimate of the suprabasal rate of oxidative synthesis at the end of exercise (Q\(_{end}\)).
ATP production from anaerobic glycolysis (L)

During exercise, glycogenolysis and the resulting lactate and H⁺ production cause disturbances in pH. These changes in pH are readily measured by ³¹P MRS data and can therefore be used to estimate ATP production from glycogenolysis; 1 mol of H⁺ resulting in 1.5 mol of ATP. This requires estimation of the flux rates: H⁺ production accompanying changes in PCr concentration via the creatine kinase reaction (H⁺\textsubscript{CK}, which is positive, i.e. H⁺ ‘consumption’, when [PCr] is falling in exercise, and negative, i.e. H⁺ generation, when [PCr] is rising in recovery), by the buffers of the muscle cytosol (H⁺\textsubscript{β} which is positive, i.e. H⁺ ‘buffering’, when pH\textsubscript{i} is falling in exercise, and negative, i.e. H⁺ ‘unbuffering’, when pH\textsubscript{i} is rising in recovery) and proton efflux from the cells (H⁺\textsubscript{efflux}). Together these sum to the total proton yield (P) during exercise:

\[ P = H⁺\textsubscript{CK} + H⁺\textsubscript{β} + H⁺\textsubscript{efflux} \]  

Equation 6

From which

\[ L = 1.5 \times P \]  

Equation 7

The number of protons consumed at the creatine kinase reaction was calculated from the time dependent changes in [PCr] using the proton stoichiometric coefficient γ (Kushmerick, 1997):

\[ H⁺\textsubscript{CK} = - \gamma D \]  

Equation 8

Protons buffered (H⁺\textsubscript{β} mM.min⁻¹) was calculated from the apparent buffering capacity, β\textsubscript{total} (mmol acid added per unit change in pH\textsubscript{i}) and from the (smoothed) rate of pH change during exercise:
\[ H^+_\beta = - \beta_{\text{total}}(dpH_i/dt) \]  
\[ \text{Equation 9} \]

where

\[ \beta_{\text{total}} = \beta_{\text{non-bicarbonate-non-Pi}} + \beta_{\text{Pi}} \]  
\[ \text{Equation 10} \]

The intrinsic cytosolic buffering capacity \( \beta_{\text{non-bicarbonate-non-Pi}} \) is calculated from initial-exercise data:

\[ \beta_{\text{non-bicarbonate-non-Pi}} = \beta_a - \beta_{\text{Pi}} \]  
\[ \text{Equation 11} \]

where the apparent \( \beta \) (\( \beta_a \)) is obtained from the initial rate of change in [PCr] (\( \Delta \text{PCr} \)) and alkanisation of pH (\( \Delta pH_i \)):

\[ \beta_a = \gamma \Delta \text{PCr}/\Delta pH_i \]  
\[ \text{Equation 12} \]

\( \beta_{\text{Pi}} \) was calculated as:

\[ \beta_x = (2.303 \times H^+ \times K \times [P_i])/(K + H^+)^2 \]  
\[ \text{Equation 13} \]

where \( K = 1.77 \times 10^{-7} \) (Conley et al., 1998). \( \beta_{\text{bicarbonate}} \) was neglected, which assumes that muscle is a closed system during short duration exercise \textit{in vivo} (Kemp et al., 1993). Proton efflux (\( H^+_{\text{efflux}} \text{ mM.min}^{-1} \)) was estimated for each time point of exercise assuming a linear pH-dependence constant \( \lambda \):

\[ H^+_{\text{efflux}} = - \lambda \Delta pH_i \]  
\[ \text{Equation 14} \]

This proportionality constant \( \lambda \) (mM.min\(^{-1}\).pH unit\(^{-1}\)) was estimated from initial recovery after exercise cessation:
\[ \lambda = -\frac{V_{\text{eff}}}{\Delta pH_i} \]  
Equation 15

At the cessation of exercise, the PCr resynthesised at the creatine kinase reaction is essentially solely a consequence of oxidative ATP production (Kemp et al., 2014). Therefore, \( H^{\text{eff}}_{\text{efflux}} \) can be calculated from the rate of proton production from creatine kinase \( (H^{\text{CK}}) \) and the rate of pH change on the other side.

\[ V_{\text{eff}} = \beta_{\text{total}} (dpH/dt) = \gamma V_{i,PCr} \]  
Equation 16

Where \( \Delta pH_i \) is very low, Equation 15 becomes unreliable, and the end-exercise rate of \( H^{\text{eff}}_{\text{efflux}} \) is simply assumed to be equal to \( H^{\text{eff}}_{\text{efflux}} \) calculated from the initial recovery data by Equation 14.

In the present experiments, where \( pH_i \) is close to steady state or changing only slowly by the end of exercise, \( L \) is a very small component of \( ATP_{\text{tot}} \).  

Statistical analyses

Relationships between variables were assessed with a Pearson correlation coefficient, where appropriate. The difference between \(^{31}\)P measures at discrete time points and across exercise intensities were compared with a two-factor (time \( \times \) intensity domain) repeated measures ANOVA. Bonferroni-corrected paired t-tests were used post hoc to identify simple effects in the case of a significant interaction. For all tests, \( \alpha = 0.05 \). Analyses were completed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA).

Results

During RI exercise, participants attained a peak power output of 47 \( \pm \) 11 W at a \( \dot{V}O_{2\text{peak}} \) of 2.00 \( \pm \) 0.48 L.min\(^{-1}\). Based on peak power output and estimated LT (\( \sim \)60-70\% \( \dot{V}O_{2\text{peak}} \)), moderate (sub-LT; 19 \( \pm \) 4 W) and heavy (supra-LT; 46 \( \pm \) 11 W) constant power exercise bouts were
assigned. The dynamics of $\dot{V}O_2$ were examined *post hoc* to confirm the appropriate intensity assignment (Whipp, 1996; Rossiter, 2011).

During moderate exercise, there was no discernable pulmonary $\dot{V}O_{2sc}$ (0.06 ± 0.12 L.min⁻¹). However, during heavy exercise the $\dot{V}O_{2sc}$ was 0.37 ± 0.16 L.min⁻¹ (*Figure 1A*), or a 22 ± 8 % increase. [PCr] did not change between 3 and 8 min of moderate intensity exercise (30 ± 8 vs. 32 ± 7 mM; $p = \text{n.s.}$). Conversely, during heavy exercise [PCr] fell from 3 to 8 min (19 ± 7 vs. 18 ± 7 mM or 12 ± 15 % fall; $p < 0.05$; *Figure 1B*).

ATP yield during moderate and heavy exercise from oxidative phosphorylation ($Q$), PCr hydrolysis ($D$), lactate production ($L$) and, consequently, $ATP_{tot}$ are presented in Table 1. $V_{[PCr]}$, calculated as described in Equation 4 is shown, along with the rate constant of PCr resynthesis ($k$) and the amplitude of PCr recovery ($A$), in *Figure 2* (moderate) and *Figure 3* (heavy).

Comparisons of $ATP_{tot}$ revealed a significant interaction (time × intensity domain; $F[1,13] = 17.2$; $p < 0.01$; $\eta^2 = 0.57$). $ATP_{tot}$ was not different between 3 and 8 min of moderate exercise ($p = \text{n.s.}$; *Figure 2, Table 1*), but $ATP_{tot}$ increased ($\Delta ATP_{tot}$) during heavy exercise from 3 to 8 min (CI Difference 1.9, 12.6 mM.min⁻¹; $p < 0.05$; *Figure 3, Table 1*), equating to a 26 ± 30 % increase in $ATP_{tot}$ from 3 to 8 min (*Figure 4A*). This percentage change in $ATP_{tot}$ was not different to that measured in both the $[PCr]_{sc}$ and the $\dot{V}O_{2sc}$ ($F[2,26] = 2.4$; $p = \text{n.s.}; \eta^2 = 0.16$) (*Figure 4A*). However, among participants the individual values of $\Delta ATP_{tot}$ during heavy exercise were not significantly correlated with the magnitude of the $\dot{V}O_{2sc}$ (*Figure 4B*).

To examine the relationship of $\dot{V}O_2$ and [PCr], a correction for the transit delay from muscle to lung was applied. $\dot{V}O_2$ data were time-corrected using 12 s difference with respect to $^{31}$P
measures (Rossiter et al., 1999; Krustrup et al., 2009). The relationship between \( \dot{V}O_2 \) and [PCr]
was linear during moderate exercise and the first 3 min of heavy exercise \( (r^2 = 0.94; \text{Figure 5}) \).
However, the slope of the [PCr]-\( \dot{V}O_2 \) relationship was significantly steeper when data from 8 min of heavy exercise was included (-67 ± 25 vs. -61 ± 25 mL.min.mM\(^{-1}\); \( p < 0.05 \)).

**Discussion**

The [PCr] slow component ([PCr]\text{sc}), like the \( \dot{V}O_2 \) slow component (\( \dot{V}O_{2\text{sc}} \)), is only present during exercise above LT. That the [PCr]\text{sc} and \( \dot{V}O_{2\text{sc}} \) are of similar magnitude (Rossiter et al., 2002) led to the argument that the \( \dot{V}O_{2\text{sc}} \) is caused by an increased phosphate cost of power production (P/W) during heavy intensity exercise. However, this is at odds with the observed dissociation between the [PCr]\text{sc} and \( \dot{V}O_{2\text{sc}} \) in endurance-trained individuals (Layec et al., 2009b; Layec et al., 2012), and both observations relied upon equivocal assumptions about the dynamic relationships between [ADP]-\( \dot{V}O_2 \) or \( \dot{V}O_2 \cdot [PCr] \) (Yoshida & Watari, 1993, 1994; Rossiter et al., 2002; Jones et al., 2008; Kemp, 2008; Wust et al., 2011). Our present data agree with previous reports that mean [PCr]\text{sc} and \( \dot{V}O_{2\text{sc}} \) magnitudes were not statistically different. Crucially, however, the data add that, among individuals, the increase in the \( \dot{V}O_{2\text{sc}} \) during heavy intensity exercise (averaging ~22%) is not correlated with the increase in the phosphate cost of power production, ATP\text{tot} (average ~26%). Thus, while the exercising limb remains likely the major source of the \( \dot{V}O_{2\text{sc}} \) (Poole et al., 1991; Rossiter et al., 2002; Bailey et al., 2010; Dimenna et al., 2010), the observed dissociation between \( \dot{V}O_{2\text{sc}} \) and ΔATP\text{tot} (Figure 4B) strongly suggests that the progressive increase in \( \dot{V}O_2 \) during heavy exercise is not solely due to contractile inefficiency (P/W). Thus, other explanations, such as a reduction in mitochondrial efficiency (P/O), should also be considered.
The primary aim of this investigation was to estimate the ATP turnover rate for exercise below and above LT and over time without assumptions about the [ADP]-\(\dot{V}O_2\) or \(\dot{V}O_2-[PCr]\) relationship. By using the most robust estimations of ATP\(_{tot}\) (Kemp et al., 1995; Walter et al., 1999; Lanza et al., 2005; Faraut et al., 2007), we provided \(^{31}\)P MRS-derived estimates of ATP yield from oxidative phosphorylation, lactate production, and PCr hydrolysis at 3 and 8 min of exercise that were unencumbered by the recently-challenged assumptions about the [ADP]-\(\dot{V}O_2\) relationship (Kemp, 2008; Wust et al., 2011; Glancy & Balaban, 2012; Kemp et al., 2014).

Unsurprisingly, there were no changes in ATP\(_{tot}\) during exercise below the lactate threshold, where negligible muscle fatigue is expected (Sargeant & Dolan, 1987; Yano et al., 2001), reflecting a steady-state condition. Conversely, during heavy exercise in which the \(\dot{V}O_{2sc}\) and \([PCr]_{sc}\) were present, ATP\(_{tot}\) was increased between 3 and 8 min of exercise. This is consistent with the suggestions that the \(\dot{V}O_{2sc}\) is consequent to increased P/W in the large locomotor muscles during supra-LT exercise (Rossiter et al., 2002), perhaps associated with muscle fatigue and a reduction in contractile efficiency. However, the lack of relationship between \(\Delta\text{ATP}_{tot}\) and \(\dot{V}O_{2sc}\) is in contrast to this postulate, and challenges the current understanding of the \(\dot{V}O_{2sc}\) aetiology (Rossiter, 2011; Poole & Jones, 2012).

Dissociation of the \(\dot{V}O_{2sc}\) and changes in the phosphate cost of exercise may have a few different explanations. It may indicate \(\dot{V}O_2\) originating from regions within the knee extensors that are not interrogated by the surface coil. While we can only speculate on this, a similar finding has been reported where the \(\dot{V}O_2\) and \([PCr]\) slow components were dissociated in endurance trained participants but not in sedentary controls, despite increasing EMG activity in both participant groups during the \(\dot{V}O_{2sc}\) (Layec et al., 2009b; Layec et al., 2012). It was
hypothesised that the exercise-trained volunteers may be better able to optimise motor unit recruitment patterns to maintain high-intensity exercise (e.g. compared to active but untrained subjects (Rossiter et al., 2002)), thereby recruiting motor unit pools that reside outside of the muscle volume being interrogated by MRS. It should be noted, however, that our surface coil interrogated a large muscle volume (~300 g) compared to alternative techniques, e.g. biopsy (~200 mg). Additionally, controversy exists whether progressive recruitment itself is even responsible for the slow component (Zoladz et al., 2008; Cannon et al., 2011; Vanhatalo et al., 2011), in which case recruitment of muscle outside the surface coil view would seem to be an unlikely explanation if the motor program and recruitment pattern is stable.

The source of the $\dot{V}O_{2sc}$ may even reside outside of the locomotor muscles. Progressive increases in respiratory (Wasserman et al., 1995; Zoladz & Korzeniewski, 2001) or cardiac work, or even work from non-power-producing musculature, such as stabilising effort during cycling (Billat et al., 1998) may contribute to a reduction in exercise efficiency during the slow component. It is unlikely that the latter source would contribute to prone knee-extension where the work of stabilising the torso is minimised by the body position, the ergometer, and the heavy strapping used to isolate quadriceps activity. Nonetheless, the work of ventilation during prone knee-extension may still contribute a meaningful proportion; particularly as the locomotor muscle mass in our study is relatively small in comparison to cycling or running.

Finally, dissociation of the $\dot{V}O_{2sc}$ and $\Delta$ATP$_{tot}$ could result from mitochondrial uncoupling (reduced P/O; Figure 5). In this scenario, an increased O$_2$ cost of ATP resynthesis may contribute to driving the increase in VO$_2$ during heavy exercise, rather than it coming exclusively from an increased ATP cost of muscle power generation.
\[ \dot{V}O_2-[PCr] \text{ relationship and mitochondrial coupling during heavy intensity exercise} \]

Without an invasive measure of \( \dot{V}O_2 \) across the volume of tissue interrogated by MRS, the relationship between whole body \( \dot{V}O_2 \) and localised [PCr] is the next best estimate for coupling of \( O_2 \) uptake and ATP turnover. Our data show that the mean \( \dot{V}O_2-[PCr] \) relationship was linear over the moderate intensity, and during the first minutes of heavy exercise \((r^2 = 0.94; \text{Figure 5})\). Importantly, this relationship became steeper \((p < 0.05)\) with the inclusion of data from the final minute of heavy exercise. With some important assumptions, these data suggest a reduced P/O between 3 and 8 minutes of heavy exercise, implicating mitochondrial uncoupling as an additional mechanism of the \( \dot{V}O_2 \) slow component.

It is important to recognise that the \( \dot{V}O_2/[PCr] \) slope reflects the combined influence of mitochondrial density, the rate constant \((k)\) of [PCr] breakdown relative to \( k \) of \( \dot{V}O_2 \), the total [creatine], and the P/O (Meyer, 1988; Kemp et al., 2014). Mitochondrial density and total [creatine] are constant during acute exercise, and therefore any divergence in \( \dot{V}O_2-[PCr] \) slope would result from changes in \( k[PCr] \) and/or P/O over the exercise intensities. While the \( k[PCr] \) was not different between 3 and 8 min of heavy intensity exercise \((p = \text{n.s.})\), there was variance among individuals (Figure 3A). Therefore, while we base our interpretation on the group mean, we cannot rule out the influence of variance in the individual changes in \( k[PCr] \) in interpreting the \( \dot{V}O_2-[PCr] \) slope. In addition, we used a fixed transit delay to phase align the \( \dot{V}O_2 \) and [PCr] measurements in the time domain. This correction provided the best fit to the kinetics that we could make, but it is a limitation for interpreting the \( \dot{V}O_2-[PCr] \) relationship. Specifically, small errors in transit delay adjustment result in non-linear distortion when plotting single participant data, although this influence is greater during the early kinetics (first 2 min) than between 3 and 8 minutes of exercise where the kinetics are slower. Finally, the progressive intramuscular
Acidification during exercise would be expected to dissociate the dynamics of \( \dot{V}O_2 \) and \([\text{PCr}]\), speeding the former, and slowing the latter (Iotti et al., 1993; Gerbino et al., 1996; Layec et al., 2013). Therefore, while substantial assumptions necessarily underlie the interpretation of the \( \dot{V}O_2\)-[PCr] relationship, it is currently the only way to examine change in P/O as a potential mechanism explaining the lack of relationship between the magnitude of the \( \dot{V}O_{2sc} \) and \( \Delta ATP_{\text{tot}} \). These data suggest that P/O is stable during moderate intensity exercise and the first 3 min of heavy intensity exercise, in agreement with the other \( ^{31}\text{P} \) MRS studies [e.g. where the \( \dot{V}O_2\)-[PCr] relationship is strikingly linear throughout the metabolic rate range (Bailey et al., 2010)], but that sustained heavy intensity exercise beyond 3 min may be accompanied by a reduction in P/O. Consequently, contrary to the prevailing hypothesis (Rossiter et al., 2002), the \( \dot{V}O_{2sc} \) may at least, in part, be a result of mitochondrial uncoupling in the active muscle during acidifying exercise.

**Potential mechanisms of mitochondrial uncoupling**

There are various mechanisms that might cause the mitochondrial trans-membrane proton gradient to dissipate during exercise. This proton 'leak' is regulated by uncoupling proteins and contributes to setting the resting P/O. If this process is augmented during exercise, the ATP yield per atomic oxygen consumed would fall. Others have shown upregulation of uncoupling proteins 2 and 3 with an acute bout of exercise (UCP2, UCP3; both expressed in skeletal muscle) and these can induce mitochondrial uncoupling, likely to minimise production of, and damage from, reactive oxygen species (ROS) (Brand et al., 2004; Bo et al., 2008; Jiang et al., 2009). This effect may be akin to the chronic uncoupling reported with ageing, posited as a protective mechanism against ROS damage (Brand et al., 2004; Amara et al., 2007), particularly as leak respiration comprises a large proportion of resting \( \dot{V}O_2 \). However, the kinetics of UCP upregulation are relatively slow in comparison with the exercise duration in our study;
upregulation of uncoupling proteins is typically present ~45-90 min post-acute exercise. Additionally, investigations into mitochondrial uncoupling have relied on relatively long bouts of exercise (> 30 min), and evidence from human muscle suggests that acute exercise may not be sufficient to elicit the same effect size for upregulation seen in the rat (Fernstrom et al., 2004). Therefore, upregulation of uncoupling proteins seems less likely to fully explain the lack of relationship between \( \dot{V}O_{2sc} \) and \( \Delta ATP_{tot} \) during heavy exercise.

Alternatively, dissociation of the \( \dot{V}O_{2sc} \) and \( \Delta ATP_{tot} \) may result from high \([H^+]\) or \([Pi]\) during exercise (Walsh et al., 2002). Low pH can reduce [ADP] from a shift in the creatine kinase equilibrium (Conley et al., 2001), and also serve to dissociate creatine kinase from the mitochondrial membrane, leading to a disruption in oxidative phosphorylation (Walsh et al., 2002). While evidence for a direct effect of acidosis is certainly not conclusive (Suleymanlar et al., 1992; Kemp et al., 2014), numerous studies show disturbances to oxidative phosphorylation through the inhibition of respiratory enzymes or reductions in the proton motive force (Hillered et al., 1984; Harkema & Meyer, 1997; Jubrias et al., 2003), but fail to result in change to P/O alone (Tonkonogi & Sahlin, 1999). Nevertheless, the variable relationships between the magnitude of the \( \dot{V}O_{2sc} \) and \( \Delta ATP_{tot} \), together with a steeper \( \dot{V}O_{2}=[PCr] \) relationship, suggest P/O change as a possible scenario during heavy exercise.

**Technical considerations and study limitations**

While limitations accompany the estimations, our study design provides advantage over previous reports of ATP turnover rate in the literature. Prior estimations have relied on extrapolation of \( V_{i[PCr]} \), which is assumed to be only affected by the [PCr] recovery amplitude. This model constrains P/O with a linear \( \dot{V}O_{2}=[PCr] \) relationship, by definition (Layec et al., 2009a), which is in contrast with recent findings (Kemp, 2008; Wust et al., 2011; Glancy &
and the observations in this study (Figure 5). Conversely, \([\text{PCr}]\) recovery dynamics may be plastic during supra-LT exercise where intracellular acidification (Yoshida & Watari, 1993, 1994), fatigue related metabolite accumulation (Jones et al., 2008), and muscle fatigue (Yano et al., 2001; Cannon et al., 2011) have been reported. While the group mean for \(k[\text{PCr}]\) resynthesis (or time constant, \(\tau = 1/k\)) is not different following sub- and supra-LT exercise in this study and others (Rossiter et al., 2002), our data suggests that \(k[\text{PCr}]\) is not constant within an individual. Therefore, in our study, \(V_{i[\text{PCr}]}\) (and, thus, \(Q\) and \(ATP_{\text{tot}}\)) were not constrained to increase in response only to changes in \([\text{PCr}]\). In other words, the augmented amplitude of \([\text{PCr}]\) during the slow component did not result in an obligatorily faster initial rate of change following the cessation of exercise; our measurement was dependent on the recovery dynamics characterised and specific to that moment in time. Consequently, the estimations provided for oxidative ATP yield in our study are devoid of the assumptions about the \(\dot{\text{VO}}_2\)-\([\text{ADP}]\) and \(\dot{\text{VO}}_2\)-\([\text{PCr}]\) relationships.

\(ATP_{\text{tot}}\) is most heavily weighted on changes in \(V_{i[\text{PCr}]}\), a measure that is sensitive to noise in the MRS signal [e.g. Figure 7 in (Rossiter et al., 2000)]; this initial rate is derived from characterisation of the kinetics of \([\text{PCr}]\) recovery. The influence of noise in \([\text{PCr}]\) recovery kinetics, particularly in the early transient, is likely the largest source of variability to resolve \(ATP_{\text{tot}}\). Conversely, the confidence in characterising \([\text{PCr}]\) off-kinetics is substantially greater than for pulmonary \(\dot{\text{VO}}_2\) or even \([\text{PCr}]\) during the on-transient. Any improvement in the characterisation of \(^{31}\text{P}\) dynamics will take a considerable leap in signal:noise and more rapid spectra acquisition.

The heterogeneous nature of skeletal muscle metabolism (Koga et al., 2007; Damon et al., 2008; Saitoh et al., 2009; Cannon et al., 2013) may have obscured the characterisation of \([\text{PCr}]\)
dynamics, and therefore ATP<sub>tot</sub>. $^{31}$P MRS measures a volume of tissue (~300 g) which may not be representative of the entire knee extensor group responsible for the power output, or the diversity of metabolic strain within this group. Finally, the unmeasured work of knee flexion is not accounted for with our ergometer. Therefore, the work of knee flexion (to lift the leg) is assumed to be constant in our experiments, but does contribute to the pulmonary VO<sub>2</sub> signal.

Conclusions

Similar to previous studies, the mean magnitude of the VO<sub>2</sub> and [PCr] slow components were not different during heavy exercise, consistent with the prevailing hypothesis for the intramuscular source of the VO<sub>2</sub>sc: an increase in the phosphate cost of force production. Although the magnitude of the VO<sub>2</sub> slow component (~22%) was similar to the increase in ATP<sub>tot</sub> (~26%) from 3 to 8 min during heavy exercise, there was no relationship detected between these measures among individuals. Therefore, our data suggest that the pulmonary VO<sub>2</sub> slow component does not solely originate from increases in the phosphate cost of power production (increased P/W). Other mechanisms, such as an increased O<sub>2</sub> cost of ATP resynthesis (reduced P/O) during acidifying exercise, may also contribute to generating the VO<sub>2</sub> slow component.

Acknowledgements

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Figure Legends

**Figure 1.** VO₂ (panel A), [PCr] (panel B), and pH (panel C) plotted as a function of time for moderate (●) and heavy (○) intensity prone bilateral knee-extension exercise. Black rectangle denotes exercise bout from time 0 to 8 min. Data points are 8 s means with error bars representing SD.

**Figure 2.** Moderate exercise recovery rate constant (k; Panel A), amplitude of PCr resynthesis (A; Panel B), initial rate of PCr resynthesis (V_{i[PCr]}; Panel C), and ATP_{tot} (Panel D) at 8 min of exercise, plotted as a function of 3 min of exercise. Dashed line is y = x.

**Figure 3.** Heavy intensity rate constant (k; Panel A), amplitude of PCr resynthesis (A; Panel B), initial rate of PCr resynthesis (V_{i[PCr]}; Panel C), and ATP_{tot} (Panel D) at 8 min of exercise, plotted as a function of 3 min of exercise. Dashed line is y = x.

**Figure 4.** Magnitudes of the VO₂_{2sc} and [PCr]_{sc} and ΔATP_{tot} from min 3 to 8 of heavy exercise expressed as a % change (Panel A). Panel B is ΔATP_{tot} during heavy exercise plotted as a function of the VO₂_{2sc}.

**Figure 5.** Relationship between pulmonary VO₂ and [PCr] during moderate (●) and heavy (○) exercise. The regression shown (solid line) was fit to moderate and the first 3 min of data from heavy exercise and extrapolated (dashed line) to 8 min of heavy exercise. Error bars represent SD. VO₂ data were phase aligned with respect to [PCr] measurements.
Tables

Table 1. Rates of ATP turnover from oxidative phosphorylation ($Q$), PCr hydrolysis ($D$), lactate production ($L$) and the sum ($\text{ATP}_{\text{tot}}$) during moderate and heavy constant power exercise at two time points. Values are presented in mM.min$^{-1}$ as means (SD).

<table>
<thead>
<tr>
<th></th>
<th>Moderate</th>
<th></th>
<th>Heavy</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>3 min</td>
<td>8 min</td>
<td>3 min</td>
<td>8 min</td>
</tr>
<tr>
<td>$Q$ (mM.min$^{-1}$) §</td>
<td>23 (14)</td>
<td>17 (13)</td>
<td>35 (17)</td>
<td>42 (13)*</td>
</tr>
<tr>
<td>$D$ (mM.min$^{-1}$)</td>
<td>0.6 (1.2)</td>
<td>0.2 (1.0)</td>
<td>1.1 (2.6)</td>
<td>0.7 (0.9)</td>
</tr>
<tr>
<td>$L$ (mM.min$^{-1}$)</td>
<td>1.0 (1.3)</td>
<td>0.3 (0.6)</td>
<td>1.5 (1.3)</td>
<td>1.3 (1.7)</td>
</tr>
<tr>
<td>$\text{ATP}_{\text{tot}}$ (mM.min$^{-1}$) #</td>
<td>24 (14)</td>
<td>17 (14)</td>
<td>38 (16)</td>
<td>44 (14)*</td>
</tr>
</tbody>
</table>

§Time × Intensity interaction; $p < 0.01$; F[1,13]=17.2; $\eta^2$=0.57
#Time × Intensity interaction; $p < 0.01$; F[1,13]=17.2; $\eta^2$=0.57
*Different from 3 min; $p < 0.05$
Figure 1

(A) VO₂ (L/min⁻¹) over time.

(B) [PCr] (mM) over time.

(C) pH over time.
Figure 2

A  
$k$ at 8 min  
$k$ at 3 min

B  
$A$ at 8 min (mM)  
$A$ at 3 min (mM)

C  
$V_{i[PCr]}$ at 8 min (mM.min$^{-1}$)  
$V_{i[PCr]}$ at 3 min (mM.min$^{-1}$)

D  
$ATP_{tot}$ at 8 min (mM.min$^{-1}$)  
$ATP_{tot}$ at 3 min (mM.min$^{-1}$)
Figure 3
Figure 5

\[ \dot{V}O_2 \ (L \cdot min^{-1}) \]

\[ [PCr] \ (mM) \]
References


