Dissociating external power from intramuscular exercise intensity during intermittent bilateral knee-extension in humans

Matthew J Davies[1], Alan P Benson[1], Daniel T Cannon[2], Simon Marwood[3], Graham J Kemp[4,5], Harry B Rossiter[1,6], Carrie Ferguson[1]

[1]School of Biomedical Sciences, Faculty of Biological Sciences & Multidisciplinary Cardiovascular Research Centre, University of Leeds

[2]School of Exercise & Nutritional Sciences, San Diego State University, San Diego, CA 92182, USA

[3]School of Health Sciences, Liverpool Hope University

[4]Magnetic Resonance & Image Analysis Research Centre, University of Liverpool

[5]Department of Musculoskeletal Biology, University of Liverpool

[6]Rehabilitation Clinical Trials Center, Division of Respiratory & Critical Care Physiology & Medicine, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance CA, 90254, USA

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Corresponding Author:
Carrie Ferguson, PhD
School of Biomedical Sciences,
Faculty of Biological Sciences &
Multidisciplinary Cardiovascular Research Centre,
University of Leeds, Leeds, LS2 9JT, UK
c.ferguson@leeds.ac.uk

Key points summary

- Continuous high-intensity constant-power exercise is unsustainable, with maximal oxygen uptake ($\dot{V}O_{2max}$) and the limit of tolerance attained after only a few minutes.
- Performing the same power intermittently reduces the O_2 cost of exercise and increases tolerance. The extent to which this dissociation is reflected in the intramuscular bioenergetics is unknown.
- We used pulmonary gas exchange and ^{31}P magnetic resonance spectroscopy to measure whole-body $\dot{V}O_2$, quadriceps phosphate metabolism and pH during continuous and intermittent exercise of different work:recovery durations.
- Shortening the work:recovery durations (16:32 s vs. 32:64 s vs. 64:128 s vs. continuous) at a work rate estimated to require 110 % peak aerobic power reduced $\dot{V}O_2$, muscle phosphocreatine breakdown and muscle acidification, eliminated the glycolytic-associated contribution to ATP synthesis, and increased exercise tolerance.
- Exercise intensity (i.e. magnitude of intramuscular metabolic perturbations) can be dissociated from the external power using intermittent exercise with short work:recovery durations.

Abstract

Compared with work-matched high-intensity continuous exercise, intermittent exercise dissociates pulmonary oxygen uptake ($\dot{V}O_2$) from the accumulated work. The extent to which this reflects differences in O_2 storage fluctuations and/or contributions from oxidative and substrate-level bioenergetics is unknown. Using pulmonary gas-exchange and intramuscular ^{31}P magnetic resonance spectroscopy, we tested the hypotheses that at the same power: ATP synthesis rates are similar; but peak $\dot{V}O_2$ amplitude is lower in intermittent vs. continuous exercise. Thus, we expected that: intermittent exercise relies less upon anaerobic glycolysis for ATP provision than continuous exercise; shorter intervals would require relatively greater fluctuations in intramuscular bioenergetics than in $\dot{V}O_2$ compared with longer intervals. Six men performed bilateral knee-extensor exercise (estimated to require 110% peak aerobic power) continuously and with three different intermittent work:recovery durations (16:32; 32:64; 64:128s). Target work duration (576s) was achieved in all intermittent protocols; greater than continuous (252±174s; p<0.05). Mean ATP turnover rate was not different between protocols (~43mM·min^{-1} on average). However, the intramuscular PCr component of ATP generation was greatest (~30mM·min^{-1}), and oxidative (~10mM·min^{-1}) and anaerobic glycolytic (~1mM·min^{-1}) components lowest for 16:32 and 32:64s intermittent protocols, compared with 64:128s (18±6, 21±10 and 10±4mM·min^{-1}, respectively) and continuous protocols (8±6, 20±9 and 16±14mM·min^{-1}, respectively). As intermittent work duration increased towards continuous, ATP production relied proportionally more upon anaerobic glycolysis and oxidative phosphorylation, and less upon PCr breakdown. However, performing the same high-intensity power intermittently vs. continuously reduced the amplitude of fluctuations in $\dot{V}O_2$ and intramuscular metabolism, dissociating exercise intensity from the power output and work done.

Abbreviations

^{31}P, phosphorus; MRS, magnetic resonance spectroscopy; ATP, adenosine triphosphate; FIDs, free induction decays; H^+, hydrogen; L^-, blood lactate; LT, lactate threshold; MR, magnetic resonance; $NADH^+$, nicotinamide adenine dinucleotide; P:O, oxygen cost of ATP resynthesis; P:W, ATP cost of force production; PCr, phosphocreatine; Pi, inorganic phosphate; pH_i, intramuscular pH; PO_2, partial pressure of oxygen; RF coil, radiofrequency coil; RIT, ramp-incremental test; $\dot{V}O_2$, oxygen uptake; $\dot{V}O_{2max}$, maximal oxygen uptake; $\dot{V}O_{2peak}$, peak oxygen uptake; $\dot{V}O_{2SC}$, slow component of oxygen uptake.

Introduction

The coupling of internal (capillary-to-myocyte) to external (capillary-to-alveolus) O_2 exchange during dynamic exercise is dependent on muscular oxidative ATP synthesis, the dynamics of the circulation, and volume of the intervening O_2 stores, predominantly in the form of oxyhaemoglobin in the venous blood. At the onset of continuous constant-power exercise, the kinetics of pulmonary oxygen uptake ($\dot{V}O_2$) are supplemented by contributions to energy transfer from utilisation of O_2 stores and, proportionally more significant, from substrate-level phosphorylation (phosphocreatine (PCr) breakdown, glycolysis/glycogenolysis accumulating lactate); termed the O_2 deficit. The O_2 deficit is associated with accumulation of products linked to muscle fatigue, such as intramuscular inorganic phosphate (Pi) and H^+ (Allen *et al.,* 2008), and hence $\dot{V}O_2$ kinetics are strongly associated with exercise tolerance (Whipp & Ward 1992; Burnley & Jones, 2007; Sperandio *et al.,* 2009; Murgatroyd *et al.,* 2011): a fast response proffering greater exercise tolerance (Murgatroyd & Wylde, 2011; Rossiter, 2011).

$\dot{V}O_2$ kinetics are intensity dependent (Özyener *et al.,* 2011). Critical power (the asymptote of the relationship between power and tolerable duration, which occurs between ~60-80 % $\dot{V}O_{2max}$; Poole *et al*., 1988; van der Vaart *et al.,* 2014) marks the individual threshold in the rate of metabolic power production below which the bodily demands for ATP resynthesis are met by wholly-aerobic energy transfer (Poole *et al.,* 1988; Jones *et al.,* 2008). During continuous exercise exceeding critical power, $\dot{V}O_2$ continues to rise (through the action of the slow component; $\dot{V}O_{2SC}$), and intramuscular PCr breakdown and Pi and H^+ accumulation are progressive (Poole *et al.,* 1988; Jones *et al.,* 2008; Vanhatalo *et al.,* 2010). During constant power exercise above critical power, where duration exceeds ~ 2 min (Hill *et al*., 2002), the limit of tolerance is commonly associated with the attainment of $\dot{V}O_{2max}$, a minimum intramuscular [PCr] and pH_i and maximum [Pi] (Jones *et al.,* 2008; Vanhatalo *et al.,* 2010). This limits the volume of work that can be accumulated during constant power exercise above critical power (Monod & Scherrer, 1965; Moritani *et al.,* 1981), where exercise can

only be continued once a reduction in power to a value equal or below critical power is made (Gaesser & Poole, 1996; Coats *et al.,* 2003; Ferguson *et al.,* 2010).

Intermittent exercise, in which periods of supra-critical-power work are interspersed with periods of recovery, dissociates the work done from systemic ($\dot{V}O_2$ and blood lactate, [L^-]) responses. Thus, the volume of work tolerated is increased, and the associated metabolic strain is reduced, using intermittent compared with continuous exercise (Astrand *et al.,* 1960; Margaria *et al.,* 1969; Turner *et al.,* 2006; Combes *et al.,* 2017). The magnitude of this mechanical-to-metabolic dissociation is dependent on the work:recovery duration, and is greatest when the work periods are short (e.g. 10-30 s; Turner *et al.,* 2006; Combes *et al.,* 2017). The effect is that homeostasis of $\dot{V}O_2$ and blood [L^-] is less disturbed during intermittent compared to continuous exercise performed at the same power and accumulating the same volume of work. By the same notion, intermittent exercise can be used to provide a greater volume of supra-critical power work in a given duration (Chidnok *et al*., 2013). This approach has been used in an attempt to enhance the stimulus for physiological adaptations by exercise training (e.g. Kemi *et al.* 2005; Helgerud *et al.* 2007; Wisløff *et al.* 2007; MacInnis *et al.* 2017).

It remains unclear the extent to which the systemic mechanical-to-metabolic dissociation by intermittent exercise (e.g. as frequently observed in pulmonary $\dot{V}O_2$; Turner *et al.,* 2006; Guiraud *et al.,* 2010; Chidnok *et al.,* 2012; Combes *et al.,* 2017) is matched by a similarly attenuated response of intramuscular phosphate metabolism. For example, intermittent exercise with short work bouts (10-30 s) and a low work:recovery ratio is hypothesised to have a relatively greater reliance on depletion of oxymyoglobin and venous oxyhaemoglobin O_2 stores compared to exercise with longer work bouts (Astrand *et al.,* 1960). This, coupled with a limb-lung vascular transient delay, temporally dissociates cardiac output and O_2 extraction responses at the lung, damping the response amplitude compared to the active muscles (Barstow & Mole, 1987; Barstow *et al.,* 1990; Rossiter, 2011; Benson *et al.,* 2013).

This infers that intramuscular oxidative phosphorylation and PCr breakdown, and thus the intramuscular bioenergetic strain, would be increased (in comparison to the pulmonary $\dot{V}O_2$ response) in short vs. longer intermittent work bouts or continuous exercise at the same power. However, this contradicts our knowledge of the progressive decrease in work efficiency during long intermittent or continuous exercise during which a $\dot{V}O_{2SC}$ is observed, consequent to greater PCr breakdown resulting from increases in both intramuscular ATP cost of force production (P:W) and O_2 cost of ATP production (P:O) (Rossiter *et al.,* 2002; Krustrup *et al.,* 2003; Turner *et al.,* 2006; Bailey *et al.,* 2010; Cannon *et al.,* 2014).

We aimed to investigate the coupling dynamics of intramuscular bioenergetics to pulmonary gas exchange during continuous and intermittent exercise at the same power. We used ^{31}P magnetic resonance spectroscopy (MRS) to measure intramuscular phosphate responses during bilateral knee-extensor exercise in continuous and intermittent exercise of different work:recovery durations in comparison to pulmonary $\dot{V}O_2$; each performed at the same power. We hypothesised that: (1) ATP synthesis rates are similar in intermittent and continuous exercise at the same power; but (2) the peak pulmonary $\dot{V}O_2$ amplitude will be lower in work-matched intermittent vs. continuous exercise. Thus, we expect that: (3) intermittent exercise relies less upon anaerobic glycolysis for ATP provision than continuous exercise, and is associated with greater exercise tolerance; despite (4) short intervals requiring relatively greater fluctuations in intramuscular bioenergetics than in systemic pulmonary gas exchange compared with longer intervals.

Materials and methods

Ethical approval

Liverpool Hope Faculty of Sciences and Social Sciences Research Ethics committee and the University of Liverpool Committee on Research Ethics approved the study, and all procedures complied with the latest version of the Declaration of Helsinki. Prior to participating all volunteers provided written informed consent.

Participants

Six healthy men (mean ± SD: age 24 ± 5 yr; height: 176 ± 7 cm; weight: 80 ± 12 kg) volunteered to participate. All participants regularly undertook exercise, and any contraindications that would have precluded involvement in the study, including contraindications to MRS, were identified using a pre-exercise assessment questionnaire.

Exercise protocols

Ergometry. All exercise tests were performed on a computer-controlled electromagnetically braked MR compatible bilateral knee-extension ergometer (MRI Ergometer Up/Down, Lode BV, Groningen, The Netherlands). As described previously, this ergometer was customised for use in a Siemens 3T MR scanner using extended carbon-fibre lever arms (Cannon *et al.,* 2014). Participants lay prone with their feet secured into plastic stirrups using Velcro straps. The stirrups were connected to the extended ergometer lever arms and attached to a drive crank for the electromagnetically braked flywheel. To isolate the work to the quadriceps Velcro strapping was also used to secure participants' hips to the patient bed, minimising contributions from the hip flexors and extensors. Using this ergometer the external resistance is only applied during knee-extension. The only work during knee-flexion is that required to lift the mass of the lower leg. The range of motion is limited by the scanner dimensions to between ~30 degrees flexion and full extension. Participants were familiarised with performing a constant knee-extension frequency of 90 kicks·min^{-1} set using a metronome. This kick frequency also allowed the flywheel speed to be maintained above the minimum operating speed and aligned MR scanner acquisitions with muscle contractions.

Familiarisation. The exercise protocols were completed in two phases – familiarisation and testing. The familiarisation phase took place in a temperature-controlled human physiology laboratory. All exercise protocols began with a period of rest (~1-3 min) and then knee-

192 extension exercise at 5 W (~2-4 min), with each of these phases continued until a steady
193 state was attained.
194
195 Participants first completed a ramp-incremental exercise test (RIT; 3 $W \cdot min^{-1}$) to the limit of
196 tolerance; defined as the point at which the participant was unable to maintain the full range
197 of motion at the target kicking frequency (90 $kicks \cdot min^{-1}$) or when the flywheel speed
198 decreased below the minimum operating speed, despite strong verbal encouragement.
199 Participants were familiarised with the protocol by repeating it until the performance (power
200 and duration) and physiologic responses ($\dot{V}O_2$ etc.) were reproducible between visits
201 (minimum of 3 repeats performed). Once familiarised, the power corresponding to 110 % of
202 RIT peak power was calculated and used in all subsequent exercise protocols. Comparison
203 of $\dot{V}O_{2peak}$ at the limit of RIT and continuous exercise was used to confirm $\dot{V}O_{2max}$ (Poole &
204 Jones, 2017). Continuous and intermittent protocols were also repeated until reproducible
205 physiological responses were obtained (typically requiring 2 repeats).
206
207 *Testing.* The collection of pulmonary gas exchange data for matching to MRS data was
208 performed in the same temperature-controlled human physiology laboratory as the
209 familiarisation phase. Following a period of rest and warm-up at 5 W, for the continuous
210 exercise protocol, power was instantaneously applied at the power equivalent to 110 % of
211 RIT peak, and the participants were required to continue the exercise to the limit of
212 tolerance. Intermittent protocols comprised periods of work at a power equivalent to 110 %
213 of RIT peak, and periods of recovery at 5 W. The three intermittent protocols performed by
214 all participants had work:recovery durations of 16:32 s, 32:64 s and 64:128 s. These
215 durations were chosen to align with MRS data acquisition, there being one ^{31}P spectrum
216 acquired every 8 s. Each intermittent protocol was continued until a total of 576 s of work
217 was accumulated (at a 1:2 work:recovery duty cycle this corresponded to a total duration of
218 28 minutes 48 seconds, allowing 216 complete ^{31}P spectra to be collected), or to the limit of

tolerance, whichever was the shorter. Only one exercise protocol was performed on a given day, with at least 24 hr between visits, and protocols were performed in a random order.

Subsequently, continuous and intermittent exercise protocols were repeated inside the bore of a 3T superconducting magnet for measurement of intramuscular phosphate responses by ^{31}P MRS using the same ergometer and the same exercise protocol as used for pulmonary gas exchange data collection.

Pulmonary gas exchange

Participants breathed through a facemask for measurement of respired gases (Zan 600, Geratherm, Germany). Volume and flow rates were sampled at 125 Hz and measured using a pneumotach; with O_2 and CO_2 gas concentrations measured using electrochemical cell and infrared gas analysers, respectively. Using BlueCherry software, gas concentration and volume signals were time-aligned for online calculation of breath-by-breath pulmonary gas exchange and ventilatory variables.

Prior to each test the flow sensor and gas analysers were calibrated according to the manufacturers' guidelines. The pneumotach was calibrated using a 3 L syringe across a range of flow rates, with the gas analysers calibrated using certified gas mixtures that spanned the expected inspired and expired ranges of both O_2 and CO_2.

^{31}P Magnetic Resonance Spectroscopy

Relative concentrations of intramuscular phosphates (ATP, PCr, Pi) were measured using a 3T superconducting magnet (Magnetom Trio, Siemens AG, Erlangen, DE), and pH_i was calculated from the chemical shift of Pi to PCr (Moon & Richards,1973). A one-pulse ^{31}P MRS acquisition was employed using a dual-tuned (^{1}H, 15 cm diameter; ^{31}P 18 cm diameter) surface RF coil (RAPID Biomedical GmbH, Rimpar, Germany) placed under the knee extensors of the right leg and positioned halfway between the hip and knee. This provided a

metabolic signal from a mid-thigh slice of the *rectus femoris*, *vastus medialis*, *vastus intermedialis* and *vastus lateralis* (Cannon *et al.,* 2014). Once in the correct position, the participants' hips were secured to the scanner bed using non-distensible Velcro straps. Participants were then moved inside the bore of the magnet and the scanning procedure commenced.

Sagittal and coronal gradient-recalled echo images of the thigh were taken to confirm placement of the RF coil in relation to the knee extensors. ^{1}H shimming was performed to optimise magnetic field homogeneity. Subsequently, a fully relaxed high-resolution unsaturated spectrum and 32-scan spectrum (repetition time of 10 s) were obtained, with this used as the reference baseline spectra. Throughout the protocol ^{31}P free induction decays (FIDs) were collected every 2 s, with four FIDs used to provide a spectrum every 8 s. The continuous and intermittent exercise protocols were aligned to ensure that each spectrum did not straddle work-recovery transitions.

Data Analyses

All breath-by-breath $\dot{V}O_2$ responses were filtered to remove any erroneous breaths (defined as those occurring outside the 99 % prediction limits of the local mean) resulting from sighs, coughs or swallowing etc. (Lamarra *et al.,* 1987). For the RIT, lactate threshold (LT) was estimated non-invasively using standard ventilatory and pulmonary gas exchange criteria (Whipp *et al.,* 1986). In both RIT and continuous constant-power exercise $\dot{V}O_{2peak}$ was identified as the greatest 12-breath (~20 s) moving average prior to the limit of tolerance.

For the intermittent responses, breath-by-breath data were linearly interpolated to provide a value every second. $\dot{V}O_2$ data were then phase-aligned to PCr to account for the limb-lung vascular transit delay (Rossiter *et al.,* 1999), and then averaged to provide a datum every 8 s – i.e. to match the intervals of ^{31}P data collection. Intermittent exercise was characterised by an expected transient phase where the amplitude of the work-recovery fluctuations in $\dot{V}O_2$

were climbing (in this study, the first 192 s), and a subsequent periodic steady-state phase where the amplitude of $\dot{V}O_2$ fluctuations stabilised between exercise and recovery phases. For this reason, to analyse the time course of the intramuscular and pulmonary responses to intermittent exercise, the first 192 s were eliminated and the subsequent $\dot{V}O_2$ and phosphate data sorted into time-bins of 384 s each, resulting in a total of 4 repeats (or bins) of intermittent work-recovery phases (Figure 1). Within each time-bin, like transitions were aligned to the onset of work at 110 % of RIT peak power and averaged to increase the signal:noise (Lamarra *et al.,* 1987; Rossiter *et al.,* 2000). The peak, nadir and peak-to-nadir amplitude of fluctuations in each variable were identified within each bin. All data were then normalised to the amplitudes measured during continuous exercise between 5 W (0 %) and peak (100 %).

Kinetic analysis of ^{31}P MRS data

This has been described in detail elsewhere (Cannon *et al*., 2014). Briefly, PCr kinetics were modelled using non-linear least-squares regression (implemented in Excel, Microsoft Office 2016). The rate of ATP turnover was estimated from the contributions of PCr breakdown (D), oxidative phosphorylation (Q) and glycogenolysis (L), which were determined from the PCr, Pi and pH_i data acquired during exercise and recovery, using methods explained in detail elsewhere (Kemp, 2015; Kemp, 2016). To improve the signal:noise ATP turnover was calculated as a mean rate throughout the work phases of the protocols (i.e. mean of the 4 bins).

Estimating ATP turnover using ^{31}P MRS *in vivo* relies on some assumptions, particularly in relation to the estimated contribution of oxidative phosphorylation (Q) (discussed in detail elsewhere; Kemp, 2015; Kemp, 2016). However, sensitivity analysis suggests that none of the calculations used depended substantially on any particular assumption. Using initial PCr breakdown rate (D) as a measure of initial ATP turnover, and initial recovery PCr resynthesis as a measure of end-exercise supra-basal oxidative ATP synthesis rate (Q) depends on only

303 the most general of assumptions about closed-loop feedback control of oxidative ATP
304 synthesis; the use of the relationship between Q and [ADP] established by analysis of
305 recovery kinetics to 'predict' Q during exercise assumes only one of several possible modes
306 of mitochondrial feedback control (Kemp, 2015), which each provide very similar results
307 during exercise of this kind. Finally, the calculated contribution of glycolytic ATP production
308 is small in the present study, and depends on uncontroversial models of cellular pH
309 buffering, and assumptions of approximately linear pH-dependence of acid efflux to which
310 the detailed results are rather insensitive (Kemp, 2015; Kemp, 2016).
311
312 **Statistics**
313 Metabolic perturbations (peak, nadir and peak-to-nadir amplitude) were initially compared
314 among the four time-bins using a one-way repeated measured ANOVA, to investigate the
315 effect of time on metabolic disturbances. Subsequently, peak continuous exercise values,
316 and final time-bin values for all intermittent protocols were compared using a one-way
317 repeated measures ANOVA to investigate the effect of exercise protocol (continuous and 3
318 intermittent protocols) on metabolic disturbances. Finally a two-way repeated measured
319 ANOVA was used to compare the relative amplitude of change ($\dot{V}O_2$ vs. PCr), and
320 investigate how this changed between intermittent protocols (16:32 vs. 32:64 vs. 64:128 s).
321 *Post hoc* Tukey-corrected pairwise comparisons were performed where appropriate.
322 Statistical significance was set at $p < 0.05$. All values are reported as mean ± SD.
323
324 **Results**
325 *Ramp incremental responses*
326 The estimated LT was 1.46 ± 0.26 $L \cdot min^{-1}$ (72 ± 2 % $\dot{V}O_{2peak}$), with the tolerable limit attained
327 at a $\dot{V}O_{2peak}$ of 2.04 ± 0.36 $L \cdot min^{-1}$ and peak power of 34 ± 7 W.
328
329 *ATP turnover and exercise tolerance during continuous and intermittent exercise*

Continuous constant-power exercise at 110 % RIT peak power (38 ± 7 W) was sustained for 252 ± 174 s, and $\dot{V}O_{2peak}$ at the limit of tolerance (2.03 ± 0.26 $L \cdot min^{-1}$) was not different from RIT $\dot{V}O_{2peak}$, confirming $\dot{V}O_{2max}$ (p = 0.891). The mean rate of ATP turnover during continuous exercise performed to the limit of tolerance was 44.7 ± 18.4 $mM \cdot min^{-1}$, with large contributions from anaerobic glycolysis (L; 33 ± 19 %) and oxidative phosphorylation (Q; 50 ± 23 %) compared with and PCr breakdown (D; 17 ± 6 %) (Table 1 and Figure 2). At intolerance in continuous exercise PCr declined to 38 ± 13 % of baseline and pH_i reached a nadir of 6.67± 0.07 (cf. 7.07 ± 0.04 at rest).

In all intermittent protocols the 576 s target of work at 110 % RIT peak power was accumulated. This equated to 327 ± 180 % more work done during intermittent exercise than with continuous exercise at the same power. Mean ATP turnover was not different among continuous and the work phases of intermittent exercise protocols (p > 0.05; Table 1). Following removal of the initial kinetic phase (first 192 s), the 4 binned-repeats of the work-recovery phases of intermittent exercise did not differ (p > 0.05) within the 16:32 s or 32:64 s intermittent protocols. In other words, the $\dot{V}O_2$, PCr and pH_i fluctuation peak, fluctuation nadir and fluctuation amplitude were constant following the removal of the initial 192 s kinetic phase (Figure 3). However, for the 64:128 s intermittent protocol peak metabolic disturbance (PCr; p < 0.05) and fluctuation amplitude ($\dot{V}O_2$; p < 0.05) increased between time-bins 1 and 4. For these reasons, the $\dot{V}O_2$, PCr and pH_i peak values used for all subsequent analyses were those from the final bin of intermittent exercise in all protocols (i.e. the values measured in the 4^{th} time-bin of Figure 1).

Absolute bioenergetic and pulmonary responses during continuous and intermittent exercise

Comparing within variables across the four different exercise protocols, the absolute $\dot{V}O_2$ increase, PCr breakdown and pH_i fall were less during short work:recovery intermittent exercise versus long work:recovery duration exercise (p < 0.05; Table 2, Figure 3). The peak values of the disturbance in $\dot{V}O_2$, PCr and pH_i during the 16:32 s intermittent protocol did not

358 reach those seen during continuous exercise (p < 0.05). Similarly, the peak values of the
359 disturbance in $\dot{V}O_2$ and pH_i during the 32:64 s intermittent protocol were less than those
360 during continuous (p < 0.05), although peak PCr was not different (p = 0.07). However, the
361 absolute peak value of the disturbance of $\dot{V}O_2$ (p = 0.06), PCr (p = 0.72) and pH_i (p = 0.08)
362 during 64:128 s intermittent exercise were not different to those at the limit of tolerance in
363 continuous exercise (Table 2, Figure 3).
364
365 *Relative fluctuations in intramuscular bioenergetics and pulmonary $\dot{V}O_2$ during intermittent*
366 *compared with continuous exercise*
367 In order to compare the relative excursion between intramuscular and pulmonary variables,
368 responses were normalised between 5 W baseline and peak values of continuous exercise.
369 Comparing between $\dot{V}O_2$ and PCr during intermittent exercise, the relative peak to nadir
370 amplitude of $\dot{V}O_2$ and PCr fluctuations increased with work bout duration (p < 0.05; Table 3),
371 with a strong inverse relationship between PCr breakdown and $\dot{V}O_2$ (r^2 = 0.88; p < 0.05).
372 However, the amplitude of the $\dot{V}O_2$ fluctuation was less than that of PCr for 16:32 and 32:64
373 s protocols (p < 0.05; Table 3; Figure 3). The relative contribution of PCr breakdown to
374 intramuscular ATP production was greatest during the short intermittent cycles (16:32 and
375 32:64 s). At the longer cycles (64:128 s and continuous) the contributions from oxidative
376 phosphorylation (Q) and anaerobic glycolysis (L) were at their greatest (p < 0.05; Figure 2).
377
378 **Discussion**
379 The major finding of this study was that the metabolic strain of exercise ($\dot{V}O_2$, intramuscular
380 PCr breakdown, pH_i) is dissociated from the external power and cellular demand for ATP
381 production by performing the exercise intermittently. While continuous constant-power
382 exercise at 110 % peak RIT power could only be sustained for ~ 4 minutes, our findings are
383 consistent with previous reports that exercise tolerance was increased by at least 3-fold, and
384 a greater volume of work accumulated, when the same power is performed intermittently
385 (Astrand *et al.* 1960; Margaria *et al.* 1969; Turner *et al.* 2006; Chidnok *et al.* 2013; Skiba *et*

386 *al.* 2014). We found that mean ATP turnover during the work phases were not different for
387 both continuous and intermittent exercise at the same external power (Table 1), such that
388 alterations in work efficiency could not explain the differences in tolerance. Nevertheless, the
389 magnitude of intramuscular metabolic fluctuations was attenuated during intermittent
390 exercise. This dissociation was greatest when the work:recovery durations were shorter
391 (Figure 3), despite the work:recovery duty cycle (1:2) and power output remaining constant
392 for all intermittent protocols.
393
394 These data support our hypotheses that ATP synthesis rates would be similar in intermittent
395 and continuous exercise at the same external power (110 % peak RIT power; hypothesis 1),
396 despite pulmonary $\dot{V}O_2$ fluctuations being lower in intermittent exercise (hypothesis 2). We
397 also found, contrary to some suggestions (Rossiter *et al.* 2002; Krustrup *et al.* 2003; Cannon
398 *et al.* 2014), that the small fluctuations in pulmonary $\dot{V}O_2$ during the shorter vs. longer
399 work:recovery durations, were not mirrored in the intramuscular responses. As intermittent
400 work interval duration increased towards matching the continuous protocol, the mean ATP
401 production relied increasingly upon anaerobic glycolysis and oxidative phosphorylation and
402 less upon PCr breakdown (hypothesis 3). On the other hand, during short work:recovery
403 intermittent exercise, the relative amplitude of the $\dot{V}O_2$ fluctuations were damped compared
404 to those of intramuscular PCr (hypothesis 4): The ratio between relative amplitudes of $\dot{V}O_2$
405 and PCr fluctuations were 53 % during 16:32 s, 69 % during 32:64 s, rising to 90 % during
406 64:128 s (Figure 3; Table 3). This is consistent with proportionally greater contributions to
407 the ATP turnover from PCr hydrolysis and suggests proportionally greater stored O_2 usage
408 during short work:recovery intermittent exercise than longer work:recovery intermittent
409 exercise or continuous constant-power exercise (Figure 2; cf. Turner *et al.* 2006). It also
410 suggests that the capacitance of the intervening energy and O_2 stores has a significant
411 impact in damping the external (pulmonary) respiratory responses to intermittent exercise
412 relative to the internal (intramuscular) bioenergetics.
413

414 *Intermittent exercise tolerance*

415 At the onset of continuous exercise, the ability of intramuscular oxidative phosphorylation to
416 meet the cellular ATP requirement is dependent on its kinetics, with any shortfall
417 compensated for by substrate-level phosphorylation (O_2 deficit). This non-oxidative ATP
418 supply is capacity-limited, and propagates a 'fatigue cascade' (Murgatroyd & Wylde, 2011).
419 This cascade leads to the accumulation of fatigue-related metabolites, exercise inefficiency
420 (reflected in the $\dot{V}O_{2SC}$), intramuscular PCr depletion and, ultimately, exercise intolerance
421 (Jones *et al.* 2008; Vanhatalo *et al.* 2010). Consequently, the rate at which intramuscular
422 oxidative phosphorylation responds to alterations in ATP demand ($\dot{V}O_2$ kinetics) is a key
423 determinant of high-intensity exercise tolerance (Whipp & Ward, 1992; Jones & Burnley,
424 2009; Murgatroyd *et al.* 2011). Mean ATP turnover was not different between protocols
425 (Table 1), and therefore the initial rate of $\dot{V}O_2$ change was the same at the onset of both
426 continuous and intermittent exercise regardless of work:recovery duration (DiMenna *et al.*
427 2010). Consequently, the amplitude of the intramuscular $\dot{V}O_2$ fluctuation, and requirement for
428 substrate-level phosphorylation, was determined by the intermittent work duration. While
429 shortening the intermittent duration resulted in a relatively greater proportional contribution
430 by PCr breakdown to overall ATP synthesis, it also resulted in increased system stability and
431 exercise tolerance. That is, $\dot{V}O_2$, PCr and pH_i fluctuations were small and there was no
432 measurable cellular contribution to the exercise task from anaerobic glycolysis. Indeed, the
433 $\dot{V}O_2$ fluctuations during the shortest intermittent protocol remained below the estimated
434 lactate threshold throughout. This cellular bioenergetics response is consistent with the
435 observations that exercise was better sustained, and more work done, during intermittent
436 compared with continuous exercise.

437

438 *Damping of pulmonary respiration by cellular bioenergetics*

439 During short work:recovery intermittent exercise the peak fluctuation in $\dot{V}O_2$ vs. PCr (17.0 ±
440 6.9 vs. 32.1 ± 20.6 %) suggests that the relative intramuscular metabolic strain is greater
441 than that extrapolated from the $\dot{V}O_2$ measured at the mouth. The dissociation between

muscle $\dot{V}O_2$ (inferred from PCr) and pulmonary $\dot{V}O_2$ (measured) during short work bouts is likely due to rapid transients in intramuscular and venous O_2 storage. The ~10 s delay after the onset of high-intensity exercise in the appearance of deoxygenated myoglobin (Richardson *et al.* 2015) suggests that venous haemoglobin deoxygenation (Turner *et al.* 2006) bears the brunt of this damping process (cf. Astrand *et al.* 1960), and may result in a narrowing of the capillary-to-myocyte PO_2 driving pressure. This finding is also consistent with slow activation of muscle oxidative phosphorylation at exercise onset (e.g. Korzeneiski & Rossiter, 2015). Given that the $\dot{V}O_2$ in this study was measured at the mouth without use of an algorithm to estimate alveolar gas exchange, there is also the potential for a contribution from changes in pulmonary O_2 stores (Beaver *et al.* 1981; Aliverti *et al.* 2004; Wüst *et al.* 2008). While the degree of this effect is unknown, any changes in end-expiratory lung volume are anticipated to be small during this prone exercise task.

Dissociating exercise intensity from power output

The phrases 'exercise intensity' and (relative) 'power output' are commonly used interchangeably. The finding that intensity and power output can be completely dissociated depending on the work:recovery duration highlights the importance of providing these two terms with distinct definitions. The dissociation here occurred to the degree that a severe intensity exercise bout (where $\dot{V}O_2$ exceeded critical power) could be reduced to moderate intensity (where $\dot{V}O_2$ remained below the lactate threshold) through shortening the duration of work intervals, despite the power output and total work done remaining constant. Thus, the term power output refers to a rate of energy transfer from the skeletal muscle to perform external work (mechanical power), while the intensity that a given power output engenders depends on the peak magnitude of the metabolic fluctuation(s) evoked during the task. By shortening the work:recovery durations, intensity (including the requirement for anaerobic glycolysis to contribute to the ATP turnover) is minimised and exercise better sustained.

In our study the fluctuation in the $\dot{V}O_2$ response to intermittent exercise was considerably damped compared to intramuscular PCr. Nevertheless, in the short-duration intermittent protocol (16:32 s), where the magnitude of this effect was greatest, there remained a large dissociation between the external power and the intramuscular metabolic strain. This was achieved by terminating the work bout before intramuscular PCr substantially decreased, and allowing PCr to increase during the intervening recovery interval. During the shortest work:recovery duration of intermittent exercise we found that the peak and nadir of the $\dot{V}O_2$ and PCr fluctuations remained below values associated with the lactate threshold and there were no net contributions from anaerobic glycolysis to meet the cellular demands for ATP turnover, despite power exceeding that achieved at $\dot{V}O_{2max}$ in the RIT. This bioenergetics behaviour is consistent with responses observed during continuous exercise at far lower powers that are termed moderate intensity (Wasserman *et al.* 1967; Rossiter *et al.* 2002). The accumulation of lactate and the associated intramuscular acidosis occurs relatively slowly after exercise onset, e.g. glycolysis itself is not activated for ~10-15 s after exercise onset (Conley *et al.* 1998; Walsh *et al.* 2008). However, any delayed activation of glycolytic flux is unlikely to be a major contributor to the relative preservation of muscle pH_i and lack of muscle acidification in this protocol because the 16 s exercise bout was repeated many times over the ~30 minute protocol; which would certainly be sufficient to identify any activation of glycolytic flux. The strong probability is that any cytosolic redox challenge consequent to increased glycolytic flux was met either by intramitochondrial transport of accumulated pyruvate (effectively reversing any lactate formation during the work bout), or of $NADH^+$, during the recovery phases of the intermittent bouts. Because sustained energy provision was not required, the very short work bouts and interspersed recovery intervals allowed aerobic energy provision to remain below the lactate threshold and the substrate-level contributions to the exercise energetics in short intermittent work bouts appear to be essentially limited to PCr breakdown (Figure 2).

We also observed (Figure 3) that during the work phases of intermittent exercise pH_i increases while PCr is falling (as H^+ is sequestered in the Lohmann reaction: ADP + PCr + H+ ⟷ ATP + Cr). This means that during short intermittent bouts, the lowest pH_i occurs during recovery where PCr is greatest and the muscle is alkalotic during the work phase when PCr is lowest. This is unlike during longer duration intermittent bouts (64:128 s) or continuous exercise where PCr and pH_i are both low during the muscular activity. Whether this alkalinising effect during short intermittent exercise is protective of muscle fatigue is currently unclear, but clearly the lesser magnitude of PCr breakdown (and Pi accumulation) is associated with increased exercise tolerance and a prolongation of work capacity. Furthermore, the influence of this effect on the cellular transduction of training responses is currently unknown (see *Implications* below).

Extending the work:recovery durations predictably increased the intramuscular metabolic strain. In the 32:64 s protocol, the peak $\dot{V}O_2$ fluctuation (1.54 ± 0.36 $L \cdot min^{-1}$) exceeded the estimated lactate threshold (1.46 ± 0.26 $L \cdot min^{-1}$), which was associated with a cellular acidosis (pH_i; 6.84 ± 0.12), and an increased contribution from anaerobic glycolysis to ATP turnover. These features are consistent with heavy-intensity exercise (where metabolic power production is between the lactate threshold and critical power). The sustained decrease in pH_i in the 32:64 s protocol demonstrates that the O_2 deficit accumulated during the work phase to the extent that anaerobic glycolysis became a necessary contributor to the energy transfer (Figure 2). The magnitudes of the intramuscular energetic strain and acidosis are consistent with those in continuous exercise at a power just below critical power (estimated to be ~60-80 % peak aerobic power during cycle ergometry; Wasserman *et al.* 1967; Rossiter *et al.* 2002; Jones *et al.* 2008). Again, the peak intramuscular acidosis occurred during recovery, rather than during the work phase of the intermittent exercise. Our data emphasise that it is not the mean metabolic response during intermittent exercise, but rather the peak of the metabolic perturbation that is likely important in determining the intramuscular metabolic strain: The mean $\dot{V}O_2$ during the 32:64 s intermittent protocol was

524 below the lactate threshold (1.18 ± 0.17 vs. 1.46 ± 0.26 $L \cdot min^{-1}$), which reflects an average
525 of the entire work:recovery cycle.
526
527 We would expect the sustained metabolic acidosis during the 32:64 s intermittent protocol to
528 be associated with a slow component in both $\dot{V}O_2$ and PCr. However, there was no
529 progressive increase in $\dot{V}O_2$ and decrease in PCr between time bins during either 16:32 s or
530 32:64 s protocols. This, together with a mean ATP turnover rate among protocols that was
531 not different, suggests that there was no change in either the efficiency of force production
532 (P:W) or mitochondrial efficiency (P:O) during the acidifying heavy-intensity intermittent
533 protocol. This has implications for work efficiency and the mechanisms contributing to the
534 $\dot{V}O_{2sc}$. Work efficiency is typically assumed constant during the early transient (e.g. first 60 s)
535 of either sub- or supra-LT exercise. However, findings in stimulated dog muscle (Wust *et al*.,
536 2011) and in some human studies (Bangsbo *et al*., 2001; Koppo *et al*., 2004) suggest that
537 work efficiency may be initially high and rapidly decline over the first ~15-30 s of contraction
538 before rebounding and levelling out after ~1-2 min. For exercise above LT, a second decline
539 in work efficiency is observed after ~2 min as the $\dot{V}O_{2sc}$ develops. Our data that ATP
540 turnover appeared greater at 16:32 s compared with 32:64 s (albeit non-significant) may
541 reflect some effect of rapid changes in work efficiency in the very early transient.
542 Subsequently, for the longer intermittent and the continuous protocol, work inefficiencies
543 associated with the $\dot{V}O_{2sc}$ became increasingly evident. We speculate that, as the peak of
544 the metabolic fluctuation in the 32:64 s protocol only exceeded the LT for a few seconds (~8
545 s, on average) at the end of each work phase, the intervening recovery was sufficient to
546 constrain any transient fatiguing processes that contribute to the $\dot{V}O_{2sc}$. Without the
547 accumulation of muscle fatigue, the drive for progressive work inefficiency in the form of a
548 $\dot{V}O_2$ or PCr slow component was absent (Cannon *et al.* 2011; Grassi *et al.* 2015; Keir *et al.*
549 2016). While prolonging the work:recovery duration increased the magnitude of metabolic
550 perturbations and exercise intensity above that seen during 16:32 s, there was still a clear

551 dissociation between the external mechanical power and the exercise intensity
552 (intramuscular metabolic strain).
553
554 During exercise with the longest work:recovery (64:128 s) protocol there was an increase in
555 the intramuscular strain (Figure 2, 3). The peak intramuscular responses during the 64:128 s
556 intermittent protocol were consistent with those during continuous exercise above critical
557 power (Jones *et al.* 2008). A progressive reduction in work efficiency was present, with the
558 $\dot{V}O_2$ and PCr fluctuations in the final work phases (bin 4; Figure 1, 3) exceeding those of the
559 first work phase (bin 1; $p < 0.05$). Despite this, we did not observe this effect in the ATP
560 turnover rate during the 64:128 s intermittent protocol. This may be influenced by the
561 necessity to calculate ATP turnover as the mean rate of the work phases to increase
562 signal:noise, which also reduced the ability to detect an inefficiency by this method. The
563 reduction in work efficiency (as reflected in the $\dot{V}O_2$ and PCr responses; Figure 3) is likely to
564 be consequent to an increase in the ATP requirement to maintain power production (Cannon
565 *et al.* 2014). While the mechanism(s) responsible for a progressive reduction in work
566 efficiency during the $\dot{V}O_{2sc}$ remain controversial, the prevailing suggestion during voluntary
567 exercise is that progressive recruitment of motor units innervating low oxidative and/or type
568 II muscle fibres may be responsible (Pringle *et al*., 2003; Krustrup *et al*., 2004). Although a
569 reduction in the mitochondrial P:O has yet to be completely ruled out (Cannon *et al.* 2014),
570 this seems unlikely (Korzeneiski & Rossiter, 2015). In the 64:128 s protocol the contribution
571 of cellular anaerobic glycolysis to ATP production became increasingly evident (Figure 2),
572 and pH_i fell during the exercise (unlike in the shorter intermittent protocols). This fall in pH_i
573 during the work phase is consequent to a metabolic acidosis and associated lactate
574 accumulation, and appeared to become more pronounced as the ~30 minute intermittent
575 exercise progressed. Although this long duration intermittent protocol led to a more extreme
576 cellular energetic strain, the intervening recovery bouts damped the magnitude of cellular
577 energetic swings, thus prolonging exercise tolerance and increasing the volume of work
578 accumulated (compared with continuous exercise at the same power output).

579 *Implications*

580 Although the 64:128 s protocol was sustainable for the target duration and total accumulated
581 work, the intramuscular and systemic metabolic responses suggest that participants were
582 close to intolerance by the end of this protocol: peak $\dot{V}O_2$ and PCr response were not
583 different from continuous exercise (Table 2, Figure 3). This greatly contrasts the 16:32 and
584 32:64 s intermittent protocols, where systemic and intramuscular response were of moderate
585 and heavy intensity respectively, and exercise could likely be sustained far beyond the ~30
586 minute protocol. This was despite accumulating the same amount of total work, in the same
587 amount of time, in all three intermittent protocols. Thus, during shorter duration
588 work:recovery bouts the internal and external bioenergetic homeostasis was better
589 maintained, and intensity reduced, during work- and duration-matched exercise.

590

591 The dissociation between power output and bioenergetic function may have important
592 implications for understanding the variability in the physiologic adaptations to intermittent
593 exercise, or for tailoring intermittent exercise training protocols to target specific
594 physiological adaptations. While intermittent exercise can be superior to traditional
595 continuous moderate-intensity exercise for increasing whole-body $\dot{V}O_{2max}$, muscle oxidative
596 capacity, angiogenesis or stroke volume (e.g. Kemi *et al.* 2005; Helgerud *et al.* 2007; Wisløff
597 *et al.* 2007; MacInnis *et al.* 2017), other studies find no difference between the training
598 interventions (e.g. Gibala *et al.* 2006; Burgomaster *et al.* 2008; Bartlett *et al.* 2012; Ellingsen
599 *et al.* 2017). In instances of no difference between training approaches, the specific power
600 and intermittent duration of the protocols used may not optimise the intramuscular energetic
601 response to promote remodelling (assuming intramuscular biogenic adaptations are a goal
602 of the training). Our data emphasise that, for example, intermittent exercise at 60 % of peak
603 aerobic power with a 60:60 work:recovery duration is likely to induce a greater intramuscular
604 bioenergetics homeostatic challenge than a protocol using 110 % of peak aerobic power and
605 a 15:15 s work:recovery intermittent protocol (cf. Gayda *et al.* 2012).

606

607 Given the protocol dependence of the dissociation between the external power and
608 intramuscular metabolic strain, intermittent exercise allows a greater mechanical power to be
609 achieved during training interventions than would otherwise be possible during continuous
610 exercise. This dissociation also ameliorates the ventilatory demands and perceived exertion
611 from the metabolic requirement of this mechanical power that would otherwise be associated
612 with high-intensity exercise. Given the mechanical load on the skeletal muscle is, in and of
613 itself, an important signal for driving skeletal muscle adaptation in the absence of a
614 metabolic challenge (Hellsten *et al.* 2008; Høier *et al.* 2010) our data have implications for
615 the optimisation of rehabilitation in clinical populations. For example, a high relative power
616 with short work:recovery durations would provide a high mechanical strain without the
617 associated metabolic response. This allows for a functional improvement by overcoming
618 pathological pulmonary or cardiovascular system limitations that would normally limit the
619 external power output that could be achieved during training. Conversely, the relative
620 importance of metabolic signalling (e.g. by AMPK) in driving beneficial muscular adaptations
621 means that the stimulus during short work bouts may not be sufficient to optimise the
622 training stimulus. Thus, our findings of dissociating muscle metabolic responses from
623 mechanical power require further systematic investigation in relation to intermittent exercise
624 training protocols.
625
626 *Conclusion*
627 Performing dynamic knee-extensor exercise at the same high-intensity power intermittently
628 reduces the O_2 cost and the intramuscular metabolic strain of performing the same power
629 during continuous exercise. Mean intramuscular ATP production rates are not different in
630 intermittent and continuous exercise at the same power output. Despite this, pulmonary $\dot{V}O_2$
631 increases less during short intermittent exercise (work:recovery 16:32 s), than during longer
632 intermittent exercise (32:64 s or 64:128 s), and PCr contributes relatively more to ATP
633 production during short vs. longer intermittent or continuous exercise. The latter suggests
634 proportionally greater stored O_2 usage during short work:recovery intermittent exercise than

longer intermittent or continuous exercise. In addition, as intermittent exercise work bout duration increases towards becoming continuous, relative ATP production relies increasingly upon anaerobic glycolysis and oxidative phosphorylation and less upon PCr breakdown. Our data are also consistent with $\dot{V}O_2$ kinetics being an important determinant of exercise tolerance, through the rate of O_2 deficit accumulation; even during intermittent exercise. The extent we could dissociate power output and exercise intensity was greatest at the shortest work:recovery durations and was observable within the intramuscular bioenergetics.

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Additional information

Competing interests

None declared.

Author contributions

MD, CF conceived the study, and all authors contributed to the design of the study. MD, GJK CF collected the data. MD, GJK, CF analysed the data, all authors contributed to the interpretation of the data. MD, CF prepared the first draft of the manuscript. All authors critically reviewed and approved the final version of the manuscript, and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Tables

Table 1. Mean ATP turnover, and contributions from phosphocreatine breakdown (D), oxidative phosphorylation (Q), and anaerobic glycolysis (L) during continuous and intermittent bilateral knee-extension exercise. Continuous exercise was performed to the limit of tolerance (252 ± 174 s). Intermittent exercise was performed with work:recovery durations of 16:32 s, 32:64 s and 64:128 s, each for a total duration of 28 minutes 48 seconds.

Protocol	ATP $mM \cdot min^{-1}$	D $mM \cdot min^{-1}$	Q $mM \cdot min^{-1}$	L $mM \cdot min^{-1}$
Continuous	44.7 ± 18.4	8.3 ± 5.7$^{(2,3)}$	19.9 ± 8.8$^{(2,3)}$	16.4 ± 14.4$^{(2,3)}$
16:32	45.0 ± 19.5	34.4 ± 15.7$^{(1,4)}$	10.5 ± 4.8$^{(1,4)}$	0.1 ± 0.0$^{(1)}$
32:64	34.8 ± 8.8	25.3 ± 6.1$^{(1)}$	8.5 ± 2.5$^{(1,4)}$	1.0 ± 1.7$^{(1)}$
64:128	49.1 ± 17.5	17.5 ± 6.1$^{(2)}$	21.4 ± 9.8$^{(2,3)}$	10.2 ± 4.3

Values are presented as mean ± SD. $^{(1)}p < 0.05$ vs. continuous; $^{(2)}p < 0.05$ vs. 16:32 s intermittent exercise; $^{(3)}p < 0.05$ vs. 32:64 s intermittent exercise; $^{(4)}p < 0.05$ vs. 64:128 s intermittent exercise.

Table 2. Absolute and relative [normalised between 5 W baseline (0 %) and the limit of tolerance during continuous exercise (100 %)] peak metabolic responses during continuous and intermittent exercise at 110 % of ramp incremental peak power. Continuous exercise was performed to the limit of tolerance (252 ± 174 s). Intermittent exercise was performed with work:recovery durations of 16:32 s, 32:64 s and 64:128 s, each for a total duration of 28 minutes 48 seconds.

		Continuous exercise	Intermittent exercise		
			16:32	32:64	64:128
$\dot{V}O_2$	$L \cdot min^{-1}$	2.03 ± 0.26	1.28 ± 0.24$^{(1, 3, 4)}$	1.54 ± 0.36$^{(1, 2, 4)}$	1.80 ± 0.31$^{(2, 3)}$
	% Continuous	100 ± 0	45.1 ± 7.0$^{(4)}$	63.7 ± 14.8$^{(4)}$	83.6 ± 13.1$^{(2, 3)}$
PCr	% Baseline	38.2 ± 13.0	73.1 ± 16.2$^{(1,4)}$	55.8 ± 16.5	45.0 ± 15.8
	% Continuous	0 ± 0	54.4 ± 27.2$^{(3, 4)}$	29.9 ± 21.7$^{(2)}$	9.6 ± 25.3$^{(2)}$
pH_i		6.67 ± 0.07	6.92 ± 0.07$^{(1, 4)}$	6.84 ± 0.12$^{(1)}$	6.77 ± 0.12$^{(2)}$
	% Continuous	100 ± 0	38.4 ± 11.3$^{(4)}$	60.0 ± 23.8	77.5 ± 31.2

Values are presented as mean ± SD. $^{(1)}p < 0.05$ vs. continuous; $^{(2)}p < 0.05$ vs. 16:32 s intermittent exercise; $^{(3)}p < 0.05$ vs. 32:64 s intermittent exercise; $^{(4)}p < 0.05$ vs. 64:128 s intermittent exercise.

Table 3. The relative amplitudes of $\dot{V}O_2$, PCr and pH_i fluctuations during intermittent bilateral knee-extension exercise compared with continuous exercise. Values are normalised between 5 W baseline (0 %) and the limit of tolerance during continuous exercise (100 %). Power is 110 % of ramp incremental peak power. Continuous exercise was performed to the limit of tolerance (252 ± 174 s). Intermittent exercise was performed with work:recovery durations of 16:32 s, 32:64 s and 64:128 s, each for a total duration of 28 minutes 48 seconds.

work:recovery duration	$\dot{V}O_2$ (%)	PCr (%)	pH_i (%)
16:32	17.0 ± 6.9	32.1 ± 20.6$^{(1)}$	21.3 ± 7.7
32:64	41.3 ± 15.0$^{(a)}$	60.2 ± 12.5$^{(1; a)}$	48.3 ± 23.9$^{(a)}$
64:128	77.2 ± 18.1$^{(a, b)}$	85.7 ± 21.3$^{(a, b)}$	74.4 ± 30.3$^{(a, b)}$

Values are presented as mean ± SD. $^{(1)}p < 0.05$ between $\dot{V}O_2$ and PCr in the same exercise protocol. Within variables (i.e. within $\dot{V}O_2$, PCr or pH_i): $^{(a)}p < 0.05$ from the 16:32 s intermittent protocol; $^{(b)}$ $p < 0.05$ vs. both 16:32 and 32:64 s intermittent protocols.

903 **Figures**

904 **Figure 1.** Schematic of the intermittent exercise protocols and time-bins used for $\dot{V}O_2$ and
905 ^{31}P MRS measures. Following a warm-up at 5W, intermittent exercise with work phases
906 performed at 110 % of ramp-incremental peak power was initiated with work:recovery
907 durations of either 16:32 s (top), 32:64 s (middle) or 64:128 s (bottom). The first 192 s of
908 each test was eliminated (grey box) to exclude a kinetic transient phase that preceded the
909 stabilisation of $\dot{V}O_2$ and ^{31}P MRS fluctuations, with like transitions in each time-bin time-
910 aligned to exercise onset and data averaged to improve signal:noise.

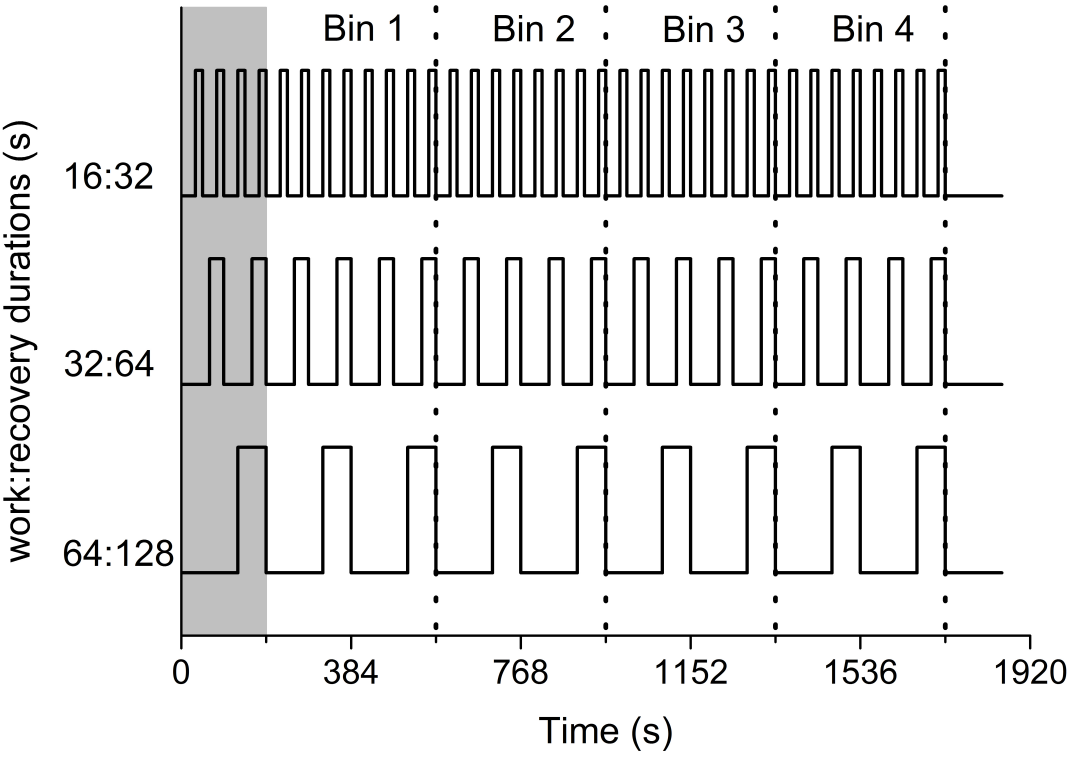

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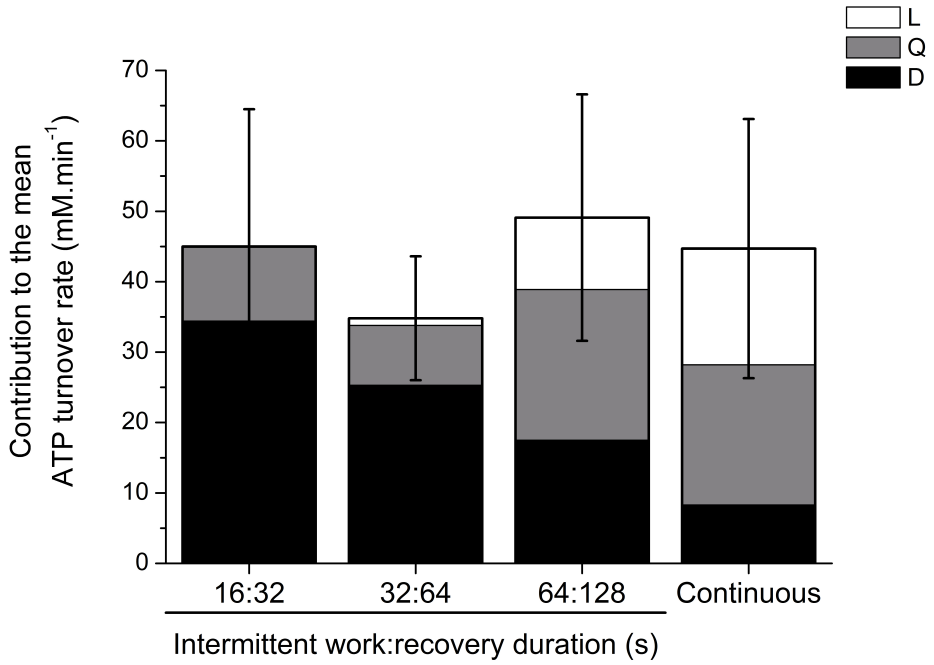
Figure 2. Contributions from phosphocreatine breakdown (D), oxidative phosphorylation (Q), and anaerobic glycolysis (L) to the mean ATP turnover rate at 110 % of ramp-incremental peak power during continuous and intermittent exercise comprising work:recovery durations of 16:32 s, 32:64 s and 64:128 s. Upper: Absolute energetic system contributions to mean ATP turnover. Lower: Relative energetic system contributions to mean ATP turnover.



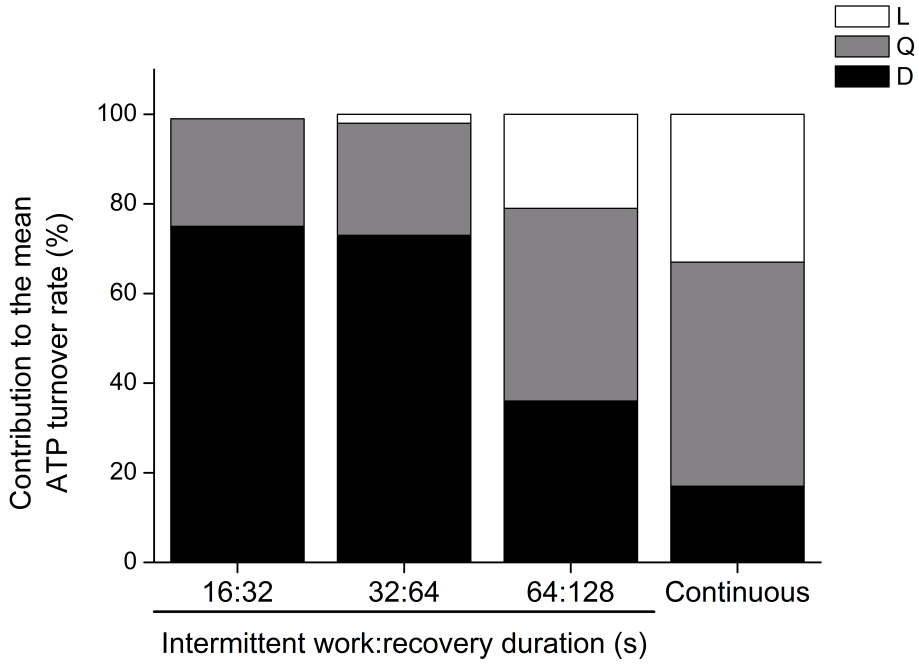


Figure 3. $\dot{V}O_2$, PCr (top row) and pH_i (bottom row) responses to work:recovery durations of 16:32 s (first column), 32:64 s (second column), 64:128 s (third column) or continuous exercise (forth column). Also displayed is the lactate threshold (LT) from the ramp-incremental exercise test (dotted line), and the $\dot{V}O_{2max}$ (top row, dashed line) and pH_i (bottom row, dashed line) attained at the limit of tolerance of the continuous exercise protocol. Grey areas indicate the exercise period performed at 110 % of ramp incremental peak power. Note in the 16:32 s protocol that $\dot{V}O_2$ never exceeds the LT, and there are only minor changes in pH_i, consistent with the 16:32 s intermittent protocol being moderate-intensity. The peak $\dot{V}O_2$ amplitude exceeds the LT in 32:64 and 64:128 s intermittent protocols and during continuous exercise, with this accompanied by a metabolic acidosis (decline in pH_i), consistent with a greater exercise metabolic strain in these protocols.