Muscle phosphocreatine kinetics in children and adults at the onset and offset of moderate-intensity exercise

Alan R. Barker, Joanne R. Welsman, Jonathan Fulford, Deborah Welford, and Neil Armstrong


The splitting of muscle phosphocreatine (PCr) plays an integral role in the regulation of muscle O2 utilization during a “step” change in metabolic rate. This study tested the hypothesis that the kinetics of muscle PCr would be faster in children compared with adults both at the onset and offset of moderate-intensity exercise, in concert with the previous demonstration of faster phase II pulmonary O2 uptake kinetics in children. Eighteen peri-pubertal children (8 boys, 10 girls) and 24 adults (8 men, 8 women) completed repeated constant work-rate exercise transitions corresponding to 80% of the P/PCr intracellular threshold. The changes in quadriceps [PCr], [P], [ADP], and pH were determined every 6 s using 31P-magnetic resonance spectroscopy. No significant (P > 0.05) age- or sex-related differences were found in the PCr kinetic time constant at the onset (boys, 21 ± 4 s; girls, 24 ± 5 s; men, 26 ± 9 s; women, 24 ± 7 s) or offset (boys, 26 ± 5 s; girls, 29 ± 7 s; men, 23 ± 9 s; women 29 ± 7 s) of exercise. Likewise, the estimated theoretical maximal rate of oxidative phosphorylation (Qmax) was independent of age and sex (boys, 1.39 ± 0.20 mM/s; girls, 1.32 ± 0.32 mM/s; men, 2.36 ± 1.18 mM/s; women, 1.51 ± 0.53 mM/s). These results are consistent with the notion that the putative phosphate-linked regulation of muscle O2 utilization is fully mature in peri-pubertal children, which may be attributable to a comparable capacity for mitochondrial oxidative phosphorylation in child and adult muscle.

31P-magnetic resonance spectroscopy; O2 uptake kinetics; oxidative capacity; maturation

FOLLOWING A “STEP” TRANSITION to a higher metabolic rate either below or above the ventilatory threshold (moderate and heavy exercise, respectively), early-pubertal children display a faster rise in phase II pulmonary O2 uptake (pVO2), which provides a close reflection of the rise in muscle O2 utilization (mVO2). Refs. 4, 26), compared with older children or adults (19, 22, 66). The more rapid adjustment of pVO2 to a new steady state reduces the magnitude of the incurred O2 deficit in children, which reflects a reduced contribution of non-oxidative energy equivalents, i.e., phosphocreatine (PCr) breakdown and anaerobic glycolysis, to the total energy turnover during exercise. Furthermore, pubertal boys display faster phase II pVO2 kinetics than girls during heavy- (21) but not moderate-intensity cycling exercise (22). However, the mechanism(s) explaining these age- and sex-related differences in the pVO2 kinetic response are largely unknown.

Although the determinants of the rise in mVO2 at the onset of exercise are the subject of considerable debate (59), the evidence suggests that factors relating to the intrinsic properties of the muscle to adjust mitochondrial respiration are the principal determinants of mVO2 rather than an O2 delivery-mediated limitation (26, 28, 65). In particular, a close kinetic coupling exists between the dynamics of muscle PCr and the rise in phase II pVO2 at the onset of knee-extensor exercise (53, 54), suggesting the control of mVO2 is mechanistically linked to the product(s) and/or reactant(s) involved in the creatine kinase splitting of PCr. Indeed, the PCr-Cr shuttle provides a model by which the dynamics of PCr are functionally coupled to the rise in mVO2 following a step change in metabolic rate and has received theoretical and empirical support (25, 37, 45, 51).

Consistent with this notion is a recent study by Barker et al. (2), which demonstrated a close correspondence between the kinetics of muscle PCr, determined by using 31P-magnetic resonance spectroscopy (31P-MRS) during quadriceps exercise and phase II pVO2 measured during cycling ergometry, both at the onset and offset of moderate-intensity exercise in 9- to 10-yr-old children. Given this close agreement between the kinetics of muscle PCr and phase II pVO2 in children and adults, one would predict an age-related slowing of the muscle PCr kinetics during growth and maturation. However, although the effect of aging on the kinetics of muscle PCr is well documented during the latter decades of human life (12, 14, 36), the kinetics of PCr in child muscle remains poorly defined. Cross-sectional studies using 31P-MRS have found the resynthesis of PCr in child muscle to be faster (57) or similar (38) to that determined in adults following maximal exercise. However, the extrapolation of muscle PCr recovery kinetics to provide an insight into the mechanisms accounting for the faster exercise onset pVO2 kinetics found in children compared with adults is complicated by a number of methodological issues.

First, similar to the pVO2 response kinetics, the temporal- and amplitude-based characteristics of the muscle PCr response are dependent on the exercise intensity domain under investigation (33, 54). During exercise where muscle pH does not fall below resting values, the breakdown of PCr follows a single-exponential function and reaches a steady-state level within 2–3 min (“moderate” exercise). In contrast, for exercise intensities that elicit a drop in pH from rest (“heavy” exercise) following the initial exponential fall in PCr, an additional delayed breakdown of PCr, termed the PCr slow component, is

Address for reprint requests and other correspondence: N. Armstrong, Children’s Health and Exercise Research Centre, St. Luke’s Campus Univ. of Exeter, Exeter, EX1 2LU, United Kingdom (e-mail: N.Armstrong @exeter.ac.uk).

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observed (33, 54). Therefore, a requirement for analyzing the kinetics of muscle PCR both within and between subject groups is that the muscle PCR and pH should conform to a particular exercise intensity domain. Second, it remains to be established whether the kinetics of muscle PCR at the onset of exercise are influenced by age and/or sex. This is particularly important since the recovery of muscle PCR at the offset of exercise has been reported to be slower than the rate of PCR splitting at exercise onset (54). Last, determination of the muscle PCR kinetics using \(^{31}\text{P-MRS}\) is confounded by a low signal-to-noise ratio brought about by large intersample fluctuation in the PCR profile (52). Repeat-exercise transitions should therefore be time aligned and averaged to enable the determination of PCR kinetic parameters to within acceptable confidence to permit meaningful physiological interpretation (52). Unfortunately, these issues have yet to be addressed in pediatric studies.

Therefore, to provide further insight into the mechanism(s) underlying the faster phase II \(\text{pVO}_2\) found in children compared with adults during moderate-intensity cycling exercise (20), the purpose of the present study was to determine, using \(^{31}\text{P-MRS}\), the kinetic changes in quadriceps muscle PCR at the onset and offset of moderate-intensity exercise in children and adults. Given our current appreciation of the child-adult differences in \(\text{pVO}_2\) kinetics and the close coupling with the kinetics of muscle PCR (2, 53), it was hypothesized that children would demonstrate more rapid muscle PCR kinetics both at the onset and offset of exercise compared with adults, with no sex differences present for both age groups.

**MATERIALS AND METHODS**

**Subjects**

Eighteen children (8 boys, 10 girls) and 16 adults (8 men, 8 women) were included in the present study. After written and verbal explanations of the study’s aims, risks, and procedures were given, all subjects and the children’s parents/guardian(s) provided informed consent to take part in the project, which was approved by the institutional ethics committee. Preexperimental questionnaires identified that all subjects were healthy and showed no contraindications to exercising inside the MR scanner. None of the subjects was involved in a formalized exercise training program, and they were considered to be low to moderately active.

Each child subject made between 6 and 10 visits to the Research Centre, with the testing of adults taking place over 2–5 sessions. All children and adults were habituated to exercising on the quadriceps ergometer at the required cadence inside a purpose-built, to-scale replica model of the MR scanner. All subjects completed a number of repeat trials on the ergometer at a range of exercise intensities identical to the actual protocols employed in the study. All subjects were well habituated to the test procedures.

**Experimental Procedures**

**Descriptive characteristics.** Subjects’ body mass was measured using a calibrated balance beam scale (Avery, Birmingham, UK) and stature and seated height using a stadiometer (Holtain, Crymych, Dyfed, UK). Subjects’ ages were calculated as the difference between the date of birth and the date of the first visit. To provide an estimation of the children’s level of biological maturity, sex-specific regression algorithms were used to determine a maturity “offset” score from the age at peak height velocity using anthropometric measurements (46). The 95% limits of agreement in using this procedure are \(\pm 1\) yr.

**Quadriceps ergometer.** The quadriceps exercise consisted of performing dynamic knee extensions and flexions with the right leg on a non-magnetic ergometer while lying prone inside the MR scanner. The right foot was fastened to a padded foot brace, which was connected to the ergometer load basket using a rope and pulley system. This provided resistance against which continuous concentric and eccentric quadriceps contractions could be performed inside the MR scanner over a distance of \(\approx 0.22\) m. To ensure interrogation of the quadriceps muscles for metabolite changes occurred in the same volume of interest and to standardize the exercise protocol both within and between subjects, the quadriceps exercise was performed at a cadence set in unison with the magnetic pulse sequence (40 repetitions/min). Alignment of the knee extensions/flexions with the pulse sequence was guided using a projected image of a vertical moving cursor set to the frequency of 40 pulses/min. The subjects were required to follow the metronomic cursor using a second vertical cursor under voluntary control of the subject. To prevent displacement of the quadriceps volume of interest relative to the surface coil and minimize adjacent muscles contributing to the exercise task, nylon straps were fastened over the subject’s legs, hips, and lower back. Power output (W) was calculated continuously during the exercise protocols, as described previously (1).

**Step-incremental test.** Each subject completed an incremental test to exhaustion inside the MR scanner for determination of the intra-cellular threshold (IT) using the ratio of \(\text{Pi}\) and PCR (IT\(_{\text{PCR}}\)). Following a 2-min baseline measurement period and starting with an initial basket load of 0.5 kg, an incremental test was undertaken whereby the basket load was increased in steps of 0.5 kg/min until subject exhaustion occurred. This was typically within 7–14 min. Using a plot of \(\text{Pi}/\text{PCR}\) vs. power output at a sample resolution of 30 s, each subject’s IT\(_{\text{PCR}}\) was identified by two investigators. The IT\(_{\text{PCR}}\) was defined as the power output at which a sudden and sustained breakpoint in \(\text{Pi}/\text{PCR}\) from an initial slow phase occurs (41). The reliability for establishing the IT\(_{\text{PCR}}\) has, within our laboratory, a coefficient of variation of 10% (1).

**Constant work rate exercise.** On separate days, each subject completed constant work rate exercise transitions inside the MR scanner with the work rate set to 80% of IT\(_{\text{PCR}}\). The exercise protocol consisted of a 2-min rest period for resting measures, then 6 min of constant work rate quadriceps exercise, followed by 6 min of rest for assessment of the recovery dynamics. Between two and four repeat constant work rate exercise transitions were performed on a given day, with at least 15 min of rest given between each test.

**MRS Measurement and Quantification**

A 1.5-T whole body MR scanner (Philips Gyroscan Intera) was used to monitor the changes in quadriceps muscle energetics. A 6-cm \(^{31}\text{P}\) transmit/receive surface coil was fastened securely to the scanner bed and positioned under the subject’s right quadriceps muscle at the midpoint between the hip and knee joints. Gradient echo images were initially acquired to ensure the quadriceps muscle was positioned correctly relative to the coil. Tuning and matching of the coil was performed to maximize energy transfer between the coil and the muscle. Subsequently, an automatic shimming protocol using the \(^1\text{H}\) signal was undertaken within a volume that defined the quadriceps muscle to optimize the homogeneity of the local magnetic field, thereby leading to maximum signal collection. \(^{31}\text{P}\) spectra were obtained using an adiabatic pulse every 1.5 s, with a spectral width of 1,500 Hz and 512 data points. Phase cycling with four phase cycles was employed, and four measurements were performed, leading to spectra acquired every 6 s to improve the signal to noise of the profile yet provide high-resolution data for kinetic analysis. Since the signal intensities for \(^{31}\text{P}\) metabolites (PCR, \(\text{Pi}\), and ATP) were significantly saturated during the test protocol, T1 correction factors were determined during the rest phase using a pulse interval of 20 s and applied to all peak intensities. The assumption that the \(^{31}\text{P}\) spectra T1 relaxation times for PCR, \(\text{Pi}\), and ATP remain constant from rest to...
steady-state exercise has recently been demonstrated during moderate calf exercise (10).

The $^{31}$P spectra areas were quantified using a nonlinear least squares peak fitting software package (MRUI Software, version 2.0) employing the AMARES fitting algorithm (47, 61). Spectral areas were fitted assuming prior knowledge of the following peaks: $P_i$, phosphodiesters, $PCr$, α-ATP (2 peaks, amplitude ratio of 1:1), γ-ATP (2 peaks, amplitude ratio of 1:1), and β-ATP (3 peaks, amplitude ratio of 1:2:1). Changes in $PCr$ were expressed as a percentage change from baseline, set to 100%, using the mean $PCr$ spectral area obtained during the 2-min rest period. The ratio between $P_i$ and $PCr$ was determined from their respective $^{31}$P spectral areas. Intracellular pH was determined using the chemical shift of the $P_i$ spectral peak relative to the $PCr$ peak:

$$pH = 6.75 + \log (\sigma - 3.27)(5.96 - \sigma)$$

where $\sigma$ represents the chemical shift in parts per million between the $P_i$ and $PCr$ resonance peaks (56). The changes in [ADP] (µM) during the constant work rate exercise bouts were estimated using the creatine-kinase reaction (3):

$$[ADP] = \frac{(0.74\times[ATP] \times [Cr])}{(1.66 \times 10^8 \times [H^+]/[PCr])}$$

where 0.74 is the monovalent ion activity coefficient, [ATP] is the concentration of ATP (in mM), [Cr] is the concentration of creatine (in mM), and 1.66 × 10⁸ is the equilibrium constant for the creatine kinase reaction. Muscle [PCr] was estimated using the ratio of $PCr$/ATP and assuming an [ATP] of 8.2 mM (34) and [Cr] was estimated from [total creatine] – [PCr], where total creatine was assumed to be 36.9 mM (3).

Muscle $PCr$ Kinetic Parameter Estimation

All $PCr$ kinetic parameters were estimated using an iterative least-squares nonlinear regression procedure with their corresponding 95% confidence intervals (95% CIs) to establish the precision of the derived point estimate (GraphPad Prism, GraphPad Software, San Diego, CA). Each separate constant work rate exercise transition was initially checked for $PCr$ sample fluctuations that were greater than ±4 SD from a moving local mean (63). The latter was determined using the average of the five $PCr$ data points before the $PCr$ data point under question. Such large $PCr$ fluctuations are considered unrelated to the underlying physiological profile and are likely to arise due to the low signal-to-noise properties of the $^{31}$P-MRS technique and/or the acute mistiming of a quadriceps contraction relative to the pulse sequence during exercise. To enhance the signal-to-noise properties and therefore the underlying features of the $PCr$ response profile before kinetic parameter estimation, each subject’s repeat constant work exercise transitions were time aligned to the onset of exercise ($t = 0$ s) and averaged, yielding a single $PCr$ response with a sample resolution every 6 s (52). Using the resulting averaged constant work response profile, each subject’s $PCr$ responses were normalized relative to the previous steady-state baseline, using the average $PCr$ value during the 2-min rest or exercise period for determination of the onset and offset kinetics, respectively.

The $PCr$ exercise onset and offset responses were initially modeled using a single exponential function including a delay term:

$$PCr_{o} = \Delta PCr_{o} \times (1 - e^{-TD/\tau})$$

where $PCr_{o}$, $\Delta PCr_{o}$, $TD$, and $\tau$ are the value of $PCr$ at a given time ($t$), the amplitude change in $PCr$ from the control baseline to a new steady state at the onset or offset of exercise, delay term, and the time constant of the response, respectively. However, preliminary analyses revealed that the average model delay term was not significantly ($P > 0.05$) different from 0 s in all subject groups, both at the onset and offset of exercise. This indicates that the splitting and resynthesis of muscle $PCr$ occurred with no discernable delay at both the onset and offset of exercise, respectively. A single exponential model with no delay term was therefore employed for all analyses:

$$PCr_{t} = \Delta PCr_{o} \times (1 - e^{-t/\tau})$$

To provide an estimation of the theoretical maximal rate of oxidative phosphorylation ($Q_{max}$), the following equation was used, assuming the linear first-order model of metabolic control (14, 35, 45):

$$Q_{max} = k_{PCr} \times [PCr]_{rest}$$

where, $k_{PCr}$ is the rate constant derived from the recovery $PCr$ time constant (1/τ), and [PCr]rest is the concentration of muscle $PCr$ at rest.

Normalization of Power Output for Quadriceps Muscle Mass

Determination of quadriceps muscle mass. To normalize the absolute power output measurements during the constant work rate exercise, each subject’s quadriceps muscle mass was determined using MRI (Philips Gyroscan Intera) as recommended (23). Briefly, two scan sequences, each consisting of 50 transverse plane images, were obtained from the right leg using a multi-slice turbo-spin-echo sequence (repetition time = 1,830 ms, echo time = 15 ms, field of view = 80 mm, matrix = 256³). Slice thickness was set at 5 mm and slice separation at 5 mm. Starting from the medial epicondyle and terminating at the head of the femur, the quadriceps muscle (rectus femoris, vastus lateralis, vastus medialis, and vastus intermedius) anatomical cross-sectional area of each slice was determined using region of interest editing software. Quadriceps muscle volume was calculated using the sum of each anatomical cross-sectional area multiplied by 10 mm (5-mm slice thickness + 5-mm slice gap). The quadriceps muscle volume was converted to mass, assuming a muscle density of 1.043 (24), since data regarding a potential age- or sex-related modulation in quadriceps muscle density is limited due to the poor reliability (intra-class correlation $r = 0.089$) of the ultrasonography technique (42). The same investigator performed all muscle mass quantification procedures with an intra-observer reliability of ~5% coefficient of variation.

Allometric modelling. Absolute power output during the constant work rate exercise bouts were corrected for child-adult differences in quadriceps muscle mass using standard log-linear allometric modeling procedures (5, 58). Potential group differences in the scaling exponent were examined by including an interaction variable in the regression model (5, 58). The interaction variable did not yield a significant contribution to the regression model ($P = 0.660$), indicating the slope exponent ($b$) describing the relationship between power output and quadriceps muscle mass was comparable between the children and adults. After verification of the model using appropriate diagnostic tools (5), a power function ratio ($Y/X^b$) was determined to scale power output ($Y$) during the constant work rate exercise for differences in quadriceps muscle mass ($X^b$).

Statistics

Potential mean differences in the $^{31}$P-MRS metabolic responses were examined using a two-way between-measures ANOVA with age (child vs. adult) and sex (male vs. female) as the model factors. If a significant age by sex interaction was observed, the main effect results were not reported. Mean differences were followed up on using planned multiple comparisons with the alpha value adjusted using the Bonferroni procedure. All results are presented as means ± SD, with rejection of the null hypothesis accepted at an alpha level of $P = 0.05$. All analyses were performed using SPSS (version 11.0).

RESULTS

Descriptive and Incremental Test Responses

The subjects’ descriptive characteristics and exercise responses during the incremental test to exhaustion are presented in Table 1.
The boys had a significantly larger maturity offset score from the age at peak height velocity compared with girls (−3.4 ± 0.4 (range −2.9 to −4.1) vs. −1.7 ± 0.5 (range −0.8 to −2.3) yr; \( P = 0.000 \)). During the step-incremental test to exhaustion, the men and women had a significantly higher peak power output and power output at the ITPi/PCr compared with the boys and girls, respectively. Sex differences were present in the adult group, with men displaying a higher peak power output and ITPi/PCr than women.

**Constant Work Rate Exercise**

Power output. The temporal and amplitude characteristics of the power output during the exercise bouts are illustrated in Fig. 1A. At the onset of exercise, power output rose instantaneously with square-wave-like kinetics and was well maintained at the target power output throughout the entire test. Absolute power output during constant work rate exercise was significantly higher in men than in boys (14 ± 2 vs. 7 ± 1 W; \( P = 0.000 \)) and in women than in girls (10 ± 1 vs. 7 ± 2 W; \( P = 0.000 \)). In the adult group, men attained a significantly higher absolute power output than women (\( P = 0.004 \), although no sex differences were present in children (\( P = 1.000 \)). The log-linear allometric model revealed a significant linear relationship between absolute power output and quadriceps muscle mass (\( b = 0.73, R^2 = 0.83, P = 0.000 \)). When power output was adjusted for quadriceps muscle mass using the power function ratio (W·kg\(^{-0.73}\)), no significant differences were present between men and boys (7 ± 1 vs. 8 ± 1 W·kg\(^{-0.73}\); \( P = 1.000 \)), women and girls (7 ± 0 vs. 7 ± 1 W·kg\(^{-0.73}\); \( P = 1.000 \)), boys and girls (\( P = 1.000 \)), or men and women (\( P = 1.000 \)), respectively. Furthermore, when power output was expressed relative to the ITPi/PCr, the children (boys, 89 ± 11%; girls, 82 ± 11%) and adults (men, 87 ± 10%; women, 88 ± 16%) exercised at a comparable intensity relative to the ITPi/PCr (\( P > 0.05 \)). Collectively, these results demonstrate that the experimental protocol was successful in exercising the subjects at a similar power-output relative to the metabolic IT and quadriceps muscle mass.

Muscle metabolic responses. The ratio of PCr/ATP was 4.38 ± 0.45 in the boys, 4.53 ± 0.61 in the girls, 5.62 ± 0.98 in the men, and 4.89 ± 0.80 in the women, which are similar to adult data previously reported from our laboratory (33). Muscle [PCr] at rest was significantly higher in the men compared with the boys (46.1 ± 8.1 vs. 35.9 ± 3.7 mM; \( P = 0.000 \)).
0.023) but similar between the women and girls (40.1 ± 6.5 vs. 37.2 ± 5.0 mM; *P* = 1.000). No differences were evident in muscle [PCr] at rest between sexes (*P* > 0.500).

The average muscle metabolic (P/PCr, [ADP]) and pH responses during the rest, exercise, and recovery phases of the quadriceps constant work rate exercise bouts are illustrated in Fig. 1 with the average values from the last 30 s of each phase reported in Table 2. No age- or sex-related differences were observed for pH between the children and adults at rest (*P* > 0.05). At the onset of exercise, an initial muscle alkalosis was evident in the pH profile for all groups, which later fell to a steady-state level. Pairwise comparisons revealed a significantly higher pH during exercise in girls compared with women (*P* = 0.017) during steady-state exercise, although a similar pH was evident between boys and men (*P* = 0.676), girls and boys (*P* = 1.000), and men and women (*P* = 1.000) during exercise. During the recovery phase, pH was significantly lower in women compared with men (*P* = 0.045), with no differences between men and boys (*P* = 1.000), girls and boys (*P* = 1.000), or girls and women (*P* = 0.140). At rest, no significant age- or sex-related differences were evident in the P i-to-PCr ratio between children and adults (*P* > 0.05). At exercise onset, P i/PCr increased with exponential properties and reached a steady-state magnitude that was independent of age and sex between the children and adults (*P* > 0.05). This was also the case for when P i/PCr was expressed as a change from resting levels. During recovery, P i/PCr decreased with exponential properties to a value close to rest, with no significant (*P* > 0.05) age- or sex-related differences present. Resting quadriiceps muscle [ADP] was significantly higher in boys than in men (*P* = 0.017) but comparable between the girls and women, boys and girls, and men and women (*P* = 1.000). At the onset of exercise, [ADP] rose with exponential-like kinetics and attained a steady-state concentration that was independent of age and sex (*P* > 0.05). This was also the case when [ADP] was expressed as a delta change from resting levels. During recovery, [ADP] decreased with exponential-like kinetics to a resting value that was higher in boys than in men (*P* = 0.020) but similar between girls and women, girls and boys, and men and women (*P* > 0.800).

**Muscle PCr Kinetics**

On average, the children completed 6 ± 2 (range 3–9) and the adults 4 ± 1 (range 2–5) repeat constant work rate exercise transitions for determination of the PCr response dynamics. A typical PCr profile for a child and adult subject at the onset and offset of exercise is shown in Fig. 2, along with the fitted nonlinear regression model and residual plots. In all cases, the single-exponential model provided an appropriate fit of the PCr dynamics, as indicated by the unsystematic and random profile of the model’s residuals.

The estimated time constants for the PCr kinetics at the onset and offset of exercise are shown in Table 3. The average 95% CIs for the estimated PCr time constants were approximately ±6 s for both the onset and offset dynamics, highlighting the good degree of statistical precision attained in the derived kinetic estimates. The pairwise comparisons found no significant (*P* = 0.616–1.000) age- or sex-related differences in the PCr kinetic time constant at the onset (boys, 21 ± 4 s; girls, 24 ± 5 s; men, 26 ± 9 s; women, 24 ± 7 s) or offset (boys, 26 ± 5 s; girls, 29 ± 7 s; men, 23 ± 9 s; women 29 ± 7 s) of exercise. The individual PCr onset and offset time constants for children and adults are shown in Fig. 3.

The estimated theoretical maximal rate of oxidative phosphorylation (Qmax) is illustrated in Fig. 4. The pairwise comparisons revealed no age- or sex-related differences (*P* = 0.216–1.000) for the estimated Qmax (boys, 1.39 ± 0.20 mM/s; girls, 1.32 ± 0.32 mM/s; men, 2.36 ± 1.18 mM/s; women, 1.51 ± 0.53 mM/s).

**DISCUSSION**

In conflict with the study’s hypothesis, we have demonstrated that the time constant describing the kinetics of muscle PCr, a putative metabolic feedback controller of mVO2, was independent of age and sex, both at the onset and offset of moderate-intensity exercise. Likewise, the steady-state rise in the reputed regulators of oxidative phosphorylation (i.e., P i/PCr and [ADP]) during exercise was strikingly similar between the children and adults. Collectively, these results support the notion that, during the transition to and recovery from quadriiceps moderate-intensity work rates, the regulation of mitochondrial respiration is fully mature in peri-pubertal children and sex independent. The physiological basis for these results is poorly understood but is likely to reside in a similar oxidative capacity of the muscle, as further evidenced by the age and sex independence of the estimated Qmax, which provides a theoretical estimate of the maximal rate of mitochondrial oxidative phosphorylation.

**Table 2. Constant work rate exercise responses**

<table>
<thead>
<tr>
<th>Variable</th>
<th>ANOVA</th>
<th>Child</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Boys (n = 8)</td>
<td>Girls (n = 10)</td>
</tr>
<tr>
<td>Rest pH</td>
<td><em>P</em> = 0.043</td>
<td>7.05 ± 0.02</td>
<td>7.06 ± 0.03</td>
</tr>
<tr>
<td>Exercise pH</td>
<td><em>P</em> = 0.003</td>
<td>7.08 ± 0.01</td>
<td>7.08 ± 0.02</td>
</tr>
<tr>
<td>Recovery pH</td>
<td><em>P</em> = 0.034</td>
<td>7.03 ± 0.02</td>
<td>7.03 ± 0.03</td>
</tr>
<tr>
<td>Rest P i/PCr</td>
<td><em>P</em> = 0.022</td>
<td>0.13 ± 0.03</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>Exercise P i/PCr</td>
<td>NS</td>
<td>0.31 ± 0.05</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>Recovery P i/PCr</td>
<td><em>P</em> = 0.008</td>
<td>0.13 ± 0.03</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>Rest ADP, μM</td>
<td><em>P</em> = 0.005</td>
<td>8 ± 5 s</td>
<td>6 ± 6</td>
</tr>
<tr>
<td>Exercise ADP, μM</td>
<td><em>P</em> = 0.016</td>
<td>21 ± 8</td>
<td>20 ± 7</td>
</tr>
<tr>
<td>Recovery ADP, μM</td>
<td><em>P</em> = 0.003</td>
<td>8 ± 5 s</td>
<td>7 ± 5</td>
</tr>
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</table>

Data are means ± SD. Rest, exercise, and recovery values were calculated during the final 30 s of their respective stage of the constant work rate protocol. Two-way between-measures ANOVA results (*P* < 0.05): *a*significant main effect for age; *b*significant main effect for sex; *c*significant age by sex interaction. NS, no significant differences observed (*P* > 0.05). Bonferroni adjusted pairwise comparisons: Significant within-age-group sex difference, *d*P < 0.05. Significant within-sex-group age difference: *e*P < 0.01.
It has previously been demonstrated that children display a faster rise in the phase II pVO\textsubscript{2} response compared with adults at the onset of moderate and heavy exercise (19, 22, 66). For example, Fawkner et al. (22) demonstrated more rapid phase II pVO\textsubscript{2} kinetics in 11- to 12-yr-old children (boys 19 ± 2 s; girls 21 ± 6 s) compared with 19- to 26-yr-old adults (men 28 ± 9 s; women 26 ± 5 s) during moderate cycling exercise, with no differences between the sexes. However, the underlying mechanisms accounting for this age-related modulation of the pVO\textsubscript{2} kinetic response have yet to be resolved, although a decline in the muscles’ potential for oxygen utilization has been implicated (20).

Of particular interest is the strong body of evidence suggesting that the PCr-Cr shuttle plays an integral role in modulating the rise in mVO\textsubscript{2} following an abrupt change in the metabolic rate (8, 37, 45, 62). In particular, the creatine kinase splitting of muscle [PCr] appears to provide a high capacitance temporal buffer for muscle [ATP] at the onset of exercise, thereby delaying the rise in putative metabolic feedback controllers to signal an increased rate of oxidative phosphorylation (37). Although the exact signal(s) to drive an increase in mVO\textsubscript{2} is currently unknown, putative feedback mechanisms implicate PCr and/or Cr, [ADP], and the phosphorylation potential (45, 62, 67).

Consistent with this supposition are studies demonstrating that a close kinetic coupling exists between the respective kinetic fall and rise in muscle PCr and phase II pVO\textsubscript{2} at the onset of moderate-intensity exercise in children (2) and adults (3, 53, 54). Given this mechanistic coupling between muscle PCr and phase II pVO\textsubscript{2}, it was hypothesized that, in line with the demonstration of faster pVO\textsubscript{2} kinetics in children compared with adults, a requisite age-related difference would be evident in the dynamics of muscle PCr. However, despite yielding similar PCr kinetic time constants for the children and adults to those reported by Fawkner et al. (22) for the phase II pVO\textsubscript{2} response during moderate-intensity cycling exercise, we found

### Table 3. Muscle PCr kinetic responses

<table>
<thead>
<tr>
<th>Variable</th>
<th>Child</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>τPCr, s</td>
<td>NS</td>
<td>21±4</td>
</tr>
<tr>
<td>95% CIs, ±s</td>
<td>5±1</td>
<td>6±1</td>
</tr>
<tr>
<td>Offset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>τPCr, s</td>
<td>NS</td>
<td>26±5</td>
</tr>
<tr>
<td>95% CIs, ±s</td>
<td>5±2</td>
<td>7±2</td>
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</tbody>
</table>

Data are means ± SD. PCr kinetics parameters at the onset and offset of moderate-intensity exercise. All kinetic parameters were estimated using a single-exponential model. τ and 95% CIs denote the time constant and the 95% confidence intervals, respectively.
no age- or sex-related differences in the PCr kinetics either at the onset or offset of exercise. In contrast, the data from the present study support a body of research that has been criticized on methodological grounds (20), demonstrating no differences in the phase II p\(\dot{V}_O^2\) time constant between children and adults during moderate-intensity cycling exercise (16, 29, 55). Collectively, therefore, these results are consistent with the notion that the control and adjustment of m\(\dot{V}_O^2\) during the step transition to a lower or higher metabolic rate may not be fundamentally different between young children and adults, at least for moderate-intensity exercise.

Previous studies examining the kinetics of muscle PCr in children and adults are sparse and yield equivocal findings. In agreement with the present paper, Kuno et al. (38) found no differences in the recovery time constant of quadriceps muscle PCr in untrained boys (12–15 yr old) compared with age-matched trained boys and adult men (25 \(\pm\) 2.6 yr old) following a knee extensor/flexion incremental test to exhaustion. The authors concluded that invariant PCr kinetics were indicative of a comparable mitochondrial oxidative capacity between child and adult muscle. In contrast, following an exhaustive calf exercise challenge, Taylor et al. (57) reported 6- to 12-yr-old children to have a faster PCr recovery half time [12 \(\pm\) 4 s (time constant \(\sim\) 17 s)] than adults [27 \(\pm\) 8 s (time constant \(\sim\) 39 s)] and attributed this to the enhanced capacity for oxidative phosphorylation in the child’s muscle as evidenced by an almost twofold higher Q\(_{\text{max}}\) (child, 91 \(\pm\) 46 mM/min vs. adult, 54 \(\pm\) 17 mM/min), assuming an [ADP] control model of mitochondrial ATP synthesis.

Although providing seminal data describing the kinetics of muscle PCr in child and adult muscle, the experimental conditions under which muscle PCr was determined in the two studies above preclude any firm conclusions to be drawn as to whether an age-related modulation of the muscle PCr kinetics may account for the faster p\(\dot{V}_O^2\) kinetics observed in children compared with adults (19, 22, 66). In particular, the studies by Kuno et al. (38) and Taylor et al. (57) were restricted to the recovery of muscle PCr following a single bout of exhaustive exercise. Given that the p\(\dot{V}_O^2\) response exhibits both temporal- and amplitude-based differences depending on the intensity of the imposed exercise and the exercise transition (i.e., onset or offset responses) (9, 48, 54), the kinetics of muscle PCr must be examined with specific reference to the exercise intensity domain and exercise transition to test p\(\dot{V}_O^2\) control theories. By exercising each subject relative to his or her predetermined metabolic IT in the present study, the muscle phosphate and pH profile were ensured to be similar both within and between subject groups, such that the onset and recovery PCr kinetics were determined during moderate-intensity exercise, i.e., under conditions of no cellular acidosis (see Fig. 1) (51, 64).

In addition, it should be considered that the distinct muscle fiber-type heterogeneity present in the calf muscle may have biased the findings presented by Taylor et al. (57). Compared with adults, the smaller calf muscle in children may result in a greater interrogation of the underlying soleus (mainly type I fibers) relative to that of the gastrocnemius muscle (mainly type IIA and IIX fibers), which may confound interpretation of muscle PCr kinetics using an unlocalized \(^{31}\text{P}-\text{MRS}\) signal (50). For example, it has been reported that the time constant for the resynthesis of muscle PCr in the cat soleus muscle (largely type I fibers, \(\tau \sim 7.2\) min) is twofold faster than the biceps brachii muscle (mostly type IIA and IIX fibers, \(\tau \sim 14.4\) min; Ref. 39). Assuming similar metabolic properties are present in human muscle fiber types that impact the recovery of muscle PCr (17), it is plausible that the slower PCr recovery kinetics observed in

![Figure 3](image-url) PCr time constants at the onset and offset of exercise. Individual PCr kinetic time constants at the onset (A) and offset (B) of exercise in the children and adult groups are shown.

![Figure 4](image-url) Theoretical maximal rate of oxidative phosphorylation (Q\(_{\text{max}}\)). The bars and error bars represent the mean and SD, respectively. No significant age- or sex-related differences in Q\(_{\text{max}}\) were identified (\(P > 0.05\)).
the adults compared with children in Taylor et al.’s (57) study were a consequence of a greater relative proportion of gastrocnemius muscle occupying the 31P-MRS signal in adults and, conversely, the soleus in children.

Lastly, an important feature of the present study was that each subject completed several repeat constant work rate exercise transitions to dampen the influence of the large sample to sample variability in the PCr response profile reducing the statistical precision of the estimated kinetic parameters (52). This allowed the determination of PCr time constants with good statistical confidence (95% CIs of approximately ±6 s), which appear suitably narrow to draw meaningful conclusions in the current paper. Unfortunately, previous studies with children (38, 57) have failed to report the error spanning their estimated kinetics parameters, which limits their physiological interpretation.

According to Meyer’s (45) linear first-order model of metabolic control, the kinetics of muscle PCr are principally determined by the mitochondrial “resistance,” that is the density and number of mitochondria in the muscle. Indeed, the recovery rate constant of muscle PCr has been reported to be linearly dependent on the mitochondrial enzyme citrate synthase in animal (49) and human muscle (44), which supports the PCr kinetic time constant reflecting the muscles’ capacity for oxidative phosphorylation. The age and sex invariant muscle PCr kinetics in the current paper may therefore be indicative of a similar oxidative capacity of the muscle. Interestingly, this supposition is consistent with muscle biopsy data showing a mixed-sex group of children (aged 6 yr) to have a similar level of citrate synthase enzyme activity in the vastus lateralis muscle compared with adolescents (aged 13–17 yr) and adult groups, although other Krebs cycle enzymes (e.g., isocitric, fumarase, and malate dehydrogenase) were found to be higher in children and adolescents (7, 27). Moreover, the volume of mitochondria expressed as a relative percentage of the total fiber volume in the vastus lateralis muscle has been reported to be comparable between 6-yr-old girls and boys and with the data of untrained adults taken from a previous study (6).

Assuming linear first-order metabolic control, an estimate of the theoretical maximal rate of oxidative phosphorylation \( Q_{\text{max}} \) can be obtained from knowledge of the PCr resynthesis rate constant \( 1/\tau \) and resting muscle [PCr] (14, 35, 45). Taking the PCr rate constant to represent the oxidative properties of the muscle (see above), the model predicts that the maximal rate of oxidative metabolism will be achieved when [PCr] is fully depleted, i.e., when the metabolic signal (PCr and/or Cr, or [ADP]) to stimulate oxidative metabolism is maximal. Since the kinetics of muscle PCr were independent of age and sex in the present study, \( Q_{\text{max}} \) would be entirely modulated by resting [PCr], which we found to be higher in the men compared with the boys (46.1 ± 8.1 vs. 35.9 ± 3.7 mM) but not between the women and the girls (40.1 ± 6.5 vs. 37.2 ± 5.0 mM) or between the sexes. However, despite men having a 1.7- and 1.5-fold higher \( Q_{\text{max}} \) than boys and women, respectively, this difference was not statistically significant (see Fig. 4) and thereby supports the notion that the theoretical maximal rate of oxidative metabolism is fully mature in peri-pubertal children. However, given inherent concerns in estimating \( Q_{\text{max}} \) (see Considerations and Limitations), this conclusion warrants further enquiry and should be interpreted with extreme caution.

As alluded to earlier, although the kinetics of muscle PCr have been implicated to play a fundamental role in signaling a rise in \( mV_{\text{O2}} \) during metabolic transitions, the precise metabolic signal(s) is unknown (51, 62). Candidate variables such as the phosphorylation potential (67), which can be estimated using the P31-to-PCr ratio (41), and [ADP] (11, 15) were determined in the present study. Indeed, it has been demonstrated in human vastus lateralis muscle fiber bundles that a decrease in PCr/Cr increases the sensitivity of the mitochondria to [ADP], thus stimulating a rise in muscle respiration, whereas an increase in PCr/Cr has the opposite effect (62). These data suggest that the time course of muscle PCr/Cr and [ADP] may be mechanistically linked in modulating \( mV_{\text{O2}} \) during metabolic transitions. In accord with previous adult data (3), at the onset of exercise, both P/PCr and [ADP] increased with exponential-like properties, which were both age and sex invariant (see Fig. 1, curves not shown for clarity). Moreover, the steady-state magnitude, either expressed in absolute terms or as a delta change from rest, was age and sex invariant. Collectively, these data therefore further support the notion that the metabolic perturbation, both during the nonsteady state and steady state, required to drive mitochondrial respiration is fully mature in peri-pubertal children.

Considerations and Limitations

Fundamental to the present study is the assumption that the kinetics of phase II pV\( \text{O2} \) provides a noninvasive window into the dynamics of \( mV_{\text{O2}} \) during the nonsteady state in children and adults. Although this assumption has been verified in adults using the direct Fick technique (26), it awaits confirmation in the pediatric population. Indeed, if child-adult differences in the cardiac output dynamics, size of the venous volume, and utilization of body \( \text{O2} \) stores were present during the nonsteady state, the association between phase II \( pV_{\text{O2}} \) and \( mV_{\text{O2}} \) may be distorted beyond acceptable limits in children. However, we do not believe this be the case. Similar to adults, the child’s \( pV_{\text{O2}} \) response at the onset of moderate-intensity exercise displays the three-phase response, providing that repeat exercise transitions are time aligned and averaged (2, 22). Moreover, when the phase II response is isolated and modelled, its resulting time constant closely corresponds with that of the breakdown of muscle PCr in peri-pubertal children (2), which is routinely used as a surrogate of \( mV_{\text{O2}} \) in human (3, 43, 53) and animal studies (40, 45) investigating aspects of metabolic control. Although indirect, this association suggests that the underlying \( mV_{\text{O2}} \) response is reflected via the phase II pV\( \text{O2} \) region and thus is acceptable to investigate aspects of metabolic control during childhood and adolescence.

Why there is a lack of an age-related modulation of the PCr kinetics in the present study is inconsistent with our current understanding of the pV\( \text{O2} \) kinetic response in children and adults. However, the potentially confounding role physical fitness may have on the PCr response parameters should be considered. Indeed, the pV\( \text{O2} \) kinetic response (and presumably muscle PCr, although see Ref. 32) in adults has been reported to be sensitive to exercise training, such that the time constant is more rapid in subjects with higher aerobic fitness (31). Despite every attempt in the present study to recruit subjects who were either low or moderately active, it is pertinent to note that the PCr time constants in some adults were similar to those...
typically found in trained endurance athletes ($\tau = \sim 10–15 \text{s};$ Ref. 30). However, it is not uncommon to observe a muscle PCR time constant of $\sim 12–15 \text{s}$ at the onset or offset of exercise in low to moderately active young adults (13, 36). Therefore, whether the muscle PCR kinetic response during moderate-intensity exercise does or become slower through the transition from child to adulthood requires further investigation, with large sample sizes and well defined subject groups.

An inherent assumption in estimating muscle [ADP] and $Q_{\text{max}}$ (specifically [PCr]$_{\text{rest}}$) in the present study is that the resting [ATP] in peri-pubertal children is similar to that observed in young adults and is sex independent. Unfortunately, for ethical reasons, data concerning a putative age- and/or sex-related modulation of [ATP] and [PCR] during growth and maturation are lacking. However, there are limited cross-sectional data in boys showing that, over the age span of 11–16 yr, resting [ATP] in the quadriceps rectus femoris muscle is age invariant, whereas a progressive age-related rise was observed for muscle [PCr]$_{\text{rest}}$ (18). Although this latter finding is in agreement with the child-adult differences for [PCr]$_{\text{rest}}$ in the present study, given the paucity of descriptive data available, the muscle [ADP] and $Q_{\text{max}}$ data should be interpreted with due caution.

The estimated $Q_{\text{max}}$ for the men in the present study is notably higher than previously reported for the quadriceps muscle using a linear model of metabolic control (14, 60). However, this measurement requires knowledge of the resting muscle [PCR], which was estimated in the present study using standard $^{31}$P-MRS procedures. Although comparative data for $^{31}$P-MRS determined quadriceps muscle [PCR] at rest is lacking, a recent review paper suggested this is close to 35 mM (34). This is substantially lower than the 46 mM (range of 34–62 mM) in the present study but is close to previous studies reporting a lower $Q_{\text{max}}$ than the present investigation (14, 60). According to Meyer’s (45) linear model of metabolic control, the substantially higher muscle [PCR] at rest in the present study would increase the “theoretical” $Q_{\text{max}}$. Indeed, since it has recently been shown that variation in resting muscle [PCR] can have profound consequences for describing mitochondrial function using models of metabolic control (34), we have very strong reservations in making interstudy comparisons with $Q_{\text{max}}$ data.

Although the girls in the present study were age-matched with the boys’ group, their level of biological maturity, as assessed using an estimated offset score from the age at peak height velocity, was significantly higher than the boys. Whether this had an impact either on the sex- or age-related comparisons is difficult to discern, especially given the lack of available data describing the muscle enzyme profile, mitochondrial content, and muscle metabolic responses during growth and maturation in girls and boys. Future research should therefore consider biological maturity as well as chronological age when investigating children’s muscle metabolic responses during exercise.

In conclusion, the present study used $^{31}$P-MRS to investigate whether the faster phase II $p$VO$_2$ kinetics, and by extension the dynamics of mVO$_2$, found in children compared with adults can be explained by an age-related modulation of the dynamics of muscle PCR, which are thought to play a principal role in regulating mVO$_2$ during metabolic transitions. However, we found no age- or sex-related differences in quadriceps muscle PCR kinetics either at the onset or offset of exercise. Moreover, the steady-state perturbation to the putative drivers of oxidative phosphorylation was similar between children and adults. Collectively, these results indicate that, following a step transition to a higher or lower metabolic rate, the control of mitochondrial oxidative phosphorylation is independent of age and sex, at least for exercise transitions below the metabolic intracellular thresholds. This is consistent with a comparable capacity for oxidative metabolism in child and adult muscle.

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GRANTS

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REFERENCES


