Age- and sex- related differences in muscle phosphocreatine and oxygenation kinetics during high-intensity exercise in adolescents and adults

Running head: PCr and HHb kinetics in adolescents and adults

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ABSTRACT AND KEYWORDS

The aim of this investigation was to examine the adaptation of the muscle phosphates (e.g. PCr and ADP) implicated in regulating oxidative phosphorylation, and oxygenation at the onset of high intensity exercise in children and adults. The hypotheses were threefold: primary PCr kinetics would be faster in children than adults; the amplitude of the PCr slow component would be attenuated in children; and the amplitude of the HHb slow component would be reduced in children. Eleven children (5 girls, 6 boys, 13 ± 1 yrs) and 11 adults (5 women, 6 men, 24 ± 4 yrs) completed two to four constant work rate exercise tests within a 1.5 T MR scanner. Quadriceps muscle energetics during high intensity exercise were monitored using ^{31}P-MRS. Muscle oxygenation was monitored using near-infrared spectroscopy. The time constant for the PCr response was not significantly different in boys (31 ± 10 s), girls (31 ± 10 s), men (44 ± 20 s) or women (29 ± 14 s, main effects: age, p=0.37, sex, p=0.25). The amplitude of the PCr slow component relative to end-exercise PCr was not significantly different between children ($23 \pm 23\%$) and adults ($17 \pm 13\%$, p=0.47). End-exercise [PCr] was significantly lower, and [ADP] higher, in females (18 ± 4 mM and 53 ± 16 μM) than males (23 ± 4 mM, p=0.02 and 37 ± 11 μM, p=0.02), but did not differ with age ([PCr]: p=0.96, [ADP]: p=0.72). The mean response time for muscle tissue deoxygenation was significantly faster in children (22 ± 4 s) than adults (27 ± 7 s, p=0.01). The results of this study show that the control of oxidative metabolism at the onset of high intensity exercise is adult-like in 13 yr old children, but that matching of oxygen delivery to extraction is more precise in adults.

Keywords: ^{31}P-magnetic resonance spectroscopy, near-infrared spectroscopy, energetics, growth, muscle, metabolism, maturation

Abbrevations: HHb – deoxyhaemoglobin/myoglobin; PCr – phosphocreatine; P_i – inorganic phosphate; $p\dot{V}O_2$ - pulmonary oxygen consumption; $\dot{V}O_2$ - oxygen consumption; TCr – total creatine; IT – intracellular threshold; a.u. – arbitrary units, CP – critical power

GRAPHICAL ABSTRACT

Phosphocreatine kinetics and muscle oxygenation during high intensity exercise in children and adults

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During high intensity quadriceps exercise, muscle [PCr], [ADP], and pH changed to a similar extent in children and adults. The PCr kinetic response was similar in all groups, however, muscle deoxygenation was faster in children than adults. Control of oxidative metabolism is similar in children and adults, but this is associated with a more precise matching of muscle oxygenation to metabolic demand in adults at the onset of high intensity quadriceps exercise.

Introduction

At the onset of a 'step' change in external work-rate, pulmonary oxygen consumption ($p\dot{V}O_2$) increases in an exponential manner (termed phase II or the primary component), following a delay of ~ 15 seconds, and provides a valid estimation of O_2 consumption within the muscle (1). During moderate intensity exercise (below the blood lactate threshold) $p\dot{V}O_2$ reaches steady-state within ~ 3 minutes in an healthy individual, whereas for exercise intensities above the lactate threshold (heavy exercise), a further progressive increase in $p\dot{V}O_2$, called the slow component, emerges after two to three minutes of exercise. The speed of the exponential rise in $p\dot{V}O_2$, at exercise onset is important, since slower $\dot{V}O_2$ kinetics incur a greater reliance on non-oxidative energy sources, i.e. muscle phosphocreatine (PCr) breakdown and anaerobic glycolysis, leading to an accumulation of metabolic byproducts and potentially a reduction in exercise tolerance (2).

Studies investigating developmental changes in the $p\dot{V}O_2$ kinetic response at exercise onset have revealed important insights into age and sex related changes in metabolic control. For example, a 2 yr longitudinal investigation in 9 boys and 13 girls between the ages of 11 to 13 yrs, reported a significant slowing of the phase II response (~ 20%) and increase in the $p\dot{V}O_2$ slow component (~ 50%) during 9 minutes of heavy intensity cycling exercise (3). While a trend towards faster phase II kinetics, and an attenuated slow component is a common feature of the $p\dot{V}O_2$ profile in young children compared to older children or adults, the mechanistic basis for this phenomenon is unknown (4).

It is generally considered that the primary rise in $p\dot{V}O_2$ at exercise onset is dependent on the rate of adaptation of metabolic pathways in the muscle and not muscle O_2 delivery (5). Empirical studies in adults have shown a close association between $p\dot{V}O_2$ kinetics and muscle PCr, the latter measured using [31]phosphorus magnetic resonance spectroscopy (^{31}P-MRS) (6-8); a relationship which has recently been established in 9-10 yr old children at the onset and offset of exercise (9). Interestingly, the $p\dot{V}O_2$ slow component is also associated with a mirror like fall in muscle PCr during exercise (7), although unlike the primary phase, the slow component is sensitive to changes in muscle O_2 delivery. For example, during hyperoxic breathing, both the muscle PCr and $p\dot{V}O_2$ slow components are significantly reduced, despite no change to the initial kinetic response (10,11).

A recent ^{31}P-MRS study by Barker and colleagues (12) has shown that the kinetics of muscle PCr during moderate intensity exercise, i.e. below the inorganic phosphate(P_i)/PCr intracellular threshold (IT), are similar between children and adults, implying that the phosphate-linked control of cellular respiration is adult-like in children during moderate intensity exercise. However, whether this conclusion can be extrapolated to exercise above the muscle P_i/PCr threshold, i.e. high intensity exercise, remains questionable. It has been shown that during high intensity exercise O_2 delivery plays an increasingly dominant role in modulating muscle metabolism (13). Moreover, high intensity exercise is associated with a fall in cellular pH from rest which might inhibit the ADP signal for oxidative phosphorylation by reducing muscle [ADP] as predicted by the creatine kinase equilibrium (14).

Given that exercising children are characterized by a higher intra-cellular pH (15-17) and a greater mass specific muscle blood flow (and thus O_2 delivery) in the vastus lateralis muscle (18), compared to older children or adults, it is conceivable that at higher exercise intensities differences in metabolic control at exercise onset might be observed between children and adults. Indeed, the data of Zanconato et al. (16), albeit during incremental exercise, support this contention. These authors found no child-adult differences in the profiles of muscle P_i/PCr or pH during moderate intensity work-rates, but during exercise above the muscles' P_i/PCr IT, noted a lower rate of change of P_i/PCr and pH in children. Thus, there might exist an exercise intensity dependence of age-related differences in metabolic control at the onset of exercise between children and adults, although no data are currently available for high intensity exercise.

The aim of this study therefore was to non-invasively examine the kinetics of muscle PCr, using ^{31}P-MRS, and muscle oxygenation, using near-infrared spectroscopy (NIRS), during high intensity quadriceps exercise in children and adults. Muscle oxygenation was monitored using the deoxyhaemoglobin/myoglobin (HHb) signal which provides information on the balance between muscle O_2 delivery and utilization within the microcirculation (19). It was hypothesized that children would display more rapid muscle PCr kinetics and a reduced muscle PCr slow component compared to adults; and that the PCr slow component would be associated with a reduced HHb slow component in children, reflective of an enhanced muscle oxygenation.

Methods

Participants

Eleven children (six boys and five girls, 13 ± 1 yrs) and eleven adults (six men and five women, 24 ± 4 yrs) volunteered to participate in the study, which was approved by the institutional ethics board. Adult participants and parents or guardians of child participants gave written, informed consent, and children provided written assent to participate. All participants were healthy and recreationally active in sports including netball, soccer, martial arts, and dance.

Stature and seated height for all participants were measured using a stadiometer (Holtain, Crymych, Dyfed, UK), and body mass was measured using a calibrated beam balance scale (Avery, Birmingham, UK). Age was calculated from the date of the first test, and maturity was estimated using the methods described by Mirwald et al. (20) and expressed as the estimated time in years from the age at peak height velocity (YAPHV).

Before data collection, each participant completed a number of familiarization sessions on a replica of the quadriceps ergometer within a scale model of the MR scanner. These sessions included rehearsal of tests identical to the tests performed during data collection. Participants visited the laboratory at the centre three to five times to complete data collection.

Experimental protocol

Exercise was conducted on a custom-built quadriceps ergometer within a 1.5 T MR scanner (Phillips Gyroscan Intera), with a 6 cm ^{31}P transmit-receive coil affixed to the bed under the right quadriceps muscle group (primarily over the rectus femoris muscle)

and a NIRS probe securely fixed over the vastus lateralis muscle. Both were positioned midway between the hip and knee joints. The ergometer and related measurement methods have been described previously (21).

Each participant first completed an incremental test to exhaustion using the right leg. The test began with a load of 5 N in the ergometer basket, and the load was increased by 5 N·min^{-1} until the participant was unable to maintain the cadence or range of motion required or until the participant was exhausted. P_i/PCr over the test was plotted against power output, allowing two independent investigators to identify an IT, as described by Barker and colleagues (21).

After this test, participants completed two to four constant work rate bouts, each separated by at least 48 hours. These constant work rate bouts consisted of two minutes of resting data collection and seven minutes of work at an intensity corresponding to 20% of the difference between the workload at the intracellular P_i/PCr threshold and the maximal workload (i.e. 20% Δ).

^{31}P-MRS measurements

Prior to exercise testing, gradient echo images were initially acquired to ascertain the position of the muscle relative to the coil. Matching and tuning of the coil was then carried out, followed by an automatic shimming protocol on the signal volume that defined the quadriceps muscle, so as to optimize the signal from the muscle under investigation.

During the test protocol ^{31}P spectra were obtained using an adiabatic pulse every 1.5 s, with a spectral width of 1500 Hz and 512 data points. Phase cycling with four phase cycles was employed, leading the acquisition of spectra every six s. The areas of the spectra acquired were then quantified using a non-linear least squares peak-fitting software package (jMRUI Software, version 2.0) (22) and the AMARES fitting algorithm (23). Spectral areas were fitted assuming presence of the following peaks: P_i, phophodiester, PCr, α-ATP (two peaks, amplitude ratio 1:1), γ-ATP (two peaks, amplitude ratio 1:1) and β-ATP (three peaks, amplitude ratio 1:2:1). In all cases, relative amplitudes were corrected for partial saturation because of the short repetition time relative to the longitudinal time constant T_1, which has been shown not to change with exercise of this intensity (24).

To calculate [PCr] and [ADP], several assumptions were necessary. In adults, the concentration of total creatine ([TCr]) was assumed to be 45 mM (25), and [ATP] was assumed to be 8.2 mM (26). In children, limited data suggests that [ATP] remains constant between 11–16 yrs (27) and is similar to adult values (28). However, no estimate of [TCr] is available for children. Eriksson & Saltin (27) demonstrated an increase in [PCr] from ~21 mM at 11 yrs to ~35 mM at 16 yrs old in boys, while Gariod et al. (28) found [PCr] to be similar in children and adults. Therefore, in the absence of reliable published estimates of [ATP] and [TCr], adult estimates were used to calculate [PCr] and [ADP] in children, which is in line with previous studies in this field (12,17,29).

To calculate [PCr], the raw P_i signal and raw PCr signal from resting ^{31}P-MR spectra were used. [PCr] + [P_i] was assumed to equal [TCr]. From this, [PCr] at rest was calculated from the ratio of the raw PCr signal to the sum of the raw P_i and PCr signals (30,31). Resting [PCr] was multiplied by the relative change in PCr to give [PCr] during exercise. [ADP] was calculated from [PCr] and pH, using equation 1 (25):

$$[ADP] = \frac{[ATP][Cr]}{[PCr][H^+]K_{CK}} \qquad \text{(equation 1)}$$

Where K_{CK} is the equilibrium constant for the creatine kinase reaction, which was assumed to be 1.66×10^9. The shift in the P_i peak relative to the PCr peak was used to calculate intracellular pH (32).

NIRS Measurements

Changes in deoxygenated haemoglobin/myoglobin were measured using a commercially available near-infrared spectroscopy device (NIRO-300, Hamamatsu Photonics KK). The NIRS system used in this investigation uses spatially resolved spectroscopy, and consists of pulsed laser diodes transmitting light at wavelengths 775, 810, 850, and 910 nm through a fibre bundle to the skin. The laser light is emitted at a pulse width of 100ns and a repetition rate of 2 kHz for each wavelength. The emitter is housed in a dark probe which also contains the detector, and thus ensures a consistent emitter-detector separation of 4 cm. Reflected and scattered light is detected by a three segment photodiode chip as described by Suzuki et al. (33). The probe housing the optodes was placed midway between the greater trochanter and lateral epicodyle of the

femur, over the vastus lateralis muscle. The probe was fixed using adhesive tape and extraneous light was excluded using layers of elastic bandage around the thigh. HHb data was interpolated to 1s intervals and expressed as a change, in arbitrary units (AU) from resting baseline.

Modeling of PCr and HHb data

PCr data, in 6 s time bins, were represented as a percent change from resting baseline. Data for each transition were filtered for outliers. Outliers were identified using a rolling mean of five previous data points – any point that lay outside four standard deviations from this local mean was deleted (6). All transitions for each subject were time-aligned and averaged and modeled using non-linear regression to fit an exponential function of the form:

$$\Delta PCr_{(t)} = PCr_{(0)} - \Delta PCr_{ss}(1 - e^{-t/\tau}) \qquad \text{(equation 2)}$$

Where $PCr_{(0)}$ is the baseline PCr concentration, ΔPCr_{ss} is the projected steady-state difference in PCr from $PCr_{(0)}$, t is time, the independent variable, and τ is the time constant for the curve (time required to reach 63% of ΔPCr_{ss}). The onset of the slow component was determined from the plateau in a plot of an iterative fit of the time constant beginning at 60 s, as previously described (6). The primary component was modeled from the start of exercise to the onset of the slow component (Figure 1). The slow component is calculated as the difference between the amplitude of the primary curve (ΔPCr_{ss}) and the average PCr over the last 30 s of exercise, and presented as a

percentage of end-exercise PCr. Finally, the amplitude of each component was represented in mM [PCr], using the calculated [PCr]. The phosphate cost of exercise ($[PCr]\cdot W^{-1}$) was calculated from end-exercise [PCr] and power output over the test.

NIRS data were interpolated to 1-s intervals and represented as a change, in arbitrary units (a.u.), from resting baseline calculated over the penultimate 30 s prior to exercise (changes in the participant's leg position immediately prior to exercise affected oxygenation during the 30 s preceding exercise) (Figure 2). Each participant's HHb profile showed an exponential-like rise following a delay at the onset of exercise, as described by other authors (31,34,35). The delay was characterized by a marked fall in HHb (increased oxygenation) in most participants. The onset of the exponential-like phase of the response was identified by two independent investigators. An exponential equation in the same form as equation 2 was fitted from the beginning of the exponential portion of the HHb response to the emergence of a "third phase". The time constant and the time delay were summed to provide a mean response time. Responses during the third phase of HHb kinetics were heterogeneous, with many participants showing a further increase in deoxygenation as described by Jones et al. (35). A number of participants, however, demonstrated a plateau or decrease in deoxygenation. The difference in deoxygenation from the end of the exponential phase to the end of exercise (calculated as the average over the final 30 s of exercise) was expressed relative to the total change in HHb.

Statistical Analysis

Results are presented as mean ± standard deviation. Each variable was tested for normal distribution using the Shapiro-Wilkes statistic. Two-by-two factorial ANOVA was used to identify group differences, with follow-up testing by planned independent two-sided t-tests (equal variances assumed) with Bonferroni correction. Resting and exercise [PCr], pH, and HHb were compared using dependent t-tests. An initial alpha level of 0.05 was used. During follow-up testing, significance was accepted at $p \leq 0.01$, due to the Bonferroni correction. Analyses were carried out using SPSS version 11.0.

Results

Participant Characteristics

Peripubertal children aged 13 ± 1 yr were compared with adults aged 24 ± 4 yrs. Boys and girls were similar in stature (boys: 1.50 ± 0.04 m, girls: 1.55 ± 0.12 m) and mass (boys: 42.6 ± 3.7 kg, girls: 44.7 ± 10.6 kg), while men were both taller (1.81 ± 0.08 m) and heavier (80.9 ± 11.8) than women (stature: 1.53 ± 0.08, mass: 54.0 ± 4.6 kg). Girls were significantly more mature than boys, (0.7 ± 1.5 YAPHV and -1.4 ± 0.1 YAPHV, respectively; $p < 0.01$).

Power output

Power output during the constant-load exercise bouts was 19 ± 4 W in men, 15 ± 1 W in women, 12 ± 2 W in girls, and 12 ± 2 W in boys. Two-by-two factorial ANOVA revealed significant main effects for age ($p < 0.01$) and sex ($p = 0.04$), and a significant

interaction effect (p=0.01). Power output was significantly higher in men than women (p=0.01) and men than boys (p<0.01), but was not different in boys and girls (p=0.53), or in women and girls (p=0.04). The overall work rate relative to the IT for P_i/PCr was 28%Δ, with no significant differences between groups. The power output did not differ from the target power output in men (18 ± 3 W, p=0.21), women (13 ± 1 W, p=0.16), boys (11 ± 2 W, p=0.32) or girls (12 ± 2 W, p=0.37).

Muscle Phosphates

The parameters of the PCr response to square-wave exercise can be seen in Table 1, with average profiles illustrated in Figure 3. The 95 % confidence intervals for the PCr time constants were ~ 6 s in all groups. The time constant and amplitude of the primary component of the response were similar across groups. End-exercise PCr was lower in females than males whether expressed in absolute terms or relative to resting baseline. The [PCr] cost of exercise was 1.5 ± 0.3 mM/W in boys, 1.8 ± 0.5 mM/W in girls, 1.0 ± 0.3 mM/W in men, and 1.6 ± 0.2 mM/W in women. ANOVA main effects demonstrated this to be significantly higher in females than males (p=0.01), and children than adults (p=0.01) with no significant interaction effect (p=0.19). Resting [ADP] was 5 ± 1 µM in boys, 6 ± 1 µM in girls, 4 ± 1 µM in men, and 4 ± 2 µM in women, which was higher in children than adults (p<0.01), with no sex (p=0.43) or interaction effects (p=0.72). End-exercise [ADP] was 38 ± 9 µM in boys, 50 ± 17 µM in girls, 37 ± 14 µM in men, and 55 ± 17 µM in women. This is significantly greater in females than males (p=0.02), but no age effects (p=0.72) or interaction effects (p=0.60) were identified.

pH

Figure 4 shows the group average pH responses for men, women, boys, and girls. Resting pH was 7.08 ± 0.02 in boys, 7.09 ± 0.02 in girls, 7.04 ± 0.02 in men, and 7.00 ± 0.02 in women. A main effect for sex was not found in resting pH ($p=0.08$), but resting pH was higher in children ($p<0.01$), and an interaction between age and sex was found ($p=0.02$). Independent t-tests revealed that resting pH was significantly higher in girls than women, ($p<0.01$) but did not find any significant differences between men and women ($p=0.01$), boys and girls ($p=0.62$), or men and boys ($p=0.04$). End-exercise pH was 6.98 ± 0.06 in boys, 6.93 ± 0.07 in girls, 6.98 ± 0.07 in men and 6.97 ± 0.03 in women, and there were no main age ($p=0.29$) or sex effects ($p=0.34$), and no interaction effects ($p=0.33$). Children's higher resting pH was reflected in a greater decline in pH in children compared with adults ($p=0.01$). The decrease in pH over the exercise bout was significant in boys (0.10 ± 0.08, $p=0.03$) and girls (0.16 ± 0.07, $p=0.01$) but not in men (0.06 ± 0.07, $p=0.11$) or women (0.03 ± 0.04, $p=0.18$).

HHb Kinetics

Muscle deoxygenation initially decreased, before increasing in an exponential-like manner for approximately 45 s. Trends toward slower responses in adults compared to children were apparent for both the time delay and the time constant (Table 2). This resulted in children having a significantly faster mean response time compared to adults. Varied responses during the final phase of the exercise bout were seen in both

adults and children (Figure 5). Thirteen participants had an upward HHb (7 children and 6 adults), 6 maintain a steady-state plateau (2 children and 4 adults) and 3 displayed a fall in HHb towards end-exercise (2 children and 1 adults).

Discussion

This is the first study to examine muscle phosphates and oxygenation during high intensity quadriceps muscle exercise in children and adults. It was hypothesized that children would display more rapid muscle PCr kinetics and a reduced muscle PCr slow component compared to adults, and that the PCr slow component would be associated with a reduced HHb slow component in children. However, none of these hypotheses were supported by the experimental results. There were no differences in PCr kinetics in children and adults, and the HHb slow component was also similar in children and adults. However, the MRT for haemoglobin/myoglobin deoxygenation was faster in children than adults, which implicates subtle age-related difference in the matching of muscle blood flow to metabolic rate at exercise onset.

Muscle Phosphates

Previous investigations of $p\dot{V}O_2$ kinetics have reported faster kinetics in young children during moderate (36,37) and high (3,37) intensity cycle ergometer and treadmill (38) exercise. In support of these findings previous investigations of PCr kinetics during the recovery from exercise have reported faster kinetics in children

compared with adults (17,29), which is indicative of a greater oxidative capacity. Current evidence supports feedback control of oxidative metabolism through ADP, with a role for PCr in buffering the rise in [ADP] (39). PCr is also thought to play a key role in transmitting the rise in [ADP] from the contractile mechanism to the mitochondria (40). The current study examined the response of some putative metabolic control substances, specifically [PCr] and [ADP]. An exponential curve was fit to the primary phase of the PCr response, and neither the time constant nor the amplitude of this curve was significantly different with age or sex. These results imply that the phosphate linked control of oxidative metabolism is similar in children and adults, and in males and females. Of interest though, is the 30% difference between the men and boys PCr time constant which while not statistically different might have biological significance. However, it is pertinent to note that the present results are consistent with an earlier study during moderate intensity exercise. Barker et al. (12) reported similar PCr kinetics in children and adults, suggesting that oxidative capacity is similar in prepubertal children and adults. Taken collectively, the phosphate-linked control of oxidative metabolism in 13-yr-old children appears to be independent of age and sex during both moderate and high intensity exercise.

Slight sex differences in [PCr] and [ADP] at rest were found. However, the magnitude of the difference – 0.3 mM between boys and girls, and 0.5 mM between men and women – was unlikely to greatly impact the control of oxidative metabolism. Further, several assumptions were made in the calculation of [PCr] and [ADP]. If any of these assumptions was inaccurate, the calculated [PCr] and [ADP] would be incorrect which a recent study has shown to have a major impact on interpreting indices of metabolic

control (25). Resting [ATP] was assumed to be 8.2 mM in both children and adults, while total creatine [TCr] was assumed to be 45 mM (25). Limited data suggest that resting [PCr] in the rectus femoris muscle might increase between 11 and 16 yrs in boys (27), although Garoid et al. (28) reported no differences in resting muscle PCr between children and adults. During exercise, [PCr] decreased, and [ADP] increased to a similar extent in children compared with adults, but end-exercise [PCr], whether expressed in absolute terms or relative to resting [PCr], was significantly lower in females compared with males. End-exercise [ADP] was also significantly greater in females.

The [PCr] cost per watt was higher in females than males, and in children than adults, suggesting either a decreased oxidative capacity or an impaired exercise efficiency in these groups. Reduced mitochondrial content is associated with a greater PCr cost for an equivalent change in exercise intensity (41), which would suggest that mitochondrial capacity is lower in children compared with adults. This is contrary to previous reports of greater oxidative capacity in children (17,29), and suggestions that oxidative capacity might be higher in women than men (42). The similar PCr kinetics in all groups in the current study also fails to support this possibility. Thus, a difference in exercise efficiency seems a more likely explanation for this finding. Sex differences in muscle efficiency have not been found for treadmill exercise in adults (43). However, sex differences in fatigue during short-term, high intensity exercise in adults are commonly reported, and have been attributed to differences in the leg vasodilatory response (44), differences in fibre-type (45), or muscle activation patterns (46). Any of these factors might have contributed to the greater PCr cost of exercise in females compared with males in the current study. The oxygen cost of supra-threshold exercise is higher in

children than adults during high-intensity exercise (47), but the causes of this are unclear. The differences in the current study seem to suggest a loss of efficiency or economy during quadriceps exercise in females compared with males, and in children compared with adults, which requires further investigation.

This is the first study to examine the slow component of the PCr response in children, and found that the amplitude of this phase of the response was not significantly different from adults, representing $23 \pm 23\%$ and $17 \pm 13\%$ of the total change in PCr respectively. An attenuated $p\dot{V}O_2$ slow component in young children compared with adults or older children is a common finding (3,37,38). Given the relationship between the $p\dot{V}O_2$ and PCr slow components, which are similar both temporally and in size (7), we hypothesized that the PCr slow component would be smaller in children than adults. The aetiology of the slow component is unresolved to date. Progressive muscle fibre recruitment, possibly increasing use of less-efficient type II muscle fibres, is often cited as a possible explanation for the slow component (48,49). Few studies have examined maturational changes in muscle fibre composition, and the findings, while inconclusive, have suggested that children may have a greater proportion of type I fibres (50). This study fails to demonstrate any difference in slow component amplitude which might support age-related differences in muscle fibre recruitment.

End-exercise pH was similar in all groups: 6.98 ± 0.06 in boys, 6.93 ± 0.07 in girls, 6.98 ± 0.07 in men and 6.97 ± 0.03 in women. While intramuscular pH depends on a number of factors, including glycolytic ATP turnover, cellular buffering (e.g. by PCr breakdown via the creatine kinase reaction), and the rate of clearance within and outside the cell,

similar end-exercise pH in children and adults provides indirect evidence that anaerobic glycolysis was contributing to the energy demands of exercise to a similar degree in these groups. It is a longstanding notion that children have lower blood lactate levels following intense short-term exercise, such as a Wingate anaerobic test (51), as well as submaximal cycling exercise (52). This observation has been cited as indirect evidence for a reduced glycolytic capacity in children. For maximal exercise tasks, previous ^{31}P-MRS studies have reported lower pH in adults compared with children (15-17). However, several recent investigations have found that clearance of lactate or protons from the cell is faster in children than adults (29,51,53). Both intracellular pH and blood lactate concentrations depend upon a balance of intracellular reactions (54) as well as transport of lactate and buffering in the bloodstream. The results of this study cannot address the balance of these factors, but imply that the overall balance is similar in children and adults during high intensity quadriceps exercise.

Muscle Oxygenation

To the best of our knowledge, this study is the first to examine the kinetics of HHb in children. In both children and adults, the response was triphasic, as previously reported for adults (19), with a delay preceding an apparently exponential increase in deoxygenation. Finally, a plateau or more gradual increase or decrease in oxygenation was seen. Initially, a decrease in HHb was seen in all participants, which is indicative of surplus oxygen delivery relative to demand within the microcirculation. This might be the result of the muscle pump, and/or might reflect mechanical expansion of small blood vessels with the onset of muscle contractions. The duration of this phase was

shorter in females compared with males. These novel findings might indicate that the matching of delivery to oxygen demand is more precise in males. Future studies should use multiple NIRS probes to investigate possible age and sex differences in muscle oxygen delivery and extraction heterogeneity.

Following this delay, an exponential-like increase in HHb was seen in all participants. There were no differences between groups in the speed of this response, as indicated by similar time constants for all groups. However, the MRT encompasses both the duration of the delay and the time constant for the exponential increase in HHb, and is a more functionally relevant measure of the speed of muscle deoxygenation kinetics. The MRT was significantly faster in children (22 ± 4 s) than adults (27 ± 7 s). In adults, there is some suggestion that greater oxygen delivery, as in upright compared to supine cycling, results in slower HHb kinetics (35). Thus children's faster kinetics may reflect an impaired oxygen delivery at exercise onset. However, it is likely that age differences in oxygen extraction also play an important role in determining the speed of the HHb response (19,55). A faster muscle deoxygenation in children suggests that children are less able to match oxygen delivery to the fuel demands of the working muscle at exercise onset.

The third phase of muscle deoxygenation was particularly interesting in children and adults, since considerable variation was evident not only in the magnitude of the change, but also in the direction of the change. In some individuals, HHb continued to increase after the exponential phase, sometimes by over 100%, while other responses showed a plateau or even a decrease. This observation reflects the considerable

interindividual variability in the ability to match oxygen delivery to oxygen demand – those who were able to maintain a plateau had the most precise matching. This phase of the response has been the focus of scant discussion in the literature, but is typically reported as either a plateau or a slow-component-like increase during cycling exercise (34,35). The amplitude of this phase of the response was expected to be lower in children than adults, based on a longitudinal study by Koch (18) which used Xenon-133 labelling to examine muscle blood flow during cycling exercise in children. Koch found that muscle blood flow decreased from 12 to 14 yrs in boys, and proposed a greater muscle blood flow in young children. However, in the present study, changes in HHb during the final phase were independent of age and sex. The nature and causes of the varied response in both children and adults warrant further investigation.

Considerations

There are several limitations to this study that merit discussion. First, the potential confounds of activity and maturation must be discussed. All individuals recruited for the study participated in recreational activities including soccer, dancing, jogging, and martial arts. However, it was not practical to quantify habitual physical activity. Differences in training or fitness are associated with changes in PCr kinetics as well as muscle blood flow (56,57). Another potential confound was the maturational status of the child participants. The boys, at 1.4 ± 0.1 yrs before peak height velocity, and the girls, at 0.7 ± 1.5 yrs after peak height velocity, were probably experiencing many of the physiological changes related to puberty, including hormonal changes. In humans, particularly males, a number of changes in muscle size and morphology are associated

with puberty, and there is some suggestion that hormones might affect muscle structure and function (58).

Exercise intensity was also assumed to be comparable in all individuals. All participants were assigned a load above their IT for P_i/PCr. However, critical power (CP), which demarcates the boundary between heavy and severe exercise (59), was not determined in this study. The [PCr] and pH responses to sub-CP exercise differ profoundly from [PCr] and pH responses to exercise above CP (59). Children have a smaller interval between the IT and CP, so it was important to minimize the risk of setting exercise intensity above CP.

In conclusion, we hypothesized that during high intensity quadriceps exercise, children would have faster PCr kinetics, a reduced PCr slow component amplitude, and a greater HHb slow component than adults. However, this study found that during exercise at an intensity greater than the IT for P_i/PCr, children and adults show similar PCr kinetics. A PCr slow component was identified in children for the first time, and found to be similar in amplitude to the slow component in adults. Consistent with the findings of Barker et al. (12) for moderate-intensity exercise, metabolic control appears to be adult-like in 13 yr old children during high intensity exercise. The kinetics of muscle HHb, measured using NIRS, were significantly faster in children than adults, implying that oxygen delivery is less closely matched to oxygen extraction in children at the onset of exercise. The results of this study suggest that skeletal muscle metabolism is similar in children and adults during high intensity exercise, and that future research should examine age-related differences in muscle oxygenation during exercise.

Acknowledgements

References

1.   Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK, Wagner PD. Muscle O2 uptake kinetics in humans: implications for metabolic control. J Appl Physiol 1996;80(3):988-998.

2.   Poole DC, Jones AM. Towards an understanding of the mechanistic bases of VO2 kinetics. In: Jones AM, Poole DC, editors. Oxygen Uptake Kinetics in Sport, Exercise and Medicine. Oxon: Routledge; 2005. p 294-328.

3.   Fawkner SG, Armstrong N. Longitudinal changes in the kinetic response to heavy-intensity exercise in children. J Appl Physiol 2004;97:460-466.

4.   Armstrong N, Barker A. Oxygen uptake kinetics in children and adolescents: a review. Pediatr Exerc Sci 2009;21:130-147.

5.   Poole DC, Barstow TJ, McDonough P, Jones AM. Control of oxygen uptake during exercise. Med Sci Sports Exerc 2008;40(3):462-474.

6.   Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR, Whipp BJ. Dynamic asymmetry of phosphocreatine concentration and O(2) uptake between the on- and off-transients of moderate- and high-intensity exercise in humans. J Physiol (Lond) 2002;541(Pt 3):991-1002.

7.   Rossiter HB, Ward SA, Howe FA, Kowalchuk JM, Griffiths JR, Whipp BJ. Dynamics of intramuscular 31P-MRS Pi peak splitting and the slow components of PCr and O2 uptake during exercise. J Appl Physiol 2002;93:2059-2069.

8.   Barstow TJ, Buchthal S, Zanconato S, Cooper DM. Muscle energetics and pulmonary oxygen uptake kinetics during moderate exercise. J Appl Physiol 1994;77(4):1742-1749.

9.   Barker AR, Welsman JR, Fulford J, Welford D, Williams CA, Armstrong N. Muscle phosphocreatine and pulmonary oxygen uptake kinetics in children at the onset and offset of moderate intensity exercise. Eur J Appl Physiol 2008;102(6):727-738.

10.   Haseler LJ, Kindig CA, Richardson RS, Hogan MC. The role of oxygen in determining phosphocreatine onset kinetics in exercising humans. J Physiol 2004;558(Pt 3):985-992.

11.   Wilkerson DP, Berger NJ, Jones AM. Influence of hyperoxia on pulmonary O_2 uptake kinetics following the onset of exercise in humans. Respir Physiol Neurobiol 2006;153(1):92-106.

12.   Barker AR, Welsman JR, Fulford J, Welford D, Armstrong N. Muscle phosphocreatine kinetics in children and adults at the onset and offset of moderate intensity exercise. J Appl Physiol 2008.

13.   Hogan MC, Richardson RS, Haseler LJ. Human muscle performance and PCr hydrolysis with varied inspired oxygen fractions: a ^{31}P-MRS study. J Appl Physiol 1999;86(4):1367-1373.

14.   Jubrias SA, Crowther GJ, Shankland EG, Gronka RK, Conley KE. Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle in vivo. J Physiol 2003;553(Pt 2):589-599.

15.   Kuno S, Takahashi H, Fujimoto K, Akima H, Miyamaru M, Nemoto I, Itai Y, Katsuta S. Muscle metabolism during exercise using phosphorus-31 nuclear magnetic resonance spectroscopy in adolescents. Eur J Appl Physiol Occup Physiol 1995;70(4):301-304.

16. Zanconato S, Buchthal S, Barstow TJ, Cooper DM. 31P-magnetic resonance spectroscopy of leg muscle metabolism during exercise in children and adults. J Appl Physiol 1993;74(5):2214-2218.

17. Taylor DJ, Kemp GJ, Thompson CH, Radda GK. Ageing: Effects on oxidative function of skeletal muscle in vivo. Mol Cell Biochem 1997;V174(1):321.

18. Koch G. Muscle blood flow after ischemic work and during bicycle ergometer work in boys aged 12 years. Acta Paediatr Belg 1974;28(Supplement):29.

19. DeLorey DS, Kowalchuk JM, Paterson DH. Effects of prior heavy-intensity exercise on pulmonary O2 uptake and muscle deoxygenation kinetics in young and older adult humans. J Appl Physiol 2004;97(3):998-1005.

20. Mirwald RL, Baxter-Jones AD, Bailey DA, Beunen GP. An assessment of maturity from anthropometric measurements. Med Sci Sports Exerc 2002;34(4):689-694.

21. Barker A, Welsman J, Welford D, Fulford J, Williams C, Armstrong N. Reliability of 31P-magnetic resonance spectroscopy during an exhaustive incremental exercise test in children. Eur J Appl Physiol 2006;98(6):556-565.

22. Naressi A, Couturier C, Castang I, de Beer R, Graveron-Demilly D. Java-based graphical user interface for MRUI, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals. Comput Biol Med 2001;31(4):269-286.

23. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. J Magn Reson 1997;129(1):35-43.

24. Cettolo V, Piorico, C, Francescato MP. T_1 measurement of ^{31}P metabolites at rest and during steady-state dynamic exercise using a clinical nuclear magnetic resonance scanner. Magn Reson Med 2006;55(3):498-505.

25. Kemp GJ, Meyerspeer M, Moser E. Absolute quantification of phosphorus metabolite concentrations in human muscle *in vivo* by ^{31}P MRS: a quantitative review. NMR Biomed 2007;20(6):555-565.

26. Harris RC, Hultman E, Nordesjo LO. Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. Scand J Clin Lab Invest 1974;33(2):109-120.

27. Eriksson O, Saltin B. Muscle metabolism during exercise in boys aged 11 to 16 years compared to adults. Acta Paediatr Belg 1974;28 suppl:257-265.

28. Gariod L, Binzoni T, Ferretti G, Le Bas JF, Reutenauer H, Cerretelli P. Standardisation of 31phosphorus-nuclear magnetic resonance spectroscopy determinations of high energy phosphates in humans. Eur J Appl Physiol Occup Physiol 1994;68(2):107-110.

29. Ratel S, Tonson A, Le Fur Y, Cozzone P, Bendahan D. Comparative analysis of skeletal muscle oxidative capacity in children and adults: a ^{31}P-MRS study. Appl Physiol Nutr Metab 2008;33(4):720-727.

30. Lanza IR, Befroy DE, Kent-Braun JA. Age-related changes in ATP-producing pathways in human skeletal muscle in vivo. J Appl Physiol 2005;99(5):1736-1744.

31. Forbes SC, Raymer GH, Kowalchuk JM, Thompson RT, Marsh GD. Effects of recovery time on phosphocreatine kinetics during repeated bouts of heavy-intensity exercise. Eur J Appl Physiol 2008;103(6):665-675.

32. Taylor DJ, Bore PJ, Styles P, Gadian DG, Radda GK. Bioenergetics of intact human muscle: A 31P nuclear magnetic resonance study. Mol Biol Med 1983;1:77-94.

33. Suzuki S, Takasaki S, Ozaki T, Kobayashi Y. A tissue oxygenation monitor using NIR spatially resolved spectroscopy. Proc SPIE 1999;3597:582-592.

34. DeLorey DS, Kowalchuk JM, Paterson DH. Relationship between pulmonary O2 uptake kinetics and muscle deoxygenation during moderate-intensity exercise. J Appl Physiol 2003;95(1):113-120.

35. Jones AM, Berger NJ, Wilkerson DP, Roberts CL. Effects of "priming" exercise on pulmonary O2 uptake and muscle deoxygenation kinetics during heavy-intensity cycle exercise in the supine and upright positions. J Appl Physiol 2006;101(5):1432-1441.

36. Fawkner SG, Armstrong N, Potter CR, Welsman JR. Oxygen uptake kinetics in children and adults after the onset of moderate-intensity exercise. J Sports Sci 2002;20:319-326.

37. Armon Y, Cooper DM, Flores R, Zanconato S, Barstow TJ. Oxygen uptake dynamics during high-intensity exercise in children and adults. J Appl Physiol 1991;70(2):841-848.

38. Williams CA, Carter H, Jones AM, Doust JH. Oxygen uptake kinetics during treadmill running in boys and men. J Appl Physiol 2001;90:1700-1706.

39. Glancy B, Barstow T, Willis WT. Linear relation between time constant of oxygen uptake kinetics, total creatine, and mitochondrial content in vitro. Am J Physiol Cell Physiol 2008;294(1):C79-87.

40. Bessman SP, Geiger PJ. Transport of energy in muscle: the phosphorylcreatine shuttle. Science 1981;211(4481):448-452.

41. Korzeniewski B, Zoladz JA. Factors determining the oxygen consumption rate (VO2) on-kinetics in skeletal muscles. Biochem J 2004 379(3):703-710.

42. Kent-Braun JA, Ng AV. Skeletal muscle oxidative capacity in young and older women and men. J Appl Physiol 2000;89(3):1072-1078.

43. Woo JS, Derleth C, Stratton JR, Levy WC. The influence of age, gender, and training on exercise efficiency. J Am Coll Cardiol 2006;47(5):1049-1057.

44. Parker BA, Smithmyer SL, Pelberg JA, Mishkin AD, Herr MD, Proctor DN. Sex differences in leg vasodilation during graded knee extensor exercise in young adults. J Appl Physiol 2007;103(5):1583-1591.

45. Wust RC, Morse CI, de Haan A, Jones DA, Degens H. Sex differences in contractile properties and fatigue resistance of human skeletal muscle. Exp Physiol 2008;93(7):843-850.

46. Clark BC, Collier SR, Manini TM, Ploutz-Snyder LL. Sex differences in muscle fatigability and activation patterns of the human quadriceps femoris. Eur J Appl Physiol 2005;94(1-2):196-206.

47. Zanconato S, Cooper DM, Armon Y. Oxygen cost and oxygen uptake dynamics and recovery with 1 min of exercise in children and adults. J Appl Physiol 1991;71(3):993-998.

48. Krustrup P, Soderlund K, Mohr M, Bangsbo J. The slow component of oxygen uptake during intense, sub-maximal exercise in man is associated with additional fibre recruitment. Pflugers Arch 2004;447(6):855-866.

49. Endo MY, Kobayakawa M, Kinugasa R, Kuno S, Akima H, Rossiter HB, Miura A, Fukuba Y. Thigh muscle activation distribution and pulmonary VO2 kinetics during moderate, heavy, and very heavy intensity cycling exercise in humans. Am J Physiol Regul Integr Comp Physiol 2007;293(2):R812-820.

50. Jansson E. Age-related fibre type changes in human skeletal muscle. In: Maughan RJ, editor. Biochemistry of Exercise IX. Champaign, IL: Human Kinetics; 1996. p 297-307.

51. Beneke R, Hutler M, Jung M, Leithauser RM. Modeling the blood lactate kinetics at maximal short-term exercise conditions in children, adolescents, and adults. J Appl Physiol 2005;99(2):499-504.

52. Pianosi P, Seargeant L, Haworth JC. Blood lactate and pyruvate concentrations, and their ratio during exercise in healthy children: developmental perspective. Eur J Appl Physiol Occup Physiol 1995;71(6):518-522.

53. Beneke R, Hutler M, Leithauser RM. Anaerobic performance and metabolism in boys and male adolescents. Eur J Appl Physiol 2007;101(6):671-677.

54. Robergs RA, Ghiasvand F, Parker D. Biochemistry of exercise-induced metabolic acidosis. Am J Physiol Regul Integr Comp Physiol 2004;287(3):R502-516.

55. Ferrari M, Mottola L, Quaresima V. Principles, techniques, and limitations of near infrared spectroscopy. Can J Appl Physiol 2004;29(4):463-487.

56. Jones AM, Wilkerson DP, Berger NJ, Fulford J. Influence of endurance training on muscle [PCr] kinetics during high-intensity exercise. Am J Physiol Regul Integr Comp Physiol 2007;293(1):R392-401.

57. Neary JP, McKenzie DC, Bhambhani YN. Effects of short-term endurance training on muscle deoxygenation trends using NIRS. Med Sci Sports Exerc 2002;34(11):1725-1732.

58. Cooper DM, Barstow TJ. Magnetic resonance imaging and spectroscopy in studying exercise in children. Exerc Sport Sci Rev 1996;24:475-499.

59. Jones AM, Wilkerson DP, DiMenna F, Fulford J, Poole DC. Muscle metabolic responses to exercise above and below the "critical power" assessed using 31P-MRS. Am J Physiol Regul Integr Comp Physiol 2008;294(2):R585-593.

Table 1

Parameters of the PCr response to high intensity exercise in children and adults

	Boys (n=6)	Girls (n=5)	Men (n=6)	Women (n=5)	ANOVA
Rest [PCr] (mM)	40.1 ± 0.6	39.8 ± 0.6	41.3 ± 0.8	40.8 ± 1.7	[a]p=0.02* [b]p=0.37
PCr τ (s)	31 ± 10	31 ± 10	44 ± 20	29 ± 14	[a]p=0.37 [b]p=0.25
Primary amplitude (mM)	-15.6 ± 4.0	-16.5 ± 3.8	-14.7 ± 3.6	-19.6 ± 3.5	[a]p=0.49 [b]p=0.09
Primary amplitude (% change)	-39 ± 10	-40 ± 9	-36 ± 9	-48 ± 8.6	[a]p=0.08 [b]p=0.67
Slow component (mM)	2.4 ± 0.4	5.4 ± 3.4	3.3 ± 2.0	3.3 ± 2.6	[a]p=0.53 [b]p=0.15
Slow component (% end-exercise)	11 ± 3	36 ± 30	16 ± 12	19 ± 14	[a]p=0.47 [b]p=0.09
End-exercise [PCr] (mM)	22.1 ± 3.9	17.8 ± 5.3	23.2 ± 4.9	17.9 ± 3.3	[a]p=0.76 [b]p= 0.02*
End-exercise PCr (%)	55 ± 10	45 ± 14	56 ± 11	44 ± 8	[a]p=0.96 [b]p= 0.03*

Data are presented as mean ± SD. Two-by-two factorial ANOVA results ($p < 0.05$): [a]significant main effect for age; [b]significant main effect for sex; *significant difference, $p < 0.05$

Table 2

Parameters of the deoxyhaemoglobin/myoglobin response to high intensity exercise in children and adults

	Boys (n = 6)	Girls (n = 5)	Men (n = 6)	Women (n = 5)	ANOVA
Time delay (s)	14 ± 2	10 ± 1	15 ± 2	13 ± 3	[a]p=0.08 [b]p=0.01*
τ (s)	9 ± 2	10 ± 4	14 ± 3	13 ± 9	[a]p=0.09 [b]p=0.80
MRT (s)	23 ± 2	20 ± 5	29 ± 2	25 ± 10	[a]p=0.02* [b]p=0.17
Final phase (%)	-7 ± 56	49 ± 42	16 ± 35	38 ± 23	[a]p=0.74 [b]p=0.06

Data are presented as mean ± SD. Two-by-two factorial ANOVA results ($p<0.05$): [a]significant main effect for age; [b]significant main effect for sex; *significant difference, $p<0.05$

FIGURE LEGENDS

Figure 1. Representative PCr response, with fit portion of the curve (solid line) and projection of the amplitude (dotted line) superimposed

Figure 2. Fitted HHb response for a representative participant (with curve superimposed) over 60 s of rest and 100 s of exercise

Figure 3. PCr response (average responses for each group) over two minutes of rest and seven minutes of exercise

Figure 4. Mean pH responses for male and female children and adults during two minutes of rest and seven minutes of high intensity exercise

Figure 5. HHb profiles from two men (middle and bottom) and one girl (top) during one minute of rest and seven minutes of exercise.