Insights Into Developmental Muscle Metabolism Through the Use of $^{31}\text{P}$-Magnetic Resonance Spectroscopy: A Review

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$^{31}\text{P}$-phosphorous-magnetic resonance spectroscopy ($^{31}\text{P}$-MRS) has become an extremely valuable technique to investigate changes in muscle metabolism noninvasively and in vivo. The purpose of this article is to critically review how $^{31}\text{P}$-MRS has contributed to current understanding of muscle metabolic function in healthy children and adolescents. In addition, an overview of the basic principles of $^{31}\text{P}$-MRS and its application to the study of muscle metabolism is provided and discussed in relation to child-specific methodological concerns when using this technique.

Studies by Eriksson and colleagues using the muscle biopsy technique some 35 years ago provided the first direct insights into pediatric exercise metabolism (see Ref. 18 for a review). Following maximal cycling exercise, a 1.6 to twofold increase in lactate and breakdown of phosphocreatine (PCr) in the rectus femoris muscle in a small group of boys between the ages of 11–16 years was documented, with no age-related changes in the hydrolysis of adenosine triphosphate (ATP). In addition, the activity of the glycolytic rate-limiting enzyme phosphofructokinase was lower in the 11 year old boys than adults. While these data are often cited to support the concept of an age-related development of anaerobic metabolism from childhood into adolescence, no biopsy data have subsequently been published to confirm or refute the muscle ATP, PCr and lactate responses during exercise, and subsequent studies of glycolytic enzymatic activities failed to corroborate Eriksson’s findings (9, 21).

Due to the ethical constraints of using the biopsy technique with healthy children, the bulk of studies conducted since Eriksson’s seminal work have employed child friendly noninvasive indices of muscle metabolism such as blood acid-base and lactate profiles (9, 44) and the respiratory exchange ratio to quantify carbohydrate and lipid substrate utilization (47). The major limitation of these methods however, is that they are removed from the muscle, provide only an indirect insight into muscle metabolism and are thus open to methodological criticisms.
To progress knowledge a number of studies have used the technique of $^{31}$P-phosphorous magnetic resonance spectroscopy ($^{31}$P-MRS). $^{31}$P-MRS permits in vivo interrogation of the muscle phosphates (PCr, ATP and inorganic phosphate, Pi) and pH during rest and exercise at a high sampling resolution (<10 s) under noninvasive conditions, and is ideally suited for studying muscle metabolism directly in young people. The purpose of this paper is to provide a critical review of how $^{31}$P-MRS has shaped current understanding of pediatric exercise metabolism in healthy children and adolescents. Before addressing this issue however, an overview of the basic principles of $^{31}$P-MRS will be presented alongside indices of muscle metabolism that can be derived from the $^{31}$P-MRS data. Where appropriate, child-specific and general methodological issues that require consideration when using $^{31}$P-MRS will be addressed.

$^{31}$P-MRS and the Study of Muscle Metabolism

Basic Principles of $^{31}$P-MRS

The technique of $^{31}$P-MRS is founded on nuclear magnetic resonance (NMR). Detailed texts are available to the interested reader (21, 39, 55) and only the basics will be summarized here. The phenomenon of NMR can be induced on any nucleus that possesses a positive or negative charge (e.g., $^{31}$P, $^1$H). When placed inside a magnetic field (B$_0$) the magnetic properties of the nucleus align against B$_0$ in one of two energy states: spin-up (low state) and spin-down (high state). At equilibrium a net excess of nuclei are positioned in the spin-up energy state—this creates a net magnetization vector (NMV) in the direction of the spin-up energy state (Figure 1a). On a 3-dimensional axis where B$_0$ is constrained to the longitudinal axis (Z), the NMV aligns itself with the Z axis and resonates on the X-Y planes due to the intrinsic angular momentum of the nucleus (Figure 1b). This angular momentum is termed the precessional frequency and is unique to the environment of a given nucleus. This forms the basis of NMR’s ability to identify nuclei of different molecular environments as different precessional frequencies are produced by the nuclei within different compounds (e.g., $^{31}$P in PCr and P$_i$).

To transform this compound specific resonance frequency into a spectrum (i.e., the spectra plot), a radio frequency pulse of exact frequency to the processional frequency of the nuclei (termed Lamour frequency) is applied and some nuclei in the spin-up phase gain energy (excitation) and move to the spin-down state. This process of nuclei moving across the energy states is called resonance and results in the NMV shifting from the longitudinal to the transverse plane (X axis), which induces a signal in a receiver coil which is positioned over the muscle of interest (Figure 1c). After the radio frequency pulse is removed (relaxation), the NMV realigns itself to B$_0$ in the longitudinal plane (termed T$_1$ recovery) whereas the NMV in the transverse plane decays (termed T$_2$ decay; Figure 1d). During this process the signal in the receiver coil decreases, which is termed the free induction decay (FID). The FID is then converted, via a Fourier transformation, into an intensity frequency spectrum, which contains the peak intensities for the frequency characteristics (expressed as a parts per million) of the molecules containing the ‘excited-relaxed’ nuclei. This is shown in Figure 2 where for $^{31}$P the resonances of P$_i$, PCr and ATP (with three peaks: $\gamma$, $\alpha$ and $\beta$) are observed in a conventional
spectrum of human muscle under resting conditions. Figure 2 also shows that not all the muscle phosphates (e.g., adenosine diphosphate, ADP) involved in energy metabolism are visible in the spectrum. This is due to the poor signal-to-noise properties of the $^{31}$P-MRS, which requires the $^{31}$P compound to have a concentration of ~0.5 mmol·L$^{-1}$ or above.

**Quantification of the Muscle Phosphates**

The spectra areas shown in Figure 2 do not provide an absolute concentration of the $^{31}$P compounds—they provide a relative signal of the $^{31}$P compounds within a given volume of tissue that consists of skin, adipose and muscle. If absolute concentrations of the muscle phosphates are desired, a common technique is to use the assumption that at rest muscle [ATP] (brackets denote concentration) is equal to 8.2 mmol·L$^{-1}$ (27), which is termed internal reference quantification. Muscle [P] and [PCr] can then be determined from the ratio of P/ATP and PCr/ATP respectively, using the β ATP resonance. The assumption behind this procedure is that the muscle [ATP] at rest does not differ markedly between individuals, nor between muscle fiber types, which appears reasonable at least in adults (27).

An advantage of quantifying absolute [PCr], [P] and [ATP] is that muscle [ADP] (μmol·L$^{-1}$), considered to be the principal feedback controller of oxidative
phosphorylation (11), can be estimated through rearranging the creatine kinase equation:

$$[\text{ADP}] = \frac{[\text{ATP}] \times [\text{Cr}]}{(1.66 \times 10^9) \times (10^{-\text{pH}}) \times [\text{PCr}]} \quad \text{Equation 1}$$

where, $1.66 \times 10^9$ is the equilibrium constant for creatine kinase (49). The assumptions behind this calculation are that muscle [ATP] remains constant during exercise, that the muscle total creatine content ([TCr]) = 42 mmol·L$^{-1}$, from which [Cr] can be determined using [TCr]-[PCr], and that the creatine kinase equilibrium remains constant during exercise, which is questionable for pH changes < 6.9 (24). Under this set of assumptions, changes in muscle [ADP] are entirely dependent on cellular pH and [PCr].

As highlighted earlier, data concerning muscle [ATP] in young people are restricted to a single study of a small group of boys aged between 11–16 years (19). This study found muscle [ATP] in the rectus femoris muscle to be age-invariant at ~5 mmol·kg$^{-1}$ wet mass (~ 7.4 mmol·L$^{-1}$, see endnote 1), which appears to support the use of the 8.2 mmol·L$^{-1}$ reference value. However, as these data are taken from a single study with poorly defined participants (no data on females or adults, lack of information regarding physical activity and biological maturity), they are by no means conclusive.
To document the developmental changes of resting muscle [ATP] and [PCr] from childhood into adulthood, Gariod et al. (22) employed an external 31P-MRS reference technique in 13 children aged 10.9 years and ten adults aged 28.8 years. This study found no significant differences in muscle [ATP] (13.6 vs. 11.9) and [PCr] (47.1 vs. 44.7), expressed as standardized arbitrary units relative to the external reference, between the children and adults respectively. As no systematic differences exist between the calibrated 31P-MRS and muscle biopsy methods to determine muscle [ATP] at rest (27), these data appear to corroborate the muscle biopsy data published by Eriksson and Saltin (19). That is, muscle [ATP] appears constant at adult-like values in children above 10 years of age. However, the studies by Eriksson and Saltin (19) and Gariod et al. (22) present conflicting data for muscle [PCr]—the latter found muscle [PCr] at rest to be similar between children and adults, whereas the former noted a 60% increase between the ages of 11–16 years (~15–24 mmol·kg⁻¹ wet mass). As the goal of assuming a resting muscle [ATP] at rest is to estimate absolute muscle [PCr] and [Pi], the experimenter is still left with the problem of 'confirming' their resting [PCr] against two studies that provide different conclusions. As a consequence, until robust conclusions can be drawn with regard to the development of resting muscle [ATP], [PCr] and [Pi] from childhood to adulthood, the assumptions required to estimate absolute metabolite concentrations using 31P-MRS in young people remain to be validated. Consequently, any inferences regarding absolute muscle metabolic changes (e.g., [ADP]) or quantitative analyses (e.g., estimating the maximal rate of oxidative [ATP] flux, see later) must be interpreted with caution in young people.

An alternative option that potentially has greater application to young people is to express the muscle phosphates as a ratio, e.g., muscle P to PCr (P/PCr). The advantages of this expression are that it does not require absolute quantification of P, and PCr and has been shown to approximate changes in the cellular [ADP] under specific conditions (11, 28). Indeed, this relationship can be predicted from equation 1 above, as assuming a constant [ATP] and pH, and that the increase in [Pi] is proportional to the increase in [Cr] during exercise (11, 28), the dynamics of muscle [ADP] can be evaluated using the P/PCr expression under these conditions:

\[
[ADP] = \frac{[ATP] \times [Cr]}{(1.66 \times 10^9) \times (10^{-pH}) \times [PCr]} \rightarrow ADP \approx \frac{P_i}{PCr}
\]  

Equation 2

That [ATP] and pH must remain constant means the P/PCr expression mirrors muscle ADP most accurately during light to moderate exercise (below the cellular ‘thresholds’, see later). However, this relationship is questionable during high-intensity exercise as the fall in cellular pH further modulates ADP relative to oxidative ATP flux via the creatine kinase reaction. Given the importance of muscle ADP as a metabolic feedback controller to stimulate oxidative phosphorylation (11, 54) however, the ratio of P/PCr against power output has great utility to explore developmental changes in mitochondrial function.

**Determination of Intracellular pH**

The 31P spectrum can also be used to quantify cellular pH to within 0.05 units accuracy (21). This is due to the specific sensitivity of the 31P spectra peaks to
pH over the physiological range. Moon and Richards (41) demonstrated that the resonance position of muscle $P_i$ relevant to a stable marker was linear between ~6.4 and 7.4 pH units, which spans the physiological range for muscle pH under resting and exercise conditions. As the muscle PCr resonance is insensitive to pH over this range, the position of the $P_i$ peak relative to muscle PCr may be used to quantify pH using the calibration equation:

$$\text{pH} = 6.75 + \log \frac{\sigma - 3.27}{5.96 - \sigma}$$

Equation 3

where, $\sigma$ represents the chemical shift in ppm between the $P_i$ and PCr resonance peaks. Acidification of the muscle results in the movement of the $P_i$ resonance toward the PCr peak, whereas for alkalosis, the $P_i$ peak displaces away from the PCr peak. As the resonance detected from the $^{31}$P-MRS spectrum predominantly reflects the changes in the muscle phosphates in the cytosol (21), the determination of pH using the $P_i$ resonance shift reflects the pH of the cytoplasm and is termed intracellular pH.

$^{31}$P-MRS and Muscle Metabolism During Exercise

It is widely accepted that $^{31}$P-MRS is the ‘gold-standard’ method for studying muscle energetics noninvasively under rest and exercise conditions. The advantages of using this technique in children are well documented (2, 14). However, the technique is open to practical and methodological limitations. Firstly, accessibility to an MR scanner, the large financial cost of testing sessions, and the requirement for trained personnel, limit its widespread use. Moreover, appropriate exercise ergometers are invariably custom made, demanding further resources and pilot work. There is also the concern that exercise performed inside the bore of an MR scanner lacks validity to the ‘real-world’. While exercise regimes recruiting a large muscle mass (e.g., calf and quadriceps) are increasingly common, the characteristics of muscle contraction (e.g., concentric and eccentric components, frequency) and body position (supine or prone) may limit direct extrapolation to upright whole-body exercise. However, as will be detailed below, the qualitative and quantitative characteristics of the muscle metabolic responses observed during exercise performed in an MR scanner closely resemble those observed during upright whole-body exercise, suggesting an acceptable degree of ‘real world’ applicability. Experimenters should be mindful that young people may be apprehensive about exercising within the constraints of the magnet bore and that the exercise protocol is likely to pose the challenge of learning a new pattern of movement. It is therefore highly recommended that participants are given time to become comfortable while lying inside the MR scanner bore and rigorously habituated to the ergometer and test protocols. In our experience, some children may require up to three separate 20 min sessions to be compliant with the test protocol and ready to perform the exercise test under experimental conditions.

Incremental Exercise to Exhaustion

Since the application of the $^{31}$P-MRS technique to study muscle metabolism in humans, one of the most commonly employed protocols is the incremental test to exhaustion. Chance et al. (11) developed the powerful utility of using the expression of muscle $P_i$/PCr against power output during incremental exercise, and given its
close relationship with muscle ADP, reasoned this expression to provide an index of mitochondrial function. That is, a muscle with a greater oxidative capacity will require a lower change in P/PCr for a given increment in power output, as a lower modulation of muscle ADP will be required to signal an increased rate of oxidative ATP synthesis to satisfy the rate of ATP hydrolysis in the muscle (11, 54). In contrast, in de-conditioned muscle with a lower oxidative capacity, such as that brought about by physical inactivity or disease, a greater change in P/PCr against power output is evident, as a greater perturbation of the muscle phosphate stores is required to stimulate oxidative metabolism through increasing the ADP signal in an attempt to satisfy the rate of ATP turnover within the muscle (see Figure 3a).

![Figure 3 — Schematic of the P/PCr response in muscle with a high (continuous lines) and low (broken lines) oxidative capacity (A). The intracellular thresholds for muscle P/PCr (o) and pH (∗) are shown in B for a 9 year old boy during incremental quadriceps exercise.](image)

Similar to the metabolic thresholds that are well documented during whole body exercise (e.g., blood lactate threshold), an intracellular threshold (IT) for muscle P/PCr (IT<sub>P/PCr</sub>) and pH (IT<sub>pH</sub>) is present at the muscle level (Figure 3b). It has been demonstrated both under control and experimental conditions (e.g., sodium bicarbonate administration), that the IT<sub>P/PCr</sub> and IT<sub>pH</sub> occur at a power output similar to the blood lactate (30) and ventilatory (51) thresholds. The metabolic ITs have been
shown to occur at a higher power output (classical ‘right-ward shift’) following a period of endurance training (33), and to correlate highly with the mitochondrial enzyme citrate synthase in adult muscle (12). Therefore, the metabolic ITs are recognized as an in vivo assessment of the oxidative capacity of the muscle and therefore mitochondrial function.

A common interpretation of the rapid changes in the muscle Pi/PCr and pH following the metabolic ITs is that the anaerobic energy contribution following this transition point is markedly accelerated (34, 57). While this interpretation is acceptable based upon the accelerated breakdown of muscle PCr and accumulation of P, following the IT, this explanation for the decline in muscle pH is misleading. Rather, cellular pH reflects a net balance of the processes involved in cellular H\(^+\) status, such as production (anaerobic glycolysis), buffering (PCr, P, and bicarbonate) and efflux (lactate-H\(^+\) cotransporter, diffusion gradients) mechanisms (48). Therefore, while cellular pH may provide an insight into the anaerobic glycolytic ATP flux during exercise, it by no means provides a direct measure, and should be interpreted as a net balance of the factors influencing cellular H\(^+\).

A recent study has demonstrated that an incremental protocol to exhaustion provides reproducible measures of muscle metabolic function in children (3). Following a thorough habituation to the exercise protocol, 14 children (7 boys, 7 girls) aged 11–12 years completed three repeat quadriceps incremental tests to exhaustion which occurred after ~7–12 min. Out of the 42 tests completed, an ITPi/PCr and ITpH was observed in 93% and 81% of the metabolic profiles respectively, with a typical error of ~10% coefficient of variation (CV; equivalent to 0.9 W) across the three trials. Exercise performance, as determined by the peak power achieved during exercise, also showed good reproducibility having a CV of 12% (equivalent to 1.8 W with peak power occurring at ~15 W) over the three tests. However, the slopes of Pi/PCr vs. power output before and after the ITPi/PCr were found to have poor reproducibility with intraclass correlation coefficients of 0.59 and 0.64 respectively. In addition, end exercise Pi/PCr over three repeat tests had a CV of 50% (0.91 Pi/PCr units), suggesting the Pi/PCr slopes and end exercise changes must be interpreted with caution when assessing developmental or disease related changes in muscle metabolism. The variable Pi/PCr responses on an individual level are also likely to limit the ability to detect changes in metabolic function using these parameters following an intervention (e.g., exercise training). In contrast, end exercise pH (1% CV, 0.05 pH units) and the slope of pH following the ITpH (intraclass correlation coefficient 0.85) displayed excellent reproducibility. Coupled with the good reproducibility of the metabolic ITs, these data suggest that sensitive changes in muscle metabolic function are detectable using \(^{31}\)P-MRS in young people. Importantly, these reliability data cohere well with the few data available in adults during an incremental protocol (8, 17, 46).

**Constant Work-Rate Exercise**

It has been argued that the ‘clues’ to the control of oxidative ATP flux reside in the non-steady-state profiles of the muscle phosphates (49). A great deal of interest has therefore focused on describing the kinetics of the muscle phosphates at the onset and offset of square-wave exercise transitions in human either in isolation, or alongside measures of oxygen uptake (\(\dot{V}O_2\); 7, 50). The outcome from the latter studies is that a close temporal coupling exists between the kinetics of muscle
PCr and $\dot{V}O_2$ at exercise onset, suggesting the creatine kinase splitting of muscle PCr, or some related function, plays an important role in shaping the adaptation of oxidative phosphorylation. Therefore, the ability of $^3$P-MRS to sample the muscle phosphates (e.g., PCr, ADP) involved in the control of oxidative phosphorylation at a high time resolution ($< 10$ s) during exercise, allows important insights into metabolic control to be addressed.

An alternative application of the kinetic profile of muscle PCr however, is that its recovery dynamics may be employed as a measure of the muscles’ oxidative capacity and thus mitochondrial function (42). Indeed, this can be appreciated from the resynthesis of muscle PCr via the creatine kinase reaction:

$$\text{ATP} + \text{Cr} \rightarrow \text{PCr} + \text{ADP} + \text{H}^+$$  \hspace{1cm} \text{Equation 4}

During recovery from exercise the provision of ATP by anaerobic glycolysis is considered to be negligible following light exercise (15). Consequently, the provision of the ATP to phosphorylate Cr to PCr is exclusively an aerobic process. Hence the recovery of muscle [PCr] provides a noninvasive index of mitochondrial function. Consistent with this notion are data from human and rat muscle demonstrating the recovery of muscle [PCr] is linearly related to the activity of the mitochondrial enzyme citrate synthase (36, 42).

The recovery of muscle [PCr] following exercise may be used to estimate the theoretical maximal rate of oxidative ATP synthesis of the muscle ($V_{\text{max}}$). Assuming a linear model of metabolic control (38), $V_{\text{max}}$ can be estimated using:

$$V_{\text{max}} = \left( \frac{1}{\tau_{\text{PCr}}} \right) \times [\text{PCr}]_{\text{rest}}$$  \hspace{1cm} \text{Equation 5}

where ($1/\tau_{\text{PCr}}$) is the recovery rate constant (see endnote 2) and $[\text{PCr}]_{\text{rest}}$ is the [PCr] at rest. The model assumes the PCr rate constant is proportional to the mitochondrial properties of the muscle (see above), and that the maximal rate of ATP synthesis will be achieved when the metabolic signal(s) (e.g., ADP) to stimulate oxidative metabolism is(are) maximal, and thus muscle [PCr] at exhaustion is close to zero.

In contrast, $V_{\text{max}}$ can be estimated using an ADP model assuming a hyperbolic relationship with the resynthesis of muscle PCr:

$$V_{\text{max}} = V_{\text{PCr}} \times \left( 1 + \frac{K_m}{[\text{ADP}]_{\text{end}}} \right)$$  \hspace{1cm} \text{Equation 6}

where, $V_{\text{PCr}}$ is the initial rate of PCr resynthesis (calculated from the product of the PCr rate constant ($1/\tau_{\text{PCr}}$) and the difference between [PCr] at rest and end-exercise). $K_m$ is the Michaelis Menten constant (endnote 3), and [ADP]$_{\text{end}}$ is the end-exercise [ADP] (11, 28). Despite these additional assumptions compared with the linear model both models appear to provide a quantitatively similar estimation of $V_{\text{max}}$, at least in healthy adults (53).

An important consideration when using the recovery of muscle [PCr] following exercise as an index of the muscles’ oxidative capacity is the influence cellular pH may have on the derived kinetic $\tau$. Numerous studies have demonstrated that muscle pH has a profound influence on the recovery rate of muscle [PCr], such that the monoexponential recovery is lengthened (i.e., slowed; 42, 53) or may be modified...
to a biexponential function (37) under conditions of moderate to high acidosis (pH < 6.95 to > 6.50). As cellular acidosis inhibits the resynthesis of muscle PCr, this complicates, if not invalidates, the assessment of the muscles’ oxidative capacity using this measure. To circumvent this issue, it has been proposed that the calculated \( \tau_{PCr} \) may be adjusted for the end-exercise pH using a correction algorithm (35). However, van der Broek et al. (53) demonstrated that on an individual basis, the relationship between muscle \( \tau_{PCr} \) and pH can range from -33 to -75 s per unit of pH across adult participants, suggesting a uniform correction factor is inappropriate. Furthermore, given that the \( \tau_{PCr} \) is required for the calculation of Vmax using the linear model (for calculation of the rate constant, 1/\( \tau_{PCr} \)) or the hyperbolic ADP model (for calculation of \( \dot{V}_{PCr} \)), under conditions of a muscle acidosis the \( V_{max} \) calculation is unlikely to provide a valid estimate of the maximal rate of ATP synthesis (53).

**\(^{31}\)P-MRS and Developmental Muscle Metabolism**

**Incremental Exercise**

Zanconato et al. (57) were the first to investigate pediatric muscle metabolism using \(^{31}\)P-MRS. This study compared the responses of 7–10 year old children (8 males, 2 females) with those of 20–42 year old adults (5 males, 3 females) during an incremental, supine, calf muscle exercise test to exhaustion. Metabolic ITs for P/PCr and pH were identified during incremental exercise, but only in 5 children (50%) and 6 adults (75%). Of the participants who displayed an IT\(_{P/PCr} \), no differences were found in the initial slope of P/PCr against exercise intensity during exercise below the metabolic ITs. However, following the metabolic ITs, children were characterized by a lower increase in P/PCr and fall in pH for a given increase in power output compared with adults, leading to a smaller rise in P/PCr (0.54 [SD 0.12] vs. 2.00 [SD 0.79]) and fall in pH (0.11 [SD 0.05] vs. 0.36 [SD 0.11]) from resting levels at exhaustion. From these data the authors concluded that during low to moderate intensity exercise (i.e., below the ITs), no appreciable differences exist in the muscle phosphate and pH responses between 7–10 year old children and adults, which is indicative of a comparable capacity for oxidative metabolism (mitochondrial function). In contrast, as during exercise above the ITs children were characterized by a lower P/PCr and pH cost per increment in exercise intensity compared with adults, the authors attributed this to a lower reliance on substrate level phosphorylation (i.e., PCr breakdown and anaerobic glycolysis) in young people during high-intensity exercise.

To investigate the muscle metabolic responses during growth and development, Kuno et al. (32) studied quadriceps muscle energetics of untrained and trained boys aged between 12–15 years, and adult men, during an incremental knee-extensor exercise task to exhaustion. No significant differences were noted for muscle PCr/(PCr+P) or pH at exhaustion between the untrained and trained children at 12, 13, 14 and 15 years of age. In contrast, muscle PCr/(PCr+P) was significantly lower in the adults (akin to a higher P/PCr) compared with all age groups, apart from the trained 15 year olds. Similar results were also found for muscle pH at exhaustion, although muscle acidosis was greater in the adults compared with all other age groups. In addition, Kuno et al. (32) analyzed the recovery of muscle [PCr] following maximal exercise and found the \( \tau_{PCr} \) kinetics to be age and training invariant,
ranging from ~40–60 s across groups. In summary, these data are in agreement with the earlier work of Zanconato et al. (57) with the authors suggesting an age-related modulation of anaerobic metabolism during high-intensity exercise, and a comparable muscle oxidative capacity between 12 year old children and young adults.

A study by Taylor et al. (52) provides a more comprehensive examination of the muscles’ oxidative capacity during growth and maturation, as inferred using the recovery of calf muscle [PCr] following incremental plantar flexor exercise in sixteen 6–12 year old children and thirty six 20–29 year old adults. At exhaustion the children had a significantly higher muscle pH (6.80 [SD 0.10] vs. 6.64 [SD 0.12]) suggesting a lower glycolytic ATP flux at maximal exercise. Interestingly, this was associated with a comparable fall in muscle [PCr] from rest (57% in children and 58% in adults) but children had a higher end exercise muscle [ADP] compared with the adults (73 μmol·L⁻¹ [SD 23] vs. 53 μmol·L⁻¹ [SD 21], respectively). As ADP is a potent stimulator of muscle respiration (11, 54), this may indicate oxidative phosphorylation was able to meet a greater proportion of the metabolic demands of exercise in the children at maximal exercise.

Conley et al. (13) have proposed that the increase in muscle acidosis during high intensity exercise acts to lower cellular [ADP] via its influence on the creatine kinase reaction, such that muscle [PCr] must fall to maintain the requisite [ADP] required to stimulate oxidative phosphorylation (see equation 1). Therefore, the children’s ability to keep muscle pH high, possibly due to a lower glycolytic flux and/or a greater rate of cellular H⁺ efflux (45), may explain their higher muscle [ADP] and greater oxidative contribution at end exercise. Consistent with this conclusion are the recovery data reported by Taylor et al. (52). Specifically, the authors found a significantly faster recovery of muscle [PCr], as assessed using the half time in children compared with adults (12 s [SD 4] vs. 27 s [SD 8]). Using an hyperbolic ADP model and assuming a resting muscle [ATP] of 8.2 mmol·L⁻¹, children were also characterized with a 1.7-fold higher Vmax compared with the adults (91 mmol·L⁻¹·min⁻¹ [SD 46] vs. 54 mmol·L⁻¹·min⁻¹ [SD 17], respectively), indicating a decline in the muscles’ oxidative capacity and maximal rate of oxidative ATP flux from childhood into adulthood.

It is pertinent to note however, that the high-intensity exhaustive experimental protocols employed by Kuno et al. (32) and Taylor et al. (52), where muscle pH was as low as ~6.50–6.90 units, and significantly lower in the adults compared with the child participants, represent a major confounding factor in interpreting their muscle PCr resynthesis and Vmax data. To overcome this issue Ratel et al. (45) examined the muscle metabolic responses in seven 10–12 year old boys and ten 24–46 year old men during 15 min of recovery following 3 min of forearm exercise at 15% of their maximum voluntary contraction. The protocol was designed to ensure the cellular pH at exhaustion was comparable between the boys and men (6.6 [SD 0.2] vs. 6.5 [SD 0.2]), thus limiting its influence on the recovery kinetics of muscle PCr. During the recovery period these authors documented a higher PCr rate constant (1.7 min⁻¹ [SD 1.2] vs. 0.7 min⁻¹ [SD 0.2]) and an ADP determined Vmax (50 mmol·L⁻¹·min⁻¹ [SD 25] vs. 29 mmol·L⁻¹·min⁻¹ [SD 8]) in the boys compared with the men, indicating a greater mitochondrial capacity of oxidative phosphorylation in the former, which is consistent with the earlier work of Taylor et al. (52) for the plantar flexor muscles.

The study by Ratel et al. (45) also estimated the efflux of cellular H⁺ during the initial 24 s of recovery, and found H⁺ efflux to be 2.8-fold higher in the boys than
men (9.72 vs. 3.49 mmol·L⁻¹·pH⁻¹·min⁻¹). As the recovery of cellular pH is dependent on proton efflux, it has been shown that H⁺ efflux determines the impact cellular pH has on the recovery of muscle PCr (53). That an individual with a higher H⁺ efflux has a smaller pH related slowing of the PCr recovery kinetics, leading to a faster resynthesis of muscle PCr. Consequently, the child-adult differences in the PCr rate constant and $V_{\text{max}}$ in the study by Ratel et al. (45) are likely to be explained, at least in part, by their differences in H⁺ efflux. Due to the impact H⁺ efflux has on the relationship between pH and $\tau_{\text{PCr}}$, the latter can only be used as a valid measure of mitochondrial function when pH at end exercise is close to resting values (53).

Petersen et al. (43) examined the dynamics of the muscle phosphates and pH during 2 consecutive minutes of sub- and supra-maximal exercise (40% and 140% of previously determined maximum work capacity (MWC)) in 9 prepubertal and 9 pubertal female swimmers. Strikingly similar muscle P/PCr and pH responses were observed during the 40% MWC bout, indicating a comparable phosphate-linked control of oxidative metabolism at this intensity. However, despite showing a 'trend' toward a higher muscle P/PCr (2.18 [SD 1.00] vs. 1.31 [SD 0.88]) and fall in pH (6.66 [SD 0.11] vs. 6.76 [0.17]) in pubertal compared with the prepubertal participants at exhaustion of the 140% MWC bout, these differences were not statistically significant. The authors concluded that substrate level phosphorylation (PCr breakdown and glycolysis) is not maturity dependent during supra-maximal exercise. However, given the magnitude of the differences, especially for P/PCr (~40%), this conclusion requires further investigation.

The study by Petersen et al. (43) also highlighted an important methodological concern regarding the use of the calf muscle to study developmental energetics using $^{31}$P-MRS. Specifically, the calf muscle is composed of two muscle groups that have profoundly different muscle fiber type populations; the soleus, which is composed of ~80–90% type I muscle fibers, and the gastrocnemius, which is composed of ~50% type I and ~50% type II muscle fibers (26). The distinct metabolic properties of type I and type II muscle fibers have been shown to result in profoundly different muscle phosphate and pH responses during muscle contractions, with a lower muscle [PCr], higher muscle pH and more rapid resynthesis of muscle PCr associated in muscle with a higher expression of type I muscle fibers (16, 40). Given the heterogeneity in calf muscle size between prepubertal children and more mature children and adults, the use of an unlocalised $^{31}$P-MRS signal is likely to result in a disproportionate sampling of the soleus and gastrocnemius muscle between groups, such that the soleus will represent a greater portion of the total $^{31}$P-MRS signal in the smaller participants, i.e., younger children (Figure 4).

In the study by Petersen et al. (43) described earlier, the prepubertal children had a mean gastrocnemius muscle thickness of 9 mm compared with 13 mm in the pubertal group, a 44% difference. Clearly, this difference in gastrocnemius muscle morphology is likely to have been further exacerbated in the child-adult comparisons performed by Zanconato et al. (57) and Taylor et al. (52). Consequently, it is plausible that the results from $^{31}$P-MRS studies utilizing the calf muscle to investigate developmental muscle energetics (43, 52, 57) may be biased to a lower P/PCr, higher pH and more rapid resynthesis of muscle PCr in the child muscle, due to the methodology adopted.

In contrast to the calf muscle, the quadriceps muscle is likely to be a more judicious choice to monitor developmental muscle energetics using $^{31}$P-MRS.
Firstly, the quadriceps muscle portions do not display the distinct muscle fiber type heterogeneity that is present between the soleus and gastrocnemius muscles (26), and hence are less susceptible to inducing biases in the muscle metabolic responses across participants heterogeneous in body size. Secondly, during moderate and high intensity quadriceps exercise, all the quadriceps muscle portions are activated and involved in energy metabolism (31), which is in contrast to calf muscle exercise where the gastrocnemius muscle is the main contributor to the power output (43). Finally, as the few muscle biopsy data in young people are taken from the rectus femoris and vastus lateralis muscles (10, 19, 23), interrogating the quadriceps muscle with $^{31}$P-MRS strengthens the comparisons that can be made between the two techniques in terms of the muscle PCr, lactate and pH profiles, and also when discussing the putative role muscle fiber type distributions and enzymatic activities may play in modulating the muscle phosphate and pH responses in young people.

Given this rationale, Barker et al. (5) investigated the muscle metabolic responses of 9–12 year old children (15 boys, 18 girls) and adults (8 men, 8 women) during incremental quadriceps exercise to exhaustion. A novel feature of this study was that MR imaging scans were used to quantify the participants’ quadriceps muscle mass to normalize power output measures using allometric models as recommended by Fowler et al. (20). No age- or sex-related differences were observed for the power output or cellular energetic state (P/PCr) at the metabolic ITs, suggesting a similar phosphate linked-regulation of mitochondrial respiration during low to moderate intensity exercise. In contrast, during exercise above the ITs both age- and sex-related differences were clearly evident. Specifically, children were characterized by a lower increase in P/PCr for a given increase in power output.
compared with adults, which was also the case for males compared with females for both age groups. This led to both age and sex-related differences in muscle P/PCr at exhaustion. In contrast, the fall in pH for a given increase in power output following the ITs was only lower in the boys compared with the men and girls, with no differences at exhaustion. Taken collectively, these data indicate that the modulation of muscle metabolism between children and adults and between sexes, is dependent upon the intensity of the imposed exercise, with a greater anaerobic component observed during supra IT exercise in adults compared with children and in females compared with males.

**Constant Work-Rate Exercise**

As discussed previously, current models of metabolic control implicate muscle PCr to play a pivotal role in matching the rate of oxidative ATP flux to the rate of ATP breakdown at the actin-myosin cross-bridges through modulating the rise in ADP within the mitochondria (49). In support a recent study by Barker et al. (6) demonstrated the kinetics of muscle PCr to be temporally related to the kinetics of VO$_2$ in children both at the onset and offset of moderate intensity exercise. As early pubertal children display faster VO$_2$ kinetics at the onset of exercise compared with older children or adults (see Ref. 1 for recent review), the authors hypothesized an age-related slowing of the phosphate controllers, specifically PCr, of oxidative phosphorylation is likely to explain the more rapid VO$_2$ kinetics found in children compared with older children or adults.

To test this hypothesis, Barker et al. (4) investigated age and sex related differences in the kinetics of the quadriceps muscle phosphates and pH during moderate intensity exercise in eighteen 9–10 year old children (8 boys, 10 girls) and 16 adults (8 men, 8 women). Each participant completed between 4–10 repeat exercise transitions to a power output corresponding to 80% of the ITs, consisting of 6 min exercise and 6 min recovery. This ensured that the kinetics of the muscle phosphates were scrutinized under conditions of no cellular acidosis (moderate exercise), which is also essential to perform a valid measurement of the muscles’ oxidative capacity using the recovery of muscle PCr. In conflict with their experimental hypotheses, the authors found no age or sex related differences in the PCr kinetics 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 steady-state rise in the reputed regulators of oxidative phosphorylation (i.e., P/PCr and [ADP]) during exercise was strikingly similar between the children and adults. The authors therefore concluded that during moderate intensity quadriceps exercise the regulation of mitochondrial respiration is fully mature in peri-pubertal children and sex independent, which is consistent with a comparable capacity for oxidative metabolism in child and adult muscle.

**So What’s New?**

Although the application of $^{31}$P-MRS to studying developmental exercise metabolism has been limited, important and novel insights can be drawn from this work, which have yet to be addressed within the literature. Specifically, from the methodologically robust studies conducted to date, it appears that child-adult and sex
differences in muscle metabolism are dependent on the intensity of the imposed exercise. That is, the metabolic ITs represent the physiological parameter that differentiates the child-adult responses. For example, the incremental studies by Barker et al. (5) and Zanconato et al. (57) revealed child-adult and sex related differences in muscle energetics only during exercise above the ITs (high intensity). In contrast during exercise below the ITs (moderate exercise), both the incremental (5, 57) and constant work-rate protocols (4, 43) found the muscle phosphate (steady-state cost or $\tau_{PCr}$) and pH responses to be indistinguishable between younger children and older children or adults. In this context it is of interest to consider the recent work of Willcocks et al. (56) published in abstract form, which provides further support to the notion of an intensity dependence of muscle metabolism between children and adults. These authors examined the kinetics of muscle PCr at the onset of high-intensity quadriceps exercise above the metabolic ITs and found 12–13 year old boys to exhibit faster muscle PCr kinetics (31 s [SD 11] vs. 45 s [SD 19]) and a reduced PCr slow component (6% [SD 1] vs. 12% [SD 12]) when compared with adult men.

Taken collectively, these data therefore raise an important question: what are the physiological mechanisms that may account for the exercise-intensity dependence of developmental muscle metabolism? It has been shown that above ~50% peak power output, the muscle PCr, $P_i$ and pH responses are dependent on muscle oxygenation as when breathing hyperoxic air (10% O$_2$), the fall in muscle [PCr] and pH and rise in [$P_i$] is greater compared with normoxia (25). Interestingly, there are some data showing a decrease in mass relative blood flow (and thus muscle oxygenation) to the vastus lateralis muscle during sub- and maximal cycling exercise from 12 to 16 years of age (29), which may explain an exercise-intensity dependence of developmental muscle energetics. Alternatively, child-adult differences in muscle recruitment patterns during incremental exercise, specifically a preferential recruitment of type I fibers in children at higher work-rates, would also account for this phenomenon. Indeed, the lower accumulation of $P_i$ and fall in PCr and pH in the child’s muscle indirectly supports a greater contribution of type I muscle fibers, which may also alleviate the requirement to recruit higher order muscle fibers (type II) due to their enhanced oxidative properties and resistance to fatigue. Lastly, and as discussed earlier, children’s ability to keep muscle pH high (possibly due to greater muscle oxygenation and/or type I fiber recruitment) during high intensity exercise allows, at least through the constraints of the creatine kinase equilibrium, ADP to rise to the requisite level to stimulate oxidative phosphorylation (13). In turn, this would reduce the anaerobic energy turnover (PCr breakdown and glycolysis) required to ensure ATP supply matched demand.

While speculative, it is hoped that the above hypotheses will pave the way for well-designed experimental studies in future years to explore the mechanistic basis for the apparent intensity-dependence of developmental changes in muscle energetics.

**Conclusion**

Detailing developmental changes in muscle metabolism has been hampered over the years by ethical and methodological constraints. This review has provided a contemporary account of how the noninvasive technique of $^{31}$P-MRS can be used to study muscle metabolism in vivo in children and adolescents, and how current
scientific understanding has been advanced through the experimental studies conducted to date. From methodologically robust studies, it can be concluded that developmental changes in muscle metabolism appear intensity-dependent. That is, during moderate intensity exercise, the phosphate linked control of oxidative ATP flux and the muscle mitochondrial capacity to support the rate of ATP turnover within the myocyte appears fully mature by the ages of 9–10 years. However, for high-intensity exercise (i.e., where cellular pH is falling) children are characterized by a lower phosphate and pH cost of contraction, reflecting an enhanced oxidative ATP flux during these work-rates. Future research is needed to investigate the physiological mechanisms (e.g., muscle oxygenation and/or fiber type recruitment) that underlie these observations, and how this relates to age, sex and maturity.

Notes
1. mmol·kg⁻¹ wet mass is converted to mmol·L⁻¹ cell water using the conversion factor of 1.47, where muscle cell water is assumed to be 0.68 L·kg⁻¹ wet mass (1/0.68 = 1.47) (Ref. 25).
2. Where τ (tau) represents a mathematical constant of the time taken to achieve 63% of a given response amplitude.
3. The Michaelis Menten constant (Kₘ) is the substrate (ADP) required to elicit 50% of the maximal rate of oxidative ATP synthesis, which is traditionally assumed to be 20 or 30 μmol·L⁻¹ (Ref. 10).

References


