Reproducibility of Muscle Metabolism Parameters in Children Determined by ^{31}P Spectroscopy

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Introduction

Exercise tests involving incrementally increasing effort up to volitional exhaustion (end exercise) are typically employed to monitor the dynamics of muscle Pi/PCr and pH in both adults and children. During such tests, non-linear changes occur in muscle Pi/PCr and pH with increasing power output, such that the variables exhibit a transition from an initial low slope to a second steeper slope as the exercise intensity increases (1). The transition to the second steeper slope is known as the intracellular threshold (IT) and is thought to represent an increased glycolytic activity status within the muscle.

As there is evidence of differences between adults and children's oxidative and glycolytic energy metabolism, the aim of the present study was to examine the practicality and reproducibility of measuring end exercise and IT values during a progressive exhaustive exercise test in children using ^{31}P-MRS. Unlike traditional laboratory tests where criteria are available to confirm maximal effort has been achieved, exhaustive exercise within the scanner bore relies heavily upon subject motivation and compliance with a novel exercise task. Thus potentially it could be particularly susceptible to subject variation when studying children. In addition, the spectral profiles of children display a lower signal to noise ratio as a direct consequence of their inherently smaller muscle size, which may induce a large degree of variability in the quantified metabolic response and preclude reliable identification of the ITs and measurement of end exercise values.

Methods

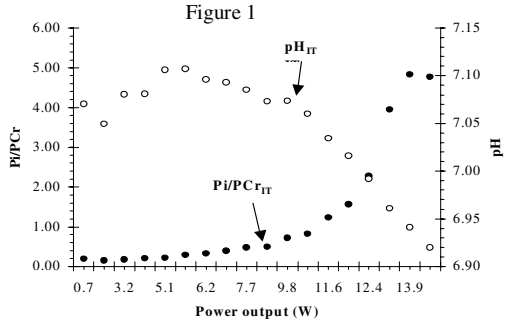
On four separate occasions, fourteen children (7 boys and 7 girls, age 11.9 ± 0.4 years, mass 37.9 ± 7.3 kg) attended the centre. On the first visit the exercise protocol was practised within a to-scale dummy scanner to ensure familiarity both with the task and the scanner environment. On the three subsequent visits, the exercise protocol was performed within a 1.5T Philips Gyroscan Clinical Intera system at the Peninsula Magnetic Resonance Research Centre at the University of Exeter. A 6cm ^{31}P transmit /receive surface coil was placed within the subject bed and the subject asked to lie upon it in a prone position such that the coil was centred at thigh level over the quadriceps muscle, with strapping placed at the back, hip and knee to ensure no movement occurred during the protocol. Initially, fast field echo (FFE) images were acquired to ensure the muscle was positioned correctly relative to the coil. An automatic shimming protocol was undertaken, specifically, within a volume that defined the quadriceps muscle to optimise the signal from the muscle under investigation and matching and tuning of the coil was carried out. Initially, an unsaturated ^{31}P spectra was acquired to allow T1 correction for those spectra obtained during the exercise protocol. Subjects were then required to perform knee extension and flexion exercise with their right leg against a pulley system, to which a variable mass was attached, via a strap fitted to the foot, at a metronomic frequency of 0.66 Hz, with all force and power measurements recorded via a non-magnetic strain gauge and shaft encoder present within the pulley system. To ensure a consistent work rate and muscle position relative to the coil during exercise, the subject was visually queued, with associated feedback, via a display visible within the scanner consisting of 2 vertical bars, one which moved at a constant frequency of 0.66 Hz triggered via the scanner, and one which displayed the relative foot position via a sensor present within the pulley. Thus, the subject was required to match the movements of these 2 bars. During the entire protocol, phosphorous spectra were acquired every 1.5 seconds, with a spectral width of 1500 Hz, and 512 data points. Phase cycling, with 4 phase cycles was employed and 20 measurements were performed, leading to a spectra acquired every 30 s, with all scanning conforming to NRPB guidelines (2). Initially, baseline measurements were acquired for 2 minutes with the subject at rest. Exercise was then begun with 0.5 kg attached to the pulley system. Thereafter, 0.5 kg was added every minute, with masses added every minute until the subject was unable to continue (volitional exhaustion). Recovery measurements were then taken for an additional 4 minutes with the subject at rest.

^{31}P spectra were quantified via peak fitting, assuming prior knowledge, using the jMRUI (version 2) software package employing the AMARES fitting algorithm (3,4). Spectra were fitted assuming the presence of the following peaks: inorganic phosphate (Pi), phosphodiester, phosphocreatine (PCr), αATP (2 peaks, amplitude ratio 1:1), γATP (2 peaks, amplitude ratio 1:1) and βATP (3 peaks, amplitude ratio 1:2:1). pH was calculated from the chemical shift of Pi relative to PCr.

Following spectral quantification each variable was plotted as a function of power output for the three incremental tests. Using plots of Pi/PCr and pH with power, ITs were identified by two experienced investigators using two methods: 1) subjective estimation and 2) an objective bilinear regression technique as employed by Marsh *et al.* (5). End exercise values were determined from the values of Pi/PCr and pH recorded at the highest power output.

Results

Figure 1



An example of plots of pH and Pi/PCr against power and the subsequent determination of the associated ITs is illustrated in figure 1.

The variation in the determination of end exercise Pi/PCr, pH, peak power (PP) and the Pi/PCr and pH ITs over the three tests was expressed as a % coefficient of variation (CV). The CV for the power output of the subjectively determined values for Pi/PCr$_{IT}$ and pH$_{IT}$ were 10.6% and 10.3%, respectively. Objective identification of the Pi/PCr IT had a CV of 16.3% whereas the pH IT was undetectable using the objective method. Reliability for PP over the three tests was 12.7%. End exercise CVs for pH$_{END}$ and Pi/PCr$_{END}$ were 0.9% and 50.0%, respectively.

Discussion

From the study it was apparent that, following a period of habituation, children were capable of undertaking the specific exercise protocol involving knee-extensor incremental tests to exhaustion with a high degree of reproducibility. As a result, values of pH$_{END}$, Pi/PCr and pH ITs when determined subjectively illustrated good reliability. In contrast, changes in Pi/PCr$_{END}$ show very poor reliability. Such a result illustrates the potential problem of ensuring subjects, and children in particular actually do exercise to exhaustion rather than terminating the exercise prematurely and thus, that caution should be employed when interpreting Pi/PCr$_{END}$ values. However, the good reliability of other parameters illustrates the potential of ^{31}P-MRS in the study of developmental exercise metabolism.

References

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