Muscle metabolism during fatiguing isometric quadriceps exercise in adolescents and adults

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***Abstract***

Children and adolescents are less susceptible to muscle fatigue during repeated bouts of high intensity exercise than adults, but the physiological basis for these differences is not clear. The purpose of the current investigation was to investigate the muscle metabolic responses, using 31-phosphorus magnetic resonance spectroscopy, during fatiguing isometric quadriceps exercise in 13 adolescents (7 females) and 14 adults (8 females). Participants completed 30 maximal voluntary contractions (6 s duration) separated by 6 s of rest. Fatigue was quantified as the relative decrease in force over the test. Fatigue was not significantly different with age (p=0.20) or sex (p=0.63). Metabolic perturbation (change in [phosphocreatine], [inorganic phosphate], and [ADP]) was significantly greater in adults compared with adolescents; no sex effects were present. Muscle pH did not differ with age or sex. PCr recovery following exercise was not significantly different with age (p=0.27) or sex (p=0.97) but a significant interaction effect was present (p=0.04). Recovery tended to be faster in boys than men but slower in girls than women, though no significant group differences were identified. The results of this study show that at a comparable level of muscle fatigue, the metabolic profile is profoundly different between adolescents and adults.

Keywords: acidosis, inorganic phosphate, pediatric exercise physiology, 31-phosporus magnetic resonance spectroscopy, anaerobic metabolism, quadriceps

***Introduction***

Muscle fatigue may be defined as the fall in the force generating capacity of the muscle (Enoka and Duchateau 2008), limiting exercise tolerance and performance. The magnitude, degree, and mechanisms of fatigue depend on the exercise task, the characteristics of the participant and the interaction between the two and may consist of both central and peripheral components. In terms of exercise modality, fatigue shows a dependence upon the type of contraction (isometric or dynamic), duration (sustained versus intermittent) and intensity of exercise (Williams and Ratel 2009).

Although muscle biopsy studies offer the opportunity to directly assess muscle composition and how it relates to fatigue properties ([Clarkson](http://www.ncbi.nlm.nih.gov/pubmed?term=Clarkson%20PM%5BAuthor%5D&cauthor=true&cauthor_uid=7421476) et al. 1980) such procedures do have some intrinsic ethical limitations, in particular when dealing with a child and adolescent group. In contrast, 31-phosphorus magnetic resonance spectroscopy (31P-MRS) offers a non-invasive window on several potential mediators of fatigue. 31P-MRS can be used to measure exercise induced alterations in intramuscular inorganic phosphate (Pi), phosphocreatine (PCr) and pH, and the recovery PCr, which may be used as an index of oxidative capacity (Walter et al., 1997). All of these parameters of muscle metabolism have been hypothesized tomediate muscle fatigue (see Fitts 2008 for a review).

A range of studies have reported that children and adolescents are less susceptible to muscle fatigue than adults (Dipla et al. 2009; Hebestreit et al. 1993; Ratel et al. 2002; Zafeiridis et al. 2005). However, little direct evidence about the mechanistic basis for age-related differences in muscle fatigue exists in this population. Hebestreit et al. (1993) reported that the half time for VO2 recovery was less in boys than men, and suggested that metabolic recovery between exercise bouts might be an important mechanism for reduced fatigue in children compared with adults. Subsequent studies have confirmed that metabolic recovery is faster in children than adults (Taylor et al. 1997; Tonson et al. 2010), although this is not a universal finding (Barker et al. 2008; Kuno et al. 1998). The importance of faster recovery kinetics between exercise bouts in determining the degree of participant fatigue was confirmed in the study conducted by Ratel et al. (2002), who reported that children and adolescents were able to maintain repeated sprint performance with less rest between sets than adults.

Gender differences in muscle fatigue are commonly reported in adults, with women showing a decreased rate of fatigue relative to men (Hunter et al. 2006; Russ and Kent-Braun 2003). This does not appear to be the case in children (De Ste Croix et al. 2009). For adults, sex differences in fatigue appear to arise both centrally and peripherally (Albert et al. 2006; Hunter et al. 2006; Kent-Braun et al. 2002; Wust et al. 2008), and may result from differences in muscle fibre type or recruitment (Hunter et al. 2006; Wust et al. 2008), use of different metabolic pathways (Russ et al. 2005; Wust et al. 2008), differences in muscle blood flow (Russ and Kent-Braun 2003; Thompson et al. 2007), or differences in central activation (Russ and Kent-Braun 2003). As such, the potential mechanisms for fatigue resistance in women compared with men are fundamentally similar to the potential mechanisms for fatigue resistance in children compared with adults.

No previous study in children or adolescents has used 31P-MRS during repetitive fatiguing exercise to investigate potential muscle metabolic contributions to fatigue. Therefore, the aim of the current study is to examine changes in muscle [PCr], [Pi], intracellular pH, and [ADP] during fatiguing exercise in adolescent and adult males and females. We hypothesize that: 1) fatigue over thirty 6 s maximal isometric quadriceps contractions will be greater in males than females and in adults than adolescents; 2) the metabolic perturbation, measured by the change in [PCr], [Pi], pH, and [ADP], will be less in adolescents than adults, and less in females than males; and 3) the recovery of muscle PCr following the fatiguing exercise will be faster in adolescents than adults.

***Materials and Methods***

Thirteen healthy adolescents, 6 boys (mean ± SD, age 13.4 ± 0.3 y, height 1.57 ± 0.07 m, body mass 46.9 ± 6.2 kg, years from age at peak height velocity (Mirwald et al. 2002) -0.6 ± 0.4 y) and 7 girls (13.3 ± 0.4 y, 1.63 ± 0.08 m, 55.9 ± 17.3 kg, 1.2 ± 0.9 y) and 14 healthy adults, 6 men (29.0 ± 7.9 y, 1.73 ± 0.05 m, 73.7 ± 12.0 kg) and 8 women (29.5 ± 9.0 y, 1.69 ± 0.07 m, 66.0 ± 6.7 kg) were recruited to take part in the study, which was approved by the institutional ethics committee. Written informed consent was obtained from adults, and from parents of adolescent participants. Adolescent participants also provided written assent.

*Ergometer and exercise test*

Prior to testing, participants visited the laboratory on a separate day for familiarization with the ergometer within a mock MR scanner set-up which included several minutes of contractions replicating the experimental task. During data collection, the entire exercise protocol was undertaken within the bore of a 1.5 T superconducting magnet (Intera, Philips, The Netherlands) at the Peninsula Magnetic Resonance Research Centre (Exeter, UK). Participants were positioned within the scanner head first in a prone position with the coil placed within the scanner bed such that the participant’s right quadriceps muscle was centred directly over it. The hips and upper legs were secured to the bed with non-distensible straps to avoid motion and the right foot placed in a padded foot cradle attached to a rope and pulley system. The system was fixed in position such that the participant’s foot was located 5-10 cm above the scanner bed with the exercise requiring contracting the quadriceps and pushing the foot down against the anchored cradle/rope assembly. A force transducer was embedded within the system such that the force generated during the contraction was recorded. After positioning, gradient echo images were acquired to confirm correct coil placement relative to the muscle under investigation. Matching and tuning of the coil and an automatic shimming protocol was then carried out. Prior to the beginning of an exercise, an unsaturated baseline spectrum was acquired with a repetition time of 20 s. Subsequently, during exercise, 31P spectra were obtained using an adiabatic pulse every 1.5 s, with a spectral width of 1500 Hz and 512 data points. Phase cycling was employed leading to a spectrum being acquired every 6 s

The fatiguing exercise protocol consisted of thirty 6 s maximum voluntary contractions (MVC), each separated by 6 s of rest. Participants were instructed to push as hard as they could during each contraction, and the importance of avoiding pacing was emphasized. Each contraction was verbally cued, and strong encouragement was provided to participants throughout each contraction. No visual feedback regarding force output was available to participants. Force was measured continuously through the protocol and data were averaged over the duration of each 6 s contraction. Within each test the peak force was identified which typically occurred within the first or second muscle contraction of the protocol. Fatigue Index was defined as the relative decrease in force from the peak contraction to the final contraction.

Fatigue Index (%) = Forcepeak – Forceend \* 100% (1)

Forcepeak

*MRS Analysis*

MRS data was analyzed using a non-linear least squares peak-fitting software package (jMRUI Software, version 4.0) (Naressi et al. 2001) and the AMARES fitting algorithm (Vanhamme et al. 1997) allowing quantification of the peak areas within the spectra assuming the presence of Pi, phosphodiester, PCr, α-ATP, γ-ATP, and β-ATP peaks. Intracellular pH was calculated from the shift in the Pi peak relative to the PCr peak (Taylor et al. 1983). A representative set of spectra can be seen in Figure 1. [Pi] and [PCr] were calculated using the unsaturated baseline spectrum assuming that [PCr] + [Pi] = [TCr] and that [TCr] = 42.5 mM (Arnold et al. 1984; Forbes et al. 2008; Lanza et al. 2005). [ADP] was calculated from [PCr] and pH, using equation 2:

[ADP] = [ATP][Cr]/[PCr][H+]KCK ­(2)

Where KCK is the equilibrium constant for the creatine kinase reaction and was assumed to be 1.66 x 109, [ATP] was assumed to be 8.2 mM (Taylor et al. 1986), and [Cr] was assumed to equal [Pi].

*PCr recovery kinetics*

To investigate the possibility that differences in recovery between contractions contribute to age differences in fatigue, the dynamics of PCr over the first three minutes of recovery was established using a monoexponential equation of the form:

PCr(t) = PCr(0) + A(1-e(-t/τ)) (3)

Where PCr(0) is the value at the end of exercise, A is the difference between the PCr at end exercise and fully recovered, t is the time from exercise cessation, and τ is the time constant for the exponential recovery of PCr (the time to reach 63% of A). 95% confidence intervals for the curve were calculated.

*Data analysis*

Data are presented as mean ± standard deviation. In order to increase signal:noise, metabolic variables and force were averaged over three contraction-relaxation cycles to give 10 binned values (one data point every 36 s) for each participant over the exercise bout. PCr recovery kinetics were fitted at 6 s temporal resolution. Mixed-model ANOVA with two between-subjects variables (age and sex) and one within-subjects variable (bin) was used to examine changes in force, [PCr], [Pi], [ADP], and pH over the course of the test. Two-by-two factorial ANOVA was used to compare fatigue index among groups. Planned t-tests with Bonferroni correction were used for post-hoc testing following two-by-two ANOVA, and Bonferroni follow up tests were used for post-hoc comparisons following mixed-model ANOVA. Statistical significance was accepted at the p<0.05 level.

***Results***

*Force*

Over the course of the exercise test, force was significantly lower in adolescents than adults, (p=0.002) but not significantly different in males and females (p=0.59) (Figure 1). No interaction between age and sex was identified (p=0.06). Force decreased significantly over the course of the test, (p<0.001), and there was a significant interaction between age and time(p<0.001). Results of follow up tests are shown on Figure 2. No significant interaction between sex and time (p=0.91) or age, sex, and time (p=0.48) was present.

The fatigue index, assessing single contractions rather than bins, gave values of 23 ± 11% in boys, 25 ± 10% in girls, 31 ± 6% in men, and 29 ± 14% in women. No significant age or sex differences in the fatigue index were identified (p=0.20 and p=0.95, respectively), and no interaction between age and sex was present (p=0.63).

*Metabolites*

At rest, no significant age, sex, or interaction effects were identified for [PCr], [Pi], [ADP], or pH (Figure 3). During exercise, [PCr] and pH significantly decreased, while [Pi] and [ADP] significantly increased in all groups. Group mean data are presented in Figure 3, while ANOVA results are represented in Table 1. Few sex differences were present. In both males and females, [Pi]1 was significantly less than [Pi]2-[Pi]10. However, in males, [Pi]2 was significantly less than [Pi]4-[Pi]10, while in females, [Pi]2 was significantly different only from [Pi]9. Over the duration of the test, [ADP] was significantly greater in adult males than adolescent males, but did not differ with age in females. Across the sexes, pH10 was significantly lower in males than females, but no significant differences between males and females were found during pH1-pH9.

*PCr recovery Kinetics*

The recovery of PCr following exercise was fitted with an exponential curve. The recovery time constant was 34 ± 10 s in boys, 39 ± 7 s in girls, 52 ± 16 s in men, and 40 ± 14 s in women. No significant group differences were seen (age: p=0.09, sex: p=0.52, interaction: p=0.13). The average 95% confidence intervals were 11 s for the PCr recovery kinetics. Considerable inter-individual variability in the kinetic response to exercise and recovery was seen in all groups.

***Discussion***

A number of previous studies have reported that children and adolescents are fatigue-resistant compared with adults. These studies have typically examined fatigue during multiple whole body sprints (Hebestreit et al. 1993; Ratel et al. 2002; Ratel et al. 2004; Ratel et al. 2006), repetitive isokinetic knee flexions (Dipla et al. 2009; Paraschos et al. 2007) or plantar flexions (Zafeiridis et al. 2005). Although studies have previously examined fatigue differences between men and boys in terms of electromyography output (Armatas et al. 2010) to our knowledge, this is the first study to examine age differences in force as well as the measurement of putative intramuscular determinants of fatigue.

The purpose of this study was to examine changes in muscle metabolism in the maintenance of force generating capacity during fatiguing exercise and compare responses in adult and adolescent males and females. Over six minutes of repeated MVC exercise, force decreased significantly in all groups, and the absolute decrease in force was greater in adults than adolescents. However, contrary to our initial hypothesis, when fatigue was expressed relative to peak force the decrease was not significantly different between adults and adolescents, or between males and females. The metabolic conditions over the exercise bout, on the other hand, differed with age – a significantly greater decrease in [PCr] and greater increase in [Pi] and [ADP] were evident in adults. Metabolic acidosis was similar in adolescents and adults, and in males and females. The subsequent PCr recovery kinetics following the test were not significantly different between groups, although a trend toward faster recovery in boys compared with men was seen.

Given the interaction between the task and individual in the development and aetiology of muscle fatigue, it is unsurprising that the degree of age dependence in fatigue varies between studies. The current study demonstrates that relative fatigue is not significantly different during repetitive maximal isometric exercise in 13 year old and 29 year old individuals, although a weak trend for greater fatigue in adults was identified (p=0.20) which warrants further investigation. Differences in metabolic enzyme activity between children and adults have been reported (Kaczor et al. 2005; Berg et al. 1986), with children showing higher activity of some aerobic pathway enzymes and lower activity of some anaerobic pathway enzymes. As well, many studies of exercise performance or tolerance in paediatric populations support the hypothesis that children tend to display a more oxidative metabolic profile during exercise (Armstrong and Barker, 2012). This would be expected to enhance fatigue resistance in the adolescent group (Bogdanis 2009), but no difference in fatigue was seen here. No sex differences in fatigue were identified, which is in keeping with some previous work during high intensity isometric exercise (Kent-Braun et al. 2002; Russ et al. 2005), but contradictory to other reports (Russ and Kent-Braun, 2003). It is likely that the nature of the exercise task, including the intensity, duration, duty cycle, and type of contraction, is critical in determining the degree of fatigue as well as the metabolic response to fatiguing exercise.

When metabolic factors are considered, a number of previous studies have indicated that children have reduced intracellular acidosis during or following intense dynamic exercise compared with adults (Barker et al. 2010; Kuno et al. 1995; Taylor et al. 1997; Zanconato et al. 1993). The current study showed that at the end of six minutes of isometric exercise, intramuscular pH, which can be broadly interpreted as an index of the glycolytic contribution to energy metabolism, was slightly decreased from resting. This decrease was significant in men, women, and boys, but not in girls and no significant difference was seen between groups. Currently, Pi and ADP are thought to be more involved in the process of fatigue development than metabolic acidosis (Westerblad et al. 2002; Westerblad and Allen, 2003).

During 6 minutes of fatiguing knee extension exercise, changes in PCr and Pi were significantly greater in adults than adolescents, but did not differ between females and males. The age difference in PCr and Pi after intense exercise is in keeping with previous work using a variety of exercise protocols (Barker et al. 2008; Barker et al. 2010; Ratel et al. 2008; Zanconato et al. 1993) and supports a large body of work in adults that has suggested that metabolic changes are important in limiting exercise tolerance and in the development of muscle fatigue during high intensity exercise (Fitts 2008). The mechanisms by which increased [Pi], decreased [PCr], or increased [ADP] might impair force production at the muscle are poorly understood but are thought to act on the contractile mechanism to reduce force output (Fitts 2008). For example, increases in intracellular [Pi] may inhibit force production via direct action on cross-bridge formation (Westerblad et al. 2002, Westerblad and Allen 2003). Alternately, these compounds might, through their action on type III and IV receptors, induce the central nervous system to reduce descending drive to the active muscles via the excitation-contraction pathway (Gandevia 2001). In the current study, differences in metabolism but not in fatigue were seen, suggesting that for this protocol, muscle fatigue might be mediated by factors other than metabolic perturbation.

One mechanism for the reduced metabolic perturbation in young people might be faster metabolic recovery between contractions. Previous studies have shown PCr recovery to be important in the maintenance of performance during repeated sprint exercise in adults (Bogdanis et al. 1996). The faster recovery of PCr following exercise in children and adolescents compared with adults has been documented by several investigators (Ratel et al. 2008; Taylor et al. 1997; Fleischman et al. 2010), although contradictory reports exist (Barker et al. 2008; Kuno et al. 1995). As well, McCormack et al. (2011) report that linear growth velocity over a year is negatively correlated with PCr τ at baseline, indicating that a relationship between growth and/or maturation and PCr recovery exists. The subjects in the current study were heterogeneous for age, but homogeneous for maturity as well as stature. It is likely that the diverse maturation of the participants in the current study contributed to variability in PCr recovery τ. No main effects for age or sex were shown. However, a significant interaction was found, with PCr recovery being ~33% faster in boys than in men, with similar values recorded in girls and women. The discrepancy between the current study and previous publications might be attributed to differences in sex, maturation, habitual physical activity, or exercise intensity. It is also possible, given the within-group variability in the current study, that statistical power was not sufficient to detect age differences. In support of this, the mean PCr recovery τ values for adolescents and adults in the current study were similar to those reported by Fleischman et al. (2010). In addition, as the rate of PCr recovery is pH dependent (Jubrias et al. 2003; van den Broek et al. 2007), it may be argued that the differences between groups could arise in differences in end exercise pH values. However, no significant differences were found between the groups and thus such a pH mediated effect can be discounted. Therefore the interaction between age and sex suggests that caution should be used in generalizing the results of investigations which have compared boys and men to groups which include girls and women.

There are several considerations in interpreting the results of this investigation. First are the potential confounding effects of maturation and physical activity. At -0.5 and 1.2 years from age at peak height velocity, boys and girls differed in maturity, and it is possible that the sex differences found in the adolescent participants reflect maturational effects. In addition, although participants were included on the basis of being active but not competing in organized sporting competitions, differences in habitual physical activity between boys and girls in early adolescence are well documented (Riddoch et al. 2004), and adults and adolescents have different lifestyles and different opportunities for activity. A pronounced difference in training between the groups would undoubtedly affect the ability to resist fatigue. Finally, it is important to acknowledge the implications of the assumptions made in this investigation; although generally accepted (Barker et al. 2010; Tonson et al. 2010), the assumed resting [ATP] and total creatine concentrations are not robustly verified in adolescents, and there is reason to treat any values dependent on these assumptions with caution. There is evidence from muscle biopsy studies that resting metabolite concentrations might differ in children and adults (Eriksson and Saltin 1974; Lundberg et al. 1979), although similar [PCr] in children and adults has also been documented (Gariod et al. 1994).

Of course, we cannot rule out the potential for differences in neuromuscular activation between adolescents and adults. Streckis et al. (2007) reported greater central fatigue in adolescents compared with adults, and postulated that a reduction is central drive reduces muscle activation and thus metabolic perturbation in young people, allowing them to recover more quickly between exercise bouts. No age differences in muscle fatigue were seen in that study. Based on the results of that study and the current findings, it could be speculated that while children experience less metabolic perturbation during fatiguing exercise, they experience a greater reduction in central drive. This may partially explain the task dependence of age differences in fatigue – a task in which fatigue is largely peripheral would result in greater fatigability in adults, while a task in which fatigue is largely central might show less of an age effect.

Overall, the results of the study do support the assertions of previous investigators (Ratel et al. 2002; Ratel et al. 2004; Zafeiridis et al. 2005) that during fatiguing exercise, muscle metabolism differs in adults and adolescents. Sex differences in fatigue in adults appear task dependent, and during this type of exercise appear to be negligible, in keeping with the findings of other investigators (Burnley et al. 2010; Kent-Braun et al. 2002). As such, this study has important implications for future research. Researchers interested in fatigue in children and adolescents should attempt to elucidate the nature of age differences in PCr recovery kinetics and to understand the role that recovery has on the maintenance of performance during repetitive high intensity exercise. Repeated brief bouts of intense exercise are common to both the natural activity patterns of children (Bailey et al. 1995) and to the sporting training that is increasingly being undertaken by young children. Thus, an understanding of the limiting factors in the performance of this type of exercise is valuable to coaches, therapists, and trainers of young people. Further, if the limitations to exercise performance during repetitive high intensity exercise differ in children and adults, there might be implications for children with diseases affecting muscle function.

In conclusion, this study examined fatigue during intermittent high intensity isometric quadriceps exercise in adolescents and adults, and reported a weak trend for greater fatigue in adults, although this did not reach significance. Greater associated metabolic perturbation in the adults was seen during fatiguing exercise, while acidosis in the muscle did not differ with age or sex. The results of this study suggest that muscle metabolism is not the sole determinant of age differences in muscle fatigue – adults and adults experienced a similar relative decrease in force despite significant differences in metabolic perturbation. Future studies should investigate central as well as peripheral contributions to fatigue in children and adolescents.

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Tables

Table 1.

Results of ANOVA analysis for metabolites measured over the course of the fatiguing protocol using 31P-MRS.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Age | Sex | Time | Age\*Sex | Age\*Time | Sex\*Time | Age\*  Sex\*Time |
| [PCr] (mM) | p=0.06 | p=0.70 | p<0.001 | p=0.11 | p<0.001 | p=0.31 | p=0.14 |
| [Pi] (mM) | p=0.07 | p=0.77 | p<0.001 | p=0.92 | p=0.008 | p=0.02 | p=0.98 |
| [ADP] (μM) | p=0.05 | p=0.64 | p<0.001 | p=0.03 | p=0.05 | p=0.99 | p=0.35 |
| pH | p=0.54 | p=0.67 | p<0.001 | p=0.44 | p=0.11 | p<0.001 | p=0.89 |

***Figure Legends***

Figure 1.

Spectra acquired during fatiguing exercise and recovery in a 13 year old female. The plot shows a decrease in the amplitude of the PCr peak and an increase in the amplitude of the Pi peak during exercise as well as the recovery of these peaks following exercise.

Figure 2.

Force during the test in adolescent and adult males and females. Each data point represents the average force over three six-second contractions. Statistical differences between age groups are marked at the top of the graph by an asterisk at the first significantly different point. Within each age group, statistical differences between data points are marked with the number of the earlier data point above the first significantly lower later data point.

Figure 3.

31P-MRS variables in adolescent and adult males and females. ANOVA results shown in Table 1. Statistical differences between age groups are marked at the top of the graph by an asterisk at the first significantly different point. Within each age group, statistical differences between data points are marked with the number of an earlier data point above the first significantly different later data point.