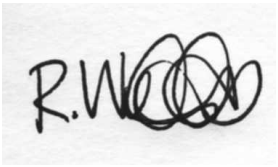
Investigation of metabolic responses to exercise in adolescents and adults during high intensity exercise and recovery

Submitted by Rebecca Willcocks to the University of Exeter as a thesis for the degree of Doctor of Philosophy by Research in Sport and Health Sciences, May 2011.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.



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Abstract

Children and adolescents are thought to use oxidative metabolism to a greater extent than adults during high intensity exercise. The studies reported in this thesis examine the nature and implications of age-related differences in muscle metabolism during high intensity exercise and recovery. Chapter 4 concluded that during heavy intensity exercise, phosphocreatine (PCr) kinetics did not differ with age or sex, while Chapter 5 revealed that during very heavy intensity exercise, the fundamental τ was slower and slow component amplitude greater in men compared with adolescent boys, indicating that exercise intensity might play a role in determining age-related differences in muscle metabolism. In Chapter 6, two bouts of very heavy intensity exercise were completed, and prior exercise reduced the PCr slow component amplitude in men but not boys. Deoxyhaemoglobin (HHb) kinetics was faster in adolescents compared with adults during both heavy and very heavy intensity exercise, indicating that matching of oxygen delivery to oxygen utilisation is less precise at the onset of exercise in adolescents compared with adults. PCr recovery from high intensity exercise was faster in boys than men, but not different in girls and women, as described in Chapter 7. The speed of PCr recovery was correlated with maturity in adolescents, but was not correlated with end-exercise [PCr] or pH. Two different tests to measure mitochondrial capacity in adolescents were evaluated in Chapter 8, and a fitted curve and gated test were both used to determine PCr recovery kinetics. Finally, in Chapter 9, age-related differences in muscle metabolism and oxygenation during fatiguing exercise were examined; a strong trend for greater fatigue in adults compared with adolescents was accompanied by greater metabolic perturbation in adults. Overall, these data show that muscle metabolism and oxygenation differs between adolescents and adults during and following very high intensity exercise.

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Abbreviations

^{31}P-MRS	31 phosphorus magnetic resonance spectroscopy	MR	Magnetic resonance
ADP	Adenosine diphosphate	MRS	Magnetic resonance spectroscopy
A_F	Fundamental amplitude	MRT	Mean response time
AMARES	Advanced Method for Accurate, Robust and Efficient Spectral fitting	MVC	Maximal voluntary contraction
ANOVA	Analysis of variance	NAD^+	Nicotinamide adenine dinucleotide
ATP	Adenosine triphosphate	NIRS	Near-infrared spectroscopy
a.u.	Arbitrary units	O_2	Oxygen
BL	Baseline	PCr	Phosphocreatine
CI	Confidence interval	P_i	Inorganic phosphate
CK	Creatine kinase	PO	Power output
CP	Critical power	Q	The PCr cost of a brief isometric contraction
Cr	Creatine	SC	Slow component
CWR	Constant work rate	SNR	Signal-to-noise ratio
D	Steady state decrease in PCr during repeated brief isometric contractions	SS	Steady state
E_1	The first bout of two exercise bouts	T	Tesla
E_2	The second bout of two exercise bouts	T_1	The longitudinal time constant for mr signal decay
EE	End-exercise	$T_{1/2}$	Half time for PCr recovery
EMG	Electromyography	τ	Time constant (time to reach 63% of the eventual steady state)
HbO_2	Oxygenated haemoglobin/myoglobin	TCr	Total creatine
HHb	Deoxygenated haemoglobin/myoglobin	TOI	Tissue oxygenation index
IT	Intracellular threshold	TW	
K_{CK}	Equilibrium constant for the creatine kinase reaction	$\dot{V}O_2$	Oxygen uptake
KE	Knee extension	V_{max}	Maximal rate of oxidative phosphorylation
KF	Knee flexion	WAnT	Wingate anaerobic test
La	Lactate	YAPHV	Years from age at peak height velocity

1 Introduction

Children and adolescents are thought to rely less on anaerobic metabolic pathways than aerobic pathways during exercise. This is supported by indirect evidence (Ratel, Tonson, Cozzone, & Bendahan, 2010) but little direct evidence is available to support or refute this proposition. Basic patterns of active play in children involve repeated brief bouts of activity (Bailey et al., 1995), a pattern that seems to be at odds with the characteristics of the aerobic energetic pathways, supposing an enhanced reliance on these pathways in children. This seeming contradiction has been the subject of considerable discussion (Inbar & Bar-Or, 1986; Van Praagh, 2000).

The supposition that children and adolescents rely on aerobic metabolism to a greater extent than anaerobic metabolism is supported by evidence from several different sources. First, muscle biopsy evidence, primarily collected in the 1970's, has suggested that children might have lower concentrations of some anaerobic substrates, higher activities of some aerobic enzyme activities, and/or lower activities of some anaerobic enzymes (Berg, Kim, & Keul, 1986; Eriksson, 1980; Haralambie, 1982; Kaczor, Ziolkowski, Popingis, & Tarnopolsky, 2005). Ethical concerns have limited the number of biopsy studies conducted in healthy paediatric populations, so a number of conclusions about exercise metabolism in childhood and adolescence have been drawn on the basis of evidence from exercise performance studies. These studies have shown that children are less susceptible than adults to muscle fatigue during repeated high intensity exercise bouts (Hebestreit, Mimura, & Bar-Or, 1993; Ratel, Bedu, Hennegrave, Dore, & Duche, 2002; Zafeiridis et al., 2005), that exercise performance recovers more quickly following high intensity exercise in children and adolescents compared with adults (Hebestreit, et al., 1993; Ratel, et al., 2002), and that blood lactate is lower in children and adolescents following intense exercise compared with adults (Beneke, Hutler, & Leithauser, 2007; Pianosi, Seargeant, & Haworth, 1995).

A number of mechanistic explanations for these phenomena have been proposed: it has been suggested that children either possess more, or recruit more, type I muscle fibres compared with adults. This might be the result of maturational differences in muscular or neural function. A sparse body of literature has failed to determine whether muscle fibre

content changes with growth and maturation (Armstrong & Barker, 2009; Eriksson, Gollnick, & Saltin, 1973; Keens, Bryan, Levison, & Ianuzzo, 1978; Lexell, Sjostrom, Nordlund, & Taylor, 1992; Mandroukas et al., 2010; Oertel, 1988). Alternately, anaerobic pathway activity might increase with maturation (Berg, et al., 1986; Kaczor, et al., 2005), and contribute to these differences. Finally, it is possible that differences in habitual physical activity in children and adults lead to a more "trained" muscle profile in children and adolescents compared with adults, which results in a greater proportion of energy production through aerobic pathways.

Recently, techniques such as 31 phosphorus magnetic resonance spectroscopy (^{31}P-MRS) and near-infrared spectroscopy (NIRS) have provided a way to measure muscle metabolism in young people directly, noninvasively and at high temporal resolution. These techniques have facilitated a number of studies that have clarified several aspects of muscle metabolism in children and adolescents. These studies have confirmed that metabolic perturbation during incremental exercise is greater in adults compared with children and adolescents (Barker, Welsman, Fulford, Welford, & Armstrong, 2010b; Kuno et al., 1995; Taylor, Kemp, Thompson, & Radda, 1997; Zanconato, Buchthal, Barstow, & Cooper, 1993), that metabolic recovery might be faster in children compared with adults (Dekerle, Williams, McGawley, & Carter, 2009; Ratel, Tonson, Le Fur, Cozzone, & Bendahan, 2008; Taylor, et al., 1997), and that when exercise is scaled to body size, performance does not differ in adults and children, but metabolism does differ (Barker, et al., 2010b)

Although ^{31}P-MRS research has clarified some issues surrounding paediatric exercise metabolism, many questions remain. In particular, high intensity exercise, recovery from exercise, and metabolism during fatiguing exercise have not been thoroughly investigated. Despite evidence from incremental exercise (Barker, et al., 2010b; Zanconato, et al., 1993) and one constant work rate (CWR) exercise study (Petersen, Gaul, Stanton, & Hanstock, 1999) suggesting that age differences in muscle metabolism are more apparent at high exercise intensities, most paediatric ^{31}P-MRS studies have involved incremental exercise (Barker et al., 2006; Barker, et al., 2010b; Kuno, et al., 1995; Taylor, et al., 1997; Zanconato, et al., 1993), moderate intensity exercise (Barker, Welsman, Fulford, Welford, & Armstrong, 2008a; Barker et al., 2008b) or exercise intensities that are not defined

relative to known thresholds of muscle metabolism (Dekerle, et al., 2009; Fleischman, Kron, Systrom, Hrovat, & Grinspoon, 2009; Petersen, et al., 1999; Ratel, et al., 2008; Tonson et al., 2010). Despite a great deal of speculation about possible metabolic contributions to fatigue resistance in children and adolescents, ^{31}P-MRS has not been used to investigate muscle metabolism during fatiguing exercise. A number of studies have examined recovery from exercise in children and adolescents (Barker, et al., 2008a; Dekerle, et al., 2009; Kuno, et al., 1995; Ratel, et al., 2008; Taylor, et al., 1997). However, metabolic changes during the preceding exercise bout have been demonstrated to influence the speed of PCr recovery (Jubrias, Crowther, Shankland, Gronka, & Conley, 2003; van den Broek, De Feyter, Graaf, Nicolay, & Prompers, 2007) and this has not been adequately investigated or controlled in a number of paediatric recovery studies (Dekerle, et al., 2009; Ratel, et al., 2008; Taylor, et al., 1997).

The purpose of this thesis is to investigate how muscle metabolism differs with age and sex during high intensity and fatiguing exercise, and to investigate recovery from exercise in young people and adults. This work has been communicated to the scientific community in a number of ways (Appendix A). Six studies (four original experiments and two studies based on post-hoc analysis of compiled data sets from the four experiments) make up this thesis:

1. Investigation of PCr kinetics during high-intensity exercise in children and adults
2. Investigation of the effect of exercise intensity on muscle metabolism and oxygenation in adolescent boys and men
3. Description of the speeding of PCr kinetics in adult men but not adolescent boys following priming exercise
4. An investigation of the influence of age, maturation, sex, and end-exercise conditions on PCr recovery kinetics in young people and adults
5. A description of a gated protocol to measure PCr recovery kinetics in adults women and adolescent girls
6. Investigation of fatigue in adolescents and adults during repeated isometric quadriceps maximal voluntary contractions

Overall, this thesis aims to elucidate the interaction of age and exercise intensity on muscle metabolism and to describe how muscle metabolism, oxygenation, and recovery might impact exercise tolerance and metabolism in young people.

2 Review of Literature

This thesis will investigate the nature and implications of age differences in metabolism during adolescence and adulthood using ^{31}P-MRS and NIRS. Before discussing the possibility that children and adolescents use aerobic pathways during exercise to a greater extent than adults, it is important to briefly delineate the metabolic pathways being discussed and describe their interaction. During exercise, energy in the form of ATP is needed to fuel muscle contraction. Very little ATP is stored within the muscle; during muscle contraction, it must be produced within the myocyte. Three pathways facilitate this ATP production. Immediately, phosphocreatine (PCr) is broken down to yield free creatine (Cr) and ATP (Equation 2.1) by the enzyme creatine kinase (CK):

$$\mathrm{PCr} + \mathrm{ADP} + \mathrm{H^+} \leftrightarrow \mathrm{ATP} + \mathrm{Cr} \qquad \text{Equation 2.1}$$

This system of ATP provision has limited capacity, but plays a very important role in muscle energetics at exercise onset. Greater capacity for ATP generation is found in the anaerobic glycolysis pathway. The net products and reactants of anaerobic glycolysis can be seen in Equation 2.2.

$$\mathrm{Glucose} + 2\mathrm{ATP} + 2\mathrm{NAD^+} \rightarrow 4\mathrm{ATP} + 2\mathrm{H_2O} + 2\,\mathrm{NADH} + 2\mathrm{Pyruvate} \qquad \text{Equation 2.2}$$

The products of anaerobic glycolysis can be used to fuel the third energy provision pathway: oxidative phosphorylation, also called aerobic metabolism or muscle respiration. Oxidative phosphorylation has a high capacity, but low power compared with anaerobic glycolysis or the breakdown of PCr. This type of ATP production takes place exclusively within the mitochondria, and involves a very complex set of enzyme-catalyzed reactions. The first chain of reactions converts amino acids (from the breakdown of protein), pyruvate (from the breakdown of carbohydrate through anaerobic glycolysis), and fatty acids (from the breakdown of lipids) into acetyl Co-A. The acetyl Co-A is used to fuel the next stage of oxidative phosphorylation, the Krebs cycle. Finally, ATP is generated through the electron transport chain.

Because oxygen is the ultimate electron acceptor during aerobic metabolism, the transport of oxygen into the cell is a critical factor in oxidative phosphorylation (Tschakovsky & Hughson, 1999). Prior to its use to generate ATP, oxygen must be taken up at the lung, transported to the active muscle within an erythrocyte (bound to haemoglobin) or dissolved in the blood, become unbound from haemoglobin and transported from the capillary into the cell, where it is bound to myoglobin for transport to the mitochondria.

The three energy pathways work together to fuel muscle contraction, but the balance of aerobic metabolism, anaerobic glycolysis, and ATP production through the creatine kinase reaction varies depending on the intensity of exercise, the muscle or muscle groups performing the exercise, the duration of exercise, and the characteristics of the person who is performing the exercise. During steady-state exercise at low intensities, most of the ATP required is produced via oxidative pathways. There is little or no accumulation of metabolic by-products generated by anaerobic glycolysis. These intensities are referred to as moderate intensities (Hogan, Gladden, Grassi, Stary, & Samaja, 1998). As exercise intensity increases, anaerobic glycolysis becomes increasingly important in ATP generation. The gas exchange threshold, when oxygen uptake is measured at the mouth, or the intracellular threshold for P_i/PCr during ^{31}P-MRS research demarcates the boundary between the moderate and heavy intensity exercise. During heavy intensity exercise, a steady state can be attained, but it is delayed compared with the attainment of steady state during moderate intensity exercise and is characterised by an elevated oxygen or phosphate cost of exercise and a decreased pH compared with moderate intensity exercise. During heavy intensity exercise, anaerobic glycolysis plays an increasingly important role in ATP generation, and an accumulation of protons lowers the intracellular pH (Robergs, 2001). At higher exercise intensities, the accumulation of metabolic by-products precludes the attainment of a steady state, and this metabolic perturbation eventually leads to fatigue and exercise cessation. This domain is referred to as the very heavy domain and occurs above the critical power (CP), which is theoretically the highest exercise intensity that can be sustained indefinitely (Whipp & Rossiter, 2005). Finally, the severe or supramaximal exercise domain encompasses exercise demanding ATP production in excess of the maximal capability of the muscle (typically measured during an incremental exercise test to exhaustion). Severe

intensity exercise can be sustained only briefly before the participant is unable to continue to meet the energetic demands of the exercise.

The physiological mechanisms that control mitochondrial ATP production have not been fully resolved, but several theories offer insight into possible metabolic control mechanisms. Several related metabolic variables, including total creatine ([TCr]), mitochondrial capacity, and the phosphorylation potential, which depends on the concentrations of ATP, ADP, and P_i during exercise, are hypothesised to be important in determining the rate of mitochondrial ATP production. Each of these factors is likely to play some part in determining the rate of ATP production, with precise control depending upon the metabolic conditions (steady-state or non steady-state) and the intensity of the exercise. A full review of proposed metabolic control mechanisms is beyond the scope of this thesis, but several important theories warrant discussion.

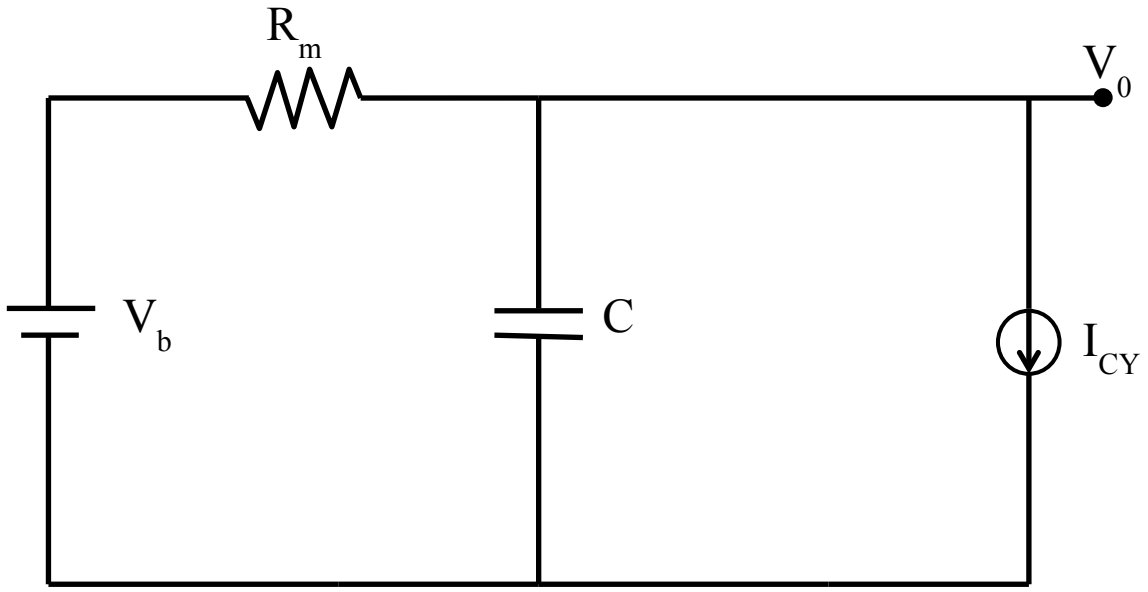

Figure 2.1. Electric analogue model, reproduced from Meyer (1988).

Ron Meyer's electric analogue model (Meyer, 1988; Figure 2.1), makes a number of predictions about metabolic control. In this model, the voltage V_b represents the free energy potential in the mitochondria, the resistor R_m depends on the number and function of mitochondria in the cell, the capacitor C depends on the creatine kinase reaction, the current I_{CY} is the cytosolic ATPase rate, and the voltage V_0 is the Gibbs free energy of ATP

hydrolysis (ΔG_{ATP}). This model predicts that metabolic control depends on the number, properties, and conditions of the mitochondria within the cell, and on the capacitance that results from the creatine kinase equilibrium. Moreover, this model predicts that the system behaves in a linear manner, such that the response of the system to perturbation is predictable. This model only applies during submaximal ATP production rates. The predictions of this model have been confirmed through both in vivo and in vitro research (Kindig, Howlett, Stary, Walsh, & Hogan, 2005, Francescato, Cettolo, & di Prampero, 2008, Glancy, Barstow, & Willis, 2008, Jones, Wilkerson, & Fulford, 2009, Roman, Meyer, & Wiseman, 2002).

Compatible with this theory is the creatine shuttle hypothesis, described by Bessman and Geiger (1981). This theory suggests that the creatine kinase equilibrium acts not only as an immediate source of energy at the contractile machinery in the myocte, but also as means of transmitting the increased ATP demand to the mitochondrion. PCr, Cr, and P_i can diffuse of between the contractile machinery and the mitochondria, and across the outer mitochondrial membrane. As well, the concentration of these creatine metabolites is much higher than the concentration of ADP in the muscle (Meyer & Wiseman, 2006), which means that the concentration gradient between the mitochondrion and the contractile machinery is amplified compared with the gradient that would result from ADP and P_i. In essence, the creatine kinase equilibrium facilitates transmission of the energy demands of exercise to the mitochondrion, and thus plays a role in metabolic control.

It is possible that metabolic rate is controlled by the availability of metabolic substrates, or by metabolic products that are generated by mitochondrial ATP synthesis. A classical view of metabolic control holds that the phosphorylation potential of the cell, determined by [ATP], [ADP], and [P_i], drives increased oxidative phosphorylation. This view is largely informed by experiments on isolated mitochondria, and might neglect to appreciate the many factors that contribute to metabolic regulation in vivo. Alternately, oxygen supply to the mitochondrion might be important in controlling increased oxidative ATP synthesis during metabolic conditions during very intense exercise or when normal physiology is disturbed by disease. If insufficient oxygen is available, this will limit mitochondrial ATP synthesis. Some investigators believe that the intracellular partial pressure of oxygen is

important in controlling metabolic rate in healthy adults (Hughson, 2009). However, a substantial body of evidence suggests that intracellular factors other than the partial pressure of oxygen are responsible for metabolic control (Grassi, 2006). A final theory that should be considered is the hypothesis that mitochondrial respiration is activated by calcium, which is released from the sarcoplasmic reticulum to stimulate muscle contraction.

Overall, the intracellular and extracellular mechanisms that control and regulate mitochondrial ATP synthesis are not resolved. Further, it is likely that a complex interaction of a number of factors allow the precise matching of ATP synthesis to ATP demand, without a single factor being unilaterally responsible for regulation of mitochondrial ATP synthesis. Thus, experiments in different conditions might reveal different aspects of metabolic control. It is possible that metabolic control mechanisms contribute to age differences in muscle metabolism, but a number of other factors determine age differences in muscle metabolism.

2.1 Age Differences in Muscle Metabolism

Children are widely supposed to rely on aerobic pathways to a greater extent than adults during exercise at a variety of intensities. Data to support or refute this idea are sparse and equivocal. A greater reliance on oxidative pathways might be due to comparatively decreased nonoxidative capability, facilitated oxidative metabolism, or a combination of the two. Investigators have attempted to elucidate the nature of metabolic differences between children, adolescents, and adults through three types of studies: muscle biopsy studies, indirect measures of muscle metabolism such as exercise performance, and direct, noninvasive measures including ^{31}P-MRS.

Muscle biopsy studies provide some evidence to support and some evidence to refute the hypothesis that children and adolescents rely more on aerobic and less on anaerobic pathways compared with adults. However, this evidence is difficult to gather because the invasive nature of this technique makes it inappropriate for use with healthy children and adolescents. Low temporal resolution also limits the utility of this technique in

investigating age differences in muscle metabolism. These studies involve the surgical removal of a small sample of muscle tissue, often from the vastus lateralis (Bogdanis, 2009). Resting [PCr] has been reported to increase with age from 11-15, while [ATP] appears to be similar throughout adolescence (Eriksson & Saltin, 1974). This work included only male participants, with 8 boys sampled in each of four age groups. A contradictory report, based on MR measurement of [PCr] in 11 year old boys and suggesting that [PCr] at rest does not change with age, was published by Gariod et al. (1994). This study was also limited to male participants, and included only 8 boys. Generalising the results of both of these studies to a general paediatric population is problematic due to the small samples, limited age range investigated, and exclusion of female participants. Similar problems are present in research that has investigated muscle enzyme activities in children and adolescents. Some of these muscle biopsy studies suggest that children might have higher activities of some aerobic pathway enzymes, such as succinate dehydrogenase (Berg, et al., 1986; Eriksson & Saltin, 1974; Haralambie, 1982) and/or lower activities of some anaerobic pathway enzymes, such as lactate dehydrogenase (Berg, et al., 1986; Eriksson & Saltin, 1974; Kaczor, et al., 2005). However, only the work of Berg et al. (1986) and Haralambie et al. (1982) included female participants, and sample sizes in these studies ranged from 7-20 participants in each group. Despite these limitations, biopsy studies of muscle substrates and enzymes provide further evidence that children and adults might differ in their aerobic or anaerobic abilities.

The fibre profile of the muscle or muscles performing the work is important in determining the relative contributions of each metabolic pathway to the energetic demands of exercise. In human skeletal muscle, each muscle fibre can be broadly categorized as a type I (slow twitch) fibre or a type II (fast twitch) fibre. These fibre types differ in their capacity for aerobic metabolism, which is reflected in the number of mitochondria within the cell, the capillary density surrounding the cell, and the amount of myoglobin in the fibre (Hogan, et al., 1998). Type I and type II fibres also contain different ATPase isoforms (Hogan, et al., 1998). In muscles with a greater volume of type I muscle fibres, aerobic metabolism supplies a greater proportion of ATP. In muscles with greater type II fibre cross sectional area, more ATP will be provided through glycolytic pathways (Hogan, et al., 1998).

Muscle fibre type in children and adolescents has been investigated through muscle biopsies, but the results of these studies have been contradictory. Several investigators have reported that the relative proportion of type I fibres decreases and the relative proportion of type II fibres decreases during childhood and adolescence and into adulthood (Glenmark, Hedberg, & Jansson, 1992; Jansson, 1996; Lexell, et al., 1992; Mandroukas, et al., 2010; Metaxas et al., 2010). However, other studies report that muscle fibre proportions are invariant with age after the first 1-2 years of life (Oertel, 1988) and that adolescents and adults have similar muscle fibre proportions (du Plessis et al., 1985). These reports discuss the number of fibres in each category; the relative cross sectional area of type I and type II fibres is less frequently reported, but appears to increase proportionately in the work of Oertel (1988), in which sexes are pooled until adulthood. In the work of du Plessis et al. (1985), type IIb (transitional) muscle fibre surface areas change from childhood to adulthood in a small (n=12) sample of girls and women but not in a large (n=68) sample of men and boys, while the relative proportions of type I and II fibres did not change in males or females. Finally, Mandroukas et al. (2010) report that both cross sectional area and number of type I muscle fibres decrease from adolescence to adulthood in wrestlers. Each muscle biopsy necessarily examines a small sample of tissue from one area of a single muscle, which included the deltoid and the vastus lateralis in the studies cited above. The results should not be uncritically extended to other muscles and muscle groups. In interpreting the results of these biopsy studies, the effect of training or activity must be considered. With endurance training or high physical activity, there is a shift from type II to type I fibres in adults (Coffey & Hawley, 2007). Greater habitual physical activity in children and adolescents might be an important mechanism for the greater proportion of type I fibres reported in some studies (Lexell, et al., 1992; Mandroukas, et al., 2010; Metaxas, et al., 2010). Another important mechanism for this difference might be the hormonal effects of puberty, particularly in males. Testosterone and other anabolic hormones promote the hypertrophy of muscle fibres, and there is some evidence from animal research that type II fibres are more sensitive to androgens than type I muscle fibres (Ophoff et al., 2009), and this has been suggested to result in selective hypertrophy of type II fibres in males during maturation (Glenmark, 1994). This would lead to an increased type II fibre cross sectional area with no change in fibre number proportions, which might affect

exercise performance but would not be reflected in the reports of Jansson et al. (1996) or Lexell et al. (1992).

The role of muscle fibre type in determining the metabolic response to exercise in children, adolescents, and adults warrants further investigation, since in addition to the studies that have implicated maturational changes in the relative proportions of type I and type II muscle fibres, some investigators have proposed that the ability to recruit type II fibres might be affected by maturation (Falk & Dotan, 2006; Falk et al., 2009; Halin, Germain, Bercier, Kapitaniak, & Buttelli, 2003). This would have a profound effect on the apparent metabolic profile of the muscle, but is not indicative of maturation within the myocyte, but rather neuromuscular maturation. This hypothesis has not been directly tested, and cannot be accepted in the absence of direct evidence. However, the consequences of this possible difference between children, adolescents, and adults will be apparent in exercise performance studies. Differences in neuromuscular maturation might be misinterpreted, in these studies, as differences in muscle metabolism that originate within the myocyte.

A number of studies have used indirect measures of muscle metabolism in childhood and adolescence to understand changes with growth and maturation. However, exercise performance is a broad indicator of muscle metabolism, and might be affected by differences in motivation, neuromuscular activation, or biomechanical differences. Furthermore, the results of these tests reflect only the balance of all metabolic processes happening throughout the active muscles, and can't easily assess the different processes involved. Thus, they are of limited value in understanding the mechanistic basis for maturational differences in muscle metabolism. Despite these limitations, these tests have provided a body of evidence in support of the hypothesis that children use aerobic metabolism to a greater extent than adults, particularly during high intensity exercise. Three important groups of studies are important to consider: those that have reported blood lactate concentrations during and following exercise, those that have examined participants' ability to maintain exercise, and those that have reported the parameters of the $\dot{V}O_2$ kinetic response to CWR exercise.

In children and adolescents, peak blood lactate accumulation following intense exercise is typically lower than in adults (Beneke, Hutler, Jung, & Leithauser, 2005; Beneke, et al., 2007; Eriksson & Saltin, 1974; Martinez & Haymes, 1992; Ratel, et al., 2002). This has been used to support the contention that the contribution of energy from glycolytic pathways is lower in children than adults. The accumulation of [La] in the blood is assumed to reflect glycolytic flux at the muscle. However, there are many factors that affect blood lactate, including the rate of efflux from the cell and intracellular and extracellular buffering and metabolism (Brooks, 1991). Thus, although suggestive of metabolic differences, interpretation of blood lactate measurements as direct indicators of glycolysis is problematic. In particular, it is not clear whether movement of lactate in the body, from intracellular to extracellular to blood compartments and around the bloodstream, is similar in rate and extent in children, adolescents and adults. As well, lactate buffering within and outside the cell has not been thoroughly examined in young people. It is likely that age differences in lactate transport and buffering exist due to the body size of children and adults. These differences will confound the physiological inferences that can be made from blood lactate measurements. Beneke and colleagues (2005, 2007) published a series of studies attempting to resolve these limitations through the use of mathematical modelling of lactate kinetics. However, the authors make a number of assumptions in their calculations. The only directly measured variables in these studies are minute-by-minute blood lactate following a WaNT, and global assumptions about muscle mass as well as the total lactate water space in the body limit the confidence that can be placed in the conclusions of these authors.

The ability of children to maintain exercise performance provides further indirect evidence that age differences in metabolism might exist. Children and adolescents have been reported to more able than adults to resist fatigue during repeated high intensity bouts of cycling (Hebestreit, et al., 1993; Ratel, et al., 2002; Ratel, Williams, Oliver, & Armstrong, 2004), running (Ratel, et al., 2004; Ratel, Williams, Oliver, & Armstrong, 2006), or knee extension and/or flexion (Dipla et al., 2009; Paraschos et al., 2007; Zafeiridis, et al., 2005). This relative fatigue resistance will be discussed in greater detail in Section 2.4, but is consistent with a greater reliance on aerobic pathways, which would entail less metabolic perturbation. Type I fibres are known to be fatigue resistant compared with type II fibres

(Hogan, et al., 1998), so this might also provide evidence that children are relying more on type I fibres in these brief exercise bouts. Streckis, Skurvydas, & Ratkevicius (2007) reported similar fatigue over a 2 minute quadriceps MVC in adolescent boys and girls and adults, although adolescents were more susceptible to central fatigue than adults during this test, indicating that the nature of the task is important in determining age differences in fatigue.

Oxygen uptake ($\dot{V}O_2$) kinetics provides some of the strongest indirect evidence for age differences in muscle metabolism. The nature of the oxygen uptake kinetic response to CWR exercise has been well documented in adults (Poole, Barstow, McDonough, & Jones, 2008) and has been described in children (Armstrong & Barker, 2009). At the onset of exercise, oxygen uptake, measured at the mouth, increases exponentially following a delay known as the cardiopulmonary phase. The exponential rise in $\dot{V}O_2$ is termed the fundamental phase of the response, and is characterised by the time constant (τ) – the time to reach 63% of the eventual steady state during moderate intensity exercise. The fundamental phase may be faster in individuals with a greater oxidative potential or greater proportion of type I muscle fibres (Pringle et al., 2003), or may be independent of muscle fibre type (Barstow, Jones, Nguyen, & Casaburi, 1996). This phase is reported to be faster in children than adults (Armon, Cooper, Flores, Zanconato, & Barstow, 1991; Fawkner, Armstrong, Potter, & Welsman, 2002; Sady, 1981; Williams, Carter, Jones, & Doust, 2001) and to slow with age in children and adolescents (Breese et al., 2010; Fawkner & Armstrong, 2004a), although conflicting reports, showing no differences in $\dot{V}O_2$ kinetics between children and adults, exist (Hebestreit, Kriemler, Hughson, & Bar-Or, 1998). Temporal resolution and mathematical modelling methodologies have improved greatly since the work of Sady et al. (1981) and Zanconato et al. (1991). While this early work has been very influential in the field, later work including the studies published by Fawkner et al. (2004a; 2002) and Breese et al. (2010) have used more rigorous methods. Faster fundamental kinetics in children and adolescents compared with adults provides evidence that children do indeed use aerobic metabolism to a greater extent during both moderate and heavy intensity CWR exercise.

During exercise in the heavy intensity domain (the demarcation between moderate and heavy exercise will be discussed in the following paragraphs), a progressive increase in the oxygen cost of exercise emerges, typically around 2-3 min after the onset of exercise(Fawkner & Armstrong, 2004a, 2004c; Jones & Poole, 2005). This is termed the slow component, and this elevated oxygen cost leads to an elevated steady state or to the cessation of exercise as a result of the individual's inability to meet the energy demands of exercise. The aetiology of the slow component is unresolved, but it emerges predominantly within the exercising muscle (Poole et al., 1991) and might reflect progressive recruitment of type II muscle fibres as fibres recruited at the onset of exercise experience fatigue (Endo et al., 2007; Krustrup, Soderlund, Mohr, & Bangsbo, 2004). Some authors have reported the absence of a slow component in children compared with adults (Armon, et al., 1991; Williams, et al., 2001). However, Armon and colleagues modelled the $\dot{V}O_2$ response to high intensity exercise using a two-term equation, with a linear and an exponential phase. The authors do not report the duration of each phase, and although the example data sets appear well-characterised by the equation used in fitting, the $\dot{V}O_2$ slow component is not thought to be linear in nature (Fawkner & Armstrong, 2004b). Caution should therefore be used in interpreting the results of this investigation. The investigation conducted by Williams et al. (2001) used treadmill running as an exercise mode. In adults (Jones & McConnell, 1999) and children (Machado, Guglielmo, Greco, & Denadai, 2009), the slow component phase of the response is smaller during treadmill running than during cycling exercise, which might have reduced the probability of detecting a slow component in young people in the study conducted by Williams et al. (2001). Despite these limitations, reports of an attenuated or absent slow component in children compared with adults and of an increasing slow component amplitude with age in children and adolescents (Breese, et al., 2010; Fawkner & Armstrong, 2004a) can be interpreted as evidence for a greater reliance on type I fibres throughout the exercise bout.

Over the past two decades, ^{31}P-MRS has been used to investigate muscle metabolism in children and adolescents. The advantages of this technique are considerable: it allows the investigation of muscle metabolites, including PCr, P_i, ATP, lactic acid or H^+, and ADP at high temporal resolution. This technique is noninvasive and can directly measure changes

in these metabolites. The primary disadvantage of magnetic resonance research is the high cost of scanning, and the limited, though increasing, availability of these magnets. Exercise mode within the magnet is also limited; the relatively small bore diameter (often 60 cm) precludes the investigation of locomotor exercise such as running. As well, the surface coil required for ^{31}P-MRS must remain fixed over the same portion of muscle throughout the exercise protocol. Despite these limitations, ^{31}P-MRS has been used to understand muscle metabolism during a variety of exercise tasks in different paediatric and adolescent populations. A number of these studies have examined incremental exercise, where the exercise intensity is progressively increased until the participant is unable to continue to exercise, within the scanner to investigate the interaction between maturation and exercise intensity. These studies have reported that the change in pH and in P_i/PCr over the test is greater in adults than children and adolescents (Barker, et al., 2010b; Kuno, et al., 1995; Taylor, et al., 1997; Zanconato, et al., 1993). This provides evidence that adults are meeting a greater proportion of the energetic demands of exercise through anaerobic pathways. Both Barker et al. (2010b) and Zanconato et al. (1993) observed that these differences emerged only during high intensity exercise, suggesting that exercise intensity might be important to consider in understanding age differences in muscle metabolism.

Zanconato et al. (1993) reported that during exercise below the IT, the rate of change of P_i/PCr was similar in children and adults, and that there was little acidosis in either group. During high intensity exercise, however, the slope of P_i/PCr and pH was significantly greater in adults than children, leading to the greater metabolic perturbation previously described. Barker et al. (2010b) described a similar pattern in the P_i/PCr and pH responses to exercise above and below the intracellular threshold during incremental exercise. The importance of exercise intensity in age differences in muscle metabolism is supported by the findings of Petersen, Gaul, Stanton, & Hanstock(1999), who asked trained 9-16 year old girls to perform 2 min of lower intensity and 2 min of very high intensity exercise. There was a trend for greater metabolic perturbation in peripubertal girls compared with prepubertal girls, indicating that the dependence on different metabolic pathways might develop with maturation during adolescence. The recruitment of a heterogeneous sample that was subsequently divided into two maturity groups rather than the recruitment of two groups known to differ in maturity weakened this study and precluded the attainment of

statistical significance for group differences. The potential intensity dependence of age-related differences in muscle metabolism is an important area for further research.

In considering exercise intensity, however, it is challenging to equalise metabolic demand between groups. In paediatric research, two primary approaches have been taken in this area: normalising the exercise intensity to body size and normalising the imposed exercise to work capability. In the former approach, the work load or exercise performance is allometrically, linearly, or simply ratio scaled to body mass, volume, or surface area, or to lean body mass or the mass, volume, or cross sectional area of the exercising muscle (Welsman & Armstrong, 2007). In the latter approach, exercise intensity is imposed relative to maximal voluntary contraction (MVC), peak oxygen uptake ($\dot{V}O_{2\,peak}$), or some other index of the ability of the participant. One such approach is the concept of domains of exercise intensity, described above (Ozyener, Rossiter, Ward, & Whipp, 2001). This method of normalising exercise intensity requires the participant to complete a ramp or incremental exercise test to allow the investigator to identify both maximum work capability and a threshold, typically the gas exchange threshold, ventilatory threshold, or intracellular threshold (IT) for P_i/PCr or pH. The IT is identified as the point where the slope of P_i/PCr decreases markedly, or the point where pH begins to decrease considerably from resting values, and demarcates the moderate and heavy intensity domains. Exercise intensities are then set relative to the power output at the intracellular threshold (for instance, 80 %IT) or as a percentage of the difference between the power output at the IT and the power output at exhaustion (for instance, 50 %Δ = PO_{IT} + 50 %(PO_{EE} – PO_{IT}), where PO_{IT} is the power output at the IT and PO_{EE} is the power output at exhaustion). The CP represents a second threshold that differentiates the heavy domain from the very heavy domain (Endo, et al., 2007). However, the CP is not typically measured in PCr kinetics studies because this parameter is time-consuming to assess. Barker et al. (2010b) demonstrated that for incremental quadriceps exercise, the power output at the intracellular threshold for P_i/PCr and the maximum power output was similar in children and adults when allometrically scaled to quadriceps muscle mass. Tonson, Ratel, Le Fur, Cozzone, & Bendahan (2008) similarly reported that isometric force in the forearm flexor muscles does not differ significantly in children and adults when scaled to muscle volume. These markers

of exercise intensity appear appropriate in normalising the exercise stimulus to body size, and provide investigators with a reliable method of investigating the interaction between exercise intensity and age in studies of paediatric exercise metabolism.

2.2 Magnetic Resonance Spectroscopy in the Study of Paediatric Exercise Metabolism

Magnetic resonance spectroscopy (MRS) can be used to directly, noninvasively investigate paediatric muscle metabolism. In MRS experiments, the active muscle is placed near the centre of the bore of the magnet with a surface coil tuned to phosphorus located adjacent to the active muscle. Within the static field, atoms with an uneven number of protons will align with the field. This alignment is perturbed with a radiofrequency pulse, and the energy emitted as the atoms return to alignment can be measured and used to generate a spectrum. The peaks of a phosphorus spectrum each correspond to a phosphorus compound and can be quantified to give information about relative concentrations of PCr, inorganic phosphate (P_i), and ATP. For more specific information about MRS, see Chapter 3.

Magnetic resonance research in a paediatric population has unique challenges. Chief among these is the lower signal-to-noise ratio (SNR), which results from the smaller muscle area over which signal can be optimised. A number of approaches have been taken to attempt to increase SNR or to compensate for low SNR, including acquiring each spectrum over a longer period (Ratel, et al., 2008; Tonson, et al., 2010), using a higher magnetic field strength (Ratel, et al., 2008; Tonson, et al., 2010), a larger muscle group (Barker, et al., 2006), and averaging several repeated exercise tests together (Barker, et al., 2008a; Willcocks, Williams, Barker, Fulford, & Armstrong, 2010). A further issue in paediatric MR research is compliance with the test and comfort within the MR environment. Compliance with the exercise test depends on several factors. First, the ergometer and test protocol must be developed with children's size and ability in mind; some commercial ergometers are not suitable for use with this population, so custom-made ergometers must be used. Second, it is important that paediatric participants are adequately familiarized with the scanner environment, the exercise modality, and the test protocol. Finally, the comfort of the children is paramount – they must be made aware of their right to withdraw from the

study at any time without penalty, and researchers should attempt to explain what to expect to reduce anxiety related to the loud noises and unfamiliar environment of the scanner. A final consideration in MR research is the high cost of scanner time; at the Peninsula MR Research Centre, the current hourly cost is ~£250. The issue of cost is not limited to paediatric research but dictates that studies must be planned carefully to optimise use of the magnet.

If the considerations described above can be met, there is a great deal of information that can be gained from ^{31}P-MRS in children and adolescents. Some of the studies which have been conducted have been described previously. Table 2.1 lists the ^{31}P-MRS studies that have been carried out in healthy children to the author's knowledge to date.

Table 2.1. ^{31}P-MRS studies that have examined changes in metabolism related to growth and maturation, 1993-2010.

Participants	Methods	Results	Critical analysis and conclusions
Zanconato et al., 1993			
8 boys, 2 girls, 9.3 ± 1.0 y; 5 men, 3 women	Dynamic plantar flexion incremental exercise to exhaustion (1.5 T, 32 s temporal resolution)	Adults performed more work when work was normalised to body weight; end-exercise (EE) P_i/PCr was higher in adults, and pH_{EE} was lower. Below intracellular threshold, slope of P_i/PCr v. power was similar in children and adults; above threshold adults had steeper slopes for both pH and P_i/PCr. Relationship between P_i/PCr and pH was not age-dependent.	Ground-breaking and well conducted study – the suggestion that age differences in metabolism are intensity-dependent is particularly interesting. The inclusion of both sexes in this report is positive, but the pooling of males and females for analysis might mask sex differences but the study was not designed to investigate possible differences between males and females.
Kuno et al., 1995			
23 untrained boys, 12-15 y; 6 sedentary adults	Dynamic knee extension incremental exercise to exhaustion (1.5 T, 8 s temporal resolution.	In untrained participants: Ratio of PCr : (PCr + P_i) was higher in adolescents than adults, as was intracellular pH at exhaustion. Recovery kinetics was similar in adolescents and adults, with mean τ ranging from ~45 to 60 s.	The values reported for intracellular pH are strikingly low for the quadriceps (~6.72 in untrained 12 year olds, 6.8 in most adolescent groups, and less than 6.6 in adults), which might explain the slow reported recovery kinetics. The similar recovery kinetics in adolescents and adults is an important finding.
Taylor et al., 1997			
15 children, 6-12 y; 125 adults, 20-85 y	Dynamic plantar flexion incremental exercise to exhaustion (2.0 T, 75 s temporal resolution); quantified metabolites assuming [ATP]=8.2 mM	At rest, PCr/ATP and PCr/P_i lower in children than adults, while pH was the same and [ADP] was higher. pH_{EE} was lower in adults (6.64 ± 0.12) than children (6.80 ± 0.10), while PCr depletion was similar in both groups. $[ADP]_{EE}$ was greater in children than adults. $T_{1/2}$ for PCr recovery was faster in children (12 ± 4 s) than adults (27 ± 8 s), as was V_{max} and the initial rate of PCr resynthesis.	Although the methodological description in this study is somewhat lacking detail, it seems well-conducted from the information provided. The sample size is a strength of this study, as is the matching of work rate during the incremental exercise test. Children and adults represented a broad age range, potentially introducing variability within groups. The significantly faster PCr recovery kinetics directly contradict the report by Kuno et al. (1995).

Ratel et al., 2008			
7 boys, 11.7 ± 0.6 y, 10 men	3 min sustained forearm contraction (15% MVC), (4.7 T, 28.8 s temporal resolution)	MVC was similar in boys and men when linearly scaled to finger flexor muscle volume. pH_{EE} was not significantly different in boys (6.6 ± 0.2) and men (6.5 ± 0.2), but boys used less PCr and had higher PCr:P_i at end exercise. Recovery kinetics were significantly faster in boys (k_{PCr} = 1.7 ± 1.2 min^{-1}) than men (k_{PCr} = 0.7 ± 0.2 min^{-1}). Proton efflux was significantly faster in boys than men.	The results of this study are very interesting, but must be interpreted in light of the significant acidosis and differences in proton efflux reported; these factors will undoubtedly influence the speed of PCr recovery in boys and men.
Barker et al., 2008			
8 boys (9.9 ± 0.4 y), 10 girls (9.8 ± 0.4 y), 8 men, 8 women	Dynamic quadriceps exercise; 6 min at 80% IT (2-4 bouts) (1.5 T, 6 s temporal resolution)	pH_{EE} was somewhat higher in children than adults but did not change from resting values in either group. Resting P_i/PCr was significantly greater in children at rest, but not different at EE. [ADP] was significantly greater in children during rest, exercise, and recovery. There were no age or sex differences in PCr kinetics at the onset or offset of exercise (Onset τ: boys = 21 ± 4 s, girls = 24 ± 4 s, men = 26 ± 9 s, women = 24 ± 7 s; offset τ: boys = 26 ± 5 s, girls = 29 ± 7 s, men = 23 ± 9 s, women = 29 ± 7 s)	This study is the first to document PCr kinetics during exercise in children, and adds more evidence to the debate surrounding the effect of growth and maturation on PCr recovery kinetics. The temporal resolution at which spectra were acquired and the emphasis on performing sufficient repeated transitions to have confidence in the parameters of the PCr kinetic response are strong points in this study. Future research should focus on the effect of exercise intensity on PCr kinetics in young people.
Tonson et al., 2010			
7 boys, 11.7 ± 0.6 y, 10 men	3 min sustained forearm contraction (15% MVC), flux through each metabolic pathway (4.7 T, 28.8 s temporal resolution). Some duplication with Ratel et al., 2007	Resting P_i/PCr lower in adults; all other resting variable similar Greater CK flux in adults; greater aerobic flux in children; more PCr depletion and P_i accumulation in adults; no differences in glycolytic flux or pH; faster proton efflux in children	This study is novel in its attempt to quantify fluxes through each of the ATP generating pathways in children. Some of the assumptions involved are unverified in children. The differences in metabolism between the forearm muscles and postural muscles such as the calf or quadriceps are not addressed. The suggestion that glycolysis does not differ with age but that CK flux does differ warrants further research.

Barker et al., 2010			
15 boys (10.8 ± 1.1 y), 18 girls (10.6 ± 1.1 y), 8 men, and 8 women	Dynamic quadriceps incremental exercise test to exhaustion (1.5 T, 30 s temporal resolution)	At rest, P_i/PCr was higher in children. Resting pH was higher in girls than women but not in boys than men. Peak power allometrically scaled to quadriceps muscle mass did not differ with age or sex. At exhaustion, P_i was higher in adults than children and in females than males, PCr was lower in adults than children and in females than males, and pH was higher in children than adults but did not differ with sex. Below the IT, the slope of P_i/PCr vs power was lower in boys than girls but did not differ with age. Above the IT, this slope was significantly greater in adults than children and in girls than boys. The slope of pH v. PO above the IT was significantly greater in men and girls than boys but similar in girls and women and men and women. At the IT for P_i/PCr, allometrically scaled power output did not differ with age and sex.	The noteworthy results of this study are: 1) further confirmation of an intensity-dependence of age differences in metabolism, and 2) evidence that the intracellular threshold and PO_{EE} for dynamic quadriceps exercise do not differ with age or sex when allometrically scaled to quadriceps muscle mass.
Fleischman et al., 2010			
23 prepubertal (10.2 ± 1.4 y) and 45 pubertal (14.3 ± 2.0 y) children, 22 young and 31 middle-aged adults	3 min dynamic quadriceps exercise; load is not clearly described (3 T, 15 s temporal resolution), measured physical activity but used questionnaires in children and accelerometers in adults.	PCr recovery τ varied significantly with age: 27.4 ± 11.7 s in prepubertal, 36.1 ± 13.2 s in pubertal; 48.0 ± 23.7 in young adults, 54.2 ± 20.9 in older adults Authors state that the load was chosen to allow "sufficient" PCr depletion and that pH was less than 6.95 and PCr:P_i less than 4 at end exercise, indicating "adequate participant effort"	There are several fundamental flaws in this ambitious study. The relationships between age, physical activity, and oxidative capacity warrant investigation, and the sample size in this study is large enough to draw strong conclusions. Unfortunately, by not reporting the degree of acidosis, these authors limit the confidence in their estimates of oxidative capacity. As well, the use of different indices of physical activity in children and adults precludes the interpretation of these data in light of possible differences in physical activity between groups.

As Table 2.1 reveals, and as discussed in Section 1.1, these studies vary considerably in the population, exercise protocol, and goals of the study. Certain trends emerge on examination. First, as previously described, incremental exercise leads to greater metabolic perturbation in adults compared with children and adolescents in all studies reported to date (Barker, et al., 2010b; Kuno, et al., 1995; Taylor, et al., 1997; Zanconato, et al., 1993). Second, in recent years there has been an increasing emphasis on the analysis of the kinetic parameters of the PCr response; the studies of Barker, Welsman, Fulford, Welford, & Armstrong (2008a), Ratel et al. (2008), and Fleischman et al. (2009) have all included PCr kinetics at the onset or offset of exercise as a primary outcome measure.

In Section 1.1, the results of a series of $\dot{V}O_2$ kinetics studies in children and adolescents were described. These studies usually report faster fundamental kinetics and a reduced slow component amplitude in young participants. The exponential phase of the pulmonary $\dot{V}O_2$ response reflects the muscle $\dot{V}O_2$ kinetic response (Krustrup, Jones, Wilkerson, Calbet, & Bangsbo, 2009), which is also reflected in the PCr response at the onset of exercise, as demonstrated by Barstow, Buchthal, Zanconato, & Cooper (1994). This was confirmed in adults by Rossiter and colleagues in an elegant series of studies published between 1999 and 2002 (Rossiter et al., 2000; Rossiter et al., 1999; Rossiter et al., 2002a; Rossiter et al., 2001; Rossiter et al., 2002b) and in children during moderate exercise by Barker, Welsman, Fulford, Welford, Williams, et al. (2008b). The PCr kinetic response is mechanistically linked to the intrinsic properties of the muscle, and is thus an important variable to investigate in an attempt to understand the metabolic differences between children, adolescents and adults.

The depletion of PCr follows a predictably exponential course at the onset of CWR exercise. The speed of this response is dictated by the mitochondrial capacity of the muscle. However, the ability of the muscle to buffer the rise in ADP through the creatine kinase reaction is also critical in determining PCr kinetics at the onset of exercise. Put simply, the speed of the response depends on the muscle's aerobic and anaerobic abilities. This relationship is predicted by Meyer's electric analogue model, which describes respiratory control in the myocyte using an electrical circuit model. The model predicts that control will depend on the number and properties of the mitochondria (analogous to a resistor), the creatine kinase reaction (analogous to a

capacitor), and the mitochondrial redox potential. Experimentally, it has been demonstrated that the speed of the PCr kinetic response depends on [creatine] and the creatine kinase equilibrium (Francescato, Cettolo, & di Prampero, 2008; Glancy, Barstow, & Willis, 2008; Jones, Wilkerson, & Fulford, 2009; Kindiget al., 2005; Roman, Meyer, & Wiseman, 2002) as well as the mitochondrial content and function of the muscle (Glancy, et al., 2008). Thus, an understanding of PCr kinetics in different intensity domains in childhood and adolescence can help investigators to understand the maturation of metabolic control.

The slow component of the PCr kinetic response is a particularly interesting phase in paediatric exercise physiology, given its possible dependence on muscle fibre recruitment (Endo, et al., 2007; Krustrup, et al., 2004). Although several studies have reported an age-related increase in the amplitude of the $p\dot{V}O_2$ slow component, no study to date has examined PCr kinetics in the heavy domain in children or adolescents. Theoretically, an attenuated PCr slow component would be expected in young people, based on previous paediatric literature and on the documented mechanistic basis for the slow component. Some research has suggested that the slow component amplitude might be dependent on the delivery of oxygen to the muscle (Haseler, Kindig, Richardson, & Hogan, 2004). Little is known about maturational differences in oxygen delivery during exercise. A study by Gunter Koch (1977) suggested that muscle blood flow to the quadriceps muscle might increase from 12-14 years, but this is an isolated report with a small sample which can't be reliably generalised. Investigation of the mechanisms underlying the development of the slow component in children and adolescents is methodologically challenging. Certainly, however, the roles of oxygen delivery and fibre recruitment in determining the amplitude of the PCr slow component in children and adolescents are important areas for further investigation.

The parameters of the PCr kinetic response are known to be malleable with intervention. This malleability has been exploited in studies designed to investigate metabolic control. Many interventions which are potentially interesting in a paediatric population, including creatine loading (Jones, et al., 2009; Smith, Montain, Zientara, & Fielding, 2004), altering oxygen delivery through hypoxia or hyperoxia (Haseler, et al., 2004), and systematic training (Jones, Wilkerson, Berger, & Fulford, 2007) are challenging to implement in a paediatric population due to the invasiveness of the

intervention or due to the commitment and compliance required from participants. Priming exercise represents an appropriate and interesting intervention to use with healthy young people. Priming exercise is also potentially externally generalisable as a model of warm-up exercise in sporting situations. A priming intervention involves a bout of heavy intensity exercise followed by a period of unloaded ergometer work or rest, followed by a second bout of heavy intensity exercise. In adults, priming exercise seems to speed the MRT for overall PCr response in the second bout (Forbes, Raymer, Kowalchuk, Thompson, & Marsh, 2008; Jones, Fulford, & Wilkerson, 2008a; Rossiter, et al., 2001). The MRT is measured by fitting a single exponential curve through both the fundamental and slow component phases of the response. This effect, as reported for both $\dot{V}O_2$ and PCr kinetics, is typically due to an increase in the amplitude of the fundamental component of the response and a decrease in the slow component amplitude; τ is seldom reported to change with priming exercise (Burnley, Jones, Carter, & Doust, 2000; Forbes, et al., 2008; Jones, Berger, Wilkerson, & Roberts, 2006; Jones, et al., 2008a; Marles et al., 2007; Rossiter, et al., 2001). In children, priming effects (a reduced slow component amplitude and faster MRT) have recently been reported for cycling exercise (Barker, Jones, & Armstrong, 2010a) but the magnitude of the priming effect in children compared with adults has never been examined, and a priming effect on PCr kinetics has not been demonstrated in children or adolescents. The mechanistic basis for the priming effect is not well understood; it is possible that facilitated oxygen delivery is important in speeding the response, or it is possible that a priming of the metabolic pathways is more important in the reported slow component reduction.

This section has described the importance of PCr kinetics research in children and adolescents, but there are some methodological challenges in this research. Compliance with the ergometer and exercise protocol is very important, as discussed in Section 2.2. The high temporal resolution required to determine the parameters of the PCr kinetic response dictates that spectra are acquired frequently during exercise compared with typical paediatric MRS studies (Table 2.1). Quality spectra rely on the muscle being in a similar position every time a spectrum is acquired. During dynamic exercise, this requires the participant to exercise at a constant rate. While good compliance vastly improves the quality of the spectra acquired, poor SNR is an inherent challenge in paediatric research, and can lead to imprecise estimates of τ. To improve confidence in

the parameters of the response, several identical tests are usually completed in both adult and paediatric PCr kinetics studies (Barker, et al., 2008a; Rossiter, et al., 2002a). These tests are averaged together, and then analysed by fitting an exponential equation to the data. For tests in the moderate domain, this exponential equation is typically fitted over the entire exercise test. However, for CWR exercise in the heavy or very heavy domains, it is important to avoid contaminating the fitted fundamental component with the slow component. Thus, the slow component is either fitted as a separate exponential term in the equation or the fitting window is limited to include only the fundamental phase. The emergence of the slow component is often identified by iteratively widening the fitting window to the point where the time constant begins to progressively lengthen (Rossiter, et al., 2002a) or where the 95% confidence intervals (CI) for the value of τ are minimised (Forbes, et al., 2008). The slow component can then be quantified as the change in PCr from the end of the fundamental phase to the end of exercise.

2.3 Near-Infrared Spectroscopy in the Study of Muscle Metabolism

To interpret PCr kinetics and other measurements of muscle metabolism during exercise at different intensities, it is necessary to consider not only the possible metabolic control parameters, but also factors extrinsic to the cell, such as oxygen delivery to the working tissue. This can be challenging in a paediatric population, since many methods of measuring muscle blood flow or oxygen delivery are invasive and thus inappropriate (Radegran, 1999). In adults, oxygen delivery to the working tissue has been shown to affect slow component amplitude (Haseler, et al., 2004), and this is the case during maximal intensity but not moderate intensity exercise in isolated dog gastrocnemius (Grassi, 2000; Grassi, Gladden, Samaja, Stary, & Hogan, 1998; Grassi, Gladden, Stary, Wagner, & Hogan, 1998). The fundamental component of the PCr kinetic response does not appear to be oxygen delivery dependent in healthy adults (Poole, et al., 2008), although several clinical populations display oxygen dependent fundamental kinetics (Poole, et al., 2008). NIRS offers a noninvasive method of examining oxygenation at the level of the muscle which is well-suited to paediatric research.

NIRS is based on the theory that the reflection and absorption of near-infrared light by haemoglobin in the small blood vessels (arterioles, capillaries, and venules) and myoglobin in the muscle depends on whether these compounds are bound to oxygen or not. The fractional reflection and refraction of this light can be measured to assess the

oxygenation of the tissue. During exercise, this can be used to quantify fractional oxygen extraction in the working tissue.

NIRS offers a promising method of investigating an important physiological variable that has previously been inaccessible in children and adolescents, but the measurement and interpretation of NIRS data can be problematic. Unless the differential pathlength factor for the tissue under consideration is known, estimates of HHb and HbO_2 can only be expressed as changes relative to resting baseline, in arbitrary units. This makes comparisons between individuals or groups impossible. The HHb kinetic response describes the temporal characteristics of the response, and consists of three phases: a delay, which reflects a close balance between oxygen delivery and utilisation and which typically lasts 5-12 s, an exponential phase with a time constant averaging 7-12 s, and a third phase which comprising the remainder of the response (DeLorey, Kowalchuk, Heenan, Dumanoir, & Paterson, 2007; DeLorey, et al., 2003; DeLorey, Kowalchuk, & Paterson, 2004; Ferreira, Townsend, Lutjemeier, & Barstow, 2005; Grassi, et al., 2003; Grassi, Quaresima, Marconi, Ferrari, & Cerretelli, 1999; Jones, et al., 2006; Jones, et al., 2008a; Koga, et al., 2007). No study to date has examined the effect of age on HHb kinetics during CWR exercise in children and adolescents. From the parameters of the response, the investigator can draw conclusions about the balance of oxygen delivery and utilisation, but NIRS is unable to distinguish between these two factors.

Paediatric NIRS investigations are sparse in the literature. Moalla, Merzouk, Costes, Tabka, & Ahmaidi (2006) used NIRS to examine the relationship between the EMG signal and NIRS signal in children and adolescents, but analysed only the time course of the oxygenation during a fatiguing isometric contraction. The reliability of NIRS in children has been documented in several abstracts (Thevenet et al., 2008; Welford, Welsman, & Armstrong, 2005) and one paper (Leclair et al., 2010), but issues affecting the validity of this measure have not been thoroughly examined. HHb kinetics has been used to examine oxygenation during CWR exercise in male adolescents (Marwood, Roche, Rowland, Garrard, & Unnithan, 2010) and young female swimmers (McNarry, Welsman, & Jones, 2010). However, there is extensive scope for further research using this technique.

2.4 Muscle Fatigue during Very High Intensity Exercise

The previous sections have documented age differences in metabolic control during exercise, particularly high intensity exercise. However, these differences are particularly interesting when their effect on exercise tolerance is considered. During very high intensity, an individual's ability to meet the demands of the task is often compromised – this is referred to as muscle fatigue. The definition of muscle fatigue varies, but a well accepted definition is that muscle fatigue is a fall in the force generating capability of the muscle (Enoka & Duchateau, 2008). The magnitude, degree, and mechanisms of fatigue depend on the characteristics of the task and of the participant, as well as an interaction between the task and the participant. Fatigue differs with the type of contraction (isometric or dynamic), intensity of exercise, duration of exercise (sustained versus intermittent) (Williams & Ratel, 2009). As well, the muscle group performing the exercise affects the degree of fatigue; muscles that are high in type I muscle fibres resist fatigue to a greater extent than more glycolytic muscles or muscle fibres (Bogdanis, 2009). Finally, fatigue differs with age (Hebestreit, et al., 1993; Kent-Braun, Ng, Doyle, & Towse, 2002; Ratel, et al., 2002), sex (Hunter, Butler, Todd, Gandevia, & Taylor, 2006; Russ & Kent-Braun, 2003; Wust, Morse, de Haan, Jones, & Degens, 2008), and training (Johansen & Quistorff, 2003).

Children and adolescents are reported to be more fatigue resistant than adults, especially during repetitive, high intensity exercise (Dipla, et al., 2009; Hebestreit, et al., 1993; Ratel, et al., 2002; Zafeiridis, et al., 2005). The studies that have demonstrated this are detailed in Table 2.2. The relationship between age and fatigue during sustained exercise is less clear, and a lack of age difference is sometimes reported (Ophoff, et al., 2009). Table 2.2 makes it apparent that despite the consistency of the reported fatigue resistance, little mechanistic research has been undertaken. The exception is an interesting study by Streckis (2007), which examined central fatigue during a sustained quadriceps muscle MVC. This study used imposed twitches to quantify the impairment in the central nervous system, and found that central fatigue was greater in children than adults, although performance declined to a similar extent in both groups. These data can be interpreted to imply that different muscle fibre recruitment patterns in children and adults are involved in reported fatigue differences. That is, children might have an attenuated ability to recruit muscle fibres fully at exercise onset and throughout

exercise. However, given the task-dependence of muscle fatigue, it important to avoid over-generalising the findings of Streckis et al. (2007). Table 2.2 also reveals a marked lack of research in females; although one study reported no sex differences in muscle fatigue in 10 year old boys and girls during a sustained submaximal contraction (Christos, Konstantinos, Dimitrios, & Eleni, 2006), this study did not include an adult comparison, and thus is not included in Table 2.2.

In addition to the potential central mechanisms of fatigue, there is good reason to speculate that metabolic factors might be important in mediating age differences in muscle fatigue. If, as previously discussed, children and adolescents preferentially utilise aerobic over anaerobic pathways to fuel high intensity exercise, young people will experience less accumulation of metabolic byproducts thought to induce fatigue, including P_i and ADP. Acidosis is no longer widely believed to be a primary mediator of muscle fatigue, but greater acidosis, if indicative of greater reliance on glycolysis, is likely correlated with a number of changes in the myocyte which lead to muscle fatigue (Westerblad, Allen, & Lannergren, 2002).

Table 2.2. Studies which have examined muscle fatigue during repetitive, high intensity exercise in children and adults, and the possible mechanisms for age differences proposed by the authors.

Participants	Methods	Results	Critical analysis and conclusions
Hebestreit et al., 1993			
8 boys (9-12 y), 8 men	2 x 30 s WAnT with 1, 2, or 10 min recovery interval	Lower peak power and relative fatigue in boys, lower relative fatigue in boys. Power recovered after 1 min in boys but was still depressed after 1 and 2 min in men. Faster $\dot{V}O_2$ recovery $T_{1/2}$ in boys.	This study demonstrates the fatigue-resistance of children during repetitive high intensity exercise for the first time. The authors speculate that different rates of recovery of performance might be due to reduced reliance on anaerobic pathways, lower mechanical and metabolic strain, or faster removal of metabolites.
Ratel et al., 2002			
11 boys (9.6 ± 0.7 y), 9 adolescent boys (15.0 ± 0.7 y), 10 men	10 x 10 s cycle sprints with 30, 60, or 300 s recovery interval	Boys were able to maintain performance regardless of recovery interval; peak power in adolescents decreased by 18.5% over the 10 sprints with 30 s recovery and by 15.3% with 60 s recovery. In adults, performance decreased by 28.5% with 30 s, 11.3% with 60 s, and significantly with 300 s. Blood lactate was significantly lower in boys.	The different recovery intervals reported allow the authors to test the hypothesis that recovery as well as exercise metabolism allows children to maintain performance. The authors believe that metabolic differences and faster PCr recovery kinetics (not measured) are the primary mechanisms for this difference.
Halin et al., 2003			
15 boys (10.5 ± 0.9 y), 12 men	30 s isometric elbow flexor MVC (last 2 s discarded)	Mean power function and muscle fibre conduction velocity were higher in men at the start of contraction, but declined more in men.	This study attempts to address the issue of muscle fibre recruitment, but it is difficult to draw conclusions based on EMG data during a sustained contraction. The authors suggest that fatigue differences might be due to metabolic or fibre recruitment differences.

Zafeiridis et al., 2005			
19 boys (11.4 ± 0.5 y), 17 adolescent boys (14.7 ± 0.4 y), 18 men	Either 4 sets of 18 max knee extension (KE) and knee flexion (KF) (60 s rest) or 2 sets of 34 max KE and KF, (120 s rest). Angular velocity was 120°/s	Boys recovered more than men between bouts, with recovery in adolescents intermediate between these values. This was true with both 30 s and 60 s recovery. Blood lactate at was higher in men than boys and adolescents at mid- and EE and higher in adolescents than boys at EE.	This study provides further evidence that age and recovery modulate muscle fatigue in young people. The authors suggest that future research should explore the relationships between recovery duration and fatigue, and the hormonal, neurological, metabolic, immunological changes during high-intensity exercise.
Ratel et al., 2006			
12 boys (11.7 ± 0.5 y), 13 men	10 X 10 s non-motorised treadmill sprints with 15 or 180 s passive recovery between.	Both peak power and mean power decreased more in men than boys with 15 s rest, and boys were able to maintain performance with 180 s rest while performance decreased significantly in men. EE blood lactate was lower in boys than men.	The authors extend previous work to running exercise, and provide further evidence that boys fatigue less than men regardless of recovery duration. However, no further evidence regarding the mechanistic basis for this phenomenon is provided.
Paraschos et al., 2007			
10 boys (10.5 ± 0.6 y), 14 men	25 consecutive max isokinetic KE at 60°/s, with EMG	Torque decreased by 25.7% in boys and 36.1% in men. Children had more antagonist activity during the contraction, while agonist activity declined more in adults.	This study provides further evidence that age differences in neuromuscular activation exist during fatiguing exercise.
Streckis et al., 2007			
7 boys (13.9 ± 0.3 y, 7 girls (13.6 ± 0.2 y), 7 men, 7 women	KE, isokinetic dynamometer. Voluntary, evoked, and superimposed isometric contractions were measured.	Voluntary activation and central activation ratio lower in adolescents than adults. Relative decrease in torque was similar in all groups over a 2 min sustained KE MVC.	This study is novel in its use of stimulated contractions to examine central fatigue, and raises the possibility that reduced metabolic fatigue is related to increased central fatigue in children and adolescents.

Dipla et al., 2009			
10 boys (11.3 ± 0.5), 10 girls (10.9 ± 0.6 y), 10 adolescent boys (14.7 ± 0.3 y), 10 adolescent girls (14.4 ± 0.7 y), 10 men, 10 women	4 sets of 18 maximal isokinetic KE and KF, 60 s rest	Increasing fatigue (F/E, and TW) with age in boys, plateau after adolescence in girls. Negative correlation between fatigue and peak torque (r=-0.68 to -0.84), and fatigue and lactate (r=-0.58 to -0.69).	Fatigue resistance decreases into adulthood, but isn't different across genders at each age.
Hatzikotoulas, et al., 2009			
15 boys (10.1 ± 1.1 y) and 15 men	20% maximal voluntary plantar flexion contraction, sustained for 10 min.	Fatigue during submaximal isometric exercise was similar in boys and men, but following this contraction, the recovery of both force and EMG was faster in boys compared with men	This study supports the hypothesis that recovery is important in age differences in fatigue, since fatigue did not differ with age during a sustained contraction.
De Ste Croix, Deighan, Ratel, & Armstrong, 2009			
16 boys (12.2 ± 0.3 y), 14 girls (12.2 ± 0.3 y), 9 men, 12 women	50 dynamic KE/KF cycles (1.56 rad/s)	Greater fatigue over the test in adults in both the flexors and extensors. No significant sex differences or interaction effects in fatigue.	This study provides further evidence that children are more fatigue resistant than adults, and the authors suggest that PCr recovery between contractions, muscle fibre recruitment, or metabolic factors might be responsible for the difference.
Armatas et al., 2010			
13 boys (10.0 ± 0.8 y), 13 men	Repetitive isometric KE MVC, test lasted until force decreased to 50% of MVC	Force declined more slowly in boys than men, while agonist activity measured by EMG increased more in men than boys. Following exercise, force recovered more quickly in boys than men.	This examines is similar to the studies of Zafeiridis et al. (2005) and Paraschos et al. (2007), but sets the key outcome variable as time to 50% decrease in MVC force rather than the decrease in MVC force at a given time. These studies strongly suggest that recovery between contractions is important in offsetting fatigue.

The studies reported in Table 2.2 strongly suggest that a primary variable that mediates the greater fatigue resistance in children and adolescents is the speed of metabolic recovery. Ratel and colleagues (2002) manipulated the recovery interval between very high intensity 10 s cycling sprints, and reported that 10 year old boys did not experience any decrease in performance over the course of 10 sprints, even with as little as 30 s of rest. Adolescent boys, on the other hand, experienced an 18.5% decrease in power output when only 30 s of recovery was provided between sprints, and a 15.3% decrease when 60 s of recovery was available. Adolescent boys were able to maintain performance with 5 min recovery between sprints, but men experienced an 28.5% decrease in power output with 30 s of recovery (significantly greater than adolescents), 11.3% decrease with 60 s recovery (not significantly different from adolescents), and an 7.4% decrease with 5 min of recovery (not significantly different from adolescents, although at $p=0.058$ it could be suggested that there is a meaningful difference, since adults experienced impaired performance whereas adolescents did not). This study provides clear evidence that the ability to recover from intense exercise differs with age, and that this difference affects performance during subsequent exercise. Further evidence is provided by the studies of Streckis et al. (2007) and Hatzikotoulas et al. (2009), which reported no age differences in fatigue when a sustained contraction was performed.

The causes of faster recovery in children and adolescents are poorly understood. It is possible that children recover more quickly because they have less to recover from (Falk & Dotan, 2006) – when recovery from maximal intensity exercise is compared, the greater metabolic perturbation described by several authors in adults might obligate a longer time to full recovery (Barker, et al., 2010b; Kuno, et al., 1995; Taylor, et al., 1997; Zanconato, et al., 1993). Alternately, greater oxidative capacity in children might lead to faster clearing of metabolic byproducts and replenishment of PCr. It is probable that both of these mechanisms are important in determining the effects of recovery time on performance.

In adults, sex has been shown to affect muscle fatigue, with women usually being less susceptible to fatigue during high intensity exercise compared with men (Hunter, et al., 2006; Russ & Kent-Braun, 2003; Wust, et al., 2008). Sex differences in muscle fatigue are predominantly peripheral in origin (Albert, Wrigley, McLean, & Sleivert, 2006;

Hunter, et al., 2006; Kent-Braun, et al., 2002; Wust, et al., 2008), and might result from differences in muscle fibre type or recruitment (Hunter, et al., 2006; Wust, et al., 2008), use of different metabolic pathways (Russ, Lanza, Rothman, & Kent-Braun, 2005; Wust, et al., 2008), or differences in muscle blood flow during isometric contraction (Russ & Kent-Braun, 2003; Thompson, Fadia, Pincivero, & Scheuermann, 2007). These sex differences are not typically apparent in children (Christos, et al., 2006; Dipla, et al., 2009). The age at which sex differences in muscle fatigue emerge is not apparent but is likely during the pubertal period.

2.5 PCr Recovery Kinetics

The speed of PCr recovery following exercise is important from a performance perspective and also from a physiological perspective. The time constant (τ) for the exponential recovery of PCr serves as a measure of muscle oxidative capacity (Meyer, 1988; Paganini, Foley, & Meyer, 1997). Glycolytic metabolism ceases within a few seconds of the end of exercise (Crowther, Kemper, Carey, & Conley, 2002b), so the speed of monoexponential recovery of PCr is determined by the oxidative capacity of the muscle. This has been used to study age-related changes in muscle function (Conley, Jubrias, & Esselman, 2000; Kent-Braun & Ng, 2000; Ratel, et al., 2008), training effects on oxidative capacity (Tartaglia et al., 2000), and alterations in disease (Argov, Löfberg, & Arnold, 2000; Radda et al., 1995). The speed of PCr recovery depends on many physiological factors, including EE pH (Jubrias, et al., 2003; van den Broek, et al., 2007), EE PCr (Bendahan, Kemp, Roussel, Fur, & Cozzone, 2003), mitochondrial capacity (Meyer, 1988; Paganini, et al., 1997), and possibly muscle fibre type (Blei, Conley, Odderson, Esselman, & Kushmerick, 1993; Crowther, Jubrias, Gronka, & Conley, 2002a).

Five studies have examined the effects of growth and maturation on PCr recovery kinetics. Of these, three have reported faster recovery in children or adolescents compared with adults (Dekerle, et al., 2009; Ratel, Tonson, Le Fur, Cozzone, & Bendahan, 2007; Taylor, et al., 1997) while two have reported no difference in the speed of recovery between children or adolescents and adults (Barker, et al., 2008a; Kuno, et al., 1995). These differences are not surprising, given the variation in age, exercise protocol, and muscle group being compared (Table 2.1). The effect of acidosis on PCr recovery kinetics is well documented and frequently discussed (Jubrias, et al.,

2003; van den Broek, et al., 2007). The use of PCr recovery kinetics as an index of oxidative capacity is reportedly only valid where pH is not decreased from the baseline value. It is likely that pH plays a confounding role in the interpretation of the data of Ratel et al. (2007), Taylor et al (1997), Kuno et al., (1995), and Fleischman et al. (2009), particularly given the age differences in proton efflux reported by Ratel et al. (2007). In that respect, the report which should be interpreted with the most confidence is that of Barker and colleagues (2008a), who reported that PCr kinetics do not differ with age or sex in children and adults following moderate exercise. The reports whose conclusions must be viewed with the most caution is that of Fleischman et al. (2010). These authors did not report the exercise stimulus or the degree of metabolic perturbation experiences by the participants; it is possible that these parameters varied between groups. Given the relevance of PCr recovery kinetics to the study of muscle metabolism in children, further work to elucidate the effects of age, sex, maturation, and exercise intensity on PCr recovery is warranted.

2.6 Conclusion and Overview

This chapter has described the evidence for and against changes in muscle metabolism with growth and maturation. A limited number of studies have directly measured metabolic substrates, enzymes, or products via muscle biopsies or magnetic resonance spectroscopy, and most of these studies have suggested that children preferentially use oxidative pathways to meet the energetic demands of exercise. This direct evidence is supported by indirect evidence, which is also suggestive of age differences in muscle metabolism. The central questions of this thesis surround the nature and implications of age differences in muscle metabolism, particularly during high intensity exercise. A series of six studies will be described and discussed.

First, PCr and HHb kinetics during heavy intensity exercise (20 %Δ) will be described in adolescent and adult males and females (Chapter 4). Similar PCr kinetics in children and adults during moderate intensity exercise were described by Barker et al. (2008a), a report which contradicts several studies that have reported age differences in $\dot{V}O_2$ kinetics in this domain (Armon, et al., 1991; Fawkner, et al., 2002; Williams, et al., 2001). Based on the work of Barker et al. (2010), Zanconato et al. (1993), and Petersen et al. (1999), age differences might be expected to emerge at higher exercise intensities. PCr kinetics during high intensity exercise have not been described in children or

adolescents, and the nature of the HHb kinetic response has not been examined in combination with PCr kinetics in young people. As well, there are reports of sex differences in $\dot{V}O_2$ kinetics in childhood (Fawkner & Armstrong, 2004), but this has not been investigated in adults or in PCr kinetics. Thus, the first study aims to describe the PCr kinetic response to high intensity exercise in male and female adolescents and adults.

This work will be extended in Chapter 5 by examining the effect of increasing exercise intensity in adolescent and adult males by comparing PCr and HHb kinetics during heavy intensity exercise with PCr and HHb kinetics during very heavy intensity exercise. $\dot{V}O_2$ kinetics in adults and in adolescents are known to differ with intensity (Lai, Camesasca, Saidel, Dash, & Cabrera, 2007; Ozyener, et al., 2001) during supra-threshold exercise, and the metabolic response to exercise above and below the CP has been shown to differ (Jones, Wilkerson, DiMenna, et al., 2008) but the very heavy exercise domain has not been extensively studied in young people. Williams et al. (2008) and Barker et al. (under review) have measured critical power in young people. In these studies, unlike similar studies in adults (Gaesser, Carnevale, Garfinkel, Walter, & Womack, 1995; Poole, Ward, Gardner, & Whipp, 1988), $\dot{V}O_{2\,peak}$ was not attained at the end of supra-CP exercise to exhaustion, indicating that the response to very high intensity exercise might differ in young people compared with adults. Chapter 5 aims to compare the metabolic responses to high intensity and very high intensity exercise in young and adult males.

The manipulability of PCr kinetics in young people will be examined in Chapter 6 via a priming intervention. In adults, priming exercise speeds the PCr (Forbes, et al., 2008; Jones, Fulford, et al., 2008) and $\dot{V}O_2$ (Burnley, et al., 2000; DeLorey, et al., 2004; Jones, et al., 2006; Marles, et al., 2007) kinetic response to exercise. This has recently been demonstrated in children (Barker, Jones, et al., 2010). The priming effect is thought to result from changes related to oxygenation, muscle metabolism, or muscle fibre recruitment (Burnley, Koppo, & Jones, 2005), each of which might differ in young people compared with adults. The effect of priming exercise on PCr kinetics has not been examined in children or adolescents, and the relative magnitude of the priming effect in adolescents compared with adults is unknown for $\dot{V}O_2$ kinetics. The aim of

Chapter 6 is to examine the magnitude of the priming effect during high intensity knee extension exercise in adolescent compared with adult males.

The speed of PCr recovery following exercise has been used by a number of investigators as an index of mitochondrial capacity in young people (Fleischman, et al., 2010; Ratel, et al., 2008) and adults (Haseler, Hogan, & Richardson, 1999; Walter, Vandenborne, McCully, & Leigh, 1997). This measure is potentially very useful in understanding the development of mitochondrial function in children and adolescents as well as alterations with training and disease in young people. However, the basic characteristics of the response depend on the metabolic state of the muscle at the end of the preceding exercise bout in adults (Jubrias, et al., 2003; van den Broek, et al., 2007), and the effects of acidosis, PCr depletion, age, sex, and maturity in young people have not been investigated. Chapter 7 examines recovery from high intensity exercise in adolescents and adults, with the hypothesis that the effect of acidosis and PCr depletion on PCr recovery kinetics will be similar in adolescents and adults but that PCr recovery kinetics will be faster in adolescents as a result of age differences in mitochondrial capacity.

Chapter 8 will describe the development of an exercise protocol to measure PCr recovery kinetics without incurring acidosis or requiring participants to exercise at a high intensity. Slade, Towse, Delano, Wiseman & Meyer (2006) and Forbes, Slade, Francis, & Meyer (2009) describe a gated exercise test wherein participants complete repeated brief isometric contractions; PCr recovery is then calculated from the degree of PCr recovery between contractions and the overall decrease in PCr. This method is promising in a paediatric population, but its feasibility and validity have not been established. Chapter 8 describes the development of this protocol and the requisite equiment and compares this method of measuring PCr recovery kinetics with PCr recovery kinetics measured following a sustained isometric contraction in adolescent and adult females.

Differences in muscle metabolism, oxygenation, and recovery have been implicated in the relative resistance to muscle fatigue seen in children and adolescents compared with adults (Dipla, et al., 2009; Hebestreit, et al., 1993; Moalla, et al., 2006; Ratel, et al., 2002). However, previous investigators have not tested these hypotheses. In fact,

although several investigators have documented greater fatigue resistance in children and adolescents compared with adults (Dipla, et al., 2009; Hebestreit, et al., 1993; Paraschos, et al., 2007; Ratel, et al., 2002; Ratel, et al., 2004, 2006; Zafeiridis, et al., 2005), the metabolic changes accompanying fatigue in young people have not been described. The purpose of the study described in Chapter 8 was to describe age and sex differences in muscle metabolism and oxygenation during fatiguing exercise.

This series of studies will elucidate the influence of age and sex on metabolic control and muscle oxygenation during high intensity exercise. As discussed throughout this chapter, there is a paucity of research examining the mechanistic basis for age differences in exercise performance in young people and adults. Through the use of novel noninvasive techniques, namely ^{31}P-MRS and NIRS, these studies will advance the paediatric literature considerably and will provide a foundation for further muscle physiology research in healthy and unhealthy children and adolescents. The overall hypothesis of this work is that adolescents will use oxidative pathways to a greater extent than adults during high intensity exercise and recovery. Specifically, PCr kinetics during exercise and recovery will be faster in adolescents than adults, and the amplitude of the slow component will be decreased in adolescents. These metabolic differences will result in a greater relative change in PCr kinetics following priming exercise in adults, and in decreased fatigue during intermittent isometric exercise in adolescents.

3 Methods

3.1 Overall Design

Participants were recruited for four different experiments (Table 3.1). Two composite data sets were compiled from these, because presentation of these analyses across studies contributes significantly to the overall aim of the thesis. Specifically, experiment 1 and experiment 2 used a similar exercise task at two different intensities. The effect of intensity was examined by comparing these two data sets. As well, data collected during recovery from high intensity exercise was pooled and examined in Chapter 7. This allowed the investigation of factors affecting PCr recovery in a relatively large sample of young people.

Table 3.1. Correspondence between chapters of the thesis and experiments conducted..

	Experiment 1: Heavy intensity exercise	Experiment 2: Two consecutive bouts of very heavy intensity exercise	Experiment 3: Gated exercise to determine PCr recovery kinetics	Experiment 4: Fatiguing intermittent isometric exercise
Chapter 4	Exercise data			
Chapter 5	Exercise data (males)	Exercise data (bout 1)		
Chapter 6		Exercise data (bouts 1 and 2)		
Chapter 7	Recovery data	Recovery data		Recovery data
Chapter 8			All data	
Chapter 9				Exercise data

The following chapter will detail methods used throughout the studies. First, the three MR-compatible ergometers and habituation methods used in these investigations will be described. Parameters and methods of MRS and NIRS data collection will be discussed. Finally, data analysis including mathematical modeling of MRS and NIRS data will be discussed.

3.2 Exercise and Habituation

3.2.1 Dynamic knee extension ergometer

This ergometer consists of a rope and pulley system (Figure 3.1). The participant's foot is secured to a padded foot cradle, and the participant secured to the scanner bed with four straps (not visible in Figure 3.1), over the distal thigh, proximal thigh, hips, and lower back. The participant exercises by moving the foot up and down within the range of motion permitted by the white frame on the scanner bed (~22 cm). The rope passes through a pulley in the frame on the scanner bed and another pulley in the frame behind the bed, and attaches to a load basket. Nonmagnetic metal masses are placed in the load basket to modify resistance. The velocity and displacement of the load basket is measured using an optical sensor embedded in the pulley in the frame behind the bed, and a load cell between the rope and the bucket measures the force exerted by the bucket and weights combined. The power output can be calculated from velocity and force.

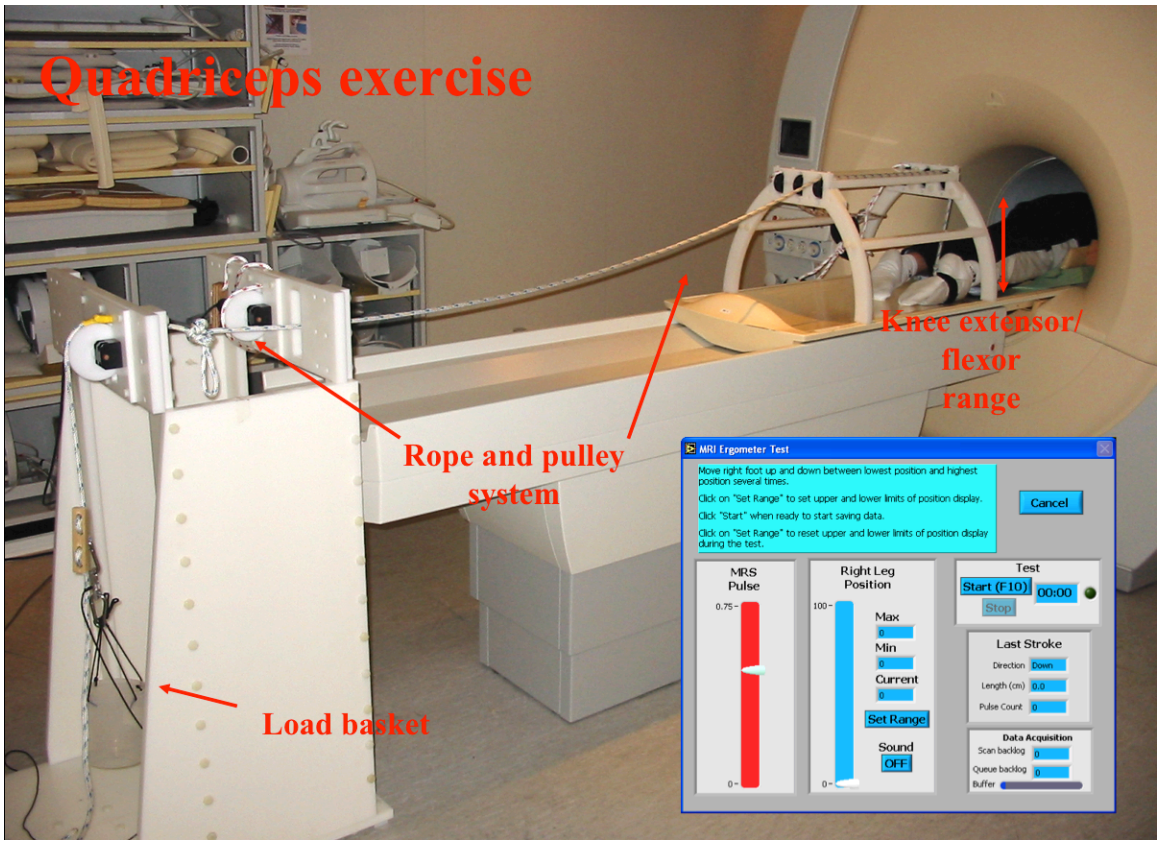

Figure 3.1. Dynamic quadriceps ergometer with participant in place within bore of MR scanner.

During dynamic ^{31}P-MRS, it is important to ensure that the same volume of muscle is sampled in each spectrum. To ensure that the leg is in the same position relative to the coil for each spectral acquisition, the dynamic quadriceps ergometer includes an integrated visual display (inset in Figure 3.1). The display is projected on the wall of the scanner room in view of the participant. The display shows a cursor within a red bar which moves vertically within the bar in time with the scanner pulses. A cursor within a blue bar is connected to the movement of the rope and pulley system via the optical sensor. Participants are asked to match the movement of the cursor within the blue bar as closely as possible to the movement of the cursor in the red bar.

Compliance with this ergometer is important. Participants must use the quadriceps muscle group and avoid recruiting extraneous muscles such as the hip flexors or the ankle dorsiflexors to move the load basket. These movements are prevented using straps to restrain the hips, and through verbal reminders from the experimenter to keep the foot plantarflexed. Participants must also maintain a consistent range of motion. The amplitude of the movement of the rope is projected on the magnet surface, allowing the experimenter to monitor compliance.

3.2.2 Isometric knee extension ergometer

The ergometer used for repetitive isometric quadriceps exercise was a modified version of the ergometer illustrated in Figure 3.1. The load basket was removed and the rope was securely affixed to the base of the frame behind the scanner bed. The participant's foot was suspended approximately 10 cm above the scanner bed, such that maximal voluntary contraction did not result in the participant's foot touching the bed. Force was measured through the load cell. Participants exercised by contracting the quadriceps muscle group to press the top of the foot toward the scanner bed. Verbal feedback and visual monitoring by the experimenter were used to encourage participants to use the quadriceps muscle group rather than the hip flexors or ankle dorsiflexors to exert force. No visual display was used with this ergometer. Participants were instructed to exert their maximal force with each contraction, and verbally encouraged throughout the test to push as hard as they could.

3.2.3 Isometric plantar flexion ergometer

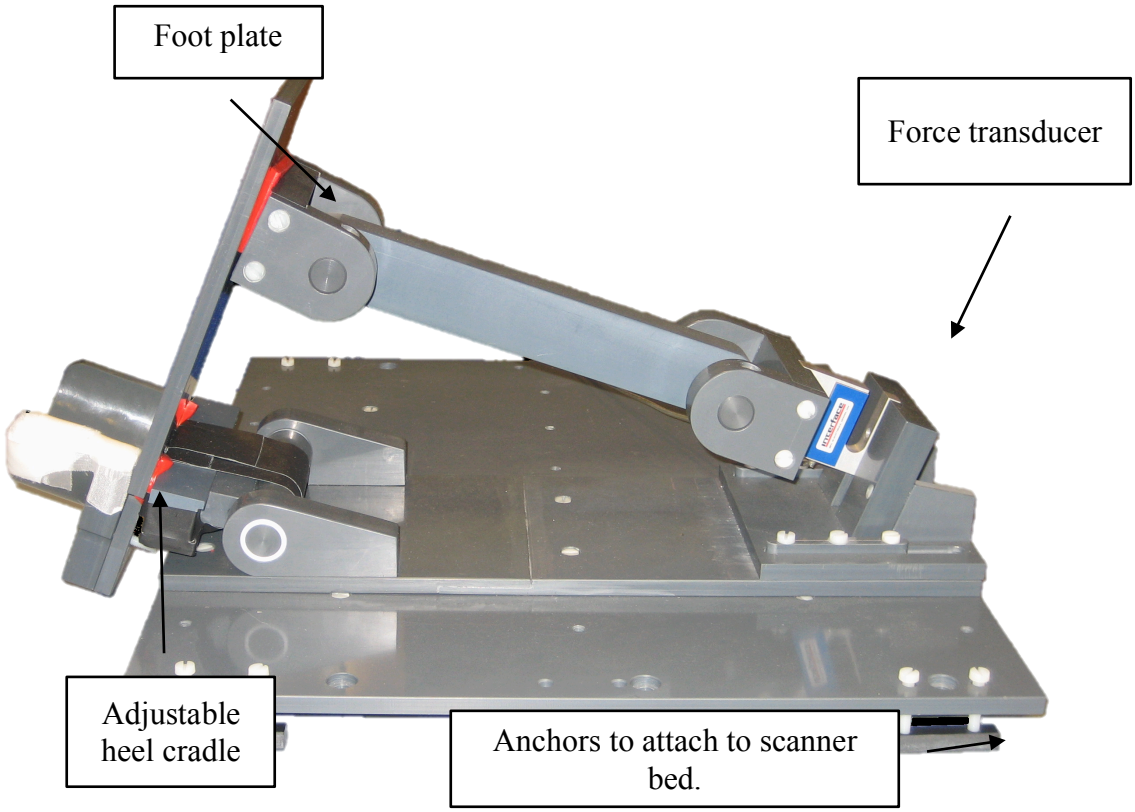

Figure 3.2. The calf ergometer, with parts labelled, and a view of a participant's foot in place in the calf ergometer prior to positioning of the participant with the calf muscle in the centre of the magnet bore.

A custom-made calf ergometer (Figure 3.2) was developed as part of the study described in Chapter 8. An existing prototype underwent iterative testing and improvement of the design to the point where it could be used to measure calf muscle metabolism during exercise and recovery. In the final version of this ergometer, the participant lay supine with the right foot secured to the ergometer with the ankle plantar-flexed at ~ 30 degrees and Velcro straps securing the participant's hips and legs. The knee was slightly flexed for the participant's comfort. The ergometer consisted of a foot pedal through which force was transmitted to a static bar perpendicular to the foot. An MR compatible force transducer (Interface, Scottsdale, AZ, USA) measured the force applied. The heel cradle of the ergometer was adjusted for each participant to ensure that the ball of the foot was positioned over the force transducer.

A visual display projected on the scanner was used to cue contractions and to provide participants with visual feedback about their force production. This display allowed a target force to be presented.

Prior to each testing session, the ergometer was positioned with the foot plate parallel to the floor. Metal masses totaling 11 kg were placed on the foot plate to calibrate the force transducer. Once the participant was positioned in the ergometer and within the scanner bore, the force reading was set to zero to account for the force of the participant's foot resting on the heel cradle.

3.3 Habituation

Prior to any testing within the MR scanner, each participant completed a habituation session within a mock MR scanner. This habituation session allowed the experimenter to acclimate the young participants to the MR scanner environment and explain the procedures. More importantly, this session allowed the adolescent and adult participants to practice the exercise tasks, which were usually novel and sometimes challenging.

To habituate participants to the dynamic exercise, a pulse generator set to 0.67 Hz triggered the visual display software. Participants were first encouraged to master matching the cursors in the blue and red bars (see Section 3.2.1) with 1-2 kg in the load basket. Once the movement was fluid, weight was increased approximately every 30 seconds to mimic the incremental test (see Section 3.6.2). Finally, participants practiced

the onset and first several minutes of a square wave exercise bout 2-4 times, to ensure that the transition from rest to exercise was smooth.

For static exercise, participants were introduced to the ergometer and the movement required. The experimenter guided the participants in producing force without recruiting extraneous muscle groups. Finally, participants were repeatedly (10-20 repetitions) asked to produce maximal and submaximal contractions after verbal (for knee extension exercise) or visual (for plantarflexion exercise) cueing.

3.4 MR data collection

MR imaging and spectroscopy techniques exploit the behavior of atoms with an uneven number of protons in a static magnetic field. These atoms align with the magnetic field. To generate a spectrum, the alignment of the atoms is disturbed through the application of a magnetic pulse at the Larmor frequency of the compound of interest, and energy is emitted as the atoms gradually return to alignment. This energy is detected by a surface coil and, through Fourier transformation, used to generate an image or spectrum. A set of phosphorus spectra acquired during exercise and recovery can be seen in Figure 3.3. In the studies described in this thesis, a gradient echo image was initially acquired, allowing the experimenters to localise the signal within the active muscle. Matching and tuning of the coil was then carried out. Finally, an automated shimming protocol to optimise the signal from the muscle under investigation was carried out. In all experiments described in this thesis, a fully saturated spectrum was initially acquired. This allows the calculation of P_i/PCr ratios at rest; partial saturation effects during the exercise protocol invalidate this calculation when the repetition time is shorter. During the exercise tests, ^{31}P spectra were obtained using an adiabatic pulse. The pulse frequency was typically 1.5 s, although in the study reported in Chapter 8, a 1 s pulse frequency was used. Phase cycling with four phase cycles was employed, leading the acquisition of spectra every 6 s or 4 s, depending on the study. Spectra were acquired with a spectral width of 1500 Hz and 512 data points.

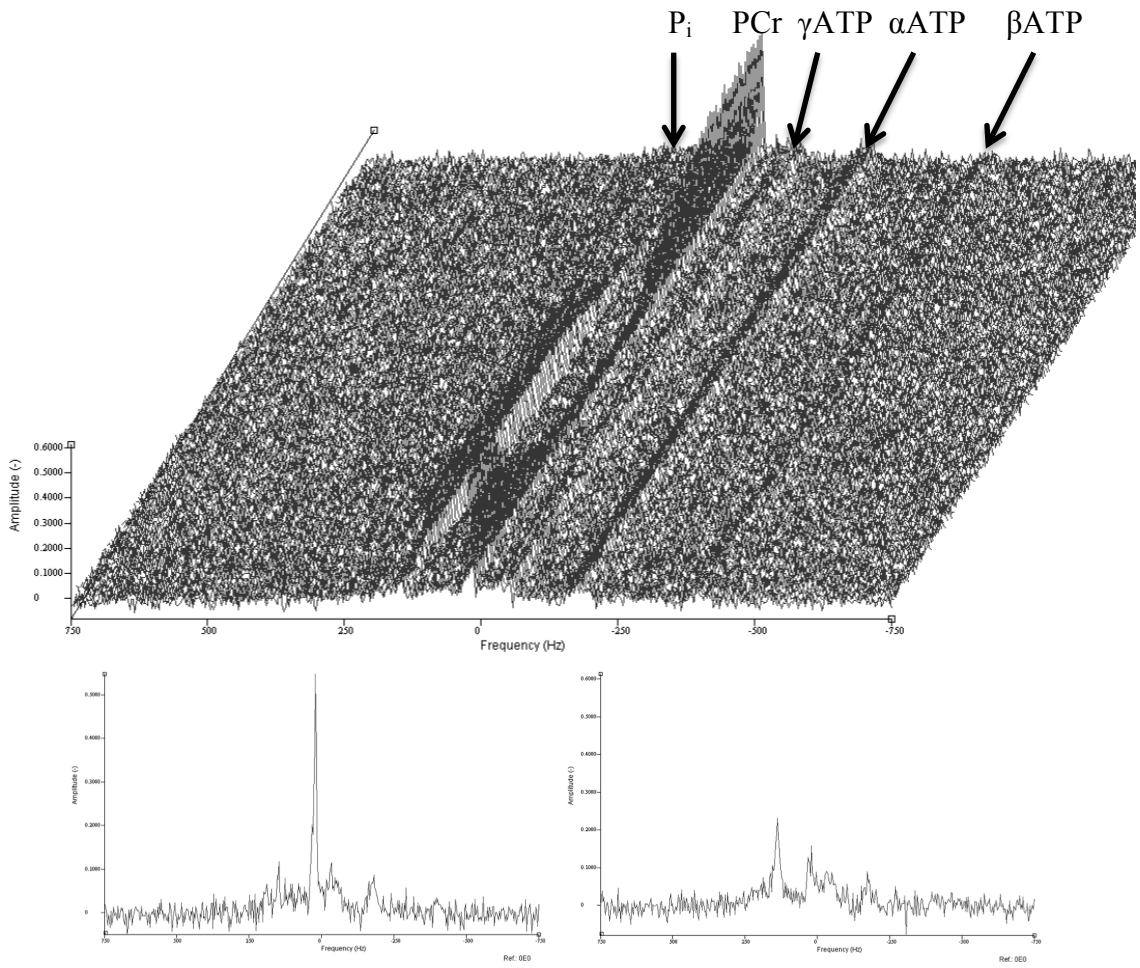

Figure 3.3. Spectra from a 13 year old girl during rest, exercise, and recovery (top). Individual spectra during rest (left) and exercise (right) are shown below. The PCr depletion and increased P_i concentration can be seen in the exercise spectrum.

The area under each peak of the spectra acquired was quantified using a non-linear least squares peak-fitting software package (jMRUI Software, version 2.0) (Naressi, Couturier, Castang, de Beer, & Graveron-Demilly, 2001) and the AMARES fitting algorithm (Vanhamme, van den Boogaart, & Van Huffel, 1997). Spectral areas were fitted assuming presence of the following peaks: P_i, phosphodiester, PCr, α-ATP (2 peaks, amplitude ratio 1:1), γ-ATP (2 peaks, amplitude ratio 1:1) and β-ATP (3 peaks, amplitude ratio 1:2:1). Where absolute concentrations of phosphorus metabolites are calculated, relative amplitudes must be corrected for partial saturation because of the short repetition time relative to the longitudinal time constant (T_1). T_1 is typically assumed to remain constant during exercise (Cettolo, Piorico, & Francescato, 2006; Frank, Wong, Haseler, & Buxton, 1999).

The chemical shift between P_i and PCr is predictably pH dependent, allowing this distance to be used to calculate intracellular pH (Taylor, Bore, Styles, Gadian, and Radda, 1983) (Equation 3.1).

$$\mathrm{pH} = 6.75 + \log\frac{(\sigma - 3.27)}{(5.69 - \sigma)} \qquad \text{Equation 3.1}$$

Where 6.75, 3.27, and 5.69 are constants associated with this relationship and σ is the chemical shift difference between P_i and PCr.

3.5 NIRS Data Collection

NIRS is based on the theory that the reflection and absorption of near-infrared light by haemoglobin in the small blood vessels (arterioles, capillaries, and venules) and myoglobin in the muscle depends on whether these compounds are bound to oxygen or not. Thus, a NIRS unit emits near-infrared light at several wavelengths through a probe placed over the region of interest (Figure 3.4). The fractional reflection and refraction of this light can be measured to assess the oxygenation of the tissue. During exercise, this can be used to quantify fractional oxygen extraction in the working tissue. Commonly available NIRS systems including the NIRO-200 and NIRO-300 give several parameters of oxygenation: the change in oxygenated haemoglobin and myoglobin (HbO_2), the change in deoxygenated haemoglobin and myoglobin (HHb), and the tissue oxygenation index (TOI). The first 2 parameters are measured using the modified Beer-Lambert method (Endo, et al., 2007), while the tissue oxygenation index requires the use of spatially resolved spectroscopy (Boushel et al., 2001). Typically, HHb kinetics are analysed to quantify the temporal characteristics of muscle oxygen extraction (DeLorey, Kowalchuk, & Paterson, 2003; Grassi et al., 2003; Jones, et al., 2006; Koga et al., 2007), because the HHb signal is not thought to be dependent on the volume of blood in the field of view of the NIRS probe.

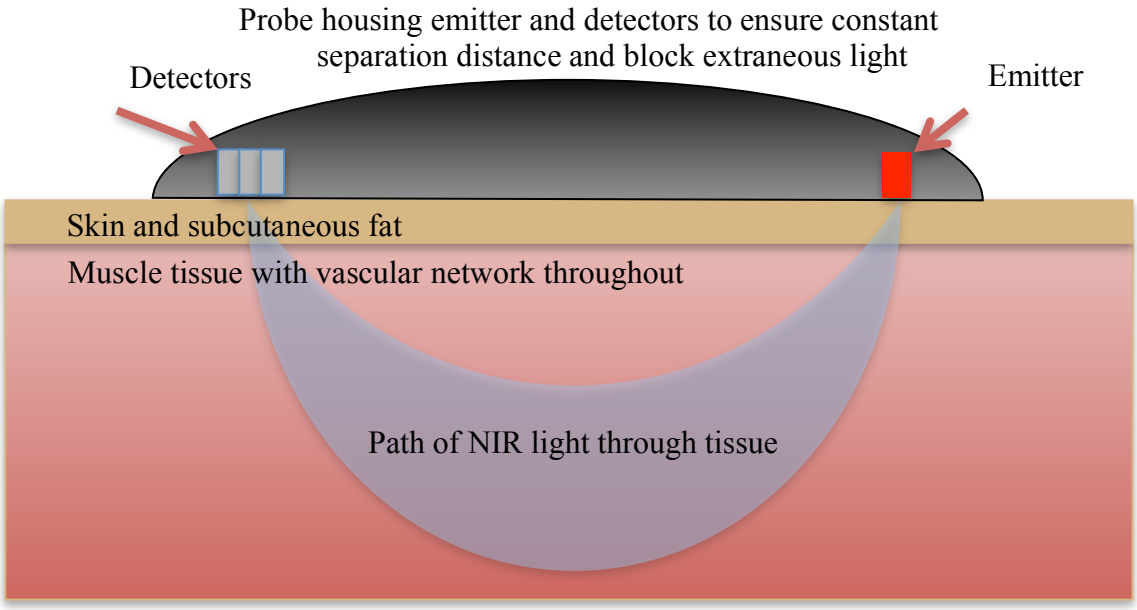

Figure 3.4. Schematic diagram of NIRS probe in place on a participant's leg. Near-infrared light is emitted by a laser (and transmitted to the emitter through optical cables), and the light reflected and refracted is measured at the detector.

The NIRS systems used in this investigation (NIRO-200 and NIRO-300, Hamamatsu photonics KK) use spatially resolved spectroscopy, and consist 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 100 ns and a repetition rate of 2 kHz for each wavelength. The emitter is housed in a dark probe that also contains the detector, and thus ensures a consistent emitter-detector separation of 4 cm for the NIRO-300 and 2.5 cm for the NIRO-200. Reflected and scattered light is detected by a 3 segment photodiode chip as described by Suzuki et al. (1999). The probe housing the optodes was placed midway between the greater trochanter and lateral epicodyle of the femur, over the vastus lateralis muscle for each study in this thesis. The probe was fixed using adhesive tape and extraneous light was excluded using layers of elastic bandage around the thigh. HHb and HbO_2 data were interpolated to 1s intervals and expressed as a change, in arbitrary units (a.u.) from resting baseline. The TOI, which is calculated as the ratio of HbO_2 to the sum of HbO_2 and HHb, was used in Chapter 6 to give an index of increased oxygen delivery following priming exercise.

3.6 Data analysis

3.6.1 Quantification of [PCr], [P_i], and [ADP]

^{31}P-MRS gives relative changes in PCr and P_i. [PCr], [P_i], and [ADP], can be calculated from these raw data. 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 (Equation 3.2; Forbes, et al., 2008; Lanza, Befroy, & Kent-Braun, 2005), with [total creatine] ([TCr]) assumed to be 45 mM in both children and adults.

$$[\mathrm{PCr}] = \frac{\mathrm{PCr}_{raw}}{(\mathrm{PCr}_{raw} + P_{iraw})} * [\mathrm{TCr}] \qquad \text{Equation 3.2}$$

Resting [PCr] was multiplied by the relative change in PCr to give [PCr] during exercise. [ADP] was calculated from [PCr] and pH, using Equation 3.3:

$$[\mathrm{ADP}] = \frac{[\mathrm{ATP}][\mathrm{Cr}]}{[\mathrm{PCr}][\mathrm{H}^+]\mathrm{K}_{\mathrm{CK}}} \qquad \text{Equation 3.3}$$

Where K_{CK} is the equilibrium constant for the creatine kinase reaction, which was assumed to be 1.66×10^9, [ATP] is assumed to be 8.2 mM, [Cr] is assumed to be approximated from [P_i], and [H^+] is calculated from intracellular pH.

Several assumptions are necessary in the calculation of [PCr], [P_i], and [ADP]. Specifically, the concentrations of [TCr] and [ATP] must be assumed. In adults, the concentration of [TCr] was assumed to be 45 mM (Kemp, Meyerspeer, & Moser, 2007), and [ATP] was assumed to be 8.2mM (Harris, Hultman, & Nordesjo, 1974). In children, limited data suggest that [ATP] remains constant between 11–16 years (Eriksson & Saltin, 1974) and is similar to adult values (Gariod, et al., 1994). However, no estimate of [TCr] is available for children. Eriksson and Saltin (1974) demonstrated an increase in [PCr] from 21mM at 11 years to 35mM at 16-years-old in boys, while Gariod et al. (1994) 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 paediatric MR studies (Barker, et al., 2008a; Ratel, et al., 2008; Taylor, et al., 1997).

3.6.2 Incremental testing and CWR intensity prescription

Prior to CWR exercise, each participant 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 $N \cdot min^{-1}$ 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. The IT was defined as the point at which the P_i/PCr v power output plot deviated from the initial slope (Figure 3.5). The IT was used to calculate a CWR intensity according to Equation 3.4.

$$x\ \%\Delta\ mass = x\ \% \cdot\ (mass_{peak} - mass_{IT}) + mass_{IT} \qquad \text{Equation 3.4}$$

Where x is the desired delta intensity, $mass_{peak}$ is the mass in the load bucket at the peak power output recorded during the testing, and $mass_{threshold}$ is the mass in the load bucket at IT.

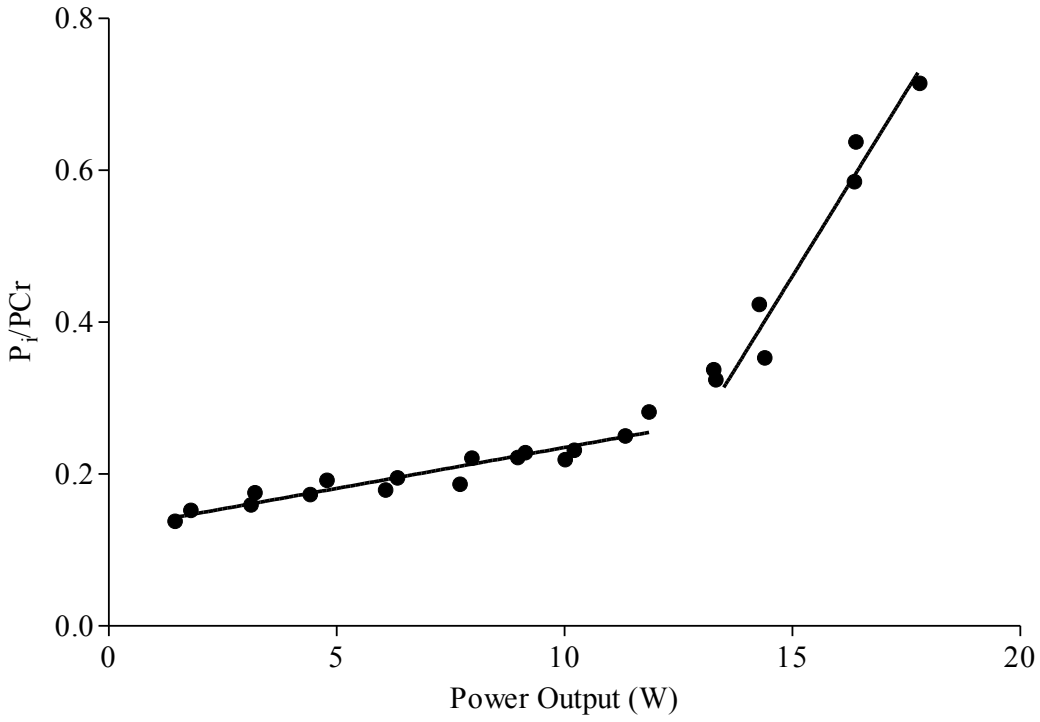

Figure 3.5. Determination of intracellular threshold for P_i/PCr (typical data set from a 12 year old boy). Regression lines are included to illustrate the two phases of the response and were not used in the identification of the threshold.

Previous research has shown that the coefficient of variation for the subjectively identified $IT_{Pi/PCr}$ over three repeated tests is 10.6%. Peak power in this study had a coefficient of variation of 12.7% (Barker et al., 2006). The ergometer and analysis procedures were identical to those utilised in the studies that make up this thesis, indicating that reliability is probably similar for the current research.

During data collection for Chapter 5 and 6, men were assigned a ramp rate of 10 $N \cdot min^{-1}$ so that the duration of the test was similar to the duration of the tests completed by boys. This did not significantly affect the mean peak power attained compared with men who completed an incremental test with a 5 $N \cdot min^{-1}$ ramp rate (Chapter 5).

3.6.3 Mathematical modelling of PCr kinetics during exercise

Chapters 4, 5, and 6 describe the analysis of PCr kinetics collected during CWR exercise. In these studies, each participant completed 2-4 transitions from rest to exercise. 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 5 previous data points – any point that lay outside 4 standard deviations from this local mean was deleted (Rossiter, et al., 2002b). All transitions for each subject were time-aligned and averaged.

The onset of the slow component during heavy intensity exercise (Chapter 4) was determined from the plateau in a plot of an iterative fit of the time constant beginning at 60 s, as previously described (Rossiter, et al., 2002b). The onset of the slow component during very heavy exercise (Chapters 5 and 6) was set to 180 s, because identification of this phase was impossible in many subjects. See Appendix E for a discussion of this modelling. To facilitate comparison of PCr kinetics at different exercise intensities, data collected during heavy intensity exercise was refit in the same way as data collected during very heavy exercise. These amendments to the modelling of PCr kinetics during heavy intensity exercise did not significantly alter the value of the time constant in boys or men.

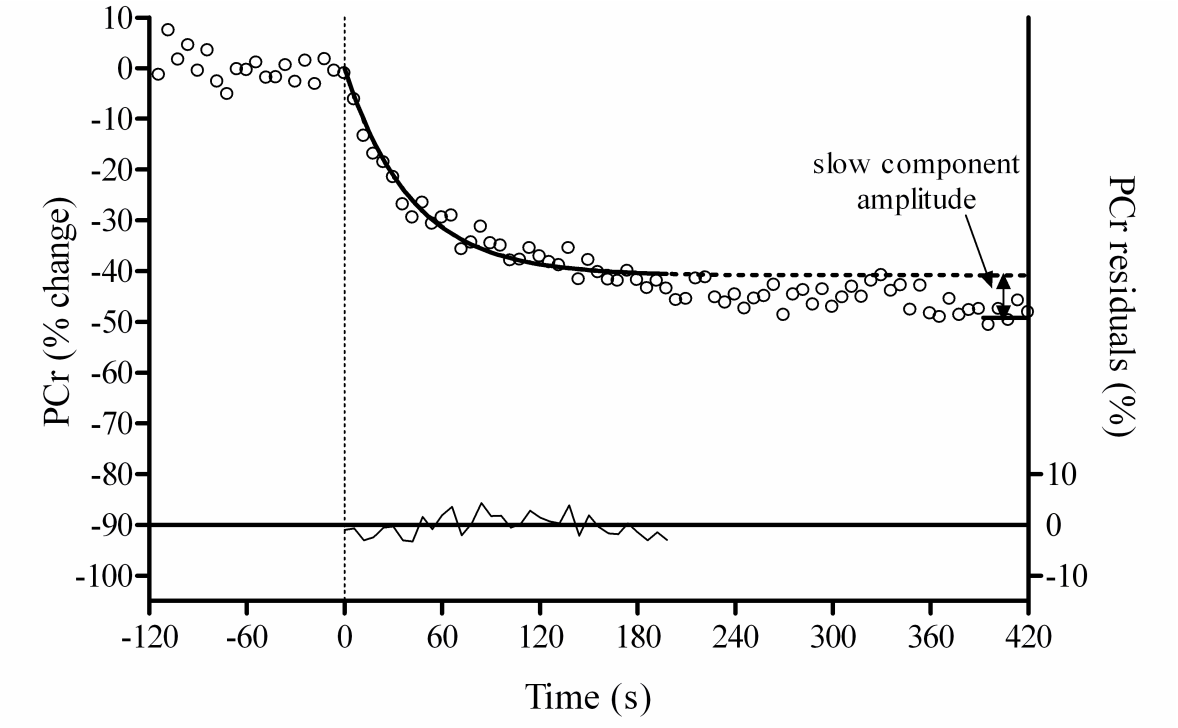

Figure 3.6. Representative PCr response from a 13 year old boy during 6 min of heavy intensity exercise. The solid curve shows the fitted portion of the curve and the dotted line shows a projection of the amplitude of the curve. The residual error is shown on the right y-axis.

The fundamental phase of the PCr response was fitted from the onset of exercise to the emergence of the slow component during heavy intensity exercise and to 120 s of exercise during very heavy intensity exercise. These data were modelled using non-linear regression to fit an exponential function of the form:

$$\Delta PCr_{(t)} = PCr_{BL} - \Delta PCr_{SS}\left(1 - e^{-\frac{t}{\tau}}\right) \qquad \text{Equation 3.5}$$

Where PCr_{BL} is the baseline PCr concentration, ΔPCr_{ss} is the projected steady-state difference in PCr from PCr_{BL}, t is time, the independent variable, and τ is the time constant for the curve (time required to reach 63 % of ΔPCr_{ss}). The primary component was modelled from the start of exercise to the onset of the slow component (Figure 3.6). 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 PCr_{EE}. Finally, the amplitude of each component was represented in mM [PCr], using the calculated [PCr].

3.6.4 Mathematical modelling of PCr kinetics during recovery

Following exercise, PCr recovery kinetics provide information about the mitochondrial capacity of the muscle. To investigate recovery kinetics, a monoexponential equation of the form:

$$PCr_{(t)} = PCr_{EE} + PCr_{ER}\left(1 - e^{-\frac{t}{\tau}}\right) \quad \text{Equation 3.6}$$

where PCr_{EE} is the relative PCr concentration prior to recovery, PCr_{ER} is PCr at the end of recovery, and τ is the recovery time constant was fitted to each data set, which consisted of 1-3 repeated transitions. Where multiple transitions were performed, data sets were averaged prior to fitting. An example fitted data set can be seen in Figure 3.7.

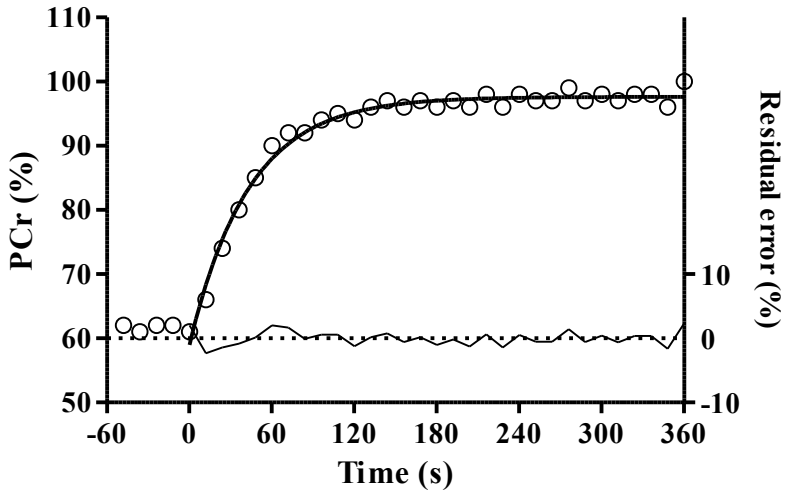

Figure 3.7. Recovery kinetics in a 13 year old boy. Residual error can be seen on the right y-axis.

3.6.5 Mathematical modelling of HHb kinetics during exercise

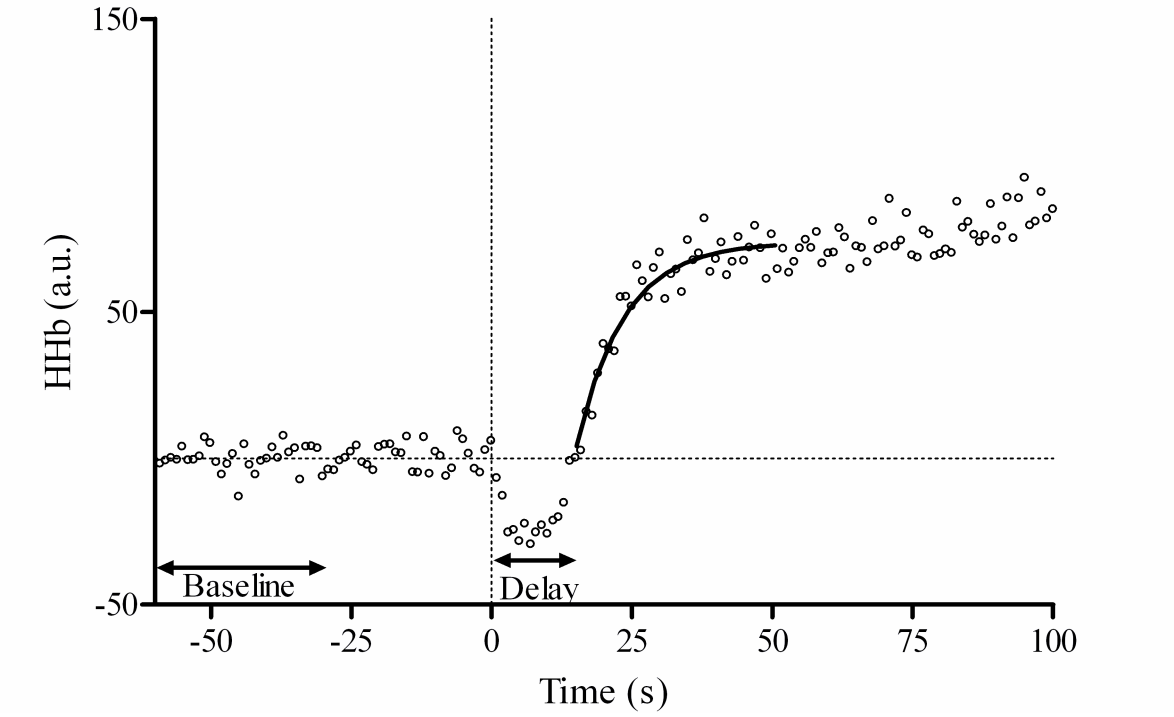

Figure 3.8. Fitted HHb response for a representative participant (with curve superimposed) over 60 s of rest and 100 s of exercise.

For each CWR bout completed by a participant, HHb data were interpolated to 1s intervals and represented as a change, in arbitrary units, 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 3.8). Each participant's HHb profile showed an apparently exponential rise following a delay at the onset of exercise, as described by other authors (DeLorey, et al., 2003; Forbes, et al., 2008; Jones, et al., 2006). The delay was characterized by a marked fall in HHb (increased oxygenation) in most participants. The onset of the exponential phase of the response was identified by two independent investigators. An exponential equation in the same form as Equation 3.5 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. (2006). 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.

3.7 Statistical analysis

All group data that appear in this thesis are presented as mean ± standard deviation. Several methods of group mean comparison were used. When there were two independent variables and four groups, such as comparisons based on age and sex, or age and exercise intensity, two-by-two ANOVA was used, with follow-up testing completed via planned t-tests with the Bonferroni correction applied. When two groups were compared, dependent or independent t-tests were used as appropriate. Finally, when two groups were compared across two conditions, repeated measures ANOVA was used to analyse data, and planned t-tests with Bonferroni correction were used for follow-up. In all cases, data were tested for normal distribution using the Shapiro-Wilkes statistic prior to analysis.

4 PCr kinetics during heavy intensity exercise in children and adults

4.1 Introduction

A recent ^{31}P-MRS study by Barker and colleagues (2008a) has shown that the kinetics of muscle PCr during moderate intensity exercise, i.e. below the 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. heavy intensity exercise, remains uncertain. It has been shown that during heavy intensity exercise O_2 delivery plays an increasingly important role in modulating muscle metabolism (Hogan, Richardson, & Haseler, 1999). Moreover, heavy intensity exercise is associated with a fall in intracellular pH from rest which might inhibit the ADP signal for oxidative phosphorylation by reducing muscle [ADP] as predicted by the creatine kinase equilibrium (Jubrias, et al., 2003).

Given that exercising children are characterized by a higher intracellular pH (Kuno, et al., 1995; Taylor, et al., 1997; Zanconato, et al., 1993) and a greater mass specific muscle blood flow (and thus O_2 delivery) in the vastus lateralis muscle (Koch, 1974), 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. (1993) and Barker et al. (2010b), 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' $IT_{Pi/PCr}$, noted a lower rate of change of P_i/PCr and pH in children. Thus, there might be 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 heavy 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 (DeLorey, et al., 2004). It was hypothesized that:

a) children would display more rapid muscle PCr kinetics and a reduced PCr slow component compared to adults;
b) the PCr slow component would be associated with a reduced HHb slow component in children, reflective of an enhanced muscle oxygenation.

4.2 Methods

4.2.1 Participants

Six boys (age 13.0 ± 0.2 y; stature 1.50 ± 0.04 m; body mass 42.6 ± 3.7 kg), 6 girls (13.0± 1.3 y; 1.55 ± 0.12 m; 44.7 ± 10.6 kg),7 men (25.4 ± 4.6 y; 1.81 ± 0.08 m; 80.9 ± 11.8) and 8 women (23.6±3.6 y; 1.53 ± 0.08; 54.0 ± 4.6 kg) volunteered to participate in the study, which was approved by the institutional ethics board. Calculations pertaining to sample size and statistical power can be seen in Appendix B. Girls were significantly more mature than boys (0.7 ± 1.5 years from age at peak height velocity (YAPHV) and -1.4 ± 0.1 YAPHV, respectively; $p<0.001$) according to the estimated maturity method as described by Mirwald et al. (2002). Adult participants and parents or guardians of child participants gave written, informed consent (Appendix C), 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).

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 3 to 5 times to complete data collection.

4.2.2 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. See Chapter 3 for full details of this exercise mode and MRS and NIRS measurements.

After completing an incremental exercise test to exhaustion within the scanner, participants completed 2 to 4 CWR bouts, each separated by at least 48 hours. These CWR bouts consisted of 2 min of resting data collection and 7 min of work at a power output corresponding to 20 % of the difference between the power output at the intracellular P_i/PCr threshold and the maximal power output (i.e. 20 %Δ).

4.2.3 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 2-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 $p \leq 0.0125$, due to the Bonferroni correction. Analyses were carried out using SPSS version 11.0.

4.3 Results

4.3.1 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, $p=0.37$).

Table 4.1. Parameters of the PCr response to heavy intensity exercise in children and adults.

	Boys (n=6)	Girls (n=5)	Men (n=6)	Women (n=5)	ANOVA
$[PCr]_{BL}$ (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 (% PCr_{BL})	-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 (% PCr_{EE})	11 ± 3	36 ± 30	16 ± 12	19 ± 14	[a]p=0.47 [b]p=0.09
$[PCr]_{EE}$ (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*
PCr_{EE} (%)	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, $p<0.05$

4.3.2 Muscle Phosphates

The parameters of the PCr response to CWR exercise can be seen in Table 4.1, with average profiles illustrated in Figure 3.6. Individual data can be seen in Appendix D. The 95 % CI 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. PCr_{EE} 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 ($p=0.19$). $[ADP]_{BL}$ 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 ($p=0.72$). $[ADP]_{EE}$ 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.

4.3.3 Intracellular pH

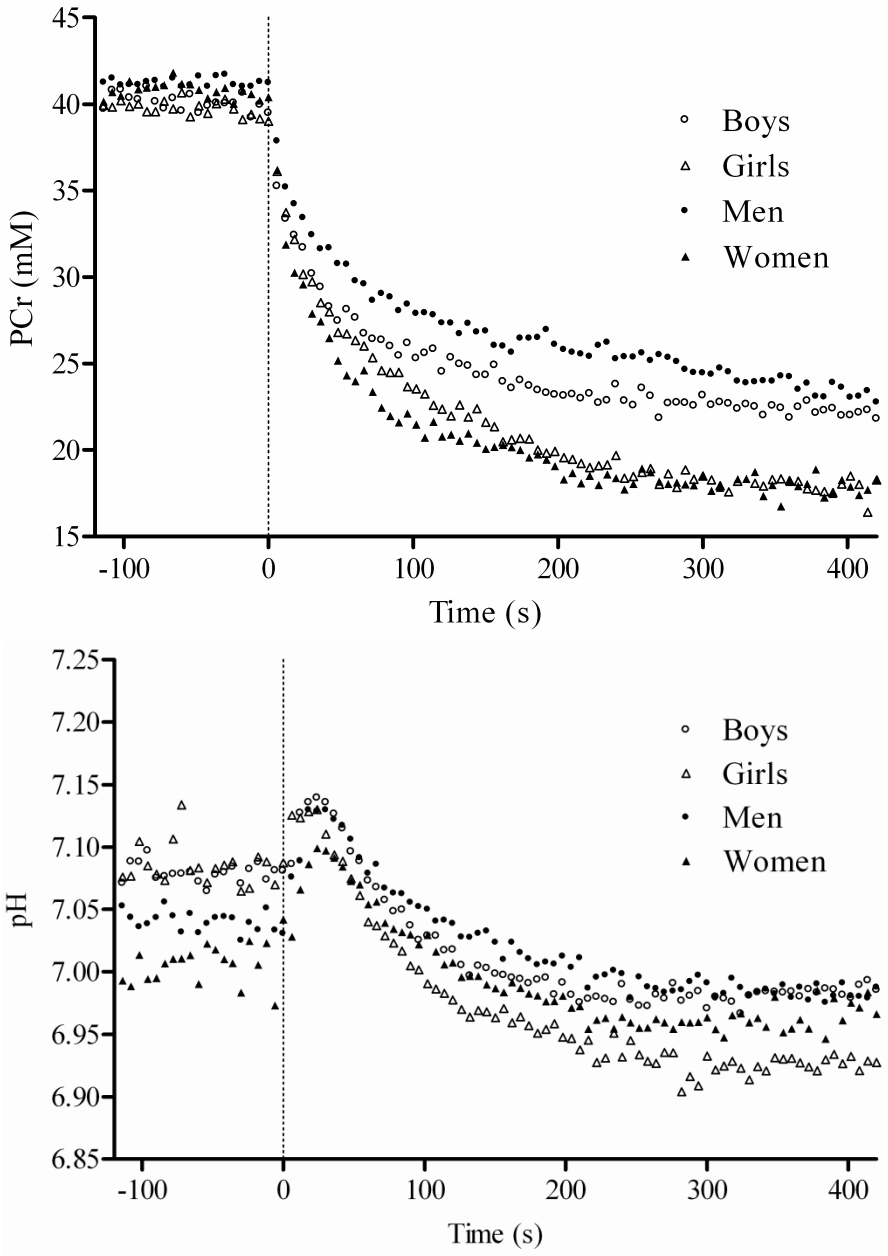

Figure 4.1. PCr and intracellular pH responses (mean over each group) over 2 min of rest and 7 min of heavy intensity exercise in boys (○), girls (Δ), men (●), and women (▲).

Figure 4.1 shows the group mean pH responses for men, women, boys, and girls. pH_{BL} 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 pH_{BL} (p=0.08), but pH_{BL} was higher in

children ($p<0.01$), and an interaction between age and sex was found ($p=0.02$). Independent t-tests revealed that pH_{BL} was significantly higher in girls than women, ($p<0.01$) there were no significant differences between men and women ($p=0.01$), boys and girls ($p=0.62$), or men and boys ($p=0.04$). pH_{EE} 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 pH_{BL} 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, $p=0.18$).

4.3.4 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 4.2). 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 4.2). 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 EE (2 children and 1 adult).

Table 4.2. Parameters of the deoxyhaemoglobin/myoglobin response to heavy intensity exercise in adolescents 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=074 [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, $p<0.05$

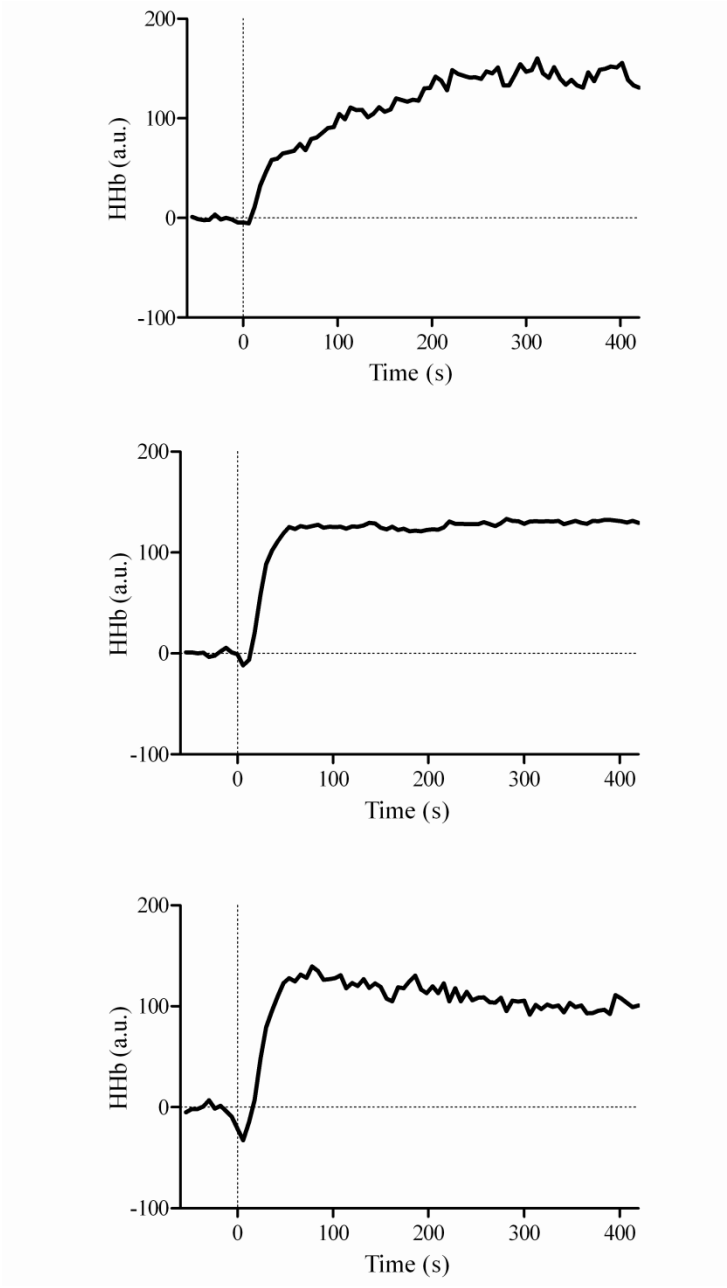

Figure 4.2. HHb profiles from 2 men (middle and bottom) and 1 girl (top) during 1 min of rest and 7 min of heavy intensity exercise.

4.4 Discussion

This is the first study to examine muscle phosphates and oxygenation during heavy intensity quadriceps muscle exercise in children and adults. It was hypothesized that children would display more rapid PCr kinetics and a reduced 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 data. 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 suggests subtle age-related differences in the matching of muscle blood flow to metabolic rate at exercise onset.

4.4.1 Muscle Phosphates

Previous investigations of p$\dot{V}O_2$ kinetics have reported faster kinetics in young children during moderate (Armon, et al., 1991; Fawkner, et al., 2002) and heavy (Armon, et al., 1991; Fawkner & Armstrong, 2004a) intensity cycle ergometer and treadmill (Williams, et al., 2001) exercise (Chapter 2). In support of these findings previous investigations of PCr kinetics during the recovery from exercise have reported faster kinetics in children compared with adults (Ratel, et al., 2008; Taylor, et al., 1997), 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] (Glancy, et al., 2008). PCr is also thought to play a key role in transmitting the rise in [ADP] from the contractile machinery to the mitochondria (Bessman & Geiger, 1981). 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 indicate 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. A discussion of considerations related to statistical power for the studies comprising this thesis can be found in Appendix B. The maturity of the current participants and the variability within each group might explain why age differences in PCr kinetics were not found in the current study despite previous literature demonstrating such differences in the $\dot{V}O_2$ kinetic response. The intensity of the exercise might also be an important factor; most $\dot{V}O_2$ kinetics research in the heavy domain has examined exercise at 40-50 %Δ, rather than 20 %Δ. These factors are discussed in Section 4.4.3. It is pertinent to note that the present results are consistent with an earlier study during moderate intensity exercise. Barker et al. (2008a) 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 heavy intensity exercise.

Significant sex differences in $[PCr]_{BL}$ and $[ADP]_{BL}$ 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]. These assumptions have been elegantly demonstrated by Kemp et al. (Kemp, et al., 2007) to profoundly impact the interpretation of measures of metabolic function. In the current study, [ATP] was assumed to be 8.2 mM in both children and adults, while total creatine [TCr] was assumed to be 45 mM (Kemp, et al., 2007). Limited data suggest that resting [PCr] in the rectus femoris muscle might increase between 11 and 16 yrs in boys (Eriksson & Saltin, 1974), although Garoid et al. (1994) 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 $[PCr]_{EE}$, whether expressed in absolute terms or relative to $[PCr]_{BL}$, was significantly lower in females compared with males. $[ADP]_{EE}$ 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 impaired exercise efficiency in these groups. Reduced mitochondrial content is associated with a greater PCr cost for an equivalent change in exercise intensity (Korzeniewski & Zoladz, 2004), 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 (Ratel, et al., 2008; Taylor, et al., 1997), and suggestions that oxidative capacity might be higher in women than men (Kent-Braun & Ng, 2000). 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 (Woo, Derleth, Stratton, & Levy, 2006). 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 (Parker et al., 2007), differences in fibre-type (Miller, MacDougall, Tarnopolsky, & Sale, 1993; Wust, et al., 2008), or muscle activation

patterns (Clark, Collier, Manini, & Ploutz-Snyder, 2005). It is likely that a combination of these factors is responsible for the current results. There is little evidence for an oxygen delivery limitation to oxygen uptake kinetics in healthy young adults (Poole, et al., 2008), suggesting that differences in muscle properties are likely to underlie sex differences in the [PCr] cost of exercise. The oxygen cost of supra-threshold exercise is higher in children than adults (Zanconato, et al., 1991), but the causes of this are unclear. The greater phosphate cost of exercise in females compared with males and in children compared with adults seems to suggest a loss of efficiency or economy during quadriceps exercise in females and in children that requires further investigation.

This is the first study to examine the slow component of the PCr response in children, The amplitude of this phase of the response was not significantly different from adults, representing 23 ± 23 % and 17 ± 13 % of the total change in PCr in children and adults, respectively. An attenuated $p\dot{V}O_2$ slow component in young children compared with adults or older children is a common finding (Armon, et al., 1991; Fawkner & Armstrong, 2004a; Williams, et al., 2001). Given the relationship between the $p\dot{V}O_2$- and PCr slow components, which are similar both temporally and in size (Rossiter, et al., 2002a), it was 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 (Endo, et al., 2007; Krustrup, et al., 2004). 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 (Jansson, 1996). 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 pH_{EE} 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 WAnT (Beneke, et al., 2005), as well as submaximal cycling exercise at the same relative exercise intensity as adults(Pianosi, et al., 1995). 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 (Kuno, et al., 1995; Taylor, et al., 1997; Zanconato, et al., 1993). Several recent investigations have found that clearance of lactate or protons from the cell is faster in children than adults (Beneke, et al., 2005; Beneke, et al., 2007; Ratel, et al., 2008), although the investigations of Beneke et al. (2005, 2007) made assumptions about the relative muscle mass and lactate space in children and adults, while the work of Ratel et al. depended on the assumption that the muscle buffering capacity is the same in children and adults. Both intracellular pH and blood lactate concentrations depend upon a balance of intracellular reactions (Robergs, Ghiasvand, & Parker, 2004) 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 heavy intensity quadriceps exercise.

4.4.2 Muscle Oxygenation

In both children and adults, the HHb response was triphasic, as previously reported for adults (DeLorey, et al., 2004), 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 utilisation 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 reflects the

overall speed of muscle deoxygenation. 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 (Jones, et al., 2006). Thus children's faster kinetics may reflect a relatively slower oxygen delivery relative to $\dot{V}O_2$ at exercise onset. However, it is likely that age differences in oxygen utilisation also play an important role in determining the speed of the HHb response (DeLorey, et al., 2004; Ferrari, Mottola, & Quaresima, 2004). A faster muscle deoxygenation in children indicates 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 (DeLorey, et al., 2003; Jones, et al., 2006). The amplitude of this phase of the response was expected to be lower in children than adults, based on a longitudinal study by Koch (Koch, 1974) 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 y 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.

4.4.3 Methodological considerations

There are several limitations to this work, which are inherent in the techniques used and in paediatric research. These include the maturational status of the young participants, differences in habitual physical activity between groups, assumptions inherent in calculating [PCr], [P_i], and [ADP], and assumptions in the use of NIRS to measure muscle oxygenation. For more in-depth discussion of these, please see Section 10.2.

The intensity of the exercise bout is also a limitation to this work; 20 %Δ was chosen to ensure that participants were working between the IT and CP. Responses to exercise above and below the gas exchange threshold and above and below CP are known to differ, so it is critical that exercise intensity is clearly defined with respect to these 2 thresholds. While this study normalized work rate to the intracellular threshold for Pi/PCr, CP was not measured and thus it is possible that some participants were working at or above this important threshold. This is more likely in children than adults due to the compressed interval between threshold and maximum power outputs. An intensity of 20 %Δ rather than the more commonly used 40 %Δ (Fawkner & Armstrong, 2004a) was used to minimize the risk of participants working at or above CP.

4.5 Conclusions

In conclusion, it was hypothesized that during heavy 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. (2008a) for moderate-intensity exercise, metabolic control appears to be adult-like in 13 yr old children during heavy 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 utilisation in children at the onset of exercise. The results of this study suggest that skeletal muscle metabolism is similar in children and adults during heavy intensity exercise, and that future research should examine age-related differences in muscle oxygenation during exercise.

5 Effect of exercise intensity on muscle metabolism and oxygenation in adolescent boys and men

5.1 Introduction

Previous research has suggested that age differences in muscle metabolism are dependent on exercise intensity (Barker, et al., 2010b; Petersen, et al., 1999; Zanconato, et al., 1993). That is, during high intensity exercise, adults or older adolescents experience greater metabolic perturbation than children or adolescents. The interaction of exercise intensity and age on PCr kinetics has never been investigated in young people. PCr kinetics do not differ between children and adults at 80 %IT (Barker, et al., 2008a). Chapter 4 introduced evidence that PCr kinetics during heavy intensity exercise do not differ in adolescent boys and girls and in adult men and women. However, a strong trend for slower kinetics in adult men compared with adolescent boys was present. It is possible that higher exercise intensities, particularly those above the CP, elicit greater child-adult differences in kinetics.

An exercise intensity of 60 %Δ was chosen to increase the likelihood that the young participants would comply with the exercise test while setting an exercise intensity that was likely to be above CP. During cycling exercise, CP has been reported to fall between 45 %Δ and 60 %Δ (for a discussion of the delta concept, see Chapter 3) (Wilkerson, Koppo, Barstow, & Jones, 2004; Pringle & Jones, 2002; Ozyener, et al., 2001; Vanhatalo, Doust, & Burnley, 2007). In 11-12 year old boys, critical power has been reported to occur at about 40 %Δ (Fawkner & Armstrong, 2003).However, measurement of quadriceps muscle CP requires several exhaustive exercise tests (Jones, et al., 2008b). This was considered an excessive commitment to ask of the participants in the current study.

There is reason to believe that exercise intensity and age interact to determine the metabolic response to exercise in young people (Chapter 2). The purpose of this study was to test the hypotheses that:

a. A significant interaction between age and intensity will be present. Specifically, slow component amplitude will be greater in men than boys during very heavy intensity exercise but not during heavy intensity exercise.

b. HHb MRT will be faster in boys than men during both heavy and very heavy intensity exercise.

5.2 Methods

5.2.1 Participants

For this study, data collected and reported in Chapter 4 were compared with data collected and reported in Chapter 6. Individual data can be seen in Appendix D. 12 adolescent boys (12.9 ± 0.3 y, -1.4 YAPHV) and 12 adult men (22.9 ± 3.7 y) participated in this study, which was approved by the institutional ethics committee. Informed consent was obtained from adult participants, and from the parent or guardian of young participants (Appendix C). Each young participant gave verbal and written assent to participate.

5.2.2 Experimental Protocol and 31P-MRS and NIRS measurements

Collection and analysis of data during heavy intensity exercise was described in Chapter 3. To investigate the effect of exercise intensity on muscle metabolism and oxygenation, a further 12 participants (6 boys and 6 men) were recruited. These participants visited the lab on 4 or 5 occasions, each separated by at least 48 hours. First, the participants were thoroughly habituated to the quadriceps ergometer used in the magnetic resonance scanner, and anthropometric measurements were taken. Second, the participants completed an incremental exercise test to exhaustion within the MR scanner. The mass was increased by 0.5 kg every minute for boys, and every 30 s for men, to ensure that time to exhaustion was similar in boys and men. P_i/PCr and pH were plotted against power output, and two independent investigators examined the plots to identify intracellular thresholds (IT). The 3rd, 4th, and 5th visits consisted of exercise at 60 %Δ for 6 min. All tests were examined for compliance, and data from any test where the power output during any minute was 20 % greater or less than the mean power output for that test was discarded.

5.2.3 MRS and NIRS data

MRS and NIRS data was collected and analysed as described in Chapter 3. During exercise, spectra were acquired every 3 s, with phase cycling employed to give a spectrum every 12 s.

5.2.4 Modelling of PCr and HHb data

The fundamental phase of the PCr kinetic response for each set of averaged transitions for each participant was fitted from 0 s to 120 s of exercise with an exponential equation (Equation 3.5). The slow component was quantified as the difference between the PCr depletion at 180 s and the PCr_{EE} (average of the last 36 s of the test) and expressed relative to PCr_{EE}. Appendix E contains a discussion of the modelling of data collected during very heavy intensity exercise. Data collected during heavy intensity exercise were refit using a fixed window to facilitate comparison with the parameters of the PCr kinetic response to very heavy intensity exercise. The first 360 s of heavy intensity exercise were included in this analysis, while the final 60 s were excluded. These amendments to the modelling of PCr kinetics during heavy intensity exercise did not significantly alter the value of the time constant in boys or men.

The HHb response consisted of 3 phases: a delay, an exponential phase, and a third phase which was variable in nature as previously described (Chapter 4). The exponential phase of the HHb response fitted using an exponential equation of the same form as Equation 3.5. This equation was fit from the onset of the exponential phase to the point where the data began to move away from a monoepxonential profile, as independently determined by 2 investigators. The HHb MRT was calculated as the sum of the delay and the time constant for the exponential phase of the response.

5.2.5 Statistical analysis

All data are represented as mean ± standard deviation. Age and exercise intensity differences were investigated with two-way ANOVA, and follow-up testing was carried out using paired or unpaired t-tests, as appropriate, with the Bonferroni correction applied.

5.3 Results

5.3.1 Incremental and CWR power output

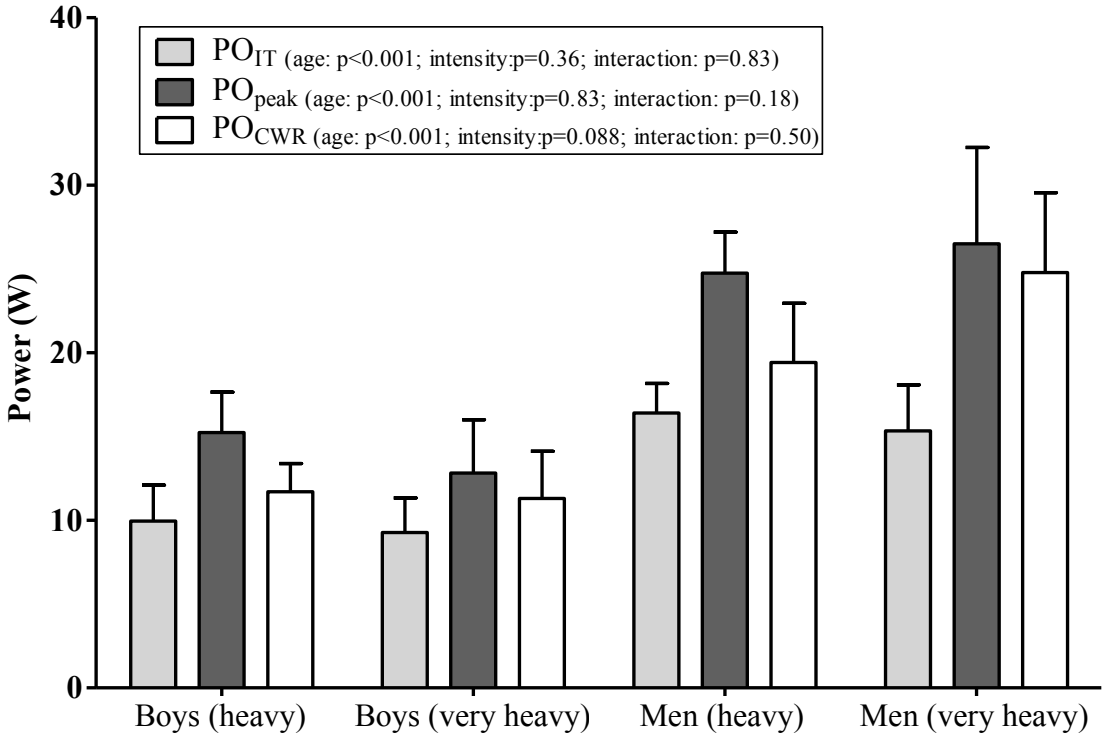

Figure 5.1 Power output for each group of participants at the intracellular threshold and peak power output during an incremental exercise test and for the mean power output attained during the CWR exercise test.

Figure 5.1 shows that no significant differences in PO_{peak}, PO_{IT}, or PO_{CWR} between intensity groups are present. A significant interaction effect for PO_{CWR} was identified, but follow-up t-tests with Bonferroni correction did not reveal any significant differences between intensity groups (boys: p=0.78; men: 0.06). Significant age differences were present (heavy: p=0.001; very heavy: p<0.001). Relative to threshold and peak power output, boys were working at 36 ± 54 %Δ and 54 ± 69 %Δ, while men were working at 34 ± 30 %Δ and 86 ± 17 %Δ, respectively. No age (p=0.42), intensity (p=0.07), or interaction (p=0.35) effects were identified.

5.3.2 PCr and HHb kinetics

Table 5.1. PCr and HHb kinetic response to heavy and very heavy intensity exercise in adolescent boys and adult men.

	Heavy		Very heavy		ANOVA		
	Boys	Men	Boys	Men	Age	Intensity	Interaction
τ (s)	28 ± 12	37 ± 22	31 ± 8	66 ± 21*†	0.005	0.05	0.04
95 % CI (s)	9 ± 7	11 ± 9	9 ± 7	22 ± 11			
A_F (%BL)	35 ± 6	33 ± 9	35 ± 5	48 ± 7*†	0.08	0.02	0.02
A_{SC} (% BL)	4 ± 2	6 ± 5	0 ± 2	12 ± 6*	0.006	0.41	0.007
A_{SC} (% EE)	8 ± 6	11 ± 12	1 ± 6	20 ± 10*	0.007	0.82	0.04
PCr_{EE} (% BL)	56 ± 11	58 ± 12	65 ± 4	37 ± 12*†	0.009	0.18	0.002
HHb DE (s)	12 ± 2	12 ± 1	12 ± 3	10 ± 2	0.70	0.27	0.36
HHb τ (s)	10 ± 2	16 ± 5	8 ± 3	18 ± 7*	<0.001	0.98	0.35
HHb MRT (s)	22 ± 2	28 ± 5	20 ± 3	29 ± 8	0.003	0.85	0.56

*- significantly different from boys at the same intensity, $p<0.013$
†- significantly different from very heavy exercise in the same age group, $p<0.013$

Table 5.1 and Figure 5.2 describe the PCr kinetic response to exercise and illustrate the age and intensity related differences. τ and A_{SC} were similar in boys during heavy and very heavy exercise, while τ was slower and A_{sc} greater during very heavy exercise at heavy exercise in men. HHb kinetics was faster in boys than men but was not affected by exercise intensity.

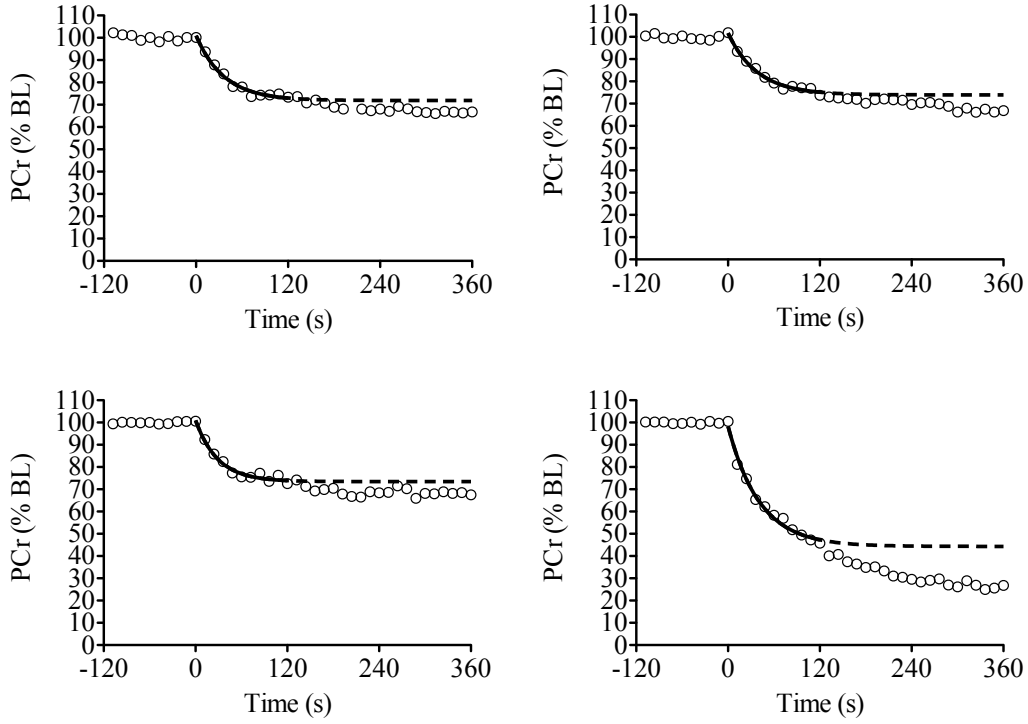

Figure 5.2. Fitted PCr kinetics during heavy (top) and very heavy (bottom) intensity exercise in 2 different representative adolescent boys (left) and 2 representative men (right). An exponential curve fitted from the onset of exercise to 120 s is superimposed.

5.3.3 Metabolic changes

Figure 5.3 shows the changes in pH from rest to EE during heavy or very heavy exercise. PCr_{EE} is shown in Table 5.1. P_i increased during exercise; P_{iEE} was significantly higher in men than boys (heavy: boys: 331 ± 99 % BL; men: 548 ± 266 %; very heavy: boys: 325 ± 87 %; men: 430 ± 54 %; age: p=0.02; intensity: p=0.37; interaction: p=0.33).

Figure 5.3. Intracellular pH during exercise in adolescent boys and men. Error bars indicate mean and SD. Age ($p<0.001$), intensity ($p<0.001$) and interaction ($p<0.001$) effects were present. *- significantly different from boys at the same intensity, $p<0.006$; †- significantly different from heavy intensity exercise in the same age group, $p<0.006$

5.4 Discussion

These data suggest that age and exercise intensity might interact to influence the metabolic response to exercise in young people and adults. Specifically, age differences were found during very heavy intensity exercise but not during heavy intensity exercise for PCr τ, the fundamental amplitude of PCr kinetics, PCr slow component amplitude, total PCr depletion, and pH. P_i after 6 min of exercise was higher in adults than adolescents, but exercise intensity did not affect P_{iEE}. Muscle oxygenation was more complex; the time constant and mean response time for HHb were faster in boys than men but were not affected by exercise intensity.

5.4.1 Metabolic responses to exercise

This is the first time that PCr kinetics during very high intensity exercise in young people has been examined. Previous investigators have described PCr kinetics during moderate intensity exercise (Barker, et al., 2008a; Barker, et al., 2008b), and Chapter 4, published as Willcocks et al. (2010) described the response to heavy intensity exercise. The absence of a slow component in young people is a surprising but not unprecedented

finding; $\dot{V}O_2$ kinetics studies have typically reported a slow component during heavy intensity exercise in children and adolescents (Barker, et al., 2010a; Fawkner & Armstrong, 2004a; Jones, et al., 2010; Lai et al., 2008; Machado, et al., 2009; Winlove, Jones, & Welsman, 2010). A monoexponential response has also been described (Armon, et al., 1991; Williams, et al., 2001), but the work of Armon and colleagues (1991) was marred by methodological flaws (see Section 2.1), and the work of Williams et al., (2001) measured kinetics during treadmill running, which has a reduced slow component compared with cycling exercise (Jones & McConnell, 1999; Machado, et al., 2009). Thus, a slow component was expected during very heavy exercise in boys. Several possible explanations for its absence, including potential sources of error in the experimental procedure, must be considered. It is possible that the $IT_{Pi/PCr}$ was misidentified in boys, resulting in this group working in the heavy or moderate rather than very heavy domain. The same experienced investigators identified intracellular thresholds in both boys and men, so this possibility is not likely to be the primary difference between boys and men. As Figure 5.1 shows, the mean power output during CWR exercise in this group was considerably greater than the mean power output at the intracellular threshold. A report by Barker et al. (under review), in which exercise above the CP in adolescents differed from the typically reported profile in adults, provides support for the veracity of these results. In the study conducted by Barker et al., $\dot{V}O_2$ did not project toward $\dot{V}O_{2\,peak}$ during exercise above CP, in keeping with the results of the current investigation. It is important to consider the possibility that the absence of a slow component is indicative of true age differences which are apparent at increasing intensities but unique to knee extension exercise in the prone position compared with upright cycling.

There are two possible mechanisms by which exercise mode might modulate the effects of age and exercise intensity on PCr kinetics. First, during this type of exercise, the quadriceps muscle contracts both concentrically and eccentrically, and is the only active muscle. During cycling or running, there is a great deal more muscle activity and the quadriceps contracts concentrically. Children have been shown to display greater co-contraction during functional tasks (Frost, Dowling, Dyson, & Bar-Or, 1997), which might result in a different muscle activation profile during cycling or running compared with the exercise in the current studies. As well, kinetics in adults is different during

concentric and eccentric contraction (Perrey, Betik, Candau, Rouillon, & Hughson, 2001) – specifically, the slow component is eliminated during eccentric work, partly due to lower metabolic demand. In this task, half of the time is spent contracting eccentrically, which might affect the amplitude of the slow component in adolescents and adults. In adults, prone or supine leg muscle exercise can induce an oxygen delivery limitation which can slow primary kinetics and/or increase the slow component amplitude (Koga et al., 1999). The interaction between exercise intensity and posture has not been elucidated, but blood flow has been implicated as the key difference between upright and supine exercise and the influence of oxygen delivery on exercise metabolism is thought to increase with exercise intensity (Grassi, 2000; Haseler, et al., 2004; Hogan, et al., 1999). Thus, facilitated muscle oxygen delivery in children, as reported during upright exercise by Koch (1974) might prevent the changes in PCr kinetics and muscle metabolism seen in adults with increasing exercise intensity.

The amplitude and τ of the fundamental phase of PCr kinetics were affected by both age and exercise intensity – the amplitude was greater, and the time constant slower, in men during very heavy intensity exercise than in men during heavy intensity exercise or boys during very heavy intensity exercise. Several investigators have proposed that children might be less able than adults to recruit higher order fibres to meet the demands of intense exercise, a hypothesis which offers an elegant explanation for the current data (Halin, et al., 2003; Ratel, et al., 2010). If men recruited proportionately more type II muscle fibres at the onset of very heavy intensity exercise, but boys were unable to alter muscle fibre recruitment in this manner, the kinetic and metabolic differences observed might result. Pringle et al. (2003) reported that the fundamental time constant during heavy and very heavy cycling exercise was significantly correlated with the type II fibre content of the vastus lateralis, a relationship that was not present during moderate intensity exercise. There is reason to suspect that muscle fibre recruitment, in addition to muscle fibre content, affects $\dot{V}O_2$ kinetics, although this evidence is necessarily indirect (Jones, Pringle, & Carter, 2005). Overall, this hypothesis is viable but considerable work is required to empirically document the possible impact of neuromuscular maturation on PCr kinetics.

Both the overall change in PCr and the degree of acidosis were significantly greater in men during very heavy exercise than in boys during very heavy exercise or in men

during heavy intensity exercise, while no differences between boys and men were apparent during heavy intensity exercise. This indicates, in keeping with the results of Barker et al., (2010b), Zanconato et al, (1993), and Petersen et al. (1999), that age differences in muscle metabolism are particularly apparent at high exercise intensities. This is compatible with the theory that boys rely on aerobic metabolism to a greater extent than anaerobic metabolism, which might be explained by different muscle fibre content or recruitment patterns, attenuated glycolytic abilities in youth, or enhanced oxidative capacity in youth. Although PCr kinetics and acidosis suggest that exercise intensity might affect muscle metabolism in boys and men, P_i was not affected by exercise intensity. P_i increased to a greater extent in men than boys during heavy and very heavy intensity exercise. Although there is some evidence that measurement of P_i using ^{31}P-MRS is unreliable in young people (Barker, et al., 2006), these results might be due to age-related differences in total creatine, in the MR visibility of the P_i pool, or to an elevated resting P_i in paediatric muscle. A different interaction between exercise intensity and age on P_i and PCr suggests that age differences in muscle metabolism at high exercise intensities might be complex, and further work is needed to elucidate the nature of these differences during different exercise protocols.

5.4.2 HHb kinetics

As discussed in Chapter 4, the time constant and MRT for HHb was faster in boys than men, indicating that fractional oxygen extraction increased more quickly in boys than men. This might be related to faster PCr kinetics in boys than men, since ANOVA revealed a significant age effect for PCr τ across both intensities in this study, or it might reflect a slower adaptation of muscle oxygen delivery at the onset of exercise. It is likely that a combination of these factors is responsible for faster deoxygenation in adolescents. Previous research has suggested that heart rate kinetics at the onset of exercise do not differ with age (Springer, Barstow, Wasserman, & Cooper, 1991), indicating that local heterogeneity of oxygen delivery or oxygen utilisation might differ in men and boys. Notably, there were no differences in HHb kinetics between heavy and very heavy intensity exercise in either age group, in keeping with some previous reports in adults (Adami, Pogliaghi, De Roia, & Capelli, 2010). HHb kinetics were similar at both intensities. This indicates that delivery of oxygen at exercise onset was sufficient to meet the demand at both intensities. It is possible that the sufficiency of oxygen delivery is unique to prone knee extension exercise, although the findings of Adami et al. (2010)

suggest that matching of oxygen delivery and utilisation is similar in cycling exercise. Muscle blood flow and oxygen delivery at the onset of exercise is an important area for future research.

5.4.3 Methodological considerations

This chapter reports for the first time PCr kinetics during very high intensity exercise in young people, a comparison of kinetics at different high intensities in boys and men, and the parameters of the HHb response during very heavy exercise. There are several methodological considerations that must be acknowledged in discussing these results; as in Chapter 4, the age and activity of the participants as well as the small sample size potentially limit the generalisability of these results. For a full discussion of this, please see Chapter 10. In addition, this study used two separate samples of boys and men rather than a within-subjects design. Unfortunately, the time commitment involved in this work prevented the recruitment of a single paediatric sample. Given the sample size, it is possible that the boys who completed very heavy intensity exercise were atypical in terms of kinetics. – that is, the lack of slow component in this group is not representative of the population Boys were recruited from the general student population at a local secondary school and none were active in competitive sport, although all were habitually active in the playground and after school, as is typical of children this age.

The interpretation of these results is complicated by the non-significant difference in exercise intensity in boys and men at during very heavy intensity exercise - boys were working at ~54 %Δ while men were working at ~86 %Δ. There was considerable variability within each group, the sources of which are twofold. First, compliance with the ergometer might have varied from trial to trial; although all participants were thoroughly habituated to the exercise prior to completing the exercise test, slight variations in the amplitude of the movement of the foot from the incremental test to the CWR tests could alter power output over the test. Second, the precision with which resistance can be determined and applied is limited by the availability of nonmagnetic weights; during the incremental test power output increased in ~1.4 W increments and during the CWR tests power could be manipulated within ~0.3 W bins. The over- and under-prescription of work rate is relatively more in boys due to their lower peak power but is unlikely to vary systematically. The difference was not statistically significant and

it is likely that young people were working at or near CP, given previous reports of this parameter (Ozyener, et al., 2001; Vanhatalo, et al., 2007). Moreover, while the magnitude of the error in setting the imposed PO is greater in boys, the direction of the error is unlikely to vary systematically. To reduce the potential for inaccurate prescription of work rate to occur, the same experienced investigator carried out all tests in both groups, and threshold identification was confirmed by a blinded second investigator who has extensive experience with this test. This process was designed to reduce subjectivity in threshold identification. Second, although the very heavy domain has seldom been investigated in young people, Barker et al. (under review) reported that the response to supra-CP exercise does not project toward $\dot{V}O_2$ peak in adolescents as it has been documented to do in adults. This indicates that the fundamental characteristics of the response might differ from the adult response in young people, and supports the results of this investigation.

5.4.4 Conclusions

The results of the current study indicate that metabolic responses to heavy and very heavy exercise differ considerably in adult men, but do not differ in adolescent boys. HHb kinetics does not support differences in oxygen delivery as a primary mechanism for this effect – deoxygenation was faster in boys than men during both heavy and very heavy exercise, but did not differ with intensity in either boys or men. If oxygenation was driving kinetics, a correspondence between HHb and PCr kinetics would be expected. Thus, it is likely that factors intrinsic to the muscle, such as muscle fibre recruitment, are responsible for the interaction of age and intensity on PCr kinetics. The first hypothesis, that there would be an age-intensity interaction effect on PCr, was accepted. The second hypothesis, that HHb kinetics would be faster in boys than men during heavy and very heavy intensity exercise was also accepted.

Further research is required to investigate the mechanisms underlying the PCr kinetic response. The effects of muscle group, contraction type, age, and exercise intensity should be investigated. CP is an important concept to examine from a developmental and methodological standpoint. CP has been examined in children several times (Barker, et al., under review; Dekerle, et al., 2009; Fawkner & Armstrong, 2002; Williams, et al., 2008) but these investigations have been exploratory and cross sectional, and have not included an adult control group to investigate how the CP and

the response to sub-CP and supra-CP exercise changes from childhood to adulthood. Longitudinal investigation of CP during childhood and adolescence would provide valuable information about this parameter of exercise tolerance. Finally, interventions including priming exercise should be used to understand how the kinetic response changes under different conditions in the same child or adolescent.

6 Prior exercise speeds PCr kinetics in adult men but not adolescent boys during very heavy intensity exercise.

6.1 Introduction

Priming exercise has been used as a model to investigate the nature of metabolic control in different populations. This intervention consists of a high intensity bout of exercise, a recovery period, and a subsequent high intensity bout of exercise. The kinetic parameters of the primed bout can be compared with the kinetics of the unprimed response to understand how subsequent exercise might be facilitated by changes in oxygen delivery or by priming of muscle metabolic pathways. PCr and $\dot{V}O_2$ kinetics, which have been demonstrated to yield very similar estimates of the speed of the adjustment of the muscle to higher energy demand (Barker, et al., 2010a; Rossiter, et al., 2001), are characterized by the time constant (τ) and, during heavy intensity exercise, by the amplitude of the slow component. The speed of the $\dot{V}O_2$ kinetic response and the slow component amplitude change with growth and maturation in childhood and adolescence (Armon, et al., 1991; Fawkner & Armstrong, 2003, 2004a; Breese et al., 2010; Williams, et al., 2001), although the same does not appear to be true for the PCr kinetic response (Chapter 4) (Barker, et al., 2008a; Willcocks, et al., 2010), which has been reported to be invariant with age during moderate and heavy intensity exercise. Results from Chapter 5 showed that the characteristics of the PCr kinetic response do differ with age in the very heavy intensity domain. The speeding of $\dot{V}O_2$ kinetics might be related to an increase in the reliance on glycolytic pathways with age.

In healthy adults, priming exercise has been demonstrated to speed the overall $\dot{V}O_2$ (Burnley, et al., 2000; Jones, et al., 2006; Rossiter, et al., 2001) and PCr (Forbes, et al., 2008; Jones, et al., 2008a; Rossiter, et al., 2001) kinetic response. This is usually predominantly attributable to a marked reduction in the amplitude of the slow component (Forbes, et al., 2008; Jones, et al., 2008a; Rossiter, et al., 2001). This pattern has recently been reported during cycling exercise in young boys (Barker, et al., 2010a). The mechanistic basis for the priming effect is not fully understood, but the effect seems to be dependent on the intensity of the prior exercise (Burnley, et al., 2000; DeLorey, et al., 2004; Raymer, Forbes, Kowalchuk, Thompson, & Marsh, 2007) and the age

(DeLorey, et al., 2004) and fitness (Buchheit, Larsen, & Ahmaidi, 2009) of the participants. The priming effect might result from an increase in oxygen delivery to the working tissue at the onset of subsequent exercise; this is supported by evidence that muscle oxygenation is higher at the onset of the second exercise bout (Barker, et al., 2010a; Hernandez, McDonald, Lai, & Gladden, 2010) but refuted by reports that the priming effect does not depend on this increase in perfusion (Forbes, et al., 2008; Saitoh et al., 2009). A second candidate mechanism is that the first bout readies the metabolic pathways within the muscle for exercise or affects muscle fibre recruitment (Jones, et al., 2008a; Layec et al., 2009). It is possible that children, by virtue of their naturally fast kinetics (Barker & Armstrong, 2010) and greater muscle blood flow during exercise (Koch, 1977), demonstrate an attenuated priming effect.

This investigation had three hypotheses:

a. The relative speeding of the PCr MRT following priming exercise would be greater in men than boys
b. The relative decrease in the PCr A_{SC} following priming exercise would be greater in men than boys
c. The HHb MRT would be faster in following priming exercise in both men and boys

6.2 Methods

6.2.1 Participants

6 boys (12.8 ± 03 y, 1.47 ± 0.04 m, 44.1 ± 8.0 kg, -1.4 YAPHV) and 6 men (21.5 ± 3.5 y, 1.82 ± 0.06 m, 83.3 ± 19.2 kg) took part in this study. Data from these participants was reported in Chapter 5, and can be found in Appendix D.

6.2.2 Experimental protocol

Chapters 3 and 5 describe the prescription of exercise intensity and the collection of MRS and NIRS data during 6 minutes of very heavy intensity exercise. During the 3rd, 4th, and 5th visits for these 6 men and 6 boys, 2 bouts of very heavy intensity exercise were completed. Participants exercised at 60 %Δ for 6 min (E_1), rested for 6 min, and then completed a second 6 minute exercise bout at 60 %Δ (E_2). As in Chapter 5, all tests were examined for compliance, and data from any test where the power output during

any minute was 20 % greater or less than the mean power output for that test was discarded. MRS and NIRS data during E_1 and E_2 were collected and modeled as described in Chapter 3. The recovery of PCr between exercise bouts was examined by fitting an exponential curve (Equation 3.6) to PCr recovery data over the 6 min between E_1 and E_2. Recovery following E_2 was also examined, but did not significantly differ from recovery following E_1 and is not reported here.

6.2.3 Statistics

All variables are expressed as mean ± standard deviation. All variables were tested for normal distribution prior to analysis using the Shapiro-Wilkes statistic. Repeated measures ANOVA was used to examine the effect of age and condition (primed vs. unprimed) and the interaction between these variables. Follow-up testing was carried out using planned independent (for age effects) and dependent (for condition effects) t-tests with Bonferroni correction. Significance was accepted at $p<0.05$ and all tests were carried out using SPSS 11.0 for Windows.

6.3 Results

6.3.1 Power output

Power output during the CWR tests was significantly higher in men (24.6 ± 4.9 W) than adolescent boys (11.3 ± 2.9 W, $p<0.001$). Men worked at 112 ± 9 % of the target 60 %Δ work rate, while boys worked at 101 ± 17 % of the target. The actual power output was not significantly different from the target in either group.

6.3.2 PCr kinetics

The goodness of fit of PCr kinetics during very heavy intensity exercise is described and illustrated in Chapter 5. The PCr kinetic response did not change with priming exercise in boys, while the slow component amplitude was significantly reduced in bout 2 in men (Table 6.1). A significant bout effect for the MRT was identified, but follow-up testing revealed only a trend for a faster response in men ($p=0.03$). Following the first exercise bout, PCr recovered monoexponentially with a time constant of 38 ± 7 s in adolescent boys and 59 ± 13 s in men ($p=0.007$).

Table 6.1. Parameters of the PCr kinetic response to very heavy intensity exercise in boys and men.

			Statistical analysis		
	Boys (n=6)	Men (n=6)	Age	Bout	Interaction
MRT (s):			p=0.002	p=0.03	p=0.04
E_1	35 ± 16	109 ± 41*			
E_2	34 ± 9	82 ± 31*			
τ (s):			p<0.001	p=0.65	p=0.78
E_1	30 ± 8	66 ± 21*			
E_2	31 ± 5	70 ± 25*			
95 % CI (s):					
E_1	9 ± 6	21 ± 11			
E_2	10 ± 5	22 ± 22			
A_F (% BL):			p<0.001	p=0.14	p=0.13
E_1	35 ± 6	48 ± 7*			
E_2	35 ± 5	57 ± 8*			
A_{SC} (% BL)			p=0.005	p=0.002	p<0.001
E_1	0 ± 2	12 ± 6*			
E_2	1 ± 2	7 ± 6†			
A_{SC} (% EE)			p=0.01	p=0.01	p=0.004
E_1	1 ± 6	20 ± 10*			
E_2	2 ± 5	11 ± 10†			

*- significantly different from boys during the same bout; †- significantly different from bout 1, p<0.013

6.3.3 Muscle phosphates and pH

Table 6.2 describes the changes in pH, PCr, P_i, and TOI during 2 bouts of very heavy intensity exercise. Metabolic perturbation (change in PCr and pH) was greater in men than boys during both bouts. Priming exercise did not affect acidosis, metabolic perturbation, or oxygenation index in boys or men, although there was a trend for greater acidosis in the first bout compared with the second in men compared with boys (p<0.001) and for greater PCr depletion in the second bout compared with the first in boys (p<0.001).

Table 6.2. Changes in muscle phosphates, pH, and oxygenation during 2 bouts of very heavy intensity exercise in boys and men.

			Statistical analysis		
	Boys (n=6)	Men (n=6)	Age	Bout	Interaction
pH:			p=0.002	p<0.001	p<0.001
BL 1	7.04 ± 0.03	7.05 ± 0.01			
EE 1	6.99 ± 0.05	6.78 ± 0.07*			
BL 2	7.02 ± 0.04	6.97 ± 0.06†			
EE 2	7.00 ± 0.04	6.86 ± 0.07*†			
PCr (% BL):			p=0.007	p<0.001	p<0.001
BL 1	100 ± 0	100 ± 0			
EE 1	65 ± 4	39 ± 10*			
BL 2	98 ± 2	95 ± 4			
EE 2	59 ± 4†	37 ± 12*			
P_i (% BL)			p=0.03	p<0.001	p=0.01
BL 1	100 ± 0	100 ± 0			
EE 1	325 ± 87	430 ± 54			
BL 2	65 ± 4†	39 ± 10†			
EE 2	335 ± 86	429 ± 69			
TOI (%)			p=0.02	p<0.001	p<0.001
BL 1	65.4 ± 4.4	64.7 ± 8.3			
EE 1	58.2 ± 9.7	28.1 ± 17.1*			
BL 2	66.8 ± 4.8	70.1 ± 7.2†			
EE 2	58.2 ± 10.4	28.7 ± 13.8*			

*-significantly different from boys at the same time point, p<0.0125; †-significantly different from E_1 BL or EE, p<0.0125

6.3.4 HHb and HbO2 kinetics

Figure 6.1 illustrates the typical HHb and HbO_2 response in adolescent boys and men during 2 bouts of very heavy intensity exercise, and the TOI, which reflects oxygenation relative to total haemoglobin, can be seen in Table 6.2. A significant bout effect (p<0.001) but no age (p=0.53) or interaction (p=0.16) effects was found for the HHb time delay (E_1: boys: 12 ± 3 s, men: 10 ± 2 s; E_2: boys: 9 ± 2 s, men: 9 ± 2 s). For the HHb τ, a significant age effect (p=0.03) but no bout (p=0.57) or interaction (p=0.06) effects were identified (E_1: boys: 8 ± 3 s, men: 18 ± 7 s; E_2: boys: 9 ± 3 s, men: 16 ± 8 s). A significant bout effect (p=0.02) but no age (p=0.06) or interaction (p=0.35) effects were identified for the HHb MRT (E_1: boys: 20 ± 3 s, men: 29 ± 8 s; E_2: boys: 18 ± 3 s, men: 25 ± 9 s). HHb was not different before (boys: p=0.44; men: p=0.10) or during (boys: p=0.72; men: p=0.95) E_2 compared with E_1. HbO_2 was significantly elevated

prior to E_2 in men (p=0.01) but not boys (p=0.09) and elevated at the end of E_2 compared with E_1 in boys (p=0.02) but not men (p=0.25).

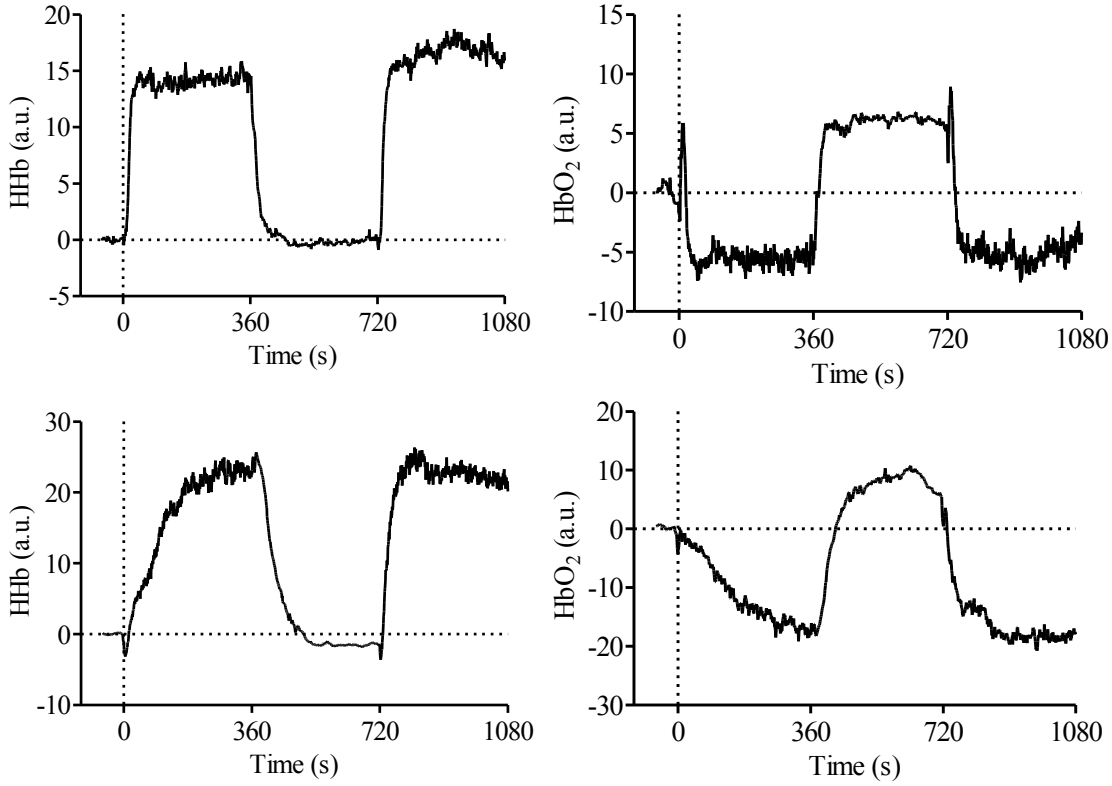

Figure 6.1.HHb (left) and HbO_2 (right) kinetics during 2 bouts of very heavy exercise separated by 6 min of recovery in a representative adolescent boy (top) and man (bottom).

6.4 Discussion

The purpose of this study was to test the hypothesis that the relative decreases in A_{SC} and PCr MRT are less in adolescent boys than adult men. This hypothesis was accepted; priming exercise resulted in a significant reduction in the slow component amplitude and a trend toward a speeding of the MRT for PCr in men, but no differences between E_1 and E_2 were apparent in adolescent boys. The third hypothesis, that the HHb MRT would be faster following priming exercise in both boys and men, was accepted. The time delay and MRT of the HHb kinetic response at the onset of exercise were reduced following priming exercise, but did not differ with age.

6.4.1 Effect of priming exercise on metabolic perturbation

Very heavy exercise resulted in significantly lower pH_{EE} and PCr_{EE} in adults compared with adolescents. However, the increase in P_i was not significantly different between groups. The differences in PCr and pH indicate that adults were probably relying on anaerobic energy sources to a greater extent than adolescents during very heavy intensity exercise. This is in keeping with the greater metabolic perturbation during supra-threshold incremental exercise reported by Barker, Welsman, Fulford, Welford, and Armstrong (2010b) and Zanconato et al. (1993). Significant bout and interaction effects, interpreted through planned follow-up t-tests, revealed that priming exercise had a profound effect on pH in men – pH was lower prior to E_2, but significantly higher at the end of E_2. This provides evidence that the balance of proton production and buffering in the myocyte was affected by priming exercise in adults. The relative concentration of PCr, on the other hand, was not affected by priming exercise in men, but was significantly lower after E_2 in boys. Finally, there were no differences in P_i between bouts or groups during exercise, but prior to E_2, P_i was significantly decreased in both boys and men.

The effect of priming exercise in adults was consistent with previous reports: in this group, E_2 was characterised by a speeding of the MRT, a reduction in slow component amplitude, and no change in the fundamental τ. In boys, on the other hand, the two bouts were virtually identical. This is a novel finding, and stands in contrast to the findings of Barker et al. (2010a), who described a speeding of the $\dot{V}O_2$ MRT and attenuation of the slow component amplitude in young boys but did not compare the response with the response in adults. The key difference between the two studies, however, is the presence or absence of a slow component: Barker et al. (2010a) reported a slow component making up 9 ± 3 % of the final response, while no slow component was present in the current study. In the current study, the amplitude and τ of a single exponential curve fitted from 0 s to 120 s were not different from the amplitude and time constant of a single exponential curve fitted over the full exercise test (see Appendix E and Chapter 3 for details of fitting, and Chapter 5 for a discussion of the possible explanations for the absence of a slow component in adolescent boys). Most adult studies have reported no effect of priming exercise on the fundamental time constant (Burnley, et al., 2000; Forbes, et al., 2008; Jones, et al., 2006; Jones, et al.,

2008a) although exceptions are sometimes reported (DeLorey, et al., 2007). In the absence of a slow component, a priming effect would be unexpected.

An elegant and plausible, though unconfirmed, explanation for these data is that adult men and adolescent boys differ in their ability to recruit higher-order muscle fibres, as discussed in Chapter 5. As discussed by Jones et al.(2008a), a decrease in the so-called recruitment threshold, or the power output at which a given muscle fibre is recruited, might underlie the changes in $\dot{V}O_2$ and PCr kinetics seen after priming exercise. This is supported by EMG data from Layec et al. (2009), Bailey, Vanhatalo, Wilkerson, DiMenna, & Jones (2009), and Dimenna, Wilkerson, Burnley, & Jones (2008). This theory posits that by lowering the recruitment threshold for a number of higher order fibres, priming exercise facilitates the recruitment of a greater muscle fibre pool at exercise onset. This reduces the strain on each fibre and reduces the need to recruit additional fibres to meet the demands of exercise, a key hypothesis for the aetiology of the slow component.

The current data are well explained by this hypothesis – in adults, the second bout has a markedly attenuated slow component and a slightly longer fundamental time constant, which may be explained by the recruitment of a greater pool of fibres at the onset of the second bout. An inability to recruit higher-order muscle fibres during childhood has been proposed by several investigators as a possible explanation for age differences in muscle metabolism (Halin, et al., 2003; Ratel, et al., 2010). A limited available fibre pool, composed primarily of oxidative fibres, in adolescents would result in faster fundamental kinetics and a reduced slow component. The identical second bout would also be explained by the adolescent participants relying on the same muscle fibres in the first and second bouts.

6.4.2 Effects of priming exercise on muscle oxygenation

It is important to consider other possible explanations for these data; one theory that has received considerable attention in the priming literature is that increased muscle blood flow during the second bout removes any oxygen delivery-related limitation, facilitating a speeding of kinetics. In the current study, NIRS was used to investigate the possibility that differences in muscle oxygenation contribute to age differences in the priming effect. HHb kinetics was investigated to examine fractional oxygen extraction. The time

delay prior to the exponential increase in HHb was reduced in both boys and men, as previously reported in adults (DeLorey, et al., 2007; Endo et al., 2005; Jones, Wilkerson, & Fulford, 2008b; Layec, et al., 2009; Saitoh, et al., 2009). The time constant for muscle deoxygenation was significantly longer in adults compared with adolescents, as described in Chapter 5. The mean response time in both groups was significantly faster during E_1 compared with E_2. The reduced time delay is significant in two respects: first, the presence of this phenomenon in both men and boys indicates that the effect of priming exercise on muscle oxygen extraction is likely independent of the effect of priming exercise on PCr kinetics. Second, the reduced time delay indicates that a mismatch between oxygen delivery and utilisation occurs earlier in the second exercise bout in both boys and men.

The TOI, measured using spatially resolved spectroscopy, fell significantly more in adults than children during both bouts of very heavy exercise. In adults, but not in children, TOI was increased prior to the start of the second bout – this was also true of HbO_2. This hyperperfusion in adults could affect metabolic parameters during the exercise bout, although it is important to note that the elevated TOI persisted for only 13 ±7 s, and TOI_{EE} was similar in both bouts. Based on the transient nature of differences in TOI and in HHb kinetics, it is reasonable to suppose that metabolic differences, rather than differences in muscle oxygenation, are responsible for the priming effect in adults in this case. This supposition is supported by several studies in adults which have concluded that oxygen delivery is not the primary mechanism for the priming effect in adults (Forbes, et al., 2008; Saitoh, et al., 2009).However, the issue is far from resolved, with several authors reporting that differences in oxygen delivery might be important in repeated bouts of exercise (Barker, et al., 2010a; Hernandez, et al., 2010; Raymer, et al., 2007).

The current study demonstrates that PCr recovery following intense exercise is faster in boys than men. It is plausible that this faster recovery is responsible for the absence of a priming effect in adolescents. However, it has been demonstrated that the $\dot{V}O_2$ (Bailey, Vanhatalo, et al., 2009; Jones, et al., 2008a) and PCr (Forbes, et al., 2008) priming effect persists after recovery durations much longer than 6 min. This implies that the priming effect is not acutely sensitive to the degree of metabolic recovery between bouts, and that a speeding of recovery kinetics of ~1/3 in adolescents is not the primary

mechanism for the age differences in priming demonstrated in this study. Extensive further discussion of the mechanisms for and implications of faster recovery kinetics in children compared with adults can be found in Chapter 7.

6.4.3 Methodological considerations

There are several possible limitations in this research that must be considered, which are discussed more extensively in Section 9.2. Briefly, these limitations include the age, maturity, and physical activity of the young participants, the possible error in assignment of imposed PO in adult and adolescent participants, the small sample (see Appendix B for a discussion of statistical power) and considerable heterogeneity in PCr kinetics within each group, and the inherent limitations of the near-infrared spectroscopy technique.

6.4.4 Conclusions

This study reports for the first time the effect of priming exercise on PCr kinetics in adolescents. In contrast to the report of Barker, Jones, et al (2010a), the results of this investigation suggest that priming exercise does not affect PCr kinetics during subsequent exercise in adolescent boys. The effects in men were similar to those previously reported – a reduction in slow component amplitude leading to a reduced MRT. The results of this study suggest that men might differ from boys in their ability to recruit higher-order muscle fibres during high intensity exercise.

7 The influence of age, maturation, sex, and EE conditions on PCr recovery kinetics in young people and adults

7.1 Introduction

As discussed in Chapters 2 and 6, faster PCr recovery in young people might influence performance during tests and activities that involve repeated bouts of exercise. The empirical support for faster recovery in young people is equivocal (see Chapter 2), and the relationship between PCr recovery and age in these studies is potentially confounded by metabolic changes, such as acidosis or PCr depletion, at the end of the exercise transition.

When pH does not decrease appreciably from resting values, PCr recovery τ has been demonstrated to provide an index of mitochondrial capacity (Glancy, et al., 2008; Paganini, et al., 1997). In adults, the influence of acidosis, resting [PCr], and $[PCr]_{EE}$ has received considerable attention in the literature (Jubrias, et al., 2003; Kemp, Van den Broek, Nicolay, & Prompers, 2010; Layec et al., 2010; van den Broek, et al., 2007). However, little is known about the effect of these factors on PCr recovery in children or adolescents. A better understanding of factors affecting PCr recovery kinetics in children and adolescents would allow this measure to be used to investigate the development of mitochondrial function.

The purpose of this study was to investigate the relationships between age, sex, maturity, and EE metabolic conditions in adolescent and adult males and females. Two hypotheses were posed:

a. PCr recovery kinetics would be faster in adolescents compared with adults,
b. A significant correlation between PCr recovery kinetics and pH_{EE} would be present in both adolescents and adults.

7.2 Methods

7.2.1 Participants

Data from 21 adolescent boys (13.1 ± 0.4 y, 1.53 ± 0.09 m, 47.7 ± 10.8 kg, -1.0 ± 0.7 YAPHV), 15 adolescent girls (13.0 ± 0.8 y, 1.58 ± 0.11 m, 50.0 ±15.0 kg, 1.0 ± 1.2

YAPHV), 20 men (25.4 ± 6.0 y, 1.78 ± 0.08 m, 78.4 ± 14.6 kg), and 17 women (26.3 ± 7.2 y, 1.67 ± 0.05 m, 60.0 ± 7.8 kg) was analysed. Data collected during exercise in these participants is also reported in Chapter 4, Chapter 5, Chapter 6, and Chapter 9. This chapter reports the metabolic response following exercise. For clarity, individual data sets are presented in Appendix D. 8 boys, 6 girls, 7 men, and 8 women completed heavy intensity exercise, 7 boys, 2 girls, and 6 men completed very heavy intensity exercise, and 6 boys, 7 girls, 7 men, and 9 women completed fatiguing intermittent isometric exercise. All of these participants and a parent or guardian provided informed consent to participate in these studies (Appendix C), which were approved by the institutional ethics committee. Data were collected and analysed as described in Chapter 3.

7.2.2 Statistical analysis

All variables are presented as mean ± standard deviation. Two-by-two ANOVA was used to identify group differences during heavy intensity exercise and following repeated MVCs. Unpaired t-tests with Bonferroni correction was used to examine differences between men and boys during very heavy intensity exercise; sex differences were not investigated at this exercise intensity due to insufficient female data. One way ANOVA was used to examine differences in recovery parameters between exercise protocols in the same group in boys, girls, and men; unpaired t-tests were used to examine differences between exercise protocols in women because only two studies included women. Simple linear regression was used to examine the relationship between pH_{EE} and PCr recovery τ and between $[PCr]_{EE}$ and PCr recovery τ. In adolescents, stepwise multiple linear regression was used to explore the influence of age, maturation, PCr_{EE}, and pH_{EE} on PCr recovery kinetics. Simple linear regression was used to examine the relationship between pH_{EE} and PCr recovery τ.

7.3 Results

7.3.1 Recovery

PCr depletion and pH_{EE} can be seen in Table 7.1, while Figure 7.1 reports mean values of PCr recovery τ for each exercise test.

7.3.2 Regression

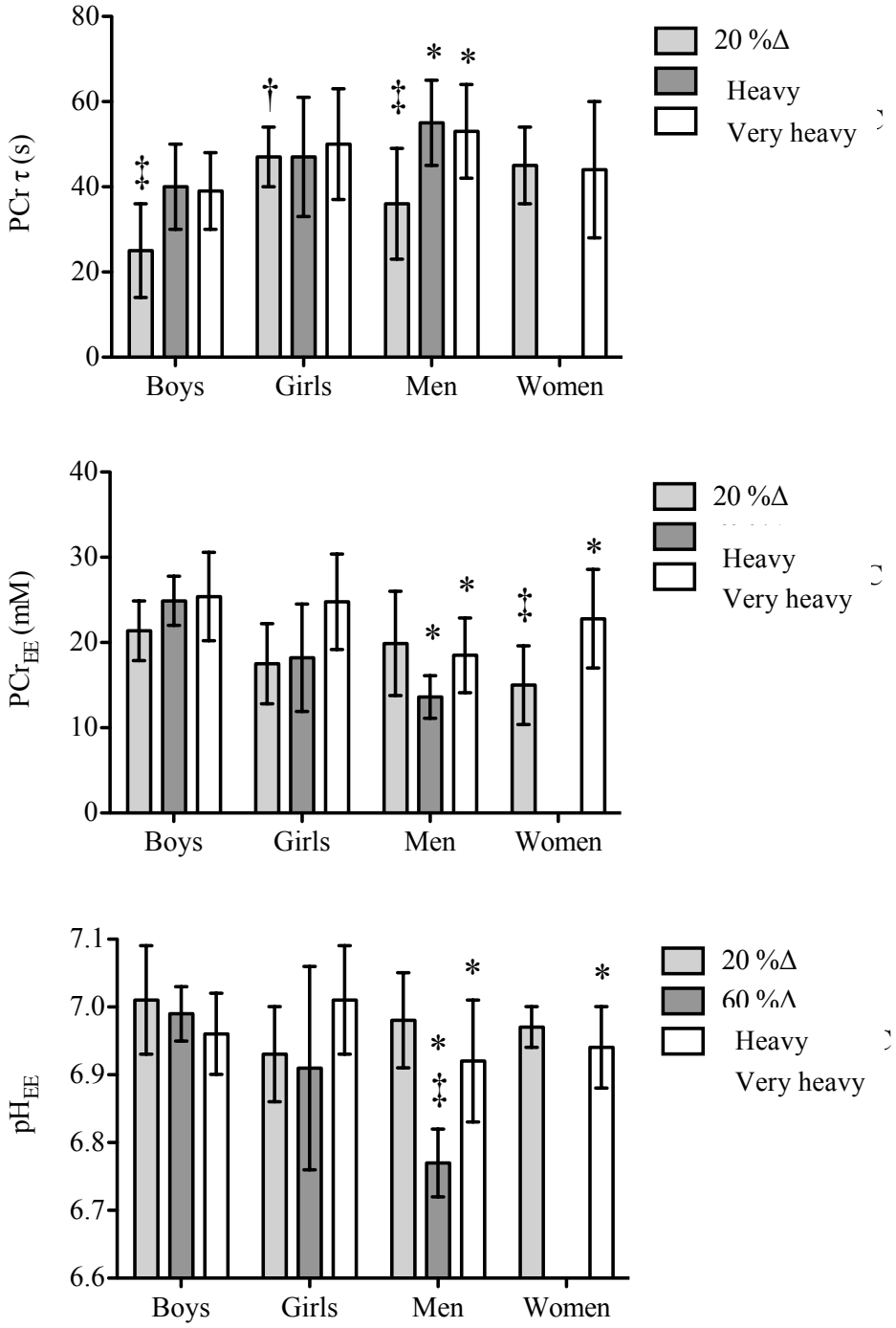

Figure 7.1. Key parameters of PCr recovery following 3 different exercise tests in adolescent boys and girls and adult men and women.* - significantly different from children of the same sex; † - significantly different from males of the same age; ‡ - significantly different from the other exercise protocols in the same sex and age group.

Stepwise multiple linear regression was carried out to investigate the influence of pH_{EE} $[PCr]_{EE}$, age, and maturity on PCr recovery kinetics within the adolescent group. Only maturity was a significant predictor of PCr recovery τ (Figure 7.2), with β=0.67, R^2=0.46, and p<0.001. Age (β=-0.12, p=0.45), pH_{EE} (β=-0.13, p=0.36), and $[PCr]_{EE}$ (β=-0.04, p=0.80) were excluded from the model.

Table 7.1. Metabolic conditions following 6 min of high intensity dynamic or isometric quadriceps exercise in adolescents and adults.

	Boys (n=21)	Girls (n=11)	Men (n=19)	Women (n=17)	ANOVA		
					Age	Sex	Interaction
PCr τ (s)	34 ± 12	49 ± 11†	48 ± 14*	44 ± 13	p=0.13	p=0.07	p=0.002
95% CI (s)	10 ± 5	20 ± 21	6 ± 3	10 ± 5			
$[PCr]_{BL}$ (mM)	40.1 ± 1.0	40.0 ± 0.7	40.3 ± 0.6	39.9 ± 1.1	p=0.81	p=0.23	p=0.47
PCr_{EE} (% BL)	61 ± 10	55 ± 15	46 ± 13*	51 ± 17	p=0.003	p=0.54	p=0.06
$[PCr]_{EE}$ (mM)	24.6 ± 3.8	21.7 ± 5.9	18.5 ± 5.3*	20.2 ± 6.8	p=0.002	p=0.65	p=0.05
pH_{EE}	6.99 ± 0.06	6.96 ± 0.09	6.90 ± 0.12*	6.96 ± 0.05	p=0.03	p=0.45	p=0.03

* - significantly different from children of the same sex
† - significantly different from males of the same age

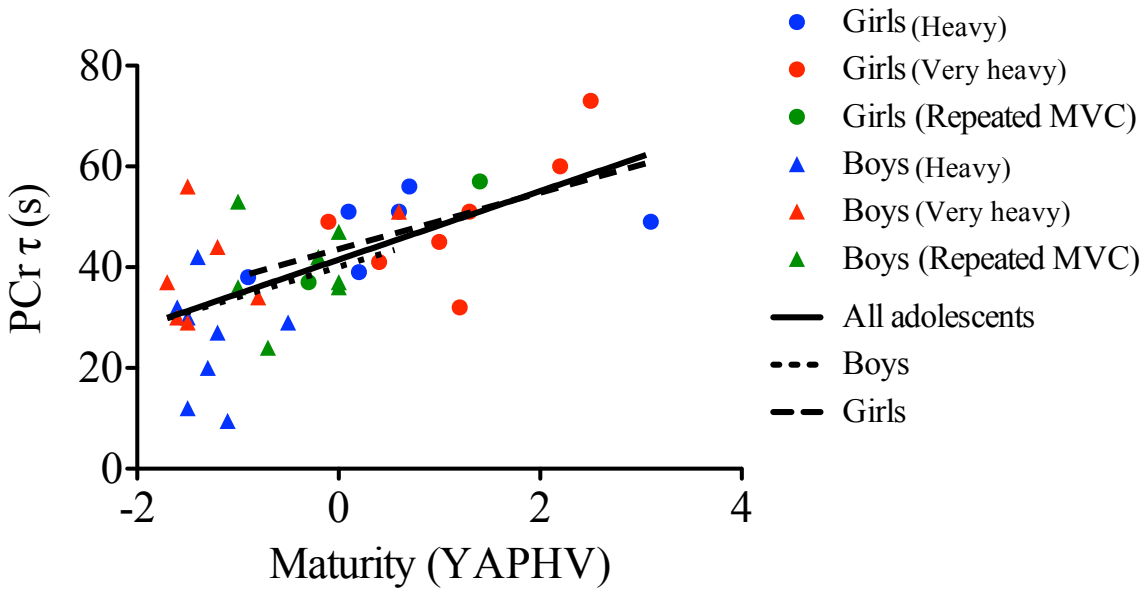

Figure 7.2. Relationship between maturity and recovery kinetics in young people. Girls were significantly more mature than boys (p<0.001). The correlation between recovery

τ and maturity was significant across the whole group (y=6.8x+41.5, R^2=0.40, p<0.0001) and in girls (y=5.6x+43.6, R^2=0.34, p=0.02) but not in boys (y=6.0x+40.1, R^2=0.10, p=0.13).

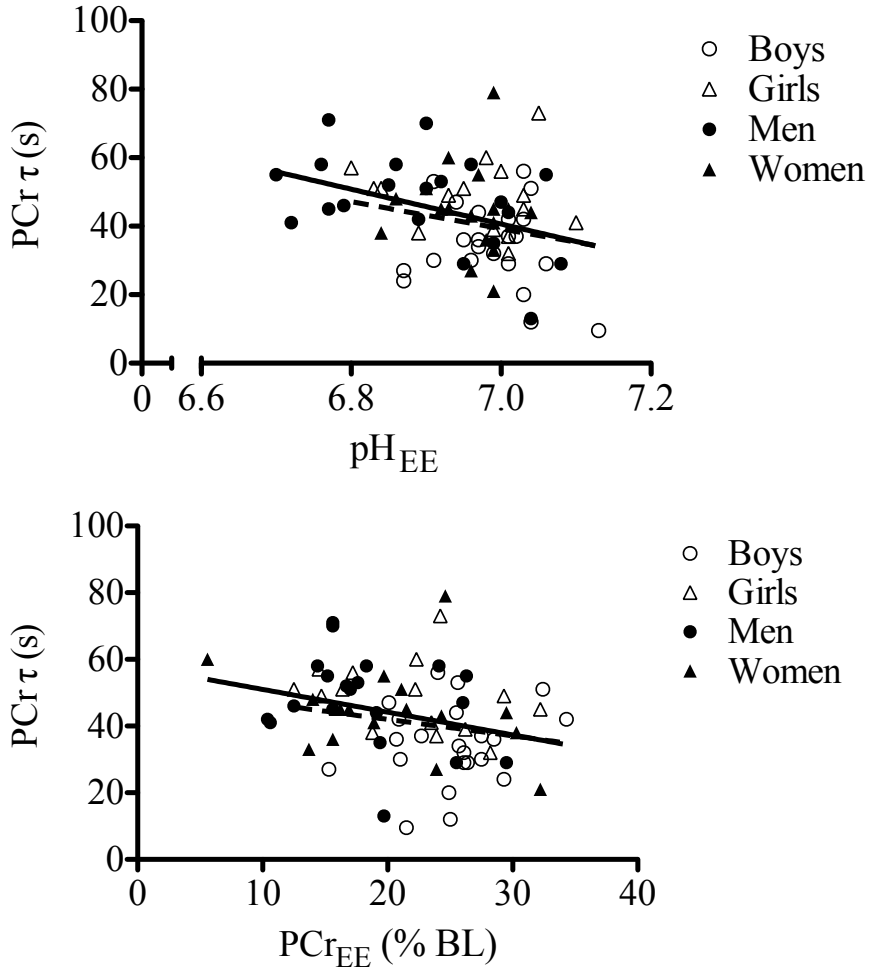

Figure 7.3. Relationship between acidosis and recovery τ (left) and $[PCr]_{EE}$ and recovery τ (right). Solid line shows linear regression over full data set (top: y=-51x+397, R^2=0.11, p=0.04; bottom: y=-0.7x+58, R^2=0.09, p=0.009), and broken line shows linear regression in adolescent participants only (top: y=-39x+315, R^2=0.05, p=0.19; bottom: y=-0.5x+52, R^2=0.04, p=0.26).

Figure 7.3 shows the relationship between PCr recovery kinetics, pH_{EE}, and $[PCr]_{EE}$. Recovery τ was very weakly but significantly correlated with both $pH_{EE}$and $[PCr]_{EE}$ when all 68 participants were included in the analysis, but was not correlated with either variable in adolescents alone.

7.4 Discussion

The time constant for the monoexponential recovery of PCr was faster in boys than in men or girls overall; on an individual study basis, PCr recovery was faster in boys than girls following heavy intensity exercise and repeated isometric MVC exercise, and faster in boys than men following heavy and very heavy dynamic exercise. Thus, the first hypothesis, that adolescents would have faster PCr recovery kinetics than adults, was not accepted. Men had greater PCr depletion following very heavy and repeated MVC exercise compared with boys. Overall acidosis and acidosis following very heavy exercise was also greater in boys than men. The second hypothesis, that acidosis would be correlated with slowed PCr recovery kinetics, was not accepted. There was no significant relationship between acidosis or PCr depletion and PCr recovery kinetics in adolescent participants. Very weak but significant negative correlations between both $[PCr]_{EE}$ and recovery τ and pH_{EE} and recovery τ in the pooled adolescent and adult data were present. Increasing maturity was significantly associated with slower PCr recovery kinetics in adolescents.

7.4.1 Factors affecting recovery kinetics

The moderate association between maturity and PCr kinetics in young people is an interesting and novel finding. This relationship supports the findings of previous studies that have reported differences in PCr recovery kinetics between children or adolescents and adults (Ratel, et al., 2008; Taylor, et al., 1997) and contributes to understanding the age- and sex- differences in PCr recovery kinetics seen in the current study. Puberty bring changes in hormones, anthropometry, and muscle morphology (Baxter-Jones & Sherar, 2007). Girls were significantly more mature than boys, and thus closer to adult in terms of their metabolic recovery from exercise. This relationship might be important in explaining a number of seemingly contradictory findings about exercise metabolism and fatigue in young people (Ratel, et al., 2010), and warrants further investigation to understand the practical implications.

The physiological implications of faster PCr recovery in the overall sample of boys compared with men or girls (Table 7.1) are important to consider, since these patterns are also evident during several of the individual exercise tests examined in this chapter.

According to Meyer's electrical analogue model (Meyer, 1988), PCr recovery kinetics offer a measure of mitochondrial capacity (Glancy, et al., 2008; Paganini, et al., 1997), which could potentially begin to elucidate the development of oxidative ability with age. Faster recovery in boys indicates that metabolic differences discussed in Chapter 2 might be related to better oxidative abilities in the muscle. Several mechanisms for this greater oxidative capacity are possible. First, an attenuated ability to utilise glycolytic pathways might obligate a reliance on oxidative pathways, leading to an adaptive up-regulation of activity in those pathways. Second, greater habitual physical activity in boys would lead to a more "trained" muscle profile, which includes faster PCr recovery kinetics (Larsen, Callahan, Foulis, & Kent-Braun, 2009; McCreary et al., 1996; Yoshida, 2002). Finally, PCr depletion in predominantly type I fibres, whether due to recruitment in the preceding bout or due to the fibre composition of the muscle itself, would result in faster recovery kinetics in boys, as only those fibres are recovrering following exercise (Jauslin et al., 2010). It is likely that an interaction between these factors is responsible for the differences seen in this chapter.

The exercise tests reported here have included concentric and eccentric exercise as well as isometric exercise. Metabolic differences between concentric, eccentric, and isometric contractions have been reported. Specifically, Ryschon, Fowler, Wysong, Anthony, & Balaban found that during concentric contractions, the free energy of ATP hydrolysis, ADP accumulation, PCr depletion, and acidosis were all higher during concentric compared with isometric or eccentric muscle actions. These authors also described a faster initial rate of PCr resynthesis following concentric exercise compared with eccentric or isometric. However, the initial rate of PCr resynthesis is heavily dependent on end-exercise [PCr], while the PCr recovery τ is not strongly related to PCr depletion (Smith, et al., 2004). Thus, the current study reports the recovery τ. To investigate the possibility that the exercise protocol played a substantial role in determining the PCr recovery kinetics, differences between protocols were examined. No differences in pH_{EE} or PCr_{EE} were found between groups in adolescents (Figure 7.1). However, very heavy intensity exercise resulted in significantly greater PCr depletion in men than heavy intensity exercise or repeated MVC exercise, while PCr depletion was significantly greater during heavy intensity exercise exercise than during repeated MVC exercise in women. In light of these differences, age and sex comparisons involving the adult groups should be made with caution.

The correlations between pH_{EE} and PCr τ and between PCr_{EE} and PCr τ in adolescents and adults together were significant but very weak. This is somewhat surprising; the relationship between pH_{EE} and PCr τ is well documented in the literature (Kemp, et al., 2010). However, the magnitude of the decrease in pH in this study was minimal – mean pH_{EE} across all participants was 6.94 ± 0.09. This is greater than the 6.88 maximum threshold for acidosis selected by Jubrias et al. (2003) and considerably less than previously reported acidosis in studies of PCr recovery kinetics (Walter, et al., 1997). The high pH values reported here are due to the muscle group, population, and exercise task used in this experiment. A further study to investigate the effects of acidosis on PCr recovery kinetics in young people might use a non-postural muscle group such as the forearm flexors, and might manipulate the exercise task to induce relatively greater or less acidosis in the same individual. In the forearm flexors of young boys, Ratel et al (2008) reported pH_{EE} of 6.6 ± 0.2. This muscle group also permits the use of higher field strength small-bore magnets to improve the signal-to-noise ratio of the collected spectra. Repeated high intensity muscle contractions are also more likely to decrease muscle pH compared with sustained contractions or slower contractions (Roussel, Bendahan, Mattei, Le Fur, & Cozzone, 2000; Walter, et al., 1997), offering another way to manipulate pH. Further research is needed to fully investigate the relationship between acidosis and PCr recovery kinetics in adolescence.

$[PCr]_{EE}$ has been linked with PCr recovery kinetics (Roussel, et al., 2000). However, a slowing or speeding of kinetics with PCr depletion is less well documented than an acidosis-related slowing. The mean relative PCr depletion was 52 ± 14%, and in the current study only a weak relationship between absolute PCr depletion and PCr recovery kinetics was found. Both PCr depletion and acidosis are related to exercise intensity, and it is difficult to separate the relative importance of these based on the current data. Future studies to investigate this relationship should follow the example of Walter et al. (1997) in the use of a number of different exercise tasks to independently manipulate [PCr] and pH at the end of exercise.

The wide 95% CI, particularly in girls, are a serious concern in the assessment and interpretation of these data. If the true value of τ cannot be estimated with acceptable precision, conclusions based on those values must be interpreted with caution.

Acceptable precision is sometimes defined as 95 % CI of <5 s (Armstrong & Fawkner, 2008). Future work in this area should endeavour to minimise these 95% CI, by using protocols that increase SNR or decrease variability in the data.

7.4.2 Conclusions

This study represents an important step forward in understanding PCr recovery kinetics in adolescence. The large sample size and inclusion of female as well as male participants are strengths of this study. Two distinct areas for future research are obvious extensions of this work. First, PCr recovery kinetics should be measured in healthy children and adolescents to understand how mitochondrial capacity develops in young people and how it can be altered with and training. Second, PCr recovery kinetics in children with cystic fibrosis, obesity, muscular dystrophy, and especially mitochondrial myopathies might offer an important clinical measure. Finally, the effects of pH on PCr recovery kinetics in children and adolescents should be elucidated through the use of exercise tests and protocols designed to manipulate acidosis and PCr depletion independently.

In summary, PCr recovery following 6 min of quadriceps exercise of different intensities and modes of contraction was significantly faster in boys than men and in boys than girls, but did not differ between women and girls or women and men. $[PCr]_{EE}$ and pH_{EE} did not systematically affect PCr recovery τ, but there was a significant slowing of PCr recovery τ with maturation in adolescents. These results support the hypothesis that oxidative capacity decreases from childhood to adulthood, and provide evidence that recovery is an important aspect of exercise metabolism for further research.

8 Measurement of PCr recovery kinetics using gated exercise in adult women and adolescent girls

8.1 Introduction

As discussed in Chapter 7, mitochondrial capacity is an important physiological property. PCr recovery kinetics could be used to investigate the efficacy of pharmacological and therapeutic interventions in children with diseases that affect muscle function as well as to develop a greater understanding of age and maturation related changes in skeletal muscle function. Measurement of mitochondrial capacity could allow the investigation of changes in muscle physiology with growth and maturation, training, disease, and treatment in young people. However, Chapter 7 demonstrated the 95 % CI for the recovery time constant can be wide, particularly in those individuals who complete only 1 exercise test, which reduces confidence in estimates of mitochondrial capacity.

Measurement of PCr recovery τ typically involves either several repeated exercise tests, which are averaged to model the kinetic response with confidence (Barker, et al., 2008b) or a sustained maximal contraction lasting 15-30 s (Slade et al., 2006). One previous paediatric study has reported the 95% CI for PCr recovery kinetics; in that study 5-10 moderate transitions were averaged together to give 6 s 95% CI (Barker, et al., 2008a). These tests are not suitable for clinical work, either due to the cost and time demands of multiple tests, or due to the maximal exertion required to perform sustained muscle contraction. A study published by Slade and colleagues (Slade, et al., 2006) used a gated exercise model to determine the PCr recovery τ in adults in a single visit and without strenuous exercise. Given the potential application of gated exercise to enable the assessment of the PCr τ within a clinical setting, the purpose of the current study was to establish the suitability of gated exercise for use within a pediatric population.

There were several phases to this investigation; First, an ergometer that would allow the performance and measurement of isometric calf muscle exercise within the magnetic resonance scanner was developed (see Chapter 3 for a description of the ergometer). This was used to develop a gated exercise protocol in adult females – several contraction and relaxation intervals were tested to find a duty cycle that was

comfortable for participants but that resulted in an overall PCr depletion. Finally, the gated exercise protocol was used in an adolescent population to assess the applicability and validity of this exercise test in young people. This also allowed comparison of the adult and adolescent responses to ensure that they were qualitatively similar and to confirm the results of Chapter 7 in a female population. The primary purpose of this investigation was to develop a gated exercise protocol in young people.

8.2 Methods

8.2.1 Participants and protocol

7 women (23.2 ± 3.4 y, 63.2 ± 5.5 kg, 1.65 ± 0.04 m) and 6 adolescent girls (13.8 ± 0.3 y, 51.3 ± 9.0 kg, 1.61 ± 0.05 m, 1.4 ± 0.5 YAPHV (Mirwald, et al., 2002)) were recruited to participate in the study, which was approved by the institutional ethics committee. All participants were healthy and recreationally active. Informed consent was obtained from all adult participants, and the consent and assent of a parent or guardian and each adolescent, respectively, was obtained (Appendix C). Participants completed 3 exercise tests on a calf ergometer whilst lying supine within a 1.5 T magnetic resonance scanner (Philips Gyroscan Intera) with a 6 cm ^{31}P transmit receive surface coil positioned beneath the belly of the right calf muscle. These tests consisted of: 1) a calf muscle maximal voluntary contraction (MVC), 2) a gated exercise test lasting 6 min; and 3) a 20 s sustained calf muscle MVC. Muscle PCr and pH was determined by ^{31}P-MRS during tests 2 and 3.

8.2.2 Measurement of MVC (Test 1)

Each participant completed 5 MVCs, each lasting 4 s and separated by 1 min of rest. The 3 trials with the highest force were averaged to determine a ‘target’ force for exercise tests 2 and 3. A visual display projected on the scanner provided force feedback to the participants.

8.2.3 Gated exercise (Test 2)

The gated exercise test consisted of 23 MVCs, each lasting 4 s, with 12 s of recovery allowed following each contraction. PCr was measured every 4 s, immediately prior to and following contraction, as well as twice during recovery. PCr data from the first 5 contractions were discarded to eliminate contractions where depletion during exercise

and resynthesis during recovery were not balanced. PCr values corresponding to the end of recovery and the end of contraction for the remaining 18 contraction cycles were averaged (Figure 8.1). These values were used, along with the time between contractions, to calculate the PCr recovery τ (Slade, et al., 2006).

$$\tau = \frac{-\Delta t}{\ln[\frac{D}{(D+Q)}]} \qquad \text{Equation 8.1}$$

where τ is the PCr recovery time constant, Δt is the recovery interval between contractions, D is the steady-state decrease in PCr (the average PCr at the end of recovery) and Q is the PCr cost of each contraction. Recovery was assumed to be monoexponential, and PCr recovery between contractions was assumed to balance PCr depletion due to contraction. Intracellular pH was determined each minute during exercise and recovery from the shift in the P_i peak relative to the PCr peak (Equation 3.3).

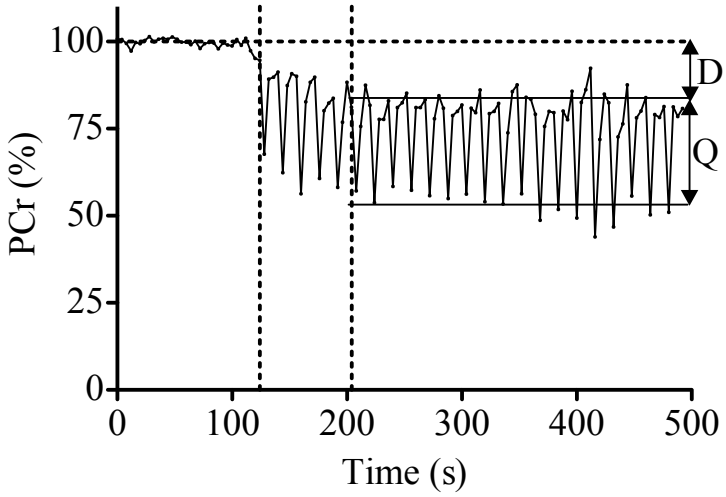

Figure 8.1. Average PCr during a gated exercise test. The beginning of exercise and the beginning of the analysed portion of the test are indicated with vertical lines, while the horizontal lines show the mean depletion of PCr at the end of each contraction and the end of each recovery period, which were used to calculate PCr τ.

Prior to the development of this protocol, several contraction and relaxation intervals were used. Initially, a 3 s contraction and 21 s recovery interval was used. The problems with this protocol were twofold: first, PCr recovered completely between contractions,

precluding analysis using Equation 8.1. Second, spectrum quality at 3 s temporal resolution at 1.5 T in the calf muscle was worse than desired. To improve SNR, spectra were then collected every 4 s, necessitating a 4 s contraction. The recovery interval was decreased, first to 16 s, then to 12 s, to ensure that PCr recovery was incomplete in all adult participants tested.

8.2.4 Sustained exercise (Test 3)

Following 5 min of rest from the gated protocol, each participant completed a sustained 20 s MVC, and PCr recovery from this contraction was monitored for 6 min. PCr recovery over the 6 min was fitted to an exponential equation (Equation 3.6). Intracellular pH was measured over 20 s of exercise and each minute during recovery.

8.2.5 31P-MR spectroscopy

^{31}P-MRS was carried out as described in Chapter 3. For this study, an adiabatic pulse was generated every 1 s. Four phase cycles were averaged, giving a spectrum every 4 s. Quantification of data proceeded as previously described (Chapter 3).

8.2.6 Data analyses

Variables were tested for normality using the Shapiro-Wilkes statistic. Potential mean differences in physiological parameters between adolescent and adult females were analysed using independent t-tests. The agreement between the PCr τ determined from the gated and sustained exercise tests were compared using Bland-Altman analysis (Bland & Altman, 1986) and dependent t-tests. The change in pH during the gated test was analysed using repeated measures ANOVA, and post-hoc testing was carried out using planned t-tests with the Bonferroni correction. All analyses were performed using GraphPad Prism 4 for Windows (GraphPad Software Inc.) with an alpha level of 0.05.

8.3 Results

8.3.1 Force and metabolic parameters of gated and sustained exercise

Table 8.1.PCr recovery parameters determined during gated exercise and following a sustained muscle contraction.

Participant	MVC (N)	PCr τ_{Gated} (s)	PCr $\tau_{Sustained}$ (s)	Gated PCr_{EE} (% BL)	Sustained PCr_{EE} (% BL)
Girls					
1	374	18	12	64	52
2	523	14	20	45	58
3	367	22	28	58	40
4	394	10	11	43	37
5	589	†	21	†	52
6	448	†	22	†	70
Mean	**431**	**16**	**19**	**53**	**50**
SD	77	5	6	10	11
Women					
1	555	16	24	53	48
2	505	12	27	67	44
3	570	15	17	64	48
4	501	18	20	62	48
5	570	26	9	67	58
6	494	18	20	56	51
7	515	11	16	48	47
Mean	**551***	**20**	**19**	**60**	**57**
SD	43	5	4	7	8

* - significantly different from girls, $p \leq 0.05$
† - analysis of these data using the gated method was not possible

Table 8.1 shows the parameters of the PCr recovery during gated exercise, and after a sustained contraction. The force and PCr responses for both the gated and sustained exercise tests for a representative participant are shown in Figure 8.2. MVC force was significantly lower in girls than women ($p=0.01$). Force was maintained throughout the gated test in both girls and women; the force on the last contraction was 99 ± 9 % of the force on the first contraction. There were no significant differences between the gated and sustained exercise tests for PCr τ ($p=0.40$) or PCr_{EE} ($p=0.06$). Mean 95 % CI for the fit of the exponential curve to PCr following recovery were ~ ± 1 s in both girls and women.

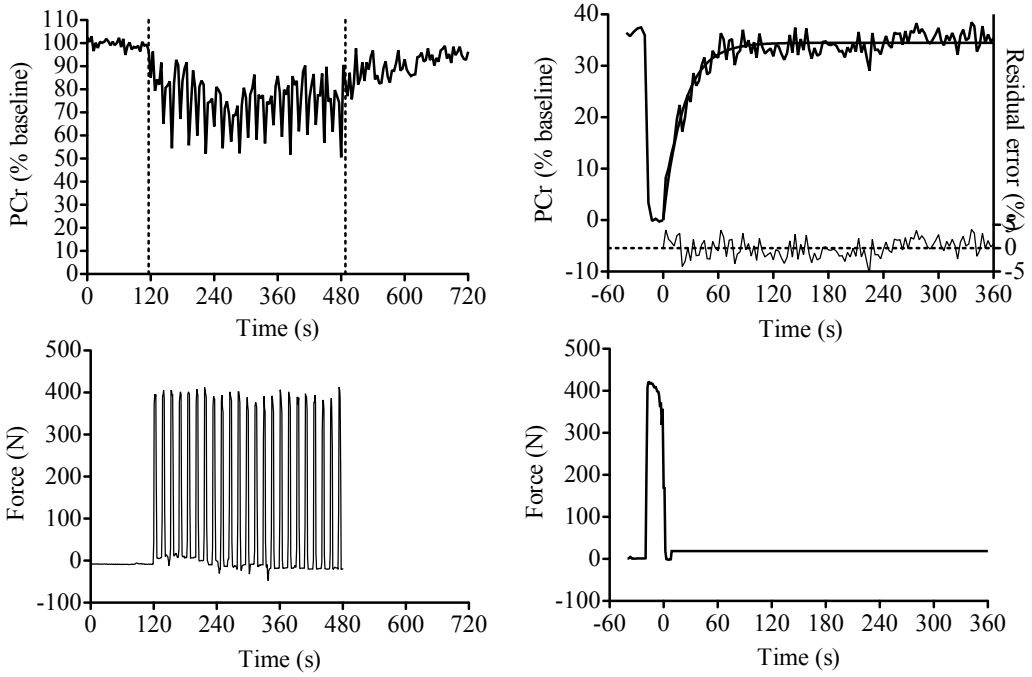

Figure 8.2.PCr (top) and force (bottom) for a 13 year old girl during a gated exercise test (left) and during 20 s of sustained exercise and 6 min of recovery (right). Dashed vertical lines indicate the beginning and end of the exercise test.

8.3.2 Intracellular pH

Intracellular pH was measured each minute during the gated exercise test (Figure 8.3). During the sustained exercise test, pH was measured every minute during rest and recovery, and averaged separately during the 20 s contraction. Repeated measures ANOVA revealed that pH changed significantly in the gated test. Post-hoc testing identified a significant increase from baseline to the first minute ($p=0.005$) and then did not change through the rest of the test ($p>0.1$ for all comparisons). However, following the 20 s contraction, pH decreased. The average nadir was 6.88 ± 0.04, a significant decrease from baseline (6.96 ± 0.06, $p<0.001$). The time of this nadir was midway (2-4 min) through recovery.

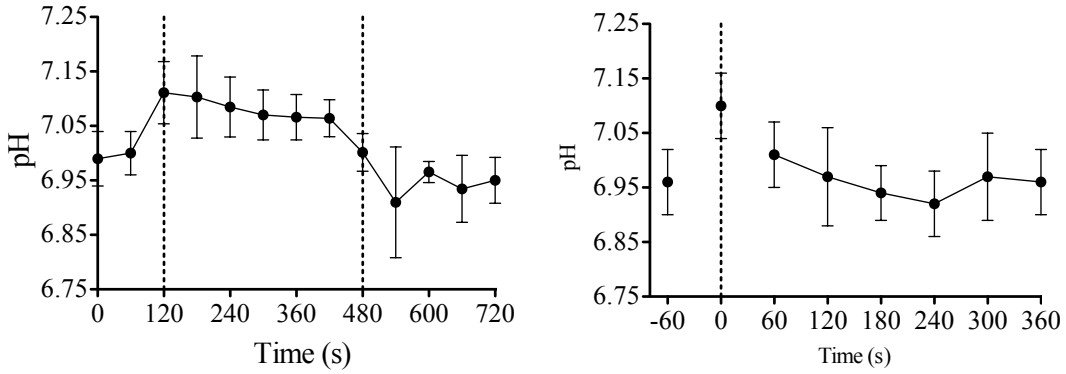

Figure 8.3. Intracellular pH (mean and SD) averaged from all participants during the gated (left) and sustained test (right).

8.3.3 Agreement between gated and sustained PCr recovery τ

Figure 8.4 illustrates the agreement between the PCr recovery τ measured following sustained exercise and using the gated protocol. Bland-Altman analysis revealed a mean bias of 2 s (p=0.37), with 95% limits of agreement (LOA) from -14 to 18 s.

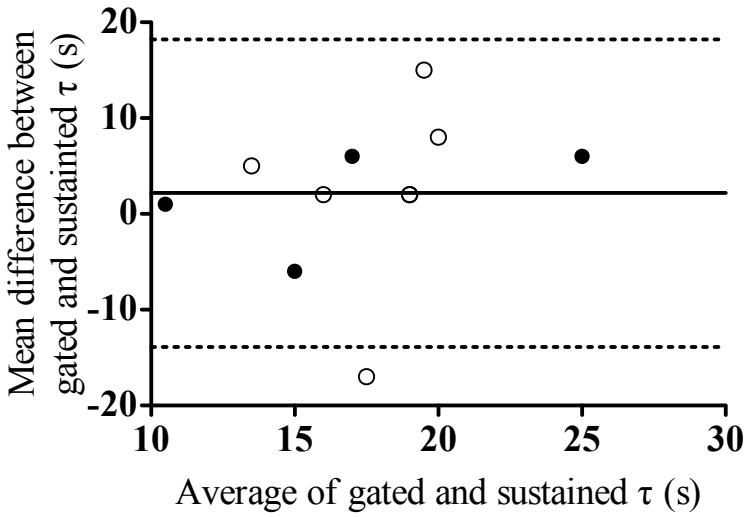

Figure 8.4.Bland-Altman plot for gated τ and τ measured following sustained isometric contraction for 4 adolescent girls (○) and 7 adult women (●). Solid line indicates the mean bias and dashed lines indicate the limits of agreement.

8.4 Discussion

The purpose of this study was to examine the feasibility of gated exercise in a paediatric population to determine PCr τ, as well as to determine the relationship between gated PCr τ and PCr τ measured following sustained exercise in the calf muscle. PCr recovery τ is an important measure of mitochondrial capacity in young people, but measurement of this variable often requires that participants complete repeated exercise tests, which are averaged together (Barker, et al., 2008b; Willcocks, et al., 2010), or a sustained exercise bout, which can be challenging. By reducing the number or intensity of tests required from participants, gated exercise might be an efficient method to measure mitochondrial capacity in young people with minimal cost, time commitment, and physical discomfort to the participant. In addition, the brief exercise bouts of the gated protocol are more similar to children's naturally sporadic activity patterns (Bailey, et al., 1995). In the current study, gated exercise was used to measure the PCr τ in 67 % of girls and 100 % of women. In addition, Bland-Altman analysis revealed a mean bias of 2 s (95 % LOA: -14 to 18 s). The correspondence between the two tests was variable: in 8 subjects, including all four girls whose data could be analysed, the two estimates of τ were within 6 s of each other. However, in three women, a large discrepancy between the estimates was present.

8.4.1 Factors affecting estimates of PCr recovery

The use of gated exercise to calculate PCr recovery τ is based on the assumption that recovery from brief exercise follows a predictable monoexponential time course. It is widely accepted that an exponential function describes PCr recovery kinetics when pH does not appreciably decrease from resting values (Forbes, Paganini, Slade, Towse, & Meyer, 2009a; Meyer, 1988), although more intense exercise might result in more complex patterns of recovery (Forbes, et al., 2009a). Assuming monoexponentiality of PCr recovery, the time for recovery and the amplitude of PCr recovery can be measured and the parameters (specifically the τ) of the exponential response can be calculated. Use of the gated method depends on the selection of an exercise intensity that results in incomplete PCr recovery between contractions. Gated exercise has been used in several muscle groups in adults (Chance, Eleff, Leigh, Sokolow, & Sapega, 1981; Forbes et al., 2009b; Slade, et al., 2006) to determine the rate of PCr recovery.

The parameters of the response to gated and sustained exercise were similar in adolescent girls and adult women. This is in keeping with the results of Chapter 7, and with some previous descriptions of PCr recovery in children, adolescents, and adults (Barker, et al., 2008a; Kuno, et al., 1995). However, given reports that PCr recovery is faster in children than adults (Dekerle, et al., 2009; Ratel, et al.; Taylor, et al., 1997), it is possible that the similarity in these responses is due to the advanced age and maturity of the participants. Future work to elucidate the influence of age and maturity on oxidative capacity measured via the recovery of PCr in non-acidotic muscle is warranted.

Anaerobic metabolism might affect the results of this investigation in two ways. First, intracellular pH is known to affect the speed of PCr recovery (Jubrias, et al., 2003; van den Broek, et al., 2007). Specifically, even a slight decrease in pH slows the PCr τ (van den Broek, et al., 2007). In the current study, pH remained slightly elevated above baseline throughout the gated exercise test. However, there was a significant, though small, decrease in pH during recovery from the sustained exercise test. This decrease is attributable to proton generation during PCr resynthesis, and represents a limitation of using the sustained exercise test to measure PCr τ. Second, glycolysis has been demonstrated to continue for several seconds following the cessation of exercise (Crowther, et al., 2002b; Forbes, et al., 2009a). The duration of prolonged glycolysis varies from study to study, likely reflecting differences in the preceding exercise duration, intensity, and muscle group. It is possible that glycolysis continued over the early stages of recovery during the gated protocol, following sustained exercise, or both. A prolonged glycolytic contribution to energy metabolism could lead to errors in the calculation of the PCr τ in the gated or sustained analysis.

Due to children's inherently smaller muscle mass, the signal-to-noise ratio of the PCr and P_i peaks in ^{31}P-MR spectra is often poor compared with spectra collected in adults. This has led previous investigators to average repeated recovery transitions together to increase confidence in the parameters of the PCr response in children (Barker, et al., 2008b). Gated exercise, which depends on averaging recovery parameters over a number of repeated contractions within a single testing session, offers another method of improving confidence. However, a low signal-to-noise ratio can also cause problems in the collection and interpretation of data during gated exercise. In two of the

adolescent participants in the current study, it was impossible to discern the parameters of the response. The signal-to-noise ratio can be improved by increasing the size of the muscle being interrogated (for instance, the quadriceps rather than the calf muscle) and/or by conducting investigations at a higher magnetic field strength, and should be considered in planning a ^{31}P-MRS study in a paediatric population.

8.4.2 Gated and fitted estimates of PCr recovery τ

There was some agreement between the PCr τ derived from gated exercise and that measured following sustained exercise in 11 adolescent girls and women. The mean bias was 2 s, and in 4 girls and 4 women, the difference was ± 6 s or less. The mean difference between the 2 estimates was 35% of the mean PCr τ.. Metabolic recovery following exercise has been reported to be slowed by 47% in Duchenne muscular dystrophy carriers compared with healthy controls (Kemp, Taylor, Dunn, Frostick, & Radda, 1993), by 67% in female athletes with cystic fibrosis compared with matched controls (Selvadurai et al., 2003), and by 48% in patients with mitochondrial myopathy compared with healthy controls (Taylor, Kemp, & Radda, 1994). Thus, this test might be useful in a clinical setting. Although the PCr τ measured following sustained exercise is generally regarded as the gold standard measurement of oxidative capacity, the potential for acidosis to affect this measure must be taken into account.

In the study published by Slade et al. (2006), the mean difference between PCr τ measured using gated and burst exercise was found to be 6 s. These authors report that the correlation between the 2 estimates of τ was high (r=0.82). However, the use of correlations to assess agreement has been criticised on the grounds that it measures association rather than agreement (Bland & Altman, 1986). Slade et al. report a slight decrease in pH following burst exercise similar to the decrease in pH found during recovery from sustained exercise in the current study. As discussed above, the assumption of monoexponential recovery is only valid where there is no significant acidosis. It is possible that the sustained exercise protocol risks underestimating mitochondrial capacity as a result of the effect of acidosis on PCr resynthesis. This would result in slower PCr recovery following sustained exercise compared with during gated exercise. 75 % of girls and 86 % of women had slower kinetics following sustained exercise, indicating that the effect of acidosis requires further investigation.

8.4.3 Methodological considerations

Several aspects of this investigation must be acknowledged as potentially problematic. First, the sample size was small, potentially limiting the generalisability of the results. Second, the young participants in the study were peripubertal (1.4 ± 0.5 YAPHV). Given the homogeneous sample, the use of this protocol with younger, smaller children and with clinical populations must be tested. Greater variability in the parameters of the response would be expected if a greater range in ages and physical fitness and ability was included in the current study; this is important to investigate and quantify, both from a physiological and methodological perspective.

Finally, the 33% failure rate of the gated protocol is a meaningful limitation of this protocol. This was due to problems related to SNR. Higher magnetic field strength is potentially a useful way to overcome that problem, but most researchers are limited in their access to higher field strength magnets. Further work to examine different exercise protocols (such as repeated very rapid contractions, sustained contractions at different intensities, and gated exercise) and different muscle groups to determine the optimal method of measuring mitochondrial capacity in a given population and experimental setting is warranted.

8.4.4 Applications and conclusions

There are many potential applications of the gated protocol in a paediatric population, particularly in the investigation of the effects of disease and treatment. Both young and adult participants reported that the gated protocol was comfortable, and compliance with the required force output was excellent during the gated protocol. Diseases such as cystic fibrosis (de Meer, Jeneson, Gulmans, van der Laag, & Berger, 1995), mitochondrial myopathy (Vorgerd & Zange, 2002), and muscular dystrophy (Kemp et al., 1993) are thought to affect mitochondrial capacity and exercise tolerance in children – changes in mitochondrial capacity with therapy or treatment could be non-invasively monitored using this protocol. As well, this type of exercise might be useful in the prediction of insulin sensitivity in children, independent of body weight (Fleischman, et al., 2009). Finally, gated exercise might be used to elucidate the development of mitochondrial capacity in young people and to investigate the effects of habitual physical activity and intensive sports training in this population.

The current study has demonstrated that gated exercise can be used to determine PCr recovery τ in adolescent girls and adult women. The gated exercise protocol was tolerated well by the participants, which is an advantage in many paediatric groups and prospectively applicable to patient groups. In most participants, the gated estimate of PCr τ corresponds well with PCr τ measured following sustained exercise. In 2 participants, poor SNR prevented determination of PCr τ using the gated protocol. Given the clinical implications of mitochondrial capacity for exercise, recovery, and fatigue, PCr recovery kinetics are important to understand in adolescents and children in health and disease. This study demonstrates that both sustained and gated exercise represents a cost-effective and well-tolerated method to measure PCr τ in young people. However, methodological considerations in the use of PCr τ as a clinical measure require further research.

9 Fatigue in adolescents and adults during repeated isometric quadriceps maximal voluntary contractions

9.1 Introduction

Fatigue, defined as a fall in the force generating capacity of the muscle (Enoka & Duchateau, 2008) has been shown to be less in children than adults during some exercise tasks (Dipla, et al., 2009; Hebestreit, et al., 1993; Ratel, et al., 2002; Zafeiridis, et al., 2005). Evidence for greater fatigue resistance in children and adolescents compared with adults was discussed in Section 2.4, but little direct information about the mechanistic basis for these differences exists. Hebestreit et al (1993) noted that the half time for $\dot{V}O_2$ 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. Hebestreit's suggestion that recovery is an important mediator of age-related differences in muscle fatigue during repetitive exercise was supported 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. This might be due to a greater reliance on oxidative metabolism in children and consequently less accumulation of metabolic byproducts including lactate. It has also been suggested that differences in neuromuscular activation contribute to greater fatigue resistance in children and adolescents; 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. Finally, Moalla et al. (2006) reported that restrictions in blood flow to working tissue caused by isometric contraction are correlated with fatigue in 13 year old boys, an effect that would be greater in adults than children due to greater muscle mass in adult males and females. Any of these mechanisms, or a combination of several factors, could underlie the reported performance differences between children and adults during repeated high intensity exercise.

The differences in physiology that underlie sex differences in muscle fatigue in adults have been explored to a greater extent. Sex differences are predominantly peripheral in origin (Albert, et al., 2006; Hunter, et al., 2006; Kent-Braun, et al., 2002; Wust, et al.,

2008), and might 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), or differences in muscle blood flow during isometric contraction (Russ & Kent-Braun, 2003; Thompson, et al., 2007). Fundamentally, the hypothesised mechanisms for greater fatigue resistance in women compared with men are similar to the hypothesised mechanisms for greater fatigue resistance in children compared with adults.

The aim of the current study is to examine changes in muscle PCr, P_i, intracellular pH, [ADP], HbO_2, and HHb during fatiguing exercise in adolescent and adult males and females to test several proposed mediators of age and sex differences in fatigue. The hypotheses of this investigation are fourfold:

a. fatigue over 30 brief isometric quadriceps contractions will be greater in men than women and in adults than adolescents.
b. metabolic perturbation, reflected in the change in PCr, P_i, pH, and ADP, will be less in adolescents than adults, and less in women than men.
c. PCr recovery kinetics following exercise will be faster in adolescents than adults.
d. The change in HbO_2 with each contraction relative to the overall change in HbO_2 will be greater in adults compared with adolescents, and in men compared with women.

9.2 Methods

9.2.1 Participants

Fourteen healthy adolescents (7 boys, 13.5 ± 0.3 y, 1.58 ± 0.06 m, 50.7 ± 11.5 kg, -0.5 ± 0.5 YAPHV; 7 girls, 13.3 ± 0.4 y, 1.63 ± 0.08 m, 55.9 ± 17.3 kg, 1.2 ± 0.9 YAPHV) and 14 healthy adults (6 men, 29.0 ± 7.9 y, 1.73 ± 0.05 m, 73.7 ± 12.0 kg; 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. Individual data from each of these participants can be seen in Appendix D, and considerations related to sample size and statistical power are addressed in Appendix B. Written informed consent was obtained from adults, and from parents of young participants (Appendix C). Adolescent participants gave written assent to participate and were reminded each visit of their right

to withdraw from the study at any time. Participants visited the lab for familiarization with the ergometer within a mock MR scanner, and on a subsequent day, visited the MR scanner to take part in the experiment.

9.2.2 Ergometer and exercise test

Participants lay prone in a 1.5 T MR scanner (Philips GyroscanIntera) The right foot was secured 5-10 centimeters above the scanner bed, such that it did not touch the scanner bed when the participant briefly performed a maximal quadriceps contraction. The foot was supported by a padded foot cradle that was attached to a rope and pulley system (Chapter 3). A force transducer was embedded within the system. The participant's hips and legs were secured to the scanner bed with non-distensible straps. The test consisted of a 6 s quadriceps MVC followed by 6 s of rest. This cycle was repeated 30 times. Each contraction was verbally cued, and strong verbal encouragement was provided to participants throughout each contraction. Force was measured continuously and data were averaged to give 1 s resolution. Fatigue was defined as the relative decrease in force from the peak contraction (typically the first or second contraction) to the final contraction.

9.2.3 Near-infrared spectroscopy

A description of the NIRS instrument can be found in Section 3.5. As described there, HHb and HbO_2 data were collected continuously and interpolated to 1 s intervals. The maximum HHb (HHb_{max}) and the minimum HbO_2 (HbO_{2min}) over 3 contraction cycles (measured using a rolling 36 s window) was recorded, and this value was used to normalize the change in HHb and HbO_2 with each contraction. The MRT for the HHb response was estimated by fitting an exponential curve to the portion of the test over the subjectively identified exponential phase of the response.

9.2.4 Magnetic resonance spectroscopy

For the details of MR data collection, see Chapter 3. Recovery of PCr was fitted using a monoexponential equation (Equation 3.6)

9.2.5 Data analysis

All variables were tested for normal distribution using the Shapiro-Wilk statistic, and 2-by-2 factorial ANOVA was used to compare groups. Planned t-tests with Bonferroni correction was used for post-hoc testing. Significance was accepted at $p<0.05$.

9.3 Results

9.3.1 Force

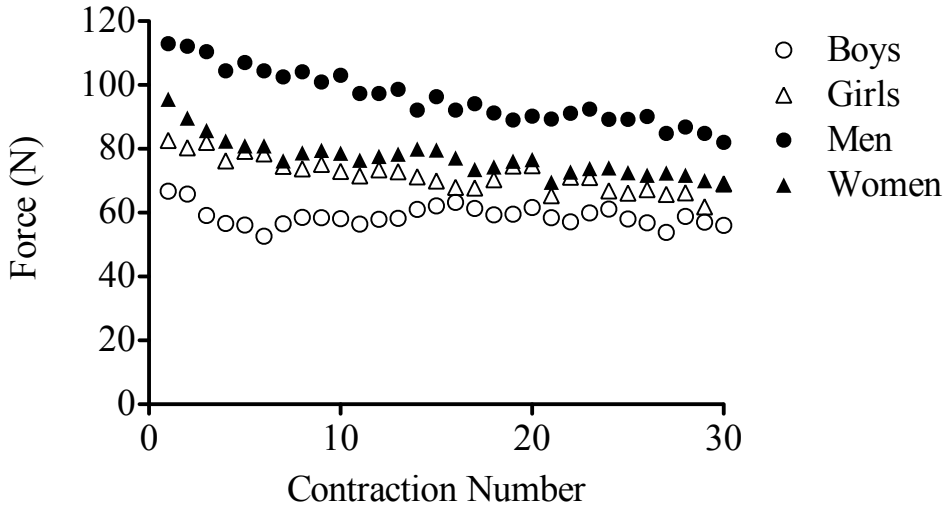

Figure 9.1. Force decreased over the test in all groups, with a greater absolute decrement in adults compared with adolescents, but similar relative fatigue.

Peak force was significantly greater in adults than adolescents ($p=0.003$), but no sex ($p=0.76$) or interaction ($p=0.14$) effects were identified (Figure 9.1). Force decreased by 19 ±11% in boys, 25 ± 10% in girls, 31 ± 6% in men, and 29 ± 14% in women, a significant decrease in all groups ($p<0.001$). There were no age ($p=0.08$), sex ($p=0.61$) or interaction effects ($p=0.35$). Force at the end of exercise was higher in adults than adolescents ($p=0.04$) but there were no sex ($p=0.62$) or interaction effects ($p=0.21$). The absolute decrease in force was significantly greater in adults than adolescents ($p=0.003$) but did not differ with sex ($p=0.76$) and there was no significant interaction effect ($p=0.14$).

9.3.2 Metabolic parameters

Metabolic parameters of the test can be seen in Figure 9.2. [PCr] (age: p=0.02, sex: p=0.51; interaction: p=0.10), [P_i] (age: p=0.03, sex: p=0.52; interaction: p=0.84), [ADP] (age: p=0.04, sex: p=0.77; interaction: p=0.08), and pH (age: p=0.05, sex: p=0.10; interaction: p=0.85), all changed to a greater extent in adults compared with adolescents.

No main age (p=0.40) or sex (p=0.83) effects were identified for the speed of PCr recovery following 6 min of exercise. PCr recovery τ was 39 ± 9 s in boys, 50 ± 13 s in girls, 53 ± 11 s in men, and 44 ± 16 s in women. A significant interaction was present (p=0.04) – recovery tended to be faster in adolescents among males and faster in adults among females. Post hoc t-tests using the Bonferroni correction did not reveal any significant differences between groups (boys/men: p=0.03, girls/women: p=0.19 boys/girls: p=0.03, men/women: p=0.25).

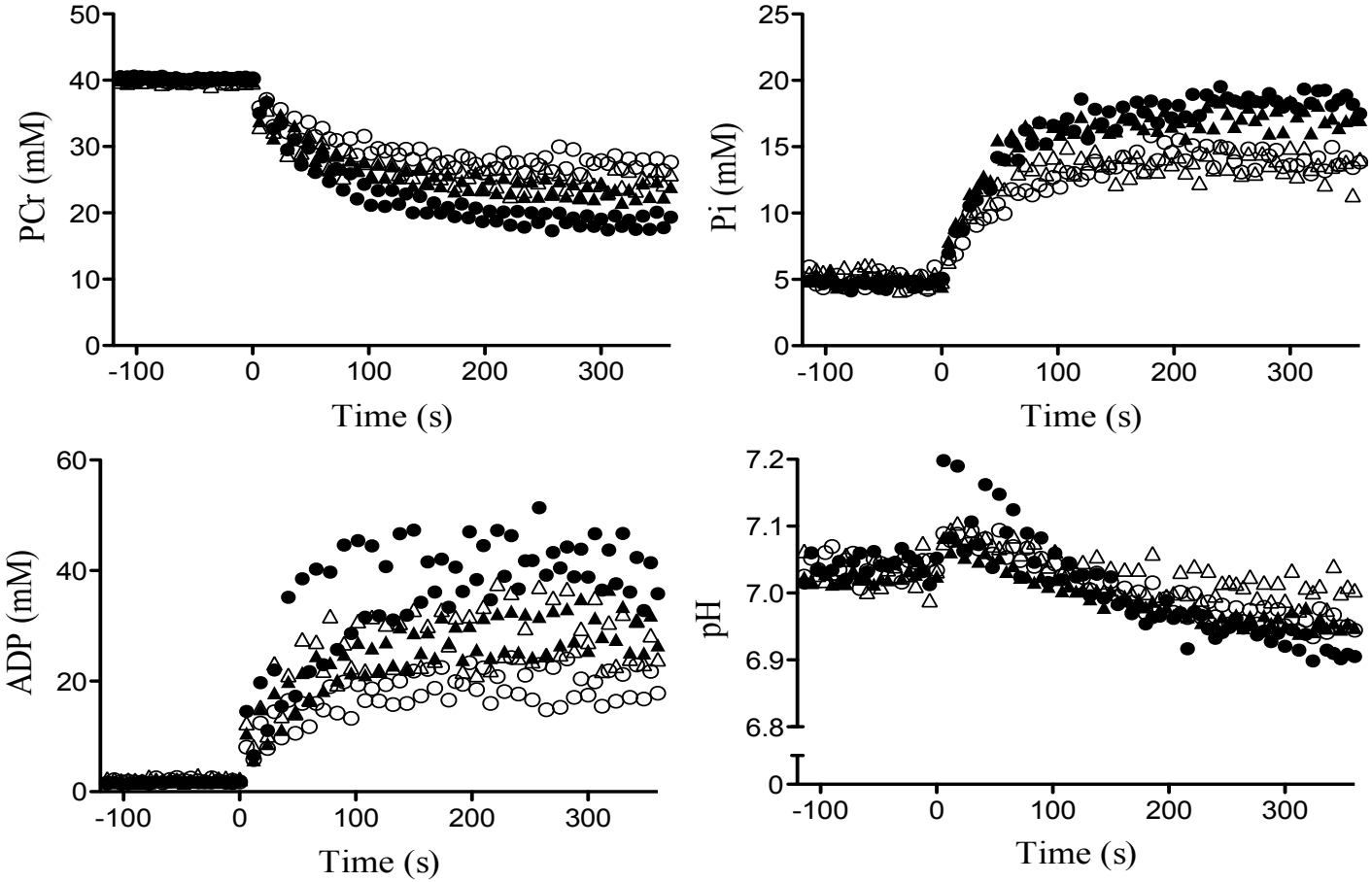

Figure 9.2.Metabolic parameters over 6 min of exercise in boys (○), girls (Δ), men (●), and women (▲); [PCr], [P_i], [ADP], and pH all changed more in adults than adolescents.

9.3.3 Muscle oxygenation

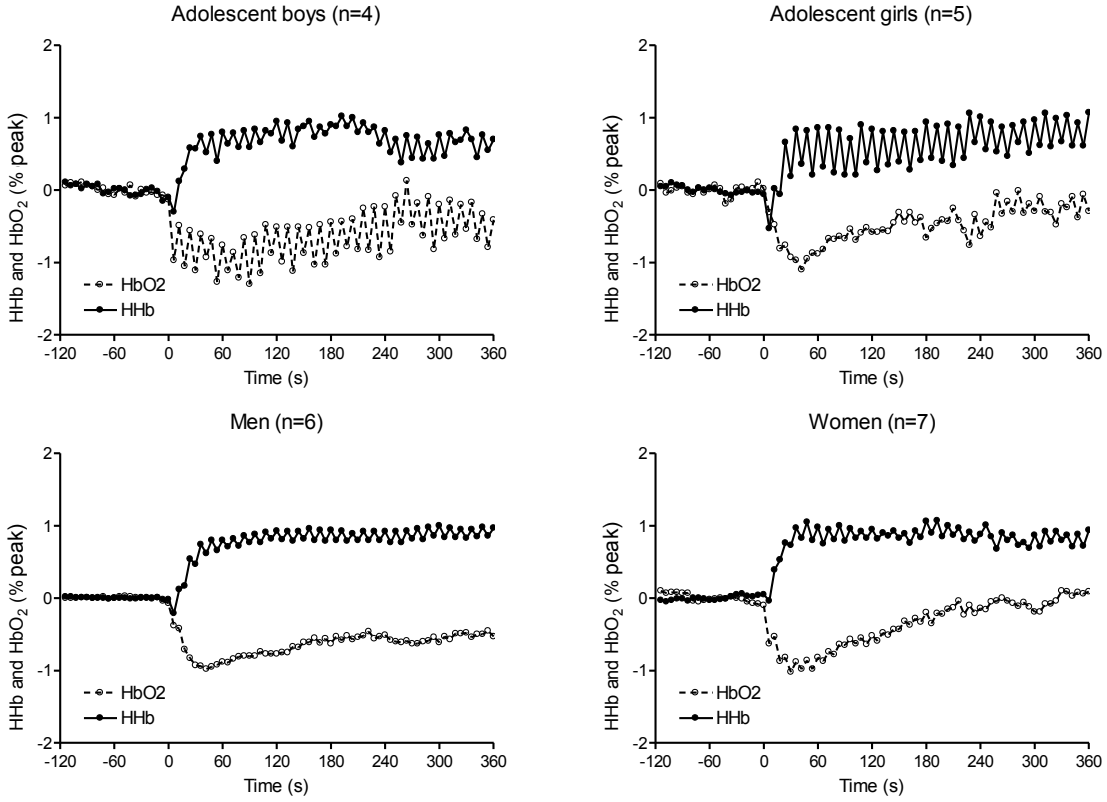

Figure 9.3. Muscle NIRS responses during 6 min of high intensity intermittent exercise.

NIRS data were only available for a subset of participants (4 boys, 5 girls, 6 men, and 7 women). The HHb and HbO_2 responses to intense exercises were similar in all groups (Figure 9.3) – the change in HHb with each contraction relative to HHb_{max} and the change in HbO_2 relative to HbO_{2min} did not differ with age (p=0.37 and p=0.15) or sex (p=0.62 and p=0.90), and there was no significant interaction effect (p=0.29 and p=0.53). The MRT for HHb was also similar in all groups (age: p=0.33, sex: p=0.20, interaction: p=0.32).

9.4 Discussion

The purpose of this study was to examine changes in muscle PCr, P_i, pH, [ADP], HHb, and HbO_2 during fatiguing exercise in adult and adolescent males and females. Over 6 min of repeated MVC, force decreased significantly in all groups, and the absolute decrease in force was greater in adults than adolescents. However, contrary to the

hypothesis, when fatigue was expressed relative to MVC, the decrease was not significantly different between adults and children, or between males and females. Thus, the first hypothesis was not accepted. The second hypothesis was that metabolic perturbation would be greater in males than females and greater in adults than adolescents. The data supported the first but not the second half of that hypothesis. Metabolic conditions over the exercise bout differed with age – a significantly greater decrease in PCr and pH and greater increase in P_i was evident in adults, and a significant age and interaction effect for the increase in [ADP] following exercise. PCr recovery kinetics following the test tended to be faster in boys than men but did not significantly differ in adolescent girls and women, so the third hypothesis was not accepted. The fourth hypothesis was not accepted. Fatigue was accompanied by similar changes in muscle oxygenation with contraction and a similar overall rate of adaptation of muscle oxygenation to the demands of exercise.

9.4.1 Muscle metabolism

A number of previous studies have reported that children and adolescents are more fatigue-resistant than adults. These studies have typically examined fatigue during multiple whole body sprints (Hebestreit, et al., 1993; Ratel, et al., 2002; Ratel, et al., 2004, 2006) or repetitive isokinetic contractions (Dipla, et al., 2009; Paraschos, et al., 2007; Zafeiridis, et al., 2005). This study is the first to examine age differences in muscle fatigue in children or adolescents during repetitive maximal voluntary isometric contractions. Given the interaction between task and individual in the development and Aetiology of fatigue, it is unsurprising that the degree of age difference 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 trend for greater fatigue in adults was identified ($p=0.08$) which warrants further investigation. Force decreased by 19 ±11% in boys, 25 ± 10% in girls, 31 ± 6% in men, and 29 ± 14% in women. 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 & Kent-Braun, 2003). The trend for greater fatigue in adults compared with adolescents might be caused by metabolic, muscle fibre, or neuromuscular factors.

This study investigated metabolic perturbation during fatiguing exercise. The change in PCr and P_i was significantly greater in adults than adolescents, but did not differ in females and males. The age difference in PCr and P_i after intense exercise is in keeping with previous work (Barker, et al.; 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 generating fatigue during high intensity exercise (Fitts, 2008). The mechanisms by which increased [P_i], decreased [PCr], or increased [ADP] might impair force production at the muscle are poorly understood. These compounds might act on the contractile mechanism to reduce force output (Fitts, 2008). 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 (Gandevia, 2001). The current work does not answer the question of the relative contribution of different pathways by which fatigue is mediated in adolescents and adults. However, these data suggest that metabolic differences might play a role in determining age differences in fatigue during repetitive high intensity exercise.

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 (Bogdanis, Nevill, Boobis, & Lakomy, 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), although contradictory reports exist (Barker, et al., 2008a; Kuno, et al., 1995). Methodological differences between these studies, specifically the inclusion or exclusion of female participants and the intensity of the preceding exercise bout, limit the interpretation of these results in the context of the current study. The current study did not reveal main effects for age or sex, but a significant interaction was found – PCr recovery τ was ~25% faster in boys than in men, but similar in girls and women. There are several possible explanations for this discrepancy. Differences in habitual physical activity between groups or differences in maturation might contribute to the effect seen, particularly in light of the significant relationship between maturation and PCr recovery τ described in Chapter 7. However, this interaction indicates that caution must be used in generalizing the results of investigations which have compared boys and men to groups which include girls and women, and that future research should include both males and females.

A number of previous studies have indicated that children experience reduced intracellular acidosis during or following intense exercise, compared with adults (Barker, et al.; Kuno, et al., 1995; Taylor, et al., 1997; Zanconato, et al., 1993). At the end of 6 min of exercise, intramuscular pH was slightly decreased from resting. This decrease was significantly different in adolescents and adults. Currently, acidosis is not believed to be an important fatigue mediator; P_i and ADP are more likely to be important in this process (Westerblad, et al., 2002). The current data do not provide evidence about the cellular mechanisms of muscle fatigue, and the possibility that differences in acidosis are important in muscle fatigue in children and adolescents remains.

9.4.2 Muscle oxygenation

The experimental hypotheses were not confirmed: there were no significant differences in muscle oxygenation or oxygen extraction measured using NIRS. Previous studies have hypothesized that both age and sex differences in fatigue might be, in part, due to greater contraction-induced ischaemia consequent to greater muscle mass in men (Thompson, et al., 2007). Thus, greater fluctuation in HbO_2 relative to the overall change in HbO_2 in adults compared with adolescents was expected, reflecting greater ischaemia with contraction and hyperaemia with relaxation. This was not the case. Changes in HHb during the test were likewise similar in all groups, indicating that muscle oxygenation is not likely to contribute to age differences in fatigue during repetitive intense isometric quadriceps contractions. The MRT for HHb at the onset of contractions did not differ with age or sex, indicating that the matching of oxygen delivery to utilization is similar in children and adults, and in males and females, during this type of exercise. Because fatigue was similar in men and women, little can be speculated regarding the causes of sex differences in muscle fatigue differences reported in other studies. However, during this type of exercise, oxygenation does not appear to vary with sex in adults, and does not appear to contribute to age differences in fatiguability.

The nature of the task deserves discussion. Typically, age differences in muscle fatigue are found during repetitive high intensity exercise where the recovery interval between bouts is between 15 and 300 s (Dipla, et al., 2009; Hebestreit, et al., 1993; Ratel, et al.,

2002; Ratel, et al., 2006; Zafeiridis, et al., 2005). During sustained tasks, children and adults are often found to be similarly fatiguable (Hatzikotoulas et al., 2009; Streckis, et al., 2007). The protocol used in this study is unique in the paediatric literature in its use of a brief recovery duration. The trend toward age differences in fatigue, the metabolic differences during exercise, and the trend for faster recovery kinetics in boys compared with men all provide evidence that the interaction between age, exercise, and muscle fatigue is complex, and further work is needed to elucidate the phenomenon of greater fatigue resistance in children and adolescents compared with adults.

There are, of course, several considerations in interpreting the results of this investigation, discussed further in Section 10.2, including the potential confounding effects of maturation and activity and is the potential for error to be introduced through the assumptions made in calculating [PCr], [P_i], and [ADP].

Overall, the results of this study support the assertions of previous investigators (Ratel, et al., 2002; Ratel, et al., 2004; Zafeiridis, et al., 2005) that age differences in muscle metabolism are present during repetitive intense exercise. 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, Vanhatalo, Fulford, & Jones; Kent-Braun, et al., 2002). 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 plays in 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 such as Duchenne muscular dystrophy.

In conclusion, this study examined fatigue during intermittent high intensity isometric quadriceps exercise in adolescents and adults, and found no significant age- or sex-

related differences in muscle fatigue. However, a strong trend for greater fatigue in adults was present. Significantly greater metabolic perturbation in the adults is likely to contribute to the trend for greater fatigue. In males, it is possible that metabolic recovery between contractions was important in reducing PCr depletion and P_i accumulation, while PCr recovery kinetics were somewhat slower in girls compared with women. Muscle oxygenation was examined, with the hypothesis that greater muscle mass in adults compared with adolescents and in women compared with men would affect oxygen delivery to the muscle during contraction, but no age or sex differences in oxygenation or oxygen extraction were found. The results of this study suggest that central mechanisms might mediate fatigue differences in young people and adults, since metabolic differences were present but did not lead to differences in fatigability. Further work is required to elucidate the relative importance of central and peripheral mechanisms of fatigue in children and adolescents.

10 Discussion and Conclusions

10.1 Overview

This thesis has described a series of studies designed to elucidate the influence of age and sex on muscle metabolism and oxygenation during high intensity exercise and recovery. As discussed in Chapter 2, research into the nature and causes of age differences in muscle metabolism during exercise is sparse and equivocal. Overall, this literature suggests that children and adolescents preferentially utilise aerobic pathways over anaerobic pathways during exercise, particularly during high intensity exercise. The current set of studies has suggested that this is true only during very high intensity exercise, although it is likely that these conclusions are specific to the mode of exercise and age of the participants.

Chapter 4 described heavy intensity exercise, and the hypotheses that adolescents would display faster fundamental PCr kinetics and a reduced PCr slow component were not confirmed. However, the HHb MRT was significantly faster in adolescents compared with adults. This work suggests that metabolic control during heavy intensity exercise might be similar in adults and adolescents. In keeping with this are the findings of Barker et al. (2008a), who found that PCr kinetics in the moderate domain do not differ in children and adults.

In Chapter 5, the PCr kinetic response to heavy and very heavy exercise in men and adolescent boys was examined. Considerable changes in the PCr kinetic response (slowed fundamental τ and increased A_{SC}) were evident between heavy and very heavy exercise in men. However, the fundamental τ and A_{SC} did not differ between heavy and very heavy intensity exercise in boys. Thus, a significant interaction between age and intensity was observed for PCr kinetics, where age differences were more pronounced during very heavy compared with heavy intensity exercise. HHb kinetics was not affected by intensity, although the MRT was faster in boys than men at both intensities. This study supports the hypothesis that age differences in muscle metabolism are more pronounced at high exercise intensities, possibly due to age differences in the ability to utilise glycolytic pathways or recruit type II muscle fibres.

The effect of age differences in muscle fibre recruitment was investigated in Chapter 6 through the use of a priming intervention. Prior exercise resulted in a reduced A_{SC} in men but not boys, suggesting that muscle fibre recruitment might be more malleable by intervention in men. This can be interpreted as evidence that adolescents are limited in their ability to recruit higher order muscle fibres, although other contributors to the priming response, including the role of muscle oxygenation, must be considered. Metabolic recovery between bouts of exercise was faster in boys than in men.

To investigate the possibility that age differences in PCr recovery contribute to age differences in the muscle metabolic response to exercise, PCr recovery data from all of the studies in the thesis (except the gated exercise protocol reported in Chapter 8) was pooled in Chapter 7. This allowed a large-scale analysis of possible mediators of PCr recovery kinetics across a variety of exercise protocols. This chapter revealed that $[PCr]_{EE}$ and pH_{EE} are both significantly correlated with PCr recovery τ across all adolescents and adults studied in this thesis. However, the correlation is very weak, possibly due to the limited range of $[PCr]_{EE}$ and pH_{EE} values observed in these studies. In adolescents, no significant correlation was found between $[PCr]_{EE}$, pH_{EE}, or age. However, maturity was significantly associated with PCr recovery τ. The overall results of this study support the hypothesis that PCr recovery kinetics change with maturity, and indicate that further research is required to elucidate these differences.

To facilitate this further research, an alternate method of measuring PCr recovery kinetics, gated exercise, was developed in Chapter 8. This method involves averaging PCr recovery over a number of brief contraction and recovery cycles, and allows the calculation of PCr recovery kinetics without strenuous or prolonged exercise. In Chapter 9, PCr recovery τ measured using gated exercise was compared with PCr recovery τ measured following a sustained contraction. These values compared well in most participants, but in two participants, it was not possible to measure τ using gated exercise, and in a further two participants, there was a large difference between gated τ and sustained τ, leading to wide 95 %CI for the two estimates of τ. Further developmental work is necessary to overcome these problems, but the gated protocol is theoretically promising in young people, and this chapter shows that it is a feasible protocol in adolescents.

Finally, in Chapter 10, the impact of muscle metabolism and recovery on exercise tolerance during high intensity repetitive isometric exercise was examined in adolescents and adults. During 6 min of repeated isometric MVC, adults experienced greater metabolic perturbation (greater decrease in [PCr] and pH and greater increase in [ADP] and [P_i]). However, the decrease in MVC over 6 min was not significantly different in adolescent and adults. PCr recovery tended to be faster in adolescent boys than adult men, but not in adolescent girls compared with adult women. No age or sex differences in muscle oxygenation were present during this exercise test. Overall, the results of this investigation do not support previous studies that have found that children and adolescents are less susceptible to muscle fatigue than adults during repetitive high intensity exercise (Dipla et al., 2009; Ratel et al., 2002; Zafeiridis et al., 2005). This can be interpreted as evidence that age differences in muscle fatigue are not directly mediated by muscle metabolism, since the current study found differences in metabolism in the absence of differences in fatigue. As suggested by Streckis et al. (2007), central factors might also play a role in determining age differences in muscle fatigue.

Several overall conclusions can be drawn from the studies in this thesis. First, age differences in PCr kinetics and in metabolic perturbation are present during intense exercise, but not during exercise at 20 %Δ, which is slightly above the intracellular threshold for P_i/PCr. This supports a limited glycolytic capacity, or an enhanced oxidative capacity, in adolescents. Second, PCr recovery kinetics appears to be dependent on both age and sex, with adolescent boys displaying faster kinetics than adult men, but women and girls displaying similar recovery kinetics. This effect might be mediated by maturation. Finally, during repeated, brief MVC exercise, age differences in fatigue are not significant despite significant age differences in metabolic perturbation, confirming that muscle fatigue is a complex, task dependent phenomenon.

The significant age differences in muscle metabolic variables during fatiguing intermittent isometric exercise and during very heavy intensity exercise support an increased reliance on oxidative pathways and a decreased reliance on anaerobic pathways in adolescents compared with adults. Faster recovery kinetics in men compared with boys supports a difference in the oxidative capacity of fibres recruited during exercise, with oxidative capacity being higher in boys than men. A majority of

previous paediatric exercise physiology has been restricted to male participants, so the inclusion of adolescent and adult females in the current work is both important and revealing. Females reach physical maturity earlier than males, and this was reflected in the similarities between adolescent females and adult females in the studies reported in this thesis. It is important to consider both the possibility that women and girls are more similar in their muscle metabolism than men and boys throughout development, and the possibility that girls and women differ during childhood but become more similar during puberty, such that the 13 year old girls who participated in these studies were already very close to adult in their metabolic responses to exercise. The interpretation of these data is complicated by the pubertal status of the participants, but it is important to understand not only how prepubertal participants respond to exercise, but how and when the response becomes more adult in nature.

If the male data is considered separately from the female data to simplify interpretation and to facilitate comparison with many previous studies, the results of this work clearly indicate that boys do not rely on anaerobic pathways to the same extent as men. However, the mechanistic basis for this effect is unclear. It is possible that anaerobic pathway enzymes are immature in this population. Certainly, the biopsy studies of Berg et al. (1986), Eriksson et al. (1974) and Kaczor et al. (2005), which showed that adolescents have lower levels of some anaerobic pathway enzymes compared with adults, support this hypothesis. Alternately, aerobic capability in boys might be greater than in men, through a maturational effect on the myocyte or through the effects of habitual physical activity. Finally, the differences seen might arise not in the intrinsic properties of the myocytes, but in how they are used during exercise. Specifically, these differences might arise from a proportionally greater recruitment of type I muscle fibres in adolescent boys compared with adults.

No direct evidence about muscle fibre type or muscle fibre recruitment in this population is available. However, age differences in muscle fibre recruitment have been proposed by several investigators (Falk & Dotan, 2006; Falk, et al., 2009; Halin, et al., 2003). Furthermore, several researchers have shown age differences in muscle fibre type in the deltoid or vastus lateralis muscle (Glenmark, et al., 1992; Jansson, 1996; Lexell, et al., 1992; Mandroukas, et al., 2010; Metaxas, et al., 2010). Differences in active muscle fibres during exercise, whether that difference is due to neurology or

muscle morphology, would explain the key findings of this thesis. Similar PCr kinetics in adolescents and adults during heavy intensity exercise exercise might reflect a similar recruited muscle fibre pool. That is, the intensity of exercise might not be high enough to obligate the progressive recruitment of a large proportion of higher order fibres during 7 minutes of exercise in adults or adolescents, so age differences in muscle fibre type or recruitment are not apparent. By contrast, during very heavy intensity exercise, adults can be presumed to be recruiting a large number of higher order muscle fibres to meet the demands of the exercise (Vollestad & Blom, 1985). In boys, the metabolic profile did not change from heavy intensity to very heavy intensity exercise, possibly because the pool of fibres available for recruitment was more homogeneous. A shift in the muscle fibre recruitment profile has been implicated in the priming effect (Jones, et al., 2008a). If this is a predominant mechanism for the priming effect and if boys do not have a broad pool of higher order fibres available for recruitment, the absence of a change in kinetics following priming exercise in adolescent boys described in this thesis would be explained. Faster PCr recovery kinetics in muscles with a higher proportion of type I muscle fibres have been described (McDonough, Behnke, Musch, & Poole, 2004). In this thesis, PCr recovery kinetics were significantly different in men and boys, but not in women and girls. The influence of maturation on PCr recovery kinetics was documented in Chapter 7; a more mature muscle fibre recruitment pattern in girls might underlie the interaction between sex and age found for PCr recovery kinetics overall. Finally, during fatiguing exercise, metabolic perturbation was greater in adults than children, and a nonsignificant trend for greater fatigue was present. These findings might be explained by a greater dependence on type II muscle fibres in adults (McDonough, Behnke, Padilla, Musch, & Poole, 2005).

Another possible explanation for the metabolic differences between adolescents and adults is a difference in the ability to transport and utilise oxygen at the mitochondrion. NIRS was used to investigate this possibility during high intensity exercise in this set of studies. NIRS results in this thesis were equivocal; there were some differences in HHb kinetics and TOI with age, but the magnitude and duration of these effects was not large, and was sometimes not accompanied by metabolic differences. Specifically, the MRT for HHb was faster in adolescents compared with adults during both heavy and very heavy intensity exercise, indicating that a mismatch between oxygen delivery and oxygen utilisation occurred earlier in young people than in adults. The difference in

MRT was 6 s in males and 5 s in females during heavy intensity exercise, and 9 s in males during very heavy intensity exercise. PCr kinetics tended to be faster, rather than slower, in young people, suggesting that this earlier imbalance did not lead to slowed PCr kinetics. NIRS captures oxygenation in the small vessels of the section of muscle under the probe, but does not take into account factors such as intracellular oxygen transport or heterogeneity of oxygen delivery within the muscle. It is possible that these factors differ with age, but these possible determinents of mitochondrial oxygen supply were not investigated in this set of studies.

Overall, these studies support both neural and myocellular differences between adolescents and adults, but do not support differences in muscle oxygenation as a primary difference between adolescents and adults during exercise. Future research should focus on manipulating muscle fibre recruitment and muscle metabolism to clearly elucidate the effect of each on exercise performance in young people.

10.2 Limitations and methodological considerations

It is important to acknowledge the limitations in this work. These include the maturational state of the participants, the differences in physical activity level in adolescents and adults, the small sample size, assumptions made in the calculation of [PCr], [P_i], and [ADP], limitations in NIRS technology, and issues related to motivation in young people.

10.2.1 Maturation

All of the young people who participated in this set of studies were adolescents, and were experiencing the physical and hormonal changes accompanying puberty. In humans, particularly males, a number of changes in muscle size and morphology are associated with puberty, and there is some suggestion that hormones may affect muscle function (Cooper & Barstow, 1996). Chapter 7 provides evidence that maturation in young people is correlated with increasing PCr recovery τ, and increasing testosterone during puberty has been associated with changes in muscle physiology (Hansen, Bangsbo, Twisk, & Klausen, 1999). Thus, it is likely that the maturity of the participants affected the results of these investigations in several ways. First, girls were more mature than the boys in each study which included a sex comparison, despite the groups being very similar in age. Thus, a more child-like response would be expected in

boys compared with girls; maturity, rather than sex differences, might explain differences in PCr kinetics and in fatigue described in Chapter 4 and Chapter 9. Second, hormonal changes during puberty differ with sex, such that adult men have greater muscle mass than women (Frontera, Hughes, Lutz, & Evans, 1991). Thus, the differences between boys and men were likely larger than the differences between girls and women as a result of hormonally mediated changes in muscle physiology in men. Finally, the maturational status of the participants in these investigations might obscure differences in muscle metabolism that exist between prepubertal children and adults – future work should include younger children. Although these limitations are important to acknowledge, the maturational status of the participants is a strength of the current work as well as a weakness; in understanding the development of muscle metabolism, it is important to understand how metabolic variables vary with age during puberty as well as understanding the state of the muscle during childhood and during adulthood. Furthermore, some of the exercise tests, particularly those described in Chapter 6 and Chapter 9, required both determination and coordination on the part of the participants. Peak height velocity typically occurs around 12 years in girls and 14 years in boys, but there is a great deal of variation in the age at peak height velocity (Malina, Bouchard, & Bar-Or, 2004). There are undoubtedly a number of prepubertal children who would cope very well with these difficult tests, but there are also a number of children in whom compliance would be poor due to their young age. Thus, for financial and practical reasons, studying adolescents was deemed to be appropriate for these studies. With these protocols established in an older population, expanding the work to include younger children is possible in the future.

10.2.2 Physical activity

Physical activity patterns and levels are known to differ with age and sex (Lodi et al., 1999; Ness et al., 2007; Sallis, Prochaska, & Taylor, 2000), and training is a strong determinant of PCr kinetics and the metabolic response to exercise (Crowther, et al., 2002a; Jones, et al., 2007). It is possible that higher physical activity levels in the young people in the current set of studies played a role in the results of these investigations. Efforts were made to minimise this effect; in each study, children who enjoyed physical activity but did not compete at a high level in any sport were recruited. Adults were recruited on the same basis. Participants reported recreational activities including soccer, dancing, jogging, and martial arts. However, it is likely that there was some bias

in both the paediatric and adult samples – that is, individuals who enjoy activity are more likely to volunteer for an exercise study than individuals who do not. The young participants in the studies reported a wide range of leisure-time physical activity – overall, the boys who completed very heavy intensity exercise (Chapter 5 and 6) reported playing outside during most of their leisure time, while several of the female participants who completed repeated high intensity isometric exercise reported primarily sedentary behaviour before and after school. In future work, quantification of physical activity using accelerometry, as investigators have done when investigating muscle metabolism in young and elderly adults (Kent-Braun, et al., 2002), is advisable.

10.2.3 Sample size

The number of participants in each study was not large; group sizes ranged from 5 to 8 participants of a given age and sex. These samples were determined with a priori power calculations (Appendix B), although a lack of comparable prior literature reduced the accuracy with which the population standard deviation could be estimated. This might have resulted in an insufficient sample to detect population differences between boys and men during heavy intensity exercise. In these groups, there was a 30 % slowing of the PCr τ in men compared with boys that did not achieve statistical significance. This was partly due to significant variation within groups. Due to the small group size, the representativeness of the sample and thus the generalisability of the research must be questioned. However, with the cost of MR scanning (see Chapter 2), very large samples for this type of work are unrealistic at the current time.

10.2.4 Assumptions in calculating [PCr], [Pi], and [ADP]

Changes in PCr were calculated assuming resting [ATP] was similar in children and adults and in males and females. Very little research has been performed on resting [ATP] in children, but biopsy data from the vastus lateralis of 33 adolescent boys (Eriksson & Saltin, 1974) and calibrated ^{31}P-MRS data from the calf muscles of 13 male children and adolescents (Gariod, 1994) indicates that resting [ATP] is likely to be similar in children and adolescents and adults. The magnitude and impact of any systematic differences, especially in the peripubertal participants in these studies is unknown. PCr was calculated with the assumption that ATP content in paediatric and adult muscle was a constant 8.2 mM, and thus the differences in [PCr] during rest and exercise must be interpreted with caution. The calculation of [PCr], [Pi], and [ADP] also

depended on the assumption that the sum of the unsaturated P_i and PCr peaks is equivalent to the concentration of total creatine in the region of interest.

10.2.5 NIRS

The limitations of spatially-resolved near infrared spectroscopy are well-known and discussed in the literature (Ferrari, et al., 2004). First, it is not possible to monitor absolute changes in HHb without a known differential path length factor. The time course of the response can still be discussed with confidence, but quantification of the initial decrease and the change in HHb following the exponential phase relative to the amplitude of the exponential phase relies on the assumption that the initial phase is similar in all individuals. This is likely not the case, and thus these quantifications are only a very rough way to discuss differences between groups. A critical assumption of NIRS is that the majority of the signal comes from haemoglobin rather than myoglobin (Mancini et al., 1994).

The principal challenge of discussing HHb kinetics is the fact that these results offer only a depiction of the balance between oxygen delivery and oxygen utilisation, and do not give any reflection of which of these factors is responsible for the bulk of the changes. This is unavoidable with the technique, and does not eliminate the value of this non-invasive measure of muscle oxygenation. However, the possibility of delivery or utilisation driving the response seen must be recognized. In all probability, both delivery and utilisation are involved in each phase of HHb kinetics. Finally, considerable regional variation in HHb kinetics has been described (Koga, et al., 2007)which is unaccounted for in the studies described in this thesis.

10.2.6 Compliance with exercise protocols

In conducting the studies reported in this thesis, participants were assumed to be giving their full effort during each test, and to be complying with experimental protocols to the best of their ability. Research has suggested that children and adolescents do not differ in their exercise abilities when exercise intensity is appropriately scaled to body size (Barker, et al., 2010b; Tonson, et al., 2008). This could be construed to imply that children are capable of exerting maximal effort to the same extent as adults. In practical terms, compliance was encouraged in a number of ways – these considerations were applied to both children and adults. First, thorough habituation was carried out; this was

important in ensuring that participants understood and could comply with the exercise task and also allowed their performance on the test within the MR scanner to be evaluated against previously determined abilities. Second, participants were reminded prior to each test that they were welcome to withdraw from the study with no penalty. Paediatric participants were assured that no one would be upset at all if they chose not to complete the test, even if they stopped in the middle of the exercise, but that they needed to give full effort throughout the test as long as they chose to participate in the study. Finally, data were examined following the test to ensure compliance; this was particularly important for repeated maximal isometric contractions because performance changed throughout the test.

10.3 Implications of this work

There are many novel aspects to the current research that considerably expand the literature surrounding muscle metabolism in children and adolescents. This is the first time that PCr kinetics during heavy intensity exercise have been reported, the first investigation of muscle metabolic parameters during fatiguing exercise in young people, and the first set of studies to combine NIRS with ^{31}P-MRS in young people. The investigation of factors affecting PCr recovery kinetics in adolescent participants (Chapter 7) is novel, as is the use of a gated exercise protocol in young people (Chapter 8). ^{31}P-MRS research lacks generalisability to sporting activities or activities of daily living, due to the constraints on the nature of the exercise that can be imposed in the magnet. However, this research indicates that male adolescents are less fatigable compared with adults and that recovery is faster in young compared with adult males. This effect appears to be predominantly due to metabolic factors. Thus, in training young athletes, shorter recovery durations might be appropriate. These studies have not, however, addressed any issues related to training in young people. Basic scientific inquiry, rather than applied research, was the primary purpose of this investigation, so the principal impact of this work is for researchers investigating both healthy children and adolescents and children and adolescents with diseases affecting muscle function.

Research can build from this work in a number of ways, detailed in Section 10.4. There are also several ways that this research can be extended to topics with considerable clinical impact. These include the use of PCr recovery kinetics as a predictor of insulin resistance (Fleischman, et al., 2009) and the use of tests developed here to understand

how muscle function is altered in diseases such as muscular dystrophy, cystic fibrosis, and type I diabetes.

10.4 Future work

There are many potential areas for further research to develop from this thesis. These areas fall into 3 basic categories: investigation of the mechanistic basis for the phenomena described in this thesis; elucidation of the age, sex, and maturation related patterns described; and extension of the work described in this thesis, both to larger samples and to studies designed to test the phenomena described here.

In the first category, there is a great deal of further work that is possible. A study of the PCr response to exercise at and above the CP would clarify the possible age differences in muscle metabolism during very heavy intensity exercise. Studies which both control (using accelerometry) and manipulate (via training) physical activity to understand how training interacts with age in young people are important to conduct to understand how muscle metabolism is affected by physical activity in young people. Manipulation of muscle fibre recruitment through contraction frequency (Crowther & Gronka, 2002) might offer insight into the question of whether neuromuscular maturation affects fibre recruitment during exercise in young people. Chapter 4 suggested that muscle efficiency or economy might differ with age or sex, which is important to investigate further. Finally, muscle fatigue is important to understand, given the implications for both basic science and real-world applications such as training young athletes or preserving functional ability in children with diseases affecting muscle function. Studies in this area should focus on the interaction between the exercise task, including the type, intensity, and duration of the contraction and the recovery time available to participants, and the age and sex of participants.

In elucidating the development of muscle metabolism with age and maturity, longitudinal studies offer an optimal way to investigate the problem. However, these studies can be difficult to implement – they require time, money, commitment from participants, and measures sensitive enough to detect small changes with growth and maturation. As an alternative to longitudinal studies, investigation of children and adolescents representing a greater range of age and maturity than described in this thesis will develop a broader picture of the development of muscle function. The feasibility of

these studies will depend on the availability of sensitive and reliable tests that are possible for young participants. The work of Fleischman et al. (2010) represents an important step in this direction, given the broad age range and large sample, but these authors have chosen an inappropriate and poorly described exercise task, limiting the conclusions that can be drawn from their work. Chapter 5 described an initial attempt to describe exercise intensity-dependent age differences in muscle metabolism, but a more robust study to describe PCr kinetics in the same individuals during moderate, heavy, and very heavy exercise is warranted.

The development of mitochondrial capacity in healthy and unhealthy children and adolescents represents a promising area for future research. However, measurement of this variable is complex, as described in Chapters 7 and 8. Thus, further work to develop measurement protocols is warranted. Another important area for research is determination of resting [ATP], [P_i], and [PCr] in children and adolescents; calibrated ^{31}P-MRS represents a non-invasive method of doing so. This technique involves using an external reference phosphorus source with a known concentration to normalise the biological phosphates present in muscle (Kemp, et al., 2007). As well, muscle oxygenation and blood flow is poorly understood in a paediatric population, largely due to methodological limitations. Blood oxygenation level dependent MR imaging might offer a means to further investigate the role of muscle oxygenation in determining the metabolic profile in young people, particularly in combination with thoughtful application of NIRS techniques. Finally, the role of muscle fibre recruitment could be investigated through studies that involve the careful use of stimulated contractions, although these studies may be challenging in a paediatric population.

This thesis consists of a number of small preliminary studies, which provide novel evidence that age differences in muscle metabolism depend on exercise intensity. However, further evidence is required to confirm these findings. Follow-up studies should use larger sample sizes, and should consider attempting to recruit more homogeneous groups of participants. This could include selecting groups of young people matched for both age and maturity (for instance, comparing early maturing boys with late maturing girls) or matching adult and adolescent groups for habitual physical activity using accelerometry. Investigating a muscle group, such as the forearm flexors,

that aren't fundamental to walking and running, could also reduce the effects of habitual activity.

10.5 Final conclusions

In conclusion, the data reported in this thesis support the hypothesis that metabolic differences between adults and adolescents are present during exercise. This thesis provides evidence that age differences in muscle metabolism increase with increasing exercise intensity. A reduced ability to recruit type II muscle fibres in young people is a compelling possible explanation for these differences, but this hypothesis is difficult to test directly. This thesis also reports that PCr recovery kinetics are faster in boys than men, but not in adolescent girls compared with women. This difference might be mediated by maturation, which was greater in girls than boys in this set of studies and which is significantly correlated with PCr recovery kinetics.

The studies reported in this thesis also lay a foundation for further investigation of the metabolic response to high intensity exercise using noninvasive techniques such as ^{31}P-MRS and NIRS. As technology continually improves, techniques such as arterial spin labelled imaging to measure muscle perfusion, ^{1}H spectroscopy to measure total creatine, and ^{13}C spectroscopy to measure muscle glycogen are also likely to be important in determining the control and nature of the metabolic response to exercise in young people. The exercise protocols described and developed here are well-suited to some of these modalities. There is also a great deal of scope for further ^{31}P-MRS research to understand how the muscles of children and adolescents respond to high intensity exercise. The research in this thesis, along with the work that will extend and expand it, will be invaluable in understanding normal paediatric exercise physiology, along with alterations in physiology related to training, sedentary lifestyles, and disease.

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Appendix A – Papers and presentations

The work contained in this thesis has been communicated to and recognised by the scientific community in the following ways:

Journal articles

Willcocks, RJ, Williams, CA, Barker, AR, Fulford, J, & Armstrong N. (2010). Age- and sex- related differences in muscle phosphocreatine and oxygenation kinetics during high-intensity exercise in adolescents and adults. *NMR in Biomedicine, 23(6),* 569-577

Willcocks, R.J., Fulford, J., Barker, A., & Williams, C.A. Measurement of mitochondrial capacity using gated exercise in women and adolescent girls: A ^{31}P-MRS study. *Under revision, Muscle & Nerve*

Willcocks, R.J., Fulford, J., Barker, A., & Williams, C.A. The influence of age and sex on muscle oxygenation and metabolism during fatiguing exercise.*In preparation*

Williams, C.A., Willcocks, R.J., Barker, A.R., Fulford, J., & Armstrong, N. Muscle phosphocreatine kinetics in adolescents and adults during recovery from high intensity exercise.*In preparation*

Conference presentations

Willcocks, R.J., Fulford, J., Barker, A.R., & Williams, C.A. (2010) Metabolic and oxygenation parameters associated with muscle fatigue in adolescent boys and men. Presented at 2010 Annual Congress of the European College of Sport Science in Antalya, Turkey, received Young Investigator Award

Willcocks, R.J., Fulford, J., Barker, A.R., Williams, C.A. (2009). Recovery from brief isometric calf exercise in young and adult females measured using ^{31}P-MRS. Presented at 2009 Paediatric Work Physiology Meeting in Lille, France.

Barker, A.R., Willcocks, R. J., Doust, J.H., & Williams, C.A. (2009). Advances in paediatric exercise science: Lessons from the past. BASES fellow symposium presented

at the British Association of Sport and Exercise Sciences Annual Conference in Leeds, UK.

Williams, C.A., Willcocks, R.J., Barker, A.R., Fulford, J., Armstrong, N. (2009). Muscle phosphocreatine kinetics in children and adults during high-intensity exercise. Presented by Dr. Williams at 2009 ACSM Annual Meeting in Seattle, WA, USA.

Willcocks, R.J., Barker, A.R., Fulford, J., Welford, D., Welsman, J.R., Armstrong, N., & Williams, C.A. (2008). Kinetics of phosphocreatine and deoxyhaemoglobin in children and adults during high-intensity exercise. Presented at 2008 ACSM Annual Meeting in Indianapolis, IN, USA.

Williams, C.A., Willcocks, R.J., Barker, A.R., Fulford, J., Welford, D., Welsman, J.R., & Armstrong, N. (2008). Recovery of muscle oxygenation and phosphocreatine in children and adults following high-intensity quadriceps exercise. Presented by Dr. Williams at 2008 ACSM Annual Meeting in Indianapolis, IN, USA.

Williams, C.A., Willcocks, R.J., Barker, A.R. (2008). Magnetic resonance spectroscopy.Session in BASES Workshop on Advanced Techniques in Paediatric Exercise Science.

Book chapters

Willcocks, RJ, Fulford, J, Barker, AR, & Williams, CA. (2010). Recovery from brief isometric calf exercise in young and adult females measured with ^{31}P-MRS. In: Baquet, G & Berthoin, S. *Children and Exercise XXV, p 155-158.*

Prizes

Young Investigator Award, European College of Sport Science Annual Meeting 2010 (Joint 5thin poster competition)

Appendix B – Sample size and statistical power

In planning the studies described in this document, *a priori* power calculations were carried out to determine the minimum sample size required to address the hypothesis. These calculations necessarily involve the use of estimates of mean differences and standard deviations over the groups. *Post hoc* inspection of experimental data revealed greater variability than expected. Thus, some investigation of the power of the analyses to detect meaningful events was warranted. The mathematical problems with *post hoc* power analysis are well known (Hoenig & Heisey, 2001). 95 % CI for the difference between groups and effect sizes can be calculated to examine the magnitude of the difference between groups in more detail than that provided through statistical hypothesis testing alone.

1. PCr kinetics during heavy intensity exercise

No a priori power calculations are available for this study; it was the first study conducted and the importance of a prior power analysis was not yet appreciated by the student. Table B1 shows the effect size and 95 %CI for the age comparison and for the sex comparison for selected variables. In cases where statistical significance was attained for a comparison, large effect sizes are also present, and the 95 %CI exclude 0. This indicates that type I error was unlikely for these comparions.

2. PCr kinetics during repeated bouts of very heavy exercise in boys and men

A priori power calculations suggested that seven participants in each group would provide sufficient statistical power to detect a 10 second difference in the PCr MRT. Eight boys were recruited, but two failed to complete the testing. Statistical significance was obtained with six, instead of seven, participants in each group. To avoid wasting money and scanner time, no further participants were recruited. See Table B1 for post hoc analyses, which confirmed the predominantly significant findings of this study.

A sex comparison was planned for this study, but limitations on available MR scanner time required that the age comparison be prioritised; thus, recruitment of female participants ceased after two girls had completed the protocol.

3. Measurement of PCr recovery kinetics using gated exercise in girls and women

A priori power calculations for this exploratory pilot study were conducted, to determine the number of participants required to detect a 5 s difference in the PCr recovery time constant between adults and adolescents. However, the data revealed that the mean recovery τ did not differ between girls and women, so power to detect that difference is not meaningful. Because there was no mean difference, these data are not included in Table B1.

It could be argued that the study did not include sufficient participants to completely demonstrate the usefulness of the gated protocol. However, the study was intended to be pilot work, and it was considered more important to devote further magnet time to investigate hypotheses related to age and sex differences in muscle metabolism than to continue to perfect this technique, in light of the high cost of scanning and the overall aim of this thesis.

4. Muscle metabolism during repeated quadriceps MVCs in children and adults

PCr_{EE} was deemed the most important variable in which to detect significant differences. A significant age difference in PCr_{EE} was detected, although poor compliance in one boy and under recruitment among girls and men resulted in a sample size of 7 participants in each of these groups rather than the desired 8.

Table B1 shows that power was acceptable for metabolic variables but possibly low for force variables; future studies with fatigue as an outcome measure should focus on powering the study to detect these differences rather than the accompanying metabolic changes. The medium-to-large effect size for the age comparison for fatigue (d=0.76) provides evidence that meaningful differences might be present that are not detected statistically.

Table B1. Effect size and 95% CI of the difference for key comparisons examined in Chapters 4, 5, 6, and 9.

	Age d=	Age 95 % CI lower and upper bound		Sex d=	Sex 95 % CI lower and upper bound	
Heavy intensity exercise						
PCr_{EE} (%BL)	0.47	-5	17	0.89	0	21
τ (s)	0.45	-21	8	0.53	-6	21
SC (%EE)	0.26	-13	22	0.79	-31	5
HHb MRT (s)	1.35	-14	-2	0.17	-7	9
Very heavy intensity exercise						
pH_{EE1}	2.50	0.06	0.27			
PCr_{EE1} (%BL)	1.98	4	38			
pH_{EE2}	2.48	0.04	0.17			
PCr_{EE2} (%BL)	1.51	-2	46			
PCr MRT_1 (s)	2.59	-121	-28			
PCr τ_1 (s)	2.46	-59	-12			
PCr SC_1 (s)	2.35	-28	-5			
PCr MRT_2 (s)	2.07	-92	-11			
PCr τ_2 (s)	2.39	-65	-12			
PCr SC_2 (s)	1.00	-19	5			
HHb MRT_1 (s)	1.72	-24	2			
HHb MRT_2 (s)	1.69	-16	1			
		Fatiguing exercise				
Decrease in force (% peak)	0.76	-16	1	0.27	-12	6
PCr_{EE} (% BL)	0.91	1	22	0.24	-15	8
P_{iEE} (% BL)	1.28	-163	-35	0.31	-47	102
pH_{EE}	0.61	-0.02	0.10	0.56	-0.10	0.02

Appendix C – Participant information and consent forms

PCr and HHb kinetics during heavy intensity exercise

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Information Sheet for Child Participants and Parents

What are you investigating?
We are interested in finding out how muscles produce energy during exercise and if children's muscle is different to adults. We also want to find out how much of your leg is muscle and how much is bone, fat, and other tissues.

How are you going to find these things out?
We will be using techniques called magnetic resonance imaging and magnetic resonance spectroscopy. These techniques allow us to take "pictures" of what the inside of your leg looks like (a bit like an x-ray but with no radiation) and when you exercise in the magnet we get images of how you are producing energy. These techniques do not use x-rays or any other potentially harmful ionising radiation, but instead use a combination of radio waves (similar to those for radio and TV transmission) and a large magnet.

Is it safe?
Worldwide millions of people have had MRI scans with no apparent side effects and it has not been found to be harmful in any way.

What tests will I be doing?
We need you to do several tests spread over 5-6 visits. On your first visit we will show you around the Centre and the Scanner and give you plenty of practice at the tests. We will also measure your height and weight. We have built a copy of the magnet in one of our laboratories in the Centre so you practice without being inside the real magnet. We will ask you to exercise on the equipment we have made specifically to go inside the magnet. It is very easy; you lie on your tummy with one foot attached to a strap and just move your foot up and down from the knee (a bit like kicking your legs in swimming). On the second visit we will make it harder by adding more weight to the strap. We will ask you to exercise for several bouts of 1 minute. Each bout will get harder, and we will ask you to do as many bouts as you can. In total you will exercise for about 10-15 minutes. On the third, fourth, and fifth visits we will ask you to do a number of exercise tests in the scanner so we can measure your muscles at the same time. One of the team will be in the scanning room during the test to answer any questions you have. The third visit will be a rerun of the test practiced on day two, but the fourth and fifth visits will involve you exercising in the magnet with a heavy weight for 6 minutes. It is possible that this will be repeated once more on a separate day.

What do I need to wear?

For all the exercise tests and scans you should wear your PE kit (shorts, t-shirt, and trainers with no metal buckles and buttons!).

When will I need to come?
Members of the research team will collect you from school and take you back after testing. You will visit us at during the lunch break and during some PE and Games lessons with another pupil volunteer.

What is it like in the scanner?
Don't forget that you will have lots of practice in the "pretend" scanner. The real scanner is quite noisy so we will ask you to wear headphones. A member of the study team will stay next to the scanner all the time you are in there but if you feel unhappy you can stop the scan at any time.

Why is the safety checklist necessary?
Because the MRI uses a large magnet is it important that nobody brings any metal too near to the scanner. Objects can be attracted to the magnet so it is important that people remove all metal objects from their bodies, such as coins, as these could fly out of their pockets and potentially hit someone. Objects within the body such as pacemakers may also function incorrectly within the magnetic field and so it is important that people with these implants are not scanned. Some people who don't like being in confined spaces (like lifts) may not like being in the scanner because it is quite a small space. We try not to recruit people who will feel unhappy in the scanner.

What if I want to drop out of the study?
You can drop out of the study at any time even while being scanned. We will not be upset or cross and neither will your teachers at school.

What will you do with the results?
All the results we collect will be stored on a computer. No one will be told your individual results. We will write the study up as a paper and present the group results to other researchers but your information will remain confidential.

What should I do if I want to participate?
If you would like to be involved in coming to the University for this exciting project please make sure you do the following:

1. Both you and a parent/guardian complete the participant consent form.
2. Get your parent/guardian to fill out the magnetic resonance imaging safety checklist form, otherwise we won't be able to put you in the scanner.
3. Get your parent/guardian to complete the pre-exercise test questionnaire. This is purely for health and safety reasons, not to make judgements on your health and fitness.
4. Return the forms to your school teacher, Mr. Martin.

We will contact you through the school when we are ready to start testing to arrange your visits.

What if I have a question?
If you have any questions regarding the scan or the study generally please contact Professor Craig Williams or Ms. Rebecca Willcocks through the Research Centre's secretary on 01392 264812 (office hours) and we will be happy to help.

We hope you would like to be part of this project, and are looking forward to seeing you in the coming weeks.

Best wishes, Prof. Craig Williams and Ms. Rebecca Willcocks

This study has been approved by the School of Sport and Health Sciences Ethics Committee

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Participant Consent Form (Ethics approval code 05G)

To be completed by the Parent/Guardian:

I consent for my child …….to attend the research centre to take part in some exercise tests. I have read the information sheet provided and had the opportunity to discuss the study with the researchers. I understand that my child will be involved in approximately 5-6 visits to the centre and will be collected and returned to school by Research Centre Staff. I understand that my child will have his or her height and weight measured. I understand that my child will be involved in magnetic resonance scanning and will exercise in the scanner on 3-4 occasions and have his or her leg volume measured. I understand that the data collected in this study will be stored confidentially and used to prepare scientific reports and presentations but my child's individual results will not be identifiable. I understand that my child can drop out of the study at any time without giving a reason.

Signed……..…….Parent/Guardian
Date……..
……

To be completed by the child participant

I am happy to take part in some exercise tests at the research centre. These have been explained to me and I understand what I will be asked to do.
I know that I will be coming to the Centre for approximately 5 visits and will have some scans taken of my legs.
I know that I can stop taking part in the study at any time, even during scans without affecting my relationship with the researchers or the school.

Signed……Child Participant
Date……..

This study has been approved by the School of Sport and Health Sciences Ethics Committee

Effect of prior exercise in young people and adults

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Participant Information for Child Participants and Parents/Guardians

Effect of prior exercise in young people and adults: A magnetic resonance study to determine whether prior exercise affects exercise metabolism in children

What are we investigating?
When someone warms up before exercise, they get the blood flowing to their muscles and get their muscles ready to exercise. This study is designed to examine how an intense warm-up bout of exercise affects children's ability to adjust to the energy demands of a subsequent bout of exercise, compared with adults. The study is particularly concerned with oxygen delivery to working muscles, and how efficiently the muscles can use that oxygen.

How are we going to find these things out?
We will use a technique called magnetic resonance (MR) spectroscopy. With this, we can see how some chemicals in your muscle are changing to give you energy to exercise. The MR scanner uses a combination of radio waves and a large magnet to find these things out.We are also going to use a technique called near infrared spectroscopy (NIRS), which uses light to find out how much oxygen is in your blood.

Why is this study being undertaken?
We would like to understand more about how children's bodies respond to exercise. There are lots of differences between children and adults when they are exercising; this study will try to understand whether an intense warm-up helps children's muscles during a later exercise test.

What tests will I be doing?
You will have to do up to five different tests:
1. On your first visit, you will visit the university to try out the exercise. You will lie on your stomach, and we will attach a rope to your foot with some weights on the end. We will ask you to lift the weights up and down with your foot (the movement is a bit like kicking a football, but you will be lying on your front). You will try this exercise with lighter weights and heavier weights, and we will measure your height and weight. If you do not like doing this exercise, you will not have to do any of the other tests.
2. The second time you come to the university, we will use the MRI scanner to measure your muscles while you do the kicking exercise. You will start kicking with only ½ kg of weight attached to your leg, and we will make the weight heavier and heavier until you can not continue to exercise. You will be in the scanner for about 30 minutes, but only exercising for about 10 of those.
3. If your second test goes well, we will ask you to come back up to three more times. For these tests, you will be in the scanner again, and you will do more kicking exercise. This time,

though, the weight that will be attached to your foot will stay the same for the whole test. The test will be: six minutes of exercise, then six minutes of rest, then six more minutes of exercise.

Is it safe?
Worldwide, millions of people have had MRI scans with no apparent side effects and it has not been found to be harmful in any way.

What do I need to wear?
For all the exercise tests and scans you should wear your PE kit (shorts, t-shirt, and trainers with no metal buckles, zips, or buttons).

What is it like in the scanner?
You will have a chance to practice in the model scanner. The real scanner is quite noisy sometimes, so you will have to wear headphones for part of the test. A member of the study team will stay next to the scanner all the time you are in there but if you feel unhappy you can stop the test at any time.

Why is the safety checklist necessary?
Because the MRI uses a large magnet is it important that nobody brings any metal too near to the scanner. Objects can be attracted to the magnet so it is important that people remove all metal objects from their bodies, such as coins, as these could fly out of their pockets and potentially hit someone. Objects within the body such as pacemakers and implants may also function incorrectly within the magnetic field and so it is important that people with these implants are not scanned. Some people who don't like being in confined spaces (like lifts) may not like being in the scanner because it is quite a small space. We try not to recruit people who will feel unhappy in the scanner.

Why do I need to complete a health questionnaire?
It is important that together with your parents you fill out the attached health questionnaire as honest and as accurately as possible. You should provide any relevant medical information that might affect your ability to take part in exercise testing. This information will help us in making sure that the risk is minimised of any future injuries or illnesses developing during the study.

What if I want to drop out of the study?
You can drop out of the study at any time even while being scanned. No one will be upset or cross with you.

What will you do with the results?
All the results we collect will be stored on a computer. No one will be told your individual results. We will write the study up as a paper and present the group results to other researchers but your information will remain confidential. Only the people who are doing the tests will be able to see your individual information. You are most welcome to request a copy of the results of the project if you wish.

What if I have a question?
If you have any questions regarding the scan or the study generally please contact Associate Professor Craig Williams (01392264890) or Ms. Rebecca Willcocks (01392264889) and we will be happy to help.

The Ethics Committee of the School of Sport and Health Sciences has reviewed and approved this project.

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Participant and Parent/Guardian Consent Form

Effect of prior exercise in young people and adults: A magnetic resonance study

To be completed by the Parent/Guardian:
I have read the information sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage. I understand that my child will be asked to visit the university up to five times to take part in exercise testing in the magnetic resonance scanner to measure muscle responses to exercise.

I know that:
1. my child's participation in this project is entirely voluntary
2. my child is free to withdraw from the project at any time without disadvantage
3. the raw data on which the results of the project depend will be retained in secure storage
4. the results of the project may be published but my child's anonymity will be preserved.

Signed……..…….Parent/Guardian

Date……..
Emergency contact phone number:

……
……
……

To be completed by the child participant :

I am happy to take part in some exercise tests at the research centre. These have been explained to me and I understand what I will be asked to do.
I know that I will have some scans taken of my legs during some exercise tests during up to five visits to the university.
I know that I can stop taking part in the study at any time, even during scans without affecting my relationship with the researchers or the school.

Signed……Child Participant

Date……..

This study has been approved by the School of Sport and Health Sciences Ethics Committee

Gated measurement of PCr recovery kinetics in adolescent girls and women

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Participant Information for Child Participants and Parents/Guardians

Calf muscle metabolism in children and adults: *A magnetic resonance study*

What are we investigating?
We are interested in how your muscles use energy while you are doing exercise that is made up of very brief muscle actions. When you use your muscles and then relax them, they have time to recover. It is this recovery between short contractions that we are interested in measuring.

How are we going to find these things out?
We will use a technique called magnetic resonance (MR) spectroscopy. With this, we can see how some chemicals in your muscle are changing to give you energy to exercise. The MR scanner uses a combination of radio waves and a large magnet to find these things out.

Why is this study being undertaken?
Children seem to be able to recover very quickly from exercise. Some scientists think that this might be because children's muscles use energy providing systems in different ways compared with adults. We would like to use a magnetic resonance scanner to investigate how your muscles recover from exercise.

What tests will I be doing?
The test that you will be doing with us involves pushing your foot against a pedal (as if you were pressing the accelerator on a car) as hard as you can for two seconds, then resting for 30 seconds. You will do this over and over again. The test will last for 15 minutes. You will do the test twice: once in a copy of the MR scanner to practice, and once in the magnet to make measurements. The tests will take place on different days. This test should not tire your muscles out too much; it will probably feel quite comfortable. You can decide that you are not happy to take part at any time during the testing. If you decide that you would prefer not to finish the testing, you will not be in any trouble and we will not be cross.

Is it safe?
Worldwide, millions of people have had MRI scans with no apparent side effects and it has not been found to be harmful in any way.

What do I need to wear?

For all the exercise tests and scans you should wear your PE kit (shorts, t-shirt, and trainers with no metal buckles, zips, or buttons).

What is it like in the scanner?
You will have a chance to practice in the "pretend" scanner. The real scanner is quite noisy sometimes, so you will have to wear headphones for part of the test. A member of the study team will stay next to the scanner all the time you are in there but if you feel unhappy you can stop the test at any time.

Why is the safety checklist necessary?
Because the MRI uses a large magnet is it important that nobody brings any metal too near to the scanner. Objects can be attracted to the magnet so it is important that people remove all metal objects from their bodies, such as coins, as these could fly out of their pockets and potentially hit someone. Objects within the body such as pacemakers may also function incorrectly within the magnetic field and so it is important that people with these implants are not scanned. Some people who don't like being in confined spaces (like lifts) may not like being in the scanner because it is quite a small space. We try not to recruit people who will feel unhappy in the scanner.

Why do I need to complete a health questionnaire?
It is important that together with your parents you fill out the attached health questionnaire as honest and as accurately as possible. You should provide any relevant medical information that might affect your ability to take part in exercise testing. This information will help us in making sure that the risk is minimised of any future injuries or illnesses developing during the study.

What if I want to drop out of the study?
You can drop out of the study at any time even while being scanned. No one will be upset or cross with you.

What will you do with the results?
All the results we collect will be stored on a computer. No one will be told your individual results. We will write the study up as a paper and present the group results to other researchers but your information will remain confidential. Only the people who are doing the tests will be able to see your individual information. You are most welcome to request a copy of the results of the project if you wish.

What if I have a question?
If you have any questions regarding the scan or the study generally please contact Associate Professor Craig Williams (01392264890) or Ms. Rebecca Willcocks (01392264889) and we will be happy to help.

The Ethics Committee of the School of Sport and Health Sciences has reviewed and approved this project.

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Participant Information for Adult Participants

Calf muscle metabolism in children and adults: A magnetic resonance study

What are we investigating?
We are interested in how your muscles use energy while you are doing exercise that is made up of very brief muscle actions. When you use your muscles and then relax them, they have time to recover. It is this recovery between short contractions that we are interested in measuring.

How are we going to find these things out?
We will use a technique called magnetic resonance (MR) spectroscopy. With this, we can see how some chemicals in your muscle are changing to give you energy to exercise. The MR scanner uses a combination of radio waves and a large magnet to find these things out.

Why is this study being undertaken?
Children seem to be able to recover very quickly from exercise. Some scientists think that this might be because children's muscles use energy providing systems in different ways compared with adults. We would like to use a magnetic resonance scanner to investigate how your muscles recover from exercise.

What tests will I be doing?
The test that you will be practicing for us involves pushing your foot against a pedal (as if you were pressing the accelerator on a car, except that the pedal will not move) as hard as you can for two seconds, then resting for 30 seconds. You will do this over and over again. The test will last for 15 minutes. You will do the test twice: once in a copy of the MR scanner to practice, and once in the magnet to make measurements. The tests will take place on different days. This test will probably not tire your muscles out too much; it should feel quite comfortable. You may decide not to take part in the project without any disadvantage to yourself of any kind.

Is it safe?
Worldwide, millions of people have had MRI scans with no apparent side effects and it has not been found to be harmful in any way.

What do I need to wear?
For all the exercise tests and scans you should wear exercise kit (shorts, t-shirt, and trainers with no metal buckles, zips, or buttons).

Why is the safety checklist necessary?

Because the MRI uses a large magnet is it important that nobody brings any metal too near to the scanner. Objects can be attracted to the magnet so it is important that people remove all metal objects from their bodies, such as coins, as these could fly out of their pockets and potentially hit someone. Objects within the body such as pacemakers may also function incorrectly within the magnetic field and so it is important that people with these implants are not scanned. Some people who don't like being in confined spaces (like lifts) may not like being in the scanner because it is quite a small space. We try not to recruit people who will feel unhappy in the scanner.

Why do I need to complete a health questionnaire?

It is important that together with your parents you fill out the attached health questionnaire as honest and as accurately as possible. You should provide any relevant medical information that might affect your ability to take part in exercise testing. This information will help us in making sure that the risk is minimised of any future injuries or illnesses developing during the study.

What if I want to drop out of the study?
You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

What will you do with the results?
All the results we collect will be stored on a computer. No one will be told your individual results. We will write the study up as a paper and present the group results to other researchers but your information will remain confidential. Only the people who are doing the tests will be able to see your individual information. You are most welcome to request a copy of the results of the project if you wish.

What if I have a question?
If you have any questions regarding the scan or the study generally please contact Associate Professor Craig Williams (01392264890) or Ms. Rebecca Willcocks (01392264889) and we will be happy to help.

The Ethics Committee of the School of Sport and Health Sciences has reviewed and approved this project.

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Participant Consent Form

Gated exercise using ^{31}P-MRS

To be completed by the Parent/Guardian:

I have read the Information sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage. I understand that my child will be asked to visit the Centre twice to take part in exercise testing in the magnetic resonance scanner to measure muscle responses to exercise.

I know that:

5. my child's participation in this project is entirely voluntary
6. my child is free to withdraw from the project at any time without disadvantage
7. the raw data on which the results of the project depend will be retained in secure storage
8. the results of the project may be published but my child's anonymity will be preserved.

Signed……Parent/Guardian
Date……..

……

To be completed by the child participant :

I am happy to take part in some exercise tests at the research centre. These have been explained to me and I understand what I will be asked to do.
I know that I will have some scans taken of my legs during some exercise tests during two visits to the Centre.
I know that I can stop taking part in the study at any time, even during scans without affecting my relationship with the researchers or the school.

Signed……Child Participant
Date……..

This study has been approved by the School of Sport and Health Sciences Ethics Committee

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Participant Consent Form
Gated exercise examined using ^{31}P-MRS

I have read the Information sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage. I understand that I will be asked to visit the Centre twice to take part in exercise testing in the magnetic resonance scanner to measure muscle responses to exercise.

I know that:

9. my participation in this project is entirely voluntary
10. I am free to withdraw from the project at any time without disadvantage
11. the raw data on which the results of the project depend will be retained in secure storage
12. the results of the project may be published but my anonymity will be preserved.

Signed……
Date……

This study has been approved by the School of Sport and Health Sciences Ethics Committee

Fatigue during repetitive isometric exercise in adolescents and adults

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Participant Information for Child Participants and Parents/Guardians

Quadriceps muscle fatigue during exercise in children and adults: A magnetic resonance study

What are we investigating?
Several studies have shown that children are more "fatigue-resistant" than adults during exercise; that is, they don't get tired out as easily as adults when they are exercising. We want to find out whether that is because children's muscles use some energy systems more than others.

How are we going to find these things out?
We will use a technique called magnetic resonance (MR) spectroscopy. With this, we can see how some chemicals in your muscle are changing to give you energy to exercise. The MR scanner uses a combination of radio waves and a large magnet to find these things out.We are also going to use a technique called near infrared spectroscopy (NIRS), which uses light to find out how much oxygen is in your blood.

Why is this study being undertaken?
We would like to understand more about how children's bodies respond to exercise so that we can help to recommend the best exercise for children. There are lots of differences between children and adults when they are exercising; this study will try to understand the reasons that children become less fatigued than adults when they do intermittent exercise.

What tests will I be doing?
The test that you will be doing with us involves pushing your foot against a pad while you are lying on your front in the MR scanner. You will visit the university twice: once to practice the exercise, and once to do the test in the MR scanner. We will measure your height and weight, and will take some resting scans of your legs to measure how much muscle you have. You can decide that you are not happy to take part at any time during the testing. If you decide that you would prefer not to finish the testing, you will not be in any trouble and we will not be cross.

Is it safe?
Worldwide, millions of people have had MRI scans with no apparent side effects and it has not been found to be harmful in any way.

What do I need to wear?
For all the exercise tests and scans you should wear your PE kit (shorts, t-shirt, and trainers with no metal buckles, zips, or buttons).

What is it like in the scanner?

You will have a chance to practice in the model scanner. The real scanner is quite noisy sometimes, so you will have to wear headphones for part of the test. A member of the study team will stay next to the scanner all the time you are in there but if you feel unhappy you can stop the test at any time.

Why is the safety checklist necessary?
Because the MRI uses a large magnet is it important that nobody brings any metal too near to the scanner. Objects can be attracted to the magnet so it is important that people remove all metal objects from their bodies, such as coins, as these could fly out of their pockets and potentially hit someone. Objects within the body such as pacemakers and implants may also function incorrectly within the magnetic field and so it is important that people with these implants are not scanned. Some people who don't like being in confined spaces (like lifts) may not like being in the scanner because it is quite a small space. We try not to recruit people who will feel unhappy in the scanner.

Why do I need to complete a health questionnaire?
It is important that together with your parents you fill out the attached health questionnaire as honest and as accurately as possible. You should provide any relevant medical information that might affect your ability to take part in exercise testing. This information will help us in making sure that the risk is minimised of any future injuries or illnesses developing during the study.

What if I want to drop out of the study?
You can drop out of the study at any time even while being scanned. No one will be upset or cross with you.

What will you do with the results?
All the results we collect will be stored on a computer. No one will be told your individual results. We will write the study up as a paper and present the group results to other researchers but your information will remain confidential. Only the people who are doing the tests will be able to see your individual information. You are most welcome to request a copy of the results of the project if you wish.

What if I have a question?
If you have any questions regarding the scan or the study generally please contact Associate Professor Craig Williams (01392264890) or Ms. Rebecca Willcocks (01392264889) and we will be happy to help.

The Ethics Committee of the School of Sport and Health Sciences has reviewed and approved this project.

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Participant Consent Form
Quadriceps muscle fatigue during exercise in children and adults: A magnetic resonance study

To be completed by the Parent/Guardian:
I have read the Information sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage. I understand that my child will be asked to visit the Centre twice to take part in exercise testing in the magnetic resonance scanner to measure muscle responses to exercise.

I know that:
13. my child's participation in this project is entirely voluntary
14. my child is free to withdraw from the project at any time without disadvantage
15. the raw data on which the results of the project depend will be retained in secure storage
16. the results of the project may be published but my child's anonymity will be preserved.

Signed……..…….Parent/Guardian
Date……..

Emergency contact phone number:
……
……
……
To be completed by the child participant :

I am happy to take part in some exercise tests at the research centre. These have been explained to me and I understand what I will be asked to do.
I know that I will have some scans taken of my legs during some exercise tests during two visits to the Centre.
I know that I can stop taking part in the study at any time, even during scans without affecting my relationship with the researchers or the school.

Signed……Child Participant
Date……..

This study has been approved by the School of Sport and Health Sciences Ethics Committee

Appendix D - Participants

Chapters 4, 5, 6, 7, and 9 report different parameters of exercise performance and metabolism from an overlapping set of participants. For clarity, this appendix presents the variables gathered from each individual organised by protocol. Participants visited the laboratory to perform four different exercise protocols.

1. Heavy intensity exercise (Table D1). Data from these participants appears in Chapter 4, Chapter 5, and Chapter 7. PCr kinetics parameters for these participants vary slightly from Chapter 4 to Chapter 5. In Chapter 5, kinetics was fitted with a fixed window to facilitate comparison between heavy and very heavy intensity exercise. Values in this table are those presented in Chapter 4.
2. Very heavy intensity exercise (Table D2). Data from these participants appears in Chapter 5, Chapter 6, and Chapter 7. PCr kinetics for these participants is discussed in detail in Appendix E, and is not repeated here.
3. Fatiguing isometric exercise (Table D3). Data from these participants appears in Chapter 7 and Chapter 9.
4. Gated and sustained exercise for the measurement of PCr recovery τ. Data from these participants appears only in Chapter 8 and is not reproduced in this appendix.

Table D1. Individual data for participants who completed 7 minutes of heavy intensity exercise.

Code	Sex	Age (y)	Target PO (W)	Actual PO (W)	PCr_{BL} (mM)	pH_{BL}	PCr_{EE} (mM)	PCr_{EE} (% BL)	pH_{EE}	τ (s)	A (% BL)	SC (% EE)	HHb De (s)	HHb τ (s)	HHb MRT (s)	τ_{REC} (s)
D808	M	13.2	11.0	13.7	40.5	7.05	24.6	61	7.03	15	67	10	16	10	26	20
D809	M	12.8	14.0	12.8	40.1	7.11	15.3	38	6.87	36	44	15	14	11	25	27
D810	M	13.2	13.7	13.0	40.6	7.09	21.0	52	6.96	41	59	15	10	10	20	30
D811	M	13.2	9.2	10.0	39.9	7.08	21.2	53	7.01	33	58	9	12	11	23	42
*D812	M	13.3	14.4	11.0	41.1	7.06	21.5	52	7.13							10
*D813	M	12.6	13.1	11.0	41.4	7.09	26.1	63	7.06							29
D814	M	12.8	8.8	9.6	39.7	7.07	26.1	66	6.99	36	71	9	16	7	23	32
D815	M	12.8	10.0	11.1	40.7	7.08	24.7	61	7.04	24	67	11	14	7	21	12
D816	F	14.8	12.8	13.8	39.6	7.07	14.8	37	6.93	32	52	40	10	8	18	49
D817	F	13.2	13.4	13.1	39.6	7.10	17.9	45	7.00	33	58	29	11	10	28	56
E36	F	11.9	12.8	12.1	38.9	7.08	26.2	67	6.99	14	74	10	12	17	21	39
*E37	F	12.5	10.9	12.2	40.5	7.06	22.5	56	6.95							51
E38	F	13.3	12.2	13.2	41.1	7.11	12.5	30	6.83	43	56	84	9	9	19	51
E39	F	11.7	9.3	9.6	40.5	7.07	19.2	47	6.89	32	52	10	10	6	13	38
*D7	M	32.5	16.3	17.6	39.3	7.03	10.6	27	6.89							42
D11	M	29.9	16.3	17.3	39.6	7.02	19.7	50	7.05	15	54	9	14	14	28	13
D12	M	21.3	18.7	23.1	40.8	7.06	16.9	41	6.90	43	57	37	14	12	26	51
D13	M	19.5	20.8	19.8	40.7	7.08	29.5	72	7.08	26	78	8	11	19	30	29
D14	M	26.7	16.0	15.6	39.6	7.03	19.2	49	7.01	61	60	24	16	13	29	44
D15	M	24.3	19.3	24.1	40.9	7.04	25.8	63	7.00	69	65	4	16	12	28	47
D16	M	23.8	17.8	16.6	40.6	7.01	25.1	62	6.95	49	71	15	16	17	33	29
D3	F	25.6	15.3	14.4	40.8	7.00	15.9	39	6.93	42	44	12	13	8	21	45
*D4	F	23.8	9.3	14.0	40.0	7.00	5.6	14	6.93							60
D5	F	18.8	12.5	14.5	39.8	7.00	21.5	54	7.01	22	57	5	14	28	42	45
D6	F	25.9	12.3	14.9	36.8	6.97	16.4	45	6.98	44	49	10	7	36	43	45
*D8	F	19.4	12.8	13.0	40.4	7.02	18.9	47	6.99							41
D9	F	26.2	14.2	15.5	39.6	7.01	18.6	47	6.99	27	64	37	10	9	19	33
D10	F	18.8	12.6	12.1	40.8	7.04	14.1	35	6.98	11	45	31	11	13	24	55

*Data for these participants is not included in Chapter 4, but is included in Chapter 7.

Table D2. Individual data for participants who completed two bouts of very heavy intensity exercise.

Code	Sex	Age	Target PO (W)	Actual PO (W)	pH_{BL}	PCr_{EE1} (% BL)	pH_{EE1}	PCr_{RE1} (% BL)	pH_{RE1}	PCr_{EE2} (% BL)	pH_{EE2}	Tau $_{R1}$ (s)	HHb DE_1 (s)	HHb τ_1 (s)	HHb MRT_1 (s)	HHb DE_2 (s)	HHb τ_2 (s)	HHb MRT_2 (s)
E432	M	12.5	13.0	12.6	7.00	68	6.91	98	7.00	58	6.94	35	10	11	21	8	14	23
E435	M	12.7	11.7	9.6	7.06	64	7.04	96	6.99	61	7.01	48	13	10	23	11	14	25
E436	M	12.5	10.0	8.9	7.07	67	7.01	97	7.03	59	7.04	29	17	7	23	11	7	19
E438	M	12.7	12.1	15.5	7.03	62	6.98	98	7.10	60	7.00	46	9	10	19	8	6	14
E439	M	13.0	6.9	8.0	7.08	58	7.01	96	6.98	53	7.03	35	11	11	22	10	9	19
E440	M	13.4	14.0	13.4	7.06	69	6.97	101	7.02	97	7.00	35	10	6	16	7	8	14
*E431	F	12.7	10.1	8.4	7.07	59	7.01					37						
*E441	F	12.7	12.6	12.7	7.02	35	6.79					57						
A1	M	21.5	21.4	23.7	7.02	69	6.97	101	7.02	65	6.99	35						
A2	M	22.3	28.4	33.0	7.03	54	6.85	98	6.90	60	6.93	80	9	25	34	13	20	33
A3	M	18.4	18.4	20.2	7.04	32	6.79	96	6.95	29	6.88	44	13	32	45	9	15	25
A4	M	19.9	19.3	23.7	7.03	26	6.72	88	6.92	27	6.84	67	8	27	35	6	25	31
A5	M	19.0	22.7	26.1	7.06	45	6.86	99	7.01	34	6.88	47	10	9	19	8	12	20
A6	M	27.9	23.0	22.5	7.05	36	6.76	95	7.05	39	6.90	57	11	14	25	7	16	23

*Data for these participants is not included in Chapter 5 or Chapter 6, but is included in Chapter 7.
NIRS data was not available for A1 due to equipment malfunction.

Code	Sex	Age	Peak force (N)	End force (N)	Decrease (N)	Decrease (%)	PCr_{EE} (% BL)	Pi_{EE} (% BL)	pH_{BL}	pH_{EE}	Recovery τ (s)
E385	M	13.4	71.0	65.3	5.7	8%	52	237	7.02	6.93	47
E387	M	13.1	67.5	55.9	11.6	17%	75	303	7.01	6.87	24
E388	M	13.7	54.6	47.4	7.2	13%	69	376	7.05	6.97	36
E395	M	13.6	78.5	50.2	28.3	36%	85	260	7.03	7.03	42
E397	M	13.7	48.3	41.6	6.7	14%	63	253	7.07	6.91	53
E398	M	12.9	88.4	59.0	29.4	33%	53	285	7.07	6.95	36
E399	M	13.6	100.1	88.1	12.0	12%	69	222	7.04	7.02	37
E389	F	12.9	71.0	49.8	21.2	30%	83	153	7.01	7.03	45
E390	F	13.3	109.4	81.6	27.8	25%	55	205	7.09	6.98	60
E391	F	12.8	65.5	59.4	6.2	9%	59	421	7.02	7.10	41
E392	F	13.1	67.8	39.8	17.1	26%	70	244	7.02	7.01	32
E393	F	13.4	65.6	48.6	28.0	41%	74	258	7.07	7.03	49
E394	F	13.8	118.8	98.7	20.1	17%	60	155	7.01	7.05	73
E396	F	13.5	93.9	68.6	25.3	27%	41	333	6.98	6.84	51
E401	M	25.8	112.3	86.7	25.6	23%	38	301	7.01	6.90	70
E405	M	23.3	79.0	51.6	27.4	35%	65	357	7.07	7.06	55
*E407	M	26.9					58	368	7.08	6.96	58
E408	M	44.8	148.1	111.6	36.5	25%	44	356	7.01	6.92	53
E410	M	27.0	151.5	99.0	52.5	35%	49	408	7.04	6.99	35
E411	M	28.6	141.6	90.5	51.2	36%	42	497	7.06	6.85	52
E414	M	24.8	85.8	59.7	26.1	30%	38	399	7.07	6.77	45
E400	F	33.2	123.2	80.1	43.0	35%	60	244	7.03	6.96	27
E402	F	25.4	94.0	49.7	44.4	47%	51	438	7.01	6.90	51
E404	F	31.0	73.0	62.9	10.1	14%	79	209	7.06	6.99	21
E406	F	22.9	70.1	48.3	21.7	31%	62	408	7.04	6.99	79
E409	F	22.8	132.5	80.7	51.7	39%	59	405	7.01	6.96	43
E412	F	49.7	68.3	57.4	10.9	16%	75	246	7.03	7.04	44
E415	F	27.3	131.4	85.2	46.2	35%	35	467	7.00	6.86	48
E416	F	23.6	90.8	77.4	13.4	15%	44	349	7.01	6.92	45

* a computer glitch resulted in a lack of ergometer data for this participant; he was not included in Chapter 9 but is included in Chapter 7.

Appendix E – Considerations in modelling PCr data acquired during very heavy intensity exercise

There are two key considerations in evaluating the application of a mathematical model to a physiological data set:

1) Is the model physiologically and theoretically appropriate and justifiable?
2) Does the model fit the data?

In the first instance, there is a substantial body of literature that suggests that the adjustment of PCr at the onset of high intensity exercise is well characterised by an exponential function (Forbes, Raymer, Kowalchuk, & Marsh, 2005; Forbes, et al., 2008; Jones, et al., 2008a; Rossiter, et al., 2001; Rossiter, et al., 2002b). This exponential fundamental phase is followed by a slow component (SC) (see Chapter 2 for a discussion of the SC), which has been identified by examining a plot of the time constant generated when the fitting window is iteratively widened from 60 s (Chapter 3; (Rossiter, et al., 2002b)) or by identifying the fitting window over which 95% CI are minimized (Forbes, et al., 2008). Physiologically, it is unlikely that there is a single time point at which the SC "begins". If the SC represents a progressive increase in the PCr cost of exercise, probably this begins at or soon after the onset of exercise. Some investigators have acknowledged this, and modeled the PCr kinetics as a double exponential with a time delay prior to the SC (Haseler, et al., 2004), and the fundamental and SC phases of the $\dot{V}O_2$ kinetic response have been fitted with a double exponential model with and without a time delay prior to the SC.

For the data reported in Chapter 5 and Chapter 6, measurement of the SC presented opposite challenges in children and adults (Table E1). In children, the SC was not evident in several participants, which has been previously described for $\dot{V}O_2$ kinetics, albeit during treadmill running (Williams, et al., 2001). Thus, the challenge was to distinguish those children in whom a monoexponential relationship between PCr and time held over the full 360 s from those children who had evidence of a SC in the response. In adults, on the other hand, the SC was apparent during visual inspection of the data, but emerged so rapidly at the onset of exercise that a plateau prior to the emergence of this component was not evident. Thus, the methods of Rossiter et al. (2002b) were not adequate to identify the SC.

The method of Forbes et al. (2008) offers an appealing alternative, but led to some drastically different fitting windows during different transitions in the same individual. Fitting windows in 3 children and 4 adults differed by more than 120 s from bout 1 to bout 2. This is physiologically interesting, since the fitting window lengthened in bout 2 in all but1of these individuals, reflecting a shift toward more monoexponential kinetics after priming exercise. However, the change calculated from 72 s to the end of exercise is not comparable to the change calculated from 312 s to the end of exercise, as in participant A4.

Thus, in comparing fitting windows between two bouts, the most rigorous approach appears to be the use of a fixed window (180 to 360 s of exercise) to estimate the change in PCr. This has the disadvantage of potentially discarding part of the SC amplitude if, as is likely, the SC emerges prior to 180 s. However, this approach most faithfully conveys the nature of the response over the slow component phase of the response.

In evaluating the goodness of fit of a model, several approaches should be taken. The fit of the model and a plot of the residual error should be visually inspected to identify any areas of consistent over- or under-prediction of the data. The 95% CI must be inspected, and the R^2 should be examined, to give an objective measure of goodness of fit. Finally, any areas where the data deviates from the model in the same direction over several time points can be identified with the runs test – a significant result on this test indicates a poor fit in some area of the data.

For the current data, visual inspection and the R^2 value indicate that the best fit is provided by the Forbes method, or by an arbitrary fitting window of 120 or 180 s. The runs test suggests that in some participants, an arbitrary window of 180 s might be too long, particularly during bout 1. Although the Forbes method is effective in indentifying the longest window over which a monoexponential fit provides an accurate representation of the data, this window overlaps with the 3-minute SC window in a number of participants. Thus, fitting the fundamental component over the first 120 s of exercise allows fit to be optimized within the constraints of these data.

Table E1. A comparison of the fit of several models to phosphocreatine measured during repeated bouts of constant work rate exercise at 60 %Δ.

	E432	E435	E436	E438	E439	E440	A1	A2	A3	A4	A5	A6	Boys	sd	Men	sd
Transition 1																
PCr_{EE} (% BL)	68	64	67	62	58	69	54	32	26	45	36	38	65	4	39	10
MRT (s)	48	26	19	28	61	27	106	134	177	71	66	102	35	16	109	41
Rossiter method:																
Fitting window (s)	132	360	228	360	216	360	168	132	132	144	96	156	276	98	138	25
Baseline (%BL)	101	99	100	101	97	102	99	100	98	97	99	98	100	2	99	1
A_F (%BL)	-28	-38	-30	-40	-38	-35	-46	-54	-37	-58	-47	-50	-35	5	-49	7
τ (s)	28	27	16	31	52	27	83	83	85	52	34	77	30	12	69	21
95% CI (s)	5.7	4.7	2.7	2.7	17.1	4.5	14.3	25.5	33.3	14.3	7.8	15.5	6	5	18	9
R^2	0.98	0.95	0.97	0.99	0.94	0.96	0.99	0.99	0.98	0.98	0.99	0.99	0.97	0.02	0.99	0.01
p (runs test)	0.82	0.25	0.76	0.002	0.046	0.078	0.97	0.99	0.85	0.054	0.26	0.025	0.33	0.37	0.52	0.46
A_{sc} (% BL)	5	-3	3	-1	1	-2	-1	14	35	-6	16	10	1	3	11	14
A_{sc} (% EE)	7%	-5%	4%	-2%	2%	-3%	-2%	44%	135%	-13%	44%	26%	1%	5%	39%	52%
Forbes method:																
Fitting window (s)	132	360	84	324	96	348	168	168	60	72	96	60	224	133	104	51
Baseline (% BL)	101	99	100	101	100	102	99	100	100	99	99	100	101	1	100	1
A_F (% BL)	-28	-38	-30	-41	-31	-34	-58	-58	-21	-46	-47	-37	-34	5	-45	14
τ (s)	28	27	16	32	23	28	95	95	28	27	34	39	26	6	53	33
95% CI (s)	5.7	4.7	2.5	2.7	14.1	4.1	21.6	21.6	16.5	8	7.8	2.7	6	4	13	8
R^2	0.98	0.95	0.99	0.99	0.92	0.97	0.99	0.99	0.98	0.99	0.99	1	0.97	0.03	0.99	0.01
p (runs test)	0.82	0.25	0.93	0.08	0.5	0.13	0.87	0.87	0.7	1	0.26	0.7	0.45	0.36	0.73	0.26
A_{sc} (% BL)	5	-3	3	-2	11	-1	-13	10	53	8	16	25	2	5	17	22
A_{sc} (% EE)	7%	-5%	4%	-3%	19%	-1%	-24%	31%	204%	18%	44%	66%	4%	9%	57%	78%

Arbitrary window (120):																
Baseline (% BL)	101	99	100	100	99	101	99	100	98	98	98	99	100	1	99	1
A_F (% BL)	-28	-40	-29	-42	-34	-37	-46	-56	-36	-54	-51	-44	-35	6	-48	7
τ (s)	29	31	15	37	35	34	84	87	81	41	44	57	30	8	66	21
95% CI (s)	8	7.3	3.3	4.9	21.6	8.8	25.5	31.4	37.2	10.2	12.7	12	9	7	22	11
R^2	0.98	0.98	0.98	1	0.91	0.98	0.99	0.99	0.98	0.99	0.98	0.99	0.97	0.03	0.99	0.01
p (runs test)	0.74	0.91	0.98	0.74	0.52	0.26	0.83	1	0.91	0.52	0.52	0.11	0.69	0.27	0.65	0.33
180-360 A_{sc} (% BL)	2	-1	3	-2	1	-1	18	15	18	9	4	10	0	2	12	6
180-360 A_{sc} (% EE)	8%	-2%	9%	2%	3%	-1%	29%	22%	33%	12%	7%	16%	1%	6%	20%	10%
Arbitrary window (180):																
Baseline (% BL)	100	99	100	100	98	102	99	100	97	96	96	97	100	1	98	2
A_F (% BL)	-29	-39	-29	-41	-38	-35	-48	-61	-41	-63	-57	-57	-35	5	-55	8
τ (s)	37	29	16	34	51	29	92	105	108	66	65	103	33	12	90	20
95% CI (s)	9.4	5.7	2.7	3.1	21.6	6.5	14.7	23.5	31.4	15.9	14.7	29.4	8	7	22	8
R^2	0.96	0.97	0.98	0.99	0.93	0.97	0.99	0.99	0.99	0.98	0.98	0.99	0.97	0.02	0.99	0.01
p (runs test)	0.1	0.71	0.92	0.04	0.14	0.11	0.86	0.92	0.81	0.1	0.2	0.009	0.34	0.38	0.48	0.42
180-360 A_{sc} (% BL)	2	-1	3	-2	1	-1	18	15	18	9	4	10	0	2	12	6
180-360 A_{sc} (% EE)	8%	-2%	9%	2%	3%	-1%	29%	22%	33%	12%	7%	16%	1%	6%	20%	10%

	E432	E435	E436	E438	E439	E440	A1	A2	A3	A4	A5	A6	Boys	sd	Men	sd
Transition 2																
PCr_{EE} (% BL)	58	61	59	60	53	65	60	29	27	34	39	35	59	4	37	12
MRT (s)	49	35	39	23	30	29	108	85	143	51	41	85	34	9	86	37
Rossiter method:																
Fitting window (s)	156	360	180	192	144	360	144	180	360	360	360	228	232	101	272	100
Baseline (% BL)	99	85	78	85	81	92	94	81	95	75	80	89	87	8	86	8
A_F (% BL)	-34	-24	-23	-24	-25	-26	-36	-52	-66	-46	-41	-53	-26	4	-49	11
τ (s)	34	36	127	35	29	24	53	100	143	46	10	75	48	39	71	46
95% CI (s)	6.3	8.2	201.9	5.3	10	6.9	8.4	12.5	12.9	23.5	17.6	4.3	40	79	13	7
R^2	0.98	0.92	0.78	0.98	0.95	0.88	0.99	1	1	0.72	0.76	1	0.92	0.08	0.91	0.13
p (runs test)	0.23	0.018	0.62	0.32	0.73	0.24	0.5	0.85	0.32	0.01	0.046	0.41	0.36	0.27	0.36	0.31
A_{sc} (% BL)	7	0	-4	1	3	1	-2	0	2	-5	0	1	1	4	-1	3
A_{sc} (% EE)	12%	0%	-7%	2%	6%	2%	-3%	0%	7%	-15%	0%	3%	2%	6%	-1%	7%
Forbes method:																
Fitting window (s)	96	336	360	264	288	216	108	228	180	312	300	228	260	95	226	76
Baseline (% BL)	100	85	79	85	80	92	95	81	88	74	79	89	87	8	84	8
A_F (% BL)	-32	-25	-20	-24	-26	-27	-33	-53	-57	-47	-43	-53	-26	4	-48	9
τ (s)	27	38	85	36	37	26	45	104	14	55	49	75	42	22	57	30
95% CI (s)	5.1	5.7	41.2	4.5	10	6.7	8	11	31.4	4.1	5.7	4.3	12	14	11	10
R^2	0.99	0.97	0.79	0.98	0.91	0.94	1	1	1	0.99	0.98	1	0.93	0.07	1.00	0.01
p (runs test)	0.79	0.65	0.15	0.26	0.5	0.55	0.99	0.82	0.23	0.51	0.97	0.41	0.48	0.24	0.66	0.32
A_{sc} (% BL)	10	-1	0	1	1	0	2	-1	4	-7	-3	1	2	4	-1	4
A_{sc} (% EE)	17%	-2%	0%	2%	2%	0%	3%	-3%	15%	-21%	-8%	3%	3%	7%	-2%	12%

Arbitrary window (120):																
Baseline (% BL)	99	96	93	99	95	101	101	94	97	87	92	97	**97**	3	95	5
A_F (% BL)	-33	-36	-25	-36	-40	-39	-42	-53	-59	-61	-57	-67	-35	5	-57	8
τ (s)	31	36	24	29	30	37	57	60	110	55	47	90	31	5	70	25
95% CI (s)	6.7	10	19	4.7	7.8	12.3	14.3	16.1	66.6	10.8	10.6	10.8	10	5	22	22
R^2	0.99	0.98	0.81	0.99	0.98	0.97	0.99	0.99	0.98	0.99	0.99	1	0.95	0.07	0.99	0.01
p (runs test)	0.26	0.74	0.52	0.11	0.74	0.91	0.11	0.52	0.52	0.26	0.74	1	0.55	0.31	0.53	0.32
180-360 A_{sc} (% BL)	3	2	3	1	-2	1	11	9	14	2	-2	7	1	2	7	6
180-360 A_{sc} (% EE)	7%	5%	7%	4%	-4%	3%	21%	13%	22%	2%	-4%	11%	2%	5%	11%	10%
Arbitrary window (180):																
Baseline (% BL)	98	96	90	99	95	102	100	93	97	87	92	97	97	4	94	5
A_F (% BL)	-35	-36	-27	-37	-40	-38	-45	-58	-59	-61	-57	-63	-36	5	-57	6
τ (s)	40	38	41	31	31	31	68	75	110	54	49	80	35	5	73	22
95% CI (s)	8.4	6.3	25.5	3.5	6.9	7.3	11	12.7	25.5	5.5	6.1	5.5	10	8	11	8
R^2	0.98	0.98	0.84	0.99	0.97	0.97	0.99	0.99	0.99	1	0.99	1	0.96	0.06	0.99	0.01
p (runs test)	0.43	0.79	0.4	0.43	0.43	0.43	0.032	0.43	0.79	0.6	0.43	0.79	0.49	0.15	0.51	0.29
180-360 A_{sc} (% BL)	3	2	3	1	-2	1	11	9	14	2	-2	7	1	2	7	6
180-360 A_{sc} (% EE)	7%	5%	7%	4%	-4%	3%	21%	13%	22%	2%	-4%	11%	2%	5%	11%	10%