# The Influence of Training Status on the Physiological Responses to Exercise of Young Girls

Submitted by Melitta Anne McNarry

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# Abstract

Exercise training represents a potent stimulus to the parameters of aerobic and anaerobic fitness in adults; whether the same is true in young girls is unclear. For some parameters, such as peak oxygen uptake, the influence of training status remains controversial whilst for other parameters, such as oxygen uptake kinetics, the influence of training status remains simply uninvestigated in young girls. Despite this lack of empirical evidence, it has been suggested for some time now that children may lack trainability and that this may be related to the presence of a maturational threshold below which significant adaptations to training cannot occur. This suggestion requires investigation, not least because the findings of some studies which appear to support this contention may in reality be a reflection of the use of an inappropriate test modality for the investigation of training status influences. The purpose of this thesis was therefore to determine the physiological trainability of girls at different stages of maturation and to investigate the interaction between training status, maturity and exercise modality. To achieve this purpose a series of 5 studies was completed, in which trained and untrained girls completed ramp incremental exercise, constant-work-rate exercise and Wingate exercise on two exercise modalities, one upper (arm crank) and one lower body (cycle). During these tests, cardiovascular, respiratory, metabolic and mechanical power parameters were assessed. In response to ramp incremental exercise, trained girls were shown to have a higher peak $\dot{V}O_2$, SV and $\dot{Q}$ at all stages of maturity, along with an altered SV and fractional muscle oxygen extraction pattern, irrespective of exercise modality. The importance of exercise modality was evident during heavy intensity constant-work-rate exercise in pre-pubertal girls, where training status was only associated with significant influences on $\dot{V}O_2$ kinetics (faster phase II time constant in trained girls) during upper body ergometry. In contrast, pubertal trained girls had faster $\dot{V}O_2$ kinetics during both exercise modalities, an influence which may suggest both central and peripheral adaptations to the delivery and utilisation of oxygen. Exercise modality was also revealed to be an important factor in the demonstration of training status influences during a 30 s Wingate test, with trained girls at all stages of maturity exhibiting higher mechanical power indices during upper body ergometry only. An influence of training status was also evident in the lower fatigue index found in the trained girls at all stages of maturity during both modalities, but no influence was found in the oxidative

contribution to the Wingate test. None of these studies revealed an influence of maturity status in determining the magnitude of training status effects. Overall, the 5 studies encompassed within this thesis demonstrate that children are trainable and that this is not moderated by maturity.

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## Symbols and Abbreviations

| | |
|---|---|
| $O_2$ | Oxygen |
| $\dot{V}O_2$ | Oxygen uptake |
| $\dot{V}O_2$ max | Maximal oxygen uptake |
| RER | Respiratory exchange ratio |
| Min | Minute |
| $\dot{Q}$ | Cardiac output |
| a - $\bar{v}O_2$ difference | arterial-venous oxygen difference |
| SV | Stroke volume |
| HR | Heart rate |
| [HHb] | Deoxygenated haemoglobin and myoglobin |
| LT | Lactate threshold |
| $CO_2$ | Carbon dioxide |
| $\dot{V}CO_2$ | Carbon dioxide output |
| $\dot{V}_E$ | Minute ventilation |
| GET | Gas exchange threshold |
| PCr | Intramuscular phosphocreatine |
| $\tau$ | Time constant |
| MLSS | Maximal lactate steady state |
| CP | Critical power |
| %$\Delta$ | % difference between GET and peak $\dot{V}O_2$ |
| $\Delta$ | Delta change |
| ATP | Adenosine triphosphate |
| NIRS | Near infrared spectroscopy |
| WAnT | Wingate anaerobic test |
| PP | Peak power |
| MP | Mean power |
| FI | Fatigue index |
| PFK | Phosphofructokinase |
| $^{31}$P-MRS | P-31 magnetic resonance spectroscopy |

| | |
|---|---|
| $pH_i$ | intracellular pH |
| $P_i$ | Inorganic phosphate |
| METs | Metabolic equivalents |
| ACSM | American College of Sports Medicine |
| T | Trained girls |
| UT | Untrained girls |
| RPM | Revolutions per minute |
| BSA | Body surface area |
| $SV_i$ | Stroke volume index |
| w | Weight of Monark flywheel |
| r | Distance from the axis of rotation to the point of suspension |
| I | Inertia of the flywheel |
| l | Length of suspending wires |
| $\omega$ | Angular velocity |
| $T_i$ | Inertial torque |
| $T_r$ | Resistive torque |
| SD | Standard deviation |
| s | Seconds |
| $A_1$ | Amplitude of the primary component |
| $\delta$ | Time delay |
| ANCOVA | Analysis of covariance |
| ANOVA | Analysis of variance |
| BMI | Body mass index |
| PHV | Peak height velocity |
| WR | Work rate |
| [lactate] | concentration of lactate |
| $b \cdot min^{-1}$ | Beats per minute |
| $l \cdot min^{-1}$ | Litres per minute |
| $ml \cdot kg^{-1} \cdot min^{-1}$ | Millilitres per kilogram per minute |
| $ml \cdot m^{-2}$ | Millilitres per metre squared |
| $ml \cdot m^{-2} \cdot min^{-1}$ | Millilitres per metre squared per minute |
| W | watts |

| N | Newtons |
|-----|-----|
| m | Metres |
| n | Sample size |
| $r$ | Pearson's correlation coefficient |
| CI | Confidence interval |
| MRT | Mean response time |

# Chapter 1

## Introduction
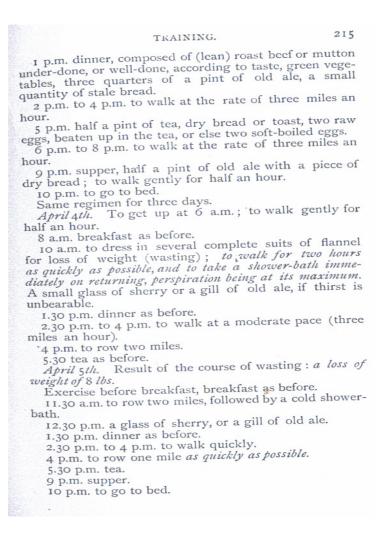
> You say 'I want to win at Olympia'. But wait. Look at what is involved… You will have to obey instructions, keep away from desserts, eat only at set hours, in both heat and cold; you must not drink cold water nor can you have a drink of wine whenever you want. You must hand yourself over to your coach exactly as you would to a doctor. Then in the contest you must gouge and be gouged; there will be times when you will sprain a wrist, twist your ankle, swallow mouthfuls of sand and be flogged. And after all that there are times when you will lose.
>
> (Epictetus, 1st–2nd century AD, *Discourses* 15, 2–5)

Training may occur under a multitude of guises and with a variety of intended outcomes but the underlying concept is the same: training is a series of practices, the object of which is to render a man or an animal, as completely and quickly as possible, fit for the performance of a given work (Lagrange, 1889). Although this definition was published more than 120 years ago, the current definition for training in the Oxford dictionary differs very little with the verb to train described as to make or become physically fit through a course of exercise and diet. The constancy in the definition of training is perhaps the least surprising realisation arising from the consideration of the history of training. Far more surprising and humbling is the realisation that many of the stipulations made of athletes today differ principally in terminology rather than meaning to those made centuries or even millennia ago, as exemplified in the extract above. Our knowledge and understanding of sport, exercise and training, and the underlying physiology, have nonetheless been revolutionised since the time of the Greek Empire when athletics were considered a supreme duty through which to approach the divine, a revolution largely occurring since the re-introduction of the Olympic Games in 1896. Since this time there have been many landmark events such as the Nobel prize winning works of Archibald Hill and Otto Meyerhof, all of which can be considered to have contributed to the remarkable feats achieved by modern day Olympians such as the 14 times gold medallist, Michael Phelps, whose training regime is certain to be a long way from that shown in Figure 1.1 detailing the preparation for a rowing "match" in the 19th century.

A feature common to the vast majority of research investigating training and performance parameters is the concentration on adult populations, a focus largely attributable to the

discouraging nature of the additional challenges facing researchers investigating paediatric physiology. The consequence of this focus is a dearth of information regarding training and performance in paediatric populations compared to adults, with questions as fundamental as whether children are physiologically trainable and, if so, how this trainability manifests itself remaining equivocal (Baxter-Jones & Mundt, 2007; Tolfrey, 2007).

**Figure 1.1** An excerpt from the *Physiology of Bodily Exercise* describing a training regime followed by a young male participant to prepare for a forthcoming rowing match (Lagrange, 1889).



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1 p.m. dinner, composed of (lean) roast beef or mutton under-done, or well-done, according to taste, green vegetables, three quarters of a pint of old ale, a small quantity of stale bread.
2 p.m. to 4 p.m. to walk at the rate of three miles an hour.
5 p.m. half a pint of tea, dry bread or toast, two raw eggs, beaten up in the tea, or else two soft-boiled eggs.
6 p.m. to 8 p.m. to walk at the rate of three miles an hour.
9 p.m. supper, half a pint of old ale with a piece of dry bread ; to walk gently for half an hour.
10 p.m. to go to bed.
Same regimen for three days.
*April 4th.* To get up at 6 a.m. ; to walk gently for half an hour.
8 a.m. breakfast as before.
10 a.m. to dress in several complete suits of flannel for loss of weight (wasting) ; *to walk for two hours as quickly as possible, and to take a shower-bath immediately on returning, perspiration being at its maximum.* A small glass of sherry or a gill of old ale, if thirst is unbearable.
1.30 p.m. dinner as before.
2.30 p.m. to 4 p.m. to walk at a moderate pace (three miles an hour).
4 p.m. to row two miles.
5.30 tea as before.
*April 5th.* Result of the course of wasting : *a loss of weight of 8 lbs.*
Exercise before breakfast, breakfast as before.
11.30 a.m. to row two miles, followed by a cold shower-bath.
12.30 p.m. a glass of sherry, or a gill of old ale.
1.30 p.m. dinner as before.
2.30 p.m. to 4 p.m. to walk quickly.
4 p.m. to row one mile *as quickly as possible.*
5.30 p.m. tea.
9 p.m. supper.
10 p.m. to go to bed.

A key tenet within paediatric physiology is that children are not mini-adults and their responses are not necessarily simply scaled down versions of adult responses (Armstrong & Welsman, 2002). Consequently the knowledge gained from the extensive research investigating training induced adaptations in adults has a very limited applicability within paediatric populations. Participation in youth sports is increasing (Anderson *et al.*, 2000; Baxter-Jones & Mundt, 2007) along with the duration, frequency and intensity of the training believed to be associated with success in these sports, whilst the age at which these

training regimes are commenced continues to decrease. Such trends emphasise the importance of increasing our understanding of the interactions between training and physiological adaptations throughout growth and maturation. Understanding these interactions may also provide an insight into the dose-response relationship thereby allowing training programmes to be tailored and orientated to child athletes. Appropriate tailoring of training programmes to paediatric populations could have significant implications on the motivation and enjoyment of participants and, consequently, on the youth drop-out rate.

A paradoxical trend in youth populations is the general decrease in physical activity and the "obesity epidemic" which considerable effort is focused on resolving (Twisk, 2007). However, the data behind these efforts are incomplete so the guidelines continue to be largely ineffectual. Although intensive training lies on the opposite end of the physical activity scale, information regarding the continuum of physical activity levels can only be beneficial in advancing and improving guidelines and remedying this epidemic.

This thesis investigates the influences of swimming-training status on the physiological responses to exercise in young girls with the intention of furthering knowledge regarding the trainability of children throughout the processes of growth and maturation.

**Chapter 2**

**Review of Literature**

*2.1 Parameters of aerobic fitness*

Aerobic fitness can be defined as the ability to deliver oxygen to the working muscles and to utilise this oxygen to provide energy during exercise. Aerobic fitness is therefore dependent on the interaction of the pulmonary, cardiovascular and haematological components of oxygen delivery as well as the oxidative mechanisms for oxygen utilisation within the muscle (Tolfrey *et al.*, 1998). Four key parameters have been identified for the assessment of an individual's aerobic fitness: maximal oxygen uptake ($\dot{V}O_2$), the lactate or gas exchange threshold, exercise economy and the kinetics of oxygen uptake (Whipp *et al.*, 1981). In adults, all of these parameters are highly sensitive to training in both healthy (reviews: e.g. Jones & Carter, 2000; Midgley *et al.*, 2006) and diseased populations (reviews: e.g. Ivey *et al.*, 2008; Meka *et al.*, 2008). However, considerably less information is available regarding the influence of training on these parameters throughout the processes of growth and maturation. The following sections discuss the evidence available detailing the influence of training on these four parameters in paediatric populations and aim to contextualise this information with regard to the more substantial information available in adult populations.

*2.1.1 Maximal and peak oxygen uptake*

The concept of a maximal oxygen uptake ($\dot{V}O_2$ max) has been one of the defining paradigms in exercise physiology for more than 75 years (Welsman *et al.*, 1997) and is generally considered to be the gold-standard measurement for the assessment of aerobic fitness (Rowland, 1990). $\dot{V}O_2$ max was first described by Hill and Lupton (1923) as "the oxygen uptake attained during maximal exercise intensity that cannot be increased despite further increases in exercise workload" and has since become the most researched variable in paediatric exercise science (Armstrong & Fawkner, 2007).

A principal criterion of the conventional $\dot{V}O_2$ max concept is the attainment of a plateau in oxygen uptake, a phenomenon rarely observed in children regardless of the leniency of the criteria used to define the attainment of a plateau (Astrand, 1952; Rivera-Brown *et al.*, 1992; Armstrong *et al.*, 1996). Early studies suggested the absence of a $\dot{V}O_2$ plateau may be related to lower levels of motivation or a decreased anaerobic capacity in children compared to adults (Paterson *et al.*, 1981; Rowland, 1989; Armstrong & Welsman, 1994; Howley *et al.*, 1995). However, more recent studies have cast doubt on these early suggestions, showing no relationship between peak heart rates, peak blood lactate concentrations or peak $\dot{V}O_2$ and the presence or absence of a $\dot{V}O_2$ plateau (Armstrong *et al.*, 1996; Armstrong & Welsman, 1997; Barker *et al.*, 2009). Consequent to the frequent absence of a $\dot{V}O_2$ plateau, the highest $\dot{V}O_2$ achieved during a progressive exercise test to exhaustion in children is commonly referred to as the peak $\dot{V}O_2$.

To aid in the determination of whether the peak $\dot{V}O_2$ achieved in a test is representative of a true exhaustive effort a number of secondary criteria have been proposed. The secondary criteria suggested for children include a respiratory exchange ratio (RER) in excess of 1.0 for treadmill exercise or 1.05 for cycle exercise, a peak heart rate in excess of 95% age predicted maximum or 195 beats·min$^{-1}$ and subjective signs of maximal effort such as facial flushing, hypernoea and sweating (Stratton & Williams, 2007). However, even when these secondary criteria are met it is argued that the only way to determine if a true maximum effort and therefore peak $\dot{V}O_2$ has been attained is to complete a supramaximal exercise test after the incremental test (Barker *et al.*, 2009). If during this test, conducted at 105% of the maximum work rate achieved during the incremental test, $\dot{V}O_2$ does not increase significantly from the value obtained in the incremental test, a true maximal effort can be considered to have been given.

Although the considerable amount of research concerning peak $\dot{V}O_2$ may suggest otherwise, it is important to highlight the limited practical applicability of peak $\dot{V}O_2$, especially within training and performance environments as it cannot be used as predictor of future performance (Matos & Winsley, 2007) or for the design of "optimal" training programmes (Bosch, 2006; Jones, 2006). Furthermore, peak $\dot{V}O_2$ appears to demonstrate a

limited adaptability to training, even in adults, as shown by studies which were unable to discriminate between individuals of homogenous performance on the basis of peak $\dot{V}O_2$ results (Wasserman *et al.*, 1973; Impellizzeri *et al.*, 2005; Meyer *et al.*, 2005). In contrast, even in the presence of a stable peak $\dot{V}O_2$, other response parameters identified as determinants of aerobic fitness may be altered by training as has been reported for both Paula Radcliffe (Jones, 2006) and Lance Armstrong (Coyle, 2005). It is therefore important to consider all of the parameters of aerobic fitness as opposed to focusing solely on peak $\dot{V}O_2$ as much of the literature available concerning children has done.

*2.1.1.1 Influence of training on peak $\dot{V}O_2$ in adults*

Aerobic training in adults results in significant improvements in peak $\dot{V}O_2$, with typical increases of 15-30% following training in an originally relatively sedentary participant (Rowell, 1993). A large inter-participant variation is present in the magnitude of the training-induced improvements, a variation at least partially attributable to genetic factors predisposing some participants to greater gains than others (Bouchard *et al.*, 1992). Other causes of this variation are the initial level of peak $\dot{V}O_2$, which demonstrates an inverse relationship with the training-induced improvement in peak $\dot{V}O_2$ (Rowell, 1993), and the quantity and quality of the training undertaken, which will influence the improvements in peak $\dot{V}O_2$ in a dose-response manner (at least until a certain point after which no further increases in peak $\dot{V}O_2$ occur) (Londeree, 1986; Mahon, 2008).

*2.1.1.2 Influence of training on the peak $\dot{V}O_2$ in children*

Many early studies investigating the influence of training on childrens peak $\dot{V}O_2$ responses provided more confusion than clarity due to methodological limitations. These methodological limitations included inappropriate training programmes, a lack of a comparable control group and/or a testing modality unspecific to the training undertaken. Consequently, and in concert with the prevailing assumptions of the time, pre-pubertal children were largely found to demonstrate minimal or no training-induced increases in peak $\dot{V}O_2$ (e.g. Kobayashi *et al.*, 1978; Mirwald *et al.*, 1981; Welsman *et al.*, 1996;

Welsman *et al.*, 1997; Tolfrey *et al.*, 1998). In contrast, more recent, methodologically sound studies indicate that pre-pubertal and pubertal children do demonstrate training-induced increases in peak $\dot{V}O_2$ (e.g. Baquet *et al.*, 2002; Nottin *et al.*, 2002b; Obert *et al.*, 2003; Rowland *et al.*, 2009a). The extent to which peak $\dot{V}O_2$ increases following training remains controversial but a recent meta-analysis suggests an increase of 5-6% is typical in children when data are appropriately analysed (Baquet *et al.*, 2003). Similar to findings in adults, there is a large inter-participant variation in the magnitude of peak $\dot{V}O_2$ increases following training, the majority (52%) of which is suggested to be related to the pre-training peak $\dot{V}O_2$ (Mahon, 2008).

The reason for the smaller improvements in peak $\dot{V}O_2$ following training in children compared to adults is unclear but may be related to the habitual level of physical activity and/or to the hormonal milieu present. It is proposed that the habitual level of physical activity and the degree of improvement observed following training may be related due to the overload principle. This principle requires that the amount of exercise performed in a training programme must exceed what the participant is accustomed to in order for adaptation to occur (Mahon, 2008). Therefore, if children exhibit a higher level of habitual physical activity than their adult counter-parts but a similar training programme is employed for both, as has frequently been done, smaller improvements would be expected in children following training. This explanation is both supported (Rowland & Boyajian, 1995) and refuted in the literature (Tolfrey *et al.*, 1998). Alternatively, a popular hypothesis to explain the child-adult disparity in training-induced improvements in peak $\dot{V}O_2$ is the "trigger hypothesis" which was first suggested by Katch (1983). He suggested that there may be a critical point during puberty below which the effects of exercise training will be minimal or will not occur at all, a viewpoint supported by others (Kobayashi *et al.*, 1978; Mirwald *et al.*, 1981; Rowland, 1985; Mercier *et al.*, 1987; Rowland, 1992; Payne & Morrow, 1993). The physiological mechanisms for this phenomenon were suggested to be changes in the hormonal environment, specifically androgens and growth hormones, which are associated with the "initiation of puberty and that influence functional development and subsequent organic adaptations" (Katch, 1983). This hypothesis led to suggestions that there may be a "golden period" during which the influence of training is maximised, a period originally proposed to be the pubertal years. However, although frequently proposed

as an explanation, there is little evidence for a maturational threshold. In contrast, evidence against a maturational threshold has been provided by a training intervention study involving groups of identical twins differing in pubertal status. In this study in which one of each of the identical twins completed a 10 week training programme whilst the other twin acted as a control, the largest improvements in peak $\dot{V}O_2$ were observed in the pre-pubertal trained twins (Weber, 1976). Further work is clearly required to resolve the mechanisms responsible for the smaller increases in peak $\dot{V}O_2$ consequent to training in children and to establish whether a "golden period" for the development of aerobic adaptations exists.

*2.1.1.3 Mechanistic basis of training influences*

The Fick equation dictates that the training-induced increases in peak $\dot{V}O_2$ must be related to an increased cardiac output ($\dot{Q}$) and/or an enhanced arteriovenous oxygen difference ($a\text{-}\bar{v}O_2$ difference). In adults, $\dot{Q}$ is increased following training due solely to an increased stroke volume (SV) as maximal heart rate (HR) is generally unchanged (Clausen, 1977; Blomqvist, 1983; Poole & Gaesser, 1985; Phillips *et al.*, 1995; Daussin *et al.*, 2007) (although some studies suggest maximal heart rate may decrease (Ekblom *et al.*, 1968; Rivera-Brown *et al.*, 1992; Goodman *et al.*, 2005)). This increased stroke volume is suggested to be related to both morphological adaptations, such as an increased left ventricular mass and volume and an increased intraventricular and posterior wall thickness, and to functional adaptations reflected by an increased preload (e.g. George *et al.*, 1991; DiBello *et al.*, 1996; Caso *et al.*, 2000; Stickland *et al.*, 2006; Venckunas *et al.*, 2008).

The $a\text{-}\bar{v}O_2$ difference is the amount of oxygen extracted from the arterial blood (i.e. fractional oxygen extraction), therefore indicating the balance between oxygen delivery and utilisation. This parameter can be estimated using near-infrared spectroscopy which measures the deoxygenated haemoglobin and myoglobin signal ([HHb]) (DeLorey *et al.*, 2003; Grassi *et al.*, 2003; Ferreira *et al.*, 2005). In adults, training-induced increases in peak $\dot{V}O_2$ are also related to an enhanced $a\text{-}\bar{v}O_2$ difference, an adaptation evident in both the elevated peak values (Volianitis *et al.*, 2004; Daussin *et al.*, 2007) and the rightward displacement of the [HHb] response pattern relative to work rate (Boone *et al.*, 2009). This

enhanced fractional oxygen extraction is facilitated by an increased mitochondrial density and oxidative enzyme activity (Holloszy, 1967; Mogensen *et al.*, 2006), and possibly by differences in muscle fibre type distribution and capillarity (Boone *et al.*, 2009).

In children, empirical evidence indicates that training-induced increases in peak oxygen uptake are similarly related to an enhanced stroke volume, and consequently cardiac output, as maximal heart rate is unaffected by training (Rowland *et al.*, 2000; Nottin *et al.*, 2002b; Obert *et al.*, 2003). There are a number of conflicting hypotheses regarding the mechanistic basis of this enhanced stroke volume in children but the current consensus is morphological rather than functional adaptations of the myocardium (Rowland *et al.*, 2009b). Morphological adaptations typically reported in children are an increased left ventricular dimension and mass (Nottin *et al.*, 2004; Rowland *et al.*, 2009a) with an increased intraventricular and posterior wall thickness also suggested (Ayabakan *et al.*, 2006), although the latter adaptations remain controversial (Nottin *et al.*, 2004; Obert *et al.*, 2009; Rowland *et al.*, 2009a). Since all of the aforementioned morphological adaptations occur in adults following training (e.g. George *et al.*, 1991; Caso *et al.*, 2000) there appears to be an age-related progression in morphological training adaptations. This age-related disparity may be associated with a biological immaturity such as a lack of testosterone (Rowland *et al.*, 1994; Obert *et al.*, 2003), thereby agreeing with the trigger hypothesis postulated by Katch (1983), or to an overload limitation either due to the lower blood pressures present in children during exercise (Nottin *et al.*, 2002b) or the shorter duration of training undertaken by children (Nottin *et al.*, 2004).

Support for morphological rather than functional adaptations is provided by studies demonstrating an enhanced stroke volume at rest and a similar response pattern in trained and untrained children (Rowland *et al.*, 2000; Nottin *et al.*, 2002b; Obert *et al.*, 2003; Rowland *et al.*, 2009a). This commonly observed response pattern is an initial rise in SV due to the mobilisation of the blood pooled in the lower extremities due to gravity (Rowland, 2009; Rowland *et al.*, 2009a) followed by a plateau at around 40-50% peak $\dot{V}O_2$ until exhaustion. Alternatively, however, stroke volume has been suggested to progressively increase until maximal exercise intensities in both trained children (Rowland *et al.*, 1998) and trained adults (Crawford *et al.*, 1985; Ferguson *et al.*, 2001; Warburton *et*

*al.*, 2002), suggesting functional adaptations also occurred in response to training. The stroke volume pattern in response to exercise and the mechanistic basis of the enhanced stroke volume generally reported in children after training therefore remain to be determined conclusively.

The other explanatory variable of peak $\dot{V}O_2$ according to the Fick equation, the $a\text{-}\bar{v}O_2$ difference, does not appear to be influenced by training in children, either at rest or at maximal exercise intensities (Rowland *et al.*, 2000; Nottin *et al.*, 2002b; Obert *et al.*, 2003). However, the pattern of the $a\text{-}\bar{v}O_2$ difference response between these two points has received very little attention in children, despite the insight it provides into the balance between oxygen delivery and utilisation. The limited available evidence suggests the $a\text{-}\bar{v}O_2$ difference increases progressively in response to exercise in healthy, untrained children (Rowland *et al.*, 1997; Turley & Wilmore, 1997a, b; Nottin *et al.*, 2002b) but no information is available concerning the influence of training on this pattern.

In conclusion, peak $\dot{V}O_2$ appears to be influenced by training in children, although to a lesser degree than in adults. Much work remains to determine the factors responsible for the smaller increase in children as well as to determine conclusively the mechanisms responsible for the enhanced peak $\dot{V}O_2$. Areas which particularly warrant further investigation are the stroke volume and $a\text{-}\bar{v}O_2$ difference response patterns and the influence of training on them.

### *2.1.2 Lactate or gas exchange threshold*

The lactate threshold (LT), determined during an incremental test, refers to the metabolic rate at which the blood lactate concentration first begins to increase above baseline levels. Originally attributed to an appreciable increase in the anaerobic contribution to the energy production, it is now accepted rather to reflect alterations to the cellular redox and phosphorylation potentials that drive mitochondrial respiration (Connett *et al.*, 1990). The onset of blood lactate accumulation is accompanied by bicarbonate buffering and, consequently, by the generation of non-metabolic carbon dioxide ($CO_2$), which causes an

increase in carbon dioxide output ($\dot{V}CO_2$) and, therefore, minute ventilation ($\dot{V}_E$). Aimed at maintaining blood gas and pH homeostasis (Davis, 1985), this ventilatory response also provides a non-invasive approach to determining the lactate threshold by consideration of the respiratory gas concentrations. A number of methods have been proposed but perhaps the most commonly used is that proposed by Beaver et al. (1986) in which the respiratory gas equivalent of the lactate threshold is referred to as the gas exchange threshold (GET). However, although a close link has long been postulated between the lactate and gas exchange thresholds, controversy continues regarding the underlying physiological mechanisms with the process described above only one of many suggested mechanisms (Wasserman *et al.*, 1973; Dickhuth *et al.*, 1999; Meyer *et al.*, 2005; Peronnet & Aguilaniu, 2006). The GET (or LT) generally occurs at 45-60% peak $\dot{V}O_2$ in adults (Jones & Poole, 2005a) and 45-70% peak $\dot{V}O_2$ in children (e.g. Cooper & Weiler-Ravell, 1984; Paterson *et al.*, 1987; Fawkner & Armstrong, 2002; Fawkner & Armstrong, 2004b, a).
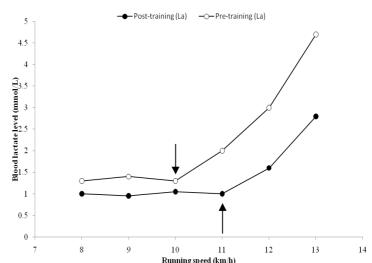
The GET is relevant within a training and performance environment due to its applicability in the design and optimisation of training programmes to an individual. The GET has been suggested to represent the optimal training intensity for improvements in endurance fitness (Weltman *et al.*, 1990; Mader, 1991), providing a high quality aerobic training stimulus without the accumulation of blood lactate which would compromise training duration (Macdougall, 1977; Weltman, 1989). Given more detailed investigation, the GET could therefore be very useful in the optimisation of training programmes to an individual, providing a scientific basis to the training regime rather than the historical and personal experience basis which is currently widely employed.

*2.1.2.1 Influence of training in adults*

In adults, the gas exchange threshold (or LT) has been shown to be a strong predictor of endurance performance (Farrell *et al.*, 1979; Tanaka & Matsuura, 1984; Coyle *et al.*, 1991; Jones & Doust, 1998) and to be highly sensitive to training which causes an increase in the percentage of peak $\dot{V}O_2$ at which the GET occurs (Davis *et al.*, 1979; Fouquet & Poty, 1982; e.g. Carter *et al.*, 1999; Fukuoka *et al.*, 2002; Kilding & Jones, 2008). Similarly, the lactate concentration at which the lactate threshold occurs is also increased by endurance

training, as illustrated in figure 2.1. This improved GET (or LT) allows a higher relative exercise intensity to be sustained without the accumulation of blood lactate, thereby delaying the onset of fatigue. Although it remains controversial, fatigue has been suggested to be associated with blood lactate accumulation either through the effects of metabolic acidosis on contractile function (Sahlin, 1992) or through an accelerated depletion of muscle glycogen (Boyd *et al.*, 1974). An increased GET is therefore a clear indicator of an enhanced endurance capacity (Jones & Carter, 2000) and may be related to a decreased rate of lactate production (Favier *et al.*, 1986). Lactate production may be reduced because of a lower rate of muscle glycogen utilisation (Saltin & Karlsson, 1971; Fitts *et al.*, 1975; Saltin *et al.*, 1976; Favier *et al.*, 1986) or due to faster oxygen uptake kinetics which would reduce the reliance on non-oxidative glycogen utilisation. Alternatively, the increased GET may be due to a training-induced improvement in the ability to eliminate lactate from the blood (Donovan & Pagliassotti, 1989; Freund *et al.*, 1992; MacRae *et al.*, 1992; Bonen *et al.*, 1997) by an increased uptake and oxidisation of lactate in the liver and muscles (Sumida *et al.*, 1993), enabled by the increased oxidative enzyme capacity of trained participants (Stallknecht *et al.*, 1998).



**Figure 2.1** Typical blood lactate response pre and post a 6 week training intervention in adults. The lactate threshold, denoted by the vertical arrows, occurs at a higher running speed following training. Adapted from Carter et al. (1999).

*2.1.2.2 Influence of training in children*

There is a paucity of information with respect to the influence of training on the GET in children. Furthermore, the information available is contradictory, with some suggesting that the GET is unaltered by training in children (Obert *et al.*, 2000; Cleuziou *et al.*, 2002) and

others suggesting the GET is increased (Paterson *et al.*, 1987; Mahon & Vaccaro, 1989; Haffor *et al.*, 1990). This discrepancy may have arisen due to assumptions on which some authors' conclusions were based. For example Paterson and colleagues (1987), who conducted a 5 year longitudinal study and reported the ventilatory threshold as a fraction of peak $\dot{V}O_2$ to increase in a cohort of boys from 11-15 years, attributed this increase to training. However, caution is advisable when considering this conclusion as no control group was studied and consequently the influence of growth and maturation cannot be accounted for. Furthermore, these authors' conclusions are based on evidence suggesting ventilatory thresholds as a fraction of peak $\dot{V}O_2$ decrease with age (Cooper *et al.*, 1984; Gaisl & Buchberger, 1984; Kanaley & Boileau, 1988), evidence refuted by more recent studies (Rowland & Green, 1988; Mahon *et al.*, 1997; Mahon *et al.*, 1998). The relationship between the GET and performance also remains unclear in children as some results indicate a similar correlation to that found in adults (Zanconato *et al.*, 1989; Hebestreit *et al.*, 2000) whilst other results cast doubt on this suggestion (Reybrouck *et al.*, 1985; Paterson *et al.*, 1987). Therefore, although it has previously been concluded that the gas exchange or ventilatory threshold can be reliably determined and studied in children (Mahon & Cheatham, 2002), little further work has been conducted to elucidate the influence of maturation or training on the threshold or, indeed, on the interaction between these two factors.

### 2.1.3 Exercise Economy

Exercise economy can be defined as the oxygen uptake required at a given absolute exercise intensity and therefore describes the relationship between oxygen consumption and exercise velocity or intensity. As exercise economy is not assessed in this thesis, only a brief summary of the influence of training is provided here.

### 2.1.3.1 Influence of training in adults

In adults, performance and exercise economy are closely related (e.g. Conley & Krahenbuhl, 1980; Daniels & Daniels, 1992; Diprampero *et al.*, 1993), with exercise economy proposed to provide a better indicator of performance than peak $\dot{V}O_2$ for elite and

near-elite runners (Costill *et al.*, 1973; Morgan *et al.*, 1989). An enhanced exercise economy (i.e. a lower $\dot{V}O_2$ for a given absolute speed or exercise intensity) is advantageous because it enables the energy demands of a given exercise intensity to be met by a lower percentage of peak $\dot{V}O_2$, thereby delaying the onset of fatigue and increasing exercise tolerance. However, whilst training is accepted to increase exercise economy in adults, less clear is the type and duration of the training required to elicit such responses.

*2.1.3.2 Influence of training in children*

Exercise economy has been investigated in children for decades, with the earliest work dating back to a study by Astrand in 1952 who reported age and sex differences in the running economy of children, with younger children and girls having lower exercise economy values (higher $\dot{V}O_2$ for a given running speed) than older children and boys respectively. However, the longevity of research into children's exercise economy is misleading as relatively few studies have been conducted in this time and consequently there remains a paucity of information about the influence of training on exercise economy in children. What little information there is provides little clarity, and almost exclusively considers running economy (e.g. Daniels *et al.*, 1978; Petray & Krahenbuhl, 1985; Unnithan *et al.*, 1996; Baquet *et al.*, 2002; Unnithan *et al.*, 2009).
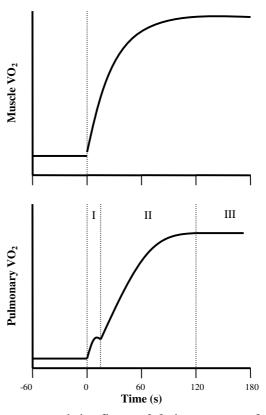
**2.1.4 Oxygen uptake kinetics**

Pulmonary oxygen uptake ($\dot{V}O_2$) kinetics following the onset of constant-work-rate exercise provide a useful assessment of the integrated capacity of the organism to transport and utilize $O_2$ to support the increased rate of energy turnover in the contracting myocytes (Whipp & Ward, 1990). The onset of exercise or an increase in exercise intensity engenders an elevated energetic demand. Transiently, this is met by intramuscular phosphocreatine (PCr) degradation and the anaerobic catabolism of glycogen. However, $\dot{V}O_2$ simultaneously rises in an exponential manner (Hill & Lupton, 1923; Henry, 1951; Mahler, 1980), thereby enabling a progressively greater proportion of the energetic demand to be met by oxidative phosphorylation.

The pulmonary $\dot{V}O_2$ response following the onset of exercise has been extensively characterised across many different exercise modalities (Linnarsson, 1974; Whipp *et al.*, 1982). The three-phase pulmonary $\dot{V}O_2$ response, first reported by Whipp and Wasserman (1972), consists of an initial rapid increase in $\dot{V}O_2$ at the onset of exercise that is typically initiated within the first breath (Jones & Poole, 2005a). During this phase, termed the cardiodynamic phase or phase I, pulmonary and muscle $\dot{V}O_2$ are temporally dissociated due to the muscle-lung transit delay. Furthermore, the amplitude of the $\dot{V}O_2$ at the muscle and mouth differ because of the influence on pulmonary $\dot{V}O_2$ of oxygen stores within the body as well as an increasing cardiac output (Whipp & Ward, 1990; Whipp *et al.*, 2005). The dissociation between muscle and pulmonary $\dot{V}O_2$ is evident in the response profiles as the cardiodynamic phase is only present in the pulmonary $\dot{V}O_2$ response; muscle $\dot{V}O_2$ increases in a mono-exponential fashion from the onset. Provided this initial cardiodynamic phase is appropriately accounted for, the subsequent muscle and pulmonary $\dot{V}O_2$ responses demonstrate a close agreement in both the time course (represented by the time constant, $\tau$) and amplitude (both primary and slow component) of the $\dot{V}O_2$ response (within +/- 10%: Barstow *et al.*, 1990; Grassi *et al.*, 1996; Krustrup *et al.*, 2009).

The cardiodynamic phase of the pulmonary $\dot{V}O_2$ response is succeeded by an exponential increase in $\dot{V}O_2$, known as phase II, which drives the $\dot{V}O_2$ towards the actual or originally projected steady state. The rate at which this exponential increase in $\dot{V}O_2$ occurs is described by the time constant ($\tau$), which reflects the time taken to achieve 63% of the phase II response. During exercise below the GET (moderate intensity exercise), this phase II exponential increase in $\dot{V}O_2$ is followed by the attainment of a steady state (phase III) where the oxygen demands and utilisation are matched. Figure 2.2 illustrates the markedly different response profiles of muscle and pulmonary $\dot{V}O_2$ to moderate intensity exercise.

**Figure 2.2** Schematic illustration of the rise in muscle and pulmonary oxygen uptake at the onset of moderate intensity exercise. Note the mono-exponential rise of muscle $\dot{V}O_2$ in comparison to the three phase response of pulmonary $\dot{V}O_2$. The vertical dashed line at $t = 0$ represents the onset of increased work rate. Subsequent dashed vertical lines denote the transition between phases of the response. See text for details of the three phases.

The response pattern illustrated in the lower panel in figure 2.2 is accurate for the description of the pulmonary $\dot{V}O_2$ response to moderate intensity exercise only. Moderate intensity exercise refers to exercise intensities below the GET or LT (see 2.1.2 "Lactate and gas exchange thresholds" for details). In this domain a steady state is achieved in approximately 2-3 minutes and the oxygen cost ("gain") of exercise is typically 9-11 ml·$O_2$.min$^{-1}$·W$^{-1}$ (Jones & Poole, 2005a).

Exercise intensities above the GET but below critical power or the maximum lactate steady state (MLSS) are described as within the heavy intensity domain. This domain is characterised by an elevated but stable blood lactate concentration with the highest blood lactate concentration at which the rate of appearance and removal of lactate are still equilibrium termed the MLSS. Alternatively, the upper boundary of the heavy intensity domain is suggested to be demarcated by critical power (CP) which is the asymptote of the power-duration curve and theoretically describes the highest intensity of exercise that can be sustained "indefinitely". The actual duration of exercise that can be sustained at critical power is controversial, with suggestions it may only be tolerable for 15-40 minutes (Housh *et al.*, 1989; Jenkins & Quigley, 1990). CP and the MLSS are broadly equivalent measures

(Poole *et al.*, 1988; Poole *et al.*, 1990; Vandewalle *et al.*, 1997), occurring at work rates of ~50%Δ in adults (50% of the difference between the GET and peak $\dot{V}O_2$, known as the "delta concept"), although some reports suggest CP may occur at a slightly but significantly higher work rate than the MLSS (Pringle & Jones, 2002; Dekerle *et al.*, 2003). In children, the critical power and MLSS are reported to occur at 65-80% peak $\dot{V}O_2$ (Fawkner & Armstrong, 2002; Fawkner & Armstrong, 2003b), however there is a considerable range especially within MLSS values, a range suggested to be methodological rather than metabolic in nature (Beneke *et al.*, 1996).
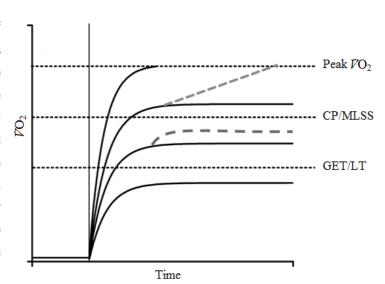
Heavy intensity exercise is further characterised by the presence of a slow component which becomes apparent after 90-150 seconds (Barstow & Mole, 1991; Paterson & Whipp, 1991). The slow component delays the attainment of a steady state until 10-15 minutes or more from exercise onset, depending on the exercise intensity and aerobic fitness of the participant. Although it remains debated whether the slow component begins at the onset of exercise (Linnarsson, 1974; MacDonald *et al.*, 1997) or develops after approximately two minutes (Barstow & Mole, 1991; Paterson & Whipp, 1991; Bearden & Moffatt, 2000), it is clear that the influence of the slow component is to elevate the $\dot{V}O_2$ above rather than towards the steady state projected from extrapolation of the response to moderate intensity exercise. Whether a slow component is present in children's responses to heavy intensity exercise has been a matter of debate, with early work suggesting no slow component was present (Armon *et al.*, 1991; Williams *et al.*, 2001). In contrast, more recent work has generally shown a slow component to be evident, albeit of a reduced amplitude compared to adults (Obert *et al.*, 2000; Fawkner & Armstrong, 2003a, 2004b).

Exercise above the CP/MLSS boundary lies within the severe intensity domain where $\dot{V}O_2$ rises until it reaches peak $\dot{V}O_2$ and blood lactate concentration rises inexorably. The presence of a slow component during severe intensity exercise depends on the relative exercise intensity within this domain; at the very highest work rates a slow component may not occur because $\dot{V}O_2$ rises in a near exponential nature to be truncated by the attainment of peak $\dot{V}O_2$. The primary component gain associated with this domain falls below the typical 9-11 ml·$O_2$·min$^{-1}$·W$^{-1}$ for moderate and heavy intensity exercise (Jones *et al.*, 2002; Pringle *et al.*, 2003; Scheuermann & Barstow, 2003).

The highest exercise intensity domain, the extreme exercise domain, refers to exercise intensities at which fatigue occurs before peak $\dot{V}O_2$ can be attained (Hill *et al.*, 2002). Due to the short duration of exercise within this domain (typically less than four times the time constant describing the exponential rise during the primary phase) no slow component is present and blood lactate concentration is generally lower than observed during severe intensity exercise.

The exercise intensity domains and their characteristic response patterns are illustrated in figure 2.3.
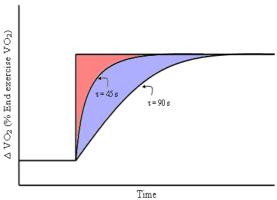
**Figure 2.3** Schematic illustration of the characteristic pulmonary $\dot{V}O_2$ responses to a square wave transition within each exercise intensity domain. The solid vertical line represents the onset of the increased work-rate and the dotted horizontal lines represent the physiological demarcating boundaries as described in the text. Phase I has been omitted from the illustration. The slow component and its characteristics are represented by the dashed lines. Adapted from Armstrong and Barker (2009).



The gain or oxygen cost of the primary component has widely been considered to remain relatively constant at ~10 ml·$O_2$·min$^{-1}$·W$^{-1}$ irrespective of the exercise intensity, with the slow component representing an elevated oxygen cost of exercise that is superimposed on the primary component. However, more recent works have shown the primary component gain to vary with exercise intensity; specifically, the primary component gain is reported to decrease with increasing exercise intensities. This decreased oxygen cost per unit work rate at higher exercise intensities means the oxygen cost of exercise does not increase linearly with exercise intensity.

A common feature regardless of the exercise domain is the lag between the instantaneous increase in energy demand and the increase in $\dot{V}O_2$ during which the initial adenosine triphosphate (ATP) demand must be met by the non-oxidative pathways of intramuscular phosphocreatine (PCr) degradation and anaerobic glycolysis. The oxygen equivalent of the energy derived from such sources during this lag, illustrated in figure. 2.4, is termed the oxygen deficit.



**Figure 2.4** Schematic illustration of the oxygen deficit and the influence of altering the speed of the $\dot{V}O_2$ kinetic response. $\tau$ is the time constant of the primary phase and the coloured area represents the oxygen deficit. Note how the shaded area increases as the $\dot{V}O_2$ response slows (increasing $\tau$).

*2.1.4.1 Influence of training in adults*

In adults, endurance training has been shown to be a potent stimulus to the $\dot{V}O_2$ kinetic response, resulting in reductions in both the phase II time constant ($\tau$) and the amplitude of the subsequent $\dot{V}O_2$ slow component (e.g. Powers *et al.*, 1985; Phillips *et al.*, 1995; Carter *et al.*, 2000; Jones & Koppo, 2005; Bailey *et al.*, 2009), adaptations which are favourable to exercise tolerance. The decreased depletion of high energy phosphates at the onset of exercise in trained participants is in agreement with a smaller oxygen deficit (Karlsson *et al.*, 1972). Such adaptations are important as they indicate a deceased intracellular perturbation and are suggested to be conducive to an increased time until exhaustion (Poole & Richardson, 1997; Demarle *et al.*, 2001). No influence of training has been reported on the primary component gain in adults (Overend *et al.*, 1992; Carter *et al.*, 2000; Berger *et al.*, 2006; Bailey *et al.*, 2009).

*2.1.4.2 Influence of training in children*

The $\dot{V}O_2$ kinetics of children have not been as comprehensively studied and the influence of endurance training on $\dot{V}O_2$ kinetics in this population remains unclear (Fawkner &

Armstrong, 2003a). The available data suggest that training neither reduces τ (Obert *et al.*, 2000; Cleuziou *et al.*, 2002) nor reduces the amplitude of the $\dot{V}O_2$ slow component (Obert *et al.*, 2000) in pre-pubertal children. No conclusions can currently be drawn regarding the influence of training on the oxygen deficit or on primary component gain as these parameters have not previously been reported in trained pre-pubertal children. Information detailing the influence of training status on the $\dot{V}O_2$ kinetics in pubertal populations is even sparser than in pre-pubertal populations, with only one study previously conducted. This study found the phase II τ to be faster but the primary component gain to be unaltered in trained adolescents during moderate intensity exercise (Marwood *et al.*, 2010).

The lack of information regarding $\dot{V}O_2$ kinetics in children is likely related to a number of methodological issues which hinder their investigation, first and foremost of which is the low signal-to-noise ratio which is detrimental to the confidence associated with the model derived parameters, especially the time constant (τ) of the primary component (Barstow & Scheuermann, 2004). A number of factors contribute to this low signal-to-noise ratio, including a small response amplitude and generally erratic breathing pattern due to a greater variability in tidal volume and the timing of breaths than observed in adults (Potter *et al.*, 1999). This low signal-to-noise ratio necessitates careful consideration of the exercise modality investigated, as a modality which stresses a small muscle mass will elicit a smaller response amplitude, thereby exacerbating the influence of the poor signal-to-noise ratio. Although challenging, these limitations do not preclude the accurate characterisation of the $\dot{V}O_2$ kinetic response in children as the signal-to-noise ratio can be significantly improved by averaging repeat transitions to an identical work-rate (Lamarra *et al.*, 1987).

The insensitivity of $\dot{V}O_2$ kinetics to training status in pre-pubertal children suggested by previous studies may be related to several methodological aspects of these studies, such as the use of only single exercise transition to characterize $\dot{V}O_2$ kinetics, the prescription of exercise intensity as a fraction of the peak $\dot{V}O_2$, the use of mixed sex cohorts, or the employment of non-specific ergometry. Accurate standardisation of the exercise intensity domain in which participants are exercising requires both the GET and peak $\dot{V}O_2$ to be considered due to the large inter-individual variation in the fraction of peak $\dot{V}O_2$ at which

the GET occurs in children (Fawkner & Armstrong, 2007). Analysis of mixed sex cohorts may have influenced the results as boys are reported to demonstrate faster $\dot{V}O_2$ kinetics and smaller slow component amplitudes (Fawkner & Armstrong, 2004c). An alternative or additional explanation for the unaltered $\dot{V}O_2$ kinetics may be the discrepancy between test and training modalities, arising, for example, due to pre-pubertal swimmers being tested on a cycle ergometer (Obert *et al.*, 2000; Cleuziou *et al.*, 2002). Considering the predominantly upper body nature of swimming (Ogita *et al.*, 1996), lower body exercise may not be sufficiently specific for the influence of training to be evident. Aside from methodological explanations, the absence of a training status influence in pre-pubertal children may be related to the presence of a maturational threshold below which significant physiological adaptations to training cannot occur (Katch, 1983). The presence of a maturational threshold has been supported by the recent study in adolescents reporting a significant influence of training on their $\dot{V}O_2$ kinetics (Marwood *et al.*, 2010) and by reports of increased concentrations of the testosterone and growth hormone in pubertal children following training but not pre-pubertal children (Zakas *et al.*, 1994; Daly *et al.*, 1998; Tsolakis *et al.*, 2003).
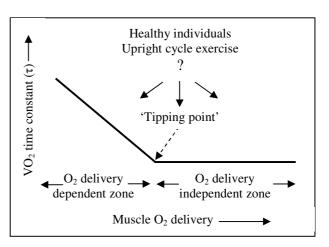
*2.1.4.3 Mechanistic basis of training adaptation in oxygen uptake kinetics*

The rate limiting determinant of the $\dot{V}O_2$ kinetic response continues to be a contentious issue, with oxygen delivery and oxygen utilisation both proposed as putative mediators. Proponents of an oxygen delivery related limitation suggest there is a specific point or site during the transport of oxygen from mouth to mitochondria that acts as a rate-limiter (Poole *et al.*, 2008a), whereas supporters of an oxygen utilisation regulator propose the finite kinetics are attributable to an intrinsic slowness of intracellular oxidative metabolism to adjust to the altered metabolic demand (Payne *et al.*, 1997).

Although there are some exercise modalities which are likely to be predominately oxygen delivery limited, such as supine and prone exercise (Hughson *et al.*, 1991; Jones *et al.*, 2006) and arm exercise above the heart level (Hughson *et al.*, 1996; Koppo & Bouckaert, 2005), the relative roles are rarely so clearly defined. A prime example of the interaction between these potential modulators is evident in disease states such as chronic heart failure

(Sietsema *et al.*, 1994), peripheral vascular disease (Paterson *et al.*, 1981) or type II diabetes patients (Stratton & Williams, 2007). Initially, oxygen delivery was thought to be the limiting factor under these conditions, but evidence now indicates all of these diseases are also associated with defects in skeletal muscle oxidative metabolism (Brass, 1996; Ventura-Clapier *et al.*, 2002; Scheuermann-Freestone *et al.*, 2003). Therefore, the $\dot{V}O_2$ kinetics in these conditions are likely to be modulated by both oxygen delivery and utilisation, an interaction which is probably also influenced by exercise intensity. The relationship between an oxygen delivery and oxygen utilisation related "control" of $\dot{V}O_2$ kinetics has recently been described with reference to a "tipping point", whereby there is a crucial point at which further reductions in oxygen delivery result in a deleterious effect on the speed of the phase II τ, as illustrated in figure 2.5 (Poole & Jones, 2005). The debate concerns where individuals lie with respect to this tipping point and the conditions or interventions which can modify this position.

**Figure 2.5** Schematic depicting the influence of altering muscle $O_2$ delivery on the speed of $\dot{V}O_2$ kinetics. Decreasing $O_2$ delivery (right to left along the x-axis) does not influence the speed of the kinetic response (shown by τ) until a specific "tipping-point". After this point, further decreases in $O_2$ delivery are associated with progressively slower kinetics. From Poole et al. (2008a).



One potential intervention which may alter an individual's position relative to the tipping point (towards the right) is endurance training as this intervention is associated with both an enhanced oxygen delivery, due to an increased muscle blood flow (Shoemaker *et al.*, 1996; Laughlin & Roseguini, 2008), and an enhanced oxygen extraction, due to an increased mitochondrial oxidative capacity (Phillips *et al.*, 1995; Krustrup *et al.*, 2004; Burgomaster *et al.*, 2008). The relative importance of these adaptations to the speeding of the $\dot{V}O_2$ kinetics remains to be conclusively determined, as oxygen delivery (Krustrup *et al.*, 2004)

and oxygen utilisation (Bailey *et al.*, 2009) have both contradictorily been reported to be the most important factors.

Relatively recent technological advances have facilitated the investigation of the mechanistic basis of $\dot{V}O_2$ kinetics in paediatric populations, the investigation of which was previously precluded by the highly invasive nature of the measurement techniques involved, such as muscle biopsies and catheterisation. These newer techniques include near-infrared spectroscopy (NIRS) and heart rate kinetics for the assessment of oxygen extraction and delivery respectively, the latter of which has been reported to reflect cardiac output kinetics (Miyamoto *et al.*, 1982; Yoshida & Whipp, 1994) and muscle blood flow (MacPhee *et al.*, 2005). NIRS measures the relative change in the concentration of oxygenated and deoxygenated haemoglobin and myoglobin within a specific, localised area of the microcirculation and myocytes of the muscle (Boushel *et al.*, 2001). The deoxygenated haemoglobin/myoglobin signal ([HHb]) indicates the balance between oxygen delivery and utilisation, thereby providing an index of fractional oxygen extraction (DeLorey *et al.*, 2003; Grassi *et al.*, 2003; Ferreira *et al.*, 2007), and is used preferentially to the oxyhaemoglobin signal as it is thought to be largely independent of changes in blood volume during exercise (Deblasi *et al.*, 1991; Mancini *et al.*, 1994; Ferrari *et al.*, 1997). The contribution of myoglobin to the signal is thought to be minimal (<10%; Seiyama *et al.*, 1988), but it remains unresolved so although the signal will be referred to as the deoxyhemoglobin signal throughout this document, it should be considered to be the combined concentration of both deoxygenated haemoglobin and myoglobin. The [HHb] response to a square-wave increase in work rate follows a similar response profile to that described for $\dot{V}O_2$. This profile consists of an initial period in which no change occurs (typically lasting 5-10 s in adults) followed by a second period of rapid increase which plateaus out to a relatively constant third phase, at least during moderate intensity exercise. The rapid increase in [HHb] is characterised by a fast time constant of 6-10 s in normal, healthy adults.

Only one study to date has reported the effect of training on the $\dot{V}O_2$ and [HHb] kinetics in adults, finding the training-induced speeding of the $\dot{V}O_2$ kinetics to be associated with an enhanced [HHb] response (Bailey *et al.*, 2009). The enhanced [HHb] response, evidenced

by a faster time delay and mean response time (time delay + time constant) during moderate intensity exercise, a faster $\tau$ during severe intensity exercise and a greater amplitude during both exercise intensities, was suggested to be indicative of an increased oxidative capacity following training. As described above, this study therefore supports oxygen utilisation as the predominant regulator of $\dot{V}O_2$ kinetics.

In spite of these technological advances, there remains a paucity of data regarding the underlying mechanisms responsible for the $\dot{V}O_2$ kinetics in pre-pubertal or pubertal children, an observation which is perhaps unsurprising given the minimal information available regarding $\dot{V}O_2$ kinetics in this population. In the sole study available to date, Marwood et al. (2010) reported that during moderate intensity exercise male adolescent football players had faster heart rate kinetics but unaltered deoxygenated haemoglobin/myoglobin ([HHb]) kinetics compared to untrained subjects. The authors interpreted these results as evidence that training enhanced both muscle $O_2$ delivery and fractional $O_2$ extraction. The mechanistic basis for any differences in $\dot{V}O_2$ kinetics during heavy intensity exercise in adolescents has yet to be investigated. The only other data available suggests that oxidative enzyme activity is increased in children following training (Eriksson *et al.*, 1973; Fournier *et al.*, 1982) but no information is currently available concerning the presence, or absence, of adaptations in muscle blood flow. Considerable research is still required in this area before conclusions can be drawn regarding the relationships between oxygen delivery and utilisation, training and the $\dot{V}O_2$ kinetic response in children.

## *2.2 Parameters of "anaerobic" fitness*

Anaerobic fitness can be described as the ability to support metabolic demands by non-oxidative mechanisms, an ability assessed through high intensity exercise. The most commonly used high intensity exercise test is the Wingate anaerobic test (WAnT) and, consequently, it is this test that will be concentrated on here. Caution is necessary when making inter-study comparisons, however, due to the somewhat bewildering array of terminology used, often indiscriminately, to describe various aspects and parameters of the response

The WAnT was devised by Cumming in 1973 and popularised by Ayalon et al.(1974), Bar-Or (1983a) and Inbar et al. (1996) from the Wingate Institute in Israel. The test originally involved pedalling on a cycle ergometer against a constant braking force at maximal effort for 30-seconds, a test heavily used in the literature. The WAnT protocol is easily adaptable to upper body exercise and studies have been published both in adults (Guglielmo & Denadai, 2000; Balmer *et al.*, 2004) and children (Blimkie *et al.*, 1988; Nindl *et al.*, 1995) assessing maximal intensity arm crank exercise. Several indices of anaerobic power and capacity can be derived from the Wingate test including peak power (PP), mean power (MP) and the fatigue index (FI) which describes the power decay over the test duration. Peak power is thought to reflect the energy generating capacity of high-energy phosphates while mean power is suggested to reflect glycolytic capacity (Inbar & Bar-Or, 1986). However, although originally proposed as an anaerobic test, the conventional 30s WAnT is not purely anaerobic, demonstrating considerable contributions by oxidative phosphorylation to energy provision towards the latter part of the test in both children (Chia, 2006) and adults (Granier *et al.*, 1995; Bediz *et al.*, 1998).

### 2.2.1 Influence of training in adults on anaerobic fitness parameters

Despite the attractiveness of the WAnT due to its short duration and minimal requirements for equipment or expertise, relatively little research has been conducted. This paucity of research may be at least partly attributable to the limited relevance of the derived parameters to people's general health status (Tolfrey, 2008).

#### 2.2.1.1 Mechanical power indices

Peak power and mean power have both been reported to be higher in trained adults (Kounalakis *et al.*, 2008; Kounalakis *et al.*, 2009) and to be increased by a training intervention (Babcock *et al.*, 1986; MacDougall *et al.*, 1998; Kin-Isler & Kosar, 2006; Norkowski & Hucinski, 2007). Furthermore, this influence of training status has been reported for both the upper and lower body (Patton & Duggan, 1987). These studies have also indicated that the specificity of the training programme is not a determinant of

significant influences being observed as both continuous aerobic (Babcock *et al.*, 1986; Kin-Isler & Kosar, 2006) and interval training (Babcock *et al.*, 1986; MacDougall *et al.*, 1998) have been shown to elicit or be associated with significantly higher PP and MP. The influence of training status on the fatigue index has not been extensively researched and remains controversial, with both a significantly lower FI in trained adults (Patton & Duggan, 1987) and no influence of training status reported (Kounalakis *et al.*, 2008).

*2.2.1.2 Oxidative Contribution to total energy expenditure*

As previously mentioned, high intensity tests are not strictly anaerobic, demonstrating a significant oxidative contribution to the total energy expenditure engendered by the 30 s WAnT.  The influence of training on the oxidative contribution to short term, high intensity exercise has not been extensively investigated but it appears that the oxidative contribution may be increased after training (Granier *et al.*, 1995). This increased oxidative contribution is likely to be related to the faster $\dot{V}O_2$ kinetics typically reported in trained participants (e.g. Powers *et al.*, 1985; Carter *et al.*, 2000; Krustrup *et al.*, 2004). An interesting detail of Granier et al.'s study is that higher oxidative contributions were only evident in middle and long distance runners, not sprinters, suggesting the type of training may be an important determinant.

*2.2.1.3 Mechanistic basis*

The limited information in adults shows training to cause a variety of adaptations in anaerobic function, including increased levels of the anaerobic substrates ATP, PCr, free creatine and glycogen as well as an increased quantity and activity of the enzymes responsible for the utilisation of these substrates (phoshofructokinase (PFK), phosphorylase and lactate dehydrogenase) (Linossier *et al.*, 1997; Rodas *et al.*, 2000; Ross & Leveritt, 2001). The higher blood lactate concentrations typically found in trained participants after a short term, high intensity exercise test (Jacobs *et al.*, 1987) support the presence of such adaptations and of an enhanced ability to derive energy from the glycolytic pathways. Depending on the specificity of the training programme, these adaptations may occur with no adaptation in aerobic substrate or enzyme concentration or activity. Such specificity is

exemplified by reports of a higher ATP and creatine concentration in the trained muscles of sprint runners and cyclists compared to their distance counterparts (Neumann, 1990). Somewhat less clear, however, is the relevance of these metabolic adaptations to performance in adults as a number of studies have reported increased substrate and enzyme concentrations without a concomitant improvement in performance (Tesch *et al.*, 1985; Jacobs *et al.*, 1987; Parra *et al.*, 2000). Confusion arises due to other studies demonstrating that sprint performance is highly correlated with both PFK activity and its ratio to citrate synthase activity (Dawson *et al.*, 1998) and, conversely, that sprint performance is improved without an enhanced energy provision from glycolysis per unit muscle mass (Boobis *et al.*, 1983). Therefore, although adaptations in anaerobic metabolism are accepted to occur following training, the functional significance of these adaptations remains questionable (Ross & Leveritt, 2001).

### 2.2.2 Influence of training in children on anaerobic fitness parameters

In comparison to the information available regarding the influence of training on children's aerobic responses to exercise, little attention has been paid to the influence of training on their anaerobic responses (Van Praagh & Dore, 2002; Chia & Armstrong, 2007; Matos & Winsley, 2007). Consequently, despite a dramatic increase in interest over the last decade, the influences of growth, maturation and training on young people's high intensity exercise responses remain poorly understood (Williams, 2008). Given the relevance to and resemblance of short-term, high-intensity exercise to children's habitual activity and play patterns, as well as the advantageous duration of such tests for children's motivation and concentration spans (Chia & Armstrong, 2007), the dearth of information available is perhaps surprising. However, although the tests themselves may be short and non-invasive, many of the methodologies required for investigating the underlying mechanisms are highly invasive and consequently the paucity of information is more understandable. A further consequence of the ethical prohibition of invasive techniques in paediatric populations is that only indirect and therefore inferential techniques can be used, techniques which cannot provide significant insights into the mechanistic basis of adaptations.

*2.2.2.1 Mechanical power indices*

Anaerobic capacity, as described by mechanical indices such as PP, MP and the FI, is suggested to be increased following training in children (Rotstein *et al.*, 1986; Obert *et al.*, 2001; Ingle *et al.*, 2006). However, there is a large inter-individual and inter-study variation in the percentage improvements seen, ranging from negligible up to ~20% improvements in PP (Obert *et al.*, 2001; Counil *et al.*, 2003) or ~10% in MP (Rotstein *et al.*, 1986). It is difficult to ascertain whether this variation is physiologically or methodologically based due to a number of inconsistencies between studies such as the length of the training programme and use of a pooled data set consisting of both boys and girls (Obert *et al.*, 2001). The specificity of the training programme required to elicit improvements in the anaerobic capacity of children is unclear, with both aerobic (Rotstein *et al.*, 1986; Obert *et al.*, 2001) and anaerobic (Grodjinovsky *et al.*, 1981; Ingle *et al.*, 2006) based training programmes reported to result in an increased PP and MP. Additionally, the specificity of the training modality is important, with a study investigating the effect of a 6-week sprint cycle or running training programme on WAnT performance (cycle) reporting significant improvements for the cyclists alone, despite both training groups undertaking a comparable volume of training (Grodjinovsky *et al.*, 1981). Further work is required to determine the effectiveness of different training regimes and modalities at increasing indices of anaerobic capacity in children, especially whether aerobic training programmes which improve both aerobic and anaerobic capacities could be designed. However, an important and fundamental question which remains to be resolved is the relevance of enhanced mechanical power indices to sports performance as it is suggested such adaptations are not meaningful from a performance standpoint (Ingle *et al.*, 2006).

*2.2.2.2 Oxidative contribution to total energy expenditure*

In concert with findings in adults, a significant oxidative contribution to the total energy expenditure engendered by a conventional 30 second WAnT has been reported in children (Chia *et al.*, 1997). The influence of training status on the oxidative contribution to the WAnT in children has not been investigated. However, given reports that $\dot{V}O_2$ kinetics are

not influenced by training status, it may be hypothesised that no influence would be evident.

*2.2.2.3 Mechanistic basis*

The basis of these training-induced adaptations in the anaerobic capacity of children remains unclear as few studies have attempted to discern the underlying mechanisms (Obert *et al.*, 2001). Amongst the proposed mechanisms is an altered muscle metabolism; unfortunately the investigation of the influence of training on muscle metabolism has been severely constrained over the years due to the ethical considerations of working with a young and vulnerable population. Consequently, the only information determined directly from the muscle dates back more than 30 years to the few muscle biopsy studies conducted in children. The earliest of these studies reported the most significant training adaptations to be evident at rest, with increased concentrations of ATP, PCr, muscle glycogen and glucose-6-phosphate, the consequence of which was reported to be an increased muscle and blood lactate concentration after maximal exertion exercise in trained participants (Eriksson *et al.*, 1973). Furthermore, an increased activity of both succinate dehydrogenase and PFK was reported in trained participants. These results led the authors to conclude that training increased the glycolytic capacity of young boys, a conclusion supported by subsequent studies which similarly demonstrated training-induced increases in the activity of the enzymes associated with glycolysis (glycogen synthase, glycogen phosphorylase, pyruvate kinase and PFK) (Fournier *et al.*, 1982; Cadefau *et al.*, 1990). However, even though considerable metabolic adaptations were observed in these studies, only small improvements in sprint running performance were found, adding to the doubt surrounding the relevance of anaerobic adaptations to sports performance. Although these muscle biopsy studies are fundamental to our current "understanding" of the training effects on anaerobic capacity, it is important to note several methodological limitations which temper the conclusions that may be drawn. Specifically, these studies all typically had relatively small sample sizes and lacked untrained controls, the latter preventing the changes observed being attributed to training *per se* as the effects of growth and maturation cannot be accounted for.

Further research is clearly required to extend these early findings but despite technological advancements such as P-31 magnetic resonance spectroscopy ($^{31}$P-MRS), which has the potential to provide non-invasive *in-vivo* insights into muscle metabolism, little work has been conducted using these techniques. In the only study to investigate the influence of training status, no differences were found in the intramuscular pH ($pH_i$) or in the ratio of PCr to inorganic phosphate ($P_i$) between sprint-trained and untrained boys, both of which are suggested to be indicators of glycolytic capacity (Kuno *et al.*, 1995). These findings therefore contradict the earlier muscle biopsy studies.

Other factors proposed as putative mediators of the training-induced enhancement of the high intensity exercise response include the mass, geometry, type and content of the exercising muscles. Lower limb muscle mass is a major determinant of the response to high intensity exercise in healthy, untrained children (Davies *et al.*, 1972; Mercier *et al.*, 1992; Santos *et al.*, 2003). Therefore, given the correlation between muscle mass and power production (e.g. Saltin & Gollnick, 1983; Hulthén *et al.*, 2001), an increased muscle mass consequent to training could partially explain the increased force production in trained children. However, whether muscle mass is increased following training in children remains controversial (increases: Sargeant *et al.*, 1985; Webb, 1990; Hansen *et al.*, 1999; unchanged: Obert *et al.*, 2001). The role of muscle mass as an explanatory variable is likely to be associated with maturity status, with muscle mass more important in pubertal than pre-pubertal populations as strength gains in the latter arise largely due to neurological adaptations, such as an increased recruitment of neurons during each contraction (Kraemer *et al.*, 1989; Ramsay *et al.*, 1990; Ozmun *et al.*, 1994). The other potential mediators have received even less attention and consequently the mechanistic basis of training-induced enhancements to the high intensity exercise response remains unclear.

## *2.3 Training criteria*

A key consideration for any discussion regarding the adaptation to training, irrespective of whether the sample population is paediatric or adult, is the type, intensity, frequency and duration of the training undertaken.

*2.3.1 Aerobic training*

Numerous attempts have been made over the years to provide guidelines as to the minimum amount of aerobic exercise required to promote and maintain health, with suggestions that training adaptations may occur if this minimum amount is exceeded. Perhaps the best known of these guidelines are those provided by the American College of Sports Medicine (ACSM) which suggest a minimum of 30 minutes, 5 days a week at a moderate intensity (defined as expending 3-6 metabolic equivalents, METs) or 20 minutes, 3 days a week at a vigorous intensity (defined as expending >6 METs) (Haskell *et al.*, 2007). The large range in these recommendations and basis on adult populations makes their applicability to children questionable. A meta-analysis of training in children suggests that while the frequency and duration suggested in the American College of Sports Medicine (ACSM) guidelines may be suitable, a higher intensity is necessary for training adaptations to occur, with an intensity in excess of 80% maximal heart rate suggested (Baquet *et al.*, 2003). However, whether generalised training guidelines can be formulated remains unclear as many training studies which satisfied the proposed criteria have failed to elicit significant improvements, while improvements have been evidenced consequent to training programmes which do not meet the recommended minimal criteria. The purpose of developing generalised training programmes is to improve standardisation between studies and thereby enable more comprehensive inter-study comparisons. It is important to note that within performance environments, however, the aim is to move away from generalised training programmes to training programmes specific to individual athletes.

*2.3.2 Anaerobic training*

Even less attention has been directed to developing guidelines for anaerobic training, probably due to the scepticism regarding its relevance to daily life. The only available guidelines suggest repeating 20-30 second bouts of exercise at 90% of maximal effort for 30-60 minutes, at least 3 times per week (Armstrong & Welsman, 1993). However, besides lacking clarity regarding what measure of maximal effort they propose, these guidelines also suggest an unfeasible amount of anerobic work be completed, especially for children. Further work is required to revise these guidelines in light of more recent research. It

should be borne in mind that predominantly aerobic training programmes have also been shown to result in glycolytic adaptations (Rotstein *et al.*, 1986; Obert *et al.*, 2001). The most effective method of training therefore remains to be elucidated but seems likely to be a mixed training programme with a focus on aerobic training.

### *2.3.3 Training modality specificity*

Training programme specificity is also an important consideration, with a large body of research in adults addressing the transferability of training-induced physiological adaptations to different modalities and forms of exercise. The significance of training specificity was demonstrated in an early study by Magel et al. (1975) in which participants completed a 10-week swimming training programme, before and after which they were assessed using a treadmill and tethered swimming test to exhaustion. Significant improvements in the peak $\dot{V}O_2$, peak $\dot{V}_E$ and test duration were reported for the tethered swimming test, none of which were evident during the treadmill test. This therefore highlights not only the specificity of training adaptations but also the importance of the specificity of the laboratory assessment to the actual training undertaken if a true reflection of training status is to be gained. More recent studies in adults have reported similar results, although the majority report training-induced adaptations to be evident even when a non-specific test modality is used, albeit of a reduced magnitude (e.g. Tordi *et al.*, 2001; Pogliaghi *et al.*, 2006). The transferable training-induced improvements are suggested to reflect central adaptations (i.e. cardiopulmonary adaptations), whereas the non-transferable improvements are thought to be local to the muscles trained and therefore to reflect peripheral adaptations (Rosler *et al.*, 1985; Loftin *et al.*, 1988; Bhambhani *et al.*, 1991; Tordi *et al.*, 2001; Pogliaghi *et al.*, 2006).

The importance of exercise mode specificity has not been extensively investigated in children. Studies involving disparate testing and training modalities, apparently unintentionally, report a similar (Mandigout *et al.*, 2001) or reduced (McManus *et al.*, 1997) magnitude of training status influences compared to those reported when similar training and testing modalities are studied. In one of the few studies to investigate the influence of exercise mode on children's exercise responses the effect of two training

programmes involving different types of exercise (cycling and aerobics) was investigated (Welsman *et al.*, 1997). Neither training programme elicited significant improvements during a treadmill test. Since no group was included in this study who completed a comparable running based training programme it is impossible to discern whether the absence of training-induced improvements is a consequence of no training effect or due to the specificity of training adaptations meaning they were not evidenced during treadmill exercise.

A later study similarly investigated the influence of two different training programmes, a sprint running programme and a continuous cycling programme, on the response to treadmill exercise in pre-pubertal boys (Williams *et al.*, 2000). In agreement with the earlier study by Welsman et al., neither training programme resulted in significant training-induced adaptations. Interpretation of these results is difficult due to the different types of training used for each exercise mode which indicate both the modality and type of training need be specific for training adaptations to be evidenced. The issue of test specificity is especially pertinent to paediatric studies due to the small magnitude of training-induced adaptations typically found; the use of non-specific tests could lead to an erroneous conclusion that children lack trainability when in fact they are trainable but appropriate tests are required for the adaptations to be shown. Further work is needed to investigate the transferability of training-induced adaptations in children. Furthermore, the extension of these studies to investigate the influence of different exercise modalities would be invaluable as the majority of training studies in children have focused on the influence of running or cycling training. This bias needs to be addressed before a comprehensive understanding of the influence of training on children throughout the processes of growth and maturation will be possible.

In light of the literature available, the aim of this thesis was to investigate the influence of swimming-training status on the physiological responses to exercise in young girls, with the intention of furthering our knowledge and understanding regarding the trainability of girls and whether this trainability is modulated by maturity status.

## *2.4 Thesis objectives*

The studies reported in this thesis were therefore designed to:

1) Determine the influence of training status on cardiovascular and metabolic response patterns in young girls

2) Determine the influence of training status on $\dot{V}O_2$, HR and [HHb] kinetics during heavy intensity exercise in young girls

3) Determine the influence of training status on the mechanical indices derived from and oxidative contribution to a 30s WAnT in young girls

4) Elucidate the importance of exercise modality in the demonstration of training status effects in young girls

5) Investigate the presence of a maturational threshold in young girls

6) Assess the influence of maturity in determining the magnitude of training status effects in young girls

These objectives were addressed in a series of 5 studies. The first study was a preliminary study, the findings of which were the basis for the subsequent studies. Studies 2 and 3 were designed to investigate further the suggestion of an influence of training status on the dynamic $\dot{V}O_2$ response (objectives 2, 4, 5, 6) whilst study 4 investigated the training status effects indicated in the cardiovascular responses (objectives 1, 4,5,6). Study 5, which meets objectives 3 to 6 above, was designed to complete the insight into the influence of training status on the physiological responses to exercise of young girls by examining the non-oxidative responses to high-intensity exercise.

# 3. General Methods

The 5 experimental investigations that comprise this thesis required the completion of 617 exercise tests. These tests were conducted at either the exercise physiology laboratory at the University of Exeter School of Sport and Health Sciences or the temporary laboratories established at Kelly College or Ivybridge Community College. In all cases, experimental procedures were approved by the University Ethics Committee prior to the initiation of testing.

## *3.1 Participants*

All participants taking part in the investigations in this thesis volunteered to participate. Participants were instructed to report to the laboratory in a rested state and having abstained from food, alcohol and caffeine for at least the preceding three hours. Testing was conducted at the same time of day (±2 hours) for each participant and participants were familiarised with the mode(s) of exercise and experimental procedures prior to the initiation of testing. All participants and their parents were provided with an information sheet that explained the purpose, procedures and potential risks and benefits of the investigation. Participants were assured that their anonymity would be protected, their data stored in a secure place and that they were free to withdraw at any point without any disadvantage to themselves. Any additional questions the participants or their parents/guardians had were answered verbally or by email. Provided the participants and their parents/guardians were satisfied they both subsequently gave written informed consent and assent, respectively.

The number of participants required to ensure sufficient power for statisitical comparisons was estimated according to the method of Vincent (Vincent, 1999). An example of the derivation of the estimated sample size, for the studies detailed in chapters 4 and 7 on the basis of finding a significant influence of training status on peak SV, is shown below. The data used were obtained from a study conducted by Nottin et al. (Nottin *et al.*, 2002a) investigating child cyclists. The mean values for stroke volume index were 63 ml·m$^{-2}$ for the cyclists and 56 ml·m$^{-2}$ for the untrained children. The standard deviation reported was 5 ml·m$^{-2}$ for both groups. The power and confidence level were set at 0.8 and 0.05, respectively.

$$N = \frac{2(SD^2)(Z_\alpha + Z_\beta)^2}{\Delta^2}$$

$$= \frac{2(5)^2 *(1.96+0.84)}{(7)^2}$$

$$= 8 \text{ participants}$$

Where N is the number of participants, SD is the standard deviation, $Z_\alpha$ is the confidence level, $Z_\beta$ is the area under the normal curve, and $\Delta$ is the difference between the two means being compared.

### *3.2 Sexual maturity and age at peak height velocity*

In all 5 studies, the pubertal stage of the participants was self-assessed using the indices of pubic hair described by Tanner (1962), which depict the 5 major stages of physical development from pre-puberty to post-puberty (see Appendix 5). Self-assessment has previously been shown to be valid and reliable (Morris & Udry, 1980; Taylor *et al.*, 2001; Schmitz *et al.*, 2004; Norris & Richter, 2005; Chan *et al.*, 2008), with the major advantage of this method over physician assessment being that a trained nurse is not required. For girls below the age of 17, the age at peak height velocity was also calculated according to the equations developed by Mirwald *et al.* (2002; Eq. 3.1) to provide an additional indicator of maturation status. These authors reported 95% confidence intervals of ± 1 year and correlation coefficients of 0.83 between maturity predicted by years to PHV and skeletal age in children of 11 years old. Therefore, years to/past PHV is provided as an additional, verification indicator only.

Years to PHV = -16.364 + (0.0002309·LLSH) + ( 0.006277 · ASH) + (0.179·L:H) + (0.0009428·AW)                                          (Eq. 3.1)

Where LLSH is the interaction factor between leg length and sitting height, ASH is the interaction between age and sitting height, L:W is the ratio of leg length to weight and AW is the interaction between age and weight.

## 3.3 Anthropometry

All participants were anthropometrically evaluated before the first test was completed. Standing and seated height were measured to 0.1 cm using a Holtain stadiometer (Holtain, Crymych, Dyfed, UK). The participant was positioned with their back against the stadiometer, unshod heels together, looking straight ahead and measurement was taken at the point of maximal inhalation. Body mass was determined using Avery beam balance scales to 0.05 kg (Avery, Birmingham, UK) with the participant in minimal clothing and without shoes. Skinfold thickness was assessed three times at four (studies 1, 2 and 3) or five (studies 3 and 4) sites around the body (bicep, triceps, subscapular, supra-iliac crest and thigh) by the same researcher for all participants using Harpenden callipers (Baty International, Burgess Hill, UK), accurate to the nearest 0.2 mm. Sites were identified according to the guidelines of Eston et al. (2009). The mean of the three measurements was taken. The mean intraobserver coefficient of variation (CV) for repeat measurements at each skinfold site calculated for the studies included in the present thesis (Table 3.1) compare favourably to the 4.6% CV previously reported in adults (Gregory *et al.*, 1991).

**Table 3.1** Intraobserver CV for repeat measurements of skinfolds at each site

|        | Bicep | Tricep | Subscapular | Supra-Illiac | Thigh |
|--------|-------|--------|-------------|--------------|-------|
| CV(%)  | 3.4   | 2.0    | 2.3         | 2.8          | 2.9   |

## 3.4 Habituation

A separate habituation session was not implemented in the studies described within this thesis. However, at the start of each session prior to any testing procedures, each participant was familiarised with the ergometers and measurement equipment. This familiarisation was done on an informal basis, with the participants allowed as long as required for them to feel comfortable with the ergometers and measuring apparatus. A separate session was not used due to the high number of repeat visits each participant was generally asked to commit to, such that an extra session did not seem a valuable use of the limited time we had with them.

## *3.5 Incremental ramp tests*

### *3.5.1 Equipment*

The incremental ramp tests described in chapters 4, 5, 6 and 7 were conducted using an electronically braked cycle or upper body ergometer (Lode Excalibur Sport, Groningen, Netherlands and Lode Angio, Groningen, Netherlands respectively). The handle bar height, seat height and crank length (cycle ergometer) and electrically controlled seat height and distance (upper body ergometer) were adapted to suit each child. In the studies involving repeat tests (chapters 5 and 6) the values were recorded so they could be replicated throughout the testing series. Studies investigating the reliability of this cycle ergometer model, which is considered the gold-standard, have reported coefficients of variation for peak power achieved during incremental tests of 6% in adults, with the variation suggested to be accountable for by day-to-day physiological variations (Earnest *et al.*, 2005; Micklewright *et al.*, 2006). No studies are available in the literature, to the best of my knowledge, that have assessed the reliability of this upper body exercise ergometer. However, given the considerable similarities between Lode cycle and arm crank ergometers we anticipate a similar reliability and validity.

### *3.5.2 Protocol*

The incremental tests were preceded by a 3 minute warm-up of unloaded pedalling during which the participants were asked to practice maintaining the pedal cadence within the desired range of 65-75 rpm or 45-55 rpm on the cycle and arm crank ergometers respectively. Immediately following this the participants performed a test to voluntary exhaustion for the determination of peak $\dot{V}O_2$. During the test the resistance increased continuously at a pre-determined rate designed to attain a test duration of 8-12 minutes and strong verbal encouragement was provided by researchers. Peak $\dot{V}O_2$ and heart rate were determined as the highest 10 second stationary means throughout the test. Peak $\dot{V}O_2$ determined by a similar protocol has previously been reported to be reliable in pre-pubertal children, with a diurnal coefficient of variation of 4.1% (Welsman *et al.*, 2005). Adolescent

girls have similarly been reported to demonstrate <5% diurnal variation in peak $\dot{V}O_2$ (Pivarnik *et al.*, 1996), in agreement with findings in adults (Katch *et al.*, 1982).

A recent study reported peak $\dot{V}O_2$ determined by an equivalent protocol to this to be as reliable in children as in adults with a coefficient of variation of less than 5%.

## *3.6 Square wave transitions*

### *3.6.1 Equipment*

In chapters 4 and 5, the same electronically braked ergometers were used as for the ramp tests. The handle bar height, seat height and crank length (cycle ergometer) and electrically controlled seat height and distance (upper body ergometer) were replicated from each participant's incremental ramp test.

### *3.6.2 Protocol*

All constant-work-rate tests involved 4 minutes of unloaded pedalling or cranking followed by an instantaneous transition to a heavy-intensity work rate calculated to require 40% of the difference between the GET and peak $\dot{V}O_2$ (40%$\Delta$) for 8 minutes. At 8 minutes the work rate instantaneously returned to an unloaded baseline at which the participants pedalled or cranked for a further 6 minutes. Throughout the cycle ergometer and upper body tests, cadences of 70 ± 5 rpm and 50 ± 5 rpm were maintained respectively. Participants were not given feedback during the test regarding their progress to prevent an anticipatory response to the transition in work rate. Transitions continued to be completed until the 95% confidence intervals associated with the $\dot{V}O_2$ $\tau$ were within ± 4 seconds for the cycle ergometer and ± 4.5 seconds for the upper body ergometer, thereby satisfying the criteria proposed by Fawkner et al. (2007). This required between 2-8 transitions to be completed for the cycle ergometer and 3-10 transitions for the upper body ergometer. To minimise the number of participant visits required, two constant-work-rate tests were completed during each visit. The tests were also completed in the same order of upper then lower body with a minimum of one hour separating the tests. This order was designed to

minimise the metabolic perturbation caused during the first test and thereby ensure complete recovery by the second test.

The test-retest reliability of the $\dot{V}O_2$ and HR responses to a step change in heavy intensity exercise was assessed for the pre-pubertal and pubertal girls during both lower and upper body exercise. The results of this analysis are summarised in Tables 3.2 and 3.3 for lower and upper body exercise, respectively, and agree with those previously reported in adults ($\dot{V}O_2$ τ: CV = 25%; end-exercise $\dot{V}O_2$: CV = 3-9%; Change in HR from baseline to end-exercise: CV = 2-10% (Wilkerson, 2006)).

**Table 3.2** Intraclass correlation coefficients and coefficients of variation for pre-pubertal and pubertal girls during repeat transitions to heavy intensity, lower body exercise

|  | $\dot{V}O_2$ τ | | End-exercise $\dot{V}O_2$ | | ΔHR | |
|  | Pre-pubertal | Pubertal | Pre-pubertal | Pubertal | Pre-pubertal | Pubertal |
|---|---|---|---|---|---|---|
| ICC | 0.90 | 0.84 | 0.87 | 0.83 | 0.76 | 0.84 |
| CV (%) | 21 | 14 | 4 | 4 | 5 | 6 |

**Table 3.3** Intraclass correlation coefficients and coefficients of variation for pre-pubertal and pubertal girls during repeat transitions to heavy intensity, upper body exercise

|  | $\dot{V}O_2$ τ | | End-exercise $\dot{V}O_2$ | | ΔHR | |
|  | Pre-pubertal | Pubertal | Pre-pubertal | Pubertal | Pre-pubertal | Pubertal |
|---|---|---|---|---|---|---|
| ICC | 0.81 | 0.70 | 0.92 | 0.92 | 0.95 | 0.95 |
| CV (%) | 23 | 20 | 6 | 5 | 6 | 6 |

### *3.7 Wingate Anserobic Tests*
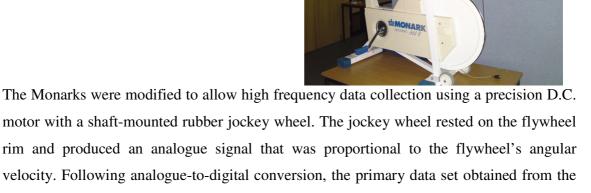
### *3.7.1 Equipment*

The Wingate tests in chapter 8 were conducted on basket loaded cycle ergometers (Monark model 814 *E*). For the upper body WAnT a Monark was mounted to a table and the pedals modified to form handles (Figure 3.1). The seat height was adjusted to suit each participant, ensuring a slight flexion in the knee during cycle WAnT and that the centre of the pedal crank was in line with the middle of the participants glenohumeral joint during upper body

WAnT. The Monark cycle ergometer has been suggested to represent the gold standard of power output measurement (Attaway *et al.*, 1992) and has been shown to be reliable and to provide good consistency (Guiraud *et al.*, 2010), indeed often being used to determine the accuracy and reliability of other ergometers (Jones & Passfield, 1998; Martin *et al.*, 1998; Micklewright *et al.*, 2006).

**Figure 3.1** Adapted Monark for upper body ergometry



The Monarks were modified to allow high frequency data collection using a precision D.C. motor with a shaft-mounted rubber jockey wheel. The jockey wheel rested on the flywheel rim and produced an analogue signal that was proportional to the flywheel's angular velocity. Following analogue-to-digital conversion, the primary data set obtained from the jockey wheel at a sample rate of 500 Hz was averaged every 10 ms to produce a 100 Hz data set as has been suggested to be optimal (Chia, 1998a).

### 3.7.2 Protocol

Each test was preceded by a standardised 3 minute warm-up, involving three "all-out" 3 second sprints at one, two and two and a half minutes against the actual test load. With the exception of during the 3 all-out sprints, participants pedalled with no basket load at a cadence of 60 rpm.

The WAnT test protocol started with three minutes seated stationary on the ergometer, followed by 30 seconds during which the participants were instructed to accelerate the unloaded flywheel to 60 rpm. Three seconds prior to the completion of this 30 second acceleration phase, the participants were given a countdown of "3-2-1-GO!" on which the load basket was dropped and the participants accelerated as fast as possible. Strong verbal encouragement was provided through-out the 30 second test. Following completion of the

30 second sprint the participant sat stationary on the ergometer for 3 minutes. Prior to the test, participants were instructed that signs of pacing would result in the test being repeated.

The WAnT test and the parameters derived from it have been reported to be reproducible in children, adolescents and adults (Inbar & Bar-Or, 1977; Coggan & Costill, 1984; Bar-Or, 1987; Naughton *et al.*, 1992; Carlson & Naughton, 1994; Inbar *et al.*, 1996). For example, in a study investigating the reliability of peak power and mean power in children, four WAnTs were conducted over four weeks with resulting coefficients of variation of 7.3% and 6.8% for PP and MP, respectively (Naughton *et al.*, 1992). These coefficients are in accord with those previously reported in adults of 6.7% and 6.5% for PP and MP, respectively (Coggan & Costill, 1984).

### 3.7.3 Optimal applied force

The optimal applied load for a WAnT test is influenced by a number of considerations such as the duration of the test and the participants' characteristics and is further dependent on whether the test is to be optimised for peak or mean values. In addition, irrespective of the load chosen, this load will not be optimal for all the muscle fibres contributing to the power production as each has a different force-velocity relationship, consequently any "optimal" load chosen should only be considered the overall optimum for an amalgam of the optima of the different muscles involved (Chia, 1998a). However, studies in pre-pubertal children have suggested peak and mean power to be relatively robust over a range of $\pm$ 10 $N.Kg^{-1}$ (Carlson & Naughton, 1994), thereby lessening the importance of this debate to some extent. After consideration of all of these factors, an applied load of 75g per kg body mass and 45g per kg body mass were chosen for the cycle and upper body WAnT respectively, in agreement with the loads applied in previous studies (Inbar *et al.*, 1974; Vandewalle *et al.*, 1987; Bar-Or, 1993).

### 3.7.4 Initiation of the Wingate from a rolling start

The Wingate tests were initiated from a rolling start to allow the collection of data during the acceleration to peak power, data which may otherwise have been lost due to the power

required to overcome the flywheel inertia. The rolling start was particularly important during the upper body WAnT in the pre-pubertal girls where a stationary start may have prevented accurate data being collected.

### 3.8 Measurement of gas exchange parameters

In all the experimental chapters in this thesis, gas exchange variables were measured on a breath-by-breath basis and displayed online (Metalyser 3B Cortex, Biophysik, Leipzig, Germany). Prior to each test the gas analyser was calibrated using gases of known concentration and the turbine volume transducer was calibrated using a 3-litre syringe (Hans Rudolph, Kansas City, MO). The delay in the capillary gas transit and analyser rise time were accounted for relative to the volume signal, thereby time aligning the concentration and volume signals. Breath-by-breath $\dot{V}O_2$ measurements during exercise have been reported to be reproducible, with coefficients of variation ranging from 5.5-12% reported in children (Wessel & Paul, 1987; Reybrouck *et al.*, 1992). More specifically, the Metalyser 3B has been reported to be reliable, with intraclass correlation coefficients of 0.97 for incremental exercise tests on a cycle ergometer (Meyer *et al.*, 2001).

### 3.9  Measurement of cardiovascular parameters

In studies 1 and 4 (chapters 4 and 7), cardiovascular parameters were non-invasively estimated by thoracic bioelectrical impedance (PhysioFlow PF-05 Lab1, Manatec Biomedical, France). This technique is based on the measurement of the transthoracic electrical impedance during cardiac ejection using a high frequency (75 kHz), low amperage (1.8 mA) alternating electrical current, which determines cardiac output according to the following equation:
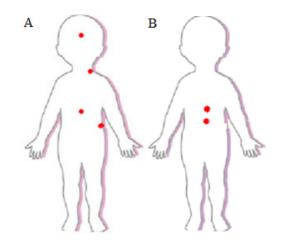
$$\text{Cardiac output} = f_c * \text{SVi} * \text{BSA} \qquad\qquad \text{(Eq. 3.2)}$$

where, $f_c$ is the heart rate based on the measurement of the R-R interval as determined by the ECG first derivative dECG/d*t* which provides a more stable signal than the ECG signal itself, BSA is body surface area calculated from the Haycock formula (Haycock *et al.*,

1978, BSA = 0.024265 * body mass0.5378 * stature0.3964) and SVi is the stroke volume index (SV/BSA).

The electrodes were positioned according to the suggestions of Welsman et al. (2005) involving the relocation of one of the electrodes normally positioned on the neck to the centre of the forehead and one of the thorax electrodes placed off centre on the lower ribs, avoiding the stomach muscles. This alternative arrangement, shown in figure 3.2, was suggested for use with paediatric populations due to their smaller body size which complicates the placement according to the original recommendations and is acceptable because electrode positioning is not crucial as long as one is on the neck and one at the base of the thorax (Welsman *et al.*, 2005).



**Figure 3.2** Positioning of Physioflow electrodes (red dots) on the ventral (A) and dorsal (B) skin surface. Note the repositioning of one electrode to the forehead and one to the lower ribs.

Prior to each test, blood pressure was measured three times using a manual sphygmomanometer with the participant seated and relaxed. The mean blood pressure was subsequently entered into the software along with the participant's age, mass and stature. The autocalibration, which involves the assessment of 30 consecutive heart beats to determine the basic curves and data required to identify stroke volume variations during exercise, was then completed with the participant seated, quiet and calm, on the ergometer.

Welsman et al. (2005) investigated the reliability of the Physioflow during cycle exercise in 20 children, reporting coeffecients of variation of 9.3% for both $\dot{Q}$ and SV and intraclass correlation coeffiecients of 0.86 and 0.83, respectively. The authors therefore concluded the Physioflow offers a reliable technique for the measurement of cardiac parameters in

children. This conclusion was similarly reached by both Charloux et al. (2000), who reported ICC for $\dot{Q}$ of 0.89 and 0.86 at rest and during exercise, respectively, and Richard et al. (2001) who found a correlation coeffiecinet for $\dot{Q}$ of 0.95 between two repeat tests. Cardiac output has also been shown to be relatively independent of electrode placement, with a bias of 0.86% incurred by the repositioning of two electrodes (Tan *et al.*, 2006).

During the constant-work-rate tests, heart rate was measured using radio telemetry and displayed online on a breath-by-breath basis (Polar S610, Polar Electro Oy, Kempele, Finland).

## *3.10 Measurement of muscle oxygenation parameters*

For studies 2, 3, 4 and 5 (chapters 5, 6, 7 and 8), the oxygenation status of the muscle was assessed using a commercially available near-infrared spectroscopy system (studies 2& 3: NIRO 300, Hamamatsu Photonics KK, Hiugashi-ku, Japan; studies 4 & 5: Portamon, Artinis Medical Systems, The Netherlands). This system consists of an emission probe which emits four wavelengths of light (776, 826, 845 and 905 nm) and a photon detector. The intensity of incident and transmitted light was recorded continuously at 2Hz and used to estimate the concentration changes relative to baseline levels for oxygenated, deoxygenated and total haemoglobin. The deoxyhemoglobin signal has been reported to be largely independent of changes in blood volume during exercise (Deblasi *et al.*, 1994; Mancini *et al.*, 1994; Ferrari *et al.*, 1997) and was subsequently used as an indicator of oxygen extraction within the field of interrogation (DeLorey *et al.*, 2003; Grassi *et al.*, 2003; Ferreira *et al.*, 2007). The contribution of myoblobin to the NIRS signal is currently unresolved, although it is believed to be relatively small (<10%, Seiyama *et al.*, 1988), therefore it is pertinent to highlight the deoxygenated haemoglobin signal described through-out this document should be considered to refer to the combined concentration of both deoxygenated haemoglobin and myoglobin. NIRS has been validated as a reliable method for the evaluation of muscle oxygenation changes, with intraclass correlation coefficients for the oxygenation range of 0.75 reported during static endurance tests (Kell *et al.*, 2004) and 0.67 during exhaustive knee extension tests (Pereira *et al.*, 2005). The

coefficient of variation for isometric handgrip exercise in adults at various intensities is reported to range from 16-23% (van Beekvelt *et al.*, 2002).

On at least one constant-work-rate transition for each exercise modality, the oxygenation status of the right vastus lateralis (bike) or right tricep brachii (upper body) was monitored. The muscle was initially cleaned and the probes placed in a rubber holder which was adhered to the skin at the midpoint of the muscle. To ensure the holder and its probes remained stationary during exercise and to minimise the interference of extraneous light, a bandage was wrapped around the arm/leg. The position of the holder relative to the fibular head or ulna head was recorded to enable accurate replication in subsequent tests. The NIRS signal was zeroed with the participant at rest in a seated position with the muscle stationary and relaxed.

The NIRO 300 has been reported to demonstrate acceptable reliability and accuracy in the non-invasive estimation of oxygenation trends within the muscle (Matcher *et al.*, 1995; Suzuki *et al.*, 1999; Komiyama *et al.*, 2001; Demura *et al.*, 2007), provided tissue oxygen saturation is not very low (Komiyama *et al.*, 2001) and the probe is carefully positioned in the same place each time (Demura *et al.*, 2007). The Portamon device has been extensively used in research but considerably less information is available regarding its reliability and validity. The limited information available suggests this device produces acceptable estimates (Shadgan *et al.*, 2009).

### *3.11 Measurement of blood lactate concentration*

In all the studies comprising this thesis, capillary blood samples were obtained from the participant's fingertip using a lancet to puncture the skin (Sarstedt) and a heparinised capillary tube to collect the blood (Sarstedt). The blood samples were subsequently analysed to determine the concentration of whole blood lactate (YSI 1500; Yellow Springs Instruments, Yellow Springs, OH or Lactate Pro; Hurstville, Australia). The samples were collected immediately on volitional termination of the incremental ramp tests and two minutes into the recovery phase of the constant-work-rate or WAnT tests. Studies investigating the reliability of the YSI lactate analyser found a high degree of reliability,

with coefficients of variation of 3% (Noordally & Vincent, 1999), and deviations from a reference sample of 5% (Medbo *et al.*, 2000). A high degree of correlation between the YSI lactate anaylser and LactatePro have been reported (r = 0.99; Pyne *et al.*, 2000) and the LactatePro shown to differ from a reference sample by 12% on average (Medbo *et al.*, 2000). The LactatePro has been reported to provide reliable measurements over a range of temperatures and altitudes and is suggested to be "at least as good" as the considerably more expensive YSI 1500 (Medbo *et al.*, 2000).

### *3.12 Determination of the gas exchange threshold and 40%Δ work rate*

The GET was determined by the V-slope method (Beaver et al. 1986) as the point at which carbon dioxide production began to increase disproportionately to $\dot{V}O_2$, as identified using purpose designed software developed using LabVIEW (National Instruments, Newbury, UK). This process involved the exclusion of the first 60 seconds of data following the onset of the ramp function and all data beyond the respiratory compensation point. A plot of $\dot{V}CO_2$ as a function of $\dot{V}O_2$ was subsequently systematically divided to produce linear regression lines. The GET was identified as the intersection between the two regression lines that minimised both the ratio of the largest standard error and the separation of the intersection point to a single linear regression line drawn through the whole selected period. The statistically selected regression lines were graphically displayed and visually verified by the researchers.

The GET was subsequently used to calculate the work rate required to elicit a response within the heavy intensity domain for the constant-work-rate tests. To calculate this, the GET was first corrected to account for the $\dot{V}O_2$ mean response time. The mean response time was assumed to approximate two-thirds of the ramp rate during incremental exercise (Whipp *et al.*, 1981). Therefore, the work rates corresponding to the GET and peak $\dot{V}O_2$ used to calculate the exercise intensity in chapter 5 were 8 W and 3 W lower than the time aligned work rates, while those in chapter 6 were 13 W and 7 W lower for lower and upper body exercise, respectively.This corrected GET was subsequently added to 40% of the difference between the corrected GET and peak $\dot{V}O_2$.
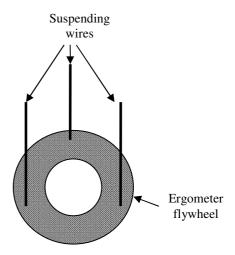
### 3.13 Determination of flywheel inertia and power output calculation

To calculate the power output generated during a WAnT, it was necessary to know the inertia of the flywheel. This was previously determined by the suspension of the flywheel from three wires, the length of which along with the weight of the flywheel and distance from the axis of rotation to the point of suspension were measured (figure 3.3) (Chia, 1998b). The mean period of oscillation was also determined by the angular displacement of the flywheel over a range of oscillations (15-200 oscillations), and subsequently the flywheel inertia was calculated according to the following equation:

$$\text{Period of oscillation (t)} = 2\pi \cdot \sqrt{\left(Il/w.r^2\right)} \qquad \text{(Eq. 3.3)}$$

where w is the weight of the flywheel, r is the distance from the axis of rotation to the point of suspension, I is the inertia of the flywheel and l is the length of the suspending wires. According to this method, the flywheel inertia was calculated to be $0.92 \text{ kg} \cdot \text{m}^{-2}$.

**Figure 3.3** Method used to determine the inertia of the flywheel. Flywheel was suspended by 3 supporting wires of equal length



The power output could then be determined and appropriately adjusted to account for the influence of the flywheel inertia and the internal force of the cycle ergometer as follows:

$$\text{Adjusted power output (P}_{adj}) = \omega \cdot (T_i + T_r) = \omega \cdot [(I \cdot (d\omega/dt)) + (L_{plus9\%} \cdot r)] \qquad \text{(Eq. 3.4)}$$

where: $\omega$ is the angular velocity, $T_i$ is the inertial torque given by the sum of the flywheel inertia plus sprocket and crank inertia (I) multiplied by the angular acceleration of the

flywheel (dω/dt), $T_r$ is the resistive torque calculated as the product of the applied force plus the frictional loss in overcoming the internal force of the ergometer ($L_{plus9\%}$, (Pirnay & Crielaard, 1979; Winter, 1991)) and the radius of the flywheel (r). The inertia of the sprocket and crank were taken as those reported by Monger et al. (1989) and the internal force of the drive train was taken as estimated in the Monark model 814 *E* manual. The adjusted power outputs calculated therefore accounted for both the accelerative and decelerative characteristics of the flywheel (Lakomy, 1986) as well as the work done in overcoming the internal force of the drive train.
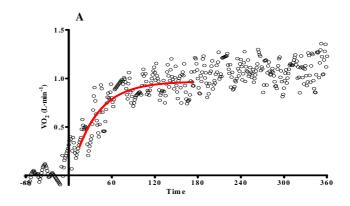
Power outputs were calculated over 1 second time periods as suggested to be appropriate by Chia  (1997) based on observations that terminal pedal cadence is ~60 rpm, meaning at least one pedal revolution is accounted for in each time interval. One second intervals also maximises the sensitivity to alterations in peak power.
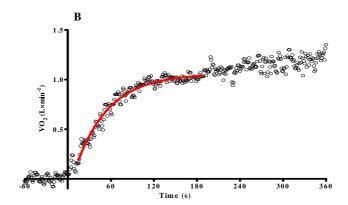
### *3.14 Analysis of $\dot{V}O_2$ response*

Initially, the breath-by-breath responses to each transition were examined to remove any errant breaths caused by coughing, swallowing, sighing etc using a 5 s moving average to identify points lying in excess of 4 SD from the local mean.  Subsequently, each transition was interpolated to 1 s intervals, time aligned to the start of exercise and ensemble averaged. The importance of ensemble averaging repeat transitions to improve the signal to noise ratio is clearly evident in Figure 3.4.

To remove the influence of phase I on the analysis of the subsequent response, the first 15 s of data were ignored. This time period was excluded on the basis of the findings of Hebestreit et al. (1998), Springer et al. (1991) and Fawkner et al. (2002).  Some researchers suggest the phase I response should be modelled with a single exponential to determine the transition from phase I to phase II.  This methodology was not adopted here because, even with breath-to-breath measurement, there are few data points in this phase to which an exponential curve could be fitted and consequently the confidence associated with the exponential model is low.  Furthermore, the identification of the transition is complicated in paediatric populations by the high level of noise and small response amplitudes.

**Figure 3.4** The $\dot{V}O_2$ response demonstrates considerable breath-by-breath variability, as shown in panel A. Ensemble averaging of multiple repeat transitions improves the signal to noise ratio, as shown in panel B following 5 transitions, thereby increasing the confidence associated with model derived parameters. The red line shows the monoexponential fit.



A single exponential model with a time delay (Eq. 3.5, figure 3.4) was then applied to the averaged response and kinetic parameters and their 95% confidence intervals determined by least squares linear regression analysis (Graphpad Prism, Graphpad Software, San Diego, CA).
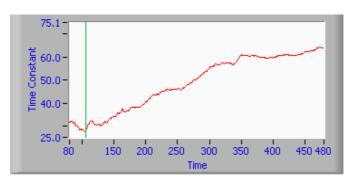
$$\Delta \dot{V} O_{2(t)} = A_1 \cdot \left(1 - e^{-(t-\delta_1)/\tau_1}\right)$$ (Eq. 3.5)

where $\Delta \dot{V}O_2$ is the increase in $\dot{V}O_2$ at time $t$ above the baseline value (calculated as the mean $\dot{V}O_2$ from the first 45 seconds of the last minute of baseline pedalling), and $A_1$, $\delta_1$ and $\tau_1$ are the primary component amplitude, time delay and time constant, respectively. The time constant is the reciprocal of the rate constant (Laughlin & Roseguini, 2008) and reflects the time taken to achieve 63% of the total response amplitude. The exponential $\dot{V}O_2$ response is generally considered to be complete after four time constants have elapsed (Ryan *et al.*, 1979). The pulmonary $\dot{V}O_2$ response described by equation 3.4 is generally

accepted to reflect the muscle $\dot{V}O_2$ response to within ±10% (Grassi *et al.*, 1996; Rossiter *et al.*, 1999; Rossiter *et al.*, 2002; Krustrup *et al.*, 2009), provided the initial phase I response is suitably accounted for.

The fitting window was constrained to exclude all data after the visually determined onset of the $\dot{V}O_2$ slow component. The onset of the $\dot{V}O_2$ slow component was determined using purpose designed LabVIEW software which iteratively fits a mono-exponential function to the $\dot{V}O_2$ data until the window encompasses the entire response. The resulting phase II time constants are plotted against time and the onset of the $\dot{V}O_2$ slow component identified as the point at which the phase II time constant consistently deviates from the previously "flat" profile, as shown in figure 3.5 (Rossiter *et al.*, 2001; Fawkner & Armstrong, 2004b).

**Figure 3.5** Visual identification of the onset of the slow component. The vertical green line represents the onset of the slow component, evidenced by the deviation from the previously "flat" profile to a progressively increasing time constant.



The $\dot{V}O_2$ slow component amplitude was subsequently determined by calculating the difference between the end exercise $\dot{V}O_2$ and the primary amplitude plus baseline $\dot{V}O_2$ and expressed both in absolute terms and relative to end exercise $\dot{V}O_2$. Although alternative methods of analysing the slow component involving the addition of a second exponential term (Barstow *et al.*, 1993; Jones & Poole, 2005b) or a linear term (Engelen *et al.*, 1996) to the single exponential described in equation 1 have been suggested, the method described here avoids the arbitrary parameterization of the slow component by models that may have no physiological basis and which often result in unphysiologically plausible values.

### 3.15 Analysis of the heart rate response

As with the $\dot{V}O_2$ responses, the HR responses to each transition were interpolated to 1 second intervals, time aligned and averaged to produce a single data set. The resulting data

set was fit with a single exponential with no time delay (Eq.3.6) with the fitting window starting at $t = 0$ and constrained to the onset of the $\dot{V}O_2$ slow component.

$$\Delta HR_{(t)} = A_1 \cdot \left(1 - e^{-(t/\tau_1)}\right)$$
(Eq. 3.6)

where $\Delta HR$ is the increase in heart rate at time $t$ above the baseline (calculated as the mean heart rate from the first 45 seconds of the last minute of baseline pedalling), and $A_1$ and $\tau_1$ are the primary component amplitude and time constant, respectively.

### 3.16 Analysis of [HHb] response

The [HHb] responses to each transition were time aligned and averaged to produce a single data set. This data set was then fit with a single exponential with a time delay (Eq. 3.5) with the fitting window commencing at $t=0$. The fitting window was constrained to the point at which the [HHb] response deviated from its original steady state to avoid distortion of the phase II response. This point was identified visually from a plot of [HHb] as a function of time.

### 3.17 Analysis of the cardiovascular and metabolic response patterns

In studies 1 and 4 (chapters 4 and 7), the most appropriate model fit to describe the cardiovascular variables as a function of percentage peak $\dot{V}O_2$ or [HHb] as a function of percentage peak work rate were identified using least-squares and maximum likelihood estimation for linear and non-linear regression, respectively. For cardiovascular variables, the linear relationship ($Y = a + bX$) was compared to the general quadratic relationship ($Y = a + bX + c(X)^2$). For the [HHb] response, the sigmoidal model ($(Y=a/(1+exp^{-(-c+dx)})$, where $a$ represents the baseline corrected amplitude and $c$ is a constant dependent upon $d$ (the slope of the sigmoid) whereby $c/d$ reveals the $x$ value that yields 50% of the total amplitude), was compared to a hyperbolic model ($(Y = ax / (b+x)$, where $a$ is the asymptotic value and $b$ is the $x$-value corresponding to 50% of the response amplitude). The best fitting model was determined on the basis of the $R^2$ values, the residual sum of squares and the F-value.

### *3.18 Allometric scaling*

In all the studies in this thesis, the influence of body size on parameter values was accounted for using analysis of covariance (ANCOVA) on log transformed data to determine the allometric relationship between body mass and peak $\dot{V}O_2$ or between body surface area and peak SV and $\dot{Q}$ (Welsman & Armstrong, 2000). Common allometric exponents were confirmed for all groups within each study and power function ratios $(Y/X^b)$ were computed. Body surface area was calculated according to the equations of Haycock et al. (1978) based on the measurement of stature and mass

**Chapter 4.**

**Study 1 - Cardiovascular responses to ramp incremental exercise in trained and untrained pre-pubertal girls: a preliminary study**

This study has been disseminated as follows:

*Poster presentation:* Winlove, M.A., Jones, A.M., Stoedefalke, K., & Welsman, J.R. (2009) Cardiopulmonary responses to ramp exercise in trained and untrained girls. The BASES Annual Conference (Leeds, 1st-3rd September, 2009)

## *4.1 Introduction*

The influence of training status on children's cardiopulmonary responses to exercise has been investigated for many years, since the study of Astrand et al. (1963) who reported a significantly higher peak oxygen uptake ($\dot{V}O_2$) in swimmers than untrained controls. Unfortunately, despite this long history of research, childrens training-induced cardiopulmonary adaptations remain poorly understood (Obert *et al.*, 2009).

Typically, exercise training is reported to be a significant stimulus to peak $\dot{V}O_2$ in children (Rowland *et al.*, 2000; Nottin *et al.*, 2002b; Obert *et al.*, 2009), although the stimulus appears less potent than in adults, eliciting increases in peak $\dot{V}O_2$ of 5-6% in children (Baquet *et al.*, 2003) compared to 15-20% in adults (Matos & Winsley, 2007). This influence of training status on peak $\dot{V}O_2$ in children is suggested to be largely attributable to an increased cardiac output ($\dot{Q}$) (Rowland *et al.*, 2000; Nottin *et al.*, 2002b), consequent solely to an increased stroke volume (SV) as peak heart rates are unaltered (Nottin *et al.*, 2002b; Obert *et al.*, 2003). This elevated stroke volume is generally attributed to influences of training status on myocardial morphology (Nottin *et al.*, 2004; Rowland *et al.*, 2009a) as it is widely accepted, with little empirical support (Pianosi, 2004), that stroke volume reaches a plateau at approximately 50-60% peak $\dot{V}O_2$ (Nottin *et al.*, 2002b; Obert *et al.*, 2003). However, the increased peak SV may alternatively be related to influences of training status on myocardial functionality as indicated by the alternative SV response

pattern of a progressive increase until exhaustion which has been reported in both trained (Rowland *et al.*, 1998) and untrained children (Pianosi, 2004). The stroke volume response pattern therefore remains to be clarified in both trained and untrained children.

The training-enhanced peak $\dot{V}O_2$ in children may alternatively or additionally be related to alterations in the arterial-venous oxygen difference ( a - $\overline{v}O_2$ difference), as indicated by the Fick equation ($\dot{V}O_2$ = cardiac output * a - $\overline{v}O_2$ difference).  The a - $\overline{v}O_2$ difference reflects the balance between oxygen delivery and utilisation and is therefore a measure of oxygen extraction. Reports indicate that the a - $\overline{v}O_2$ difference is not altered by training at rest or at peak exercise (Rowland *et al.*, 2000; Nottin *et al.*, 2002b; Obert *et al.*, 2003) but only one study has reported the response pattern of the a-vO$_2$ difference to ramp incremental exercise in children, suggesting it rises progressively until exhaustion (Nottin *et al.*, 2002b). The pattern described by Nottin et al. (2002) was based on just four data points during an entire ramp incremental test however, and therefore the a - $\overline{v}O_2$ difference response pattern also remains to be elucidated in detail in both the trained and untrained state.

The purpose of this cross-sectional study was to examine the influence of training status on the cardiopulmonary response patterns to ramp incremental exercise in pre-pubertal girls. We hypothesised that the stroke volume response pattern would demonstrate a linear increase in the trained girls in contrast to a plateau in the untrained girls. We also hypothesised that the a - $\overline{v}O_2$ difference response pattern would not be influenced by training status, increasingly linearly in both groups.

### *4.2 Methods*

### *4.2.1 Participants and anthropometry*

Ten swimming trained girls (mean age of 10.2 ± 1.0 years) and 9 healthy girls that were not involved in any regular exercise training (mean age of 10.2 ± 0.7 years) participated in this study. The trained girls, all county and regional level competitive swimmers with a mean training of 5 (± 1.6) hours/week for 2.6 (± 1.5) years, were recruited from a local swimming

club. The untrained girls were volunteers from local schools. Sexual maturity was assessed by self reporting using the indices of pubic hair described by Tanner (1962). Age to peak height velocity was estimated to provide an additional indicator of physical maturity according to the equations of Mirwald *et al*. (2002) which are based on the measurement of standing and seated height, weight, and date of birth as described below.

An anthropometrical evaluation was performed before the first test for all participants. Standing and seated height were measured to 0.1cm using a Holtain stadiometer (Holtain, Crymych, Dyfed, UK) and body mass determined using Avery beam balance scales to 0.05kg (Avery, Birmingham, UK). Skinfold thickness was assessed three times at four sites around the body (bicep, triceps, subscapular and supra-iliac crest) by the same researcher for all participants using Harpenden callipers (Baty International, Burgess Hill, UK), accurate to the nearest 0.2mm. The average of the three measurements was taken. Table 4.1 presents the participants' physical characteristics.

Participants were asked to arrive at the laboratory in a rested and fully hydrated state, at least 3 hours postprandial and to refrain from caffeinated drinks in the 6 hours prior to the test. The methods employed during this study were approved by the institutional ethics research committee and all participants and their parents/guardians gave written informed consent and assent respectively.

**Table 4.1** Anthropometric characteristics of participants

|  | Trained (N = 10) | Untrained (N = 9) |
|---|---|---|
| Age (y) | 10.2 ± 1.0 | 10.2 ± 0.7 |
| Stature (m) | 1.4 ± 0.1 | 1.4 ± 0.1 |
| Weight (kg) | 35.1 ± 5.7 | 34.9 ± 6.3 |
| BMI (kg·m$^{-2}$) | 17.8 ± 1.4 | 17.3 ± 2.8 |
| Sum of Skinfolds (mm) | 22.2 ± 7.3 | 15.0 ± 13.8 |
| PHV (yrs) | -1.6 ± 0.8 | -1.6 ± 0.5 |

*Values are mean ± S.D. PHV, years to peak height velocity. No significant differences were present*

*4.2.2 Exercise Protocol*

Each participant completed an incremental ramp test to volitional exhaustion on an electronically braked cycle ergometer (Lode Excalibur, Netherlands). The handle bar height, seat height and crank length were adapted to suit each child. After a three minute warm-up consisting of unloaded pedalling, the resistance increased by 10-14 W·min$^{-1}$ to attain a test of ~8-12 minutes in duration. Participants were instructed to maintain a cadence of 70 ± 5 rpm. Maximal tests were considered to have been achieved if in addition to subjective indications such as sweating, hyperpnoea and facial flushing, the respiratory exchange ratio was > 1.0 and there was a consistent reduction in cadence despite strong verbal encouragement

*4.2.3 Exercise Test Measurements*

Gas exchange variables (Metalyser 3B Cortex, Biophysik, Leipzig, Germany) were measured on a breath-by-breath basis and displayed online. Prior to each test the gas analyser was calibrated using gases of known concentration and the turbine volume transducer was calibrated using a 3-litre syringe (Hans Rudolph, Kansas City, MO). The delay in the capillary gas transit and analyser rise time were accounted for relative to the volume signal, thereby time aligning the concentration and volume signals.

Cardiac output, stroke volume and heart rate were determined non-invasively through-out the exercise test using a thoracic bioelectrical impedance device (PhysioFlow, PF-05 Lab1, Manatec Biomedical, France), previously validated in both adult (Charloux *et al.*, 2000; Richard *et al.*, 2001) and paediatric populations (Welsman *et al.*, 2005). The electrodes were positioned on the forehead, neck, xiphoid process and on the left hand side lower ribs, avoiding the abdominal muscles, as suggested to be appropriate for paediatric populations (Welsman *et al.*, 2005). Prior to testing, blood pressure was measured in triplicate using a manual sphygmomanometer with the participant seated at rest by the same researcher throughout the study. This mean blood pressure was entered into the Physioflow following auto-calibration which was conducted with the child seated, immobile and relaxed, on the cycle ergometer.

*4.2.4 Data Analysis*

The $\dot{V}O_2$ data were interpolated to 1-s intervals and peak $\dot{V}O_2$ was taken as the highest 10-s stationary average during the test. The gas exchange threshold was determined by the V-slope method (Beaver *et al.*, 1986) as the point at which carbon dioxide production began to increase disproportionately to $\dot{V}O_2$ as identified using purpose designed software developed using LabVIEW (National Instruments, Newbury, UK). Peak stroke volume and cardiac output were taken as the highest 15-s average during the test. The $a-\bar{v}O_2$ difference was estimated by rearrangement of the Fick equation:

$$a-\bar{v}O_2 \text{ difference} = \dot{V}O_2/\dot{Q} \qquad\qquad (\text{Eq. } 4.1)$$

Baseline values for all the cardiopulmonary parameters were taken as the 30 second average from 90 to 120 seconds during unloaded pedalling.

*4.2.5 Statistics*

The allometric relationships between body mass and peak $\dot{V}O_2$ and between body surface area and peak SV and $\dot{Q}$ were determined using analysis of covariance (ANCOVA) on log transformed data (Welsman & Armstrong, 2000). From the values of the regression slopes (allometric exponents) confirmed as common to all groups, power function ratios ($Y/X^b$) were computed. Body surface area was calculated according to the equations of Haycock et al. (1978). Following identification of a normal distribution, homogenous variation and an absence of skewness or kurtosis, a one way ANOVA was used to analyse the influence of training status. To test for linear or non-linear behaviour of the cardiovascular and $a-\bar{v}O_2$ difference variables as a function of percentage peak $\dot{V}O_2$, the linear relation ($Y = a + bX$) was compared to the general quadratic relation ($Y = a + bX + c(X)^2$) using least-squares estimation in linear regression for individual participants. Non-linear models were fit to the data using maximum likelihood estimation in nonlinear regression (Graphpad Prism, Graphpad Software, San Diego, CA).

## 4.3 Results

All the girls were found to be pre-pubertal according to Tanner stages and years to peak height velocity.

Baseline and peak respiratory and cardiovascular variables, which were not influenced by training status, are presented in Table 4.2 and 4.3, respectively. All participants were accepted to have given a peak effort. Log-linear ANCOVA identified a common exponent for all participants of b = 0.56 (SE, 0.19) for peak $\dot{V}O_2$ , b = 0.51 (SE, 0.17) for peak $\dot{Q}$ and b = 0.46 (SE, 0.14) for SV. No influence of training status was evident in the fraction of peak $\dot{V}O_2$ at which the GET occurred, either in absolute (trained: 0.9 ± 0.2 L·min$^{-1}$; untrained: 0.8 ± 0.1 L·min$^{-1}$, $P > 0.05$) or relative terms (trained: 58 ± 7%; untrained: 57 ± 5%, $P > 0.05$).

**Table 4.2** Physiological responses to unloaded pedalling in trained and untrained girls
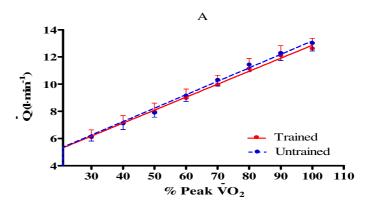
|  | Trained girls (n = 10) | Untrained girls (n = 9) |
|---|---|---|
| Baseline $\dot{V}O_2$ (L·min$^{-1}$) | 0.56 ± 0.08 | 0.55 ± 0.09 |
| Baseline $\dot{V}O_2$ (mL·min$^{-1}$·kg$^{-1}$) | 15.03 ± 2.21 | 15.10 ± 3.52 |
| Baseline $\dot{V}O_2$ (mL·min$^{-1}$·kg$^{0.56}$) | 71.13 ± 7.39 | 71.15 ± 14.72 |
| Baseline $\dot{Q}$ (L·min$^{-1}$) | 6.2 ± 1.3 | 6.01 ± 0.8 |
| Baseline $\dot{Q}$ (L·min$^{-1}$·m$^{-2}$) | 5.3 ± 0.9 | 5.2 ± 0.8 |
| Baseline $\dot{Q}$ (L·min$^{-1}$·m$^{-0.51}$) | 5.7 ± 1 | 5.6 ± 0.7 |
| Baseline SV (mL) | 56.9 ± 9.7 | 54.7 ± 6.0 |
| Baseline SV (mL·m$^{-2}$) | 48.9 ± 5.7 | 47.2 ± 5.1 |
| Baseline SV (mL·m$^{-0.46}$) | 53.0 ± 7.3 | 51.0 ± 4.9 |
| Baseline HR (b·min$^{-1}$) | 111 ± 10 | 103 ± 15 |
| Baseline a - $\overline{v}O_2$ difference (mL·dL$^{-1}$) | 9.1 ± 2.2 | 9.1 ± 1.6 |
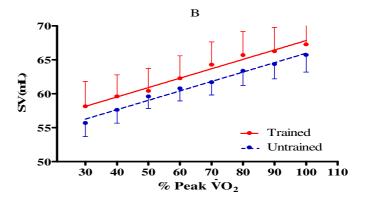
*Values are mean ± S.D. $\dot{V}O_2$, oxygen uptake; $\dot{Q}$ , cardiac output; SV, stroke volume; HR, heart rate, a-$\overline{v}O_2$ difference, arterial-venous oxygen difference. No significant differences were present*

Group averages of the cardiac output, stroke volume and a - $\overline{v}O_2$ difference responses to ramp exercise are shown in Figure 4.1 as a function of percentage peak $\dot{V}O_2$. The

cardiovascular variables are presented as absolute values for simplicity as the response patterns were not altered by using scaled variables. Cardiac output rose progressively in both trained and untrained participants and was best fit by a linear model ($R^2$: T, 0.95, UT, 0.94). Similarly, SV increased from baseline to peak $\dot{V}O_2$ with a statistically superior fit with the linear model ($R^2$: T, 0.78, UT, 0.77). The a-$\overline{v}O_2$ difference response differed with training; the trained girls demonstrated a non-linear relation with negative curvature ($R^2$: 0.88) whereas untrained girls demonstrated a linear relationship with percentage peak $\dot{V}O_2$ ($R^2$: 0.70).

**Figure 4.1** Mean cardiovascular and a-$\overline{v}O_2$ difference responses to ramp incremental exercise. Graph A shows the cardiac output ($\dot{Q}$), graph B the stroke volume (SV) and graph C the arterial-venous oxygen difference (a-$\overline{v}O_2$ difference) as a function of percentage peak oxygen uptake ($\dot{V}O_2$) in trained and untrained girls. The cardiovascular variables are presented as absolute values (± SEM) as the response patterns were not altered by using scaled variables.
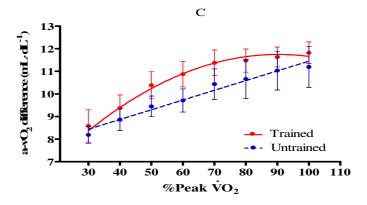
**Table 4.3** Peak physiological responses to ramp incremental exercise in trained and untrained pre-pubertal girls

| | Trained girls (n = 10) | Untrained girls (n = 9) |
|---|---|---|
| Peak $\dot{V}O_2$ (L·min$^{-1}$) | 1.54± 0.30 | 1.4 2± 0.16 |
| Peak $\dot{V}O_2$ (mL·min$^{-1}$·kg$^{-1}$) | 44.41 ± 7.88 | 40.90 ± 4.77 |
| Peak $\dot{V}O_2$ (mL·min$^{-1}$·kg$^{0.56}$) | 210.87 ± 34.82 | 193.40 ± 16.19 |
| Peak $\dot{Q}$ (L·min$^{-1}$) | 13.1 ± 2.4 | 13.3 ± 1.5 |
| Peak $\dot{Q}$ (L·min$^{-1}$·m$^{-2}$) | 11.3 ± 1.3 | 11.5 ± 1.4 |
| Peak $\dot{Q}$ (L·min$^{-1}$·m$^{-0.51}$) | 12.1 ± 1.7 | 12.3 ± 1.3 |
| Peak SV(mL) | 69.1 ± 10.3 | 66.8 ± 7.1 |
| Peak SV (mL·m$^{-2}$) | 59.4 ± 6.0 | 57.6 ± 5.5 |
| Peak SV (mL·m$^{-0.46}$) | 64.4 ± 7.6 | 62.4 ± 5.4 |
| Peak HR (b·min$^{-1}$) | 191 ± 7 | 198 ± 12 |
| Peak $a\text{-}\overline{v}O_2$ difference (mL·dL$^{-1}$) | 11.8 ± 1.8 | 10.7 ± 1.5 |

*Values are mean ± S.D. $\dot{V}O_2$ oxygen uptake; $\dot{Q}$, cardiac output; SV, stroke volume; HR, heart rate, $a\text{-}\overline{v}O_2$ difference, arterial-venous oxygen difference. No significant differences were present*

## *4.4 Discussion*

This study produced several novel findings. Firstly the estimated $a\text{-}\overline{v}O_2$ difference response pattern differed with training status. Secondly, estimated stroke volume rose linearly throughout exercise in both trained and untrained children. Thirdly swimming training for 5 hours per week was not sufficient to elicit significant changes in prepubertal children's peak cardiopulmonary responses to exercise.

This is the first study to investigate the influence of training status on the $a\text{-}\overline{v}O_2$ difference response pattern, demonstrating a linear rise in untrained girls but a non-linear rise in the trained girls. The linear rise in the untrained girls may agree with previous results but the small number of data points used in the previous study limit comparisons (Nottin *et al.*, 2002b). Despite this influence of training status on the response patterns, neither the baseline nor peak $a\text{-}\overline{v}O_2$ difference was influenced by training status, in accord with previous findings (Rowland *et al.*, 2000; Nottin *et al.*, 2002b; Obert *et al.*, 2003).

The mechanistic basis for an influence of training status on the response pattern of $a-\bar{v}O_2$ difference is unclear. The present results suggest that an altered oxygen delivery is unlikely to be associated with the different $a-\bar{v}O_2$ difference response patterns given the very similar $\dot{Q}-\dot{V}O_2$ relationships found in the trained and untrained girls. This therefore suggests the influence of training status on the $a-\bar{v}O_2$ difference response pattern may be a reflection of a training status related difference in the utilisation of oxygen, a difference which may arise due to an enhanced oxidative capacity which has previously been reported in trained children (Eriksson *et al.*, 1973; Fournier *et al.*, 1982). The utility of this training status influence on the $a-\bar{v}O_2$ difference response pattern is also elusive however, given the similarity in peak values. Whilst it is possible this disparity is an indication of the presence of a maturational threshold which limits the influences of training status that can be evidenced pre-puberty (Katch, 1983), it is beyond the scope of the current study to draw such conclusions. This limitation arises partly due to the cross-sectional nature of this study which precludes the differences observed being attributed to training *per se*, as they may purely be a reflection of genetic traits which predisposed these children to success in swimming.

The stroke volume pattern reported here agrees with previous findings in trained children (Rowland *et al.*, 1998), yet contradicts the more commonly reported pattern of an initial rise and subsequent plateau (Nottin *et al.*, 2002b; Obert *et al.*, 2003) and has not previously been reported in untrained children. No explanation is currently available for the progressively increasing SV in either trained or untrained children. In adults, an enhanced ventricular filling response (Rowland, 2009) and high blood volume (Krip *et al.*, 1997) have been proposed as explanations for a progressively increasing stroke volume until exhaustion. However, the relevance of such suggestions to the paediatric population is limited as children are not "mini-adults" (Armstrong & Welsman, 2002).

Prior to a discussion regarding possible physiological explanations for the lack of influence of training status on peak $\dot{V}O_2$, SV and $\dot{Q}$, it is important to highlight the possible methodological explanation that this finding is a reflection of the limited power of the

current study. This limited power is important when considering the influence of training status on absolute or relative physiological values but is considerably less important when investigating the influence of training status on response patterns, as was the principle aim of this study.

The lack of influence of training status on the peak $\dot{V}O_2$, SV and $\dot{Q}$ in the current study is surprising, contrasting with many (e.g. Rowland *et al.*, 1998; Rowland *et al.*, 2000; Nottin *et al.*, 2002b; Obert *et al.*, 2003) but not all previous studies (e.g. Kobayashi *et al.*, 1978; Mirwald *et al.*, 1981; Shephard, 1992; Welsman *et al.*, 1996). The absence of training status effects may be related to a disparity between the training (swimming) and testing (cycling) modalities. The transferability of training status related influences in children remains equivocal, with some suggesting no transferability (Welsman *et al.*, 1997; Williams *et al.*, 2000) whilst others report significant improvements in peak $\dot{V}O_2$ irrespective of test modality (McManus *et al.*, 1997; Mandigout *et al.*, 2001).

Alternatively, the results may indicate that 5 hours/week swimming-training is insufficient to enable significant influences of training status to be observed in peak cardiopulmonary responses. Whilst plausible, it is important to note the training volume of the current participants exceeded that outlined by Baquet et al. (2003) as necessary for significant influences of training status to be observed. The current results therefore highlight the current lack of understanding regarding the dose-response relationship between training and its effect in children.

## *4.5 Conclusion*

In summary, the current results failed to demonstrate any significant influence of swimming training status in pre-pubertal girls. Nonetheless, the SV and a-vO$_2$ difference response patterns did differ qualitatively between the trained and untrained girls, suggesting this may be an area worthy of further investigation. The importance of exercise mode also requires further investigation as the disparity between training and testing modes is perhaps one of the most plausible explanations for the current results.

## Chapter 5

## Study 2 - Influence of training status and exercise modality on pulmonary $O_2$ uptake kinetics in pre-pubertal girls

This study has been disseminated as follows:

*Publication:* Winlove, M.A., Welsman, J.R., & Jones, A.M. (2010). Influence of training status and exercise modality on pulmonary $O_2$ uptake kinetics in pre-pubertal girls. European Journal of Applied Physiology, 108(6): 1169-1179

*Poster Presentation:* Winlove, M.A., Welsman, J.R., & Jones, A.M. (2010). Influence of training status and exercise modality on pulmonary $O_2$ uptake kinetics in pre-pubertal girls. The 25th Paediatric Work Physiology Conference (Le Touquet, France, 30th September – 2nd October 2009). Awarded 2nd place in the Young Investigator Award.

### *5.1 Introduction*

Pulmonary oxygen uptake ($\dot{V}O_2$) kinetics provide a useful, non-invasive assessment of the integrated capacity of the organism to transport and utilize $O_2$ to support the increased rate of energy turnover in the contracting myocytes (Whipp & Ward, 1990). In adults, the influence of factors such as exercise intensity and exercise modality on $\dot{V}O_2$ kinetics have been well documented (for a detailed review see: (Jones & Poole, 2005b)). Exercise training is known to be a potent stimulus to $\dot{V}O_2$ kinetics in this population, resulting in reductions in both the phase II time constant ($\tau$) and the amplitude of the subsequent $\dot{V}O_2$ 'slow component' (Powers *et al.*, 1985; Casaburi *et al.*, 1987; Phillips *et al.*, 1995; Carter *et al.*, 2000; Koppo *et al.*, 2004; Jones & Koppo, 2005; Berger *et al.*, 2006; Bailey *et al.*, 2009).

The $\dot{V}O_2$ kinetic response in children has been less comprehensively described and the influence of endurance training on $\dot{V}O_2$ kinetics in this population is unclear (Fawkner & Armstrong, 2003a). The available data suggest that training neither reduces $\tau$ (Obert *et al.*,

2000; Cleuziou *et al.*, 2002) nor reduces the amplitude of the $\dot{V}O_2$ slow component (Obert *et al.*, 2000) in children. This apparent insensitivity to training clearly contrasts with findings in adults (Phillips *et al.*, 1995; Norris & Petersen, 1998; Carter *et al.*, 2000; Cleuziou *et al.*, 2003; Koppo *et al.*, 2004). However, it is possible that this is a consequence of methodological limitations in paediatric studies such as the use of only single exercise transition to characterize $\dot{V}O_2$ kinetics, the prescription of exercise intensity as a fraction of the peak $\dot{V}O_2$, the use of mixed sex cohorts, or the employment of non-specific ergometry. The relatively small $\dot{V}O_2$ response amplitudes and relatively large inter-breath variability in $\dot{V}O_2$ in children requires that several repeat transitions are performed for $\dot{V}O_2$ kinetics to be confidently characterized (Fawkner & Armstrong, 2007). Moreover, the relatively high inter-individual variability in the fraction of peak $\dot{V}O_2$ at which the gas exchange threshold (GET) occurs in children compared to adults (Fawkner & Armstrong, 2007) means that it is important to consider both the GET and the peak $\dot{V}O_2$ when attempting to standardise the exercise intensity domain in which participants are exercising. There are suggestions that boys and girls exhibit subtle differences in $\dot{V}O_2$ kinetics, with boys suggested to exhibit faster $\dot{V}O_2$ kinetics and a smaller slow component amplitude (Fawkner & Armstrong, 2004c). Therefore the analysis of both sexes together might be inappropriate. Another explanation for the reported insensitivity of children to training might be that some previous studies utilized non-specific testing modalities. Previous studies have all involved investigation of the effects of swimming training on $\dot{V}O_2$ kinetics during cycle ergometer exercise (Obert *et al.*, 2000; Cleuziou *et al.*, 2002). Considering the large contribution of the upper body to swimming (Ogita *et al.*, 1996), a test modality such as upper body ergometry, which at least demonstrates a commonality of muscles exercised, might be more likely to demonstrate training influences on the physiological responses to exercise.

The purpose of this cross-sectional study was to examine the influence of training status and exercise modality on the $\dot{V}O_2$ and HR kinetics of pre- and early-pubertal girls during heavy-intensity exercise. We hypothesized that the $\dot{V}O_2$ and HR kinetics would be faster and that the $\dot{V}O_2$ slow component amplitude would be smaller in trained swimmers compared to an age-matched untrained control group. We also hypothesized, given the

large contribution of the upper body to swimming, that training status-related differences would be more evident during upper body than during cycle ergometer exercise.

## 5.2 Methods

### 5.2.1 Participants

Eight endurance trained (T) girl and 8 untrained (UT) girls aged 10-12 yrs participated. The T girls, all competitive swimmers with a mean training volume of 8 ($\pm$ 2.5) hrs·wk$^{-1}$, were recruited from a local swimming club. The UT girls were volunteers from local schools. Sexual maturity was assessed by self-report using the indices of pubic hair described by Tanner (1962). Age to peak height velocity was estimated to provide an additional indicator of physical maturity according to the equations of Mirwald *et al*. (2002).

An anthropometrical evaluation was performed before the first test for all participants. Standing and seated height were measured to 0.1 cm using a Holtain stadiometer (Holtain, Crymych, Dyfed, UK) and body mass determined using Avery beam balance scales to 0.05 kg (Avery, Birmingham, UK). Skinfold thickness was assessed three times at four sites around the body (bicep, triceps, subscapular and supra-iliac crest) by the same researcher for all participants using Harpenden callipers (Baty International, Burgess Hill, UK), accurate to the nearest 0.2mm. The average of the three measurements was taken.

Participants were asked to arrive at the laboratory in a rested and fully hydrated state, at least 3 hours postprandial and to refrain from consuming caffeinated drinks in the 6 hours prior to the test. The methods employed during this study were approved by the institutional research ethics committee and all participants and their parents/guardians gave written informed consent and assent, respectively.

### 5.2.2 Measurements of peak $\dot{V}O_2$ and GET

On the first two visits to the laboratory, exercise mode-specific peak $\dot{V}O_2$ and gas exchange threshold (GET) were determined using an incremental ramp test to voluntary exhaustion

on both cycle (Lode Excalibur, Netherlands) and upper body (Lode Angio, Netherlands) ergometers. Gas exchange variables (Metalyser 3B Cortex, Biophysik, Leipzig, Germany) and heart rate (Polar S610, Polar Electro Oy, Kempele, Finland) were measured on a breath-by-breath basis and displayed online. Prior to each test the gas analyser was calibrated using gases of known concentration and the turbine volume transducer was calibrated using a 3-litre syringe (Hans Rudolph, Kansas City, MO). The delay in the capillary gas transit and analyser rise time were accounted for relative to the volume signal, thereby time aligning the concentration and volume signals. The handle bar height, seat height and crank length (cycle ergometer) and electrically controlled seat height and distance (upper body ergometer) were adapted to suit each child and the values recorded so they could be replicated throughout the testing series.

After a three minute warm-up consisting of unloaded pedalling or arm cranking, the resistance increased by 12 $W \cdot min^{-1}$ and 5 $W \cdot min^{-1}$ for cycle and upper body exercise, respectively, to attain a test of ~8-12 minutes in duration. Participants were instructed to maintain a cadence between 70 and 50 rpm on the cycle and upper body ergometer, respectively. Maximal tests were considered to have been achieved if in addition to subjective indications such as sweating, hyperpnoea and facial flushing, the respiratory exchange ratio (RER) was > 1.05 and there was a consistent reduction in cadence despite strong verbal encouragement. The data were interpolated to 1-s intervals and peak $\dot{V}O_2$ was taken as the highest 10-s stationary average during the test. The GET was determined by the V-slope method (Beaver *et al.*, 1986) as the point at which carbon dioxide production began to increase disproportionately to $\dot{V}O_2$ as identified using purpose designed software developed using LabVIEW (National Instruments, Newbury, UK).

### 5.2.3 Constant work rate tests

The participants returned to the laboratory on a number of other occasions to complete step-change exercise tests on the upper body and cycle ergometers. Where multiple tests were performed on the same day, at least 1 hour separated the tests and the tests were ordered such that the first test involved a smaller muscle mass (upper body), thereby resulting in a smaller metabolic perturbation and faster recovery. On average, 4 cycle and 7 upper body

transitions were completed, dependent on the number of transitions required to obtain 95% confidence intervals of < 4 s for cycling or < 4.5 s for upper body exercise. All constant-work-rate tests consisted of 4 minutes of unloaded pedalling or cranking followed instantaneously by a transition to a work rate extrapolated to require 40% of the difference between the GET and peak $\dot{V}O_2$ (40%$\Delta$) for 8 minutes. On completion, the work rate instantaneously returned to no load and the participants pedalled or cranked for a further 6 minutes. Throughout the cycle ergometer and upper body tests, cadences of 70 ± 5 rpm and 50 ± 5 rpm were maintained respectively. Fingertip blood samples were taken 1 minute after completion of the loaded phase and assayed for blood lactate concentration ([lactate]; YSI 1500, Yellow Springs Instruments, Yellow Springs, OH).

### 5.2.4 $\dot{V}O_2$ Kinetics Analysis

Initially, the breath-by-breath responses to each transition were examined to remove any errant breaths caused by coughing, swallowing, sighing etc using a 5 s moving average to identify points lying in excess of 4 SD from the local mean. Subsequently, each transition was interpolated to 1 s intervals, time aligned to the start of exercise and averaged.

To remove the influence of phase I on analysis of the subsequent response, the first 15 s of data were ignored. A single exponential model with a time delay (Eq. 5.1) was then applied to the averaged response and parameters and their 95% confidence intervals determined by least squares linear regression analysis (Graphpad Prism, San Diego, CA).

$$\Delta \dot{V} O_{2(t)} = A_1 \cdot \left(1 - e^{-(t-\delta_1)/\tau_1}\right) \qquad \text{(Eq. 5.1)}$$

where $\Delta\dot{V}O_2$ is the increase in $\dot{V}O_2$ at time $t$ above the baseline value (calculated as the mean $\dot{V}O_2$ from the first 45 seconds of the last minute of baseline pedalling), and $A_1$, $\delta_1$ and $\tau_1$ are the primary component amplitude, time delay and time constant, respectively.

The fitting window was constrained to exclude all data after the visually determined onset of the $\dot{V}O_2$ slow component, as described in section 3.14. The amplitude of the $\dot{V}O_2$ slow component was subsequently determined by calculating the difference between the end

exercise $\dot{V}O_2$ and the primary amplitude plus baseline $\dot{V}O_2$. This was expressed both in absolute terms and relative to end exercise $\dot{V}O_2$.

### 5.2.5 HR Kinetics Analysis

As with the $\dot{V}O_2$ responses, the HR responses to each transition were interpolated to 1 second intervals, time aligned and averaged to produce a single data set. The resulting data set was fit with a single exponential with no time delay (Eq. 5.2) with the fitting window starting at $t = 0$ and constrained to the onset of the $\dot{V}O_2$ slow component.

$$\Delta HR_{(t)} = A_1 \cdot \left(1 - e^{-(t/\tau_1)}\right) \qquad \text{(Eq. 5.2)}$$

where $\Delta HR$ is the increase in heart rate at time $t$ above the baseline (calculated as the mean heart rate from the first 45 seconds of the last minute of baseline pedalling), and $A_1$ and $\tau_1$ are the primary component amplitude and time constant, respectively.

### 5.2.6 Statistics

The allometric relationship between body mass and peak $\dot{V}O_2$ was determined using ANCOVA on log transformed data (Welsman & Armstrong, 2000). Following identification of a normal distribution, homogenous variation and an absence of skewness or kurtosis a two way ANOVA with repeated measures was used to analyse training status and exercise mode effects. Subsequent independent or paired samples t-tests with a Bonferonni correction were employed as appropriate to identify the location of significant effects. All data are presented as means ± SD. Statistical significance was accepted when $P < 0.05$.

### 5.3 Results

The physical characteristics of the participants are presented in Table 5.1. All children reported a maturity level of 1 or 2, indicting the study population was pre- or early-pubertal. Both groups had equal numbers of maturity stage 1 and 2 participants.

**Table 5.1** Morphological characteristics of participants

|  | Trained | Untrained |
|---|---|---|
| Age (y) | 11.4 ± 0.7 | 11.5 ± 0.6 |
| Stature (m) | 1.48 ± 0.06 | 1.52 ± 0.05 |
| Mass (kg) | 39.9 ± 6.9 | 43.2 ± 8.6 |
| Sum of skinfolds (mm) | 36.4 ± 14.6 | 43.8 ± 25.6 |
| Years to PHV (y) | -0.46 ± 0.5 | -0.31 ± 0.45 |

*Values are mean ± SD. No significant differences were present. N = 8*

Responses to the peak tests on each mode did not differ between groups before or after allometric scalling ($P > 0.05$). Peak values achieved on the cycle ergometer were significantly higher than the respective values achieved on the upper body ergometer within each group ($P < 0.005$), with the exception of peak RER, peak blood [lactate] and the fraction of peak $\dot{V}O_2$ at which the GET occurred ($P > 0.05$).

**Table 5.2** Peak physiological responses to exercise on a cycle and upper body ergometer in trained and untrained girls

|  | Cycle Ergometry | | Upper body Ergometry | |
|---|---|---|---|---|
|  | Trained | Untrained | Trained | Untrained |
| Peak $\dot{V}O_2$ (L·min$^{-1}$) | 1.69 ± 0.23 | 1.60 ± 0.30 | 1.13 ± 0.13 [#] | 1.09 ± 0.19 [##] |
| Peak $\dot{V}O_2$ (mL·kg$^{-1}$·min$^{-1}$) | 42.9 ± 4.9 | 37.8 ± 7.7 | 29.0 ± 5.5 [#] | 25.4 ± 2.9 [##] |
| Peak $VO_2$ (mL·kg$^{-0.56}$·min$^{-1}$) | 311 ± 30 | 285 ± 47 | 260 ± 33 [#] | 242 ± 31 |
| Peak HR (b·min$^{-1}$) | 192 ± 7 | 195 ± 8 | 177 ± 6 [#] | 180 ± 17 [##] |
| Peak RER | 1.11 ± 0.08 | 1.18 ± 0.13 | 1.07 ± 0.05 | 1.16 ± 0.12 |
| Peak blood [lactate] (mM) | 5.0 ± 0.9 | 5.3 ± 1.2 | 3.7 ± 0.6 | 3.9 ± 0.7 |
| Peak WR (W) | 142 ± 12 | 132 ± 25 | 59 ± 6 | 58 ± 11 |
| GET (L·min$^{-1}$) | 0.84 ± 0.06 | 0.88 ± 0.17 | 0.53 ± 0.07 [#] | 0.57 ± 0.10 [##] |
| GET (% peak $\dot{V}O_2$) | 51 ± 7 | 58 ± 3 | 50 ± 9 | 54 ± 5 |

*Values are mean ± SD. $\dot{V}O_2$, oxygen uptake; HR, heart rate; RER, respiratory exchange ratio; blood [lactate], blood lactate concentration; WR, work rate; GET, gas exchange threshold. N = 8*

*[#]　　　Significant difference between modes in trained children (P < 0.01)*

*[##]　　Significant difference between modes in untrained children (P < 0.01)*

In Table 5.3, the $\dot{V}O_2$ responses to the constant-work-rate tests on the cycle ergometer and upper body ergometer are presented. Between-group differences were evident in baseline

$\dot{V}O_2$, with UT demonstrating a higher baseline value regardless of the exercise modality ($d$ = 2.23 and 1.63 for cycle and upper body exercise, respectively; $P < 0.01$). The UT had a significantly longer $\dot{V}O_2$ phase II $\tau$ (i.e. slower kinetics) during upper body exercise compared to T ($d = 2.62$; $P < 0.001$) and also compared to their respective cycle ergometer response ($d = 1.91$; $P < 0.01$). There was no correlation ($P > 0.05$) between the upper body and cycle ergometer phase II $\tau$ in T (r = -0.36) UT (r = -0.33) or when all the girls were analysed together (r = -0.21). The $\dot{V}O_2$ response of a representative participant from both groups is illustrated in Figure 5.1 for both cycle and upper body exercise. No training status-related differences were evident in the other $\dot{V}O_2$ related parameters during constant-work-rate exercise as shown in Table 5.3. No significant correlation was evident between peak $\dot{V}O_2$ and the phase II $\tau$ in T or UT girls or when the all the girls were analysed together for either cycle exercise (r = -0.47, -0.37 and -0.36) or upper body exercise (r = -0.18, -0.43 and -0.46).

**Table 5.3** Oxygen uptake kinetics and blood [lactate] during heavy-intensity exercise on a cycle and upper body ergometer in trained and untrained girls

|  | Cycle Ergometry | | Upper Body Ergometry | |
|---|---|---|---|---|
|  | Trained | Untrained | Trained | Untrained |
| Baseline $\dot{V}O_2$ (L·min$^{-1}$) | 0.44 ± 0.04 | 0.55 ± 0.06 [*] | 0.34 ± 0.02 [#] | 0.40 ± 0.05 [*,#] |
| Phase II time delay (s) | 11 ± 3 | 12 ± 3 | 12 ± 2 | 9 ± 4 |
| Phase II $\tau$ (s) | 25 ± 5 | 25 ± 7 | 25 ± 3 | 37 ± 6 [*,#] |
| 95% confidence interval (s) | 3 ± 1 | 4 ± 0 | 4 ± 1 | 4 ± 0 |
| Phase II amplitude (L·min$^{-1}$) | 0.83 ± 0.16 | 0.64 ± 0.17 | 0.45 ± 0.07 [#] | 0.51 ± 0.18 |
| Phase II gain (mLO$_2$·min$^{-1}$·W$^{-1}$) | 9.2 ± 0.9 | 8.9 ± 2.7 | 12.2 ± 2.5 [#] | 12.5 ± 1.9 [#] |
| Slow component amplitude (L·min$^{-1}$) | 0.08 ± 0.04 | 0.06 ± 0.03 | 0.05 ± 0.4 | 0.05 ± 0.02 |
| Slow component amplitude (% end exercise $\dot{V}O_2$) | 10 ± 4 | 9 ± 5 | 9 ± 5 | 8 ± 2 |
| End-exercise $\dot{V}O_2$ (L·min$^{-1}$) | 1.35 ± 0.19 | 1.26 ± 0.17 | 0.83 ± 0.09 [#] | 0.95 ± 0.23 [#] |
| $R^2$ | 0.79 | 0.89 | 0.88 | 0.78 |
| Blood [lactate] (mM) | 3.9 ± 0.9 | 4.1 ± 1.4 | 2.7 ± 0.6 [#] | 3.3 ± 1.0 |

*Values are mean ± SD $\dot{V}O_2$, oxygen uptake; $\tau$, time constant; blood [lactate], blood lactate concentration. N = 8*

[*]　　*Significant difference between trained and untrained within exercise modality (P < 0.01)*

[#]　　*Significant difference between modes within training status group (P < 0.01)*
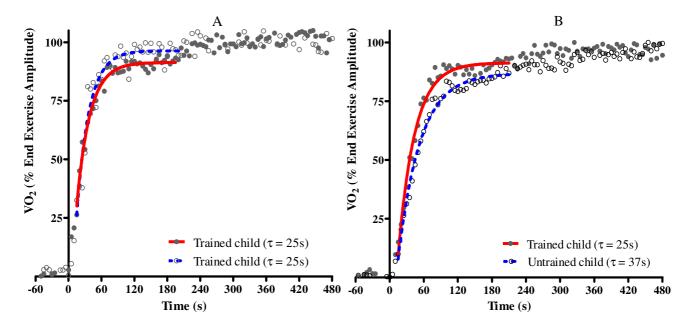
The higher work rates employed during cycle exercise resulted in higher absolute $\dot{V}O_2$ values both at the end of the primary phase and at the end of exercise ($P < 0.001$).

However, the $\dot{V}O_2$ primary component gain was higher during upper body exercise ($P <$ 0.01). T had a higher end-exercise blood [lactate] during cycle compared to upper body exercise ($d = 1.62$; $P < 0.01$). A $\dot{V}O_2$ slow component was evident in all the girls' responses with the exception of one trained girl who failed to show a significant deviation from the steady state during upper body exercise. There were no differences in the absolute or relative magnitude of the $\dot{V}O_2$ slow component with training status or exercise modality.

**Figure 5.1** Pulmonary oxygen uptake response to a step increment in work rate from an unloaded baseline to a heavy intensity work rate (40%Δ) in a representative trained (closed circles) and untrained participant (open circles) during cycle exercise (A) and upper body exercise (B). The data are expressed as a percentage of the end exercise amplitude with the solid and dashed lines represent the mono-exponential model fiT. For clarity, data are displayed as 5-s bin averages



The parameters derived from the modelling of the HR responses to constant-work-rate exercise are presented in Table 5.4. UT had higher baseline HR regardless of the exercise modality ($d = 2.55$ and 1.50 for cycle and upper body exercise, respectively; $P < 0.01$). On both modalities, the HR response was faster in T than UT ($d = 1.65$ and 1.64 for cycle and upper body exercise, respectively; $P < 0.01$) but there was no influence of exercise
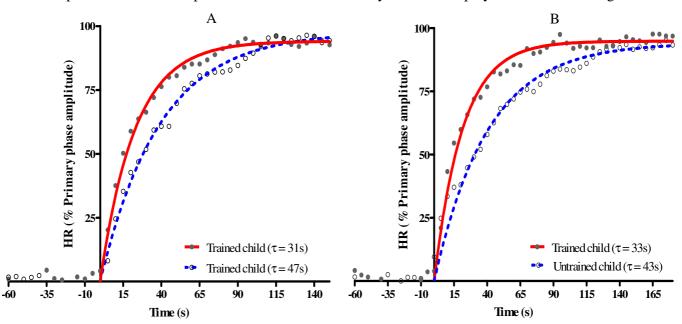
modality in either group. The HR response of a representative participant from both groups is illustrated in Figure 5.2 for both cycle and upper body exercise. No correlation was found for either exercise mode between the $\dot{V}O_2$ phase II $\tau$ and the HR $\tau$ in either trained (cycle: r = 0.53; upper body: r = 0.22) or untrained (cycle: r = 0.44; upper body: r = 0.14) girls or when all the girls were analysed together (cycle: r = 0.35; upper body: r = 0.19).

**Table 5.4** Heart rate kinetics during heavy-intensity exercise on a cycle and upper body ergometer in trained and untrained girls

| | Cycle Ergometry | | Upper Body Ergometry | |
| --- | --- | --- | --- | --- |
| | Trained | Untrained | Trained | Untrained |
| HR baseline (b·min$^{-1}$) | 91 ± 8 | 112 ± 9 [*] | 93 ± 6 | 101 ± 5 [*,#] |
| HR phase II $\tau$ (s) | 31 ± 11 | 47 ± 9 [*] | 33 ± 8 | 43 ± 4 [*] |
| HR phase II amplitude (b·min$^{-1}$) | 62 ± 8 | 54 ± 10 | 48 ± 7 [#] | 41 ± 10 [#] |
| End-exercise HR (b·min$^{-1}$) | 169 ± 9 | 179 ± 9 | 151 ± 12 [#] | 157 ± 17 [#] |
| 95% confidence interval (s) | 1 ± 0 | 2 ± 1 | 1 ± 0 | 2 ± 1 |

*Values are mean ± SD. HR, heart rate; τ, time constant. N = 8*

*\*        Significant difference between trained and untrained within exercise modality (P < 0.01)*

*#        Significant difference between modes within training status group (P < 0.01)*

**Figure 5.2.** Heart rate response to a step increment in work rate from an unloaded baseline to a heavy intensity work rate (40%Δ) in a representative trained (closed circles) and untrained participant (open circles) during A) cycle and B) upper body exercise. Data expressed as a percentage of the primary phase amplitude and the solid and dashed lines represent the mono-exponential model fit. For clarity, data are displayed as 5-s bin averages

## 5.4 Discussion

This is the first study to investigate the influence of training status and exercise modality on the $\dot{V}O_2$ and HR kinetic responses during step transitions to a heavy intensity work rate in children. We hypothesised that adaptations to swimming training would be evident in the $\dot{V}O_2$ and HR kinetics measured during constant-work-rate exercise tests and that the effects would be more evident during upper body than during cycle ergometry. The results are partly consistent with our hypotheses as training status effects were evident in the $\dot{V}O_2$ kinetic response to upper body ergometry and in the HR kinetic response to both exercise modalities. Specifically, in response to the transition to a heavy-intensity work rate, trained girls had faster $\dot{V}O_2$ (upper body only) and HR kinetics (both modalities) compared to untrained girls. There was no effect of training status on the physiological responses to incremental exercise for either exercise modality.

### 5.4.1 Peak physiological responses

The peak physiological responses observed in the present study during both cycle and upper body ergometry demonstrate the insensitivity of peak values to training in children. Whilst the absence of an influence of training status on peak $\dot{V}O_2$ is not a novel finding during cycle ergometry (Shephard, 1992; Welsman *et al.*, 1996), the physiological responses to upper body ergometry have not previously been investigated. This study therefore demonstrates that even when a more relevant, if not entirely sport-specific, exercise modality is used, incremental tests to the limit of tolerance are inappropriate for the investigation of training status differences, at least in pre- and early-pubertal girls. A limitation of this study is that no supra-maximal tests were conducted to verify if the peak $\dot{V}O_2$ values obtained from the incremental ramp tests were true peak values. The HR, RER and blood [lactate] criteria that are commonly applied for the acceptance of a $\dot{V}O_2$ 'max' both in adult and paediatric populations has recently been challenged (Poole *et al.*, 2008b; Barker *et al.*, 2009). Consequently, the peak $\dot{V}O_2$ values reported here cannot be definitively stated to reflect a maximal effort and are presented for consideration within the

limits of reliability reported for peak $\dot{V}O_2$ determined using this traditional methodology (Welsman *et al.*, 2005).

The similar peak $\dot{V}O_2$ values reported here between the T and UT girls may largely reflect genetic factors and/or that the appropriate hormonal milieu required for training adaptations in peak $\dot{V}O_2$ to be manifest is not sufficiently developed before puberty (Katch, 1983). It is widely accepted that stroke volume (and hence cardiac output) is a primary determinant of peak $\dot{V}O_2$ (Nottin *et al.*, 2002b; Obert *et al.*, 2003). It is interesting to note, therefore, that several studies have been unable to demonstrate changes in myocardial morphology following training in children (Nottin *et al.*, 2004; Obert *et al.*, 2009; Rowland *et al.*, 2009a). Since changes in cardiac structure including an increased thicknesses of the intraventricular and posterior walls thickness are commonly reported in adults (e.g. George *et al.*, 1991; Caso *et al.*, 2000), this might indicate that structural adaptations to cardiac muscle with training is somehow impeded in children. This age-related disparity may be related to a biological immaturity such as a lack of testosterone (Rowland *et al.*, 1994; Obert *et al.*, 2003) or to an overload limitation, either due to the lower blood pressures present in children during exercise (Nottin *et al.*, 2002b) or the shorter duration of training undertaken by children (Nottin *et al.*, 2004). Although training has been reported to increase testosterone and growth hormone levels from puberty onwards, no difference in the levels of these hormones has been shown pre-puberty (Zakas *et al.*, 1994; Daly *et al.*, 1998; Tsolakis *et al.*, 2003).

Consistent with previous training studies in pre-pubertal children, we found no influence of training status on the fraction of peak $\dot{V}O_2$ at which the GET occurred (Obert *et al.*, 2000; Cleuziou *et al.*, 2002). These findings contrast with those commonly reported in adults where training increases the fraction of peak $\dot{V}O_2$ at which the GET occurs (Carter *et al.*, 2000; Boone *et al.*, 2008). The reason/s for this discrepancy is unclear from the present results. Exercise modality did not influence the percentage of peak $\dot{V}O_2$ at which the GET occurred, a finding which agrees with some adult studies (Koga *et al.*, 1996; Schneider *et al.*, 2002) but contrasts with others (Davis *et al.*, 1976; Bhambhani *et al.*, 1998).

### 5.4.2 Sub-maximal physiological responses: cycle exercise

In agreement with previous studies, swimming training status did not alter the primary component $\dot{V}O_2$ kinetics nor affect the $\dot{V}O_2$ slow component amplitude in absolute or relative terms during cycle ergometer exercise (Obert *et al.*, 2000; Cleuziou *et al.*, 2002). The novelty of this study therefore lies not in the cycle ergometry results but in the confidence associated with those results, as this is the first study in children to employ a stringent and controlled methodology, the result of which was confidence intervals well within those suggested by Fawkner and colleagues (2007) for both the phase II $\tau$ and primary phase amplitude.

Our results are not, however, in agreement with those obtained in adults which show a reduced phase II $\tau$ during cycle exercise subsequent to training (e.g. Koppo *et al.*, 2004; Berger *et al.*, 2006; Figueira *et al.*, 2008; Bailey *et al.*, 2009) or consequently with our hypothesis. The cause of this disparity is not readily apparent but may be related to either an age-dependent change in the muscles' potential for oxygen utilisation (Fawkner & Armstrong, 2004a) or to the shorter duration of training in children. Alternatively, the failure of this and previous studies to demonstrate differences in the $\dot{V}O_2$ kinetic response in relation to training or training status during cycle ergometry may be related to inappropriate testing modalities. Swimming is widely accepted to have a significant upper body contribution (Ogita *et al.*, 1996) and hence the testing of swimmers on a cycle ergometer might not be sufficiently specific. Whilst there is a variable contribution of the lower body to swimming, the present results could suggest there was very little effect of training on the legs of the participants tested. Additionally, the lack of difference in $\dot{V}O_2$ kinetics between the T and UT children during cycle exercise might reflect a similar level of habitual physical activity involving walking, running or cycling in the two groups.

### 5.4.3 Sub-maximal physiological responses: upper body exercise

To our knowledge, this is the first study to characterise $\dot{V}O_2$ kinetics during upper body exercise in children. The phase II $\tau$ values reported here for UT children are similar to those of Smith et al. (2006b) and considerably faster than those reported by Koga et al. (1996)

and Koppo et al. (2002) for adults. No data are available for comparison with the trained girls phase II τ, even within the adult literature. The primary phase gain for both trained and untrained girls is within the range reported in adult studies (Koppo *et al.*, 2002; Smith *et al.*, 2006b).

This is the first study to report a significant training status-related difference in the $\dot{V}O_2$ kinetics of prepubertal girls. Specifically, the phase II τ was 32% faster for upper body ergometry in the T compared to the UT girls, a finding that emphasises the importance of test mode specificity and indicates that an exercise modality involving the upper body is more appropriate for testing swimmers. Considering that upper body ergometry does not precisely represent the movements involved in swimming, it is likely that greater differences between T and UT girls would be demonstrated if $\dot{V}O_2$ kinetics were to be characterised during a swimming test. Further work is required to develop methodologies to enable such measurements.

Resolution of the mechanisms responsible for the faster $\dot{V}O_2$ kinetics found in the swimmers of this study is impeded by the cross-sectional nature of the study. The comparison of T and UT participants means it is impossible to attribute the differences observed to training *per se*, as the differences may purely be a reflection of genetic traits which predisposed these children to success in swimming.

If physiological adaptations to training did contribute to the differences observed, the faster $\dot{V}O_2$ kinetics could potentially be related to an increased muscle $O_2$ delivery, greater muscle oxidative capacity, and/or to differences in muscle fibre type distribution or recruitment subsequent to training. HR kinetics, which may provide a crude estimate of muscle blood flow kinetics (MacPhee *et al.*, 2005), were faster for both exercise modalities in the T girls. However, the $\dot{V}O_2$ and HR kinetics were not related in either group suggesting that $O_2$ oxygen delivery was not a limiting factor, a conclusion which agrees with adult studies (see Poole et al (2008a), for review). Endurance training has been shown to enhance muscle oxidative capacity in adults through increases in mitochondrial volume and oxidative enzyme activities (Holloszy, 1967; Mogensen *et al.*, 2006). Whether similar adaptations to training are present in pre-pubertal children remains to be resolved, with information

regarding the muscle oxidative capacity of T and UT children almost non-existent (Mahon, 2008). The limited information available suggests that training may increase muscle oxidative enzyme activity in children (Eriksson *et al.*, 1973; Fournier *et al.*, 1982). An increased mitochondrial volume following endurance training would be predicted to result in faster $\dot{V}O_2$ kinetics (Meyer, 1988). In the present study, we are unable to ascertain the physiological mechanism(s) which led to faster $\dot{V}O_2$ kinetics in T compared to UT girls. However, as in adults, this is likely a function of an enhanced integrated capacity to both transport and utilize $O_2$ (Jones & Koppo, 2005; Poole *et al.*, 2008a).

It is possible that the recruitment of muscle fibres of different types may influence the pulmonary phase II $\tau$, since it has been suggested that type I fibres have a faster time constant for the rise in $\dot{V}O_2$ (Crow & Kushmerick, 1982; Krustrup *et al.*, 2008). Indirect evidence for an influence of muscle fibre type on $\dot{V}O_2$ kinetics has been provided by the demonstration of a negative correlation between the type I fibres percentage in the vastus lateralis and the pulmonary phase II $\tau$ during cycle exercise (Pringle *et al.*, 2003). Such a negative association is pertinent to the current results because some studies report that endurance training induces an increase in the percentage of type I fibres (Saltin & Gollnick, 1983; Russell *et al.*, 2003) and/or alters the recruitment pattern to reduce the contribution of type II fibres. Therefore, the faster $\tau$ in the current study may be related to training-induced differences in the metabolic properties of the muscle fibres recruited to meet the exercise demand.

There was no significant correlation between the peak $\dot{V}O_2$ and the phase II $\tau$ in either the T or the UT group for either exercise mode. This is consistent with some previous studies in both paediatric (Cleuziou *et al.*, 2002; Fawkner *et al.*, 2002) and adult (Carter *et al.*, 2000) populations, but differs from other studies which have suggested that higher peak $\dot{V}O_2$ values are associated with faster phase II $\dot{V}O_2$ kinetics in children (Cooper *et al.*, 1985)and adults (Powers *et al.*, 1985; Chilibeck *et al.*, 1996). Given that both peak $\dot{V}O_2$ and $\dot{V}O_2$ kinetics are acknowledged as parameters of oxidative metabolic function, a relationship between the two might be expected at least in a sample of subjects with heterogeneous aerobic fitness (Poole *et al.*, 2005). That this was not the case in the present study might be a function of the relatively small sample size but might also reflect differences in the

factors which are predominantly limiting, i.e., central cardiovascular factors for peak $\dot{V}O_2$ and intrinsic muscle metabolic factors for $\dot{V}O_2$ kinetics (Poole *et al.*, 2008a).

### *5.4.4 Influence of exercise modality on $\dot{V}O_2$ kinetics*

To our knowledge, this is the first study to investigate the influence of exercise modality on the $\dot{V}O_2$ kinetics of T and UT children. The slower phase II $\tau$ during upper body ergometry compared to cycle ergometry in the UT girls is consistent with previous reports in adults (Koga *et al.*, 1996; Koppo *et al.*, 2002; Schneider *et al.*, 2002). However, in the T girls there was no difference in the phase II $\tau$ between exercise modalities.

The musculature of the arm and leg differ in the percentage of type I muscle fibres and the capillary-to-fibre ratio, both being lower in the arm musculature (Johnson *et al.*, 1973; Turner *et al.*, 1997). Furthermore, the muscle perfusion pressure has been suggested to be lower during arm exercise due to a reduced "gravitational assist" to muscle blood flow compared to upright leg exercise (Koga *et al.*, 1999; Jones & Burnley, 2005; Koppo & Bouckaert, 2005). These factors could negatively influence the rate of adjustment of $\dot{V}O_2$ to an increased metabolic demand and, therefore, the modality-related differences in the phase II $\tau$ in UT girls may be a reflection of these differences. Given the physiological differences in arm and leg musculature including a higher proportion of type II fibres in the upper body, it is perhaps surprising that no differences were found in the amplitude of the $\dot{V}O_2$ slow component between exercise modalities. However, it is important to note that upper body exercise involves a contribution of accessory muscles, such as the shoulders and back, both in the generation of power and for stabilisation; it is possible that these factors obscure the relationship between fibre recruitment and $\dot{V}O_2$ kinetics (Smith *et al.*, 2006a).

### *5.5 Conclusions*

In conclusion, incremental exercise tests did not reveal any influence of training status on GET or peak $\dot{V}O_2$ regardless of exercise modality in pre-pubertal girls. However, T girls had faster phase II $\dot{V}O_2$ kinetics compared to UT girls during upper body but not cycle

ergometry. As a consequence, the $\dot{V}O_2$ kinetics of T girls was not different between the exercise modalities, whereas UT girls had slower phase II $\dot{V}O_2$ kinetics during upper body exercise compared to leg exercise. These data therefore highlight the importance of test specificity in revealing training status differences and demonstrate, for the first time, that $\dot{V}O_2$ kinetics are faster in trained compared to untrained pre-pubertal girls

**Chapter 6**

**Study 3 - Influence of training status and exercise modality on pulmonary O$_2$ uptake kinetics in pubertal girls**

This study has been disseminated as follows:

*Publication:* McNarry, M.A., Welsman, J.R., & Jones, A.M. (2010). Influence of training status and exercise modality on pulmonary O$_2$ uptake kinetics in pubertal girls. European Journal of Applied Physiology. DOI: 10.1007/s00421-010-1681-6.

*Poster Presentation:* Winlove, M.A., Welsman, J.R., & Jones, A.M. (2010). Influence of training status and exercise modality on pulmonary O$_2$ uptake kinetics in pubertal girls. The 15th Annual Conference of the European College of Sport Science (Antalya, Turkey, 23rd-26th June 2010). Awarded 3rd place in the Young Investigator Award.

## *6.1 Introduction*

Following a sudden increase in the external work rate, pulmonary oxygen uptake ($\dot{V}O_2$) increases in a predictable, exercise-intensity dependent manner. Below the gas exchange threshold (GET), the $\dot{V}O_2$ response is characterised by 3 phases: an initial cardiodynamic phase which reflects the rapid elevation in cardiac output and pulmonary blood flow, a second phase during which $\dot{V}O_2$ increases exponentially (reflecting the increasing muscle $\dot{V}O_2$; (Grassi *et al.*, 1996; Krustrup *et al.*, 2009) and a final steady-state phase which is typically achieved after 2-3 minutes of constant-work-rate exercise (Whipp & Wasserman, 1972; Whipp *et al.*, 1982). Above the GET the attainment of a steady state is delayed, or even precluded, by the presence of a supplementary "slow component" of $\dot{V}O_2$ (Whipp & Wasserman, 1972; Barstow & Mole, 1991).

The $\dot{V}O_2$ kinetic response has been demonstrated to be highly adaptive in adults, with one of the most potent interventions being exercise training. Endurance training has been shown to result in a faster phase II time constant ($\tau$) and a reduced contribution of the slow

component to the total increase in $\dot{V}O_2$ (Powers *et al.*, 1985; Koppo *et al.*, 2004; Bailey *et al.*, 2009). The temporal features of the $\dot{V}O_2$ response have similarly been shown to be influenced by training status in pre-pubertal children, provided that an appropriate test modality is used (chapter 5), although this remains controversial (Obert *et al.*, 2000; Cleuziou *et al.*, 2002).

There is a dearth of studies investigating the influence of training status on $\dot{V}O_2$ kinetics in pubertal populations. In the only previous study, adolescent male football players demonstrated significantly faster $\dot{V}O_2$ kinetics during moderate intensity cycle exercise (Marwood *et al.*, 2010). Whether training status similarly influences the $\tau$ or slow component of $\dot{V}O_2$ during heavy intensity exercise in adolescents is unknown. This question warrants investigation given reports in adults that the effects of training status on $\dot{V}O_2$ kinetics may be exercise-intensity dependent (Carter *et al.*, 2000; Krustrup *et al.*, 2004; Bailey *et al.*, 2009).

The mechanistic bases for training status-related differences in $\dot{V}O_2$ kinetics are unclear. While $\dot{V}O_2$ kinetics is accepted to be regulated by both muscle $O_2$ delivery and $O_2$ utilisation, the relative importance of each factor in different populations, at different intensities and in different exercise modalities remains a topic of debate (Tschakovsky & Hughson, 1999; Jones & Burnley, 2005; Poole *et al.*, 2008a). In adults, it appears that the training-induced speeding of the phase II $\tau$ may be predominantly related to an enhanced $O_2$ extraction at the muscle (Krustrup *et al.*, 2004; Bailey *et al.*, 2009) due to an increased mitochondrial volume and oxidative enzyme activity (Phillips *et al.*, 1995; Krustrup *et al.*, 2004) and/or to an enhanced matching of perfusion to metabolic demand (Murias *et al.*, 2010). Bulk muscle blood flow has also been shown to be enhanced following training (Shoemaker *et al.*, 1996; Laughlin & Roseguini, 2008) and therefore cannot be eliminated as a potential explanatory variable (McKay *et al.*, 2009). Information on the extent to which training status-related differences in $\dot{V}O_2$ kinetics are related to central or peripheral factors in adolescents is sparse. Marwood et al. (2010) have recently reported that during moderate intensity exercise male adolescent football players had faster heart rate kinetics but unaltered deoxygenated haemoglobin/myoglobin ([HHb]) kinetics compared to untrained

subjects. The authors interpreted these results as evidence that training enhanced both muscle $O_2$ delivery and fractional $O_2$ extraction (Marwood *et al.*, 2010). The mechanistic basis for any differences in $\dot{V}O_2$ kinetics during heavy intensity exercise in adolescents has yet to be investigated, with available techniques necessarily indirect due to the ethical considerations associated with testing young populations.

A fundamental limitation of many of the studies investigating the influence of training status on $\dot{V}O_2$ kinetics in adolescents is a lack of commonality between the muscles trained and the muscles tested. Indeed, the importance of exercise modality to the investigation of training status influences on $\dot{V}O_2$ kinetics has previously been reported in pre-pubertal girls where effects were evident in upper body but not lower body exercise (Chapter 5).

The purpose of this cross-sectional study was to assess the influence of training status on the kinetics of $\dot{V}O_2$, HR and muscle deoxygenation in pubertal girls during heavy-intensity exercise. We hypothesised that, in accordance with findings in adults, the $\dot{V}O_2$ kinetics would be faster and that the $\dot{V}O_2$ slow component would be relatively smaller in trained swimmers compared to an age-matched untrained control group. We also hypothesised, given the large upper body contribution to swimming (Ogita *et al.*, 1996), that training status-related differences would be more evident during upper body (arm crank ergometer) than during lower body (cycle ergometer) exercise.

### *6.2 Methods*

### *6.2.1 Participants*

Eight endurance-trained (T) girls and 8 untrained (UT) girls aged 13-15 years participated in this study. The T group, all competitive swimmers with a mean training volume of 12 (± 2) hours/week and who had been swimming for an average of 5.2 (± 0.7) years, were recruited from a local swimming club. The UT group comprised volunteers from local schools. Sexual maturity was assessed by self-report using the indices of pubic hair described by Tanner (1962). All children reported a maturity level of 3 or 4, indicating the study population was pubertal. Age to peak height velocity was estimated to provide an

additional indicator of physical maturity according to the equations of Mirwald *et al.* (2002).

All participants were anthropometrically evaluated during the first visit to the laboratory. Standing and seated height was measured to 0.1 cm using a Holtain stadiometer (Holtain, Crymych, Dyfed, UK) and body mass was determined to 0.05 kg using Avery beam balance scales (Avery, Birmingham, UK). Skinfold thickness was assessed three times at four sites around the body (biceps, triceps, subscapular and supra-iliac crest) by the same researcher for all participants using Harpenden callipers (Baty International, Burgess Hill, UK), accurate to the nearest 0.2 mm. The average of the three measurements was taken. Table 6.1 presents the participants' physical characteristics.

Participants were asked to arrive at the laboratory in a rested and fully hydrated state, at least 3 hours postprandial and to have avoided consuming caffeinated drinks for 6 hours prior to the test. The methods employed during this study were approved by the institutional research ethics committee and all participants and their parents/guardians gave written informed consent and assent, respectively.

**Table 6.1** Physical characteristics of participants

|                              | **Trained**     | **Untrained**    |
| ---------------------------- | --------------- | ---------------- |
| Age (y)                      | 14.2 ± 0.7      | 14.5 ± 1.3       |
| Stature (m)                  | 1.66 ± 0.04     | 1.61 ± 0.06      |
| Mass (kg)                    | 54.0 ± 5.1      | 58.7 ± 12.1      |
| Sum of skinfolds (mm)        | 34.0 ± 10.7     | 48.7 ± 23.3      |
| Estimated years past PHV (y) | 2.0 ± 0.4       | 2.4 ± 0.6        |

*Values are mean ± SD. PHV, peak height velocity. No significant differences were present. N = 8*

### 6.2.2 Incremental Test

On the first two visits to the laboratory, exercise mode-specific peak $\dot{V}O_2$ and gas exchange threshold (GET) were determined using a ramp incremental test to voluntary exhaustion on both cycle (Lode Excalibur, Netherlands) and upper body (Lode Angio, Netherlands) ergometers. The handle bar height, seat height and crank length (cycle ergometer) and

electrically controlled seat height and distance (upper body ergometer) were adjusted to suit each participant and the values recorded so they could be replicated throughout the testing series.

After a three minute warm-up consisting of unloaded pedalling or arm cranking (equivalent to 10W at 70 rpm according to the manufacturer's guidelines), the resistance increased by 20 $W \cdot min^{-1}$ and 10 $W \cdot min^{-1}$ for cycle and upper body exercise, respectively, to attain a test of ~8-12 minutes in duration (Buchfuhrer *et al.*, 1983). Participants were instructed to maintain a cadence within the range of 70 ± 5 and 50 ± 5 rpm on the cycle and upper body ergometer, respectively. Maximal tests were considered to have been achieved if in addition to subjective indications such as sweating, hyperpnoea and facial flushing and there was a consistent reduction in cadence despite strong verbal encouragement. The peak $\dot{V}O_2$ was defined as the highest 10-s stationary average during the test. The GET was determined by the V-slope method (Beaver *et al.*, 1986) as the point at which carbon dioxide production began to increase disproportionately to $\dot{V}O_2$ as identified using purpose written software developed using LabVIEW (National Instruments, Newbury, UK).

### *6.2.3 Constant Work Rate Tests*

The participants returned to the laboratory on a number of other occasions to complete "step" tests on the upper body and cycle ergometers for the determination of $\dot{V}O_2$ kinetics. Where multiple tests were performed on the same day, at least 1 hour separated the tests and the tests were ordered such that the first test involved a smaller muscle mass (upper body), thereby resulting in a smaller metabolic perturbation and faster recovery. On average, 3 cycle and 4 upper body transitions were completed, depending on the number of transitions required to obtain 95% confidence intervals of < 4 s for the phase II $\dot{V}O_2$ τ. All constant-work-rate tests consisted of 4 minutes of unloaded pedalling or cranking followed by an 'instantaneous' transition to a work rate calculated to require 40% of the difference between the GET and peak $\dot{V}O_2$ (40%Δ) for 8 minutes. At 8 minutes the work rate returned to an unloaded baseline at which the participants pedalled or cranked for a further 6 minutes. Throughout the cycle ergometer and upper body tests, cadences of 70 ± 5 rpm and 50 ± 5 rpm were maintained, respectively.

### 6.2.4 Measurements

Throughout all the tests, gas exchange variables (Metalyser 3B Cortex, Biophysik, Leipzig, Germany) and heart rate (Polar S610, Polar Electro Oy, Kempele, Finland) were measured on a breath-by-breath basis and displayed online. Prior to each test the gas analysers were calibrated using gases of known concentration and the turbine volume transducer was calibrated using a 3-litre syringe (Hans Rudolph, Kansas City, MO). The delay in the capillary gas transit and analyser rise time were accounted for relative to the volume signal, thereby time-aligning the concentration and volume signals. During each transition, fingertip blood samples were taken 1 minute after completion of the loaded phase and assayed for blood lactate concentration (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH).

For at least one transition for each exercise modality, the oxygenation status of the right *m. vastus lateralis* (cycle) or right *m. tricep brachii* (upper body) was monitored using a commercially available near-infrared system (NIRO 300, Hamamatsu Photonics KK, Hiugashi-ku, Japan). This system consists of an emission probe which emits four wavelengths of light (776, 826, 845 and 905 nm) and a photon detector. The intensity of incident and transmitted light was recorded continuously at 2 Hz and used to estimate the concentration changes relative to baseline levels for oxygenated, deoxygenated and total haemoglobin. The [HHb] signal was used as an indicator of $O_2$ extraction within the field of interrogation (DeLorey *et al.*, 2003; Grassi *et al.*, 2003; Jones *et al.*, 2006; Ferreira *et al.*, 2007). The contribution of myoglobin to the NIRS signal is currently unresolved (Seiyama *et al.*, 1988; Masuda *et al.*, 2010). Therefore, the [HHb] signal described throughout this paper should be considered to refer to the combined concentration of both deoxygenated haemoglobin and myoglobin.

The muscle was initially cleaned and the probes placed in a rubber holder which was adhered to the skin at the midpoint of the muscle. To ensure the holder and its probes remained stationary during exercise and to minimise the interference of extraneous light with the near-infrared signal a bandage was wrapped around the arm/leg. The position of the holder relative to the fibular head or ulna head was recorded to enable accurate

replication in subsequent tests. The NIRS signal was zeroed with the participant at rest in a seated position with the muscle stationary and relaxed.

### 6.2.5 $\dot{V}O_2$ Kinetics Analysis

Initially, the breath-by-breath responses to each transition were examined to remove any errant breaths caused by coughing, swallowing, sighing etc, using a 5 s moving average to identify points lying in excess of 4 SD from the local mean. Subsequently, each transition was interpolated to 1 s intervals, time aligned to the start of exercise and averaged.

To remove the influence of phase I on analysis of the subsequent response, the first 15 s of data were ignored. A mono-exponential model with a time delay (Eq. 6.1) was then applied to the averaged response and kinetic parameters and their 95% confidence intervals determined by least squares linear regression analysis (Graphpad Prism, Graphpad Software, San Diego, CA).

$$\Delta \dot{V}O_{2(t)} = A_1 \cdot \left(1 - e^{-(t-\delta_1)/\tau_1}\right)$$
(Eq. 6.1)

where $\Delta\dot{V}O_2$ is the increase in $\dot{V}O_2$ at time $t$ above the baseline value (calculated as the mean $\dot{V}O_2$ from the first 45 s of the last minute of baseline pedalling), and $A_1$, $\delta_1$ and $\tau_1$ are the primary component amplitude, time delay and time constant, respectively.

The fitting window was constrained to exclude all data after the visually determined onset of the $\dot{V}O_2$ slow component. This approach therefore avoids any possible influence of arbitrarily parameterizing the slow component. The onset of the $\dot{V}O_2$ slow component was determined using purpose designed LabVIEW software which iteratively fits a mono-exponential function to the $\dot{V}O_2$ data until the window encompasses the entire response. The resulting phase II time constants are plotted against time and the onset of the $\dot{V}O_2$ slow component identified as the point at which the phase II time constant consistently deviates from the previously "flat" profile (Rossiter et al. 2001). The amplitude of the $\dot{V}O_2$ slow component was subsequently determined by calculating the difference between the end exercise $\dot{V}O_2$ and the primary amplitude plus baseline $\dot{V}O_2$. This was expressed both in

absolute terms and relative to end exercise $\dot{V}O_2$. The functional gain of the primary $\dot{V}O_2$ response was also calculated by dividing the primary phase amplitude by the change in work rate.

### 6.2.6 [HHb] & HR Kinetics Analysis

The [HHb] and HR responses to exercise were also modelled. The responses to each transition were interpolated to 1 s intervals, time aligned and averaged to produce a single data set. The resulting [HHb] response was fitted with a mono-exponential with a time delay (Eq. 6.1) whereas the HR response was modelled by a mono-exponential without a time delay (Eq. 6.2). For both responses the fitting window started at $t = 0$ and was constrained to the onset of the "slow component".

$$\Delta HR_{(t)} = A_1 \cdot \left(1 - e^{-(t/\tau_1)}\right)$$
(Eq. 6.2)

where $\Delta$HR is the increase in heart rate at time $t$ above the baseline (calculated as the mean heart rate from the first 45 s of the last minute of baseline pedalling), and $A_1$ and $\tau_1$ are the primary component amplitude and time constant, respectively. The [HHb] time delay (TD) and $\tau$ were summed, giving the [HHb] mean response time (MRT), which provides information on the overall [HHb] response over the fundamental phase of the response.

### 6.2.7 Statistics

The allometric relationship between body mass and peak $\dot{V}O_2$ was determined using analysis of covariance (ANCOVA) on log transformed data (Welsman & Armstrong, 2000). From the values of the regression slopes (allometric exponents) confirmed as common to all groups, power function ratios ($Y/X^b$) were computed. Following identification of a normal distribution, homogenous variation and an absence of skewness or kurtosis a two way ANOVA with repeated measures was used to analyse training status and exercise mode effects. Subsequent independent or paired samples t-tests with a Bonferonni correction were employed as appropriate to identify the location of significant effects. Pearson product-moment correlation coefficients were used to assess the strength of

relationships between variables. All data are presented as means ± SD. Statistical significance was accepted when $P < 0.05$.

## 6.3 Results

The physiological responses to ramp incremental exercise on the cycle and upper body ergometers are summarised in Table 6.2. The T girls demonstrated significantly higher absolute ($d = 2.07$ and $2.28$ for cycle and upper body exercise, respectively) and relative peak $\dot{V}O_2$ for both exercise modalities ($d = 3.24$ and $2.08$ for cycle and upper body exercise, respectively). Allometric scaling did not alter the difference in peak $\dot{V}O_2$ between T and UT girls for either exercise modality. Similarly, peak work rate was significantly higher in the T girls for both cycle ($d = 1.98$) and upper body ergometry ($d = 2.59$). Peak heart rate and the fraction of peak $\dot{V}O_2$ at which the GET occurred were not affected by training status. Cycle ergometry elicited significantly higher response values compared to upper body ergometry, with the exception of peak heart rate and the fraction of peak $\dot{V}O_2$ at which the GET occurred. The difference between T and UT girls peak $\dot{V}O_2$ was less during cycle (~24% higher in T) than during upper body (~32% higher in T) ergometry.

**Table 6.2** Peak physiological responses to exercise on a cycle and upper body ergometer in trained and untrained girls

| | Cycle Ergometry | | Upper body Ergometry | |
|---|---|---|---|---|
| | Trained | Untrained | Trained | Untrained |
| Peak $\dot{V}O_2$ (L·min$^{-1}$) | 2.51 ± 0.27 | 1.98 ± 0.26 [*] | 1.88 ± 0.26 [#] | 1.36 ± 0.21 [*#] |
| Peak $\dot{V}O_2$ (mL·kg$^{-1}$·min$^{-1}$) | 46.6 ± 5.0 | 34.5 ± 2.2 [*] | 35.0 ± 6.0 [#] | 24.0 ± 4.9 [*#] |
| Peak HR (b·min$^{-1}$) | 194 ± 5 | 196 ± 6 | 186 ± 10 | 180 ± 15 |
| Peak blood [lactate] (mM) | 7.8 ± 1.5 | 6.5 ± 2.1 | 5.1 ± 1.8 | 5.4 ± 2.1 |
| Peak WR (W) | 227 ± 23 | 180 ± 26 [*] | 99 ± 9 [#] | 71 ± 13 [*#] |
| GET (L·min$^{-1}$) | 1.51 ± 0.23 | 1.12 ± 0.26 | 1.00 ± 0.29 [#] | 0.69 ± 0.29 [#] |
| GET (% peak $\dot{V}O_2$) | 60 ± 5 | 62 ± 4 | 53 ± 10 | 56 ± 12 |

*Values are mean ± SD. $\dot{V}O_2$, oxygen uptake; HR, heart rate; blood [lactate], blood lactate concentration; WR, work rate; GET, gas exchange threshold. N = 8*

*\* Significant difference between trained and untrained children within an exercise modality (P < 0.01)*

*# Significant difference between modes within trained or untrained children (P < 0.01)*

### 6.3.1 $\dot{V}O_2$ kinetics

The parameters determined from the monoexponential modelling revealed a significant influence of training status on the $\dot{V}O_2$ kinetics, as presented in Table 6.3 and illustrated in Figure 6.1. Specifically, the phase II $\tau$ was significantly lower and the phase II amplitude significantly greater in the trained girls during both cycle ($\tau$: $d = 1.64$; amplitude: $d = 1.84$) and upper body ergometry ($\tau$: $d = 1.94$; amplitude: $d = 2.65$). The $\dot{V}O_2$ slow component response was not affected by training status for either exercise modality. The temporal aspects of the $\dot{V}O_2$ response were not affected by exercise modality in contrast to the amplitude related parameters, such as the baseline $\dot{V}O_2$, phase II amplitude and end-exercise $\dot{V}O_2$, which were significantly higher during cycle ergometry. Exercise modality also influenced the primary gain, with upper body ergometry associated with a higher $O_2$ cost per watt compared to cycle ergometry. A significant correlation was evident between the phase II $\tau$ during cycle and upper body ergometry (r = 0.57; $P < 0.05$).

**Table 6.3** Oxygen uptake kinetics and blood [lactate] during heavy-intensity exercise on a cycle and upper body ergometer in trained and untrained girls
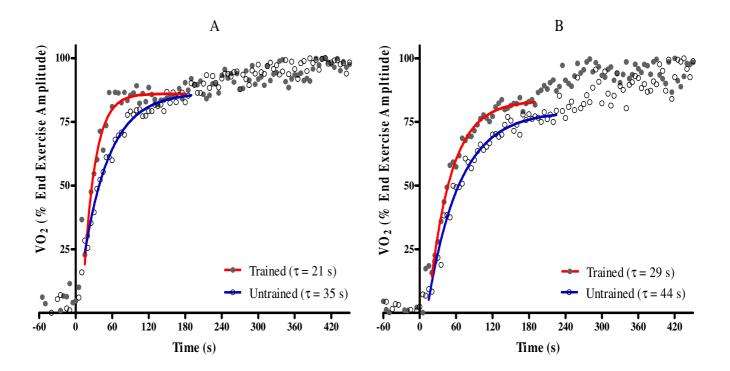
| | Cycle Ergometry | | Upper Body Ergometry | |
|---|---|---|---|---|
| | Trained | Untrained | Trained | Untrained |
| Baseline $\dot{V}O_2$ (L·min$^{-1}$) | $0.58 \pm 0.09$ | $0.64 \pm 0.10$ | $0.36 \pm 0.05$ [#] | $0.42 \pm 0.07$ [#] |
| Phase II time delay (s) | $12 \pm 3$ | $10 \pm 4$ | $13 \pm 4$ | $9 \pm 6$ |
| Phase II $\tau$ (s) | $21 \pm 6$ | $35 \pm 11$ [*] | $29 \pm 8$ | $44 \pm 8$ [*] |
| 95% confidence interval (s) | $2 \pm 0$ | $3 \pm 0$ | $2 \pm 0$ | $4 \pm 0$ |
| Phase II amplitude (L·min$^{-1}$) | $1.33 \pm 0.24$ | $0.92 \pm 0.22$ [*] | $0.86 \pm 0.13$ [#] | $0.50 \pm 0.15$ [* #] |
| Phase II gain (mLO$_2$·min$^{-1}$·W$^{-1}$) | $9.3 \pm 0.7$ | $8.2 \pm 1.0$ | $13.1 \pm 1.3$ [#] | $12.1 \pm 1.9$ [#] |
| Slow component amplitude (L·min$^{-1}$) | $0.12 \pm 0.10$ | $0.14 \pm 0.10$ | $0.07 \pm 0.05$ | $0.08 \pm 0.06$ [#] |
| Slow component amplitude (% end) | $8 \pm 4$ | $14 \pm 8$ | $7 \pm 4$ | $13 \pm 10$ |
| End-exercise $\dot{V}O_2$ (L·min$^{-1}$) | $2.03 \pm 0.29$ | $1.71 \pm 0.26$ | $1.29 \pm 0.15$ [#] | $1.00 \pm 0.20$ [* #] |
| $R^2$ | 0.94 | 0.90 | 0.95 | 0.88 |
| Blood [lactate] (mM) | $3.5 \pm 0.5$ | $5.6 \pm 1.0$ [*] | $2.9 \pm 0.7$ | $4.0 \pm 1.0$ |

*Values are mean ± SD. $\dot{V}O_2$, oxygen uptake; $\tau$, time constant; blood [lactate], blood lactate concentration. N = 8*

\*      *Significant difference between trained and untrained children within an exercise modality (P < 0.01)*

\#      *Significant difference between modes within trained or untrained children (P < 0.01)*

**Figure 6.1** Pulmonary oxygen uptake response to a step increment in work rate from an unloaded baseline to a heavy intensity work rate (40%Δ) in a representative trained (closed circles) and untrained participant (open circles) during A) cycle and B) upper body exercise. The data are expressed as a percentage of the end exercise amplitude. The trained girl's data are shown as closed circles and the untrained girl's data are shown as open circles. The solid and dashed lines represent the mono-exponential model fit to the data. Note the faster $\tau$ in the trained participant during both exercise modes. For clarity, data are displayed as 5-s bin averages



### 6.3.2 [HHb] kinetics
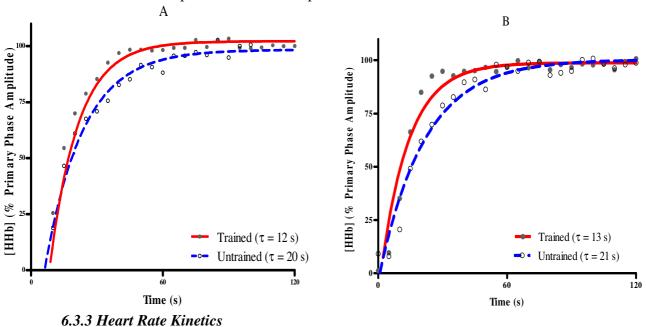
The [HHb] kinetics, summarised in Table 6.4 and illustrated in Figure 6.2, were influenced by training status, with the $\tau$ ($d$ = 1.85 and 1.54 for cycle and upper body exercise, respectively) and MRT ($d$ = 1.78 and 1.37 for cycle and upper body exercise, respectively) being significantly shorter in the trained participants irrespective of exercise modality. The influence of exercise modality was limited, with the only difference being the shorter [HHb] time delay and greater amplitude during upper body ergometry.

**Table 6.4** Deoxyhemoglobin kinetics during heavy intensity exercise on a cycle and upper body ergometer in trained and untrained girls.

| | Cycle Ergometry | | Upper Body Ergometry | |
|---|---|---|---|---|
| | Trained | Untrained | Trained | Untrained |
| [HHb] TD (s) | 6 ± 2 | 7 ± 2 | 3 ± 1 [#] | 3 ± 2 [#] |
| [HHb] τ (s) | 12 ± 2 | 20 ± 6 [*] | 13 ± 3 | 21 ± 7 [*] |
| 95% confidence interval (s) | 3 ± 0 | 2 ± 1 | 2 ± 1 | 2 ± 1 |
| [HHb] MRT (s) | 17 ± 2 | 27 ± 8 [*] | 16 ± 3 | 24 ± 8 [*] |
| [HHb] Amplitude (AU) | 71 ± 57 | 102 ± 59 | 166 ± 108 [#] | 200 ± 114 [#] |

*Values are mean ± SD. [HHb], deoxyhemoglobin/myoblobin; τ, time constant; MRT, mean response time. N = 8*

[*]        *Significant difference between trained and untrained children within an exercise modality (P < 0.01)*

[#]        *Significant difference between modes within trained or untrained children (P < 0.01)*

**Figure 6.2** Deoxyhemoglobin response to a step increment in work rate from an unloaded baseline to a heavy intensity work rate (40%Δ) in a representative trained (closed circles) and untrained participant (open circles) during A) cycle and B) upper body exercise. Data are expressed as a percentage of the primary phase amplitude, in 5-s bin averages. The solid and dashed lines represent the mono-exponential model fit to the data.



*6.3.3 Heart Rate Kinetics*

The HR kinetics, summarised in Table 6.5 and shown in Figure 6.3, were significantly affected by training status: both a lower baseline (*d* = 2.40 and 1.91 for cycle and upper body exercise, respectively) and a shorter τ (*d* = 2.42 and 4.47 for cycle and upper body
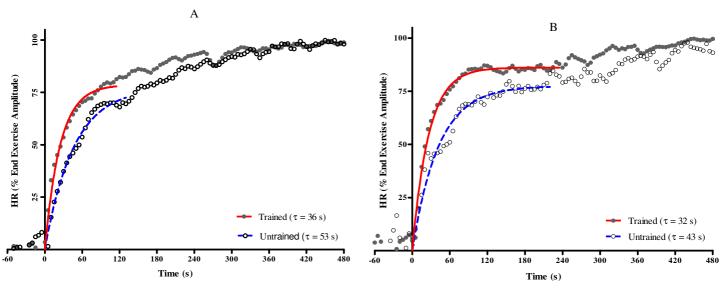
exercise, respectively) were evident in the trained girls. The trained status was also associated with a larger HR amplitude during upper body ergometry ($d = 1.6$). Exercise modality influenced the amplitude but not the kinetics of the HR response, with a greater amplitude and a higher baseline combining to elicit a significantly higher end exercise HR during cycle ergometry. The HR $\tau$ during cycle and upper body exercise were significantly correlated (r = 0.69; $P < 0.01$).

**Table 6.5** Heart rate kinetics during heavy-intensity exercise on a cycle and upper body ergometer in trained and untrained girls

| | Cycle Ergometry | | Upper Body Ergometry | |
|---|---|---|---|---|
| | Trained | Untrained | Trained | Untrained |
| HR baseline (b·min$^{-1}$) | 93 ± 8 | 114 ± 10 [*] | 87 ± 6 | 99 ± 7 [*#] |
| HR $\tau$ (s) | 36 ± 5 | 53 ± 9 [*] | 32 ± 3 | 43 ± 2 [*] |
| 95% confidence interval (s) | 1 ± 0 | 2 ± 1 | 1 ± 1 | 2 ± 1 |
| HR amplitude (b·min$^{-1}$) | 65 ± 9 | 58 ± 6 | 58 ± 9 | 37 ± 17 [*#] |
| End-exercise HR (b·min$^{-1}$) | 170 ± 13 | 182 ± 10 | 159 ± 16 | 147 ± 17 [#] |

*Values are mean ± SD. HR, heart rate; τ, time constant. N = 8*

\*       *Significant difference between trained and untrained children within an exercise modality (P < 0.01)*

\#       *Significant difference between modes within trained or untrained children (P < 0.01)*

**Figure 6.3** Heart rate response to a step increment in work rate from an unloaded baseline to a heavy intensity work rate (40%Δ) in a representative trained (closed circles) and untrained participant (open circles) during A) cycle and B) upper body exercise. Data are expressed as a percentage of the end exercise amplitude, in 5-s bin averages . The solid and dashed lines represent the mono-exponential model fit.

## 6.4 Discussion

This study is the first to investigate the influence of training status and exercise modality on the $\dot{V}O_2$, HR and [HHb] kinetics of pubertal girls. Additionally, this is the first study to report the influence of training status in adolescents on $\dot{V}O_2$ kinetics during heavy intensity exercise. Consistent with our hypotheses, training status significantly influenced the $\dot{V}O_2$, HR and [HHb] kinetics. Specifically, in response to the transition to a heavy-intensity work rate, trained girls exhibited a smaller phase II $\dot{V}O_2$ τ, HR τ and [HHb] τ (i.e. faster kinetics) although there was no difference in the relative magnitude of the $\dot{V}O_2$ slow component between trained and untrained girls. However, contrary to our hypothesis, the influence of training status was equally evident during both upper and lower body exercise.

Prior to discussing the possible physiological mechanisms responsible for the influence of training status on the $\dot{V}O_2$ kinetics during both exercise modalities, it is important to acknowledge the limitations of the current study. The most significant impediment to interpretation of the results is the cross-sectional design of this study which precludes the attribution of the differences observed to training *per se*. While the trained girls were most certainly "trained" (i.e. 12 hours of swimming training per week for 5 years), the observed differences in their responses might also reflect genetic traits which predisposed these adolescents to success in swimming.

### 6.4.1 Ramp Incremental Exercise

The influence of training status was clearly evident in the incremental ramp test responses, with the trained girls having a significantly higher peak $\dot{V}O_2$ and work rate during both cycle and upper body ergometry. Although an influence of training status on peak cycle ergometry responses is not a novel finding (Mahon and Vaccaro 1989; Rowland et al. 1991), the current results contrast previous findings in pre-pubertal girls during upper body ergometry (Chapter 5). Indeed, the current cycle ergometry results also contrast those reported in pre-pubertal girls in chapter 5. These contradictory results suggest the presence of a maturational threshold in the influence of training status on peak $\dot{V}O_2$ responses, a concept largely attributable to Katch (1983).

A higher peak $\dot{V}O_2$ in trained children is largely attributable to an enhanced peak cardiac output consequent solely to an enhanced stroke volume as peak heart rates do not differ (Nottin *et al.*, 2002b; Rowland *et al.*, 2002; Obert *et al.*, 2003). The mechanistic basis of the higher peak stroke volume in trained children remains equivocal, with evidence to suggest both morphological (Nottin *et al.*, 2004; Ayabakan *et al.*, 2006; Obert *et al.*, 2009; Rowland *et al.*, 2009a) and/or functional differences (Rowland *et al.*, 1998). In the current study, the trained swimmers' peak $\dot{V}O_2$ was 24% and 32% higher than the untrained girls during cycle and upper body ergometry, respectively. This difference is considerably larger than the 7-12% difference found in other training studies in adolescents (Mahon & Vaccaro, 1989; Rowland *et al.*, 1991). This difference likely arises from the longitudinal nature of the previous studies and, consequently, the considerably shorter training history of the trained populations. Support for this conclusion is provided by other cross-sectional studies in pubertal populations which reported a 17-25% difference in peak $\dot{V}O_2$ between footballers and untrained adolescents during cycle ergometry (Marwood *et al.*, 2010).

The absence of a training status effect on the GET, either in absolute terms or as a fraction of peak $\dot{V}O_2$, contrasts with previous findings in adolescents (Marwood *et al.*, 2010) and adults (Simon *et al.*, 1986; Boone *et al.*, 2008) but agrees with reports in pre-pubertal children (Obert *et al.*, 2000; Cleuziou *et al.*, 2002; Chapter 5). The explanation for the discrepancy is currently unclear, although it could be related to the lower glycolytic activity reported in children and adolescents (Zanconato *et al.*, 1993; Kuno *et al.*, 1995; Taylor *et al.*, 1995). When expressed in absolute values, the GET was higher during cycle than upper body ergometry, in agreement with previous paediatric (Chapter 5) and adult studies (Davis *et al.*, 1976; Koga *et al.*, 1996; Schneider *et al.*, 2002). However, the influence of exercise modality was removed when the GET was expressed as a fraction of peak $\dot{V}O_2$, as has been previously reported (Koga *et al.*, 1996; Schneider *et al.*, 2002; Chapter 5).

### 6.4.2 Constant-work-rate tests: influence of training status

Training status significantly affected the primary component $\dot{V}O_2$ kinetics during cycle ergometry, in agreement with previous work in both adolescents (Marwood *et al.*, 2010)

and adults (Powers *et al.*, 1985; Koppo *et al.*, 2004; Figueira *et al.*, 2008). Although this significant influence on the cycle ergometry response is in contrast to previous findings in pre-pubertal children (Obert *et al.*, 2000; Cleuziou *et al.*, 2002; Chapter 5), the significant influence of training status on the upper body $\dot{V}O_2$ primary component response is in agreement with the pre-pubertal literature (Chapter 5). No studies are available in adolescents or adults for comparison to the current upper body ergometer results.

The shorter $\dot{V}O_2$ $\tau$ in the trained swimmers may be related to an enhanced delivery and/or fractional extraction of $O_2$. The faster HR $\tau$ in trained participants is in agreement with previous research in both adolescent (Marwood *et al.*, 2010) and pre-pubertal (Chapter 5) populations, although this is the first study to investigate the HR kinetics during heavy intensity cycle and upper body ergometry in adolescents. If HR kinetics are accepted to provide a crude estimate of muscle blood flow kinetics, as suggested during knee extension exercise (MacPhee *et al.*, 2005), these results suggest that bulk $O_2$ delivery to the muscle was enhanced in the trained state. This would be consistent with previous studies conducted in adult populations reporting faster conduit artery blood flow kinetics and greater vascular conductance following training (Shoemaker *et al.*, 1996; Krustrup *et al.*, 2004). However, it is important to note that an increased bulk $O_2$ delivery in the trained state does not necessarily imply that $O_2$ availability was limiting in the untrained state. Indeed such a suggestion seems unlikely given the evidence available in young adults (DeLorey *et al.*, 2004; Jones *et al.*, 2006; Wilkerson *et al.*, 2006).

Alternatively, the smaller phase II $\tau$ may be related to the influence of training status on muscle fractional $O_2$ extraction (as reflected by the [HHb] response). Specifically, the [HHb] $\tau$ and MRT were significantly shorter in trained girls, although the TD was unaffected by training status. Whilst the latter finding is in agreement with previous reports, the faster $\tau$ and MRT observed in the present study is in contrast to the study of Marwood et al. (2010) in which the $\tau$ and MRT were reported to be unaffected by training status. The [HHb] response is generally accepted to reflect fractional $O_2$ extraction at the muscle (DeLorey *et al.*, 2003; Grassi *et al.*, 2003; Ferreira *et al.*, 2007); therefore the shorter [HHb] $\tau$ in the trained swimmers indicates a more rapid increase in $O_2$ extraction towards the new steady-state. In adults, a training-induced reduction of the [HHb] $\tau$ (Bailey *et al.*, 2009) has

been attributed to an enhanced muscle oxidative capacity consequent to an increased mitochondrial volume and oxidative enzyme activity (Holloszy, 1967; Mogensen *et al.*, 2006). Although an increased muscle oxidative capacity has been reported in trained children (Eriksson *et al.*, 1973; Fournier *et al.*, 1982), there is insufficient information available regarding the effects of training on muscle fibre type and oxidative capacities in children and adolescents to allow conclusions to be drawn as to the mechanisms responsible for the enhanced $O_2$ extraction kinetics in the trained girls. Therefore, although the current results do not permit the complete elucidation of the factors limiting $\dot{V}O_2$ kinetics in adolescents, it is likely that the faster $\dot{V}O_2$ kinetics in the trained girls are a function of both a faster $O_2$ delivery and greater $O_2$ extraction, as similarly concluded in adults (Jones & Koppo, 2005; Poole *et al.*, 2008a; McKay *et al.*, 2009).

The influence of training status was isolated to the primary component, with no influence evident on the amplitude of the slow component during either exercise modality whether expressed in absolute or relative terms. The absence of a training status influence is in agreement with studies in pre-pubertal children (Obert *et al.*, 2000; Cleuziou *et al.*, 2002; Chapter 5) but contrasts with the reduction typically seen with training in adults (e.g. Powers *et al.*, 1985; Koppo *et al.*, 2004; Bailey *et al.*, 2009). The explanation for this seemingly age-related influence of training status on the slow component is not readily apparent. However, in light of the putative association between the slow component and muscle fibre type recruitment (Poole *et al.*, 1994; Whipp, 1994), future investigations into the basis for this age-related effect may benefit from the inclusion of techniques (EMG, MRI) to assess differences in muscle activity between trained and untrained populations.

### 6.4.3 Constant-work-rate tests: influence of exercise modality

The influence of exercise modality on the $\dot{V}O_2$, HR and [HHb] kinetics has not previously been examined in a pubertal population. One of the most striking features of this study was the lack of exercise modality effect on the $\dot{V}O_2$ τ, a finding which contrasts with the longer phase II τ reported during upper compared to lower body exercise in adults (Koppo *et al.*, 2002; Schneider *et al.*, 2002). Typically, a longer upper body $\dot{V}O_2$ τ is attributed to a lower percentage of type I fibres in the upper body musculature (Gollnick *et al.*, 1972; Johnson *et*

*al.*, 1973; Turner *et al.*, 1997) in combination with a decreased perfusion pressure due to a reduced "gravitational assist" (Koga *et al.*, 1999; Koppo & Bouckaert, 2005). Since there is no evidence to suggest that either of these factors would be different in adolescents compared to adults, the explanation for this discrepancy is unclear. Exercise modality did not influence the [HHb] or HR $\tau$ values, in contrast to the longer HR $\tau$ reported in adults during upper compared to lower body exercise (Koga *et al.*, 1996; Schneider *et al.*, 2002). An explanation for this difference between pubertal and adult populations is not apparent from the current results. Exercise modality did, however, influence the [HHb] TD which was significantly shorter during upper body ergometry, indicating a reduced period of matching of $O_2$ delivery and utilisation.

In contrast to our previous study in pre-pubertal children (Chapter 5), there was no interaction between exercise modality and training status in the current study, with the influence of training status evident during both exercise modalities. Although conclusions are limited by the cross-sectional nature of these studies, it is possible that this difference between pre-pubertal and pubertal girls is attributable to the presence of a maturational threshold (Katch, 1983) and/or the longer training history and greater training volume reported by the pubertal girls. This greater training load may have satisfied the minimum threshold stimulus required to elicit significant alterations in the $\dot{V}O_2$ kinetic response to both lower and upper body ergometry (Berger *et al.*, 2006; Bailey *et al.*, 2009). In contrast, the lower training load of the pre-pubertal children may have only surpassed the threshold stimulus required for upper body exercise, a threshold which is likely to be reduced relative to the respective lower body threshold due to differences in habitual activity.

### *6.5 Conclusion*

In conclusion, training status significantly influenced the physiological responses of pubertal girls to both ramp incremental and constant-work-rate tests in two exercise modalities. Specifically, the $\dot{V}O_2$ kinetics of the trained girls was significantly faster than the untrained girls during both upper and lower body exercise. The faster HR and [HHb] kinetics in the trained girls may indicate that both central and peripheral factors are influenced by training status and contribute to the shorter $\dot{V}O_2$ $\tau$ in the trained state. These

results contrast with the minimal influences generally observed in pre-pubertal populations, a difference likely attributable to both a greater stage of maturation and a longer training history in adolescents. Unlike pre-pubertal populations, the specificity of the exercise test modality to the training modality is not crucial in revealing the influence of training status in adolescents

**Chapter 7**

**Study 4 - Influence of training and maturity status on the cardiopulmonary responses to ramp incremental cycle and upper body exercise in girls**

This study has been disseminated as follows:

*Publication:* McNarry, M.A., Welsman, J.R., & Jones, A.M. (2010). Influence of training and maturity status on the cardiopulmonary responses to ramp incremental cycle and upper body exercise in girls. Under review with JAP.

### *7.1 Introduction*

More young females train for competitive sport today than ever before (Thompson & Baxter-Jones, 2002). However, the influence of training on girls' physiological responses to exercise remains poorly understood, due to a relative lack of research in this population compared to adults and boys (Obert *et al.*, 2009).

In pre-pubertal girls, the peak value for pulmonary oxygen uptake ($\dot{V}O_2$) is generally reported to be increased following training (e.g. Obert *et al.*, 1996; Obert *et al.*, 2009; Rowland *et al.*, 2009a), but there are exceptions to these findings (Welsman *et al.*, 1996; Welsman *et al.*, 1997; Tolfrey *et al.*, 1998). The only studies that have investigated pubertal girls are in direct disagreement, with early studies showing significant improvements in relative peak $\dot{V}O_2$ (Burkett *et al.*, 1985; Rowland *et al.*, 1991) but a more recent study reporting no change (Stoedefalke *et al.*, 2000). The explanation for this discrepancy in pubertal girls is unclear but may be related to the pooling of data from boys and girls (Rowland *et al.*, 1991), despite the possibility that training status differences are sex-dependent (Obert *et al.*, 2003), or the inclusion of participants from a wide range of ages and maturity stages (Burkett *et al.*, 1985). Alternative explanations include differences in the baseline fitness levels of the participants recruited or exercise modalities employed in these studies. In the study of Rowland et al. (Rowland *et al.*, 1991) the participants were described as predominantly obese, a condition which would be expected to predispose these

participants to greater influences of training, especially when peak $\dot{V}O_2$ is expressed relative to body mass as it was in this study. Alternatively, or additionally, the discrepancy between previous studies may be related to a disparity in the training and testing modalities. Specifically, a number of studies employed a training regime with one exercise modality, yet tested participants using a different modality (Stoedefalke *et al.*, 2000). The issue of test specificity is especially pertinent to paediatric studies due to the smaller training-induced adaptations compared with adults (Pate & Ward, 1996). An age group in which there is an even starker lack of research is the early post-pubertal age group, i.e. 16-18 year old girls. Indeed, to our knowledge, there are no studies reporting the influence of training status on girls' physiological responses to exercise in this age group, despite the preponderance of this population participating in sport.

An elevated peak $\dot{V}O_2$ in trained children has generally been attributed to an enhanced peak stroke volume (SV), and consequently peak cardiac output ($\dot{Q}$) (Nottin *et al.*, 2002b; Rowland *et al.*, 2002; Obert *et al.*, 2003). Such training effects may be associated with functional and/or morphological adaptations to the myocardium. Although morphological adaptations are more commonly reported (Bianchi *et al.*, 1998; Nottin *et al.*, 2002b; Rowland *et al.*, 2009a), evidence of a different SV response pattern in trained children may also be indicative of functional myocardial adaptations (Rowland *et al.*, 1998). It is pertinent to note that no studies are currently available which have investigated the influence of training on the cardiovascular responses to exercise of pubertal girls.

The increased peak $\dot{V}O_2$ in trained girls may alternatively be related to an enhanced muscle fractional oxygen ($O_2$) extraction. However, no influence of training on peak muscle fractional oxygen extraction has been reported in pre-pubertal children (Rowland *et al.*, 2000; Nottin *et al.*, 2002b; Obert *et al.*, 2003) or pubertal boys (Rowland *et al.*, 2009b). No information is available regarding the effects of training on muscle fractional $O_2$ extraction during ramp incremental exercise in pubertal girls. In adults, the deoxygenated haemoglobin signal ([HHb]) from near-infrared spectroscopy (NIRS), which appears to reflect muscle fractional $O_2$ extraction (e.g. DeLorey *et al.*, 2003; Grassi *et al.*, 2003), has been shown to be influenced by training status, demonstrating a rightward shift during

incremental exercise in trained adults (Boone *et al.*, 2009). It has been suggested that this is indicative of differences in muscle $O_2$ delivery and/or muscle fibre type distribution (8).

The interaction between the influence of training status and sexual maturity remains largely unknown, although a relationship may be expected due to the more favourable hormonal milieu present beyond puberty (Zakas *et al.*, 1994; Daly *et al.*, 1998; Tsolakis *et al.*, 2003). The presence of a "golden period" or maturational threshold has been discussed for nearly 30 years without resolution (Katch, 1983), with some studies suggesting that the influence of training increases with sexual maturity (Kobayashi *et al.*, 1978; Mirwald *et al.*, 1981; Ostojic *et al.*, 2009) and others reporting no relationship between the two variables (Weber *et al.*, 1976; Danis *et al.*, 2003).

The purpose of the present cross-sectional study was to investigate the influence of, and interaction between, training status and sexual maturity on the cardiopulmonary and metabolic responses to ramp incremental exercise in intensively swimming-trained and untrained girls. Since swimming has been considered to be a predominantly upper body exercise, two exercise modalities, one upper (arm crank) and one lower body (cycle), were used to address concerns regarding the commonality of muscles stressed during training and testing. We hypothesized that significantly higher peak values would be evident in the trained girls' pulmonary gas exchange and cardiovascular responses in all three maturity groups Further, we hypothesised that the trained girls would exhibit altered SV and [HHb] response patterns to incremental exercise: specifically that the SV response would be more linear and the [HHb] response shifted to the right relative to untrained girls. Finally, we hypothesised that the magnitude of difference between trained and untrained girls would increase with increasing maturity and be larger during upper than lower body exercise.

## *7.2 Methods*

### *7.2.1 Participants and anthropometry*

In total, 21 (11 trained and 10 untrained) pre-pubertal, 30 (14 trained and 16 untrained) pubertal, and 18 (8 trained and 10 untrained) post-pubertal girls participated in this study.

The pre-pubertal and pubertal trained girls (T) were competing at British regional or national level, with the majority of the post-pubertal girls competing at international level. The pre-pubertal girls had been training for a mean of $2.5 \pm 1$ years and reported a mean training volume of $14 \pm 3$ hours/week. The pubertal and post-pubertal girls had been training $5 \pm 1.5$ years and $8 \pm 2$ years, respectively, with training volumes of $18 \pm 4$ and $22 \pm 3$ hours/week respectively. All the trained girls trained and competed throughout the swimming season, which runs ~50 weeks a year. The age-matched UT girls were volunteers from local schools who reported little regular physical activity and limited recreational sports participation. Sexual maturity was assessed by self-report using the indices of pubic hair described by Tanner (Tanner, 1962).

An anthropometrical evaluation was performed before the first test for all participants. Standing and seated height were measured to 0.1 cm using a Holtain stadiometer (Holtain, Crymych, Dyfed, UK) and body mass determined using Avery beam balance scales to 0.05 kg (Avery, Birmingham, UK). Skinfold thickness was assessed three times at five sites around the body (bicep, triceps, subscapular, supra-iliac crest and thigh) by the same researcher for all participants using Harpenden callipers (Baty International, Burgess Hill, UK), accurate to the nearest 0.2mm. The mean of the three measurements was taken. Percentage body fat was subsequently estimated based on the equations of Slaughter et al. (1988).

Participants were asked to arrive at the laboratory in a rested and fully hydrated state, at least 3 hours postprandial and to refrain from consuming caffeinated drinks in the 6 hours prior to testing. The methods employed during this study were approved by the institutional research ethics committee and all participants and their parents/guardians gave written informed consent and assent, respectively.

### 7.2.2 Experimental procedures

Each participant completed two ramp incremental tests to volitional exhaustion on separate days, one on a cycle ergometer (Lode Excalibur Sport, Netherlands) and one on an upper body ergometer (Lode Angio, Netherlands). Prior to each test, the handle bar height, seat

height and crank length (cycle ergometer) and the seat height and distance (upper body ergometer), were adjusted to suit each child.

After a 3 minute warm up, the resistance increased at a pre-determined rate to attain a test duration of 8-12 minutes. The rate for cycle ergometry was 15 W·min$^{-1}$, 20 W·min$^{-1}$ or 25 W·min$^{-1}$ for pre-pubertal, pubertal and post-pubertal girls, respectively, and 6 W·min$^{-1}$, 10 W·min$^{-1}$ or 14 W·min$^{-1}$, respectively, for the upper body ergometry. Throughout the tests, the girls were instructed to maintain a cadence of 70 and 50 rpm for the cycle and upper body ergometers, respectively. Peak efforts were considered to have been given if, in addition to subjective indications such as sweating, hyperpnea and facial flushing, there was a consistent reduction in cadence despite strong verbal encouragement.

### 7.2.3 Experimental measures

Throughout each test, gas exchange variables (Metalyser 3B Cortex, Biophysik, Leipzig, Germany) and heart rate (Polar S610, Polar Electro Oy, Kempele, Finland) were measured on a breath-by-breath basis and displayed online. Prior to each test, the gas analyzers were calibrated using gases of known concentration and the turbine volume transducer was calibrated using a 3-litre syringe (Hans Rudolph, Kansas City, MO). The delay in the capillary gas transit and analyzer rise time were accounted for relative to the volume signal, thereby time aligning the concentration and volume signals.

Cardiac output and stroke volume were determined non-invasively throughout the exercise test using a thoracic bioelectrical impedance device (PhysioFlow, PF-05 Lab1, Manatec Biomedical, France), previously validated in both adult (Charloux *et al.*, 2000; Richard *et al.*, 2001) and paediatric populations (Welsman *et al.*, 2005). Whilst the device has not previously been used during upper body exercise, it has been validated in a wide range of populations from healthy to diseased participants, during a number of perturbations and irrespective of electrode placement, therefore we do not perceive any issues with its application to upper body exercise. The electrodes were positioned on the forehead, neck, xiphoid process and on the left hand side lower ribs, avoiding the abdominal muscles, as suggested to be appropriate for young participants (Welsman *et al.*, 2005). Prior to testing,

blood pressure was measured by the same researcher in triplicate using a manual sphygmomanometer with the participant seated at rest. The mean systolic/diastolic blood pressure was entered into the Physioflow following auto-calibration which was conducted with the participant seated at rest on the ergometer.

Additionally, the oxygenation status of the right *m. vastus lateralis* (cycle) or right *m. tricep brachii* (upper body) was monitored using a commercially available near-infrared system (Portamon, Artinis Medical Systems, The Netherlands). This system consists of an emission probe which has three light sources and emits 2 wavelengths of light (760 and 850 nm) and a photon detector. The intensity of incident and transmitted light was recorded continuously at 10 Hz and used to estimate the concentration changes relative to baseline levels for oxygenated, deoxygenated and total haemoglobin. The [HHb] was used as an indicator of $O_2$ extraction within the field of interrogation (DeLorey *et al.*, 2003; Grassi *et al.*, 2003; Ferreira *et al.*, 2007). The contribution of myoglobin to the NIRS signal is currently unresolved (Seiyama *et al.*, 1988; Masuda *et al.*, 2010). Therefore, the [HHb] signal described throughout this paper should be considered to refer to the combined concentration of both deoxygenated haemoglobin and myoglobin. The muscle was initially cleaned and the portable probe strapped to the skin at the midpoint of the muscle using physiotherapists' tape (Kinesio Tex Gold). To ensure the device remained stationary during exercise and to minimize the interference of extraneous light with the near-infrared signal, a bandage was wrapped around the arm/leg, enclosing the probe.

### 7.2.4 Data Analysis

The gas exchange data were interpolated to 1-s intervals and peak $\dot{V}O_2$ was taken as the highest 10-s stationary average during the test. The gas exchange threshold (GET) was determined by the V-slope method (Beaver *et al.*, 1986) as the point at which carbon dioxide production began to increase disproportionately to $\dot{V}O_2$ as identified using purpose designed software developed using LabVIEW (National Instruments, Newbury, UK). The kinetics of the initial $\dot{V}O_2$ adjustment to the ramp incremental test were also analyzed by determination of the mean response time (MRT). This was calculated as the time from the onset of the ramp forcing function to the intersection point between the baseline $\dot{V}O_2$ and a

backwards extrapolation of the slope of $\dot{V}O_2$ as a function of time, as illustrated in figure 7.1. The baseline $\dot{V}O_2$ was defined as the average $\dot{V}O_2$ during the last minute of unloaded pedalling prior to the onset of the ramp function.
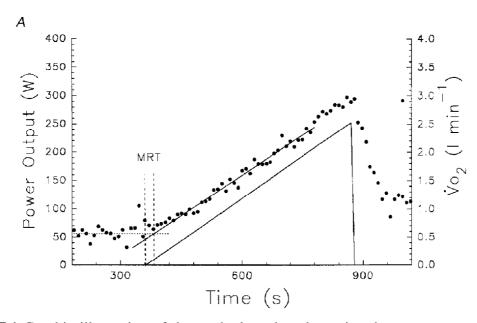


**Figure 7.1** Graphic illustration of the method used to determine the mean response time (MRT). The solid line represents the ramp incremental increase in power output and the closed circles represent the $\dot{V}O_2$ response. Reproduced from Barstow et el. (2000).

Peak stroke volume and cardiac output were taken as the highest 15-s mean value measured during the test. The peak $a - \overline{v}O_2$ difference was estimated by rearrangement of the Fick equation:

$$\text{Peak } a - \overline{v}O_2 \text{ difference} = \text{peak } \dot{V}O_2 / \text{ peak } \dot{Q} \qquad \text{(Eq. 7.1)}$$

To identify the most appropriate model fit describing the cardiovascular variables as a function of percentage peak $\dot{V}O_2$, the linear relationship ($Y = a + bX$) was compared to the general quadratic relationship ($Y = a + bX + c(X)^2$) using least-squares and maximum likelihood estimation for linear and non-linear regression, respectively (Graphpad Prism, Graphpad Software, San Diego, CA). The best fitting model was determined on the basis of the $R^2$ values, the residual sum of squares and the F-value.

The most suitable model to describe the [HHb] response was determined by comparing the sigmoidal model ($Y=a/(1+exp^{-(-c+dx)})$), where $a$ represents the baseline corrected amplitude and $c$ is a constant dependent upon $d$ (the slope of the sigmoid) whereby $c/d$ reveals the $x$ value that yields 50% of the total amplitude, to a hyperbolic model ($Y = ax / (b+x)$), where $a$ is the asymptotic value and $b$ is the $x$-value corresponding to 50% of the response amplitude (Boone *et al.*, 2009, 2010). Curve fitting was conducted using the [HHb] response normalized to the end exercise amplitude as a function of percentage peak power. The best fitting model was determined on the basis of the $R^2$ values, the residual sum of squares and the F-value.

The influence of body size was accounted for using analysis of covariance (ANCOVA) on log transformed data to determine the allometric relationship between body mass and peak $\dot{V}O_2$ and between body surface area and peak SV and $\dot{Q}$ (Welsman & Armstrong, 2000). Common allometric exponents were confirmed for all groups and power function ratios ($Y/X^b$) were computed. Body surface area was calculated according to the equations of Haycock et al. (1978).

Following identification of a normal distribution, homogenous variation and an absence of skewness or kurtosis a two way ANOVA with repeated measures was used to analyze training status and exercise modality effects. Subsequent independent or paired samples t-tests with a Bonferroni correction were employed as appropriate to identify the location of significant effects. The interaction of training status and sexual maturity status was investigated for those parameters influenced by training status using a factorial ANOVA. Pearson product-moment correlation coefficients were used to assess the strength of relationships between variables. All data are presented as means ± SD. Statistical significance was accepted when $P<0.05$.

### *7.3 Results*

Anthropometric characteristics were similar for the trained and untrained girls within each sexual maturity group (Table 7.1). The age and stature of the girls in each maturity group was significantly higher relative to the younger group. All the girls in the pre-pubertal

group were characterized as Tanner stage 1, whilst the pubertal girls were stages 3 and 4, and the post-pubertal girls were stage 5.

**Table 7.1** Participants' anthropometric characteristics

| | Pre-pubertal | | Pubertal | | Post-pubertal | |
|---|---|---|---|---|---|---|
| | Trained | Untrained | Trained | Untrained | Trained | Untrained |
| | N = 11 | N = 10 | N = 14 | N = 16 | N = 8 | N = 10 |
| Age (y) | $11.4 \pm 1.0$ | $11.9 \pm 0.9$ | $14.4 \pm 0.8$ [*] | $14.2 \pm 0.6$ [*] | $16.6 \pm 0.6$ [*#] | $16.9 \pm 0.8$ [*#] |
| Stature (m) | $1.48 \pm 0.07$ | $1.50 \pm 0.06$ | $1.64 \pm 0.07$ [*] | $1.60 \pm 0.06$ [*] | $1.67 \pm 0.4$ [#] | $1.69 \pm 0.6$ [*#] |
| Mass (kg) | $44.3 \pm 3.8$ | $43.6 \pm 6.6$ | $55.4 \pm 6.0$ [*] | $54.9 \pm 7.0$ [*] | $59.4 \pm 7.6$ [#] | $61.2 \pm 6.2$ [#] |
| Sum of skinfolds (mm) | $65.9 \pm 14.9$ | $59.2 \pm 13.2$ | $60.0 \pm 16.8$ | $67.3 \pm 18.6$ | $54.3 \pm 16.5$ | $69.8 \pm 32.3$ |
| Percentage body fat | $26.3 \pm 7.2$ | $25.3 \pm 5.9$ | $27.4 \pm 9.8$ | $31.3 \pm 9.0$ | $23.8 \pm 11.3$ | $26.9 \pm 9.4$ |

*Values are mean ± S.D.*

\* *Significant difference relative to previous maturity stage within trained or untrained girls (P<0.01).*

\# *Significant difference between pre- and post-pubertal girls (P<0.01).*

### 7.3.1 Effects of training

The differing ramp rates between maturity stages resulted in similar total test durations in both trained and untrained girls during cycle (T: Pre, $11.5 \pm 1.9$ vs. Pub, $13.5 \pm 2.1$ vs. Post, $12.3 \pm 2.0$ min; UT: Pre: $9.7 \pm 1.4$ vs. Pub, $9.5 \pm 1.6$ vs. Post, $9.2 \pm 1.8$ min; all P>0.05) and upper body ergometry (T: Pre, $10.1 \pm 2.0$ vs. Pub, $12.8 \pm 1.6$ vs. Post, $9.8 \pm 1.5$ min; UT: Pre: $9.8 \pm 2.1$ vs. Pub, $8.3 \pm 1.6$ vs. Post, $8.4 \pm 1.0$ min; all P>0.05).

As shown in Table 7.2, peak $\dot{V}O_2$ was significantly higher in the trained girls in all three maturity groups for both exercise modalities (*d*; cycle: Pre, 1.96; Pub, 1.78; Post, 1.76; upper: Pre, 1.07, Pub, 1.67, Post, 2.68). These differences existed when either ratio or allometric scaling was applied. In contrast, the influence of training status on the GET was dependent on the method of expression. When expressed as absolute $\dot{V}O_2$, the GET was higher in the trained girls in all maturity groups during both cycle and upper body ergometry. However, when expressed as a fraction of peak $\dot{V}O_2$, the differences were no longer significant, with the exception of the pre-pubertal maturity group where a significantly higher GET was still present in the trained girls. Further influences of training

status were evident in the shorter MRT for both exercise modalities for all maturity groups (*d*; cycle: Pre, 1.49; Pub, 2.30; Post, 2.11; upper: Pre, 2.02, Pub, 2.30, Post, 1.91).

**Table 7.2** Peak pulmonary gas exchange parameters for ramp incremental exercise on a cycle and upper body ergometer in trained and untrained girls according to sexual maturity status

| | Pre-pubertal | | Pubertal | | Post-pubertal | |
|---|---|---|---|---|---|---|
| | Trained | Untrained | Trained | Untrained | Trained | Untrained |
| | N = 11 | N = 10 | N = 14 | N = 16 | N = 8 | N = 10 |
| *Cycle ergometry* | | | | | | |
| Peak $\dot{V}O_2$ (L·min$^{-1}$) | 2.11 ± 0.14 | 1.74 ± 0.24 * | 2.54 ± 0.33 # | 1.99 ± 0.30 * | 2.85 ± 0.27 † | 2.35 ± 0.31 *† |
| Peak $\dot{V}O_2$ (mL·kg$^{-1}$·min$^{-1}$) | 49.1 ± 4.7 | 40.1 ± 3.9 * | 46.0 ± 5.3 | 36.3 ± 3.8 * | 48.4 ± 4.9 | 38.7 ± 7.6 * |
| Peak RER | 1.10 ± 0.08 | 1.12 ± 0.10 | 1.23 ± 0.08 | 1.19 ± 0.07 | 1.27 ± 0.15 | 1.19 ± 0.10 |
| GET (L·min$^{-1}$) | 1.14 ± 0.13 | 0.92 ± 0.15 * | 1.35 ± 0.10 # | 1.09 ± 0.18 * | 1.63 ± 0.25 † | 1.31 ± 0.24 *† |
| GET (% peak $\dot{V}O_2$) | 53 ± 6 | 53 ± 6 | 54 ± 8 | 56 ± 5 | 57 ± 8 | 56 ± 8 |
| MRT (s) | 35 ± 14 | 56 ± 15 * | 29 ± 9 | 56 ± 14 * | 25 ± 5 | 49 ± 15 * |
| *Upper body ergometry* | | | | | | |
| Peak $\dot{V}O_2$ (L·min$^{-1}$) | 1.60 ± 0.14 | 1.38 ± 0.27 * | 2.00 ± 0.30 # | 1.55 ± 0.25 * | 2.30 ± 0.15 † | 1.66 ± 0.30 * |
| Peak $\dot{V}O_2$ (mL·kg$^{-1}$·min$^{-1}$) | 36.8 ± 5.8 | 31.7 ± 4.5 * | 36.2 ± 5.4 | 28.3 ± 5.1 * | 39.1 ± 3.4 | 27.5 ± 6.8 * |
| Peak RER | 1.04 ± 0.09 | 0.99 ± 0.08 | 1.14 ± 0.11 | 1.11 ± 0.07 | 1.14 ± 0.15 | 1.16 ± 0.06 |
| GET (L·min$^{-1}$) | 0.90 ± 0.14 | 0.65 ± 0.11 * | 1.04 ± 0.20 | 0.74 ± 0.19 * | 1.17 ± 0.18 † | 0.82 ± 0.23 * |
| GET (% peak $\dot{V}O_2$) | 57 ± 4 | 48 ± 5 * | 52 ± 7 | 48 ± 11 | 51 ± 7 | 49 ± 9 |
| MRT (s) | 33 ± 10 | 66 ± 22 * | 33 ± 11 | 55 ± 12 * | 31 ± 7 | 51 ± 13 * |

*Values are mean ± S.D . $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; gas exchange threshold; MRT, mean response time.*

\* *Significant difference between trained and untrained girls within an exercise modality and maturity group (P<0.0*

\# *Significant difference relative to previous maturity group within trained or untrained girls (P<0.01)*

† *Significant difference between pre- and post-pubertal girls within trained or untrained children (P<0.01)*

The estimated peak a - $\overline{v}O_2$ difference was not influenced by training status for either exercise modality in the pre-pubertal or pubertal girls or during cycle ergometry in post-pubertal girls. However, a higher peak $\dot{Q}$ was observed in the trained girls and this reached significance in both the pubertal and post-pubertal girls (*d*; cycle: Pre, 0.6; Pub, 1.06; Post, 1.43; upper: Pre, 0.64, Pub, 1.18, Post, 1.55). An elevated $\dot{Q}$ may be attributable solely to an increased peak SV in the trained girls during both cycle (*d* = Pre, 0.59; Pub, 1.1, Post, 1.53) and upper body ergometry (*d* = Pre, 0.51; Pub, 0.94, Post, 1.71) as peak HR was not influenced by training status, as summarized in Table 7.3.

**Table 7.3** Peak cardiovascular parameters for ramp incremental exercise on a cycle and upper body ergometer in trained and untrained girls according to sexual maturity status

| | Pre-pubertal | | Pubertal | | Post-pubertal | |
|---|---|---|---|---|---|---|
| | Trained | Untrained | Trained | Untrained | Trained | Untrained |
| | N = 11 | N = 10 | N = 14 | N = 16 | N = 8 | N = 10 |
| *Cycle ergometry* | | | | | | |
| Peak $\dot{Q}$ (L·min$^{-1}$) | 16.4 ± 2.7 | 14.8 ± 2.8 | 20.9 ± 3.1 $^{\#}$ | 17.5 ± 3.4 $^{*}$ | 21.3 ± 3.9 $^{\dagger}$ | 17.1 ± 2.1 $^{*}$ |
| Peak $\dot{Q}$ (L·min$^{-1}$·m$^{-2}$) | 12.4 ± 2.0 | 11.1 ± 2.5 | 13.2 ± 2.1 | 11.1 ± 1.7 $^{*}$ | 12.8 ± 1.8 | 10.1 ± 1.4 $^{*}$ |
| Peak SV (mL) | 84 ± 13 | 76 ± 15 | 110 ± 17 $^{\#}$ | 91 ± 18 $^{*}$ | 115 ± 9 $^{\dagger}$ | 98 ± 13 $^{*\dagger}$ |
| Peak SV (mL·m$^{-2}$) | 63 ± 9 | 57 ± 13 | 69 ± 12 | 58 ± 10 $^{*}$ | 70 ± 7 | 58 ± 8 $^{*}$ |
| Peak HR (b·min$^{-1}$) | 197 ± 9 | 195 ± 7 | 194 ± 8 | 192 ± 8 | 189 ± 7 | 190 ± 11 |
| Peak a - $\bar{v}O_2$ diff (mL·dL$^{-1}$) | 13.1 ± 2.3 | 12.2 ± 2.9 | 12.8 ± 2.3 | 11.7 ± 2.4 | 13.9 ± 2.5 | 12.6 ± 2.5 |
| *Upper body ergometry* | | | | | | |
| Peak $\dot{Q}$ (L·min$^{-1}$) | 16.3 ± 3.6 | 14.3 ± 2.7 | 19.7 ± 3.7 | 15.4 ± 3.7 $^{*}$ | 22.0 ± 3.0 $^{\dagger}$ | 18.2 ± 2.1 $^{*\dagger}$ |
| Peak $\dot{Q}$ (L·min$^{-1}$·m$^{-2}$) | 11.9 ± 2.2 | 10.7 ± 2.5 | 12.5 ± 2.5 | 9.7 ± 1.8 $^{*}$ | 13.4 ± 2.4 | 10.8 ± 1.4 $^{*}$ |
| Peak SV (mL) | 87 ± 18 | 79 ± 14 | 108 ± 19 $^{\#}$ | 89 ± 22 $^{*}$ | 124 ± 18 $^{\dagger}$ | 100 ± 11 $^{*\dagger}$ |
| Peak SV (mL·m$^{-2}$) | 64 ± 12 | 59 ± 13 | 68 ± 12 | 56 ± 10 $^{*}$ | 74 ± 12 | 59 ± 8 $^{*}$ |
| Peak HR (b·min$^{-1}$) | 177 ± 16 | 180 ± 18 | 181 ± 12 | 180 ± 16 | 175 ± 12 | 170 ± 15 |
| Peak a - $\bar{v}O_2$ diff (mL·dL$^{-1}$) | 10.3 ± 1.7 | 9.9 ± 2.5 | 10.7 ± 2.7 | 10.2 ± 2.9 | 11.0 ± 2.3 | 8.6 ± 1.2 $^{*}$ |

*Values are mean ± S.D. $\dot{Q}$, cardiac output; SV, stroke volume; HR, heart rate; a - $\bar{v}O_2$ diff, arterial-venous oxygen difference*

\*   *Significant difference between trained and untrained girls within an exercise modality and maturity group (P<0.01)*

\#   *Significant difference relative to previous maturity group within trained or untrained girls (P<0.01)*

†   *Significant difference between pre- and post-pubertal girls within trained or untrained children (P<0.01)*

The SV and [HHb] response patterns were influenced by training status in all maturity groups, with SV demonstrating a linear response in trained girls (cycle: Pre, 100%, $R^2$ = 0.73, Pub, 86%, $R^2$ = 0.87, Post, 100%, $R^2$ = 0.85; upper body: Pre, 75%, $R^2$ = 0.69, Pub, 92%, $R^2$ = 0.80, Post, 100%, $R^2$ = 0.83) compared to a curvilinear response in untrained girls (cycle: Pre, 80%, $R^2$ = 0.81, Pub, 93%, $R^2$ = 0.79, Post, 90%, $R^2$ = 0.77; upper body: Pre, 80%, $R^2$ = 0.70, Pub, 93%, $R^2$ = 0.77, Post, 88%, $R^2$ = 0.75) (Figure 7.2). The response pattern was not influenced by the method of expressing SV, therefore absolute values are presented. The response pattern was also not influenced by maturity status or exercise
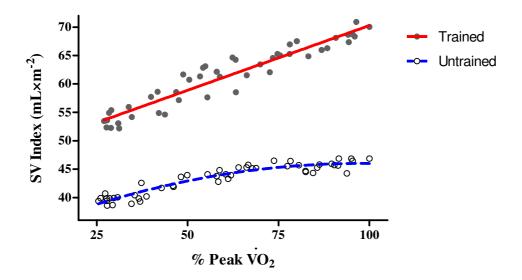
modality, therefore the SV response illustrated in Figure 7.2 is representative of all participants during both exercise modalities.
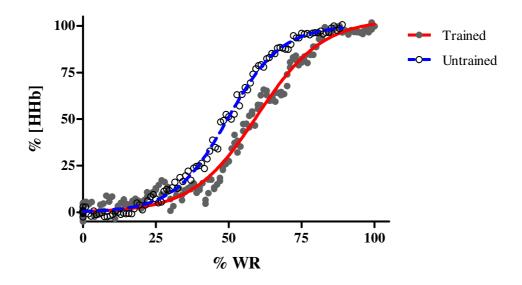
**Figure 7.2** The stroke volume response pattern for a representative trained and untrained girl during cycle ergometry (see text for details). The trained girls are represented by the closed circles and solid line whilst the untrained girls are shown by the open circles and dashed line.



The sigmoidal model was accepted as a superior fit of the [HHb] data based on higher $R^2$ values (cycle: Pre T, 0.86, Pre UT, 0.91, Pub T, 0.92, Pub UT, 0.94, Post T, 0.96, Post UT, 0.94; upper body: Pre T, 0.92, Pre UT, 0.94, Pub T, 0.95, Pub UT, 0.94, Post T, 0.96, Post UT, 0.96), lower residual sum of squares and an insignificant F-test for all participants during both exercise modalities. The influence of training status on the [HHb] response manifested as a significant rightward shift of the response, as indicted by the significantly higher work rate corresponding to 50% of the [HHb] amplitude (c/d) during both cycle (Pre: T, 61 ± 8 vs. UT, 48 ± 9, $d = 1.63$; Pub: T, 57 ± 8 vs. UT, 49 ± 6, $d = 1.22$; Post: T, 62 ± 6 vs. UT, 48 ± 11 W, $d = 1.60$; all $P<0.01$) and upper body exercise (Pre: T, 50 ± 15 vs. UT, 34 ± 11, $d = 1.24$; Pub: T, 63 ± 15 vs. UT, 50 ± 11, $d = 1.02$; Post: T, 69 ± 6 vs. UT, 52 ± 10 W, $d = 2.12$; all $P<0.01$), as illustrated in Figure 7.3 (neither maturity nor exercise modality influenced the response pattern, thus only the response to cycle exercise is shown). This rightward shift was correlated with parameters of aerobic fitness in the pre-pubertal girls during both cycle (peak $\dot{V}O_2$: r = 0.63; GET: r = 0.59; both $P<0.05$) and

upper body ergometry (peak $\dot{V}O_2$: r = 0.48; GET: r = 0.52; MRT: r = -0.49; both P<0.05) and in the post-pubertal girls during cycle ergometry (peak $\dot{V}O_2$: r = 0.71; *P*<0.01).

**Figure 7.3** Deoxy [HHb+Mb] response as a function of relative work rate for a representative trained (solid circles) and untrained (open circles) girl during cycle ergometry.



### 7.3.2 Interaction between maturity and training status

The magnitude of training status differences was similar in all three maturity groups and, consequently, neither the pulmonary nor the cardiovascular parameters revealed any interaction between training status differences and maturity. A significant interaction between training status and maturity was found for peak work rate during both modalities, however, with a greater difference between trained and untrained pubertal (cycle: 21%; upper body: 24%) and post-pubertal (cycle: 14%; upper body: 28%) girls compared to pre-pubertal girls (cycle:11%; upper body: 8%).

### 7.4 Discussion

In agreement with our hypothesis, the main findings of the current study were that a higher peak $\dot{V}O_2$, $\dot{Q}$ and SV existed in the trained participants in all three maturity groups,

although SV and $\dot{Q}$ were only significantly higher in the trained pubertal and post-pubertal girls. Interestingly, the stroke volume response pattern was dependent on training status, with a linear response in trained girls in comparison to a non-linear response in untrained girls (i.e. initial rise followed by a plateau until exhaustion) across all three maturity groups. The pattern of the deoxyhemoglobin response was also influenced by training status, demonstrating a significant rightward shift in the sigmoidal response in trained compared to untrained girls in all three maturity groups. Finally, no interactions between the magnitude of training differences and maturity status were evident in the cardiopulmonary or metabolic responses to exercise. These findings therefore suggest that there is no maturational threshold beyond which training status effects become more manifest.

The higher peak $\dot{V}O_2$ in the trained girls agrees with some previous studies in pre-pubertal (Obert *et al.*, 1996; McManus *et al.*, 1997; Rowland *et al.*, 2009a) and pubertal girls (Burkett *et al.*, 1985; Rowland *et al.*, 1991; Chapter 6), but this is the first study to investigate the influence of training status on the peak $\dot{V}O_2$ in early post-pubertal girls. An increased peak $\dot{V}O_2$ in trained participants is generally associated with an increased peak SV and consequently an increased peak $\dot{Q}$ (Nottin *et al.*, 2002b; Rowland *et al.*, 2002; Obert *et al.*, 2003), as we found in the current study for pubertal and post-pubertal girls and for which there was a trend in the pre-pubertal girls. Also, in agreement with previous studies (Nottin *et al.*, 2002b; Obert *et al.*, 2003; Rowland *et al.*, 2009b), peak HR and $a\text{-}\bar{v}O_2$ difference were not influenced by training status (with the exception of post-pubertal upper body ergometry), suggesting that the increased peak $\dot{V}O_2$ was probably solely related to an elevated SV and $\dot{Q}$.

The significant influence of training status on the relative GET in pre-pubertal girls contrasts previous reports in pre-pubertal children (chapter 5; Obert *et al.*, 2000; Cleuziou *et al.*, 2002), whilst the lack training status effect in pubertal girls disagrees with an earlier study in adolescents . No studies are available which have specifically investigated the influence of training status in post-pubertal girls but the current findings differ from those in adults (Simon *et al.*, 1986; Boone *et al.*, 2008). The reason/s for these contradictory

results is/are unclear; however, they do not support the concept of a maturational threshold. The faster MRT exhibited by the trained girls in all three maturity groups during both exercise modalities agrees with studies reporting faster $\dot{V}O_2$ kinetics in trained pre-pubertal children (chapter 5), pubertal adolescents (chapter 6) and adults (Powers *et al.*, 1985; Barstow *et al.*, 2000; Koppo *et al.*, 2004; Figueira *et al.*, 2008).

The quantitative differences in SV according to training status in children are widely attributed to morphological adaptations of the myocardium, including an increased left ventricular dimension and mass and intraventricular and posterior wall thickness (e.g. Bianchi *et al.*, 1998; Nottin *et al.*, 2002b; Rowland *et al.*, 2009a). However, the current results indicate both quantitative and qualitative differences according to training status, thereby suggesting an additional or alternative functional basis for the higher SV in trained children. The qualitative differences were observed in the SV response pattern which demonstrated the commonly reported initial increase in SV until 40-50% peak $\dot{V}O_2$ followed by a plateau until exhaustion in the untrained girls but a progressive, linear increase in SV until exhaustion in the trained girls. These differences were independent of maturity status and exercise modality. Although a progressively increasing SV is less commonly reported, it has been observed previously in trained pre-pubertal children (Rowland *et al.*, 1998), pubertal boys (Unnithan *et al.*, 1997) and adults (e.g. Crawford *et al.*, 1985; Warburton *et al.*, 2002). A common feature amongst these studies is the high training status of the subjects, especially when compared to training intervention studies. The mechanistic basis of these functional differences with training status is unclear. Whilst an enhanced diastolic filling has been suggested as the most likely mechanism in adults, the relative contribution of upstream and downstream factors to this effect remain to be resolved (Rowland, 2009), and the appropriateness of this explanation to younger populations is unclear.

The superior fit of the sigmoidal compared to hyperbolic model to the deoxyhemoglobin response in trained and untrained girls during both exercise modalities is in agreement with adult studies (Boone *et al.*, 2009). This sigmoidal [HHb] response reflects the non-linear relationship between $O_2$ delivery and utilization at the muscle due to a relatively more rapid increase in $O_2$ delivery at low compared to higher exercise intensities (Ferreira *et al.*, 2007).

This variable rate of $O_2$ delivery to utilization may be related to 1) the mechanical effects of muscular contraction; 2) an alteration in the balance between vasodilating and vasocontricting forces; and/or 3) changes in muscle fibre type recruitment, with increasing exercise intensity (Boone *et al.*, 2009, 2010). Consistent with previous findings in adults (Boone *et al.*, 2009), the [HHb] response pattern relative to percentage peak power showed a rightward shift in the trained participants, irrespective of maturity status or exercise modality. Furthermore, a relationship between the pattern of fractional $O_2$ extraction and training status is supported, at least in pre-pubertal and post-pubertal girls, by the correlations between [HHb] *c/d* and parameters of aerobic fitness. Similar findings in adults (Boone *et al.*, 2009), have been suggested to be associated with the enhanced oxidative capacity (Holloszy, 1967; Mogensen *et al.*, 2006) and/or greater percentage of type I muscle fibres reported in trained participants (Costill *et al.*, 1976; Trappe *et al.*, 2006). The applicability of these mechanisms to the rightward shift observed in the current population is unclear. However, an elevated muscle $O_2$ delivery and/or greater matching of local perfusion to metabolic rate in trained children may delay the requirement for accelerated $O_2$ extraction during incremental exercise. At the same time, an increased muscle oxidative capacity which has previously been reported in trained children (Eriksson *et al.*, 1973; Fournier *et al.*, 1982), may contribute to the observed responses.

The lack of interaction between the magnitude of training differences and maturity status in all the response parameters with the exception of the peak work rate was unexpected. We hypothesized that the difference between trained and untrained girls would increase from pre-pubertal to pubertal to post-pubertal girls. Instead, we found the magnitude of differences between trained and untrained girls to be similar in all three maturity groups (e.g. absolute cycle peak $\dot{V}O_2$: pre, 18%; pub, 22%; post, 18% higher in trained girls). Although the possible conclusions that can be drawn are limited by the cross sectional nature of this study, the present findings agree with Weber et al. (1976) and Danis et al. (2003) and suggests that there is no "golden" period during which training has an especially pronounced effect or a maturational threshold below which significant physiological adaptations to training cannot occur (Katch, 1983). It is possible that previous reports of the presence of a maturational threshold are actually a reflection of an insufficient training volume in the younger participants, therefore artificially indicating an influence of

maturation. However, even when younger and older participants completed the same training programme, controversy remained regarding the presence of a maturational threshold, therefore suggesting training volume is unlikely to explain the contradictory results. Whether the presence, or absence, of a maturational threshold is sex or sport dependent remains to be determined. However, it is possible that the influence of the hormones associated with the onset of puberty may be more important in boys or in more "anaerobic" sports. The present results indicate that significant influences of training status are evident in girls' physiological responses to exercise even before puberty is reached and that reaching puberty is not associated with greater influences of training status being manifest.

## *7.5 Conclusion*

In conclusion, training status significantly influenced the cardiopulmonary responses to exercise across all maturity stages, independent of exercise modality. Specifically, peak $\dot{V}O_2$ was higher in the trained girls during both upper and lower body exercise, an elevation attributable to the higher peak SV and $\dot{Q}$ in trained girls. The linear increase observed in SV in the trained girls may indicate both morphological and functional influences of training status on the myocardium which contribute to the higher peak SV. Furthermore, training status influenced the metabolic responses to incremental ramp exercise, with a relatively slower increase in estimated muscle fractional $O_2$ extraction in the trained girls. Neither exercise modality nor maturity status influenced the magnitude of the differences between trained and untrained girls. These findings challenge the notion that there is a "golden period" or maturational threshold regulating the influence of training status on the physiological responses to exercise in young people

# Chapter 8

## Study 5 - The influence of training and maturity status on girls' responses to high intensity upper and lower body exercise

This study has been disseminated as follows:

*Publication:* McNarry, M.A., Welsman, J.R., & Jones, A.M. (2010). The influence of training and maturity status on girls' responses to high intensity upper and lower body exercise. In Press with APNM.

### *8.1 Introduction*

The high-intensity nature of the Wingate Anaerobic test (WAnT) is highly relevant to the habitual activity and play patterns of young people (Bailey *et al.*, 1995). Nonetheless, relatively few studies have examined children's physiological response to high-intensity exercise and, consequently, the influences of growth, maturation and training status on 'anaerobic' exercise performance in young people remain poorly understood (Williams, 2008). There is a particular dearth of such information in young girls. Therefore, although a significantly higher peak power (PP) and mean power (MP) and lower fatigue index (FI) have been reported in trained young boys (Grodjinovsky *et al.*, 1981; Rotstein *et al.*, 1986; Counil *et al.*, 2003; Ingle *et al.*, 2006), it remains to be resolved whether similar influences of training status are present in young girls.

Training status has been reported to significantly influence the physiological responses to exercise in pre-pubertal girls (McManus *et al.*, 1997, although these effects may be confined to PP {McManus, 1997 #43; Bencke *et al.*, 2002) or to the sport investigated (Bencke *et al.*, 2002). In contrast, for adolescent girls no influence of training status on WAnT test performance has been reported (Siegler *et al.*, 2003). This latter finding might be considered surprising given the significant effects of training status found in women during anaerobic tests (Serresse *et al.*, 1989; Liljedahl *et al.*, 1996). Furthermore, this finding contradicts the notion of a 'maturational threshold', below which significant

physiological adaptations to training cannot occur (Katch, 1983). This concept has been debated for many years with regard to aerobic exercise responses, with some studies supporting the concept (Kobayashi *et al.*, 1978; Mirwald *et al.*, 1981) and others refuting it (Weber *et al.*, 1976; Danis *et al.*, 2003). The possibility that a maturational threshold for training status exists in the physiological response to high-intensity exercise has not been investigated.

The WAnT was originally devised as an anaerobic test but recent studies have reported a significant contribution of oxidative phosphorylation to ATP resynthesis during the test in both adults (Granier *et al.*, 1995; Calbet *et al.*, 1997; Bediz *et al.*, 1998) and children (Chia *et al.*, 1997; Chia, 2006). Furthermore, this oxidative contribution has been reported to be greater in trained adults compared to their untrained counterparts (Granier *et al.*, 1995), an effect that may be related to the faster $\dot{V}O_2$ kinetics of trained adults (e.g. Powers *et al.*, 1985; Koppo *et al.*, 2004; Figueira *et al.*, 2008). The influence of training status on the oxidative contribution to the WAnT in young people has yet to be investigated.

The purpose of the present cross-sectional study was to investigate the influence of training status on the responses to upper-body (arm crank) and lower-body (cycle) WAnT in pre-pubertal, pubertal and post-pubertal girls. We hypothesised that the trained girls would exhibit a significantly higher PP, MP and oxidative contribution and a lower FI, with the difference between trained and untrained girls increasing with maturity. We also hypothesised that the differences associated with training status would be more pronounced during upper than lower-body exercise due to the predominantly upper body nature of swimming (Ogita *et al.*, 1996).

## *8.2 Methods*

### *8.2.1 Participants*

In total, 18 pre-pubertal (8 trained, 10 untrained), 24 pubertal (9 trained, 15 untrained) and 18 post-pubertal (8 trained, 10 untrained) girls participated in this study. The trained girls (T) were all regional, national or international level swimmers. The pre-pubertal girls had

been training for a mean of 2.5 ($\pm$ 1) years and reported a mean training volume of 14 ($\pm$ 3) hrs·wk$^{-1}$. The pubertal and post-pubertal girls had been training 5 ($\pm$ 1.5) years and 8 ($\pm$ 2) years respectively, with training volumes of 18 ($\pm$ 4) and 22 ($\pm$ 3) hrs·wk$^{-1}$, respectively. The training programme was predominantly aerobic although short, high intensity repetitions were also completed. In accordance with the long-term athlete development programme, the younger maturity groups were completing non-specific swimming training programmes whilst the post-pubertal swimmers were at the early stages of tailoring their training for specific swimming events. There was no bias amongst this group towards sprint, middle or long distance swimming events. The untrained (UT) group comprised volunteers from local schools who did not participate in any form of organised sport outside the national curriculum. Sexual maturity was assessed by self-report using the indices of pubic hair described by Tanner (1962). Age to peak height velocity was estimated to provide an additional indicator of physical maturity according to the equations of Mirwald *et al.* (2002), which are based on the measurement of standing and seated height, weight, and date of birth as described below.

### 8.2.2 Anthropometry

An anthropometrical evaluation was performed before the first test for all participants. Standing and seated height were measured to 0.1 cm using a Holtain stadiometer (Holtain, Crymych, Dyfed, UK) and body mass (BM) was determined using Avery beam balance scales to 0.05 kg (Avery, Birmingham, UK). Skinfold thickness was assessed three times at five sites on the body (bicep, triceps, subscapular, supra-iliac crest and thigh) by the same researcher for all participants using Harpenden callipers (Baty International, Burgess Hill, UK), accurate to the nearest 0.2mm. The mean of the three measurements was taken. Percentage body fat and fat free mass (FFM) were subsequently estimated based on the equations of Slaughter et al. (1988).

Participants were asked to arrive at the laboratory in a rested and fully hydrated state, at least 3 hours postprandial and to refrain from consuming caffeinated drinks in the 6 hours prior to the test. The methods employed during this study were approved by the

institutional research ethics committee and all participants and their parents/guardians gave written informed consent and assent, respectively.

### 8.2.3 Experimental protocols and measures

The Wingate tests were conducted on two identical basket loaded cycle ergometers (Monark model 814 *E*), one of which was modified to allow upper body exercise. The seat height was adjusted to suit each participant, ensuring a slight flexion in the knee during the cycle WAnT and that the centre of the pedal crank was in line with the middle of the participants' glenohumeral joint during the upper body WAnT.

Each participant completed two WAnTs, one upper (UP) and one lower (LO) body, on separate days. The WAnT was preceded by a standardised 3 minute warm-up performed at a steady pace at the minimum ergometer resistance. This was interspersed at 1 min, 2 min and 2.5 minutes with a 3 s, all-out sprint against the actual test resistance to familiarise the participants with the test protocol. The resistance was set at 0.075 $kg \cdot kg^{-1}$ BM and 0.045 $kg \cdot kg^{-1}$ BM for the LO and UP WAnT, respectively, based on the guidelines of Bar-Or (1983b). After a 2 minute rest, the WAnT itself commenced with 3 minutes sitting stationary on the ergometer for the assessment of baseline responses. Following this, participants were asked to accelerate the unloaded flywheel to 60 rpm and a 3 s countdown was given. On "GO!", the participants accelerated as fast as possible and the load basket was dropped. Participants were asked to pedal as fast as they could for the entire 30 s test and warned beforehand that signs of pacing would result in the test being repeated. Strong verbal encouragement was provided through-out the 30 s test.

Throughout the WAnT, gas exchange variables (Metalyser 3B Cortex, Biophysik, Leipzig, Germany) and heart rate (Polar S610, Polar Electro Oy, Kempele, Finland) were measured on a breath-by-breath basis and displayed online. Prior to each test the gas analyser was calibrated using gases of known concentration and the turbine volume transducer was calibrated using a 3-litre syringe (Hans Rudolph, Kansas City, MO). The delay in the capillary gas transit and analyser rise time were accounted for relative to the volume signal, thereby time aligning the concentration and volume signals.

*8.2.4 Data analysis*

Power output variables were corrected for flywheel inertia and internal resistance (Chia *et al.*, 1997) and reported for each second of exercise. Peak power (PP) was defined as the highest 1-s value and mean power (MP) was defined as the mean power output over the entire test. The fatigue index (FI) was calculated as the change in power output relative to PP = (PP – end power / PP)*100).

Breath-by-breath data were interpolated to 1 s intervals and the peak $\dot{V}O_2$ was defined as the highest 3 s average. The relative contribution of oxidative phosphorylation to the total energy expenditure during the 30 s WAnT was calculated by determining the area under the curve of $\dot{V}O_2$ as a function of time, described by non-linear regression. This $VO_2$ was subsequently converted to the oxidative energy cost of exercise by multiplying by 20.92 $J \cdot mL \cdot O_2^{-1}$ and expressed relative to the total work done for the 30 s WAnT. Mechanical efficiency values of 13% (Kavanagh & Jacobs, 1988) and 30% (Bar-Or, 1996) were employed to allow comparison to previous paediatric studies (Chia *et al.*, 1997).

To determine the kinetics of the $\dot{V}O_2$ response, the interpolated data were modelled using a mono-exponential function without a time delay, as reported by Calbet et al. (2003) (Graphpad Prism, Graphpad Software, San Diego, CA):

$$\Delta VO_{2(t)} = A \cdot \left(1 - e^{-\left(\frac{t}{\tau}\right)}\right)$$

(Eq. 8.1)

Where $\Delta \dot{V}O_2$ is the increase in $\dot{V}O_2$ at time *t* above the baseline value (calculated as the mean $\dot{V}O_2$ from the first 45 s of the last minute of baseline), A and $\tau$ are the amplitude and time constant, respectively.

*8.2.5 Statistical analyses*

Following identification of a normal distribution, homogenous variation and an absence of skewness or kurtosis, a two way ANOVA with repeated measures was used to analyse training status and exercise mode effects. Subsequent independent or paired samples t-tests

were employed as appropriate to identify the specific location of significant effects. The interaction of training status and sexual maturity stage was investigated using a factorial ANOVA. The influence of body size was accounted for using ANCOVA on log transformed data to determine the allometric relationship between body mass and peak $\dot{V}O_2$, PP and MP (Welsman & Armstrong, 2000). The allometric relationship was also determined between estimated fat free mass (FFM) and PP and MP. All data are presented as means ± SD. Statistical significance was accepted when $P < 0.05$.

## 8.3 Results

Anthropometric characteristics, presented in Table 8.1, did not differ significantly between T and UT girls within each maturity group. All the girls in the pre-pubertal group were Tanner stage 1, whilst the pubertal girls were stages 3 and 4 and post-pubertal were stage 5.

**Table 8.1** Participants' anthropometric characteristics

| | Pre-pubertal | | Pubertal | | Post-pubertal | |
|---|---|---|---|---|---|---|
| | Trained | Untrained | Trained | Untrained | Trained | Untrained |
| | N = 8 | N = 10 | N = 9 | N = 15 | N = 8 | N = 10 |
| Age (y) | 11.2 ± 1.0 | 11.9 ± 0.9 | 14.2 ± 0.8 | 14.2 ± 0.6 | 16.6 ± 0.6 | 16.7 ± 0.8 |
| Stature (m) | 1.48 ± 0.08 | 1.50 ± 0.06 | 1.66 ± 0.05 | 1.60 ± 0.06 | 1.67 ± 0.04 | 1.69 ± 0.06 |
| Mass (kg) | 43.2 ± 3.1 | 43.6 ± 6.6 | 56.9 ± 6.7 | 54.9 ± 7.0 | 59.4 ± 7.6 | 61.7 ± 6.7 |
| Sum of skinfolds (mm) | 67.0 ± 17.4 | 59.2 ± 13.2 | 63.6 ± 15.3 | 67.3 ± 18.6 | 54.3 ± 16.5 | 69.8 ± 32.3 |
| Body fat (%) | 26.7 ± 8.5 | 25.3 ± 5.9 | 29 ± 10 | 31 ± 9 | 24 ± 11 | 27 ± 9 |

*Values are mean ± S.D.*

\*    *Significant difference between pre-pubertal and pubertal girls within trained or untrained children (P<0.01)*

†    *Significant difference between pubertal and post-pubertal girls within trained or untrained children (P<0.01)*

## 8.2.1 Influence of training status

The influence of training status on PP and MP was dependent on exercise modality in all maturity groups with no influence evident during cycle ergometry but significantly higher

values being present in the T girls during upper body ergometry (*d*; PP: Pre, 1.58, Pub, 1.41, Post, 1.40; MP: Pre, 1.88, Pub, 1.66, Post, 2.26), as summarised in Table 8.2 and shown in Figure 8.1.

**Table 8.2** Mechanical power indices in trained and untrained girls at 3 stages of maturity during a lower and upper body WAnT

| | Pre-pubertal | | Pubertal | | Post-pubertal | |
|---|---|---|---|---|---|---|
| | Trained | Untrained | Trained | Untrained | Trained | Untrained |
| | N = 8 | N = 10 | N = 9 | N = 15 | N = 8 | N = 10 |
| *Cycle WAnT* | | | | | | |
| PP (W) | 325 ± 41 | 359 ± 72 | 496 ± 90 [#] | 454 ± 109 [#] | 487 ± 106 | 541 ± 20 |
| PP (W·kg⁻¹BM) | 7.3 ± 1.1 | 8.3 ± 1.6 | 8.9 ± 1.2 | 8.3 ± 1.5 | 8.5 ± 1.4 | 8.8 ± 1.3 |
| PP (W·kg⁻¹FFM) | 9.5 ± 1.2 | 11.1 ± 2.2 | 12.4 ± 2.8 | 12.4 ± 1.7 | 10.2 ± 1.8 | 12.2 ± 1.6 |
| MP (W) | 258 ± 42 | 274 ± 70 | 400 ± 60 [#] | 352 ± 77 [#] | 388 ± 55 | 421 ± 35 |
| MP (W·kg⁻¹BM) | 5.9 ± 0.9 | 6.3 ± 1.5 | 7.1 ± 1.0 [#] | 6.4 ± 1.0 | 6.8 ± 0.8 | 6.9 ± 0.9 |
| MP (W·kg⁻¹FFM) | 7.8 ± 0.8 | 8.4 ± 2.2 | 10.0 ± 2.0 | 9.6 ± 1.2 | 8.5 ± 1.3 | 9.4 ± 0.7 |
| FI (%) | 28 ± 11 | 42 ± 10 [*] | 30 ± 13 | 42 ± 9 [*] | 32 ± 8 | 44 ± 10 [*] |
| *Upper body WAnT* | | | | | | |
| PP (W) | 163 ± 20 [†] | 124 ± 29 [*†] | 230 ± 42 [†] | 173 ± 41 [*†] | 245 ± 41 [†] | 190 ± 40 [*†] |
| PP (W·kg⁻¹) | 3.8 ± 0.6 [†] | 2.9 ± 0.7 [*†] | 4.0 ± 0.4 [†] | 3.4 ± 0.9 [*†] | 4.1 ± 0.4 [†] | 3.1 ± 0.8 [*†] |
| PP (W·kg⁻¹FFM) | 4.8 ± 3.9 [†] | 3.9 ± 1.0 [†] | 5.6 ± 0.9 [†] | 4.5 ± 0.6 [*†] | 5.7 ± 1.1 [†] | 4.3 ± 0.9 [*†] |
| MP (W) | 130 ± 23 [†] | 85 ± 26 [*†] | 184 ± 37 [†] | 123 ± 38 [*†] | 200 ± 30 [†] | 150 ± 15 [*†] |
| MP (W·kg⁻¹) | 3.0 ± 0.5 [†] | 2.0 ± 0.6 [*†] | 3.2 ± 0.4 [†] | 2.5 ± 0.9 [*†] | 3.4 ± 0.3 [†] | 2.5 ± 0.8 [*†] |
| MP (W·kg⁻¹FFM) | 3.8 ± 0.6 [†] | 2.6 ± 1.0 [*†] | 4.5 ± 0.9 [†] | 3.3 ± 0.5 [*†] | 4.6 ± 0.8 [†] | 3.4 ± 0.9 [*†] |
| FI (%) | 35 ± 12 | 50 ± 16 [*] | 32 ± 14 | 46 ± 16 [*] | 32 ± 6 | 44 ± 12 [*] |

*Values are mean ± S.D. PP, peak power; MP, mean power; FI, fatigue index; BM, body mass; FFM, fat free mass*
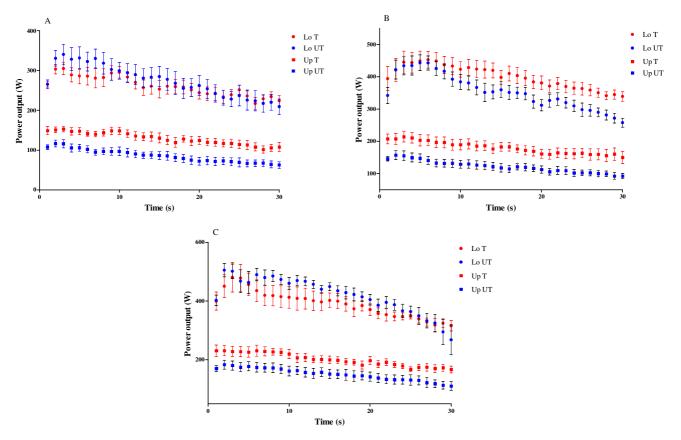
*\*      Significant difference between trained and untrained children within a maturity group (P<0.05)*

*\#      Significant difference compared to previous maturity stage within training status group (P<0.05)*

*†      Significant difference between exercise modalities within training status and maturity status group (P<0.05)*

These differences remained significant subsequent to ratio or allometric scaling with the exception of PP in the pre-pubertal girls. In contrast, irrespective of exercise modality, the trained girls in all maturity groups exhibited a lower fatigue index (*d*; cycle: Pre, 1.38, Pub, 1.15, Post, 1.35; upper body: Pre, 1.13, Pub, 1.63, Post, 1.26). During upper but not lower body ergometry, the trained girls in all three maturity groups demonstrated a significantly greater total work done (KJ; Pre: T, 3.9 ± 0.7 vs. UT, 2.6 ± 0.8, *d* = 1.77; Pub: T, 5.5 ± 1.1 vs. UT, 3.7 ± 1.1, *d* = 1.67; Post: T, 6.0 ± 0.9 vs. UT, 4.5 ± 1.2, *d* = 1.43; all *P*<0.05).

**Figure 8.1** Mean power output responses for (a) pre-pubertal, (b) pubertal and (c) post-pubertal girls during lower body (Lo) and upper body (Up) exercise. Trained girls are shown with closed and untrained girls with open symbols.



Trained girls achieved a significantly higher peak $\dot{V}O_2$ during upper body ergometry in all three maturity groups (*d*: Pre, 1.72, Pub, 2.54, Post, 2.37) and during lower body ergometry in pubertal and post-pubertal girls (*d*: Pre, 1.66, Pub, 2.04, Post, 1.72), as shown in Table 8.3. The trained girls in all maturity groups had faster $\dot{V}O_2$ kinetics during the 30 s WAnT for both exercise modalities (*d*; cycle: Pre, 1.04, Pub, 2.40, Post, 3.44; upper body: Pre,

2.06, Pub, 2.11, Post, 3.73), as shown in Figure 8.2. Despite this, the oxidative contribution to total energy expenditure was only influenced by training status in the post-pubertal girls during lower body ergometry. The $\dot{V}O_2$ τ was significantly related to peak $\dot{V}O_2$ during upper body exercise in all three maturity groups (Pre, r = -0.73; Pub, -0.52; Post, -0.48; all $P$<0.05) and during lower body exercise in pubertal (r = -0.46; $P$<0.05) and post-pubertal girls (r = -0.66; $P$<0.01).

**Table 8.3** Peak oxygen uptake and oxidative contribution to energy provision in trained and untrained girls at 3 stages of maturity during a lower and upper body WAnT

| | Pre-pubertal | | Pubertal | | Post-pubertal | |
|---|---|---|---|---|---|---|
| | Trained | Untrained | Trained | Untrained | Trained | Untrained |
| | N = 8 | N = 10 | N = 9 | N = 15 | N = 8 | N = 10 |
| *Cycle WAnT* | | | | | | |
| Peak $\dot{V}O_2$ (L·min$^{-1}$) | 1.8 ± 0.3 | 1.6 ± 0.3 | 2.2 ± 0.3 | 1.8 ± 0.2 $^*$ | 2.5 ± 0.2 | 2.2 ± 0.2 $^{*\#}$ |
| Peak $\dot{V}O_2$ (mL·kg$^{-1}$·min$^{-1}$) | 43 ± 6 | 38 ± 5 | 38 ± 7 | 34 ± 4 $^*$ | 43 ± 4 | 35 ± 7 $^*$ |
| Oxidative 13% (%) | 14 ± 3 | 14 ± 2 | 13 ± 3 | 12 ± 2 | 15 ± 2 | 10 ± 2 $^*$ |
| Oxidative 30% (%) | 33 ± 7 | 32 ± 5 | 30 ± 6 | 27 ± 5 | 34 ± 4 | 23 ± 5 $^*$ |
| $\dot{V}O_2$ τ (s) | 15 ± 6 | 20 ± 4 | 9 ± 5 | 17 ± 2 $^*$ | 8 ± 3 | 18 ± 3 $^*$ |
| *Upper body WAnT* | | | | | | |
| Peak $\dot{V}O_2$ (L·min$^{-1}$) | 1.6 ± 0.2 $^\dagger$ | 1.1 ± 0.3 $^{*\dagger}$ | 2.1 ± 0.2 $^\#$ | 1.4 ± 0.2 $^{*\dagger\#}$ | 2.1 ± 0.4 $^\dagger$ | 1.5 ± 0.3 $^{*\dagger}$ |
| Peak $\dot{V}O_2$ (mL·kg$^{-1}$·min$^{-1}$) | 36 ± 7 $^\dagger$ | 25 ± 5 $^{*\dagger}$ | 36 ± 4 | 27 ± 7 $^{*\dagger}$ | 35 ± 6 $^\dagger$ | 23 ± 5 $^{*\dagger}$ |
| Oxidative 13% (%) | 21 ± 3 $^\dagger$ | 25 ± 6 $^\dagger$ | 20 ± 5 $^\dagger$ | 20 ± 7 $^\dagger$ | 16 ± 4 | 16 ± 2 $^\dagger$ |
| Oxidative 30% (%) | 49 ± 6 $^\dagger$ | 58 ± 13 $^\dagger$ | 46 ± 11 $^\dagger$ | 47 ± 15 $^\dagger$ | 37 ± 9 | 37 ± 5 $^\dagger$ |
| $\dot{V}O_2$ τ (s) | 12 ± 4 | 20 ± 4 $^*$ | 10 ± 4 | 17 ± 3 $^{*\#}$ | 10 ± 3 | 19 ± 2 $^*$ |

*Values are mean ± S.D. $\dot{V}O_2$, oxygen uptake; Oxidative 13%, oxidative contribution assuming 13% mechanical efficiency; Oxidative 30%, oxidative contribution assuming 30% mechanical efficiency*
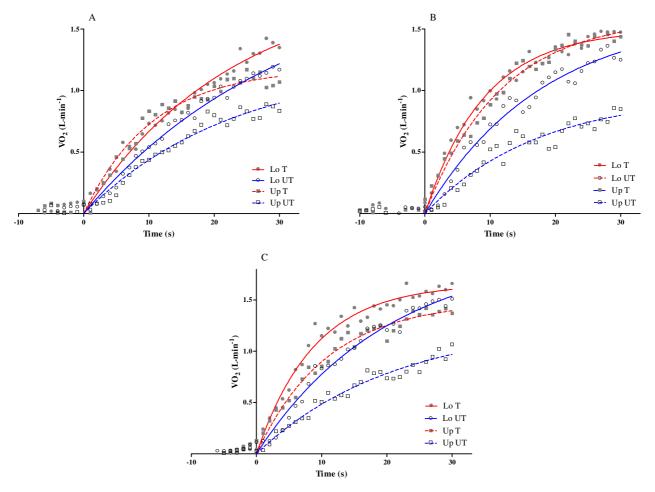
*\*       Significant difference between trained and untrained children within a maturity group (P<0.01)*

*\#       Significant difference compared to previous maturity stage within training status group (P<0.05)*

*†       Significant difference between exercise modalities within training status and maturity status group (P<0.05)*

**Figure 8.2** Mean $\dot{V}O_2$ responses for (a) pre-pubertal, (b) pubertal and (c) post-pubertal girls during lower bodo (Lo) and upper body (Up) exercise. Trained girls are shown with closed and untrained girls with open symbols.



### 8.3.2 Interaction of training status with maturity

No interaction was evident between maturity and training status for the mechanical power or $\dot{V}O_2$ related parameters, with statistically similar differences between trained and untrained girls being evident at all three stages of maturity and for both modes of exercise (Tables 8.2, 8.3).

### 8.4 Discussion

The main finding of the present study was that training status significantly influenced both the mechanical power and the $\dot{V}O_2$ responses of girls to high-intensity exercise across three

stages of maturity.  Moreover, the magnitude of these training status differences was not modulated by maturity. These data therefore suggest that there is no maturational threshold which must be surpassed for significant influences of training status to be manifest (Katch, 1983).

### 8.4.1 Influence of training status

The current results broadly agree with previous studies reporting significant influences of training status in pre-pubertal girls (McManus *et al.*, 1997; Bencke *et al.*, 2002). The effects of training status were greater in our study compared to that of McManus et al. (1997), who reported effects on PP only, perhaps as a consequence of the more trained status of the present participants. Bencke et al. (2002) reported significant influences of swim-training status during lower body exercise, whilst we found significant influences during upper body exercise only. The explanation for this discrepancy is obscure.

In adolescent girls, no influence of training status on any mechanical power parameter has been reported (Siegler *et al.*, 2003). These findings contrast with the current results for both pubertal and post-pubertal girls. Direct comparisons to this previous study are hindered by the absence of a maturity assessment of the ~ 16 year old girls, who may have been late-pubertal or post-pubertal. The discrepancy with the present findings may also be attributable to an insufficient training stimulus in the study of Siegler et al. (2003) since all participants were involved in regular football training with a subset undertaking additional resistance and plyometric training. Alternatively, or additionally, a discrepancy between the training modalities and the test modality (cycle) may explain the contradictory results (Grodjinovsky *et al.*, 1981).

In contrast to the results of the present study, the FI has previously been reported to be unaffected by training status in girls (Bencke *et al.*, 2002; Siegler *et al.*, 2003). A lower FI in our trained participants indicates a superior ability to maintain power output near the peak power output as the test proceeds. These results suggest that whilst PP and MP may be influenced by both aerobic (Rotstein *et al.*, 1986; Obert *et al.*, 2001) and anaerobic (Grodjinovsky *et al.*, 1981; Ingle *et al.*, 2006) based training programmes, aerobic training

is more effective at reducing the FI. The mechanistic basis for this is unclear but may be related to alterations in oxidative capacity and fatigue resistance in type II muscle fibres (Jones & Carter, 2000).

Before considering the possible mechanistic basis of the aforementioned training status related differences, it is appropriate to highlight the cross-sectional design of this study. A fundamental advantage of this design is that it allows the investigation of the physiological effects of long-term, intensive training programmes, the replication of which is very challenging using longitudinal intervention based studies. However, the compromise is that it precludes the elucidation of whether the training status differences are attributable to training *per se* or are a reflection of the participants' genotypes, or uncontrolled factors such as sampling bias or non-physiological learning effects.

The mechanistic basis of training-status related enhancements in the mechanical power indices of children remain unclear (Obert *et al.*, 2001), although a number of putative mechanisms have been proposed including changes in muscle metabolism, muscle mass and/or muscle fibre type. Suggestions of an altered muscle metabolism are based on early muscle biopsy studies which reported increased concentrations of adenosine triphosphate, phosphocreatine (PCr) and muscle glycogen, along with an increased activity of several glycolytic enzymes in trained children (Eriksson *et al.*, 1973; Fournier *et al.*, 1982; Cadefau *et al.*, 1990). However, more recent studies failed to find any influence of training status on intramuscular pH or the ratio of PCr to inorganic phosphate, both of which have been suggested to be indicators of glycolytic capacity (Kuno *et al.*, 1995). Lower limb muscle mass is a major determinant of the mechanical power response to high-intensity cycle exercise in healthy, untrained children (Davies *et al.*, 1972; Mercier *et al.*, 1992; Santos *et al.*, 2003). Whether upper body muscle mass is similarly influential in determining the mechanical power response to high intensity upper body exercise is unknown. A potential role of muscle fibre type distribution and/or recruitment in determining training status related differences has been suggested on the basis of reports in adults suggesting an increased percentage of type I muscle fibres in trained participants (Saltin & Gollnick, 1983; Russell *et al.*, 2003). However, due to ethical constraints, no information is presently available in young people to corroborate or refute this possibility. Thus evidence regarding

the mechanistic basis of training status related differences in the mechanical power indices of young people is inconclusive.

This is the first study to investigate the influence of training status on the oxidative contribution to high-intensity exercise in young people. In agreement with previous studies in both children (Chia *et al.*, 1997; Chia, 2006) and adults (Granier *et al.*, 1995; Bediz *et al.*, 1998; Calbet *et al.*, 2003), a significant oxidative contribution to the WAnT test was observed. However, contrary to our hypothesis and to previous findings in adults (Granier *et al.*, 1995), the influence of training status was limited to lower body exercise in post-pubertal girls; no influence was evident in the oxidative contribution to either upper or lower body WAnT exercise in pre-pubertal or pubertal girls. This finding is surprising considering the significantly faster $\dot{V}O_2$ kinetics observed in the trained girls at all three stages of maturity, which one would anticipate would result in a greater oxidative contribution to meet the energy demands. The explanation for the apparent lack of training status on the oxidative contribution to the WAnT may be related to an equal influence of training status on both the oxidative (Eriksson *et al.*, 1973; Fournier *et al.*, 1982) and glycolytic components of energy provision (Eriksson *et al.*, 1973; Fournier *et al.*, 1982; Cadefau *et al.*, 1990), such that the overall balance is not altered by training status. Further studies investigating the influence of training status on oxidative and glycolytic components of energy provision are required in young people.

As hypothesised, the influence of training status was significantly more pronounced during upper than lower body exercise, a finding most likely attributable to the predominantly upper body nature of swimming (Ogita *et al.*, 1996). This finding, which agrees with previous reports in young boys (Grodjinovsky *et al.*, 1981), highlights the importance of the exercise modality in revealing training status effects in the response to high-intensity exercise in biologically immature populations. A failure to account for disparities between the training and testing modalities may explain the absence of training status influences on the high-intensity exercise response of girls previously reported (McManus *et al.*, 1997; Siegler *et al.*, 2003).

*8.4.2 Interaction of training status with maturity*

The interaction between training status and maturity during high-intensity exercise in young populations has not previously been investigated. Contrary to our hypothesis, no interaction was found between the magnitude of training status related differences and maturity for any parameter during either lower or upper body exercise. This finding contrasts with the classic theory of Katch (1983) which suggests the presence of a maturational threshold below which significant physiological adaptations to training are not manifest. These findings have potentially important implications for youth training programmes, indicating that training benefits can be obtained even before puberty. Further research is required to elucidate whether these conclusions are specific to girls and/or swimming, as it may be anticipated that the changes in the hormonal milieu associated with the onset of puberty (Zakas *et al.*, 1994; Daly *et al.*, 1998; Tsolakis *et al.*, 2003) would have a more significant impact in boys and/or in strength/power related sports. Furthermore, it must be determined if the manifestation of significant influences of training status during pre-puberty are associated with additional benefits during adulthood. It should be emphasised that any such benefit would need to be balanced with the increased chance of burnout or injury typically associated with intensive training at a young age (Hemery, 1988; Hollander *et al.*, 1995; Baxter-Jones & Helms, 1996; Starosta, 1996; Salguero & Gonzalez-Boto, 2003).

## *8.5 Conclusion*

In conclusion, this study has demonstrated significant influences of training status on the mechanical power indices during upper body WAnT, irrespective of maturity status in 11-17 year old girls. Specifically, PP and MP were both higher and the FI was lower in swim-trained pre-pubertal, pubertal and post-pubertal girls relative to their untrained counterparts during a 30 s all-out upper body exercise test. The dichotomy in the influence of training status between the upper and lower body highlights the importance of exercise modality in revealing training status influences. The present results indicate the presence of a significant oxidative contribution to energy provision during a WAnT test in girls. However, this oxidative contribution is not influenced by training status despite significantly faster $\dot{V}O_2$ kinetics in the trained girls. Finally, this study suggests that the

influence of training status on high-intensity exercise performance is similar regardless of maturity stage, providing evidence against the concept of a maturational threshold in girls' responses to high-intensity exercise.

## Chapter 9

## Summate discussion

The influence of training status across the stages of growth and maturation was previously largely uncharacterised, especially in girls and in the pubertal maturity stage. The purpose of the present thesis was to address this paucity of data by investigating an array of physiologic responses to provide an integrated insight into the influence of training status in girls prior to, during and post puberty. The individual elements of this multi-faceted approach have produced numerous novel findings, as described in chapters 4-8. The purpose of the following discussion is to consider the findings and their implications as a whole.

### *9.1 Training volume and the influence of training status*

Fundamental to any study investigating the influence of training status or a training intervention is the frequency, intensity and duration of the underlying or implemented training programme. Although the dose-response relationship between exercise and its impact is receiving increasing interest, the main focus is to develop guidelines regarding the minimum amount of exercise required to "promote and maintain health" (Haskell *et al.*, 2007). Consequently, the minimum amount of training required to elicit significant physiological adaptations and its interaction with maturity status, sport, gender and prior training remains unclear, the primary recommendations for children being to exceed the minimum recommended amounts of exercise (30 minutes, 5 days a week; Haskell *et al.*, 2007) and to exercise above 80% maximal heart rate (Baquet *et al.*, 2003).

In the present series of studies, the influence of training status on peak $\dot{V}O_2$ was assessed in three independent and separate groups of pre-pubertal swimmers (chapters 4, 5 and 7), but only in one study (chapter 7) was peak $\dot{V}O_2$ shown to be higher in the trained girls compared to their untrained counterparts. The same testing methodology was implemented in all three studies, with cycle exercise common to all three and upper body exercise common to studies 2 and 4 (chapters 5 and 7). Although the participants in study 1 (chapter

4) were marginally younger, lighter and smaller, these anthropometric differences should not influence comparisons as all participants were of the same maturity, according to Tanner stages (1962) and years to peak height velocity, and consideration of the allometrically scaled peak $\dot{V}O_2$ does not alter the findings. A key difference amongst these studies, however, was the training volume of the swimmers: study 1, $5 \pm 2$ hr·wk$^{-1}$; study 2, $8 \pm 3$ hr·wk$^{-1}$; study 3, $14 \pm 3$ hr·wk$^{-1}$. These training volumes all exceed the minimum recommended amount of excercise (Baquet *et al.*, 2003; Haskell *et al.*, 2007), despite which only at the highest training volume was a significant influence of training observed on peak $\dot{V}O_2$. Thus, the guidelines proposed appear inappropriate for pre-pubertal populations, a conclusion supported by previous studies which similarly failed to find an influence of training status, despite also satisfying the criteria (Welsman *et al.*, 1997; Tolfrey *et al.*, 1998). This incongruity is not surprising given that the ACSM guidelines are derived from studies in adults. However, the real implications of these findings lie within youth performance training programmes, suggesting that in excess of 8 hours a week of swimming training is required for significant influences of training status to be evident in girls' peak $\dot{V}O_2$.

However, there are a number of important caveats to this conclusion, including the relevance of peak $\dot{V}O_2$ to performance. Although peak $\dot{V}O_2$ is the most widely measured parameter in paediatric exercise science (Armstrong & Fawkner, 2007), this does not confer value, and indeed, peak $\dot{V}O_2$ is noted to be of limited value in other training and performance environments (Bosch, 2006; Jones, 2006; Matos & Winsley, 2007). A further caveat is that no measure of training intensity was taken in the current studies. Given the interaction between training intensity and duration, this limits the conclusions that can be drawn regarding the importance of training volume in determining the influence of training status. Finally, it is important to highlight that quantity should not be confused with quality and the latter should always be the primary aim of a training programme.

If the lack of influence of training status on peak $\dot{V}O_2$ in studies 1 and 2 (chapters 4 and 5) *is* associated with an insufficient training stimulus this may be related to high baseline fitness in the untrained participants. The magnitude of training-induced improvements is suggested to be inversely related to the initial baseline fitness level (Eliakim *et al.*, 1996;

Tolfrey *et al.*, 1998; Mandigout *et al.*, 2001), therefore a relatively high level of fitness in the untrained girls is likely to reduce the magnitude of training status differences and necessitate a greater training stimulus for significant effects to be observed. Although a high level of baseline fitness in the untrained participants would be a contradiction to the general trends for decreasing fitness and physical activity in children (Tremblay & Willms, 2000; Wedderkopp *et al.*, 2004; Dollman *et al.*, 2005), it is pertinent to note the role of self-selection for these studies especially in the control participants and the potential bias this inherently causes.

## *9.2 Maturity and the influence of training status*

For many years debate has surrounded the concept that there is an optimal age or maturational stage at which training will be most effective. Although arguably this concept was first suggested by Ekblom (1969), the best known proponent is Katch who suggested that there is a critical or trigger point during maturation prior to which minimal training-related adaptations will occur (Katch, 1983). Many subsequent studies purported to support the presence of a maturational threshold (Kobayashi *et al.*, 1978; Mirwald *et al.*, 1981; Rowland, 1985; Mercier *et al.*, 1987; Payne & Morrow, 1993; Rowland, 1997) but equal numbers refuted the concept (Daniels & Oldridge, 1971; Eisenmann & Golding, 1975; Weber *et al.*, 1976; Daniels *et al.*, 1978; Savage *et al.*, 1986; Danis *et al.*, 2003), resulting in little consensus as to the interaction between training and maturity.

The results of studies 4 and 5 (chapters 7 and 8) challenge the conventional dogma as the influence of training status was equally potent at all stages of maturity with no interaction evident between the magnitude of training status differences and maturity stage for any measured parameter. In contrast, juxtaposition of studies 2 and 3 (chapters 5 and 6) reveals a significant interaction for the phase II $\dot{V}O_2$ $\tau$ during cycle exercise as no influence of training status was evident in pre-pubertal girls whilst the trained pubertal girls had a significantly faster $\tau$ than their untrained counterparts. The phase II $\tau$ during upper body exercise, which was faster in both pre-pubertal and pubertal trained girls, showed no interaction with maturity. Therefore, the interaction between the magnitude of training status differences and maturity appears to be dependent on exercise modality. It is difficult

to explain why the presence of a maturational threshold, which would presumably be related to favourable changes in the hormonal milieu, would be specific to the lower or upper body. We suggest the explanation may lie not with the presence of a maturational threshold but in the accumulative effect of training. The predominantly upper body nature of swimming (Ogita *et al.*, 1996) means the lower body receives a considerably lower training stimulus. When combined with a higher baseline fitness of the lower body due to habitual activity such as locomotion, a longer duration of training, as found in pubertal girls, would be required for significant influences to be evident.

Overall, the conclusion that must be drawn from the present studies is that there is no maturational threshold or trigger point in the influence of training status on girls' responses to exercise. This conclusion has implications within a training and performance environment, suggesting that significant influences of training status can be obtained even before puberty. It is important to highlight however, that further research is required to elucidate whether these conclusions are specific to girls and/or swimming, as it may be anticipated that the changes in the hormonal milieu associated with the onset of puberty would have a more significant impact in boys and/or in strength related sports. Furthermore, whilst physiological influences of training status may be evident in pre-pubertal girls, whether there is any additional benefit of gaining these influences when pre-pubertal over gaining them at a more mature stage is unknown. Moreover, any benefit will need to be balanced with the increased chance of burnout or injury typically associated with intensive training at a young age (Hemery, 1988; Hollander *et al.*, 1995; Baxter-Jones & Helms, 1996; Starosta, 1996; Salguero & Gonzalez-Boto, 2003).

## *9.3 Exercise modality and the influence of training status*

The majority of studies investigating the influence of training or training status in children have relied on cycle or treadmill erogmetry. A consequence of this narrow focus is that the importance of the specificity of the exercise modality to the training modality and the transferability of training status influences have not been extensively investigated. Furthermore, interpretation of the limited number of studies available is complicated either by the use of two modalities for training, neither of which was the test modality (Welsman

*et al.*, 1997), or by the use of two different modalities and two different types of training such that it is not possible to ascertain the influence of each factor (Williams *et al.*, 2000). Evidence from studies inadvertently involving disparate training and testing modalities is no more conclusive, with some reporting significant influences of training status (Mandigout *et al.*, 2001), some a significant but reduced influence (McManus *et al.*, 1997), and others no influence of training status (Obert *et al.*, 2000; Cleuziou *et al.*, 2002). A pivotal difference between these studies may be in the degree of disparity between the training and testing modalities: Mandigout et al. and McManus et al.'s studies involved lower body training and lower body testing whereas Obert et al. and Cleuziou et al. tested swimmers, a predominantly upper body training mode (Ogita *et al.*, 1996), using a lower body testing modality. Resolution of the importance of exercise modality in the determination of training status influences is important, as the use of inappropriate test modalities may lead to erroneous conclusions as to the trainability, or otherwise, of children.

Evidence from the present studies suggests that the interaction between exercise modality and the influence of training status may be more complicated that originally hypothesised, with additional influences from maturity and exercise protocol determining its importance. An influence of maturity is evident when studies 2 and 3 (chapters 5 and 6) are considered together: in the pre-pubertal girls in study 2, exercise modality was very important in demonstrating training status differences whereas the influence of training status was equally evident during both modalities in the pubertal girls. It is difficult to discern the basis for this disparity and, a physiological change associated with maturity seems an improbable explanation. We suggest this "maturity" influence may be a reflection of a lower level of fitness in the untrained pubertal girls relative to the untrained pre-pubertal girls (see Table 9.1), and/or the accumulation of a greater training volume in pubertal girls, which has been suggested to be an important factor (Conley *et al.*, 1984; Pate *et al.*, 1992; Jones, 1998). An influence of exercise modality is suggested when studies 2, 4 and 5 (chapters 4, 7 and 8) are considered together. In studies 2 and 5, responses to an instantaneous change in metabolic demand were studied using constant-work-rate and Wingate tests, respectively, with the influence of training status shown to be dependent on exercise modality. In study 4, responses to a gradual increase in metabolic demand were

studied using a ramp incremental test, with the influence of training status demonstrated to be independent of exercise modality. This discrepancy according to exercise protocol is most likely a reflection of the relevance of the protocols to the daily activities of the untrained children; copious metabolic transitions occur each day (Barstow & Scheuermann, 2005; Jones & Poole, 2005b) and children's play patterns are characterised by sporadic bursts of high intensity exercise (Bailey *et al.*, 1995; Berman *et al.*, 1998; Baquet *et al.*, 2007). In contrast, the exercise stresses associated with a ramp incremental test are rarely, if ever, experienced in everyday life (Armstrong *et al.*, 1990; Bailey *et al.*, 1995). Consequently, the difference between trained and untrained girls is maximised during the ramp incremental test protocol, reducing the importance of exercise modality in the detection of training status effects.

**Table 9.1** Synopsis of results from studies 2 and 3 (chapters 5 and 6) highlighting the lower "fitness" in the untrained pubertal than pre-pubertal girls.

| | Cycle | | Upper body | |
|---|---|---|---|---|
| | Pre-pubertal | Pubertal | Pre-pubertal | Pubertal |
| Peak $\dot{V}O_2$ (L·min$^{-1}$) | $1.60 \pm 0.30$ | $1.98 \pm 0.26$ [*] | $1.09 \pm 0.19$ | $1.36 \pm 0.21$ [*] |
| Peak $\dot{V}O_2$ (mL·kg$^{-1}$min$^{-1}$) | $37.8 \pm 7.7$ | $34.5 \pm 2.2$ | $25.4 \pm 2.9$ | $24.0 \pm 4.9$ |
| Peak $\dot{V}O_2$ (mL·kg$^{-b}$min$^{-1}$) | $284 \pm 47$ | $160 \pm 23$ [*] | $242 \pm 31$ | $204 \pm 32$ [*] |
| $\dot{V}O_2$ τ (s) | $25 \pm 7$ | $35 \pm 11$ [*] | $37 \pm 6$ | $44 \pm 8$ [*] |

*Mean ± S.D. $\dot{V}O_2$, oxygen uptake; τ, phase II time constant.*

*\* Significant difference between pre-pubertal and pubertal girls within exercise modality; P < 0.05*

### *9.4 Predictors of performance*

Whilst key parameters of aerobic fitness have been identified (Whipp *et al.*, 1981), the relevance of these and other parameters to performance remains unclear, especially within young populations. To the best of our knowledge, only two studies have reported the relationship between physiological parameters and swimming performance in young people. The earlier of these studies found the mean power adjusted for thoracic cross-sectional area to be a significant predictor of 50m front crawl performance, accounting for 46%, whilst the peak $\dot{V}O_2$ adjusted for thoracic cross-sectional area was a significant predictor of performances over 100, 200 and 400m, accounting for 77%, in pre-pubertal

boys (Duché *et al.*, 1993). A more recent study also reported peak $\dot{V}O_2$, estimated by backwards extrapolation from data obtained during the first 20 seconds of recovery, to be the major physiological determinant of 400m swimming performance in boys (Jurimae *et al.*, 2007). A caveat to the conclusions drawn from these studies is the limited number of physiological parameters assessed, for example, despite the importance of parameters such as $\dot{V}O_2$ kinetics and exercise economy to determining aerobic fitness, their relationship with swimming performance remains unknown. Further, both studies investigated the relationship between physiological variables and performance in boys, consequently the applicability of these predictors to girls is unknown. We endeavoured to address these issues by analysing the results from the present studies relative to performance using multiple regression analysis.

**Table 9.2** Pearson's product moment correlations describing the relationship between the parameters assessed during ramp incremental exercise (chapters 4 and 7) and performance according to FINA points. Only those parameters for which there were significant correlations are shown

| | FINA Points | |
|---|---|---|
| | Cycle ergometry | Upper body ergometry |
| Peak $\dot{V}O_2$ (L·min$^{-1}$) | 0.55 [**] | 0.69 [**] |
| Peak $\dot{V}O_2$ (L·kg$^{pfr}$·min$^{-1}$) | 0.57 [**] | 0.67 [**] |
| GET (L·min$^{-1}$) | 0.60 [**] | 0.59 [**] |
| GET (L·kg$^{pfr}$·min$^{-1}$) | 0.51 [**] | 0.62 [**] |
| Peak WR (W) | 0.71 [**] | 0.69 |
| $S_1$ Gain (mL·O$_2$·W$^{-1}$·min$^{-1}$) | -0.37 [*] | N/A |
| MRT (s) | -0.44 [*] | N/A |
| Peak $\dot{Q}$ (L·min$^{-1}$) | 0.54 [**] | 0.57 [**] |
| Peak $\dot{Q}$ (L·m$^{-2}$min$^{-1}$) | N/A | 0.58 [**] |
| Peak SV (mL) | 0.58 [**] | 0.58 [**] |
| Peak SV (mL·m$^{-2}$) | N/A | 0.54 [**] |

*$\dot{V}O_2$, oxygen uptake; GET, gas exchange threshold; pfr, power factor ratio; WR, work rate; S1 gain, gain below the GET; $\dot{Q}$, cardiac output; SV, stroke volume. * P < 0.05; ** P < 0.01*

Performance was defined according to the FINA points for each participant's best event. FINA points allow comparisons between events and individuals and are defined each year

based on the latest world record approved by FINA. The closer the participant's best time is to this world record, the higher their points score.

**Table 9.3** Pearson's product moment correlations describing the relationship between the parameters assessed during constant-work-rate exercise (chapters 5 and 6) and performance according to FINA points. Only those parameters for which there were significant correlations are shown

|  | FINA Points | |
| --- | --- | --- |
|  | Cycle ergometry | Upper body ergometry |
| $\dot{V}O_2$ phase II amplitude (L·min$^{-1}$) | 0.67 ** | 0.78 ** |
| $O_2$ deficit (L) | 0.58 * | 0.68 * |
| HR τ (s) | N/A | -0.77 ** |
| HR amplitude (b·min$^{-1}$) | N/A | 0.56 * |

$\dot{V}O_2$, oxygen uptake; $O_2$, oxygen; HR, heart rate; τ, time constant * P < 0.05; ** P < 0.01

Participants at all stages of maturity were assessed together. Initially, it was determined which variables assessed during the ramp, WAnT and constant-work-rate tests were significantly correlated with performance according to Pearson's product moment correlations (see Tables 9.2, 9.3, 9.4). Subsequently, for cycle and upper body exercise independently, all significant correlates were made available for stepwise multiple regression to determine the parameters which best predicted performance. Parameters influenced by body size were entered originally in absolute terms and subsequently in scaled terms. Finally, cycle and upper body ergometer responses were analysed together by stepwise multiple regression.

It is important to highlight a number of confounding factors with regard to this methodology and, consequently, the derived predictors of performance. These confounding factors include the compilation of performances from a range of strokes and distances and the reliance on overall performance measures which incorporate non-swimming elements (e.g. starts and turns) as well as swimming. Furthermore, the temporal separation of the swimming performance and the laboratory testing may be associated with physiological and/or technical changes which could influence the accuracy of the prediction, as would the relative novelty of the laboratory tests and limited habituation. An advantage of the current

methodology compared to the use of a time trial as part of the testing series, however, is that psychological and race preparatory factors can be assumed to be optimal for performance.

**Table 9.4** Pearson's product moment correlations describing the relationship between the parameters assessed during WAnT exercise (chapter 8) and performance according to FINA points. Only those parameters for which there were significant correlations are shown

| | FINA Points | |
| --- | --- | --- |
| | Cycle ergometry | Upper body ergometry |
| PP (W) | 0.49 * | 0.62 ** |
| PP (W·kg$^{-1}$) | N/A | 0.50 * |
| MP | 0.48 * | 0.54 ** |
| Work done (J) | 0.48 * | 0.55 ** |
| End Power (W) | 0.54 ** | N/A |
| Peak $\dot{V}O_2$ (L·min$^{-1}$) | 0.49 * | 0.49 * |
| Peak $\dot{V}O_2$ (L·kg$^{-1}$·min$^{-1}$) | 0.68 ** | N/A |
| Blood [lactate] (mMol) | 0.44 * | N/A |
| Aerobic work done (J) | 0.60 ** | N/A |
| Peak HR (b·min$^{-1}$) | N/A | -0.52 * |
| Rest HR (b·min$^{-1}$) | N/A | -0.45 * |
| HR Amplitude (b·min$^{-1}$) | -0.49 ** | N/A |
| $\dot{V}O_2 \tau$ (s) | -0.61 ** | N/A |

*PP, peak power; MP, mean power; $\dot{V}O2$, oxygen uptake; blood [lactate], blood lactate concentration; HR, heart rate; $\tau$, time constant.*
*\* P < 0.05; \*\* P < 0.01*

For cycle and upper body exercise independently, stepwise regression revealed the peak work-rate achieved during the ramp incremental tests, investigated in chapters 4 and 7, to be the major predictor of swimming performance, explaining 50% and 49% of the variance respectively. Additionally, when both cycle and upper body results were considered together, the peak work-rate during upper body exercise remained the major determining parameter (49%). However, when peak work rate was adjusted to account for body size using either body mass or fat free mass it was no longer a significant predictor of performance for either modality independently or combined. Instead, peak SV (40%) and peak $\dot{V}O_2$ (20%) were identified as significant predictors of performance during cycle

exercise, and peak $\dot{V}O_2$ was identified during upper body ergometry (47%). These findings therefore agree with the previous studies of Duché et al. (1993) and Jürimäe et al. (2007). This agreement suggests that there is no influence of gender on the predictors of performance determined from a ramp incremental test. It also indicates that the standard of swimmers is not an important factor in performance prediction as the previous studies involved swimmers of below county standard, as highlighted in Table 9.5, compared to the predominantly national level swimmers in the current studies. When both exercise modalities are considered together, the peak $\dot{V}O_2$ achieved during upper body (47%) and cycle ergometry (19%) are identified as the significant predictors of performance. The considerably greater contribution of upper body peak $\dot{V}O_2$ to predicting performance agrees with the predominantly upper body nature of swimming previously reported (Ogita *et al.*, 1996). The current findings extend those of the previous studies, not only by the consideration of upper body ergometry, but also by the demonstration that peak $\dot{V}O_2$ is the most important predictor of performance for both short (50m and 100m) and longer (200m +) distance events across the range of strokes.

**Table 9.5** Performance times in previous studies to investigate swimming performance predictors in children compared to 2010 Devon County Qualifying Times. Note the considerably slower times of the previous studies swimmers.

|  | Devon County 2010 | Jürimäe et al. (2007) | Duche et al. (1993) |
|---|---|---|---|
| 11 yr old male 200m front crawl (s) | 174 | N/A | 188 ± 31 |
| 11 yr old male 400m front crawl (s) | 360 | N/A | 399 ± 79 |
| 12 yr old male 400m front crawl (s) | 330 | 402 ± 54 | N/A |
| 14 yr old male 400m front crawl (s) | 294 | 354 ± 42 | N/A |

Regression analysis of the results of study 5 (chapter 8) showed that, in contrast to the findings of Duché et al.(1993), MP was not a significant predictor of performance during either exercise modality, irrespective of whether it was expressed in absolute or relative terms. This discrepancy may arise due to the different distance specialisations under consideration. In the study of Duché et al. MP was found to be a significant predictor for 50m front crawl performance, but not 100, 200 or 400m front crawl performance. In the present studies, performance was calculated according to each participant's best event, with only two girls reporting a 50m event as their best. Therefore, the lack of significance of MP

as a predictor in the present study actually agrees with Duché et al. when considered against the comparable distances. This finding that MP is only a predictor for sprint 50m events is not surprising given the similarity between the duration over which the MP is typically determined and the time taken to complete 50m, a similarity which indicates ATP resynthesis is likely to be supported using similar energetic pathways.

The best predictor of swimming performance identified from the parameters assessed during the cycle WAnT was peak $\dot{V}O_2$ adjusted for body size, which accounted for 45% of the performance variation. This finding agrees with those from the ramp incremental test and supports suggestions of a large aerobic contribution to the WAnT test (Chia *et al.*, 1997). Previous studies investigating performance predictors in children assessed only the mechanical indices of WAnT performance (Duché *et al.*, 1993; Jurimae *et al.*, 2007), therefore no studies are available for comparison. The importance of peak $\dot{V}O_2$ as a performance predictor may have been accentuated in the present studies due to the predominantly long distance specialisations of the participants, but further work is required to determine whether there is an interaction between swimming event distances and performance predictors in young people.

Regression analysis of the upper body WAnT results revealed peak power and resting heart rate as performance predictors. This role of PP agrees with previous studies which found a significant relationship between PP and 50m swimming performance (Sharp *et al.*, 1982; Hawley & Williams, 1991), but extends those findings to suggest the importance of PP is not specific to sprint events but swimming performance in general. Resting bradycardia in trained children has previously been reported and associated with a higher aerobic fitness (e.g. Rowland *et al.*, 1987; Obert *et al.*, 1998; Obert *et al.*, 2003; Nottin *et al.*, 2004).

When the cycle and upper body Wingate responses are considered together, upper body mean power (accounting for 19%), along with the cycle blood lactate and upper body resting HR previously mentioned, are identified as significant predictors. Previous studies have similarly shown a strong correlation between upper body mean power and performance (Inbar & Bar-Or, 1977; Williams & Hawley, 1989; Hawley & Williams, 1991; Hawley *et al.*, 1992), but this is the first study to identify upper body mean power as a

significant predictor of performance as previous studies have found lower body mean power to be more important (Hawley *et al.*, 1992). Once again, this may be attributable to the longer distance of the majority of the events investigated here compared to the 50m sprints investigated previously and supports suggestions of a predominantly upper body nature of swimming (Holander *et al.*, 1988; Ogita *et al.*, 1996).

The final set of parameters analysed in relation to performance were those determined during the constant-work-rate tests. Whilst training is accepted to be a potent stimulus to $\dot{V}O_2$ kinetics in adults (Powers *et al.*, 1985; Phillips *et al.*, 1995; Carter *et al.*, 2000; Krustrup *et al.*, 2004), and a similar phenomenon is suggested here and elsewhere in young people (Marwood *et al.*, 2010), the relevance of these training status related adaptations to performance is less clear. Studies in adults indicate that performance improvements are not always related to faster $\dot{V}O_2$ kinetics or a reduced slow component amplitude (Billat *et al.*, 1998; Norris & Petersen, 1998; Demarle *et al.*, 2001; Billat *et al.*, 2002). Faster $\dot{V}O_2$ kinetics may nonetheless be important, however, due to their role in reducing the oxygen deficit, which has been correlated with increased time to exhaustion in some studies (Demarle *et al.*, 2001), but not others (Billat *et al.*, 2002). There are currently no studies available that have investigated the relationship between $\dot{V}O_2$ kinetic parameters and performance in children. Stepwise regression analysis revealed that neither the phase II $\tau$ nor the slow component amplitude were significant predictors of performance in young girls. The phase II amplitude was the only significant predictor during both exercise modalities, a finding most likely to be a reflection of the higher work rate associated with the trained girls.

Unfortunately, the arrangement of the present studies is unsuitable for the analysis of which parameters are the best predictors of performance overall. This therefore represents an issue for future research in young populations. Nonetheless, a number of implications for the application of the present results to the training environment are demonstrated by this and previous chapters. One such implication is the importance of training programmes being individualised to each swimmer, an implication highlighted by the variety of performance predictors reported above. If training had a universal, replicable effect it would be anticipated that a small number of performance predictors would have been strongly

indicated in the above analysis, however, this was certainly not the case. To aid in the individualisation of training programmes many questions need to resolved, such as the key factor(s) around which programmes should be orientated; whilst the current investigations suggest this factor is not maturity per se it needs to be determined if stroke, distance, upper/lower body contribution or some other as yet unrealised factor such as the duration of training history represents the cornerstone. A common misconception of coaches is that training falls into either an aerobic or anaerobic zone and that these zones are separate and can be trained independently. The present findings demonstrate that in reality exercise is rarely so convieniently segregated, with the influence of a predominantly aerobic training programme evident across the "zones". This demonstration could have significant implications for the type of training coaches ask their swimmers to undertake. The greatest implication of the current findings however is that training is equally effective at all stages of maturity and thus, physiologically, training programmes do not need to be stratified on the basis of maturity. However, it is crucial that this finding is balanced with the psychological and social implications of a similar training intensity and volume in mentally immature and mature swimmers. Furthermore, it is important to maintain a perspective of the importance of childhood successes in the overall sporting career of a performer as few who are successful at a young age tend to persevere to the age and maturity at which the major competitions and successes can be obtained.

### 9.5 Study Limitations

There are a number of limitations to the present studies; some which are inherent to the study design and others which have arisen during analysis or interpretation. The most severe limitation was the cross-sectional design of the studies. This precludes attributing the training status differences to training *per se*, as they may be a reflection of participant's genetic phenotypes. The dependence of the influence of training status on genetics and heredity is suggested to be substantial but the exact percentage remains unclear with estimations for relative peak $\dot{V}O_2$ ranging from 7% (Weber *et al.*, 1976) to 50% (Bouchard, 1986) and 70% (Danis *et al.*, 2003). However, whilst this is a significant disadvantage, cross-sectional studies do also present numerous advantages, the foremost of which is that they allow comparisons between extreme groups. This advantage is especially pertinent to

studies of training effects as a limitation of many training intervention studies is the volume and duration of training implemented. Although the majority of studies implement training programmes which exceed the minimum criteria suggested by the ACSM (Haskell *et al.*, 2007) and/or by Baquet et al. (2003), replication of the training volumes undertaken by real athletes, such as the average 22 hours/week training completed by the post-pubertal girls in studies 4 and 5 (chapters 7 and 8), is unfeasible. This advantage is especially applicable to young populations where the magnitude of training status related differences is reduced (Pate & Ward, 1996) and ensuring participant adherence to a training programme is challenging at best.

A further inherent limitation of the present studies is the non-invasive and, consequently, indirect methodologies that must be employed with young people (National Institutes of Health, 1993). These constraints are largely responsible for the paucity of data available on children and adolescents and it is only with the relatively recent technological advances enabling indirect methodologies that significant advances in our understanding of paediatric physiology have been made. Unfortunately, even with these technological advances many questions are currently unanswerable, such as the influence of training status on muscle mitochondrial density and enzymatic profiles, leaving us to rely on a limited number of early studies.

Finally, further insights into the influence of training status may have been afforded if an additional lower body trained group and/or a whole body swimming ergometer had been investigated. Although the inclusion of a lower body trained group was considered, the decision was to focus on the swim trained group due to the pressures of time constraints and child recruitment. Whilst an upper body ergometer is more relevant to swimming than a cycle ergometer, it is, nonetheless, not ideal. The most specific ergometer currently available for testing swimmers is the swim bench, however, there are numerous disadvantages to this including the imprecision of applied workloads, which would have been unacceptable for studies 2 and 3 (chapters 5 and 6). Additionally, the swim bench ergometer does not address the issues surrounding the isolation of the upper or lower body for testing and neither has it been validated as a reliable and accurate ergometer.

## *9.6 Future Directions*

During the process of compiling and writing this thesis, many directions for future research have become evident. The number and diversity of issues and questions that remain to be elucidated reflect the ethical constraints which have severely restricted the investigation of paediatric physiology for many years, the recent alleviation of many of which heralds a promising era in which considerable advances in our knowledge and understanding will hopefully occur.

A prime example of these technological advances is the widening availability of magnetic resonance spectroscopy and imaging devices and the increasing number of exercise protocols being developed for use in conjunction with them. $^{31}$P-magnetic resonance spectroscopy (MRS) allows the continuous in-vivo interrogation of muscle phosphates during exercise, thereby allowing an unparalleled insight into muscle metabolism in children (Barker *et al.*, 2006). Despite this considerable potential, only one study has investigated the influence of training status using MRS  (Kuno *et al.*, 1995). In this study, the intracellular pH and the ratio of (PCr):(PCr + Pi) were not influenced by training status, although the small sample sizes, with only 3 trained boys in some age-groups, constrain the conclusions that can be drawn. A rapidly increasing number of studies in adults have shown significant influences of training status (e.g. Walker *et al.*, 1998; Forbes *et al.*, 2008; Norrbrand *et al.*, 2008; Larsen *et al.*, 2009), further demonstrating the sensitivity and application of MRS. Issues to which MRS studies may provide clarification include whether training influences the utilisation of muscle substrates during exercise and/or recovery. Is this influence on the magnitude or time-course of the response? With the increasing diversity of protocols that can be implemented within the bore of the magnet, concerns as to the relevance of these testing modalities to training will need to be addressed. MRI may also reveal the influence of training status on the fibre architecture, the perfusion, mechanical properties and the contractile activity of muscles. Such investigations may provide important insights as to the progression of training status influences throughout growth, maturation and senescence in those muscles both voluntarily (e.g. m. *Vastus lateralis*) and involuntarily (e.g. myocardium) involved in exercise. For example, the occurrence of morphological and functional adaptations of myocardium can be

investigated by MRI, contributing evidence towards the debate as to the functional or morphological basis of the enhanced stroke volume in trained participants.

The current results indicate that exercise modality is an important consideration when investigating training responses. Indeed, it is not implausible that the prevailing concept of a reduced trainability in children may be related to the use of inappropriate testing modalities for revealing training status or intervention effects. Further work is warranted to determine if the choice of exercise modality is equally important for all sports or whether it is accentuated in swimmers due to the prone position or the predominantly upper body nature of the exercise. It seems probable that a swim bench related ergometer is the most appropriate test apparatus possible in the laboratory setting, but the reliability and replicability of results from this system have not been established and it is questionable how much the action actually replicates that of swimming. There are also concerns about the unfamiliarity of the action involved for non-swimmers and the impact this may have on the control group.

One of the important outcomes of the present studies was the suggestion that no maturational threshold is present in girls' physiological responses to exercise. This finding may suggest that girls should be encouraged to train even harder before puberty. However, it remains to be established whether the gains made at this early age yield significant advantages in adulthood. The answer to this question is undoubtedly multifaceted, involving consideration of both the physiological and also the psychological impact of substantial training volumes at young ages; "burnout" and over-training must be avoided if physiological advantages are to be manifest in adulthood. Additionally, whether the absence of a maturational threshold is specific to girls and/or aerobically trained participants needs to be determined, as does the accuracy of this statement to a wider selection of physiological parameters, such as PCr kinetics, muscle fibre type distribution and enzymatic profiles and substrates.

Whilst theoretically interesting, it is also important to consider the practical implications and relevance of performance predictors. The central question is whether research can identify specific performance predictors to enable the specialisation and tailoring of

training programmes to optimise these parameters and, consequently, performance. A multitude of questions remain, however, which are likely to be sport-specific. Within a swimming context, these questions include whether the performance predictors are dependent on performance level, stroke specialisation, training cycle phase and even stroke technique. It may be hypothesised that performance predictors will be related to the event distance but this remains to be confirmed. In a more general context, interesting questions to address would be the similarity in performance predictors across sports; do weight bearing sports share a greater commonality in performance predictors than weight bearing and non-weight bearing sports? Is there a commonality in performance predictors for all sprint or endurance events? Do performance predictors vary depending on age or gender or anthropometrical characteristics?

If the current trends for decreasing physical activity and increasing obesity are to be prevented from escalating, it is crucial to develop our understanding of the dose-response relationship between training and physiological adaptations across the age range. The present studies indicate that in excess of 8 hours/week swimming training is required before significant influences on peak $\dot{V}O_2$ are observed. However, it remains to be determined if this is a representative finding and whether the training volume required is mediated by age, baseline fitness, genetics, training activity and/or gender. In addition, it is necessary to establish which are the most important parameters to improve by training; peak $\dot{V}O_2$ may be the most studied but that does not imply it is the most important. Identification of the most relevant parameters would allow investigation of the exercise modalities and regimes most effective at eliciting significant improvements. Whilst these questions are receiving an increasing degree of attention, the emphasis remains on adult populations.

Finally, research is required to determine the influence of training status on the recovery from exercise in children. The recovery from exercise of pulmonary and cardiovascular parameters is highly sensitive to adaptation due to the considerable hemodynamic and cardiopulmonary changes that occur during the recovery phase (Bar *et al.*, 2007). Despite this, these responses remain severely under-investigated. Although heart rate recovery following exercise has been used for decades as an indicator of physical fitness, it is only subsequent to an improved understanding of exercise physiology that the importance of the

recovery of other parameters, such as oxygen uptake, has been recognised (Bar *et al.*, 2007). Many areas remain uninvestigated in children, such as whether $\dot{V}O_2$ or HR kinetics recover from exercise faster in trained children and whether training influences the symmetry of the on and off $\dot{V}O_2$ kinetic responses.

## *9.7 Final Conclusions*

In summary, the studies in this thesis have demonstrated that whilst children are not mini-adults, the child-adult differences are not related to a lack of trainability or the presence of a maturational threshold, as proposed by conventional dogma.

The present studies have demonstrated significant influences of training status on an array of parameters, from those that effect exercise responses centrally, such as stroke volume and cardiac output, to those that exert their effect peripherally, such as oxygen extraction. The effects of these central and peripheral training status-related enhancements have been observed in the magnitude and speed of the overall responses, as indicated by peak $\dot{V}O_2$ and $\dot{V}O_2$ kinetics. In contrast to the independence of training status effects from maturity, exercise modality and training volume have been highlighted as important factors in the demonstration of training status effects.

This thesis has investigated only a tiny area of the field and, whilst it has furthered our understanding in a number of respects, it has also led to the identification of many more questions. Given the increasing participation in sport and awareness of the role of exercise training in combating the impending obesity epidemic, furthering our understanding of the underlying physiology, and the influence of training on it, is vital. This understanding may be most imperative in paediatric and adolescent populations as children represent the future and ensuring, and optimising, their healthy growth and development ought to be a prime concern of all societies (WHO, 2010).

# Chapter 10

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**Chapter 11**

**Appendices**

UNIVERSITY OF
EXETER

School Of Sport And Health Sciences
St Luke's Campus
Heavitree Road
EXETER
EX1 2LU

Telephone - 01392 264812
Fax - 01392 264706
Email -
K.A.Stoedefalke@ex.ac.uk

**Heart size, fitness and training in children**

**Information Sheet**

**What is the project about?**
We are interested in finding out how big you heart is and whether or not this is related to how fit you are and how this compares with children who aren't involved in swim training. We plan to measure your fitness and heart size so we can see how your age and swim training affect your heart size and function, fitness levels and swim performance.

**Who is taking part in the study?**
We need boys and girls aged 9 to 12 years from the Exeter City Swim Club or local schools to take part in the project.

**What will I be asked to do?**
If you volunteer for this study we will ask you to visit the University for once session.

Firstly, we will ask you to do a test on a stationary exercise bike. The test will start off easy and get harder and harder. You will need to breathe through a mouthpiece to allow us to measure all the air that you breathe in and out. We will ask you to exercise as long as you can. The exercise tests usually last 10-15 minutes. At the end of the test we will take a fingertip blood sample to measure lactate levels. Lactate levels give us an indication of how hard you were working during the exercise test. During the exercise test we will use a technique called thoracic bioelectrical impedance to allow us to see what your heart is doing and how it is working when you do some exercise. It works by electrodes stuck on your skin picking up signals in your body as your heart beats. Again this method is not dangerous and you cannot feel it.

We would also like to assess how mature you are. To do this we will ask you to look at some line drawings of children at different stages of growth and get you to say which one is most like you. Specifically, the line drawings will ask you to identify whether or not you have pubic hair and if you do, which drawing is most representative of your development. In addition, if you are a girl, we will want to know whether or not you have started your periods. The reason for this assessment is that children of the same age can be at different

stages of maturity. You will do the assessment in an enclosed room, on your own. When you are done we ask that you please place the completed forms into and envelope, seal the envelope and give it to the researcher. If you do not feel comfortable with doing this, you do not have to, but you can still take part in the study.

**What do I need to wear?**

For all of the exercise tests and scans you should wear your PE kit (shorts and t-shirt).

**When will I do it?**

You will complete the tests at a time suitable for you and your parents – either on your own or with other friends who are involved if this is possible. The tests will take place at the Children's Health & Exercise Research Centre at the University of Exeter (St Luke's Campus in Heavitree Road). If it is difficult for you to get to us we can arrange transport to and from the Centre with two members of the research team.

**What if I want to drop out of the study?**

Whether or not you take part in the study is entirely up to you. You can drop out of the study at any time and 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 computer. We are happy to tell you about your results but no one else will be told. We will write the study up as a paper and present the results to other researchers but your personal information will remain confidential.

**Do you have any further questions?**

If you have any questions regarding the scan or the study generally please contact Dr. Jo Welsman on 01392 264903 or Dr Kerstin Stoedefalke on 01392 262812 and we will be happy to talk to you. If you would like to participate please complete the consent form attached and return it to us via the swimming club/school. We will contact you via the club/school when we are ready to start testing.

**With best wishes,**

Dr. Kerstin Stoedefalke                                     Dr. Joanne Welsman
+44 (0) 1392 262812                                         +44 (0) 1392 264812
K.A.Stoedefalke@ex.ac.uk                                   J.R.Welsman@ex.ac.uk

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

*Appendix 1B*

UNIVERSITY OF
EXETER

School Of Sport And
Health Sciences
St Luke's Campus
Heavitree Road
Exeter, EX1 2LU

**Influence of training and exercise modality on the oxygen uptake kinetics of girls**

Telephone:1392 264803
Email: sshs-school-office
@exeter.ac.uk
www.exeter.ac.uk/sshs

**Information sheet**

Dear Participant and Parent/Guardian,

May I begin by thanking you for taking the time to read this information sheet, it will provide you with the details of our forthcoming study and hopefully provide you with the information you require to help you decide if you want to allow your daughter/s to participate in our study. It is important to say at this point that the decision about whether to take part is entirely up to you and your daughter(s) and that your daughter(s) will not be disadvantaged in the future with regard to other studies should you decide not to participate.

**What is the study about?**
The aim of the current study is to investigate the differences in the fitness of young girls. We want to carry out a comparison between girls of a normal activity level (i.e. school P.E. lessons) with those involved in intense training. The girls in our training group will be swimmers from a local swimming club and the girls in our non-trained group will be children from the local schools. We would also like to look into the effect that two different modes of exercise (a stationary bike and a stationary arm crank) have on the results of these tests. This will be the first study to look at the effect of different exercise machines on trained and untrained girls. We hope this research will improve our understanding of children's fitness and help us to improve the physical activities that occur in schools.

**What types of participants are needed?**
We are looking for two groups of girls, one group of around 10 girls of 10-12 years of age and another group of around 10 girls of 13-15 years of age, who are not involved in any form of organised sport or physical activity outside of that provided as part of the National Curriculum. The children must have no pre-existing injuries or illnesses.

**What will the participants be asked to do?**
The participants will be asked to visit School of Sport and Health Sciences on St. Luke's campus on several occasions (roughly 7-9 times). The visits will involve:
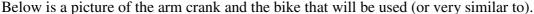
First two visits:
We will measure how tall and heavy the participant is and we will also measure the thickness of the skin and fat on their arm, back and stomach. We also need to measure how

physically mature they are. This will be done by showing the participant some drawings of children at different stages of growth and asking them to record which is most like them. This will be done on their own, in a private room and their scores will put in a sealed envelope which only the researcher will open. Specifically the drawings will ask them to identify whether they have pubic hair or not and if they do, which drawing is most like them. We will also ask them to record whether or not they have started their periods. The reason for this assessment is that children of the same age can be at very different levels of maturity, there is no right or wrong level but the results can help us understand the results of the exercise tests. If you or your daughter does not feel comfortable with this they do not have to do it but will still be able to take part in our study.

During these visits the participant will also be asked to complete two incremental exercise tests. One of these tests will be done on the stationary bike and one on the arm crank. These tests will start off easy and get harder and harder and will be stopped when the participant says they can't keep going. During these tests the participant will be breathing into a mask to allow us to measure the air that they breathe in and out. This mask does not make breathing any harder and the participant can talk through it and remove it at any time they feel uncomfortable about wearing it. The participant will also have a heart rate monitor strap on to allow us to record the rate at which their heart beats throughout the test. The tests will last approximately 10 minutes.

Following Visits:
These visits will all involve completing the same type of test multiple times on each exercise machine. The test is called a constant work rate test and during this test the participant starts pedalling with no resistance and then 4 minutes after they started pedalling the resistance is increased to a participant-specific level. This resistance will be hard work but will not be the highest resistance against which they are capable of exercising. They will do 8 minutes at this resistance and will then go back to 6 minutes of unloaded pedalling for recovery. During these tests the participant will again be asked to breathe into the mouth mask and will have the heart rate monitor strap on. We also want to measure what happens to the oxygen in the participants muscle which we can do using a small electrode. This is like a plaster which is stuck to the skin on the leg or arm. This method is completely safe and they will not feel it. At the end of the test we will take a fingertip blood sample to measure the amount of lactate in their muscles. This gives us an idea of how hard they were working because it is only produced during hard work. If the participant is not happy to have a fingertip blood sample they will not be forced to have one and not having one does not mean they can't participate in the rest of the tests.

Below is a picture of the arm crank and the bike that will be used (or very similar to).

**Why do I have to come in so many times?**
The reason so many visits are required is because we are looking at the way in which you breathe and that varies a lot between tests. We therefore have to do the test a lot of times so we can tell where the real breathing pattern is and what was a cough or a sigh etc.

**Will I need to do anything special for the study?**
The only things we ask our participants to do is to not drink alcohol (if over 18) or undertake strenuous activity in the 12 hours before the tests. We also ask that you do not eat or drink caffeinated drinks in the three hours before your test. These requests are simply to make sure we get the most accurate response to exercise rather than, for example, the response to digestion or being tired from previous activity.

**When will the study take place?**
The study will take place during school holiday time to ensure the minimum disruption to the participant's learning or other commitments. The girls will be asked to come to participate in the study in groups with their friends. Each exercise test will last approximately 30 minutes in total, including the warm up and warm down periods. Visits to the laboratory will last no longer than three hours.

**What happens if they want to drop out of the study?**
If at any point during any of these tests the participant decides they no longer wish to partake the test will be safely stopped and the participant given time to decide if they wish to stop participating in the entire study or to try again. The participant will not be disadvantaged in any way by choosing to stop participating and will not be forced to continue if they do not want to.

**What will the information be used for?**
The results collected will be written up for other scientists, both nationally and internationally, who are interested in children's fitness. Any results used will always be anonymous and in no way will the individual participant be identifiable. You are more than welcome to a copy of the results and the resulting papers should you wish. On collection the data will be assigned a numeric code to ensure anonymity and will be securely stored within the University of Exeter so only those mentioned below will be able to gain access to it.

**What if participants or parents/guardians have any questions?**
If you have any questions about our study, either now or in the future, please do not hesitate to contact us:

Dr. Joanne Welsman                                          Melitta Winlove
Telephone: 01392 262887                                  07739351081
Email: J.R.Welsman@exeter.ac.uk                    maw203@ex.ac.uk

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

*__Appendix 1C__*

UNIVERSITY OF
EXETER

School Of Sport And Health Sciences
St Luke's Campus
Heavitree Road
Exeter, EX1 2LU

## Influence of training and exercise modality on the oxygen uptake kinetics of girls

Telephone:1392 264803
Email: sshs-school-office@exeter.ac.uk
www.exeter.ac.uk/sshs

## Information sheet

Dear Participants and Parent/Guardian,

May I begin by thanking you for taking the time to read this information sheet, it will provide you with the details of our forthcoming study and hopefully provide you with the information you require to help you decide if you want to allow your daughter/s to participate in our study. It is important to say at this point that the decision about whether to take part is entirely up to you and your daughter(s) and that your daughter will not be disadvantaged in the future with regard to other studies should you decide not to participate.

**What is the study about?**
The aim of the current study is to investigate the differences in the fitness of young girls. We want to carry out a comparison between girls of a normal activity level (i.e. school P.E. lessons) with those involved in intense training. We would also like to look into the effect that two different modes of exercise (a stationary bike and a stationary arm crank) have on the results of these tests. This research will improve our understanding of children's fitness and help us to improve the physical activities that occur in schools.

**What types of participants are needed?**
We are looking for around 10 girls of 13-15 or 18+ years of age who are involved in swimming training of at least 6+ hours a week. They must have been undergoing such training for more than a year. The children must have no pre-existing injuries or illness.

**What will the participants be asked to do?**
The participants will be asked to visit the School of Sport and Health Sciences on St. Luke's campus on several occasions (roughly 7 times). The visits will involve:
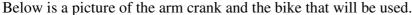
First Two Visits:
We will measure how tall and heavy the participant is and we will also measure the thickness of the skin and fat on their arm, back, stomach and thigh. We also need to measure how physically mature they are. This will be done by showing the participant some drawings of children at different stages of growth and asking them to record which is most like them. This will be done on their own, in a private room and their scores will put in a sealed envelope which only the researcher will open. The reason for this assessment is that children of the same age can be at very different levels of maturity, there is no right or

wrong level but the results can help us understand the results of the exercise tests. If you or your daughter does not feel comfortable with this they do not have to do it but will still be able to take part in our study.

During these visits the participant will be asked to complete two incremental exercise tests. One of these tests will be done on the stationary bike and one on the arm crank. These tests will start off easy and get harder and harder and will be stopped when the participant says they can't keep going. During these tests the participant will be breathing through a mouthpiece (like a snorkel) to allow us to measure the air that they breathe in and out. The participant will also have a heart rate monitor strap on to allow us to record the rate at which their heart beats throughout the test. The test will last approximately 10 minutes.

Following Visits:
These visits will all involve completing the same type of test. The test involves the participant initially pedalling against no resistance and then after 4 minutes the resistance increases to a participant-specific level. This resistance will be hard work but will not be the highest resistance against which they are capable of exercising. They will do 8 minutes at this resistance and will then go back to 6 minutes of unloaded pedalling for recovery. During these tests the participant will again be asked to breathe through the snorkel mouthpiece and will have the heart rate monitor strap on. We also want to measure what happens to the oxygen in the participants muscle which we can do using a small electrode. This is like a plaster which is stuck to the skin on the leg or arm. This method is completely safe and they will not feel it. At the end of the test we will take a fingertip blood sample to measure the amount of lactate in their muscles.

Below is a picture of the arm crank and the bike that will be used.



**Will I need to do anything special for the study?**
The only things we ask our participants to do is to not drink alcohol (if over 18) or undertake strenuous activity in the 12 hours before the tests. We also ask that you do not eat or drink caffeinated drinks in the three hours before your test. These requests are simply to make sure we get the most accurate response to exercise rather than, for example, the response to exercise and digestion.

**When will the study take place?**
The study will take place at a mutually agreed convenient time. The study will not interfere with training times or with upcoming competitions. The girls will be asked to come to the School of Sports and Health Sciences in groups with their friends. Each exercise test will

last approximately 30 minutes in total, including the warm up and warm down periods. Visits to the laboratory will last no longer than three hours.

**What happens if they want to drop out of the study?**
If at any point during any of these tests the participant decides they no longer wish to partake the test will be safely stopped and the participant given time to decide if they wish to stop participating in the entire study or to try again. The participant will not be disadvantaged in any way by choosing to stop participating and will not be forced to continue if they do not want to.

**What will the information be used for?**
The results collected will be written up for other scientists, both nationally and internationally, who are interested in children's fitness. Any results used will always be anonymous and in no way will the individual participant be identifiable. You are more than welcome to a copy of the results and the resulting papers should you wish. On collection the data will be assigned a numeric code to ensure anonymity and will be securely stored within the University of Exeter so only those mentioned below will be able to gain access to it.

**Will the study disrupt their training?**
The study will be conducted at times to ensure that no disruption to training or competition is encountered. It is hoped that the results of this study will benefit the participants in providing information they would not otherwise have access to.

**What if participants or parents/guardians have any questions?**
If you have any questions about our study, either now or in the future, please do not hesitate to contact us:

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

Dr. Joanne Welsman                                    Melitta Winlove
Telephone: 01392 262887                               07739351081
Email: J.R.Welsman@exeter.ac.uk                       maw203@ex.ac.uk

Once again we thank you for your time and look forward to your response.
We hope you will want to participate!

UNIVERSITY OF
EXETER

School Of Sport And Health Sciences
St Luke's Campus
Heavitree Road
Exeter, EX1 2LU

Telephone:1392 264803
Email: sshs-school-office@exeter.ac.uk
www.exeter.ac.uk/sshs

### Influence of training and exercise mode on cardiovascular and short term power responses

### Information sheet for Parents/Guardians of Participants

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate.

## What is the Aim of the Project?

This project forms part of a research orientated post graduate degree which is intended to provide meaningful and relevant information about children's physiology.

The aim of this project is to investigate how the heart responds to exercise and whether this response differs between intensively trained children and children of a more common activity level. We also want to see how the body provides the energy it needs for a short, sharp burst of exercise and, again, the effect that training has on this. We will use two modes of exercise, the arm crank and the stationary cycle, to look at this so we can assess differences in these responses by the upper and lower body. Information from this study will enhance our understanding of the effects of training on young people's fitness and the way in which age influences the effects of training.

## Who can take part in this study?

We are looking for two groups of volunteers aged 10-18 years of age, both male and female. The first group will be individuals who are **not** involved in any form of organized, regular training outside of normal PE and games lessons at school. The second group will be swimmers aged 10-18 years of age who are training at least 8 hours a week and who have been undergoing such training for at least one year. Participants can be male or female. Participants in either group must be free from current injuries or illness.

## What will Participants be Asked to Do?

Participants will be asked to make 4 visits to our temporary laboratory set up in the school.

On the first visit, we will measure height, sitting height and weight. We will also measure the thickness of the skin and fat on the arm, back, stomach and leg using calipers. As young people grow and mature at very different rates we also need to estimate maturity so we can fairly compare the results of the tests. This is be done by giving each individual 5 standard drawings of children at different stages of maturity for pubic hair development and asking them to record which is most like them. This is done individually, in private and the scores are put in a sealed envelope which only the researcher will open. This form of assessment is

routine for studies where understanding levels of maturity is critical to understanding the results of the exercise tests.

On the first and second visit (24+ hours apart) participants will be asked to complete the same exercise test on two modes of exercise - the stationary cycle (visit 1) and the arm crank (visit 2). The arm crank is a cycle that uses your arms rather than your legs to turn the "pedals".

Stationary cycle                                        Arm crank



Visits 1 and 2

The first type of test is a progressive exercise test. This starts off very easy and gets harder and harder, like pedalling up a hill. The test is stopped when the participant cannot keep going. The test lasts approximately 10 minutes. Whilst the final stages of this test are uncomfortable the discomfort is very short and participants recover within minutes of completing the test.

During these tests the participant will be asked to:

- Wear a face mask so we can measure the air that they breathe in and out. This mask does not make breathing any harder and they can talk through it and remove it at any time they feel uncomfortable about wearing it.
- Wear a heart rate monitor strap around the chest to allow us to record how fast the heart is beating throughout the test.
- Have 6 small electrodes placed around the upper body (e.g. back and side) so we can see how the heart works in more detail during exercise. These electrodes are just like sticky plasters.
- Have one more small electrode stuck on their arm (arm exercise) or leg (cycle exercise) so we can measure how oxygen is used in the muscles.
- Allow a fingertip blood sample (only a few drops of blood) to be taken after the test is finished so we can measure how hard they were working using the amount of lactate produced by the body.

All of these methods are used routinely in research with young people. These visits will take approximately 1 hour in total.

Visit 3 and 4

The participant's will complete a 30 seconds "all-out" exercise test, one mode on each visit. This test allows us to see how much power an individual can produce and how quickly they get tired. During this test the participants will be asked to:

- Wear a facemask like they did during the incremental tests

- Allow a fingertip blood sample after the exercise..

These visits will last approximately 1 hour.

Trained participants will also be asked to keep a training log for 1 month and provide a list of personal best times. This information gives us a way to describe the quantity of training done to other scientists.

**Can Participants Change their Mind and Withdraw from the Project?**

Participants may withdraw from any part of or the entire project at any time and without any disadvantage to them of any kind.

**What Information will be collected and what use will be made of it?**

Results will <u>always be anonymous</u> and no individual participant will be identifiable. This is ensured by the assignment of a numeric code. All data collected will be securely stored within the University of Exeter so only the researchers mentioned below will be able to gain access to them. Data will remain securely stored after dissemination of the results to inform future research. The results collected will be written up for other scientists, both nationally and internationally, who are interested in young people's fitness. A general summary of the results will be available as will copies of any resulting papers.

**Will participants need to do anything special for the study?**

We also ask that they do not eat or drink caffeinated drinks (e.g. coca-cola, tea coffee) in the three hours before your test. We also ask that they do not participate in unaccustomed exercise in the 24 hours prior to their sessions with the University.

**What if Participants have any Questions?**

If you have any questions about our project, either now or in the future, please feel free to contact either:-

Melitta Winlove                          or                          Dr Jo Welsman
07739 3351081                                                      01392 262887
maw203@ex.ac.uk                                                 J.R.Welsman@exeter.ac.uk

**What to do next….**

If all your questions have been answered to your satisfaction, please complete the attached consent forms and health questionnaire and return to the teacher/coach as indicated. Participants will be contacted either directly or via the teacher/coach to arrange the testing sessions.

Once again we thank you for your time and look forward to your response.

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

*Appendix 1E*

**UNIVERSITY OF EXETER**

School Of Sport And
Health Sciences
St Luke's Campus
Heavitree Road
Exeter, EX1 2LU

Telephone:1392 264803
Email: sshs-school-
office@exeter.ac.uk
www.exeter.ac.uk/sshs

## Influence of training and exercise mode on cardiovascular and short term power responses

## Information sheet for Participants

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether to participate. If you want to participate, we thank you. If you decide not to take part that is fine and won't mean you can't take part in any other future studies.

### What is the Aim of the Project?
We are interested in the whether training affects the way in which the body responds to exercise. We are especially interested in how the heart responds to exercise and how your muscles fuel different exercise challenges. We want to look at these things using two different types of exercise, the bike and the arm crank (bike you "pedal" with your arms) because swimming training uses both the arms and the legs.

### Who can take part in this study?
We are looking for males and females aged 9-18 years of age. We want two groups of people: a group who are not involved in any regular sports training, i.e. most of the activity they do is in P.E. lessons at school and a group who are involved in swimming training. The swimmers need to have been training for at least 8 hours a week for more that 1 year. All participants must not have any current illness or injury.

### What will I be asked to do?
In total, we will ask you to come to in four times; each visit will last approximately 1 hour.

### Visit 1 (bike)
In the laboratory set up at your school, we will measure how tall and heavy you are and measure how much fat there is on your arms, back, tummy and leg using a fold of skin and fat. We will also ask you to look at 5 standard drawings (showing how pubic hair develops) and mark which is most similar to you. This is so we know how mature you are. You will do this on our own and only the researchers named below will see the answer.

You will then do a test on the bike that is like pedaling up hill. It will start off very easy and get harder. The test will last around 10 minutes and will be stopped when you say you can't keep going. The end of this test feels like the end of a cross country run but this feeling will only last a minute or so. During this you will have a mask on so we can measure your breathing. This will not change how you breathe or make it more difficult. There will also be a strap across your chest which tells us your heart rate. You will also have 7 small sticky

plasters attached to your body (for example on your leg, back and side). These tell us how your heart and muscles respond. They do not hurt. At the end of the test we will ask to take a couple of drops of blood from a fingertip so we can see how hard you worked.

## Visit 2 (arm crank)

You will complete the same test as on visit 1 but using the arm crank instead of the bike.

## Visit 3 (bike) & 4 (arm crank)

You will be asked to do a 30 second test where you work as hard as you can for the *whole* 30 seconds. During these tests you will have a strap across your chest and a mask on like the previous tests. Like after the first two tests, we will ask to take a couple of drops of blood.

Swimmers will also be asked to keep a training log for a month and give us a list of PBs. This provides us with an idea of the volume and hours of training you are involved in.

These pictures show the type of bike and arm crank you will be using:

## Can I Change My Mind and Withdraw from the Project?

You may withdraw from participation in any part or the entire project at any time and without any disadvantage to yourself of any kind. If you do withdraw we will use the results we have already collected but no one will be able to identify them as yours.

## What information will be collected and what use will be made of it?

The results collected will be written up for other scientists, both nationally and internationally, who are interested in children's fitness. In no way will you or your results be identifiable. You are welcome to a summary of your results if you would like them.

## Will I need to do anything special for the study?

We ask that you do not eat or drink caffeinated drinks (e.g. coca-cola, tea, coffee) in the 3 hours before your test. We also ask that you do not participate in activity you do not normally do in the 24 hours before your sessions with the university.

## What if I have a question/s?

If you have any questions please feel free to contact either:-

|  |  |  |
|---|---|---|
| Melitta Winlove | or | Dr Jo Welsman |
| 07739 3351081 |  | 01392 262887 |
| maw203@ex.ac.uk |  | J.R.Welsman@exeter.ac.uk |

**What to do next….**
Please fill in the attached consent forms and health questionnaire and return to your teacher/coach as directed. One of the researchers listed will be in contact with you through your teacher or coach to arrange the exercise tests.

Once again we thank you for your time and look forward to your response.

Melitta Winlove                             Dr Jo Welsman

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

*__Appendix 2A__*

UNIVERSITY OF
EXETER

School Of Sport And
Health Sciences
St Luke's Campus
Heavitree Road
Exeter, EX1 2LU

Telephone:1392 264803
Email: sshs-school-
office@exeter.ac.uk
www.exeter.ac.uk/sshs

## __Heart size, fitness and training in children__

### __Consent Form__

To be completed by the Parent/Guardian

I consent for my child…………………………to attend the Research Centre to take part in a longitudinal study investigating heart size and training and how it relates to endurance fitness in children.  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 up to three visits to the Centre each year.  I understand that my child will be involved in magnetic resonance scanning.  In addition I understand that my child will complete an exercise test on both a cycle ergometer and a swim bench.  I also understand that my child will have a fingertip blood sample taken after the exercise tests.  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…………………

Name of school your child attends……………………………………………

Home phone number/e-mail …………………………………………………….

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 I will be coming on three separate occasions each year and on one occasion will have some scans taken of my heart. I also know that each year, for six years, I will be invited back to repeat the same tests.

I know that I can stop taking part in the study at any time, even during the scan or exercise tests, without affecting my relationship with the researchers or the swimming club.

Signed…………………………….Child Participant

Dr Kerstin Stoedefalke                          Dr Joanne Welsman

+44 (0) 1392 262812                          +44 (0) 1392 264812

K.A.Stoedefalke@ex.ac.uk                    J.R.Welsman@ex.ac.uk

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

*Appendix 2B*

UNIVERSITY OF
EXETER

School Of Sport And
Health Sciences
St Luke's Campus
Heavitree Road
Exeter, EX1 2LU

Telephone:1392 264803
Email: sshs-school-
office@exeter.ac.uk
www.exeter.ac.uk/sshs

**Influence of Exercise Mode on the Oxygen Uptake Kinetics of
Trained and Untrained Girls**

**Consent form for Participants**

I have read the Information Sheet concerning this study and understand what it is about and
what is involved. All my questions have been answered to my satisfaction. I understand
that I am free to ask for more information at any stage.

I know that:

1) I will be asked to perform two maximal effort tests, one on the stationary bike and
   one on the arm crank.

2) I will be asked to complete total of roughly 6 additional exercise tests using two
   different types of exercise.

3) I will be asked to allow a fingertip prick for drops of blood to be collected.

4) During the exercise test my heart rate and breathing will both be monitored
   continuously, as will changes in oxygen levels in the muscle.

5) I will be asked to assess my level of physical maturity with reference to standard
   drawings.

6) My participation is entirely my own choice.

7) I am free to stop taking part in the study at any time without any problems.

8) My results will be kept safe in secure storage;

9) The results of the study may be published but no one will know which results were
   mine.

I agree to take part in this project.

.............................................................                ……………
          (Signature of participant)                                        (Date)

*Appendix 2C*

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EXETER

School Of Sport And
Health Sciences
St Luke's Campus
Heavitree Road
Exeter, EX1 2LU

Telephone:1392 264803
Email: sshs-school-
office@exeter.ac.uk
www.exeter.ac.uk/sshs

**Influence of Exercise Mode on the Oxygen Uptake Kinetics of
Trained and Untrained Girls**

**Assent form for Parent**

I have read the Information Sheet concerning this study and understand what it is about and the methods involved. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

1) My child will be asked to perform two maximal effort tests, one on the stationary bike and one on the arm crank.

2) The duration of this incremental test is determined by the child.

3) My child will be asked to complete roughly 6 additional exercise tests using two different exercise modalities.

4) My child will be asked to provide fingertip blood samples.

5) During the exercise test heart rate and the participant's breathing will both be monitored continuously, as will changes in oxygen levels in the muscle.

6) My child will be asked to assess their level of maturity with reference to standard drawings.

7) My child's participation is entirely voluntary.

8) My child is free to withdraw from the study at any time without any disadvantage;

9) Any raw data on which the results of the project depend will be retained in secure storage;

10) The results of the study may be published but my child's anonymity will be preserved.

I give permission for my child to take part in this project.


........................................................................              …………….
   (Signature of parent/guradian)                                   (Date)

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School Of Sport And
Health Sciences
St Luke's Campus
Heavitree Road
Exeter, EX1 2LU

**Influence of Exercise Mode on the Oxygen Uptake Kinetics of
Trained and Untrained Girls**

Telephone:1392 264803
Email: sshs-school-
office@exeter.ac.uk
www.exeter.ac.uk/sshs

**Consent form for Participant**

I have read the Information Sheet and understand what I am being asked to do. All my
questions have been answered to my satisfaction. I understand that I am free to ask for
more information at any stage.

I know that:

1) I will be asked to perform two maximal effort tests, one on the stationary bike and
   one on the arm crank.

2) I will be asked to complete two additional tests that require a maximal effort for 30
   seconds.

3) I will be asked to allow a fingertip prick for drops of blood to be collected for
   analysis.

4) During the exercise test my heart rate and breathing will both be monitored
   continuously, as will changes in oxygen levels in the muscle. The pattern of my
   heart will also be monitored throughout the first two maximal tests.

5) I will be asked to assess my level of physical maturity with reference to standard
   drawings.

6) My participation is entirely my own choice.

7) I am free to stop taking part in the study at any time without any problems.

8) My results will be kept safe in secure storage;

9) The results of the study may be published but no one will know which results were
   mine.

I agree to take part in this project.


........................................................................                    ……………
            (Signature of participant)                                                (Date)

UNIVERSITY OF
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**Influence of Exercise Mode on the Oxygen Uptake Kinetics of
Trained and Untrained Girls**

**Assent form for Participant**

School Of Sport And
Health Sciences
St Luke's Campus
Heavitree Road
Exeter, EX1 2LU

Telephone:1392 264803
Email: sshs-school-
office@exeter.ac.uk
www.exeter.ac.uk/sshs

I have read the Information Sheet concerning this study and understand what it is about and the methods involved. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that ……………………………… (child's name):

1) Will be asked to perform two maximal effort tests, one on the stationary bike and one on the arm crank.

2) Will be asked to complete two additional tests that require a maximal effort for 30 seconds one on the stationary bike and one on the arm crank.

3) Will be asked to provide fingertip blood samples after each exercise test.

4) Will be asked to assess their level of maturity with reference to standard drawings.

5) Will have her heart rate, heart function, breathing and muscle oxygen levels monitored continuously during the exercise tests.

6) Can take part in this study if they want to but is free to withdraw from the study at any time without any disadvantage or giving a reason.

I also understand that:

7) Any raw data on which the results of the project depend will be retained in secure storage.

8) The results of the study may be published but their anonymity will be preserved.

I give permission for ………………………….. (child's name) to take part in this project.

.........................................................................                              ……………

       (Signature of Parent/Guardian)                                                   (Date)

UNIVERSITY OF
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## HEALTH SCREEN FOR CHILD VOLUNTEERS (PARENTAL FORM)

**Name of child:** ...............…………………

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is:

(i) To ensure their own continuing well-being
(ii) To avoid the possibility of individual health issues confounding study outcomes.

Your answers to the questions in this questionnaire, on behalf of your child, are **strictly confidential**.

**Please complete this brief questionnaire to confirm your child's fitness to participate:**

1) **At present,** does your child have any health problem for which they are:
| | | |
|---|---|---|
| (a) On medication, prescribed or otherwise | Yes | No |
| (b) Attending a general practitioner | Yes | No |
| (c) On a hospital waiting list | Yes | No |

2) **In the past two years,** has your child had any illness that required them to:
| | | |
|---|---|---|
| (a) Consult your family GP | Yes | No |
| (b) Attend a hospital outpatient department | Yes | No |
| (c) Be admitted to hospital | Yes | No |

3) **Has your child ever** had any of the following:

| | | |
|---|---|---|
| (a) Convulsions/epilepsy | Yes | No |
| (b) Asthma | Yes | No |
| (c) Eczema | Yes | No |
| (d) Diabetes | Yes | No |
| (e) A blood disorder | Yes | No |
| (f) Head injury | Yes | No |
| (g) Digestive problems | Yes | No |
| (h) Heart problems | Yes | No |
| (i) Problems with bones or joints | Yes | No |
| (j) Disturbance of balance/coordination | Yes | No |
| (k) Numbness in hands or feet | Yes | No |
| (l) Disturbance of vision | Yes | No |
| (m) Ear / hearing problems | Yes | No |
| (n) Thyroid problems | Yes | No |
| (o) Kidney or liver problems | Yes | No |
| (p) Allergy to nuts | Yes | No |

**If YES to any question, please describe briefly if you wish (for example, to confirm problem was/is short-lived, insignificant or well controlled.)**

**Thank you**

UNIVERSITY OF EXETER

**Influence of Exercise Mode on the Oxygen Uptake Kinetics of Trained and Untrained Girls**

**Maturity Assessment**

School Of Sport And
Health Sciences
St Luke's Campus
Heavitree Road
Exeter, EX1 2LU

Telephone:1392 264803
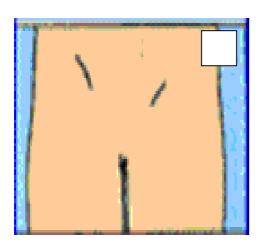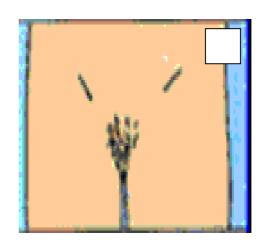Email: sshs-school-office@exeter.ac.uk
www.exeter.ac.uk/sshs

- Please look at the five pictures below and read the sentence next to the picture carefully.

- Then choose the picture that matches your stage of development and mark with a tick in the corner of the picture.

There is no right or wrong answer so just choose the one that looks the most similar.

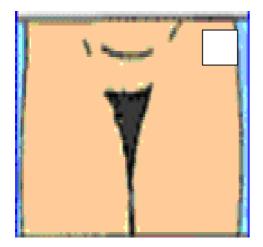Once you have finished return the form to the University staff in the envelope provided.

ALL INFORMATION WILL BE HANDLED WITH THE STRICTEST OF CONFIDENCE, AND WILL ONLY BE VIEWED BY THE RESEARCH TEAM.
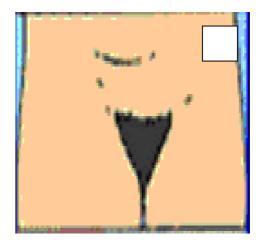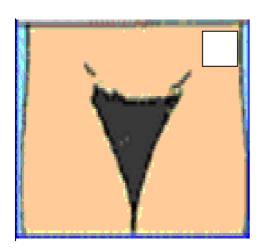
There is no pubic hair at all.

There is a little pubic hair.

Moderate amount of darker, curly and coarser hair extending outwards.



Pubic hair is coarse and curly, looks like an adults but covers a smaller area.



Coarse, thick, curly pubic hair extends to the inner thighs as an upside down triangle.