

# **Pulmonary O<sub>2</sub> Uptake Kinetics and Motor Unit Recruitment in Young People**

Submitted by Brynmor C. Breese to the University of Exeter as a thesis for the degree of Doctor of Philosophy by Research in Sport and Health Sciences, July 2011.

This thesis is available for Library use on the understanding that it is copyright material and that no quotation from this thesis may be published without proper acknowledgment.

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



A handwritten signature in blue ink, appearing to read 'Brynmor Breese', is written over a light blue grid background. The signature is fluid and cursive, with the first name 'Brynmor' and the last name 'Breese' clearly distinguishable.

.....

## Abstract

The primary objective of this thesis was to examine the influence of alterations in muscle recruitment on pulmonary O<sub>2</sub> uptake ( $\dot{V}_{O_2}$ ) kinetics during exercise above the gas exchange threshold (GET) in young people. In the first experimental chapter, the phase II time constant ( $\tau$ ) slowed over a 2-yr period in 14-16 yr old boys ( $25 \pm 5$  s vs.  $30 \pm 5$  s,  $P = 0.002$ ) and there was a greater relative  $\dot{V}_{O_2}$  slow component amplitude (Rel.  $A'_2$  (%)) during heavy-intensity exercise ( $9 \pm 5\%$  vs.  $13 \pm 4\%$ ,  $P = 0.036$ ). In the second study, 'work-to-work' transitions yielded similar phase II  $\dot{V}_{O_2}$  kinetics during unloaded-to-moderate exercise (U→M) between 11-12 yr old boys and teenagers ( $19 \pm 5$  s vs.  $22 \pm 7$  s,  $P = 0.32$ ) but the phase II  $\tau$  was significantly lengthened in the latter group at the onset of moderate-to-very heavy exercise (M→VH:  $30 \pm 5$  s vs.  $45 \pm 11$  s,  $P = 0.011$ ). There were no differences in the phase II  $\tau$  between teenagers and adult men during M→VH exercise ( $P = 0.46$ ). In the third study, increasing pedal rate from 50 rev·min<sup>-1</sup> to 115 rev·min<sup>-1</sup> significantly ( $P < 0.01$ ) lengthened the phase II  $\tau$  ( $32 \pm 5$  s vs.  $42 \pm 11$  s) and increased the relative  $\dot{V}_{O_2}$  slow component amplitude ( $10 \pm 3\%$  vs.  $16 \pm 5\%$ ) during VH cycling in untrained teenagers but the same parameters were unaltered by pedal cadence in trained junior cyclists (phase II  $\tau$ :  $26 \pm 4$  s vs.  $22 \pm 6$  s, and Rel.  $A'_2$ :  $14 \pm 5\%$  vs.  $17 \pm 3\%$ ,  $P > 0.05$ ). The fourth study reported that a reduced relative  $\dot{V}_{O_2}$  slow component amplitude in younger boys compared to men ( $11 \pm 4\%$  vs.  $16 \pm 3\%$ ,  $P = 0.015$ ) coincided with a lower percentage change in the integrated electromyogram (iEMG) of the *m. vastus lateralis* from minute 2 to minute 6 of exercise ( $\Delta iEMG_{6,2}$ :  $7 \pm 25\%$  vs.  $49 \pm 48\%$ ,  $P = 0.030$ ), suggesting that alterations in motor unit recruitment might be involved in restricting the O<sub>2</sub> cost of exercise above the primary amplitude in children compared to adults. The final experimental chapter tested this hypothesis, but no statistically significant differences were reported for the relative

$\dot{V}_{O_2}$  slow component amplitude between 10-12 yr old boys and men ( $15 \pm 7\%$  vs.  $19 \pm 4\%$ ,  $P = 0.145$ ). In boys, an excess  $\dot{V}_{O_2}$  temporally coincided with a significant increase in the transverse relaxation time ( $T_2$ ) of the *m. vastus lateralis* from the  $\dot{V}_{O_2}$  slow component time delay ( $SC_{td}$ ) to minute 6 of exercise ( $41.5 \pm 2.4$  ms vs.  $45.2 \pm 2.3$  ms,  $P = 0.001$ ), thereby consistent with the notion that delayed muscle fibre activation might contribute to the development of the  $\dot{V}_{O_2}$  slow component in youth. In conclusion, this thesis has demonstrated that maturational changes in the  $\dot{V}_{O_2}$  kinetic response to heavy-intensity exercise are extended into adolescence. During intense submaximal exercise, the recruitment of higher-order (type II) muscle fibres might be principally involved in modulating  $\dot{V}_{O_2}$  kinetics as children mature but this effect is attenuated in teenage subjects engaged in regular endurance training.

## **Acknowledgements**

First and foremost, I would like to thank my principal supervisor Associate Professor Craig Williams for your dedicated academic support, tutelage, and encouragement throughout a challenging yet rewarding four years at the Children's Health and Exercise Research Centre (CHERC). Your guidance and generosity has contributed significantly to an enjoyable and fulfilling time spent studying here in Exeter. I am also greatly appreciative to Professor Neil Armstrong for your mentorship and time devoted towards providing informative feedback throughout the write-up process. To Dr Alan Barker, I would like to extend my gratitude for your knowledgeable contribution to the experimental chapters and the constructive comments you have always provided on article drafts.

A number of individuals have devoted considerable time and energy toward facilitating the collection of data during this project. I would especially like to thank David Childs for his unreserved patience and skill in providing technical support; Dr Jonathon Fulford for his time spent operating the MR scanner; to the staff and pupils of Ivybridge Community College and Wynstream Primary School for their participation in supporting this work; and to my friends and colleagues within CHERC for their assistance in supervising the children during many laboratory sessions. I would also like to thank the division of Sport and Health Sciences, College of Life and Environmental Sciences for their financial assistance.

Finally, I would like to acknowledge my parents, Jeffrey and Rena, for their unconditional support and affection in providing me with the opportunities in life their hard work over the many years has earned.

## Table of Contents

<b>Title page</b>	<b>i</b>
<b>Abstract</b>	<b>ii</b>
<b>Acknowledgements</b>	<b>iv</b>
<b>Table of contents</b>	<b>v</b>
<b>Definitions of abbreviations and symbols</b>	<b>ix</b>
<b>List of figures</b>	<b>xii</b>
<b>List of tables</b>	<b>xvi</b>
<b>List of equations</b>	<b>xvii</b>
<b>Publications and conference presentations</b>	<b>xviii</b>
<b>Chapter One: Introduction</b>	<b>1</b>
1.1 Current perspectives on $\dot{V}_{O_2}$ kinetics in young people	<b>1</b>
1.2 Objectives of this thesis	<b>5</b>
<b>Chapter Two: Literature Review</b>	<b>7</b>
2.1 Oxygen uptake kinetics during exercise	<b>7</b>
2.1.1 <i>The three phase <math>\dot{V}_{O_2}</math> response</i>	<b>7</b>
2.1.2 <i>Modelling the phase II <math>\dot{V}_{O_2}</math> response in children</i>	<b>10</b>
2.1.3 <i>Relationship between <math>\dot{V}_{O_2}</math> dynamics at the muscle vs. mouth</i>	<b>12</b>
2.2 Influence of exercise intensity on $\dot{V}_{O_2}$ responses	<b>13</b>
2.2.1 <i>The <math>\dot{V}_{O_2}</math> slow component</i>	<b>13</b>
2.2.2 <i>Exercise intensity domains</i>	<b>15</b>
2.3 Oxygen uptake kinetics in children and adolescents	<b>17</b>
2.3.1 <i>Equating the exercise intensity in children</i>	<b>17</b>
2.3.2 <i>Age influences during moderate exercise</i>	<b>19</b>
2.3.3 <i>Youth responses during heavy/very heavy exercise</i>	<b>20</b>
2.3.4 <i>Age influences on <math>\dot{V}_{O_2}</math> kinetics during severe exercise</i>	<b>24</b>
2.3.5 <i>Summary of age-related differences in <math>\dot{V}_{O_2}</math> kinetics</i>	<b>25</b>
2.4 Physiological interpretation of phase II $\dot{V}_{O_2}$ kinetics	<b>26</b>
2.4.1 <i>Muscle <math>O_2</math> delivery inertia</i>	<b>27</b>
2.4.1.1 <i>Influence of enhancing muscle <math>O_2</math> availability</i>	<b>28</b>

2.4.1.2	<i>The ‘tipping point’ hypothesis</i>	30
2.4.1.3	<i>Muscle fibre type-specific influences</i>	32
2.4.2	<i>Muscle metabolic inertia</i>	34
2.4.2.1	<i>NADH availability</i>	34
2.4.2.2	<i>Inhibition of mitochondrial respiration</i>	35
2.4.3	<i>The PCr-Cr shuttle hypothesis</i>	36
2.4.3.1	<i>Phosphate linked modulation in children</i>	38
2.4.4	<i>Experimental predictions</i>	39
2.5	<i>Influence of muscle fibre type on <math>\dot{V}_{O_2}</math> kinetics</i>	41
2.5.1	<i>Cross-sectional studies</i>	41
2.5.2	<i>The ‘size’ principle of orderly recruitment</i>	43
2.5.3	<i>Reducing muscle fibre recruitment heterogeneity</i>	44
2.5.3.1	<i>Manipulated pedal cadence</i>	44
2.5.3.2	<i>Glycogen depletion studies</i>	46
2.5.3.3	<i>‘Work-to-work’ exercise transitions</i>	47
2.5.4	<i>Muscle fibre type/recruitment in youth</i>	51
2.6	<i>Motor unit recruitment and the <math>\dot{V}_{O_2}</math> slow component</i>	53
2.6.1	<i>Delayed muscle fibre activation</i>	53
2.6.2	<i>Integrated electromyogram (iEMG) activity</i>	54
2.6.3	<i>T<sub>2</sub>-weighted magnetic resonance imaging</i>	57
2.7	<i>Statement of hypotheses</i>	60
<b>Chapter Three: General methods</b>		<b>62</b>
3.1	<i>Subject recruitment</i>	62
3.2	<i>Experimental measures</i>	63
3.2.1	<i>Pulmonary gas exchange</i>	64
3.3	<i>Exercise testing protocols</i>	64
3.3.1	<i>Ramp exercise</i>	64
3.3.1.1	<i>Calculation of peak <math>\dot{V}_{O_2}</math> and gas exchange threshold (GET)</i>	65
3.3.2	<i>Constant work rate exercise</i>	66
3.4	<i>Data analysis procedures</i>	68
3.4.1	<i>Breath-by-breath <math>\dot{V}_{O_2}</math> responses</i>	68
<b>Chapter Four: Longitudinal changes in pulmonary O<sub>2</sub> uptake kinetics during heavy-intensity exercise in 14-16 yr old boys</b>		<b>71</b>
4.1	<i>Introduction</i>	71
4.2	<i>Methods</i>	74
4.2.1	<i>Participants</i>	74
4.2.2	<i>Experimental procedures.</i>	74

4.2.3	<i>Data analysis procedures.</i>	76
4.2.4	<i>Statistical analyses</i>	76
4.3	Results	76
4.4	Discussion	79
4.4.1	<i>Phase II <math>\dot{V}O_2</math> response</i>	79
4.4.2	<i><math>\dot{V}O_2</math> slow component</i>	82
4.5	Conclusion	86
<b>Chapter Five: The influence of age on pulmonary O<sub>2</sub> uptake and muscle deoxygenation kinetics during very heavy-intensity exercise transitions initiated from an elevated baseline work rate</b>		<b>87</b>
5.1	Introduction	87
5.2	Methods	90
5.2.1	<i>Participants</i>	90
5.2.2	<i>Experimental procedures</i>	90
5.2.3	<i>Experimental measures</i>	92
5.2.4	<i>Data analysis</i>	94
5.2.5	<i>Statistical analyses</i>	96
5.3	Results	97
5.3.1	<i>Step exercise responses</i>	98
5.3.2	<i>HR kinetics</i>	101
5.3.3	<i>Muscle [HHb] kinetics</i>	102
5.3.4	<i>Muscle iEMG activity</i>	104
5.4	Discussion	105
5.5	Conclusion	109
<b>Chapter Six: The effect of pedal rate on pulmonary O<sub>2</sub> uptake kinetics during very heavy-intensity exercise in trained and untrained teenage boys</b>		<b>110</b>
6.1	Introduction	110
6.2	Methods	113
6.2.1	<i>Participants</i>	113
6.2.2	<i>Experimental procedures</i>	113
6.2.3	<i>Data analysis procedures</i>	115
6.2.4	<i>Statistics</i>	115
6.3	Results	116
6.3.1	<i>Ramp exercise</i>	116
6.3.2	<i>Step exercise</i>	117
6.4	Discussion	120
6.5	Conclusion	124
<b>Chapter Seven: The influence of age on electromyogram activity and the slow component of pulmonary O<sub>2</sub> uptake during very heavy-intensity exercise in humans</b>		<b>126</b>
7.1	Introduction	126

7.2	Methods	127
	7.2.1 <i>Statistical analyses</i>	128
7.3	Results	128
	7.3.1 <i>Step exercise <math>\dot{V}_{O_2}</math> response</i>	128
	7.3.2 <i>iEMG activity</i>	130
7.4	Discussion	132
7.5	Conclusions	134
<b>Chapter Eight: The influence of thigh muscle recruitment on the <math>\dot{V}_{O_2}</math> slow component during very heavy-intensity exercise in boys and men</b>		<b>135</b>
8.1	Introduction	135
8.2	Methods	137
	8.2.1 <i>Participants</i>	137
	8.2.2 <i>Exercise protocols</i>	138
	8.2.3 <i>Cardio-respiratory measures</i>	139
	8.2.4 <i>Determination of <math>T_2</math> using MRI</i>	139
	8.2.5 <i>Data analysis procedures</i>	141
	8.2.6 <i>Statistical analysis</i>	141
8.3	Results	142
8.4	Discussion	146
8.5	Conclusion	149
<b>Chapter Nine: Summary, conclusions and recommendations</b>		<b>150</b>
9.1	Summary of experimental chapters	151
	9.1.1 <i>Chapter 4</i>	151
	9.1.2 <i>Chapter 5</i>	152
	9.1.3 <i>Chapter 6</i>	153
	9.1.4 <i>Chapter 7</i>	154
	9.1.5 <i>Chapter 8</i>	154
9.2	Advances on current perspectives in paediatric exercise physiology	155
	9.2.1 <i>Age-linked modulation of <math>\dot{V}_{O_2}</math> kinetics: Dependence on muscle fibre recruitment</i>	156
	9.2.2 <i><math>\dot{V}_{O_2}</math> slow component: Mechanistic basis and future applications</i>	159
9.3	Future study recommendations	162
<b>References</b>		<b>165</b>
<b>Appendix 1</b> Worked calculations of sample size (n)		<b>192</b>
<b>Appendix 2</b> Ethics approval certificates		<b>193</b>



## Definitions of abbreviations and symbols

ADP	adenosine di-phosphate
APHV	age at peak height velocity
ATP	adenosine tri-phosphate
$C_{(a-\bar{v})O_2}$	arterial and mixed venous oxygen content difference
CI	95% confidence interval
CO <sub>2</sub>	carbon dioxide
Cr	creatinine
CP	critical power
e	exponential constant (base of natural logarithm)
GET	gas exchange threshold
G <sub>p</sub>	Primary $\dot{V}_{O_2}$ gain
G <sub>tot</sub>	$\dot{V}_{O_2}$ gain at end exercise
<sup>1</sup> H	hydrogen
HbO <sub>2</sub>	muscle oxygenated haemoglobin
HHb	muscle deoxyhaemoglobin
iEMG	integrated electromyogram
L-NAME	nitro-L-arginine methyl ester
La	lactate
LT	lactate threshold
MLSS	maximal lactate steady state
MRT	mean response time
$m\dot{V}_{O_2}$	muscle oxygen uptake
MVC	maximal voluntary contraction

$n$	study sample size
NIRS	near-infrared spectroscopy
NAD <sup>+</sup>	nicotinamide adenine di-nucleotide (oxidised form)
NADH	nicotinamide adenine di-nucleotide (reduced form)
O <sub>2</sub>	oxygen
<sup>31</sup> P	phosphorous
PCr	phosphocreatine
PDH	pyruvate dehydrogenase
P <sub>i</sub>	inorganic phosphate
<sup>31</sup> P-MRS	31P-magnetic resonance spectroscopy
PO <sub>2</sub>	partial pressure of oxygen
$\dot{Q}$	cardiac output
RER	respiratory exchange ratio
rev·min <sup>-1</sup>	revolutions per minute
SD	standard deviation
$t$	time
$t^{1/2}$	time to reach 50% of the change in a given variable from baseline to its asymptote
$\tau$	time constant (time taken to attain 63% of the response amplitude)
T <sub>2</sub>	exponential decay in magnetisation of tissue water hydrogen nuclei in the transverse plane
TD	time delay
$\dot{V}_{CO_2}$	carbon dioxide output
$\dot{V}_E$	minute ventilation

$\dot{V}_{O_2}$	pulmonary oxygen uptake
$\dot{V}_{O_2 \text{ peak}}$	pulmonary peak oxygen uptake
$\dot{V}_{O_2 \text{ leg}}$	oxygen uptake across the lower limb
$\Delta$	change or difference in a value (delta)
[X]	denotes the concentration of a variable

## List of Figures

### Chapter One

- Figure 1.1 Group mean  $\dot{V}_{O_2}$  profile during cycling exercise below (a) and above (b) the estimated LT in children and adults. 2

### Chapter Two

- Figure 2.1 Schematic of the muscle (a) and pulmonary oxygen uptake (b) profiles. 8
- Figure 2.2 An example  $\dot{V}_{O_2}$  response in a child during a single step exercise transition from unloaded pedalling to 40W (a) and after time-aligning and averaging six repeated transitions of the same work rate (b). 11
- Figure 2.3 Schematic illustration of the  $\dot{V}_{O_2}$  response profiles within specific exercise intensity domains. 16
- Figure 2.4 Mathematical modelling of  $\dot{V}_{O_2}$  data in response to H exercise ( $\Delta$  40%) in an 11 yr-old child. 22
- Figure 2.5  $\dot{V}_{O_2}$  data fitted using a single-exponential model (>phase I) within a predetermined fitting window excluding the  $\dot{V}_{O_2}$  slow component. 23
- Figure 2.6 Longitudinal changes in  $\dot{V}_{O_2}$  kinetics during H intensity cycling exercise ( $\Delta$  40%) in 22 children. 24
- Figure 2.7 Age modulation of  $\dot{V}_{O_2}$  kinetic parameters during H/VH step exercise transitions in children ( $n = 30$ ), teenagers ( $n = 31$ ), and adults ( $n = 52$ ). 26
- Figure 2.8 Schematic of the ‘tipping point’ hypothesis, which outlines the dependence of phase II  $\dot{V}_{O_2}$  kinetics in relation to  $O_2$  delivery. 30
- Figure 2.9 An example  $\dot{V}_{O_2}$  profile during H-intensity cycling exercise in two adult participants with contrasting muscle fibre type distributions. 41
- Figure 2.10 Schematic illustrating the fibre-type dependency on muscle force/velocity relationships. 44
- Figure 2.11 Hypothetical model illustrating the influence of muscle fibre recruitment on  $\dot{V}_{O_2}$  kinetics during exercise above 50

the GET.

Figure 2.12	Axial MR image of the left thigh musculature in a 30 yr-old male subject before (a) and after (b) performing 6-min of high-intensity cycling exercise.	58
-------------	--	----

### Chapter Three

Figure 3.1	Non-invasive determination of the blood lactate threshold during ramp incremental ( $15 \text{ W}\cdot\text{min}^{-1}$ ) exercise in a 12-yr-old boy.	66
------------	---	----

Figure 3.2	Calculation of exercise power output from linear extrapolation of the $\Delta \dot{V}_{\text{O}_2} / \Delta \text{WR}$ response below the GET during incremental exercise in a child participant.	67
------------	---	----

Figure 3.3	Procedures for isolating the phase II $\dot{V}_{\text{O}_2}$ model fitting window during H/VH exercise transitions	69
------------	--	----

### Chapter Four

Figure 4.1	$\dot{V}_{\text{O}_2}$ response normalised per unit increment in work rate on test occasion 1 (●) and test occasion 2 (○) in a representative subject.	78
------------	--	----

Figure 4.2	Mean $\dot{V}_{\text{O}_2}$ profile ( $n = 14$ ) during heavy-intensity exercise on test occasion 1 (●) and test occasion 2 (○).	79
------------	--	----

### Chapter Five

Figure 5.1	Schematic illustration of the experimental protocol including each exercise condition (U→M, U→VH, and M→VH).	91
------------	--	----

Figure 5.2	Modelling of the muscle [HHb] response during VH exercise in a single child participant	95
------------	---	----

Figure 5.3	Group mean $\dot{V}_{\text{O}_2}$ responses in B (●), T (○), and M (●) during step exercise in each condition.	100
------------	--	-----

Figure 5.4	Group mean $\pm$ (S.E.) time constant ( $\tau$ ) for the phase II $\dot{V}_{\text{O}_2}$ response in B (●), T (○), and M (●) during step exercise in each condition	100
------------	---	-----

Figure 5.5	Heart rate response in an 11-yr-old boy following the onset of step exercise in U→VH (●) and M→VH (○).	101
------------	--	-----

Figure 5.6	Muscle deoxyhaemoglobin/myoglobin response in a	104
------------	---	-----

representative 13-yr old boy at the onset of U→VH (●) and M→VH (○) exercise.

## Chapter Six

- Figure 6.1 Pulmonary  $\dot{V}_{O_2}$  response to very heavy-intensity during 50 rev·min<sup>-1</sup> (●) and 115 rev·min<sup>-1</sup> (○) pedalling in a representative UT (a) and T (b) participant. **119**
- Figure 6.2 Mean  $\dot{V}_{O_2}$  responses to very heavy-intensity exercise during 50 rev·min<sup>-1</sup> (●) and 115 rev·min<sup>-1</sup> (○) pedalling in UT (a) and T (b) participants. **120**

## Chapter Seven

- Figure 7.1 Group mean  $\dot{V}_{O_2}$  responses in relation to age during VH constant work rate exercise. **130**
- Figure 7.2 Group mean ± (S.E.) integrated electromyogram (iEMG) activity of the *m. vastus lateralis* during VH constant work rate exercise. **131**
- Figure 7.3 Relationship between the relative  $\dot{V}_{O_2}$  slow component amplitude and percent increases in iEMG of the *m. vastus lateralis* in each group. **131**

## Chapter Eight

- Figure 8.1 Representative  $\dot{V}_{O_2}$  responses in a child (●) and adult (○) subject during VH exercise in (a) with group mean  $\dot{V}_{O_2}$  profiles shown in (b). **144**
- Figure 8.2 Group mean (± SE) change in  $T_2$  above rest in each muscle of the upper thigh at the  $\dot{V}_{O_2}$  slow component time delay ( $SC_{td}$ , closed bars) and at minute 6 (open bars) **145**
- Figure 8.3 Relationship between the relative  $\dot{V}_{O_2}$  slow component amplitude and percent increases in  $T_2$  of the *m. quadriceps femoris* in boys and men. **146**

## Chapter Nine

- Figure 9.1 Mean ± (SD) phase II  $\tau$  values during heavy-intensity exercise on test occasion 1 (●) and 2 (○) resolved using a double-exponential function (*model 1*) and a single-exponential model within a fitting window that excluded the  $\dot{V}_{O_2}$  slow component (*model 2*). **151**

Figure 9.2	Hypothetical model illustrating the influence of exercise training on $\dot{V}O_2$ kinetics within specific segments of the muscle fibre pool during transitions above the GET.	<b>159</b>
Figure 9.3	Relationship between the relative $\dot{V}O_2$ slow component amplitude and years from peak height velocity (PHV) in twenty-five youth subjects during very-heavy intensity cycling exercise.	<b>161</b>

## List of Tables

### **Chapter Four**

Table 4.1	Participant descriptive statistics and peak exercise responses on separate test occasions over a 2-yr interval	<b>77</b>
Table 4.2	Pulmonary O <sub>2</sub> uptake kinetics during heavy-intensity step exercise	<b>78</b>

### **Chapter Five**

Table 5.1	Participant descriptive characteristics and incremental exercise responses	<b>97</b>
Table 5.2	Pulmonary O <sub>2</sub> uptake kinetics during step transitions in relation to age.	<b>99</b>
Table 5.3	NIRS derived muscle deoxygenation (HHb) kinetics during step exercise in youth participants	<b>103</b>

### **Chapter Six**

Table 6.1	Physical characteristics of participants.	<b>116</b>
Table 6.2	Oxygen uptake kinetic responses during very heavy-intensity exercise at disparate pedal rates.	<b>118</b>

### **Chapter Seven**

Table 7.1	Pulmonary O <sub>2</sub> uptake and integrated electromyogram (iEMG) response during very heavy-intensity constant work rate exercise	<b>129</b>
-----------	---	------------

### **Chapter Eight**

Table 8.1	Pulmonary O <sub>2</sub> uptake kinetics during very heavy-intensity exercise in boys and men.	<b>143</b>
Table 8.2	Resting T <sub>2</sub> values for consecutive exercise bouts interspersed with 60-min recovery.	<b>143</b>

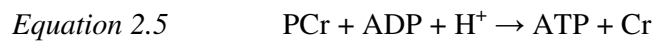


## List of Equations

*Equation 2.1*       $\dot{V}_{O_2} = \dot{Q} \cdot C_{(a-\bar{v})} O_2$

*Equation 2.2*       $\dot{V}_{O_2(t)} = \Delta \dot{V}_{O_2 SS} \cdot (1 - e^{-(t-TD)/\tau})$

*Equation 2.3*       $\dot{V}_{O_2(t)} = A_1 \cdot (1 - e^{-(t-TD_1)/\tau_1}) + A_2 \cdot (1 - e^{-(t-TD_2)/\tau_2})$



*Equation 3.1*      
$$N = \frac{2SD^2(1.96 + 0.84)^2}{\Delta^2}$$

*Equation 3.2*       $\Delta \dot{V}_{O_2} (t) = \Delta \dot{V}_{O_2} \cdot (1 - e^{-(t-TD)/\tau})$

*Equation 8.1*       $S = S_0 \exp^{(-TE/T_2)}$

## Publications and Conference Presentations

### **Peer-reviewed journal articles**

**Breese, B.C.**, Armstrong, N., Barker, A.R., & Williams, C.A. (2011). The effect of pedal rate on pulmonary O<sub>2</sub> uptake kinetics during very heavy intensity exercise in trained and untrained teenage boys. *Respir Physiol Neurobiol*, 177, 149-54.

**Breese, B.C.**, Williams, C.A., Barker, A.R., Welsman, J.R., Fawkner, S.G., & Armstrong, N. (2010). Longitudinal change in the oxygen uptake kinetic response to heavy-intensity exercise in 14- to 16-year-old boys. *Pediatr Exerc Sci*, 22, 314-25.

### **Articles under review**

**Breese, B.C.**, Barker, A.R., Armstrong, N., Jones, A.M., & Williams, C.A. (2010). The effect of baseline metabolic rate on pulmonary O<sub>2</sub> uptake kinetics during very heavy intensity exercise in boys and men.

### **Conference presentations**

**Breese, B.C.**, Barker, A.R., Armstrong, N., Fulford, J., & Williams, C.A. (2011). The influence of quadriceps muscle recruitment on the  $\dot{V}_{O_2}$  slow component during very heavy-intensity exercise in boys and men. Presented at the XXVII<sup>th</sup> International Symposium of Pediatric Work Physiology, Mawgan Porth, Cornwall, UK.

**Breese, B.C.**, Barker, A.R., & Williams, C.A. (2010). The effect of initial baseline metabolic rate on pulmonary O<sub>2</sub> uptake kinetics during severe-intensity exercise in boys aged 11-13 years. Presented at the BASES Annual Conference, University of Glasgow, UK.

**Breese, B.C.**, Armstrong, N., Barker, A.R., & Williams, C.A. (2009). The effect of pedal rate on pulmonary O<sub>2</sub> uptake kinetics during severe-intensity exercise in trained teenage cyclists. Presented at the XXV<sup>th</sup> International Symposium of Pediatric Work Physiology, Le Touquet, France.

**Breese, B.C.**, Williams, C.A., Barker, A.R., Welsman, J.R., Fawkner, S.G., & Armstrong, N. (2008). Longitudinal change in the  $\dot{V}_{O_2}$  kinetic response to heavy-intensity exercise in adolescent boys. Presented at the BASES Annual Conference, Brunel University, UK.

## Chapter One

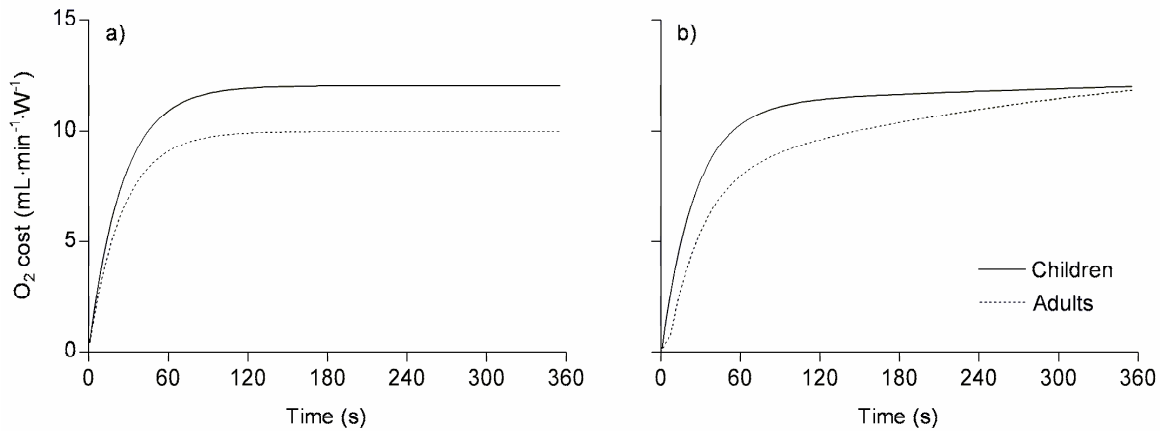
### INTRODUCTION

#### 1.1 Current perspectives on $\dot{V}_{O_2}$ kinetics in young people

The term  $\dot{V}_{O_2}$  kinetics may be defined as the rate at which an individual can supply and utilise the  $O_2$  required for oxidative ATP resynthesis in response to a step change in exercise work rate. Using breath-by-breath measurement of pulmonary gas exchange, the investigator is able to establish the rate of change in  $\dot{V}_{O_2}$  under non-steady state exercise conditions where the bulk of information pertaining to the control of oxidative metabolism resides (Whipp & Ward, 1990).

Earlier quantification of the  $\dot{V}_{O_2}$  kinetic response in youth principally involved cycling transitions initiated from rest or 'light' pedalling to exercise work rates below the individual's lactate threshold [or its non-invasive equivalent, the gas exchange threshold (GET)]. These studies revealed that whilst children demonstrated an increased  $O_2$  cost or 'gain' per unit increment in work rate ( $\Delta\dot{V}_{O_2}/\Delta WR$ ), the time constant ( $\tau$ ) for the exponential rise in  $\dot{V}_{O_2}$  toward the steady-state amplitude was independent of age (Armon, Cooper, Flores, Zanconato, & Barstow, 1991; Cooper, Berry, Lamarra, & Wasserman, 1985; Springer, Barstow, Wasserman, & Cooper, 1991). Indeed, as originally noted by Armon et al. (1991), age differences in the  $\dot{V}_{O_2}$  kinetic response were amplified at exercise work rates engendering a sustained metabolic acidosis. Specifically, an increased primary  $\dot{V}_{O_2}$  gain in younger children tended to ablate the development of a slow rise in  $\dot{V}_{O_2}$  over time (termed the  $\dot{V}_{O_2}$  slow component) compared to adult counterparts (Armon, et al.,

1991). This  $\dot{V}_{O_2}$  response profile, later demonstrated in boys aged 11-12 yr during treadmill running (Williams, Carter, Jones, & Doust, 2001), is widely acknowledged to characterise youngsters  $\dot{V}_{O_2}$  kinetic response to exercise above the GET (figure 1.1).



**Figure 1.1** Group mean  $\dot{V}_{O_2}$  profile during cycling exercise below (a) and above (b) the estimated LT in children and adults. The  $\dot{V}_{O_2}$  data are normalised per unit increment in exercise work rate. Adapted from Armon et al. (1991).

In a series of later investigations, Fawkner and colleagues (2004a, 2004b; 2002) averaged  $\dot{V}_{O_2}$  responses from repeated step exercise transitions in order to resolve the phase II  $\tau$  within narrower 95% confidence intervals. These studies reported an age-linked modulation of phase II  $\dot{V}_{O_2}$  kinetics during cycling transitions in the moderate- and heavy-intensity domains (Fawkner & Armstrong, 2004a, 2004b; Fawkner, Armstrong, Potter, & Welsman, 2002). Furthermore, mathematical modelling revealed the development of a  $\dot{V}_{O_2}$  slow component amplitude that increased (when normalised relative to the end-exercise  $\dot{V}_{O_2}$  amplitude) over a 2-yr duration in children aged 11 to 13 yr (Fawkner & Armstrong, 2004a). However, no data have been published to date to confirm or refute the hypothesis that longitudinal changes in  $\dot{V}_{O_2}$  kinetics observed throughout early childhood are

extended into adolescence. Rather, the bulk of published studies have implemented cross-sectional designs in order to compare  $\dot{V}_{O_2}$  kinetics between pre-pubertal children and adult subjects.

Since the aforementioned faster adaptation of  $\dot{V}_{O_2}$  toward the steady-state amplitude would be expected to reduce the reliance on energy liberation from anaerobic glycolysis and muscle phosphocreatine (PCr) breakdown, it can be appreciated that children's widely acknowledged diminished capacity to support ATP turnover via substrate level phosphorylation (Barker, Welsman, Fulford, Welford, & Armstrong, 2010; Eriksson, 1980; Tonson, et al., 2010; Zanconato, Buchthal, Barstow, & Cooper, 1993) is reflected in their  $\dot{V}_{O_2}$  kinetic response to exercise. This proposal is consistent with differences in muscle enzymatic activities that favours an enhanced oxidative and/or reduced glycolytic potential in younger compared to older counterparts (Berg, Kim, & Keul, 1986; Eriksson, Gollnick, & Saltin, 1973; Kaczor, Ziolkowski, Popinigis, & Tarnopolsky, 2005) that, in turn, might be influenced by a decline in type I muscle fibre expression with increasing age (Jansson, 1996). From this perspective, it is pertinent to note that children's reduced muscle PCr breakdown and attenuated fall in muscle pH during high-intensity exercise (Barker, Welsman, et al., 2010; Zanconato, et al., 1993) is similar to muscle metabolic responses found in adults with a higher proportion of type I muscle fibres (Mizuno, Secher, & Quistorff, 1994). Furthermore, the characteristic  $\dot{V}_{O_2}$  kinetic response observed in children during heavy-intensity cycling (figure 1.1) is also reported in adult subjects with a greater percentage of type I muscle fibres in the *m. vastus lateralis* (Barstow, Jones, Nguyen, & Casaburi, 1996; Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003)

The well established size principle (Henneman & Mendell, 1981) posits an orderly recruitment of motor units with progression from type I to type II fibre activation dependent upon the intensity of muscle contractile activity. For example, type I motor units consisting of smaller motoneurons that innervate fewer oxidative muscle fibres are predominantly recruited to augment muscle force production during low-intensity exercise (Gollnick, Piehl, & Saltin, 1974; Krstrup, Soderlund, Mohr, & Bangsbo, 2004b). Conversely, substrate utilisation is increased within type II muscle fibres as a greater proportion of the total motor unit pool is activated during exercise at higher work rates (Gollnick, et al., 1974; Krstrup, Soderlund, Mohr, et al., 2004b). Since type II muscle fibres are reported to have slower  $\dot{V}_{O_2}$  kinetics and an increased  $O_2$  cost per unit of tension development (Crow & Kushmerick, 1982; Krstrup, et al., 2008), their recruitment from the onset of exercise and/or progressively throughout the exercise bout has been implicated in the development of the  $\dot{V}_{O_2}$  slow component (Gaesser & Poole, 1996; Krstrup, Soderlund, Mohr, et al., 2004b; Whipp, 1994; Wilkerson & Jones, 2006). Likewise, a lengthened phase II  $\tau$  during exercise above, compared to below, the GET (Poole & Jones, 2005a) has been interpreted to reflect the oxidative response kinetics of type II muscle fibres that are ‘unveiled’ onto the pulmonary  $\dot{V}_{O_2}$  signal earlier into the exercise transition (Jones, Pringle, & Carter, 2005b; Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003). A logical hypothesis could therefore be that faster phase II  $\dot{V}_{O_2}$  kinetics and a reduced  $\dot{V}_{O_2}$  slow component in younger children might be linked to an attenuated progression through the muscle fibre recruitment hierarchy as exercise intensity is increased above the GET. However, due principally to ethical restrictions in obtaining muscle biopsies in children, the putative factors linked to muscle fibre recruitment that might contribute to an age-dependent modulation of  $\dot{V}_{O_2}$  kinetics during exercise awaits further investigative scrutiny.

## 1.2 Objectives of this thesis

In light of the foregoing introduction, the first objective of this thesis is to provide an extensive review of the available literature on how the study of  $\dot{V}_{O_2}$  kinetics in youth has contributed to current perspectives in paediatric exercise metabolism (chapter 2). It will become apparent that whilst the  $\dot{V}_{O_2}$  kinetic parameters can be resolved within acceptable limits of statistical confidence in children, there remains considerable scope to extend the existing knowledge base with regard to developmental influences on oxidative function. Chapter 4 will therefore investigate whether longitudinal changes in  $\dot{V}_{O_2}$  kinetics persist into adolescence in order to provide a quantifiable index for maturational influences on the  $\dot{V}_{O_2}$  kinetic parameters during heavy-intensity exercise.

Chapter 5 will explore the effects of an experimental model purported to influence muscle fibre recruitment on  $\dot{V}_{O_2}$  kinetics through initiating step exercise from an elevated baseline metabolic rate in male subjects of different maturity status. Chapter 6 will then investigate the interaction between trained status and manipulated pedal cadence on  $\dot{V}_{O_2}$  kinetics in order to explore the potential for aerobic fitness to influence the muscle  $O_2$  utilisation potential at disparate muscle contraction velocities likely to influence fibre recruitment during exercise. Chapters 7 and 8 will focus on age-linked differences in the  $\dot{V}_{O_2}$  slow component amplitude with particular focus on the temporal association between an excess  $\dot{V}_{O_2}$  and markers of muscle activity, for example, changes in the surface electromyogram (EMG) of the *m. vastus lateralis* (chapter 7) and quadriceps  $^1H$  transverse relaxation time ( $T_2$ ) during very-heavy intensity exercise (chapter 8).

Chapter 9 will synthesise the key experimental findings and draw upon the novel contributions to studying developmental aspects of pulmonary  $\dot{V}_{O_2}$  kinetics, including future recommendations on how the methodologies utilised throughout this thesis might be extended in order to advance our understanding of oxidative metabolism within the field of paediatric exercise physiology.



## Chapter Two

### LITERATURE REVIEW

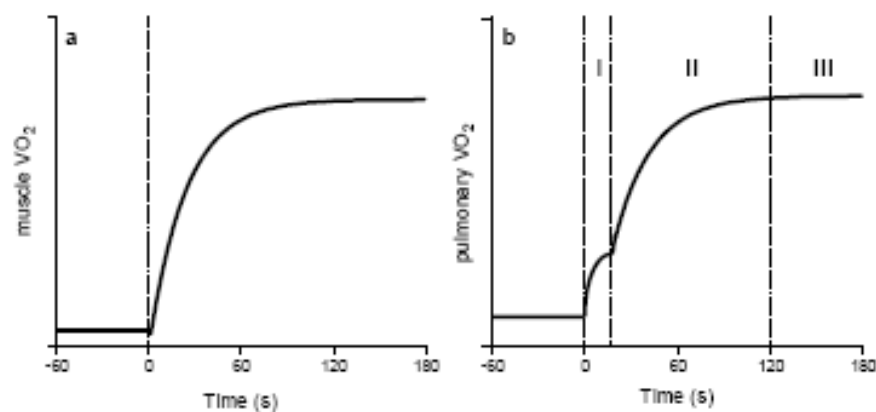
Upon a step increase to a higher metabolic rate, muscle  $O_2$  consumption ( $m\dot{V}_{O_2}$ ) rises following an exponential time course until the rate of oxidative ATP resynthesis is coupled to the rate of muscle ATP turnover resulting in a  $\dot{V}_{O_2}$  steady state during exercise below the lactate threshold (LT). The finite  $\dot{V}_{O_2}$  kinetic response mandates an obligatory contribution from muscle phosphocreatine (PCr) breakdown and glycolysis to augment the ATP requirement during the non-steady-state. The rapidity at which mitochondrial respiration is therefore able to adjust to an increase in metabolic demand is intimately related to the accumulated  $O_2$  deficit at the onset of exercise, with the kinetics of  $m\dot{V}_{O_2}$  providing an important index on the control of oxidative phosphorylation during growth and maturation.

#### **2.1 Oxygen uptake kinetics during exercise**

##### *2.1.1 The three phase $\dot{V}_{O_2}$ response*

Although simultaneous measurements of blood flow combined with arterial and venous blood sampling permit the resolution of  $m\dot{V}_{O_2}$  kinetics in humans, such techniques are invasive and ethically restricted in youth populations. Pulmonary  $O_2$  uptake ( $\dot{V}_{O_2}$ ) kinetics therefore provides a non-invasive tool to examine developmental influences on muscle oxidative function during childhood and adolescence (Armstrong & Barker, 2009). However, when estimating  $m\dot{V}_{O_2}$  kinetics based on pulmonary  $\dot{V}_{O_2}$  measurements there are several caveats that must be taken into consideration. Figure 2.1a illustrates that  $m\dot{V}_{O_2}$

increases instantaneously without any discernible delay in response to an abrupt increase in metabolic demand toward the steady-state requirement for the work rate (Behnke, et al., 2002). The mean rate of  $m\dot{V}_{O_2}$  is accurately reflected at the mouth (via the  $\dot{V}_{O_2}$  signal) under steady-state conditions in which the rate of mitochondrial ATP synthesis is coupled to the rate of muscle ATP turnover. During the non-steady state, however,  $\dot{V}_{O_2}$  is temporally dissociated from  $m\dot{V}_{O_2}$  due to the intervening blood volume between the muscle venous circulation and the pulmonary capillaries, and in magnitude due to the utilisation of  $O_2$  stores and possibly myoglobin desaturation in response to an abrupt increase in work rate (Whipp & Ward, 1990). Finally, the rate of change in  $\dot{V}_{O_2}$  will be dissociated relative to  $m\dot{V}_{O_2}$  as a given value for the arterial-venous  $O_2$  content difference ( $C_{(a-v)}O_2$ ) established at the muscle will be associated with a higher cardiac output ( $\dot{Q}$ ) by the time a reduced mixed-venous  $O_2$  tension ( $PO_2$ ) arrives at the lung (Whipp & Ward, 1990).



**Figure 2.1** Schematic of the muscle (a) and pulmonary oxygen uptake (b) profiles. The vertical dashed line at  $t = 0$  s represents an abrupt increase in external work rate. The three phase  $\dot{V}_{O_2}$  response is illustrated in (b). From Armstrong & Barker (2009) with permission. See text for further explanation.

The net consequence is that a mono-exponential increase in  $m\dot{V}_{O_2}$  is modified at the lung incorporating two intervening  $\dot{V}_{O_2}$  phases that precede the establishment of a  $\dot{V}_{O_2}$  steady-state (figure 2.1b). The seminal work of Brian J. Whipp and colleagues (1982) originally revealed a three-phase  $\dot{V}_{O_2}$  response when averaging several repetitions of moderate-intensity exercise (i.e. below the lactate threshold, LT) in order to eliminate the influence of inter-breath fluctuations on the  $\dot{V}_{O_2}$  signal. The authors proposed that an immediate rise in  $\dot{V}_{O_2}$  (phase I) was mediated via an increased  $\dot{Q}$  (and hence pulmonary blood perfusion) since an altered mixed-venous  $PO_2$  would not influence  $\dot{V}_{O_2}$  at the lung due to the muscle-to-lung transit delay at the onset of exercise. This is exemplified in the following Fick equation:

$$\dot{V}_{O_2} = \dot{Q} \cdot C_{(a-\bar{v})}O_2 \quad \text{Equation 2.1}$$

The initial phase I response has therefore been termed the cardio-dynamic region as the rise in  $\dot{V}_{O_2}$  (~ 15 s) reflects an increased  $\dot{Q}$  that occurs indiscriminately of any changes in muscle  $O_2$  utilisation at the onset of exercise. The demonstration that markedly slower changes in  $\dot{V}_{O_2}$  during phase I are manifest under exercise conditions which constrain the initial stroke volume response (for example, initiating exercise transitions from a higher work rate or in the supine posture) is consistent with this proposal (Hughson & Morrissey, 1982; Karlsson, Lindborg, & Linnarsson, 1975; Weiler-Ravell, Cooper, Whipp, & Wasserman, 1983). It is not until after the muscle-to-lung transit delay has elapsed that the influence of an expanded  $C_{(a-\bar{v})}O_2$  reflecting muscle  $O_2$  utilisation is signalled at the pulmonary capillaries. The increased end-tidal  $PCO_2$  and reduced  $PO_2$  elicits a rapid

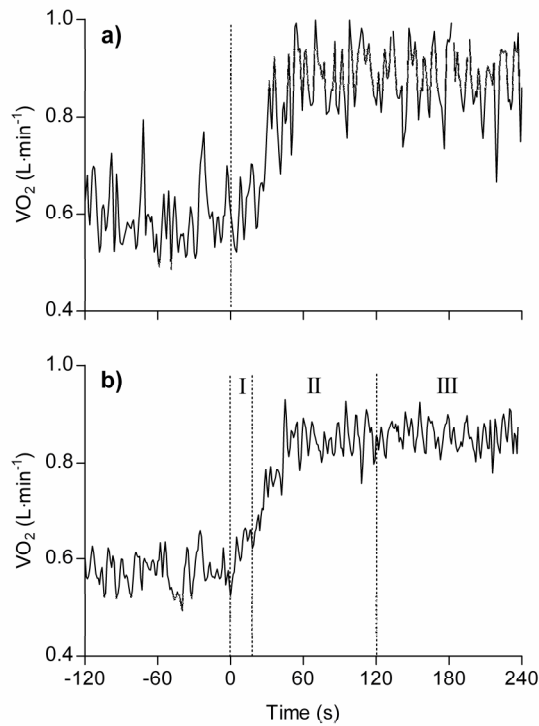
exponential rise in  $\dot{V}_{O_2}$  (phase II) toward the steady-state  $\dot{V}_{O_2}$  requirement (phase III) (Whipp & Ward, 1990; Whipp, et al., 1982).

### 2.1.2 Modelling the phase II $\dot{V}_{O_2}$ response in children

In order to provide an acceptable estimation of  $m\dot{V}_{O_2}$  kinetics, the exponential properties of phase II  $\dot{V}_{O_2}$  must be isolated and further characterised using appropriate mathematical modelling techniques (Armstrong & Barker, 2009). This is complicated in youth as an inherently low  $\dot{V}_{O_2}$  amplitude and larger inter-breath fluctuations conspire to produce a  $\dot{V}_{O_2}$  response with low signal-to-noise properties (Potter, Childs, Houghton, & Armstrong, 1999). Figure 2.2a illustrates a single exercise transition in a child in which the phase II  $\dot{V}_{O_2}$  region is not visually discernible as the increment in  $\dot{V}_{O_2}$  above baseline is low ( $\sim 0.3$  L $\cdot$ min $^{-1}$ ) in relation to the large breath-to-breath noise. It is only after enhancing the signal-to-noise ratio (through ensemble averaging repeated exercise transitions) that the three-phase  $\dot{V}_{O_2}$  response originally described by Whipp et al. (1982) can be revealed (figure 2.2b). The resulting  $\dot{V}_{O_2}$  response can be characterised using the following mathematical expression:

$$\dot{V}_{O_2(t)} = \Delta\dot{V}_{O_2SS} \cdot (1 - e^{-(t-TD)/\tau}) \quad \text{Equation 2.2}$$

where  $\dot{V}_{O_2(t)}$ ,  $\Delta\dot{V}_{O_2SS}$ , TD and  $\tau$  represent the value of  $\dot{V}_{O_2}$  at a given time, the amplitude change in  $\dot{V}_{O_2}$  from baseline to a new steady-state, time delay and the time constant, respectively.



**Figure 2.2** An example  $\dot{V}O_2$  response in a child during a single step exercise transition from unloaded pedalling to 40W (a) and after time-aligning and averaging six repeated transitions of the same work rate (b). Note how enhancing the  $\dot{V}O_2$  signal-to-noise properties reveal the phase II  $\dot{V}O_2$  portion in (b) that is purported to reflect  $m\dot{V}O_2$  kinetics at the onset of exercise.

Previous studies in children aged 9-12 yr have revealed that averaging 4-10 step exercise transitions yields 95% confidence intervals (CI) in the phase II  $\dot{V}O_2$   $\tau$  within approximately  $\pm 5$  s (Barker, Welsman, Fulford, Welford, Williams, et al., 2008; Fawkner, Armstrong, Potter, et al., 2002). A further consideration in the model described in equation 2.2 is to resolve the phase II  $\dot{V}O_2$   $\tau$  without contamination from the cardio-dynamic (phase I) response. This is most conveniently achieved by visually identifying the phase I – II interface and constraining the single-exponential fit to start at a time point that excludes the phase I portion. However, as illustrated in figure 2.2, the inherent breath-to-breath noise often obscures the duration of phase I in children despite averaging multiple exercise

transitions. Previous investigators have therefore either assigned an exponential term to characterise phase 1 (Williams, et al., 2001) or elected to omit the initial 15-25 s of data following the onset of exercise (Barker, Welsman, Fulford, Welford, & Armstrong, 2008; Fawcner, Armstrong, Potter, et al., 2002) in order to eliminate this portion of the response from the model fitting window. The latter approach might be considered justified in children since the duration of phase I is reported to approximate ~ 15 s in youth populations (Hebestreit, Kriemler, Hughson, & Bar-Or, 1998; Springer, et al., 1991) and in adults the capillary-to-lung mean transit time is ~ 10-12 s following the onset of exercise (Krustrup, Jones, Wilkerson, Calbet, & Bangsbo, 2009).

### *2.1.3 Relationship between $\dot{V}_{O_2}$ dynamics at the muscle vs. mouth*

Whilst averaging  $\dot{V}_{O_2}$  responses over repeated exercise transitions permit the resolution of phase II  $\dot{V}_{O_2}$  kinetics in children, ethical restrictions in directly measuring  $m\dot{V}_{O_2}$  has confined physiological inferences regarding the control of oxidative metabolism to be made at more remote sites (e.g. the lungs) relative to exercising muscle in youth populations. It is therefore important to experimentally verify that the phase II  $\dot{V}_{O_2}$   $\tau$  resolved from mathematical modelling provides an accurate representation of the rate at which  $m\dot{V}_{O_2}$  rises following the onset of exercise.

Grassi et al. (1996) performed simultaneous measurements of alveolar  $\dot{V}_{O_2}$  and leg  $\dot{V}_{O_2}$  ( $\dot{V}_{O_2 \text{ leg}}$ ) to examine the agreement between pulmonary  $\dot{V}_{O_2}$  and  $m\dot{V}_{O_2}$  kinetics during moderate-intensity cycling in six adults. Leg blood flow was measured using constant-infusion thermodilution and combined with arterial and femoral venous blood sampling to

resolve the kinetics of leg  $\dot{V}_{O_2}$  (assumed to reflect  $m\dot{V}_{O_2}$ ) using the Fick principle. The authors reported that the time taken to attain 50% of the steady-state  $\dot{V}_{O_2}$  amplitude above baseline pedalling (i.e. the response half-time,  $t^{1/2}$ ) was similar between  $\dot{V}_{O_2 A}$  and  $\dot{V}_{O_2 leg}$  at exercise onset ( $26 \pm 3$  vs.  $28 \pm 6$  s). During knee-extensor contractions, the  $\tau$  for phase II  $\dot{V}_{O_2}$  was also reported not to significantly differ from that of  $\dot{V}_{O_2 leg}$  in adults exercising above the ventilatory threshold (Koga, et al., 2005). However, it is conceivable that estimates for  $m\dot{V}_{O_2}$  kinetics were confounded in these studies due to the transit time for blood between the  $O_2$  sampling sites that was unaccounted for.

Krustrup et al. (2009) used similar techniques to quantify  $m\dot{V}_{O_2}$  responses but importantly incorporated the femoral artery-to-vein transit time in their calculations of  $m\dot{V}_{O_2}$  dynamics. The authors reported similar phase II  $\dot{V}_{O_2}$  kinetics in relation to  $m\dot{V}_{O_2}$  ( $\tau + TD$ ) during low- ( $30 \pm 3$  vs.  $30 \pm 3$  s) and high-intensity ( $32 \pm 3$  vs.  $29 \pm 4$  s) knee-extensor exercise in seven men (Krustrup, et al., 2009). The amplitude changes in  $\dot{V}_{O_2}$  above rest were also similar between the mouth and muscle over the primary component region. These findings therefore confirmed that the rate and amplitude of muscle  $O_2$  utilization were accurately reflected in the phase II  $\dot{V}_{O_2}$  signal at the onset of exercise.

## **2.2 Influence of exercise intensity on $\dot{V}_{O_2}$ responses**

### *2.2.1 The $\dot{V}_{O_2}$ slow component*

Whipp & Wasserman (1972) originally examined the influence of exercise work rate (50 – 175 W) on breath-by-breath  $\dot{V}_{O_2}$  responses in six adults. The authors reported that the

attainment of a  $\dot{V}_{O_2}$  steady-state was delayed or even unattainable at progressively higher exercise intensities due to the development of a secondary  $\dot{V}_{O_2}$  component that distorted a simple mono-exponential characterisation of the response. Moreover, an increased  $\Delta \dot{V}_{O_2}$  from the 3<sup>rd</sup> to 6<sup>th</sup> minute of exercise was manifest under conditions in which blood lactate concentration [La] remained elevated throughout the exercise bout. These results presented an early indication that an additional exponential term should be incorporated into the  $\dot{V}_{O_2}$  model fitting procedure at exercise intensities above the LT.

Casaburi et al. (1989) initially demonstrated that a double exponential model provided an improved fit of the  $\dot{V}_{O_2}$  response at work rates associated with an elevated [La] compared to a single-exponential function. The assumption, however, in this study that the primary and slow phases were initiated simultaneously (i.e. after a common time delay) from the onset of exercise was unfounded. It is therefore pertinent that modeling the  $\dot{V}_{O_2}$  slow component using an independent delay term (equation 2.3) reduced the squared error of the model residuals when fitted to  $\dot{V}_{O_2}$  data at higher exercise intensities (85-100 %  $\dot{V}_{O_2 \text{ max}}$ ) (Barstow & Mole, 1991). These findings confirmed that a  $\dot{V}_{O_2}$  slow component of delayed-onset was superimposed upon the primary  $\dot{V}_{O_2}$  response rather than elicited close to the onset of exercise (Barstow, 1994; Poole, Barstow, Gaesser, Willis, & Whipp, 1994). This reinforced the use of a mono-exponential function in conjunction with a constrained fitting window to resolve the primary  $\dot{V}_{O_2}$  parameters under conditions in which a  $\dot{V}_{O_2}$  slow component was observed.

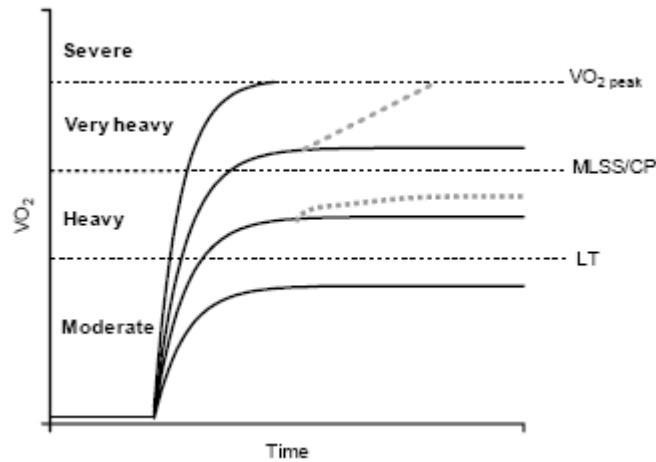


$$\dot{V}_{O_2(t)} = A_1 \cdot (1 - e^{-(t-TD_1)/\tau_1}) + A_2 \cdot (1 - e^{-(t-TD_2)/\tau_2}) \quad \text{Equation 2.3}$$

where  $\dot{V}_{O_2(t)}$  represent the value of  $\dot{V}_{O_2}$  at a given time,  $A_1$  and  $A_2$ ,  $\tau_1$  and  $\tau_2$ , and  $TD_1$  and  $TD_2$  are the amplitudes, time constants, and independent time delays of each exponential respectively. The metabolic consequence of the  $\dot{V}_{O_2}$  slow component is that the end-exercise  $O_2$  cost in relation to work rate (i.e. the  $\dot{V}_{O_2}$  gain) is extended above the predicted value based on the  $\Delta \dot{V}_{O_2} / \Delta WR$  relationship established below the LT.

### 2.2.2 *Exercise intensity domains*

In 1988, David Poole and colleagues extended the earlier work of Wassermann & Whipp (1972) and examined the  $\dot{V}_{O_2}$  slow component in relation to the critical power (CP) defined as the asymptote of the power/time-to-exhaustion hyperbola. The authors reported that the CP represented an important demarcation point at which the  $\dot{V}_{O_2}$  slow component and blood [La] would eventually stabilise leading to the delayed attainment of a  $\dot{V}_{O_2}$  steady-state below the participants'  $\dot{V}_{O_2 \max}$ . In contrast, exercising subjects at a power output ~15W above their CP precluded the establishment of a  $\dot{V}_{O_2}$  steady-state with an inexorable rise in  $\dot{V}_{O_2}$  and blood [La] over time resulting in the attainment of  $\dot{V}_{O_2 \max}$  (Poole, Ward, Gardner, & Whipp, 1988). Whipp & Rossiter (2005) have therefore proposed a schema to provide a frame of reference for investigating  $\dot{V}_{O_2}$  kinetics within discrete exercise intensity domains that are bounded by specific physiological parameters (figure 2.3).



**Figure 2.3** Schematic illustration of the  $\dot{V}_{O_2}$  profile within specific exercise intensity domains. Note that the primary  $\dot{V}_{O_2}$  amplitude results in the attainment of  $\dot{V}_{O_2 \max}$  during severe exercise whereas the  $\dot{V}_{O_2}$  slow component will eventually elicit  $\dot{V}_{O_2 \max}$  in the very heavy domain. Exercise below the CP enables the  $\dot{V}_{O_2}$  slow component to asymptote at a submaximal steady-state  $\dot{V}_{O_2}$  value that exceeds the predicted  $O_2$  cost from linear extrapolation of the  $\Delta\dot{V}_{O_2}/\Delta WR$  response established in the moderate domain. See text for further details. Reproduced from Armstrong & Barker (2009).

The moderate domain (M) is defined as all exercise intensities that fall below the LT or its non-invasive equivalents termed the gas exchange threshold (GET) or ventilatory threshold (VT). During moderate-intensity exercise  $\dot{V}_{O_2}$  rises, following phase I, with mono-exponential kinetics to attain a  $\dot{V}_{O_2}$  steady-state equivalent to an  $O_2$  cost of  $\sim 10 \text{ mL}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$  in healthy adults. For exercise above the LT but below the CP (heavy-intensity, H), the achievement of a steady state is delayed and the  $O_2$  cost of exercise is elevated over time ( $\sim 12\text{-}13 \text{ mL}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$ ) due to the development of the  $\dot{V}_{O_2}$  slow component. This excess  $\dot{V}_{O_2}$  represents a non-linear increase in the  $\Delta\dot{V}_{O_2}/\Delta WR$  relationship that alters the  $\dot{V}_{O_2}$  response from a simple mono-exponential function of time.

During exercise above the CP but below  $\dot{V}_{O_2 \max}$  (very-heavy intensity, VH), the  $\dot{V}_{O_2}$  slow component rises rapidly following the primary exponential phase resulting in the eventual

attainment of  $\dot{V}_{O_2 \max}$  and thereby restricting the tolerable duration of exercise in this domain. For exercise work rates estimated to require a  $\dot{V}_{O_2}$  at or above  $\dot{V}_{O_2 \max}$  (severe-intensity, S), the primary  $\dot{V}_{O_2}$  response elicits the attainment of  $\dot{V}_{O_2 \max}$  with the development of a restricted  $\dot{V}_{O_2}$  slow component due to the lower tolerable duration of exercise (~ 2 min).

## **2.3 Oxygen uptake kinetics in children and adolescents**

### *2.3.1 Equating the exercise intensity in children*

The profound temporal- and amplitude-based differences in the  $\dot{V}_{O_2}$  kinetic response (figure 2.3) indentifies the importance of prescribing exercise within equivalent intensity domains in order to derive valid physiological inferences regarding developmental influences on oxidative metabolism. Unfortunately, the paediatric literature is replete with exercise studies that have arbitrarily selected exercise work rates in relation to  $\dot{V}_{O_2 \text{ peak}}$ . This is problematic since there is considerable inter-subject variability in the occurrence of the LT as a fractional percentage of the  $\dot{V}_{O_2 \text{ peak}}$  in children (Reybrouck, Weymans, Stijns, Knops, & van der Hauwaert, 1985). It is therefore accepted that exercise work rate should be normalised in relation to the LT [or its non-invasive equivalent, the gas exchange threshold (GET)] to ensure similar blood acid-base and gas exchange responses during exercise in the M domain (Armstrong & Barker, 2009).

The difficulty in equating the metabolic stress above the LT is compounded in child populations due to lower absolute differences in  $\dot{V}_{O_2}$  between the LT and  $\dot{V}_{O_2 \text{ peak}}$  that compresses the range of work rates spanning the H-to-VH-intensity domains. Furthermore,

the intervening CP (the boundary that delineates the H and VH domains, figure 2.3) cannot be routinely measured in children due to the requirement for repeated exhaustive exercise bouts in order to establish this parameter. Previous investigators have therefore utilised the 'delta' ( $\Delta$ ) concept to prescribe exercise work rates as a percentage difference between the LT and  $\dot{V}_{O_2 \text{ peak}}$  in order to explore child-adult differences or longitudinal changes in  $\dot{V}_{O_2}$  kinetics (Armon, et al., 1991; Fawkner & Armstrong, 2004a; Williams, et al., 2001). However, without formal identification of the CP, selecting exercise work rates in the region of  $\Delta 40\text{-}50\%$  might render some participants exercising in the H and others in the VH domain.

To resolve these concerns, Fawkner & Armstrong (2002) exercised seventeen children (mean age  $10.3 \pm 0.4$  yr) to volitional exhaustion at three constant power outputs and estimated CP as the intercept of the linear power – time<sup>-1</sup> relationship. The authors reported CP to occur at  $\sim 70\text{-}82\%$   $\dot{V}_{O_2 \text{ peak}}$  in children [similar to previous reports in adults, e.g. Poole et al. (1988)] and later confirmed that exercise work rates estimated to require a  $\dot{V}_{O_2}$  of  $40\%\Delta$  resulted in most children exercising in the H domain (Fawkner & Armstrong, 2003). However, in order to resolve an age dependency on  $\dot{V}_{O_2}$  kinetics in the VH domain it is important to ensure that selected work rates are above the CP in children. From this perspective, it is pertinent to consider the data from two recent studies that has shed some light on this issue in youth (Barker, Bond, Toman, Williams, & Armstrong, in press; Williams, Dekerle, McGawley, Berthoin, & Carter, 2008). In these investigations, the authors estimated CP as described in Fawkner & Armstrong (2002) and predicted the  $\dot{V}_{O_2}$  required at this intensity from extrapolation of the linear relationship between  $\dot{V}_{O_2}$  and

power output during ramp exercise. This analysis revealed CP to occur at a  $\dot{V}_{O_2}$  corresponding to  $31 \pm 19\% \Delta$  (Barker et al., in press) and  $38 \pm 11\% \Delta$  (Williams et al., 2008) in a mixed sex group of adolescents. Moreover, exercising subjects 10% above this intensity resulted in an exorable rise in blood [La] over time and reduced the tolerable duration of exercise to ~50% of the time-to-exhaustion recorded at or 10% below CP (Barker, et al., in press; Williams, et al., 2008). These findings therefore support the utility of prescribing power outputs  $\geq 60\% \Delta$  in order to reinforce confidence that young people are exercising in the VH-intensity domain.

### 2.3.2 *Age influences during moderate exercise*

Cooper et al. (1985) initially reported similar  $\dot{V}_{O_2}$  kinetics between children ( $\tau_p = 27$  s) and teenagers ( $\tau_p = 28$  s) during rest-to-exercise transitions below the estimated LT. However, using a mono-exponential model in this study constrained to start at  $t = 0$  s (i.e. with no delay term) may have confounded interpretation of an age-dependency on  $\dot{V}_{O_2}$  kinetics during M exercise. Similar phase II  $\dot{V}_{O_2}$  kinetics were later demonstrated between children and adults when incorporating mathematical descriptors into the model fit to account for the muscle-to-lung transit delay (Springer, et al., 1991; Williams, et al., 2001). However, as noted by Fawkner and Armstrong (2002), the low number of repeated exercise transitions in these studies may have been insufficient to overcome the low  $\dot{V}_{O_2}$  signal-to-noise responses in children. Fawkner et al. (2002) therefore utilized the procedures outlined by Lamarra et al. (1987) to estimate the number of repeated exercise transitions required in order to yield  $\tau_p$  values within acceptable 95% confidence limits. The authors reported markedly faster phase II  $\dot{V}_{O_2}$  kinetics in boys compared to men ( $\tau_p = 19 \pm 2$  s vs.  $28 \pm 9$  s)

and therefore concluded an enhanced potential for oxidative metabolism in youth during exercise below the GET (Fawkner, Armstrong, Potter, et al., 2002).

### 2.3.3 Youth responses during heavy/very heavy exercise

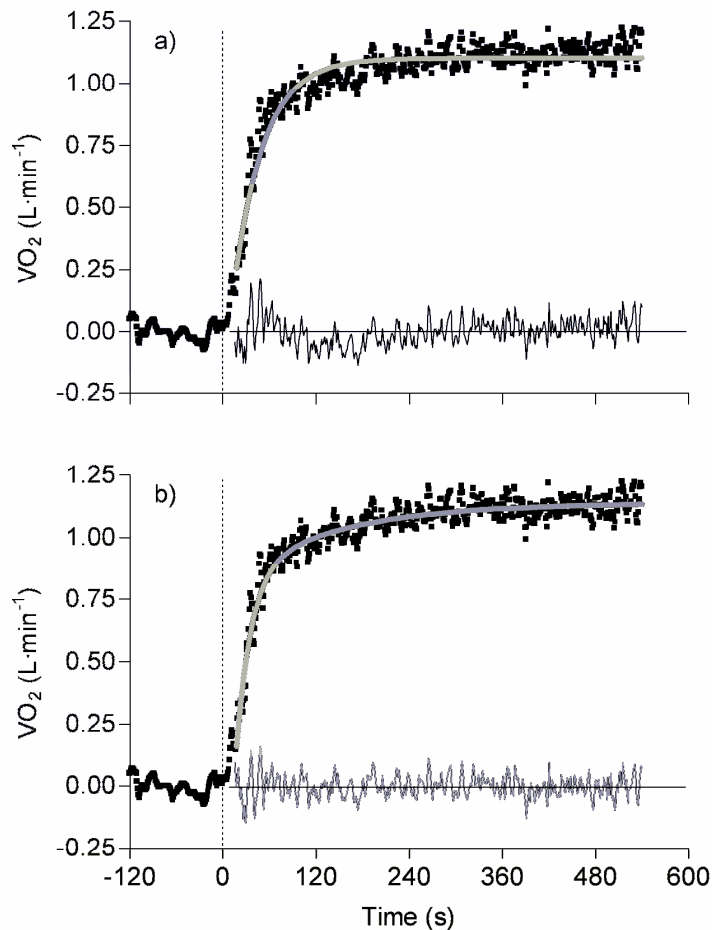
In the first study to investigate the  $\dot{V}_{O_2}$  slow component in youth, Armon et al. (1991) exercised six children at work rates above the GET in order to examine age influences on  $\dot{V}_{O_2}$  kinetics during heavy/very heavy exercise. The authors modeled  $\dot{V}_{O_2}$  data from the 3<sup>rd</sup> to 6<sup>th</sup> minute of exercise ( $\dot{V}_{O_2, 6-3}$ ) using linear regression to derive  $b$  coefficient values that were subsequently normalized per body mass to quantify the  $\dot{V}_{O_2}$  slow component. The  $\dot{V}_{O_2, 6-3}$  slope increased in adults from  $\Delta 25\%$  to  $\Delta 75\%$  (0.48 to 1.76 mL·kg<sup>-1</sup>·min<sup>-1</sup>) resulting in a greater total  $\dot{V}_{O_2}$  gain ( $G_{tot}$ ) at these work rates compared to M-intensity exercise. In contrast, children demonstrated a lower mean  $\dot{V}_{O_2, 6-3}$  slope (~ 0.24 mL·kg<sup>-1</sup>·min<sup>-1</sup>) and an invariant  $G_{tot}$  at  $\Delta 75\%$  compared to 80% GET (12.7 vs. 13.1 mL·kg<sup>-1</sup>·min<sup>-1</sup>). Williams et al. (2001) subsequently reported a similar  $\dot{V}_{O_2}$  response in 11-12 yr old boys during H treadmill running ( $\Delta 50\%$ ). In this study, children attained a greater  $G_{tot}$  compared to adults through an increased primary  $\dot{V}_{O_2}$  gain ( $G_p$ ) since the  $\dot{V}_{O_2}$  slow component contributed <1% to the total  $\Delta \dot{V}_{O_2}$  above rest in boys. The authors also reported a ~28% speeding in the  $\tau_p$  in boys ( $15 \pm 1$  s) compared to men ( $19 \pm 2$  s) at the onset of H exercise.

Earlier reports were interpreted to suggest that children, unlike adults, did not produce a  $\dot{V}_{O_2}$  slow component during exercise above the GET (Armon, et al., 1991; Williams, et al., 2001). However, the study of Williams et al. (2001) was conducted in treadmill running and therefore comparison with cycling exercise is complicated due to the different

contraction regimens involved between these test modalities (Carter, et al., 2000). Furthermore, it was conceivable that an arbitrary linear term fitted between the 3<sup>rd</sup> and 6<sup>th</sup> minute of exercise in Armon et al. (1991) misrepresented the actual  $\dot{V}_{O_2}$  slow component amplitude in children given the considerable inter-subject variability in the TD for this parameter (Barstow & Mole, 1991; Ozyener, Rossiter, Ward, & Whipp, 2001; Paterson & Whipp, 1991). An additional consideration is the intensity of the imposed exercise bout when attempting to detect a  $\dot{V}_{O_2}$  slow component amplitude in youth. For example, in the case of Hebestreit et al. (1998), the reduced tolerable duration of exercise at work rates prescribed at or above children's  $\dot{V}_{O_2\text{ peak}}$  (i.e. during severe exercise, see section 2.3.4) would have likely restricted the development of a delayed-onset  $\dot{V}_{O_2}$  slow component under these circumstances. Indeed, perusal of the individual  $\dot{V}_{O_2}$  responses from Armon et al. (1991), as noted by (Fawkner & Armstrong, 2004a), revealed a positive  $\dot{V}_{O_2\text{ 6-3}}$  slope in 73% of children studied during intense ( $\Delta 50\%$ ) submaximal exercise. Likewise, Obert et al. (2000) reported a pronounced linear rise in  $\dot{V}_{O_2}$  (mean slope =  $0.86 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) superimposed onto the primary  $\dot{V}_{O_2}$  component in twenty-three 10-13 yr old children during constant work rate exercise at 90% of the peak work rate attained from an incremental ramp test. However, in this study, the modelling of a single exercise transition confounded meaningful interpretation of the resolved phase II  $\tau$  in children.

In light of the aforementioned concerns, Fawkner & Armstrong (2004b) were prompted to apply different mathematical models in order to establish an optimum curve fitting procedure that appropriately characterized the  $\dot{V}_{O_2}$  responses in sixty-two 10-15 yr old children during H ( $\Delta 40\%$ ) cycling exercise. The authors reported that a double-exponential

model (equation 2.3) resulted in a superior fit of the  $\dot{V}_{O_2}$  response in 77% of children as indicated by a significant reduction in the sum of squared model residuals compared to a single-exponential function (figure 2.4).

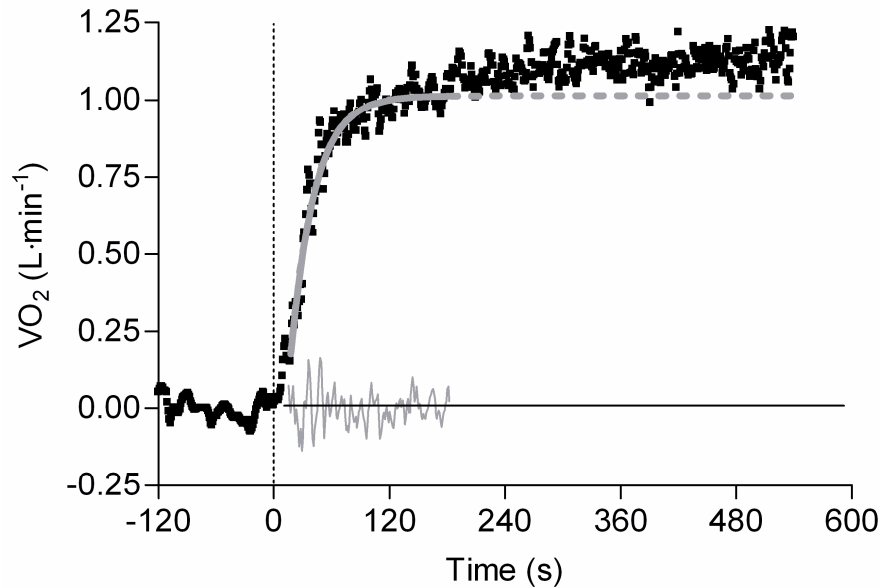


**Figure 2.4** Mathematical modelling of  $\dot{V}_{O_2}$  data in response to H exercise ( $\Delta$  40%) in an 11 yr-old child. Solid grey lines denote the model fit and the corresponding residual plots. Data are modeled using a single-exponential (>phase I) to the end of exercise (a) and using a double-exponential function as described in *equation 2.3* (b). Note the improved fit of the  $\dot{V}_{O_2}$  response from analysis of the residual plots in (b) compared to (a).

Fawkner & Armstrong (2004b) therefore confirmed a similar two-component  $\dot{V}_{O_2}$  response in children compared to adults exercising in the H domain. However, as cautioned by Whipp & Rossiter (2005), including an additional exponential term within a single model (figure 2.5b) can increase the inter-dependency between the estimated phase II and



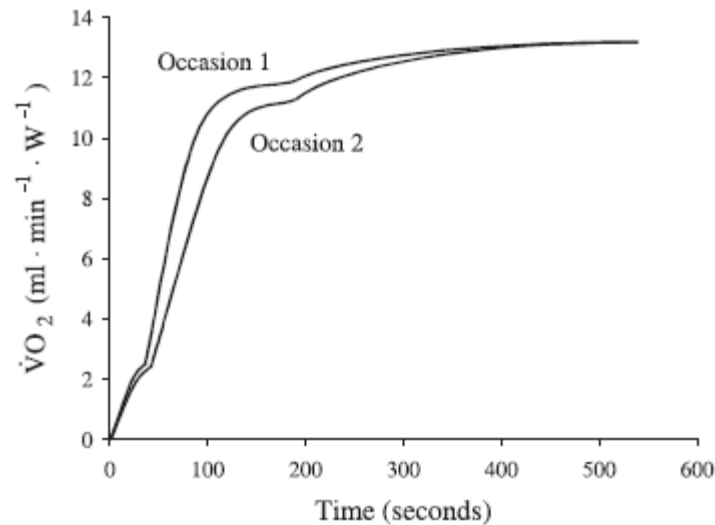
slow component parameters from the model fit. The procedures outlined by Rossiter et al. (2001) have therefore been recommended to independently model the phase II  $\dot{V}_{O_2}$  region within a predetermined fitting window excluding the  $\dot{V}_{O_2}$  slow component in order to resolve the  $\tau_p$  with greater statistical confidence in children (figure 2.5).



**Figure 2.5**  $\dot{V}_{O_2}$  data fitted using a single-exponential model (>phase I) within a predetermined fitting window excluding the  $\dot{V}_{O_2}$  slow component. Dashed grey line represents the phase II model fit extended to illustrate the magnitude of the  $\dot{V}_{O_2}$  slow component (TD = 180 s). This modelling technique reduced the 95% CI window for the  $\tau_p$  to within 4 s compared to 6 s using a double-exponential function.

Using the model illustrated in figure 2.6, Fawkner & Armstrong (2004a) provided the only study to date to investigate longitudinal changes in  $\dot{V}_{O_2}$  kinetics during H exercise in 22 pre-pubertal children (13 boys and 9 girls). Over a 2-yr period, the authors reported a proportionally greater contribution from the  $\dot{V}_{O_2}$  slow component to the overall  $\Delta\dot{V}_{O_2}$  above baseline in boys and girls. Despite a similar  $G_{tot}$  between test occasions, children demonstrated a reduced  $G_p$  and an extended  $\tau_p$  on the 2<sup>nd</sup> compared to the 1<sup>st</sup> laboratory visit

(figure 2.6). These findings were consistent with earlier cross-sectional evidence in terms of an age-related modulation in  $\dot{V}_{O_2}$  kinetics during H/VH exercise (Armon, et al., 1991; Williams, et al., 2001).



**Figure 2.6** Longitudinal changes in  $\dot{V}_{O_2}$  kinetics during H intensity cycling exercise ( $\Delta$  40%) in 22 children. Mean  $\dot{V}_{O_2}$  responses are plotted normalized per unit increase in work rate on test occasion 1 (aged 11 yr) and test occasion 2 (aged 13 yr). From Fawcner and Armstrong (2004a) with permission.

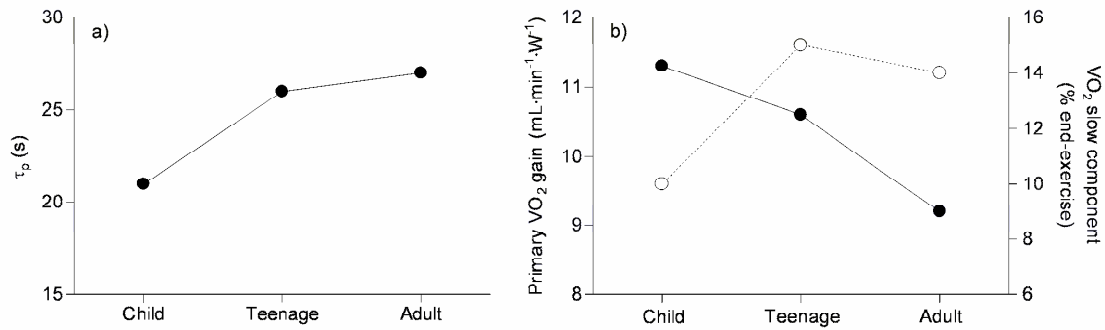
#### 2.3.4 Age influences on $\dot{V}_{O_2}$ kinetics during severe exercise

Hebestreit et al. (1998) investigated the  $\dot{V}_{O_2}$  kinetic response during S exercise in nine boys (aged 9-12 yr) and eight men (aged 19-27 yr). Modeling of the  $\dot{V}_{O_2}$  responses revealed similar phase II  $\dot{V}_{O_2}$  kinetics between boys and men exercising at 100%  $\dot{V}_{O_2 \text{ peak}}$  ( $\tau = 28 \pm 6$  vs.  $28 \pm 4$  s) and 130%  $\dot{V}_{O_2 \text{ peak}}$  ( $\tau = 20 \pm 4$  vs.  $21 \pm 6$  s) with no age-related differences reported in the accumulated  $O_2$  deficit ( $\text{mLO}_2 \cdot \text{kg}^{-1}$ ) during S exercise. These data were interpreted to indicate a similar energy liberation from  $O_2$ -independent mechanisms (muscle PCr hydrolysis and anaerobic glycolysis) in children compared to adults at higher exercise intensities (Hebestreit, et al., 1998). However, an invariant  $G_p$  at

50% and 100%  $\dot{V}_{O_2 \text{ peak}}$  in boys (10.9 vs. 10.4 mL·min<sup>-1</sup>·W<sup>-1</sup>) but not in men (9.7 vs. 8.3 mL·min<sup>-1</sup>·W<sup>-1</sup>) suggested an augmented oxidative ATP flux over the primary exponential phase during exercise  $\geq \dot{V}_{O_2 \text{ peak}}$  in children.

### 2.3.5 Summary of age-related differences in $\dot{V}_{O_2}$ kinetics

Based on the available literature, there is a consensus that younger children demonstrate an enhanced capacity to rapidly transport and utilize O<sub>2</sub> in response to a step increase in exercise work rate compared to older counterparts. An augmented  $\Delta \dot{V}_{O_2} / \Delta WR$  response from the onset of exercise above the GET also results in a reduced  $\dot{V}_{O_2}$  slow component in youth compared to adults. Unfortunately, differences in the procedures used to model  $\dot{V}_{O_2}$  responses allied to inconsistencies in the selection of exercise intensities have confounded interpretation of an age-dependent modulation in oxidative metabolism. Figure 2.7 therefore provides an overview of the  $\dot{V}_{O_2}$  kinetic parameters in relation to age during H/VH cycling exercise. A total of nine studies were reviewed ( $n = 113$ ) that used similar modeling procedures to resolve the  $\tau_p$  with the  $\dot{V}_{O_2}$  slow component amplitude normalized in relation to the total  $\Delta \dot{V}_{O_2}$  above baseline pedaling. In order to reduce the potential confounding influence of mixed sex groups, data are presented for male subjects only with mean  $\dot{V}_{O_2}$  values grouped in relation to chronological age (child: 8-12 yr, teenage: 13-17 yr, and adult: >21 yr).



**Figure 2.7** Age modulation of  $\dot{V}O_2$  kinetic parameters during H/VH step exercise transitions in children ( $n = 30$ ), teenagers ( $n = 31$ ), and adults ( $n = 52$ ). Mean  $\tau_p$  values are presented in (a) with age differences in the primary  $O_2$  cost of exercise ( $\bullet$ ) and relative  $\dot{V}O_2$  slow component amplitude ( $\circ$ ) shown in (b). See text for further details.

Figure 2.7 reveals an overall 29% slowing of the  $\tau_p$  with increasing age and 23% reduction in the  $G_p$  in adults compared to children. There is also a proportionally greater contribution from the  $\dot{V}O_2$  slow component to the overall  $\Delta\dot{V}O_2$  in older compared to younger counterparts reaching maturity during adolescence. Unfortunately, to date, the physiological mechanism(s) that underpin developmental alterations in  $\dot{V}O_2$  kinetics are unresolved with few experimental data available in youth. The proceeding sections will therefore present an overview of the physiological factors proposed to regulate the phase II  $\dot{V}O_2$  (and by inference  $m\dot{V}O_2$ ) response in adults.

## 2.4 Physiological interpretation of phase II $\dot{V}O_2$ kinetics

Further insight into the delayed matching of oxidative ATP resynthesis to the rate of ATP turnover occurring within the myocyte with increasing age can be explored using the general equation for oxidative phosphorylation:



where, NADH and NAD<sup>+</sup> represent the reduced and oxidized forms of the nicotinamide adenine dinucleotide (NAD) carriers. From equation 2.4, the factors that could potentially limit the rise in  $m\dot{V}_{O_2}$  at the onset of exercise therefore include: 1) the transport and delivery of O<sub>2</sub> to mitochondria; 2) ‘feed-forward’ provision of oxidizable substrate (in the form of NADH) into the electron transport chain; and/or 3) ‘feedback’ control linked to the rise in ADP and P<sub>i</sub> released from ATP hydrolysis at the myofibrils. The latter two mechanisms implicate that intracellular factors restrict the O<sub>2</sub> utilisation potential whereas the former identifies a specific rate-limiting step in the O<sub>2</sub> cascade from the lungs (alveoli) to mitochondria controlling  $m\dot{V}_{O_2}$  kinetics. Although data are limited in children, there have been several experimental attempts to manipulate one of the components listed in equation 2.4 in order to further elucidate the factors principally regulating oxidative metabolism in adults.

#### *2.4.1 Muscle O<sub>2</sub> delivery inertia*

Several previous studies have demonstrated that restricting muscle O<sub>2</sub> supply can alter  $\dot{V}_{O_2}$  kinetics during exercise in humans. For example, breathing hypoxic gas mixtures has been demonstrated to extend the  $\tau_p$  during cycling exercise compared to normoxia in adults and children (Engelen, et al., 1996; Springer, et al., 1991). Furthermore, reducing the “gravitational assist” to muscle blood flow under conditions in which the recruited muscle mass is at or above the heart level has been demonstrated to slow  $\dot{V}_{O_2}$  kinetics during arm cranking (Koppo, Bouckaert, & Jones, 2002; Winlove, Jones, & Welsman, 2009) and exercise in the supine compared to upright body posture (Jones, Berger, Wilkerson, & Roberts, 2006; Koga, et al., 1999; M. J. MacDonald, Shoemaker, Tschakovsky, &

Hughson, 1998). These findings therefore indicated that diffusive components of muscle O<sub>2</sub> delivery, shown to contribute to the limitation of  $\dot{V}_{O_2 \max}$  in humans (Richardson, Tagore, Haseler, Jordan, & Wagner, 1998; Wagner, 2000), might constrain  $\dot{V}_{O_2}$  kinetics at the onset of step transitions to a higher exercise work rate.

Although restricting muscle O<sub>2</sub> delivery can potentially slow  $\dot{V}_{O_2}$  kinetics, evidence that O<sub>2</sub> availability is rate limiting infers that enhancing O<sub>2</sub> transport should speed the rate of adjustment in  $\dot{V}_{O_2}$  toward the projected steady-state requirement (Poole, Barstow, McDonough, & Jones, 2008). From this perspective, Grassi et al. (1998) have demonstrated that pump-perfusing isolated canine muscle in order to remove any delay in muscle O<sub>2</sub> delivery across rest-to-exercise transitions resulted in an unchanged  $\tau$  during contractions requiring 60%  $\dot{V}_{O_2 \max}$ , with only a ~ 10% speeding evident at 100%  $\dot{V}_{O_2 \max}$  (Grassi, et al., 2000). Using the same experimental preparation, enhancing peripheral O<sub>2</sub> diffusion did not influence  $\dot{V}_{O_2}$  kinetics following pharmacological intervention and hyperoxia to promote O<sub>2</sub> offloading at the muscle (Grassi, Gladden, Stary, Wagner, & Hogan, 1998). These findings therefore implicated a metabolic inertia independent of alterations in muscle O<sub>2</sub> supply in regulating  $\dot{V}_{O_2}$  kinetics at the onset of exercise.

#### *2.4.1.1 Influence of enhancing muscle O<sub>2</sub> availability*

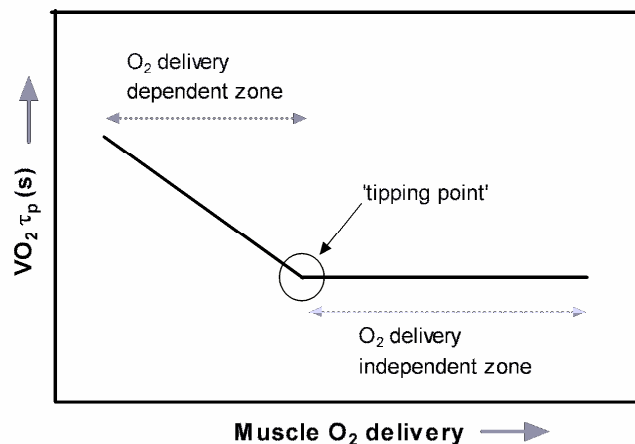
It has been suggested that prior or ‘priming’ exercise that induces a metabolic acidosis (i.e. above the LT) increases muscle vasodilation and accentuates a rightward shift in the HbO<sub>2</sub> dissociation curve thereby increasing muscle O<sub>2</sub> availability in humans following the onset of a second exercise bout (Jones, Koppo, & Burnley, 2003). Earlier reports that prior H

exercise speeded  $\dot{V}_{O_2}$  kinetics during step transitions above (but not below) the GET were therefore interpreted to suggest that an inadequate muscle  $O_2$  perfusion might limit  $\dot{V}_{O_2}$  kinetics at higher exercise work rates (Gerbino, Ward, & Whipp, 1996; M. MacDonald, Pedersen, & Hughson, 1997). It was unclear however whether a priming-induced speeding of the overall  $\tau$ , resolved using a monoexponential function extended to end-exercise, was linked to a shortened  $\tau_p$  or to changes in the primary and/or  $\dot{V}_{O_2}$  slow component amplitudes.

Burnley et al. (2000, 2002) therefore delineated the primary  $\dot{V}_{O_2}$  phase from the  $\dot{V}_{O_2}$  slow component using double-exponential modeling procedures in order to examine priming influences on the  $\tau_p$  during exercise above the GET. The authors reported that an overall speeding of  $\dot{V}_{O_2}$  kinetics following prior H exercise resulted from an increased primary and lower  $\dot{V}_{O_2}$  slow component amplitude rather than to changes in the  $\tau_p$  which remained unchanged (Burnley, Doust, Ball, & Jones, 2002; Burnley, Jones, Carter, & Doust, 2000). These data therefore indicated that phase II  $\dot{V}_{O_2}$  kinetics were independent of muscle  $O_2$  availability during upright exercise in the H/VH domains in young adults. The demonstration of an unaltered  $\tau_p$  following the administration of recombinant human erythropoietin or under hyperoxic conditions during exercise further corroborated the view that muscle  $O_2$  delivery was not rate limiting even at exercise work rates requiring  $\geq \dot{V}_{O_2 \text{ peak}}$  (Wilkerson, Berger, & Jones, 2006; Wilkerson, Rittweger, Berger, Naish, & Jones, 2005).

### 2.4.1.2 The ‘tipping point’ hypothesis

Although interventions purported to enhance muscle O<sub>2</sub> delivery have been demonstrated not to speed the  $\tau_p$  in humans (Burnley, et al., 2002; Burnley, et al., 2000; c.f. Scheuermann, Hoelting, Noble, & Barstow, 2001; Wilkerson, et al., 2006; Wilkerson, et al., 2005), it is important to consider that participants selected in these investigations were predominately young men with  $\dot{V}_{O_2 \max}$  values of  $\sim 50 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ . Previous studies have elicited faster phase II  $\dot{V}_{O_2}$  kinetics when increasing muscle oxygenation at the onset of exercise in older men (aged  $\sim 65\text{-}70$  yr) with a lengthened  $\tau_p$  ( $\sim 40$  s) compared to younger counterparts (DeLorey, Kowalchuk, & Paterson, 2004; Gurd, et al., 2009). Poole & Jones (2005) have therefore proposed a model illustrated in figure 2.8 that identifies a ‘tipping point’ in relation to which altering O<sub>2</sub> delivery might influence the  $\tau_p$  during exercise.



**Figure 2.8** Schematic of the ‘tipping point’ hypothesis, which outlines the dependence of phase II  $\dot{V}_{O_2}$  kinetics in relation to O<sub>2</sub> delivery. Adapted from Poole and Jones (2005). The proximity to the tipping point of an exercising healthy individual during upright cycling is currently debated [see Poole et al. (2008) for a review]

The model proposes that an exercising individual will be positioned in the ‘O<sub>2</sub> delivery independent zone’ under circumstances in which enhancing muscle O<sub>2</sub> availability does not



elicit a speeding of phase II  $\dot{V}_{O_2}$  kinetics (e.g. during upright exercise). In contrast, restricting  $O_2$  delivery to mitochondria (i.e. moving from right-to-left on the horizontal axis) in the supine posture or under hypoxic exercise conditions ‘sensitizes’ the  $\tau_p$  to alterations in muscle  $O_2$  supply once the tipping point has been reached. This is exemplified when considering the interaction between body posture and priming influences on  $\dot{V}_{O_2}$  kinetics during H-intensity exercise transitions (DiMenna, Wilkerson, Burnley, Bailey, & Jones, 2010; Jones, et al., 2006). It has been demonstrated, for example, that a lengthened phase II  $\tau$  during supine compared to upright cycling is corrected when transitions in the former condition are primed to alleviate a reduction in muscle  $O_2$  perfusion extant in the supine posture (DiMenna, Wilkerson, et al., 2010; Jones, et al., 2006).

Whilst figure 2.8 indicates that  $O_2$  delivery might be rate *limiting* under specific circumstances, Tschakovsky & Hughson (1999) had proposed that muscle  $O_2$  supply would *regulate*  $\dot{V}_{O_2}$  kinetics in *all* exercise conditions. Specifically, at a constant rate of ATP utilization during steady-state exercise, alterations in the intracellular  $PO_2$  dictates the reduction in the cellular energetic state (i.e. the sum of ATP + PCr) required to maintain a given rate of oxidative ATP flux (Hughson, Tschakovsky, & Houston, 2001). Wilson and colleagues [see (1994) for a review] originally demonstrated *in vitro* that a fall in the intracellular  $PO_2$  below 30 mmHg mandated a greater perturbation of the phosphorylation potential ( $[ATP]/[ADP] \cdot [Pi]$ ) to drive mitochondrial respiration. The demonstration that muscle PCr breakdown is reduced during hyperoxic gas inspiration compared to normoxia during steady-state plantar flexor contractions is consistent with this proposal (Haseler, Richardson, Videen, & Hogan, 1998). It is therefore conceivable, since intracellular  $PO_2$  has been reported to fall from rest (30 mmHg) to below 5 mmHg within the initial 20 s of

muscle contractions (Richardson, et al., 2006; Richardson, Noyszewski, Kendrick, Leigh, & Wagner, 1995), that muscle O<sub>2</sub> availability could modulate the cellular energetic state during transitions from lower to higher metabolic rates.

To further explore this proposal, Haseler et al. (2004) compared muscle PCr kinetics under different conditions of fractional inspired O<sub>2</sub> during plantar flexor contractions at ~ 60 % maximum power output. The authors reported that increasing the driving pressure for capillary-to-myocyte O<sub>2</sub> diffusion did not elicit a speeding of the muscle PCr  $\tau$  during exercise (Haseler, Kindig, Richardson, & Hogan, 2004). Jones et al. (2008) have also reported an unchanged muscle PCr  $\tau$  during high-intensity knee-extension exercise despite increased muscle oxygenation following prior exercise. These studies demonstrated that a sparing in muscle [PCr] across the rest-to-exercise transient was principally related to a reduction in the PCr slow component and not due to the initial kinetic fall in the cellular energetic state. These findings were therefore consistent with an invariant  $\dot{V}_{O_2} \tau_p$  under conditions of enhanced O<sub>2</sub> availability (Burnley, et al., 2002; Burnley, et al., 2000; Wilkerson, et al., 2006; Wilkerson, et al., 2005) and hence supported the notion that under 'normal' exercise conditions participants were positioned to the right of the tipping point in figure 2.8.

#### *2.4.1.3 Muscle fibre type-specific influences*

The Fick equation dictates that the rise in  $\dot{V}_{O_2}$  at exercise onset is the product of blood flow delivery ( $\dot{Q}_{O_2}$ ) and the  $C_{(a-\bar{v})}O_2$  difference across exercising muscle. A pronounced time delay (~ 19 s) before a reduction in the microvascular  $PO_2$  (an index of fractional muscle O<sub>2</sub> extraction) has therefore been interpreted to indicate that  $\dot{Q}_{O_2}$  is matched to the

rate of  $O_2$  utilization at the onset of contractions in animal muscle (Behnke, et al., 2002). However, there is evidence to indicate a fibre type-specific dependency on the  $\dot{Q}_{O_2}$ -to- $\dot{V}_{O_2}$  relationship [see Poole, Barstow, McDonough, & Jones (2008) for a recent review]. For example, studies utilizing phosphorescence quenching have demonstrated a faster reduction in microvascular  $PO_2$  (shorter TD and  $\tau$ ) in fast-twitch compared to slow-twitch animal muscle during electrical stimulation. Furthermore, the amplitude change in microvascular  $PO_2$  across the rest-to-exercise transient was greater in muscle comprising predominately of type II compared to type I fibres (Behnke, McDonough, Padilla, Musch, & Poole, 2003; McDonough, Behnke, Padilla, Musch, & Poole, 2005). These findings could have profound implications on restricting muscle  $O_2$  availability at the onset of H/VH exercise in humans (Engelen, et al., 1996; Jones, et al., 2006; Koga, et al., 1999). If, for example, the external power output required an increased contribution from type II motor units, then limiting  $O_2$  supply (e.g. under hypoxia or during supine exercise) might be expected to exert an especially pernicious influence on blood-to-myocyte  $O_2$  diffusion in muscle fibres already disadvantaged in terms of a lower  $\dot{Q}_{O_2}/\dot{V}_{O_2}$  response. Conversely, enhancing muscle  $O_2$  availability (e.g. under hyperoxia or following prior exercise) might improve  $\dot{Q}_{O_2}$ -to- $\dot{V}_{O_2}$  matching within higher-order fibre pools and augment the rise in  $\dot{V}_{O_2}$  at the onset of exercise. Although this appears not to influence the  $\tau_p$ , it might explain the elevated  $G_p$  and reduced  $\dot{V}_{O_2}$  slow component amplitude following interventions presumed to increase muscle  $O_2$  supply (Burnley, et al., 2000; Wilkerson, et al., 2006). Based on these considerations, a recent review (Poole, et al., 2008) has therefore proposed that type I and II muscle fibres might operate concomitantly on the right ( $O_2$  independent)

and left ( $O_2$  dependent) respectively of the proposed tipping point for muscle  $O_2$  delivery illustrated in figure 2.8.

#### 2.4.2 *Muscle metabolic inertia*

The lack of studies reporting a speeding of phase II  $\dot{V}_{O_2}$  kinetics in response to interventions that enhance muscle  $O_2$  availability has been interpreted to suggest that intrinsic metabolic factors principally limit the rate at which  $\dot{V}_{O_2}$  can adapt to an increase in metabolic demand (Grassi, 2005). This proposal has received further support from experimental techniques that permit an insight into the  $\dot{Q}_{O_2}/\dot{V}_{O_2}$  relationship during exercise. In human muscle, for example, there is an initial delay prior to the desaturation of oxyhemoglobin estimated using near-infrared spectroscopy (NIRS) at the onset of M and H cycling exercise (DeLorey, Kowalchuk, & Paterson, 2003; Grassi, et al., 2003). This would predict that an increased blood flow response (due to the rapid action of the muscular pump and active vasodilation) can satisfy the rise in  $\dot{V}_{O_2}$  at the onset of exercise therefore suggesting that  $O_2$  delivery is not rate limiting under these conditions. Considerable research interest has therefore been devoted into elucidating the precise loci of an intracellular inertia that might restrict the rate of oxidative metabolism.

##### 2.4.2.1 *NADH availability*

One possibility is that the transport of acetylcarnitine units into the tricarboxylic acid cycle might restrict the availability of reduced NADH substrate for oxidation in the electron transport chain (Timmons, Gustafsson, Sundberg, Jansson, & Greenhaff, 1998). Timmons et al. (1998) initially reported that arterially infusing dichloroacetate (DCA) to up-regulate the pyruvate dehydrogenase (PDH) enzyme resulted in a marked reduction in the anaerobic

energy expended during submaximal exercise in humans. The authors interpreted a reduced blood [La] and attenuated fall in muscle [PCr] during exercise following DCA infusion to infer that muscle oxidative metabolism was speeded in this condition. However, in human and canine muscle, DCA infusion has been shown not to speed either  $m\dot{V}_{O_2}$  or phase II  $\dot{V}_{O_2}$  kinetics during high-intensity exercise ( $\sim 80\text{-}110\% \dot{V}_{O_2 \text{ peak}}$ ) therefore indicating that an intracellular oxidative inertia is unlikely to reside at the PDH complex (Grassi, et al., 2002; Jones, Koppo, Wilkerson, Wilmshurst, & Campbell, 2004). The demonstration, however, of a reduced primary  $\dot{V}_{O_2}$  and muscle PCr amplitude has been interpreted to suggest that a sparing of the  $O_2$  deficit might be linked to a reduced ATP cost of muscle contraction (i.e. improved metabolic efficiency) following activation of the PDH enzyme (Rossiter, et al., 2003).

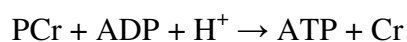
#### *2.4.2.2 Inhibition of mitochondrial respiration*

It is known that nitric oxide (NO) is an important regulator of mitochondrial respiration by acting as an inhibitor of cytochrome c oxidase in competition with  $O_2$  within the electron transport chain (Brown, 2000). Additionally, endothelium-dependent NO synthesized by nitric oxide synthase (NOS) is reported to function as part of a feedback mechanism enabling a higher intracellular  $PO_2$  to be maintained through active vasodilatation and  $\dot{V}_{O_2}$  inhibition (Kindig, McDonough, Erickson, & Poole, 2001). This has prompted several investigations to inhibit NOS production by injecting the L-arginine analogue nitro-L-arginine methyl ester (L-NAME) in order to investigate an oxidative metabolic inertia in animal and human muscle (Grassi, Hogan, Kelley, Howlett, & Gladden, 2005; Jones, Wilkerson, Koppo, Wilmshurst, & Campbell, 2003; Jones, Wilkerson, Wilmshurst, & Campbell, 2004; Kindig, et al., 2001; Wilkerson, Campbell, & Jones, 2004).

The administration of L-NAME has been demonstrated to result in faster phase II  $\dot{V}_{O_2}$  kinetics at the onset of M and H step exercise with more profound influences at higher work rates requiring  $\sim 105\% \dot{V}_{O_2 \text{ peak}}$  ( $\sim 44\%$  reduction in the  $\tau_p$ ) (Jones, Wilkerson, et al., 2003; Jones, Wilkerson, et al., 2004; Wilkerson, et al., 2004). Interestingly, these effects in response to L-NAME were not reported during contractions requiring  $\sim 60\% \dot{V}_{O_2 \text{ peak}}$  in isolated canine gastrocnemius muscle with a known higher type I muscle fibre proportion (Grassi, et al., 2005). It is therefore conceivable that an NO-linked inhibition of mitochondrial respiration might exert an especially pernicious influence on the oxidative functioning in higher-order type II muscle fibres during exercise in humans. This might be anticipated since an increased NOS activity in type II muscle (Kobzik, Reid, Bredt, & Stamler, 1994) would be expected to mitigate a lower  $\dot{Q}_{O_2}/\dot{V}_{O_2}$  response in order to preserve intracellular  $PO_2$  in these higher-order fibre pools.

### 2.4.3 *The PCr-Cr shuttle hypothesis*

It is known that the splitting of muscle PCr catalyzed by the creatine kinase (CK) enzyme serves to buffer changes in [ADP] and maintain cellular ATP/ADP homeostasis in response to an increase in metabolic demand. This process can be summarized in the following reaction in which the donation of a phosphate bond from muscle PCr to ADP restores myofibrillar ATP concentration:



*Equation 2.5*

The location of creatine kinase adjacent to the myofibrils provides an important spatial buffer between the sites of energy production and utilisation. Furthermore, the products and/or reactants involved in the creatine kinase splitting of muscle PCr are intimately involved in adjusting the rate of oxidative phosphorylation by regulating the rate of ADP delivery to mitochondrion (Bessman & Geiger, 1981; Kindig, Howlett, Sary, Walsh, & Hogan, 2005; Mahler, 1985; Rossiter, Howe, & Ward, 2005; Walsh, et al., 2001). Specifically, the Cr liberated from energy buffering at the myofibrils is transduced to the inter-mitochondrial space where ATP formed via oxidative metabolism donates a phosphate bond to yield PCr + ADP. The formed [ADP] enters the inner mitochondria to provide the requisite substrate in order to stimulate oxidative phosphorylation. Finally, cellular ATP/ADP is continually maintained as PCr formed via mitochondrial creatine kinase is shuttled to the cytoplasm in order to buffer increases in ADP arising from the cross-bridge formation between actin and myosin (Bessman & Geiger, 1981).

The fundamental tenet of the Cr shuttle hypothesis is that the rate of PCr hydrolysis in buffering ADP levels across a metabolic transient dictates the kinetics of Cr release into the inter-mitochondrial space and hence regulates the rate of ADP provision that drives mitochondrial respiration. Mahler (1985) presented initial evidence of a direct proportionality between  $m\dot{V}_{O_2}$  and PCr kinetics in electrically stimulated isolated amphibian muscle. Kindig et al. (2005) have also demonstrated a faster kinetic fall in intracellular  $PO_2$  (an analogue to  $m\dot{V}_{O_2}$  in this model) following acute creatine kinase inhibition in contracting single amphibian myocytes. Collectively, these findings suggest a fundamental role of the creatine kinase reaction in buffering cellular ATP/ADP

concentrations and thereby attenuating the rise in the putative phosphate signaling of oxidative ATP flux in response to a step increase in metabolic demand.

To explore the control determinants of  $m\dot{V}_{O_2}$  during exercise in humans, Harry B. Rossiter and colleagues (1999; 2002) simultaneously measured pulmonary gas exchange and muscle PCr breakdown using 31-phosphorus magnetic resonance spectroscopy ( $^{31}\text{P}$ -MRS). The authors reported that modeling the phase II  $\dot{V}_{O_2}$  response incorporating a delay term yielded a  $\tau_p$  that was similar to that of muscle PCr kinetics during M and H knee-extension exercise. These data further supported resolving the phase II  $\dot{V}_{O_2}$   $\tau$  to infer the kinetics of muscle PCr (and by extension that of  $m\dot{V}_{O_2}$ ) during step exercise transitions from a lower to a higher work rate. Furthermore, there existed a dynamic coupling between the fall in [PCr] and rise in  $\dot{V}_{O_2}$  irrespective of whether exercise intensity was below or above the intracellular threshold (IT) for muscle pH (analogues to the LT parameter) (Rossiter, et al., 1999; Rossiter, Ward, Kowalchuk, et al., 2002).

#### *2.4.3.1 Phosphate linked modulation in children*

Barker et al. (2008a) have provided the only study to date to investigate the kinetic relationship between phase II  $\dot{V}_{O_2}$  and muscle PCr breakdown during M-intensity exercise in youth. Eighteen 9-11 yr old children completed repeated square-wave cycling and knee-extension exercise bouts for the determination of the phase II  $\dot{V}_{O_2}$   $\tau$  and muscle PCr  $\tau$  at constant power outputs below the IT for muscle pH. The authors interpreted similar phase II  $\dot{V}_{O_2}$  and muscle PCr kinetics in children ( $\tau = 23 \pm 4$  vs.  $23 \pm 5$  s) to suggest that a progressive lengthening in the  $\tau_p$  with increasing age might be principally linked to an age-



related modulation of the putative metabolic feedback controllers of oxidative metabolism (Barker, Welsman, Fulford, Welford, Williams, et al., 2008).

To explore the aforementioned proposal, Barker et al. (2008b) compared the kinetics of muscle PCr breakdown between eighteen children (8 boys, 10 girls) and sixteen adults (8 men, 8 women) in response to M constant work rate exercise. In contrast to their experimental hypothesis, the authors reported a similar muscle PCr  $\tau$  in boys compared to men ( $\tau = 21 \pm 4$  vs.  $26 \pm 9$  s) and in girls compared to women ( $\tau = 24 \pm 5$  vs.  $24 \pm 7$  s). These data were interpreted to suggest a similar muscle oxidative capacity in children and adults exercising below the LT (Barker, Welsman, Fulford, Welford, & Armstrong, 2008). This poses an important theoretical question as to whether age differences in muscle metabolic responses to exercise might preside under conditions in which the external work rate obligates the recruitment of higher-order type II motor units. From this perspective, it is pertinent that muscle PCr kinetics were  $\sim 42\%$  slower in men compared to boys ( $\tau = 44 \pm 20$  vs.  $31 \pm 10$  s) at the onset of H knee extension transitions (Willcocks, Williams, Barker, Fulford, & Armstrong, 2010) with previous reports of a reduced fall in intramuscular [PCr] in children compared to adults during incremental exercise above but not below the IT for muscle pH (Barker, Welsman, et al., 2010).

#### *2.4.4 Experimental predictions*

The electric analog model of respiratory control originally proposed by Meyer (1988) predicts that the  $\tau$  for the exponential reduction in [PCr] and related rise in  $\dot{V}_{O_2}$  at exercise onset is dependent upon an interaction between the concentration and/or activity of oxidative enzymes (mitochondrial resistance) and resting [PCr] (metabolic capacitance).

Based on the theoretical predictions of Meyer's (1988) model, it is therefore conceivable that an enhanced oxidative enzymatic profile and/or reduced [PCr] in children would predictably result in faster phase II  $\dot{V}_{O_2}$  kinetics in youth.

Utilizing the muscle biopsy procedure, Haralambie et al. (1982) have shown increased tricarboxylic acid cycle enzyme activities in teenagers (aged 13-15 yr) compared to adults. Resting [PCr] in the *m. rectus femoris* has also been reported to progressively increase (~15 to 24 mM·kg<sup>-1</sup>) from the age of 11-to-16 yr (Eriksson & Saltin, 1974). One mechanism that might account for metabolic differences with age could be linked to changes in muscle fibre type distribution during growth. Glenmark et al. (1992) have reported a longitudinal decline in the proportion of type I muscle fibres over an 11-yr period in 55 individuals aged 16-27 yr. Moreover, the percentage of type II muscle fibres has been reported to increase (~ 15%) in the *m. vastus lateralis* between the ages of 5-to-20 yr (Lexell, Sjostrom, Nordlund, & Taylor, 1992).

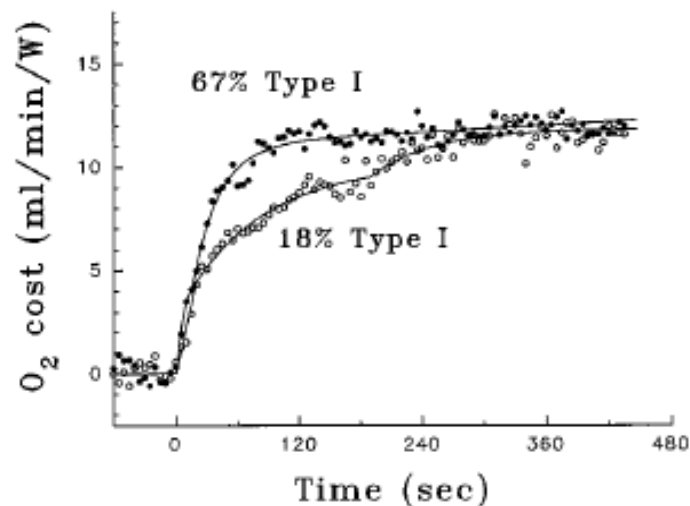
The functional significance of differences in muscle fibre type and/or recruitment on  $\dot{V}_{O_2}$  kinetics during exercise is emphasized when considering the biochemical differences between muscle fibre populations. Type II muscle fibres have an increased [PCr] at rest (Sahlin, Soderlund, Tonkonogi, & Hirakoba, 1997; Soderlund & Hultman, 1991) and lower mitochondrial content per fibre volume compared to type I myocytes (Howald, Hoppeler, Claassen, Mathieu, & Straub, 1985) which would predispose higher-order muscle fibres to slower  $\dot{V}_{O_2}$  kinetics according to Meyer's (1988) electric analog model. This is exemplified in the study of Crow & Kushmerick (1982) in which electrical stimulation of the extensor digitorum longus muscle (comprising type IIa and IIx fibres) elicited a

markedly slower  $\dot{V}_{O_2} \tau$  (~138 s) compared to type I soleus muscle (~36 s). The proceeding sections will therefore examine the relationship between muscle fibre type and  $\dot{V}_{O_2}$  kinetics in adults and explore non-invasive interventions purported to influence muscle fibre recruitment patterns during exercise.

## 2.5 Influence of muscle fibre type on $\dot{V}_{O_2}$ kinetics

### 2.5.1 Cross-sectional studies

Barstow et al. (1996) originally investigated the relationship between muscle fibre type and  $\dot{V}_{O_2}$  kinetics in ten adult participants during H-intensity ( $\Delta 50\%$ ) exercise. Although no influences of muscle fibre type were observed on the  $G_{tot}$ , the authors reported that the proportion of type I muscle fibres in the *m. vastus lateralis* correlated significantly with the  $G_p$  and relative  $\dot{V}_{O_2}$  slow component amplitude ( $r = 0.78$  and  $-0.83$ , respectively). It was



**Figure 2.9** An example  $\dot{V}_{O_2}$  profile during H-intensity cycling exercise in two adult participants with contrasting muscle fibre type distributions. Note that the contribution of the primary and slow components to the end-exercise  $O_2$  cost is modified dependent on the % of type I fibres in quadriceps muscle. From Barstow et al. (1996), with permission.

therefore considered that alterations in muscle fibre recruitment (toward increased type II fibre activation) might restrict the rise in  $\dot{V}_{O_2}$  at exercise onset and therefore require a greater proportional contribution from the  $\dot{V}_{O_2}$  slow component to attain the end-exercise  $\dot{V}_{O_2}$  amplitude. However, since the  $G_p$  and  $\dot{V}_{O_2}$  slow component were also correlated with  $\dot{V}_{O_2 \text{ peak}}$ , it was difficult to discriminate between the co-dependent influences of aerobic fitness and muscle fibre type *per se* on  $\dot{V}_{O_2}$  kinetics.

Pringle et al. (2003) extended the earlier work of Barstow et al. (1996) using similar procedures to examine the interaction between exercise intensity and muscle fibre type on  $\dot{V}_{O_2}$  kinetics in fourteen young adults. Importantly in this study, participants were of similar aerobic fitness (mean  $\dot{V}_{O_2 \text{ peak}} = 47.9 \pm 2.3 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) and performed repeated exercise transitions at each intensity (M, H, and VH) to enhance the  $\dot{V}_{O_2}$  signal-to-noise response. Consistent with previous reports (Barstow, et al., 1996), the authors reported that the % of type I fibres in the *m. vastus lateralis* correlated negatively with the relative  $\dot{V}_{O_2}$  slow component amplitude and positively with the  $G_p$  during H and VH exercise. Furthermore, Pringle et al. (2003) also noted that a slower  $\tau_p$  was manifest during exercise above but not below the GET in participants with a lower compared to higher type I fibre proportion. These findings were interpreted to reflect the oxidative response characteristics of higher-order muscle fibres with inherently slower  $\dot{V}_{O_2}$  kinetics (Crow & Kushmerick, 1982) that were unveiled on the phase II  $\dot{V}_{O_2}$  signal at the onset of H/VH exercise (Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003).

### 2.5.2 *The 'size' principle of orderly recruitment*

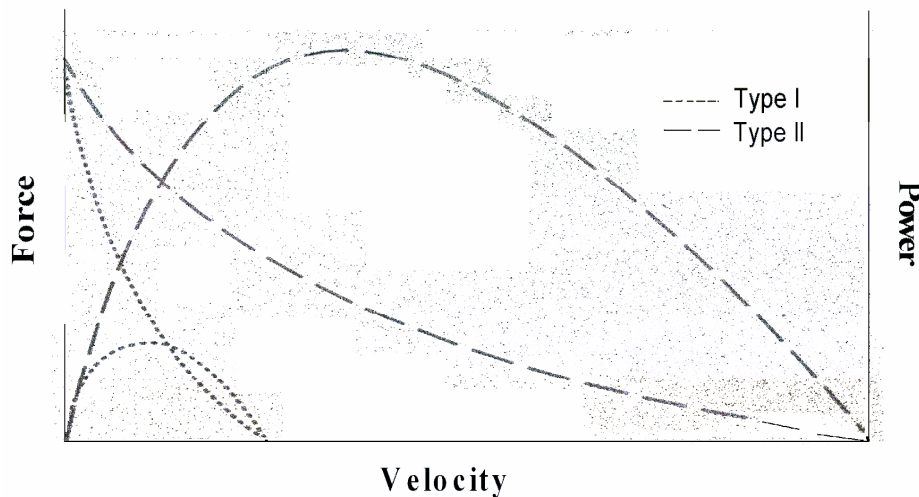
The well established size principle posits an orderly recruitment of motor units based on motoneurone size and the intensity of muscle contractile activity (Henneman & Mendell, 1981). For example, smaller motoneurons consisting of thin nerve axons and innervating fewer muscle fibres have a lower threshold for depolarization and are therefore recruited at low force outputs (i.e. during M exercise). Conversely, larger motor units require greater central nervous stimulation and are therefore recruited as the requirement for muscle force production is increased. This pattern of motor unit recruitment dictates that increasing exercise intensity will accentuate a greater drive through the recruitment hierarchy from muscle fibres with higher-to-lower cellular oxidative capacity and metabolic efficiency (i.e. type I-IIa-IIx).

Previous analyses from muscle biopsy specimens has revealed that type I muscle fibres are predominantly recruited at low cycling power outputs ( $\sim 31\text{-}50\% \dot{V}_{O_2 \text{ peak}}$ ), with reductions in glycogen content and [PCr] reported in both principal fibre types (I and II) in response to high-intensity ( $\sim 80\text{-}91\% \dot{V}_{O_2 \text{ peak}}$ ) exercise (Gollnick, et al., 1974; Krstrup, Soderlund, Mohr, et al., 2004b; Vollestad & Blom, 1985). It is therefore apparent that  $\dot{V}_{O_2}$  responses during H/VH transitions would conflate the oxidative response characteristics of a heterogeneous muscle fibre population. This has prompted several studies to experimentally reduce muscle fibre recruitment heterogeneity in order to reveal the  $\dot{V}_{O_2}$  amplitude and  $\tau$  characteristics in contrasting segments of the total muscle fibre pool during exercise in humans.

### 2.5.3 Reducing muscle fibre recruitment heterogeneity

#### 2.5.3.1 Manipulated pedal cadence

It is widely acknowledged that muscle force-velocity relationships are fibre-type dependent under conditions in which the muscle length is changing during dynamic contractions [see Sargeant (1999) for a review]. For example, the rate of force decline is restrained in type II fibres as muscle shortening velocity is increased enabling power output to be optimized at higher contraction frequencies (figure 2.10). In contrast, a leftward shift in the muscle



**Figure 2.10** Schematic illustrating the fibre-type dependency on muscle force/velocity relationships. Note that increasing muscle contraction velocity (moving from left-to-right on the x-axis) would be expected to enhance the contribution of type II motor units at higher external power outputs. From Sargeant (1999), with permission.

force-velocity curve (i.e. in type I fibres) diminishes the potential for lower-order muscle fibres to contribute to the external power output at higher contraction frequencies (Sargeant, 1994). Several previous investigations have therefore manipulated pedal rate in order to examine the putative influence of muscle fibre recruitment on  $\dot{V}_{O_2}$  kinetics during exercise above the GET (Barstow, et al., 1996; Dimenna, Wilkerson, Burnley, Bailey, &

Jones, 2009a, 2009b; Pringle, Doust, Carter, Tolfrey, & Jones, 2003; Vercruyssen, Missenard, & Brisswalter, 2009).

Barstow et al. (1996) originally reported that altering pedal cadence did not influence either the phase II  $\dot{V}_{O_2}$  parameters or the  $\dot{V}_{O_2}$  slow component amplitude during H transitions in adults (although there was a tendency for the  $G_p$  to reduce at 90 rev·min<sup>-1</sup> compared to pedaling at 45 rev·min<sup>-1</sup>). There was evidence however to indicate that the relative exercise intensity was not appropriately equated between pedal rate conditions. For example, the  $\Delta \dot{V}_{O_2}$  from baseline to end-exercise was reduced (~24%) at 90 rev·min<sup>-1</sup> compared to 45 rev·min<sup>-1</sup> with participants evidencing a lower blood [La] at the higher cadence. It was also conceivable that 90 rev·min<sup>-1</sup> pedaling might have enabled type I muscle fibres to shorten close to their optimal velocity for power generation and efficiency and therefore influence  $\dot{V}_{O_2}$  responses at the mouth (i.e. the potential for differences in muscle fibre recruitment was limited due to the narrow range of selected pedal cadences). The observation that cycling efficiency is enhanced in participants with a higher type I fibre proportion pedaling at 80 rev·min<sup>-1</sup> is indirectly consistent with this proposal (Coyle, Sidossis, Horowitz, & Beltz, 1992).

Using a modified experimental approach, Pringle et al. (2003) investigated the  $\dot{V}_{O_2}$  kinetic response over an extended range of pedal rates (35-115 rev·min<sup>-1</sup>) in order to amplify differences in muscle fibre recruitment at slow and fast pedal frequencies. The authors also elected to normalize exercise work rate in relation to pedal rate-specific GET and  $\dot{V}_{O_2 \text{ peak}}$  values obtained from separate ramp exercise tests in each cadence condition. Although differences between pedal rates were non-significant, there was a tendency for the  $\tau_p$  to

lengthen at 115 rev·min<sup>-1</sup> compared to 35 rev·min<sup>-1</sup> (30 ± 3 vs. 24 ± 2). Furthermore, despite a similar O<sub>2</sub> cost (G<sub>tot</sub>) at end-exercise between pedal rates, there was a greater proportional contribution from the  $\dot{V}_{O_2}$  slow component that counteracted a reduced G<sub>p</sub> at 115 rev·min<sup>-1</sup> compared to 35 rev·min<sup>-1</sup>. This investigation therefore demonstrated that a non-invasive exercise intervention (i.e. increasing pedal frequency) could reveal the characteristic  $\dot{V}_{O_2}$  kinetic profile (i.e. reduced G<sub>p</sub> and increased  $\dot{V}_{O_2}$  slow component amplitude) previously linked to an increased type II fibre recruitment from studies using muscle biopsies (Barstow, et al., 1996; Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003).

#### 2.5.3.2 Glycogen depletion studies

A number of studies have utilized prior exercise and dietary regimens in order to amplify the metabolic properties of either type I or II muscle fibres on the pulmonary  $\dot{V}_{O_2}$  signal during subsequent exercise. For example, Krstrup et al. (2004a) reported an increased O<sub>2</sub> cost of M-intensity exercise following selective glycogen depletion of type I muscle fibres at an equivalent cycling power output (mean = 139 ± 4 W). The authors also revealed a  $\dot{V}_{O_2}$  slow component in the M domain under conditions of elevated fast-twitch fibre activation thereby directly linking an excess  $\dot{V}_{O_2}$  with the recruitment of higher-order muscle fibres in humans (Krstrup, Soderlund, Mohr, & Bangsbo, 2004a).

During high-intensity exercise (~ 90%  $\dot{V}_{O_2 \text{ peak}}$ ), previous studies have reported no effect on either the primary or slow component  $\dot{V}_{O_2}$  amplitudes following prolonged low-intensity exercise (~ 90-120 min at 60%  $\dot{V}_{O_2 \text{ peak}}$ ) to reduce type I muscle fibre recruitment



(Bouckaert, Jones, & Koppo, 2004; Perrey, Candau, Rouillon, & Hughson, 2003). It was conceivable however that type I fibres might have oxidized free fatty acids (FFA) under conditions of glycogen deprivation thereby restricting alterations in muscle fibre recruitment in the experimental condition (Bouckaert, et al., 2004). This prompted Carter et al. (2004) to utilize an intermittent exercise protocol ( $10 \times 1$  min bouts at  $120\% \dot{V}_{O_2 \text{ peak}}$ ) in order to reduce the influence of type II fibre recruitment on  $\dot{V}_{O_2}$  responses during H exercise (Note: it was reasoned that a reduced capacity to metabolize FFA in higher-order muscle fibres would enforce a greater proportional contribution from the type I fibre population to the external power output). The authors reported that elevating type I fibre activation resulted in predictable changes in  $\dot{V}_{O_2}$  kinetics (i.e. an increased  $G_p$  and reduced  $\dot{V}_{O_2}$  slow component) during step ( $\Delta 50\%$ ) exercise transitions. However, whilst alternating substrate availability in specific muscle fibre pools can potentially influence  $\dot{V}_{O_2}$  kinetics during exercise, the use of such experimental interventions is impractical in youth subjects due to the requirement for prolonged exercise bouts and restricted dietary intakes.

#### 2.5.3.3 'Work-to-work' exercise transitions

Hughson & Morrissey (1982) originally investigated the influence of initiating step exercise transitions from an elevated baseline work rate (so called 'work-to-work' exercise) using modeling techniques to resolve phase II  $\dot{V}_{O_2}$  kinetics in six adults. Participants were required to complete a single exercise transition from rest to 80% LT (*step 1→3*) and in a subsequent protocol perform two additive steps to an equivalent exercise work rate (*step 1→2* and *step 2→3*). The authors reported a lengthened  $\tau_p$  in the upper compared to lower

region of the M domain ( $\tau_p = 61 \pm 11$  vs.  $30 \pm 8$  s). These results therefore challenged the notion of an invariant  $\tau_p$  within the moderate domain and hence indicated that additional factor(s) could be rate limiting oxidative metabolism below the LT. However, resolving the  $\tau_p$  in response to a single exercise bout in each step condition cast uncertainties over physiological inferences drawn from the modeled parameters in this study.

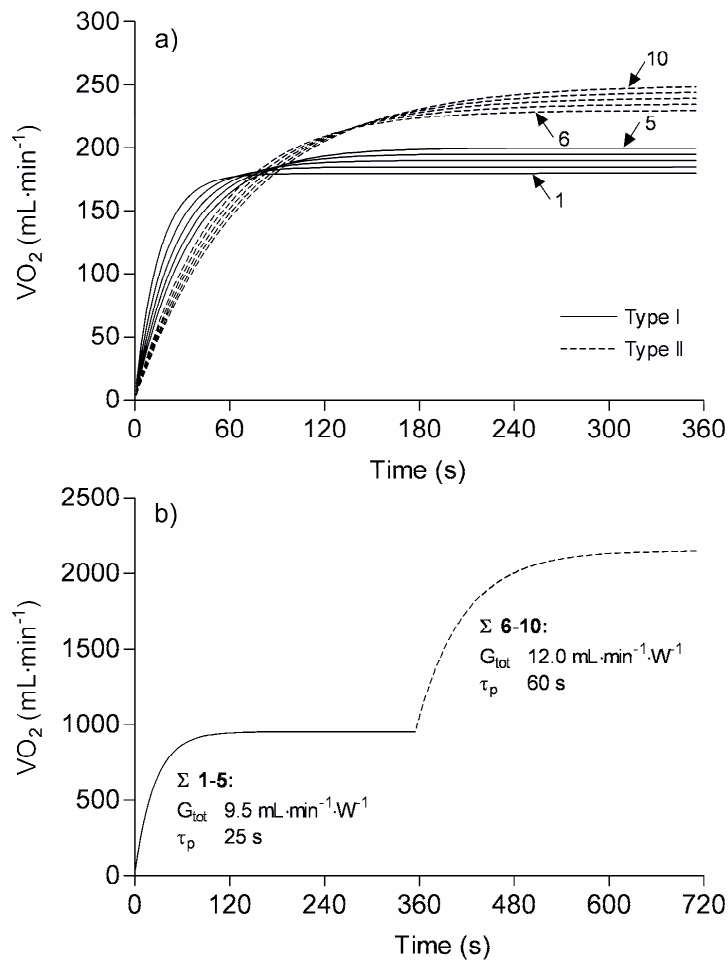
Brittain et al. (2001) further explored work-to-work exercise influences on  $\dot{V}_{O_2}$  kinetics using  $\dot{V}_{O_2}$  data averaged over multiple exercise transitions in order to yield  $\tau_p$  values within narrower confidence limits. The authors employed similar exercise protocols to that described previously (Hughson & Morrissey, 1982) but importantly elected to initiate exercise transitions from unloaded pedaling as opposed to rest. Brittain et al. (2001) demonstrated a lengthened  $\tau_p$  and an elevated  $G_{tot}$  in the upper compared to lower region of M domain. Furthermore, the authors cited previous demonstrations that  $O_2$  delivery was not rate limiting in this domain (Burnley, et al., 2000; Gerbino, et al., 1996) coupled with reducing the influence of parasympathetic removal on exercise HR (owing to prior baseline pedaling) to indicate that intrinsic metabolic factors were principally restricting  $\dot{V}_{O_2}$  kinetics in *step 2*→*3*. The findings were therefore interpreted to suggest that muscle fibre pools with a reduced oxidative capacity and a greater  $O_2$  cost of exercise (reflecting lower metabolic efficiency) might have been recruited at the onset of work-to-work exercise.

Brittain et al. (2001) initially described the potential for work-to-work exercise to delineate the oxidative properties of lower- and higher-order muscle fibres through establishing an orderly pattern of motor unit recruitment in accord with the size principle. The force exerted per pedal crank revolution at M exercise power outputs [estimated to require <20%

MVC, Sjogaard (1978)] would have been unlikely however to evoke the recruitment of type II motor units during exercise. Wilkerson & Jones (2006) therefore explored the influence of altering baseline work rate on  $\dot{V}_{O_2}$  kinetics during S-intensity exercise transitions in an attempt to reveal the metabolic properties of higher-order muscle fibres (i.e. type IIa and IIx). In this study, seven men (mean age =  $26 \pm 4$  yr) completed step exercise transitions to 100%  $\dot{V}_{O_2 \text{ peak}}$  elicited from either unloaded pedaling, M, or H baseline work rates (U→S, M→S, and H→S). The authors reported that progressively slower phase II  $\dot{V}_{O_2}$  kinetics from U→S to H→S were not associated with alterations in HR kinetics but were manifest under conditions in which the integrated electromyogram (iEMG) of the *m. vastus lateralis* was elevated at the onset of exercise. It was therefore suggested that M→S transitions might have amplified the metabolic properties of type II muscle fibres that were presumably recruited to augment force production across the work-to-work exercise transient. However, exploring the influence of work-to-work exercise on the  $\dot{V}_{O_2}$  slow component was restricted due to the lower tolerable duration of exercise at S work rates.

In a subsequent investigation, Wilkerson and Jones (2007) explored the influence of manipulating baseline work rate on  $\dot{V}_{O_2}$  kinetics during H-intensity ( $\Delta 40\%$ ) exercise elicited from unloaded pedaling (U→H) and M exercise (M→H). The authors also included unloaded-to-moderate transitions (U→M) to provide reference  $\dot{V}_{O_2}$  gain and  $\tau$  values during exercise predominantly involving type I fibre activation. Consistent with previous reports, the phase II  $\dot{V}_{O_2}$  kinetics were slower during M→H compared to U→M and U→H exercise. Of particular interest, however, was that initiating H exercise from an elevated

baseline work rate increased the  $G_p$  and reduced the  $\dot{V}_{O_2}$  slow component amplitude (i.e.  $\dot{V}_{O_2}$  kinetics reverted toward a mono-exponential response). These findings were interpreted to suggest that reducing type I fibre recruitment at the onset of work-to-work



**Figure 2.11** Hypothetical model illustrating the influence of muscle fibre recruitment on  $\dot{V}_{O_2}$  kinetics during H-intensity exercise. Note that the contribution of *motor units 1*→*5* and *motor units 6*→*10* to the external power output yields a net  $\dot{V}_{O_2}$  response that would be indistinguishable from a double-exponential profile expressed at the mouth (a). Dividing a full transition into two step transitions drives an orderly recruitment sequence therefore amplifying the  $\tau$  and  $\dot{V}_{O_2}$  gain characteristics of the lower- and higher-order motor units and resulting in a lower  $\dot{V}_{O_2}$  slow component response in the 2<sup>nd</sup> step (b). Adapted from Brittain et al. (2001) and Wilkerson & Jones (2007). See text for further details.

exercise enabled the oxidative response characteristics of higher-order muscle fibres to be unveiled earlier in the transition (i.e. over the primary  $\dot{V}_{O_2}$  region) resulting in a lower  $\dot{V}_{O_2}$  slow component response (figure 2.11).

Wilkerson & Jones (2007) therefore proposed a model illustration to identify how the recruitment of a heterogeneous population of muscle fibres might influence the  $\dot{V}_{O_2}$  response during H/VH exercise. Specifically, the requisite  $O_2$  cost of M exercise ( $\sim 8-10 \text{ mL}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$ ) can be attained with relatively rapid  $\dot{V}_{O_2}$  kinetics if type I fibre recruitment were principally contributing to the external power output. In contrast, a  $\dot{V}_{O_2}$  slow component could be elicited above the GET if the protracted response of higher-order muscle fibres with slower  $\dot{V}_{O_2}$  kinetics and a greater  $O_2$  cost were superimposed on the fundamental  $\dot{V}_{O_2}$  response. The intensity of exercise in relation to CP will dictate whether the  $\dot{V}_{O_2}$  slow component can eventually asymptote at a submaximal  $\dot{V}_{O_2}$  (H exercise) or increase  $\dot{V}_{O_2}$  toward maximum if there is a requirement to recruit additional motor units over time (VH exercise).

#### 2.5.4 *Muscle fibre type/recruitment in youth*

Serial sections of muscle biopsy tissue stained histochemically for myofibrillar ATPase activity has returned equivocal results in terms of alterations in muscle fibre type composition with increasing age (Bell, MacDougall, Billeter, & Howald, 1980; Glenmark, et al., 1992; Lexell, et al., 1992). For example, Lexell et al. (1992) have demonstrated that increases in mean fibre diameter were accompanied by an increased proportion of type II muscle fibres in the *m. vastus lateralis* from autopsied muscle biopsy specimens obtained in

male subjects aged between 5 to 37 yr. In contrast, it has been reported that muscle fibre type distribution is established at near adult proportions in 6-yr old children (Bell, et al., 1980) with some evidence of conversion from type IIa to IIx during adolescent growth (Colling-Saltin, 1980; Fournier, et al., 1982). In order to resolve these discrepancies in the literature, Jansson (1996) provided a comprehensive review of 42 studies ( $n = 1,112$ ) and concluded that, on average, the % of type I fibres declined from ~58% to ~48% between the ages of 10 yr to 20 yr in healthy, untrained male subjects. However, the extent to which relatively small changes in muscle fibre type distribution might contribute to the observed differences in the primary  $\dot{V}_{O_2}$  parameters and the  $\dot{V}_{O_2}$  slow component between adults/teenagers and younger children (see section 2.3.3) is unknown. From this perspective, it is pertinent to note that Pringle et al. (2003) have shown a 32% speeding in the phase II  $\tau$  and 7% reduction in the relative  $\dot{V}_{O_2}$  slow component amplitude in adults classified as having a high compared to low proportion of type I fibres in the *m. vastus lateralis* (mean =  $67 \pm 2$  vs.  $43 \pm 4\%$ ) during heavy-intensity ( $\Delta 40\%$ ) cycling.

Given the aforementioned findings, an important theoretical question is whether an elevated type I fibre expression might ‘desensitise’ the  $\dot{V}_{O_2}$  kinetic response in younger children to interventions purported to enhance higher-order (type II) muscle fibre recruitment during exercise. However, to date, the majority of the available literature on the influence of muscle recruitment during exercise in youth has focused on the development of force during isometric maximal voluntary contractions (MVC). For example, utilisation of the twitch interpolated technique, in which a supramaximal electrical stimulation is applied during the peak of an MVC, has revealed a greater increment in force output above that elicited by voluntary effort in 10- compared to 16- yr old children (Blimkie, 1989; Paasuke,

Ereline, & Gapeyeva, 2000). Likewise, an attenuated decay in the EMG mean power frequency (which is sensitive to the accumulation of H<sup>+</sup> and K<sup>+</sup> ions) during sustained isometric contractions has been interpreted to reflect a lower involvement of fatigue-sensitive motor units in boys compared to men (Halin, Germain, Bercier, Kapitaniak, & Buttelli, 2003). Collectively, these results might be suggested to reflect a limited capability in children to voluntarily activate their higher-threshold (type II) motor units which might explain their lower peak torque per unit of muscle cross sectional area compared to adults (Falk & Dotan, 2006; Falk, et al., 2009). However, the influence of alterations in muscle fibre recruitment on metabolic responses during intense submaximal exercise is absent in young people relative to adult counterparts. There is therefore clearly scope to apply the non-invasive experimental models reviewed in section 2.5.3 in order to extend the existing knowledge base on muscle fibre type energetics in paediatric populations.

## **2.6 Motor unit recruitment and the $\dot{V}_{O_2}$ slow component**

### *2.6.1 Delayed muscle fibre activation*

The demonstration that an excess  $\dot{V}_{O_2}$  temporally coincides with a fall in intramuscular [PCr] during high-intensity knee-extension contractions (Rossiter, Ward, Kowalchuk, et al., 2002) suggests that the  $\dot{V}_{O_2}$  slow component predominately reflects an increased muscle ATP turnover to sustain constant work rate exercise. It is therefore pertinent that ATP consumption per unit of isometric tension development is greater in fast-twitch compared to slow-twitch animal muscle (Crow & Kushmerick, 1982) with an increased O<sub>2</sub> cost of ATP resynthesis (i.e. lower P:O ratio) demonstrated in isolated type II compared to type I mitochondria (Willis & Jackman, 1994). However, the extent to which differences in muscle fibre type efficiency contribute to the development of the  $\dot{V}_{O_2}$  slow component

during dynamic exercise in humans remains conjectural. From this perspective, it is pertinent that peak thermodynamic efficiencies are obtained at faster shortening velocities and higher relative forces in single type II compared to type I human muscle fibres (He, Bottinelli, Pellegrino, Ferenczi, & Reggiani, 2000).

Krustrup et al. (2004b) investigated the involvement of muscle fibre-specific recruitment on the  $\dot{V}_{O_2}$  slow component through obtaining muscle biopsies during high-intensity cycling ( $\sim 80\% \dot{V}_{O_2 \text{ peak}}$ ) in seven men. Single muscle fibres were assayed in relation to myofibrillar ATPase activity and analysed for changes in muscle glycogen and PCr concentration at separate time intervals throughout exercise compared to rest. The authors reported that additional type I and II muscle fibres were recruited from *minute 3* to *minute 6* in temporal coherence with an increased  $\dot{V}_{O_2}$  ( $\sim 140 \text{ mL}$ ) during constant work rate (CWR) exercise. However, a greater net reduction in [PCr] in type II fibre pools intimated a shift toward increased activation of higher-order motor units as intense exercise proceeded. Furthermore, the  $\dot{V}_{O_2}$  slow component was exclusively manifest under conditions of type II muscle fibre recruitment but not during M exercise ( $50\% \dot{V}_{O_2 \text{ peak}}$ ) in which type I fibres independently contributed to the external power output. These findings were therefore consistent with an increased  $O_2$  cost over time linked to the serial recruitment of type II muscle fibres during high-intensity exercise in humans. `

### 2.6.2 *Integrated electromyogram (iEMG) activity*

Surface electromyography (EMG) provides an estimate of electrical activity within superficial muscle regions located adjacent to the positioning of electrodes over active muscle (De Luca, 1997). Several previous studies have reported a temporal link between



increases in surface EMG activity and the development of the  $\dot{V}_{O_2}$  slow component during CWR exercise above the GET (Burnley, et al., 2002; DiMenna, Wilkerson, Burnley, & Jones, 2008; Saunders, et al., 2000; Shinohara & Moritani, 1992). However, interpretations of these data are problematic since the EMG signal is unable to discriminate between increases in electrical muscle activity arising from additional muscle fibre recruitment and/or increased firing frequency of active motor units (i.e. rate-coding) (De Luca, 1997). This identifies an immediate consideration as to whether a concurrent rise in  $\dot{V}_{O_2}$  and EMG activity reflects an  $O_2$  cost incurred from delayed muscle fibre activation and/or is attributable to rate-coding of motor units that were already recruited from the onset of exercise.

Despite reports linking type II muscle fibre recruitment in relation to the  $\dot{V}_{O_2}$  slow component (Barstow, et al., 1996; Krstrup, Soderlund, Mohr, et al., 2004a; Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003; Pringle, Doust, Carter, Tolfrey, & Jones, 2003), confirmation of this proposal based on surface EMG has remained elusive in humans. For example, it has been suggested that a shift in the EMG power density spectrum toward higher frequencies might reflect increased type II fibre recruitment during exercise (Wretling, Gerdle, & Henriksson-Larsen, 1987). Borrani et al. (2001) originally provided evidence of a similar time onset between an increase in the muscle EMG mean power frequency (MPF) and the  $\dot{V}_{O_2}$  slow component elicited during H-intensity treadmill running. In contrast, a number of studies have reported that the  $\dot{V}_{O_2}$  slow component develops independent of any changes in the MPF during exercise above the GET (Burnley, et al., 2002; c.f. Scheuermann, et al., 2001; Perrey, Betik, Candau, Rouillon, & Hughson, 2001; Tordi, Perrey, Harvey, & Hughson, 2003). However, the potential for increases in

muscle temperature and metabolite accumulation to influence the MPF signal have largely confounded physiological interpretation of this parameter (Bigland-Ritchie, Donovan, & Roussos, 1981; Brody, Pollock, Roy, De Luca, & Celli, 1991).

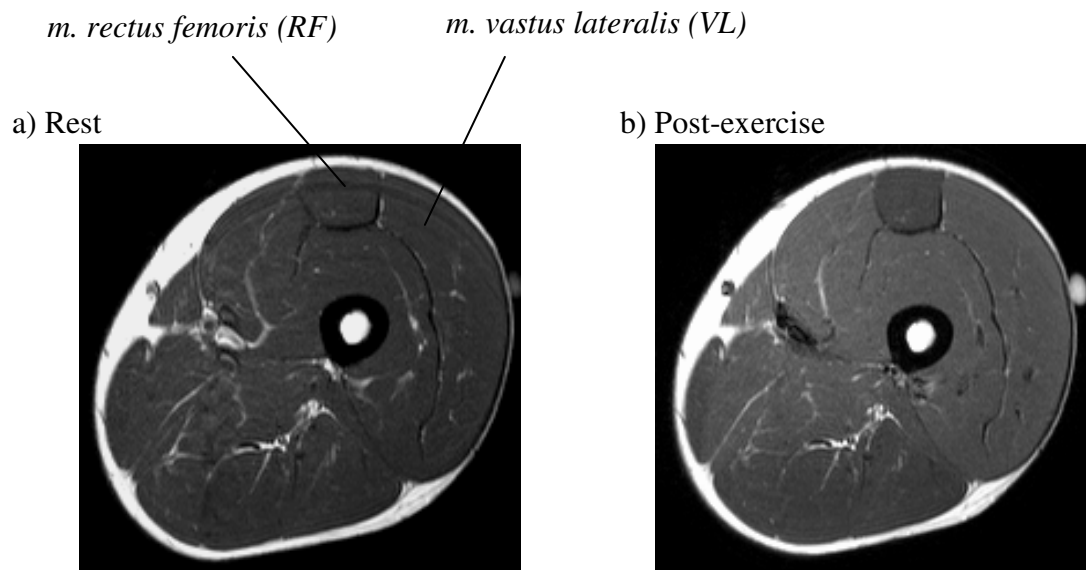
Notwithstanding limitations in elucidating muscle fibre-type specific recruitment, the demonstration that muscle EMG increases over time exclusively under exercise conditions in which an excess  $\dot{V}_{O_2}$  develops is consistent with an intramuscular origin for the  $\dot{V}_{O_2}$  slow component (Poole, et al., 1991). There is considerable debate, however, as to whether additional muscle fibre recruitment is an obligatory symptom for a progressive reduction in muscle contractile efficiency during CWR exercise (Jones, Pringle, & Carter, 2005a; Zoladz, Gladden, Hogan, Nieckarz, & Grassi, 2008). For example, if a high proportion of the total motor unit pool were recruited from the onset of intense exercise (Krustrup, Soderlund, Mohr, Gonzalez-Alonso, & Bangsbo, 2004), then  $O_2$  may be continually utilized in fatiguing muscle fibers due to recovery processes that increase the ATP turnover per unit of external work done. This is consistent with a recently reported increased  $\dot{V}_{O_2}$  gain (analogous to a  $\dot{V}_{O_2}$  slow component response) despite a gradual decline in external power output during ‘all-out’ voluntary exercise in humans (Vanhatalo, Poole, DiMenna, Bailey, & Jones, 2011).

An alternative explanation is that pronounced metabolite accumulation (e.g.  $P_i$  and  $H^+$ ) in fatiguing muscle fibres could mandate the recruitment of previously quiescent motor units resulting in a greater  $O_2$  cost to sustain the exercise work rate (Poole & Jones, 2005b). Under these circumstances, increases in the muscle EMG over time might be expected to principally involve the activation of motor units residing higher in the recruitment hierarchy

with a lower metabolic efficiency (i.e. type II). It is therefore pertinent that increasing muscle  $O_2$  availability following prior exercise is demonstrated to elicit a commensurate reduction in the  $\dot{V}_{O_2}$  slow component and muscle EMG activity (Bailey, Vanhatalo, Wilkerson, Dimenna, & Jones, 2009; Burnley, et al., 2002; DiMenna, et al., 2008). This might be interpreted to suggest that a priming-induced facilitation in matching  $O_2$  delivery to the rate of  $O_2$  utilization could reduce the rate of fatigue development and therefore negate the requirement for delayed motor unit activation during exercise. However, whether similar mechanisms linked to an attenuated change in muscle EMG over time are involved in reducing the  $\dot{V}_{O_2}$  slow component in children compared to adults has not been previously investigated.

### *2.6.3 $T_2$ -weighted magnetic resonance imaging*

Given that the pulmonary  $\dot{V}_{O_2}$  signal will summate the metabolic response activities of different muscles engaged in the exercise task, it is important that methodologies with a higher spatial resolution are sought in order to comprehensively examine the influence of muscle recruitment patterns on the  $\dot{V}_{O_2}$  slow component. Functional magnetic resonance imaging (MRI) has been used in order to provide ‘visual mapping’ of individual muscle recruitment within the upper thigh region during exercise in humans (Endo, et al., 2007; Ray & Dudley, 1998; Reid, Foley, Jayaraman, Prior, & Meyer, 2001; Richardson, Frank, & Haseler, 1998). Using this technique, an exercise-induced slowing of the muscle proton ( $H^+$ ) transverse relaxation time ( $T_2$ ) induces an increased image contrast in the recruited muscle(s) during an exercise task (Meyer & Prior, 2000). This change in muscle  $T_2$  is well



**Figure 2.12** Axial MR image of the left thigh musculature in a 30 yr-old male subject before (a) and after (b) performing 6-min of high-intensity cycling exercise. In this example, a 19% lengthening in the transverse relaxation time ( $T_2$ ) above rest in the VL (compared to only 4% in RF) induces an enhanced tissue contrast in the former thereby signalling an increased contribution to the external power output during exercise.

correlated to surface iEMG recordings (Adams, Duvoisin, & Dudley, 1992) and increases proportionally in relation to graded exercise intensity (Fisher, Meyer, Adams, Foley, & Potchen, 1990; Jenner, Foley, Cooper, Potchen, & Meyer, 1994).

Saunders et al. (2000) originally investigated the influence of  $T_2$  changes on the  $\dot{V}_{O_2}$  slow component amplitude in sixteen adults during CWR exercise above the GET ( $\Delta 60\%$ ). The authors reported that an excess  $\dot{V}_{O_2}$  (~285 mL) temporally coincided with increases in EMG and  $T_2$  values in the *m. vastus lateralis*. Furthermore, changes in  $T_2$  and  $\dot{V}_{O_2}$  from 3-15 min were positively correlated suggesting a mechanistic link between increases in muscle activation and an excess  $\dot{V}_{O_2}$  during high-intensity exercise. However, differences in the  $\dot{V}_{O_2}$  slow component and distribution of the  $T_2$  values within the upper thigh

musculature were not defined in relation to the physiological parameter demarcating the transition between the H and VH exercise intensity domains.

Endo et al. (2007) explored the interaction between  $T_2$  changes in ten muscles and the  $\dot{V}_{O_2}$  slow component in relation to exercise intensity below and above the estimated CP in eleven adults. Consistent with Saunders et al. (2000), increases in  $T_2$  and  $\dot{V}_{O_2}$  from minute 3 to minute 6 were elicited during exercise above but not below the GET. However, the authors further noted that an elevated  $\dot{V}_{O_2}$  slow component amplitude in VH compared to H ( $\sim 95 \text{ mL}\cdot\text{min}^{-1}$ ) coincided with a greater inter-muscle variance in  $T_2$  values as exercise proceeded. It was therefore suggested that elevated muscle recruitment heterogeneity might reflect greater involvement of the higher-order type II muscle fibers as indicated by an increased  $T_2$  signal from minute 3 to minute 6 in specific individual muscles but not in others. This might be considered a reasonable assumption since increases in motor unit firing frequency (rate-coding) would not be expected to influence the muscle  $T_2$  signal during exercise. However, it is conceivable that quantifying the difference in  $\dot{V}_{O_2}$  between minute 3 and minute 6 of exercise might have misrepresented the actual  $\dot{V}_{O_2}$  slow component amplitude in previous studies (Endo, et al., 2007; Saunders, et al., 2000). Furthermore, most of the investigations conducted to date have principally involved young adults, with no data currently available on the potential contribution from alterations in muscle activation on the development of the  $\dot{V}_{O_2}$  slow component in child or adolescent subjects. Given ethical restrictions in obtaining muscle biopsies in youth, this research question can therefore be most conveniently addressed using non-invasive procedures such as muscle EMG and  $T_2$  changes during intense constant work rate exercise.

## 2.7 Statement of hypotheses

The preceding literature review has presented evidence to support an age-linked modulation of  $\dot{V}_{O_2}$  kinetics and explored the physiological factors proposed to regulate the rate of oxidative metabolism following the onset of exercise in healthy adults. This analysis has identified an emerging consensus that differences in muscle fibre type and/or recruitment patterns influences the  $\tau$  and amplitude of the primary  $\dot{V}_{O_2}$  response during H/VH-intensity exercise. Likewise, studies utilising markers of muscle activation have linked the recruitment of higher-order (type II) fibres to the development of the  $\dot{V}_{O_2}$  slow component. Therefore, in light of the available evidence, the studies presented in chapters 4-8 are intended to investigate the extent to which differences in muscle fibre recruitment might contribute to age-related differences in  $\dot{V}_{O_2}$  kinetics during VH-intensity exercise. Specifically, the following experimental hypotheses will be tested:

1. During H-intensity exercise, the phase II  $\tau$  and relative  $\dot{V}_{O_2}$  slow component amplitude will increase longitudinally in 14-to-16 yr old boys.
2. Step transitions above the GET initiated from an elevated baseline work rate will lengthen the phase II  $\tau$  and increase the overall  $\dot{V}_{O_2}$  gain compared to step exercise elicited from unloaded pedalling in teenagers and adults but not in younger boys.
3. During VH-intensity exercise, pedalling at fast compared to slow cadences will lengthen the phase II  $\tau$  and increase the  $\dot{V}_{O_2}$  slow component in untrained teenagers but will not alter the same  $\dot{V}_{O_2}$  kinetic parameters in trained junior cyclists.

4. In younger boys, a reduced  $\dot{V}_{O_2}$  slow component will coincide with an attenuated change in iEMG of the *m. vastus lateralis* from the 2<sup>nd</sup> to 6<sup>th</sup> minute of constant work rate exercise compared to teenagers and men.
5. A reduced  $\dot{V}_{O_2}$  slow component will be temporally associated with a lower change in quadriceps T<sub>2</sub> over time during VH-intensity exercise in 10-12 yr old boys compared to men.

## Chapter three

### GENERAL METHODS

#### 3.1 Subject recruitment

Prior to data collection, local schools were approached and pupils were invited to take part in exercise testing following verbal explanation of the study's experimental procedures and number of laboratory visits. Informed assent/consent was then obtained from each child volunteer and their parent(s)/guardian(s) after written information had been provided detailing the study's objectives and associated risks and benefits. Preliminary health questionnaires identified that all subjects were asymptomatic and therefore showed no medical contraindications to vigorous exercise. Subjects recruited for exercise testing reported, in most cases, moderate-to-high physical activity levels that included participation in team sports at local school and/or club level. In chapter six, junior cyclists were engaged in formalised training programmes in accord with the subject inclusion criteria specified for this particular investigation. In chapters 5-8, subjects' descriptive statistics (stature, sitting height, chronological age and body mass) were entered into sex-specific algorithms in order to provide an estimate of age at peak height velocity (APHV) using the procedures originally described by Mirwald et al. (2002). These techniques are accurate in predicting APHV to within  $\pm 1$  yr in 95% of cases (Mirwald, Baxter-Jones, Bailey, & Beunen, 2002). Each individual's chronological age on the first laboratory visit was then subtracted from their predicted APHV in order to derive an offset score in years as a marker for biological maturity. Throughout the proceeding experimental chapters, subjects referred to as 'boys' or 'younger children' were therefore categorised as having a maturity offset score of  $\leq -1.5$  years from APHV (range = -2.9 to -1.6 years). The terms 'adolescents' or 'older children'



are used interchangeably to describe teenage subjects with a maturity offset score of  $\geq -0.5$  years from PHV (range = -0.4 to +3.1 years).

An important consideration when conducting research in children is that sampling is adequate to ensure that recruitment does not needlessly commit more pupil time than is necessary. As such, a requirement of the local research ethics committee was to estimate *a priori* the minimum sample size ( $n$ ) required in order to reduce the probability of type II errors. The procedures outlined in Vincent (1999) pp. 142 were therefore used as described in equation 3.1 in order to determine the  $n$  required to achieve 80% statistical power at  $p = 0.05$ .

$$N = \frac{2SD^2(1.96 + 0.84)^2}{\Delta^2} \quad \text{Equation 3.1}$$

where  $\Delta$  denotes the difference between mean scores and SD represents the pooled standard deviation for variables of interest from previously published data. Worked calculations of  $n$  are provided in appendix 1 (pp. 192). Certificates of ethical approval are also provided in appendix 2 (pp. 193-195).

### **3.2 Experimental measures**

Data contained in chapters 4-8 were collected either at exercise physiology laboratories housed within the Children's Health and Exercise Research Centre (CHERC) or at an offsite laboratory facility situated at Ivybridge Community College. In chapter 8, additional exercise tests and subject scans were performed at the MRI Unit, Peninsula College of Medicine and Dentistry (PCMD).

### 3.2.1 Pulmonary gas exchange

Breath-by-breath changes in gas exchange and ventilation were measured during each exercise test using a standard algorithm (Beaver, Wasserman, & Whipp, 1973) and displayed on-line using a computer system. In chapters 4 and 6, gas fractions of O<sub>2</sub> and CO<sub>2</sub> were continuously drawn from a low dead space (90 mL) mouthpiece-turbine assembly and determined by mass spectroscopy (EX671, Morgan Medical, Kent, UK). For exercise protocols administered in chapters 5 and 7-8 this analysis was performed using a metabolic cart including a DVT turbine digital transducer to measure expired volume and an electrochemical cell (O<sub>2</sub>) and ND infrared (CO<sub>2</sub>) analyzers that measured expired gas concentrations (Matalyser 3B Cortex, Biophysik, Leipzig, Germany). In all cases, the gas analysers were calibrated before each exercise test with gases of known concentration and the volume sensor was calibrated using a 3-litre syringe (Hans Rudolph, Kansas City, MO) over a range of flow speeds. The sum of the gas-transport and analyzer-response delay terms was determined, and appropriate adjustments were made in the software.

## 3.3 Exercise testing protocols

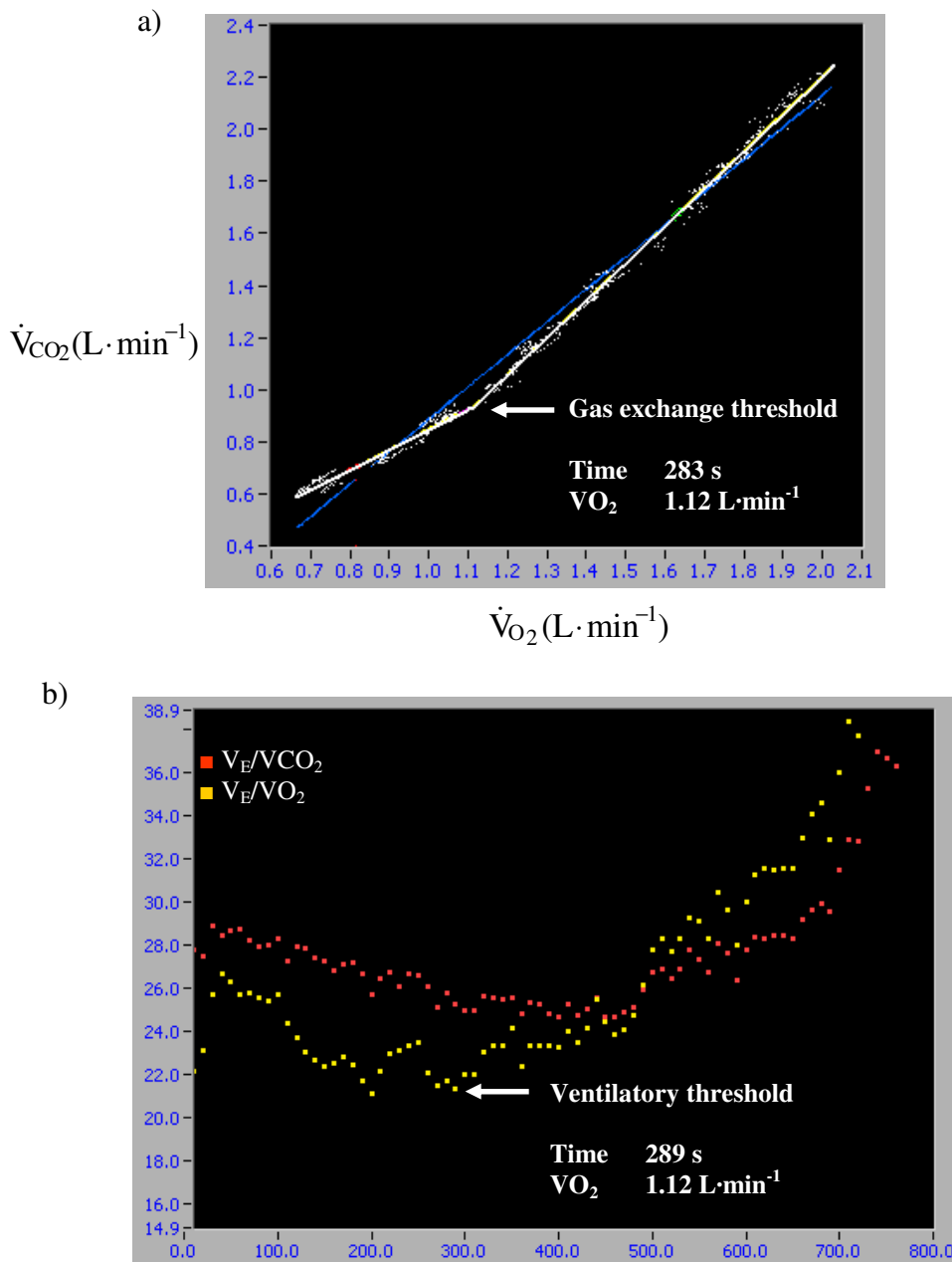
### 3.3.1 Ramp exercise

Preliminary exercise testing involved the performance of a ramp incremental cycle test to the limit of tolerance in order to determine  $\dot{V}_{O_2 \text{ peak}}$  and non-invasively estimate the GET from changes in expired gas concentrations and ventilation. All exercise tests were performed on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) with the seat height, handlebar height, and crank length adapted to each subject and subsequently maintained throughout the testing period. After 3-min of unloaded

pedaling, the work rate was continuously increased by  $15 \text{ W}\cdot\text{min}^{-1}$  in boys (aged  $\leq 12$  yr) and  $30 \text{ W}\cdot\text{min}^{-1}$  in teenagers and adult men to attain a test duration of 8-12 min in each individual. Participants were instructed to cycle at a preferred pedal rate of between 70-80  $\text{rev}\cdot\text{min}^{-1}$  throughout the test, with exhaustion defined as a drop in cadence below 60  $\text{rev}\cdot\text{min}^{-1}$  for 5 consecutive seconds at which point the test was terminated. In the study included in chapter 6, participants were asked to maintain pedal rates of 50  $\text{rev}\cdot\text{min}^{-1}$  and 115  $\text{rev}\cdot\text{min}^{-1}$  with verbal instruction provided if/when they deviated by more than  $\pm 5 \text{ rev}\cdot\text{min}^{-1}$ .

### *3.3.1.1 Calculation of peak $\dot{V}_{O_2}$ and gas exchange threshold (GET)*

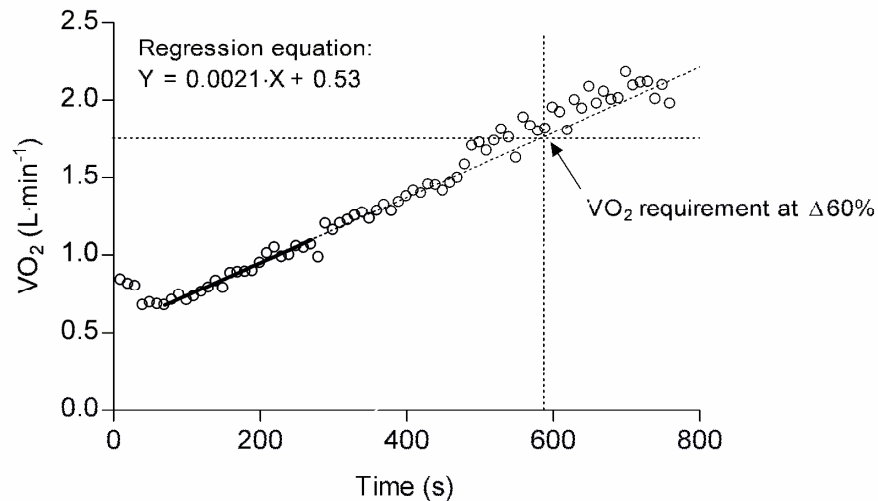
The  $\dot{V}_{O_2 \text{ peak}}$  was taken as the highest recorded 10-s stationary average value during the incremental test which has recently been shown to reflect a maximum  $\dot{V}_{O_2}$  in 93% of children performing ramp exercise (Barker, Williams, Jones, & Armstrong, 2009). The GET was determined using the V-slope method (Beaver, Wasserman, & Whipp, 1986) as the first disproportionate increase in  $\text{CO}_2$  output ( $\dot{V}_{\text{CO}_2}$ ) relative to the increase in  $\dot{V}_{O_2}$ , and subsequently verified from visual inspection of the increase in the ventilator equivalent for  $\dot{V}_{O_2}$  ( $\dot{V}_E/\dot{V}_{O_2}$ ) with no increase in  $\dot{V}_E/\dot{V}_{\text{CO}_2}$  (figure 3.1). The test-retest reproducibility for measuring the GET using these procedures with children in our laboratory has a CV of 5-8 % (Fawcner, Armstrong, Childs, & Welsman, 2002).



**Figure 3.1** Non-invasive determination of the blood lactate threshold during ramp incremental (15 W·min<sup>-1</sup>) exercise in a 12-yr-old boy. Note that an inflection time point reflecting a disproportionate increase in  $\dot{V}_{CO_2}/\dot{V}_{O_2}$  in (a) is corroborated in the ventilatory equivalents for  $\dot{V}_{CO_2}$  and  $\dot{V}_{O_2}$  in (b).

### 3.3.2 Constant work rate exercise

In order to examine  $\dot{V}_{O_2}$  kinetic responses, the external work rate on the cycle ergometer was abruptly increased to elicit a step transition in exercise intensity that remained constant throughout the remainder of exercise. The selected exercise work rates were estimated to



$$\begin{aligned} \dot{V}_{O_2 \text{ peak}} &: && 2.18 \text{ L}\cdot\text{min}^{-1} \\ \dot{V}_{O_2 \text{ at GET}} &: && 1.12 \text{ L}\cdot\text{min}^{-1} \text{ (from figure 3.1)} \\ \text{Target } \dot{V}_{O_2} &: && (2.18 - 1.12) \times 0.60 + 1.12 = 1.76 \text{ L}\cdot\text{min}^{-1} \end{aligned}$$

Time at which target  $\dot{V}_{O_2}$  occurred:  
 $(1.76 - 0.53)/0.0021 = 586 \text{ s (9.8 min)}$

Target power output:  
 $9.8 \times 15 = 147 \text{ W}$   
 where 15 is the ramp rate of  $15 \text{ W}\cdot\text{min}^{-1}$

Correction for  $\dot{V}_{O_2}$  kinetic lag during incremental exercise:  
 $147 \text{ W} - (15 * 0.75) = \underline{136 \text{ W}}$

where 0.75 is the correction factor used for child and adult subjects based on a  $\dot{V}_{O_2}$  mean response time of 45 s that has been reported to be independent of body size (Cooper, Weiler-Ravell, Whipp, & Wasserman, 1984).

**Figure 3.2** Calculation of exercise power output from linear extrapolation of the  $\Delta \dot{V}_{O_2} / \Delta \text{WR}$  response below the GET during incremental exercise in a child participant. Note: the actual  $\dot{V}_{O_2}$  attained at the end of 6-min constant work rate exercise would inevitably exceed the predicted  $\dot{V}_{O_2}$  steady-state amplitude ( $1.76 \text{ L}\cdot\text{min}^{-1}$ ) if the child were to evince a  $\dot{V}_{O_2}$  slow component.

require a fraction of the  $\dot{V}_{O_2}$  at the GET (M exercise) or to target a specific  $\dot{V}_{O_2}$  requirement expressed as a percentage of the difference ( $\Delta$ ) between the GET and  $\dot{V}_{O_2 \text{ peak}}$  (H/VH exercise) following adjustment of the  $\dot{V}_{O_2}$  lag time during ramp exercise (figure 3.2). The work rates estimated to require  $\Delta 60\%$  were prescribed for VH-intensity exercise

for all participants included within chapters 5, 7-8. In chapters 4 and 6, work rates estimated to require  $\Delta 40\%$  and  $\Delta 70\%$  were prescribed for H- and VH-intensity exercise in teenage subjects, respectively.

### 3.4 Data analysis procedures

#### 3.4.1 Breath-by-breath $\dot{V}_{O_2}$ responses

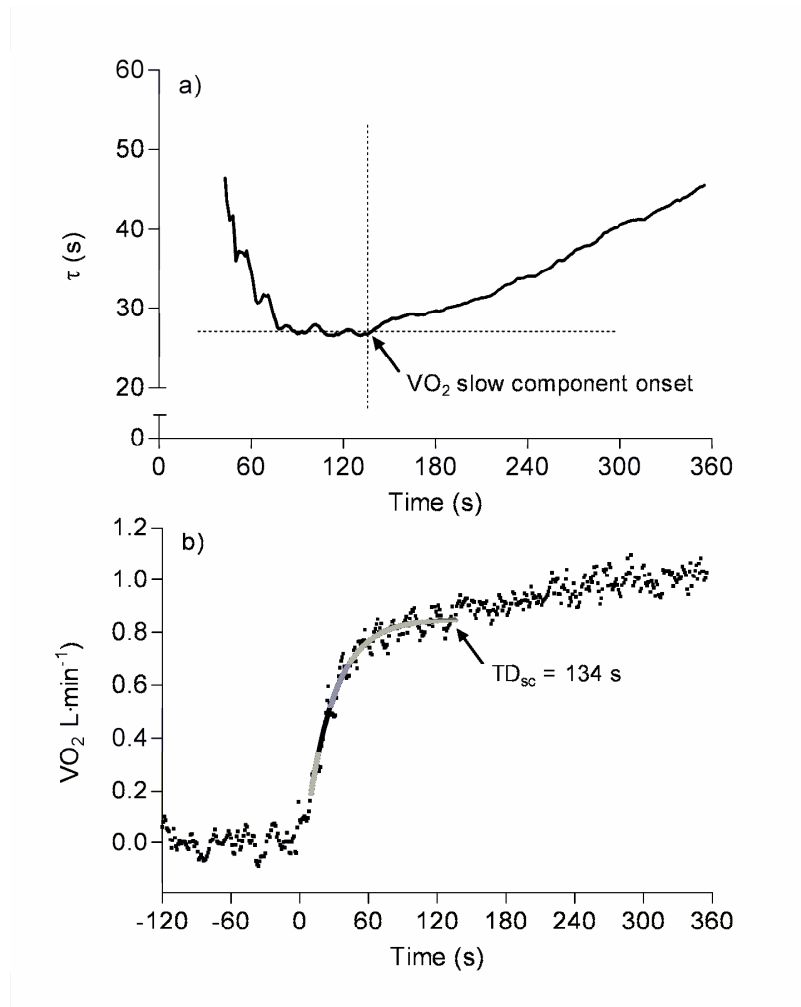
The breath-by-breath  $\dot{V}_{O_2}$  data from each step exercise transition were initially examined to exclude errant breaths by removing values lying more than four standard deviations from the local mean determined using a 5-breath rolling average. Filtered  $\dot{V}_{O_2}$  data were subsequently linearly interpolated to provide second-by-second values and identical repetitions of each step exercise bout were time aligned to the start of exercise and averaged together to enhance the  $\dot{V}_{O_2}$  signal-to-noise response.

The first 15 s of data after the onset of exercise were deleted to remove the phase I (cardio-dynamic) response, and the phase II portion of the  $\dot{V}_{O_2}$  response was modelled using the non-linear equation:

$$\Delta \dot{V}_{O_2} (t) = \Delta \dot{V}_{O_2} \cdot (1 - e^{-(t-TD)/\tau}) \quad \text{Equation 3.2}$$

where  $\Delta \dot{V}_{O_2} (t)$ ,  $\Delta \dot{V}_{O_2}$ , TD and  $\tau$  represent the value of  $\dot{V}_{O_2}$  at a given time (t), the amplitude change in  $\dot{V}_{O_2}$  from baseline to its asymptote, time delay and the time constant of the response, respectively. For exercise above the GET, a purpose-designed software program developed with LabVIEW (National Instruments, Newbury, UK) was used in

order to identify an optimal fitting window with which to estimate the phase II  $\dot{V}_{O_2}$  response parameters. The fitting window was iteratively widened by 1 s intervals, starting



**Figure 3.3** Procedures for isolating the phase II  $\dot{V}_{O_2}$  model fitting window during H/VH exercise transitions. The point at which the individually plotted  $\tau$  values diverge from a plateau and progressively increase thereafter is defined as the time onset for the  $\dot{V}_{O_2}$  slow component (a). A single-exponential model (> phase I) constrained at the  $\dot{V}_{O_2}$  slow component TD is then applied to resolve the phase II parameters of the response (b).

from a 60 s fitting window and finishing with a fitting window that encompassed the entire data set (Rossiter, et al., 2001). The estimated  $\tau$  for each fitting window was plotted against time to allow the beginning of the  $\dot{V}_{O_2}$  slow component to be determined through visual inspection. The onset of the  $\dot{V}_{O_2}$  slow component was defined as the point at which a plateau in the estimated  $\tau$  was followed by a progressive increase in the estimated  $\tau$ .

The phase II parameter estimates from *equation 3.1* were then resolved by least-squares non-linear regression (GraphPad Prism, GraphPad Software, San Diego, CA). The amplitude of the  $\dot{V}_{O_2}$  slow component was calculated from the difference between the mean of the final 30 s at end-exercise and the phase II asymptote and expressed in relative terms against the end-exercise  $\dot{V}_{O_2}$ . To provide information on the ‘overall’  $\dot{V}_{O_2}$  kinetics [mean response time (MRT)], *equation 3.1* with TD constrained to 0 s (i.e. no delay term) was fit from the onset to the end of exercise. For all conditions the functional ‘gain’ of the primary  $\dot{V}_{O_2}$  response was calculated by dividing the asymptotic primary amplitude by the increment in work rate above baseline ( $\Delta \dot{V}_{O_2} / \Delta WR$ ). Likewise, the  $\dot{V}_{O_2}$  gain at end-exercise was calculated in a similar manner.



## Chapter Four

### **LONGITUDINAL CHANGES IN PULMONARY O<sub>2</sub> UPTAKE KINETICS DURING HEAVY-INTENSITY EXERCISE IN 14-16 YR OLD BOYS**

#### **4.1 Introduction**

At the onset of a step transition in exercise there is an almost immediate increase in cardiac output which occurs prior to the arrival at the lungs of venous blood from the exercising muscles. This cardiodynamic phase (phase I) which lasts about 15-20 s is independent of oxygen uptake at the muscle ( $m\dot{V}O_2$ ) and reflects an increase in pulmonary blood flow with exercise. Phase II, the primary component, is a rapid exponential increase in pulmonary oxygen uptake ( $\dot{V}O_2$ ) that arises with hypoxic and hypercapnic blood from the exercising muscles arriving at the lungs. Phase II kinetics are described by the time constant ( $\tau$ ) which is the time taken to achieve 63% of the change in  $\dot{V}O_2$ . In phases I and II, adenosine triphosphate (ATP) resynthesis cannot be fully supported by oxidative phosphorylation and the additional energy requirements of the exercise are met from oxygen stores, phosphocreatine (PCr) and glycolysis.

During moderate-intensity exercise (i.e. exercise below the GET),  $\dot{V}O_2$  reaches a steady state in children within about 2 min with an oxygen cost (gain) of about  $10 \text{ mL}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$  above that found during unloaded pedalling. During heavy-intensity exercise [i.e. exercise above the GET but below critical power or maximal lactate steady state], the primary phase II gain is similar to that observed during moderate-intensity exercise but the oxygen cost increases over time as a slow component of  $\dot{V}O_2$  is superimposed upon the primary component and the achievement of a steady state is delayed.

Recent studies using  $^{31}\text{P}$ -magnetic resonance spectroscopy ( $^{31}\text{P}$ -MRS) have revealed a close kinetic coupling between muscle PCr, a surrogate of muscle  $\text{O}_2$  consumption (Meyer, 1988), and  $\dot{V}\text{O}_2$  during both moderate-intensity and heavy-intensity exercise in adults (Rossiter, Ward, Kowalchuk, et al., 2002) and moderate-intensity exercise in children (Barker, Welsman, Fulford, Welford, Williams, et al., 2008). The mechanisms underlying the slow component remain speculative but appear to be a function of muscle fibre distribution, motor unit recruitment and the matching of oxygen delivery to active muscle fibres (Gaesser & Poole, 1996). Therefore, the study of  $\dot{V}\text{O}_2$  kinetics to an imposed heavy-intensity exercise stimulus has the potential to provide important information on developmental changes in exercise metabolism.

Armon et al. (1991) were the first to examine child-adult differences in  $\dot{V}\text{O}_2$  kinetics during 6 min of cycling exercise across a range of exercise intensities above the GET. They concluded that, unlike adults, a single exponential was sufficient to describe the  $\dot{V}\text{O}_2$  responses of a mixed group of boys and girls. Although their methodology of using an arbitrary linear term set from 3-6 min is likely to have misrepresented the actual magnitude of the  $\dot{V}\text{O}_2$  slow component (Fawkner & Armstrong, 2004b), Armon et al. did report a positive linear term in 73% of the children's responses above the GET. They observed children to exhibit faster exercise onset  $\dot{V}\text{O}_2$  kinetics and to consume a greater percentage of the final  $\dot{V}\text{O}_2$  during the initial exponential phase compared to adults (Armon, et al., 1991). Williams et al. (2001) also reported a negligible  $\dot{V}\text{O}_2$  slow component, comprising 0.9% of the total  $\dot{V}\text{O}_2$  response, in children during 6 min of heavy-intensity treadmill

running, and concluded that a single exponential model was sufficient to resolve the kinetic response parameters. However, given that the same authors reported that the  $\dot{V}O_2$  slow component is markedly reduced during treadmill running compared to cycling exercise (Carter, et al., 2000), it is difficult to ascertain whether this finding is reflective of the child's physiology or the exercise modality employed.

To overcome these methodological concerns, Fawkner & Armstrong (2004a) longitudinally investigated the  $\dot{V}O_2$  kinetic response during heavy-intensity cycling exercise in pre-pubertal children over a 2-yr period. These authors used an iterative curve fitting procedure to firstly identify the onset of the  $\dot{V}O_2$  slow component, and then fitted a single exponential model to the phase II response only. In contrast to earlier reports, there was a discernible  $\dot{V}O_2$  slow component manifest in pre-pubertal children, and, over the 2-yr interval, the phase II  $\tau$  and  $\dot{V}O_2$  slow component were significantly increased (Fawkner & Armstrong, 2004a). This study was the first to report the 95% confidence intervals for children's phase II  $\tau$  during heavy-intensity exercise and to eliminate the possible confounding effects of inter-subject variability by using a longitudinal design to study appropriately age-related influences on  $\dot{V}O_2$  kinetics in children.

There are few data on  $\dot{V}O_2$  kinetics during heavy-intensity exercise in older children. A recent investigation reported that both the phase II  $\tau$  and  $\dot{V}O_2$  slow component during heavy-intensity cycling exercise in male adolescents aged 14-17 yrs were comparable to adult data reported previously by others (Lai, et al., 2008). However, no previous investigation has examined whether the age-related changes in  $\dot{V}O_2$  kinetics during heavy-

intensity exercise evidenced in pre-pubertal children (Fawkner & Armstrong, 2004a) persist or increase through the teen years. Therefore, the purpose of this study was to longitudinally investigate changes in the  $\dot{V}O_2$  kinetic response to heavy-intensity cycling exercise in 14-16 yr old boys. We hypothesised that both the phase II  $\tau$  and  $\dot{V}O_2$  slow component would significantly increase over a 2-yr period.

## **4.2 Methods**

### *4.2.1 Participants*

Fourteen healthy boys completed all tests on two occasions, separated by a 2-yr interval. Written, informed consent was obtained from each participant and their parents. Ethical approval was granted by the Institutional Research Ethics Committee. Participants were instructed to visit the laboratory on a minimum of four occasions over a 2-wk period, and testing took place at approximately the same time of day for each participant. On each visit, participants' stature was measured with a Seca 220 stadiometer (Vogel & Halke, Hamburg, Germany) and body mass determined by use of Seca electronic scales (Vogel & Halke). Due to the prevailing sociological climate in the UK at the time of the study regarding maturity screening we were unable to assess the participants' maturational status.

### *4.2.2 Experimental procedures.*

The same investigators collected the data using the same apparatus on both test occasions. All exercise tests were performed on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) with the seat height, handlebar height, and crank length adapted to each child and subsequently maintained throughout the testing period. The Lode ergometer was calibrated according to the manufacturer's

recommendations and had a baseline pedalling resistance equivalent to 10 W at 70 rpm. During all exercise tests, gas-exchange variables were measured and displayed online by use of an EX670 mass spectrometer and analysis suite (Morgan Medical, Rainham, UK) that was calibrated according to the manufacturer's instructions. Expired volume was measured using a turbine flowmeter (Interface Associates) with a dead space volume of 90 mL. Volume calibration was achieved by using a handheld 3-L calibration syringe (Hans Rudolph, Kansas City, MO) over a range of flow speeds. The sum of the gas-transport and analyzer-response delay terms was determined, and appropriate adjustments were made in the software. All calibration procedures were repeated before each experimental test.

On the first visit, participants completed an incremental ramp test to voluntary exhaustion for determination of  $\dot{V}O_{2\text{peak}}$  and GET. After a 3-min baseline of unloaded pedalling, the resistance increased continuously by 25 and 40 W·min<sup>-1</sup> that resulted in the termination of exercise between 8-10 min for each participant on test occasions 1 and 2, respectively. Participants pedalled at a cadence of 70 ± 5 rpm, and were actively encouraged to continue until voluntary exhaustion. Maximal effort was considered to have been given if, in addition to subjective indications of intense effort (e.g., excessive hyperpnoea, facial flushing, sweating, discomfort), respiratory exchange ratio reached a value > 1.00. All participants satisfied these criteria.  $\dot{V}O_{2\text{peak}}$  was taken as the highest recorded 10-s stationary average value during the maximal exercise test. The GET was determined non-invasively as the first disproportionate increase in CO<sub>2</sub> output ( $\dot{V}CO_2$ ) relative to the increase in  $\dot{V}O_2$  using methods previously described from our laboratory (Fawkner & Armstrong, 2004a).

On subsequent visits, subjects completed a series of ‘step’ exercise transitions consisting of 6 min of unloaded pedalling, followed instantaneously by a work rate that, by extrapolation from the ramp response, corresponded to 40% of the difference ( $\Delta$ ) between the  $\dot{V}O_2$  at the GET and  $\dot{V}O_{2\text{ peak}}$  for 9 min. This intensity has been shown with boys to lie within the heavy-intensity domain (Fawkner & Armstrong, 2003). A pedal cadence of 70 rpm was maintained throughout. A single transition was completed on each visit and at least three and in most cases four transitions were completed in total to obtain 95% confidence intervals within  $\pm 5$  s for the phase II  $\tau$ .

#### *4.2.3 Data analysis procedures.*

All procedures stated in section 3.3.1 on the treatment and modelling of breath-by-breath  $\dot{V}O_2$  data in response to step exercise were completed.

#### *4.2.4 Statistical analyses*

Paired samples t-tests were used to identify significant differences in  $\dot{V}O_2$  responses between both test occasions. Statistical significance was set at an alpha level of 0.05.

### **4.3 Results**

Participants’ physiological responses to incremental exercise are presented in Table 4.1. The  $\dot{V}O_{2\text{ peak}}$  and GET increased in absolute terms ( $L \cdot \text{min}^{-1}$ ) over the 2-yr study period. No significant changes were found for  $\dot{V}O_{2\text{ peak}}$  normalised per body mass ( $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) or for the GET when expressed in relation to  $\dot{V}O_{2\text{ peak}}$ . Based on the ramp exercise responses, the power outputs calculated to require  $\Delta 40\%$  were  $149 \pm 35$  and  $177 \pm 23$  W on test occasions 1 and 2, respectively.

**Table 4.1** Participant descriptive statistics and peak exercise responses on separate test occasions over a 2-yr interval

	Test Occasion 1	Test Occasion 2	<i>P</i> value
Age (yr)	14.1 ± 0.2	16.0 ± 0.2	0.001
Body mass (kg)	62.9 ± 10.2	71.6 ± 11.1	0.001
Stature (m)	1.72 ± 0.10	1.80 ± 0.11	0.001
$\dot{V}O_{2\text{ peak}}$ (L·min <sup>-1</sup> )	3.14 ± 0.46	3.78 ± 0.41	0.001
$\dot{V}O_{2\text{ peak}}$ (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	50 ± 5	54 ± 8	0.106
GET (L·min <sup>-1</sup> )	1.56 ± 0.25	1.91 ± 0.33	0.003
GET (% $\dot{V}O_{2\text{ peak}}$ )	50 ± 5	49 ± 3	0.712
$\dot{V}O_2$ at $\Delta$ 40% (L·min <sup>-1</sup> )	2.20 ± 0.32	2.66 ± 0.34	0.001
$\dot{V}O_2$ at $\Delta$ 40% (% $\dot{V}O_{2\text{ peak}}$ )	70 ± 3	70 ± 3	0.786

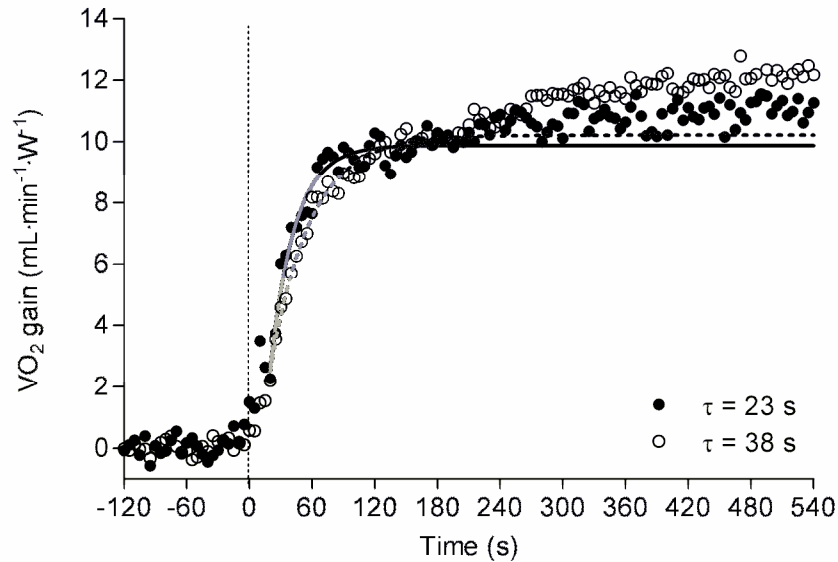
Data are presented as mean ± SD. GET, gas exchange threshold;  $\Delta$  40%,  $\dot{V}O_2$  requirement at 40 % of the difference between the GET and  $\dot{V}O_{2\text{ peak}}$ .

Table 4.2 presents the  $\dot{V}O_2$  kinetic parameters on each test occasion. The  $\dot{V}O_2$  response in a representative participant is presented in figure 4.1 with mean  $\dot{V}O_2$  profiles on each test occasion shown in figure 4.2. Over the 2-yr duration, the phase II  $\tau$  was significantly slowed and there was a significantly greater proportional contribution from the  $\dot{V}O_2$  slow component to the end-exercise  $\dot{V}O_2$  above baseline pedalling. There were no significant differences in the O<sub>2</sub> cost of exercise per unit increment in work rate over the primary exponential phase between test occasions. The end-exercise  $\dot{V}O_2$  gain was significantly greater on test occasion 2 compared to test occasion 1. The end-exercise amplitude exceeded the estimated  $\dot{V}O_2$  requirement at  $\Delta$ 40% but there were no differences in percentage utilisation of  $\dot{V}O_{2\text{ peak}}$  between test occasions 1 and 2 (78 ± 4% vs. 81 ± 7%, respectively).

**Table 4.2** Pulmonary O<sub>2</sub> uptake kinetics during heavy-intensity step exercise

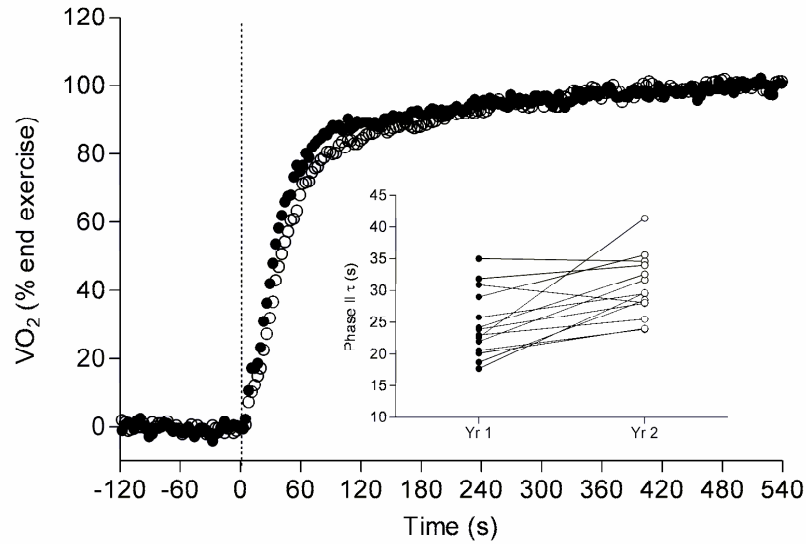
Variable	Test Occasion 1	Test Occasion 2	<i>P</i> value
Baseline $\dot{V}O_2$ (L·min <sup>-1</sup> )	0.72 ± 0.10	0.85 ± 0.12	0.002
Phase II time delay (s)	12 ± 3	13 ± 4	0.232
Phase II time constant (s)	25 ± 5	30 ± 5	0.002
Primary $\dot{V}O_2$ amplitude (L·min <sup>-1</sup> )	1.56 ± 0.34	1.92 ± 0.28	0.002
Primary gain (mL·min <sup>-1</sup> ·W <sup>-1</sup> )	10.5 ± 0.8	10.8 ± 0.7	0.157
$\dot{V}O_2$ slow component amplitude (L·min <sup>-1</sup> )	0.16 ± 0.08	0.28 ± 0.10	0.003
Relative $\dot{V}O_2$ slow component amplitude (%)	9 ± 5	13 ± 4	0.036
End-exercise $\dot{V}O_2$ (L·min <sup>-1</sup> )	2.44 ± 0.46	3.05 ± 0.32	0.001
Total $\dot{V}O_2$ gain (mL·min <sup>-1</sup> ·W <sup>-1</sup> )	11.6 ± 0.6	12.4 ± 0.7	0.001

Data are presented as mean ± SD.



**Figure 4.1**  $\dot{V}O_2$  response normalised per unit increment in work rate on test occasion 1 (●) and test occasion 2 (○) in a representative subject. The solid and dashed grey lines denote the phase II model fit extended to illustrate the magnitude of the  $\dot{V}O_2$  slow component. The onset of step exercise is indicated by the vertical dotted line.





**Figure 4.2** Mean  $\dot{V}O_2$  profile ( $n = 14$ ) during heavy-intensity exercise on test occasion 1 (●) and test occasion 2 (○). The  $\dot{V}O_2$  data are expressed as a percentage of the end-exercise amplitude above baseline pedalling. The onset of step exercise is indicated by the vertical dotted line. *Insets:* individual phase II  $\tau$  values over the 2-yr duration of the study.

#### 4.4 Discussion

This is the first study to investigate longitudinally the  $\dot{V}O_2$  kinetic response at the onset of heavy-intensity cycling exercise in teenage boys. The principal findings were that over a 2-yr interval the phase II  $\tau$  significantly slowed by  $\sim 5$  s and the  $\dot{V}O_2$  slow component amplitude increased by  $\sim 4\%$  when expressed relative to the end-exercise  $\dot{V}O_2$ . These results suggest an age-dependent influence on the physiological mechanisms regulating oxidative metabolism at the onset of heavy-intensity cycling exercise in males between the ages of 14-16 yr.

##### 4.4.1 Phase II $\dot{V}O_2$ response

Data from the present study demonstrate a similar trend in the phase II  $\tau$  to that previously reported from a longitudinal investigation in pre-pubertal children at the onset of heavy-

intensity cycling exercise (Fawkner & Armstrong, 2004a). Using the same apparatus, methods and analytical techniques over the same time period as the present study, Fawkner & Armstrong demonstrated that in a group of 13 pre-pubertal boys (mean age = 10.6 [0.3] yr) the phase II  $\tau$  significantly increased from 17 [5] to 22 [5] s over a 2-yr period (Fawkner & Armstrong, 2004a). In the present study, the phase II  $\tau$  significantly increased from 25 [5] to 30 [5] s over a 2-yr time interval therefore evincing a progressively slower phase II response during heavy-intensity exercise with increasing age.

On the first test occasion with younger boys, we were able to assess pubic hair to estimate stage of maturation but, due to the prevailing sociological climate in the UK at the time of other tests in the series, we were unable to repeat the procedure on subsequent occasions. In an earlier longitudinal study of young people within the same age range as our participants, we demonstrated an independent and positive maturational influence on  $\dot{V}O_{2\text{ peak}}$  even with age, body size and fatness controlled for (Armstrong & Welsman, 2001) but we and others have documented that, as in the present study, there was no relationship between young people's  $\dot{V}O_{2\text{ peak}}$  and either the phase II  $\tau$  or the slow component during heavy-intensity exercise (Cleuziou, et al., 2002; Fawkner & Armstrong, 2004a). It is possible, however, that maturational changes have an independent influence on the  $\tau$  and/or slow component over and above those due to chronological age, but this awaits further investigation. Adult data were not collected in the current study and inter-study comparisons of  $\dot{V}O_2$  kinetic data must be made with extreme caution, but it is of interest to note that the mean phase II  $\tau$  obtained on the second test occasion in the present study is similar to values previously reported in adults (Ozyener, et al., 2001) and in 14-17 yr old males (Lai, et al., 2008) during cycling exercise within the same intensity domain.

It is not readily apparent why the phase II  $\tau$  slows with age during the teen years but the primary kinetics is considered to be principally dependent upon the mitochondrial potential to generate the required ATP for exercise with a possible contribution from O<sub>2</sub> transport to the site of utilization at higher work rates (Armstrong & Barker, 2009). There is limited evidence of a tendency for O<sub>2</sub> delivery (muscle blood flow per unit tissue) to decrease from the ages of 12 to 16 yr (Koch, 1984). The extent to which O<sub>2</sub> delivery limits phase II  $\dot{V}O_2$  kinetics during heavy-intensity exercise is clouded by contradictory data and it seems most likely that O<sub>2</sub> only becomes rate limiting when severely restricted by disease or old age (Fawcner & Armstrong, 2008b). However, whether O<sub>2</sub> delivery decreases with age during childhood and limits the kinetic response to heavy-intensity exercise remains to be determined.

There are several competing theories for the control of oxidative phosphorylation at the onset of exercise [see Poole, Barstow, McDonough, & Jones (2008) for a recent review]. A popular contention, however, is that the rate of  $\dot{V}O_2$  at exercise onset is regulated by the exchange of intramuscular phosphates between the splitting of ATP at the myofibrils and its subsequent synthesis at the mitochondria (Meyer, 1988; Walsh, et al., 2001). Upon a step increase in metabolic demand, ATP levels are temporally buffered by the breakdown of muscle PCr catalyzed by the creatine kinase reaction as described in *equation 2.5*.

The subsequent release of creatine (Cr) and its rephosphorylation by creatine kinase within the mitochondria provides the requisite ADP substrate in order to stimulate oxidative phosphorylation, and is termed the 'PCr-Cr shuttle' mechanism (Bessman & Geiger, 1981).

Rossiter et al. (2002) have reported a dynamic symmetry between the rate of PCr breakdown and the rise in phase II  $\dot{V}O_2$  during the on-transient response at the onset of high-intensity exercise in adults. This suggests that faster phase II  $\dot{V}O_2$  kinetics reported in children compared to adults might be explained by an age dependent change in the putative phosphate linked controller(s) of mitochondrial oxidative phosphorylation. Whether there is an age-dependent effect on the exchange of intramuscular phosphates regulating mitochondrial oxidative phosphorylation during heavy-intensity exercise is presently unclear. However, a recent study from our laboratory (Willcocks, et al., 2010) reported a non-statistically significant trend for faster muscle PCr kinetics in 13 yr old boys compared to men at the onset of high-intensity quadriceps exercise (PCr  $\tau$  = 31 vs. 45 s), which is consistent with the  $\dot{V}O_2$  kinetics data presented herein. The mechanisms underlying the more rapid muscle PCr kinetics in younger children await resolution, although enhanced mitochondrial properties and/or lower stores of resting muscle PCr have been implicated (Armstrong & Barker, 2009).

#### 4.4.2 $\dot{V}O_2$ slow component

As hypothesized, for exercise above the GET, there was a discernible  $\dot{V}O_2$  slow component response in boys exercising within the heavy-intensity domain. This supports the existence of a  $\dot{V}O_2$  slow component in youth which has been shown previously in pre-pubertal children (Fawcner & Armstrong, 2004a, 2004b, 2004c) and in teenage participants (Lai, et al., 2008) exercising in this domain. The relative contribution of the  $\dot{V}O_2$  slow component to the total gain in  $\dot{V}O_2$  also increased significantly over the 2-yr interval. As stated earlier, cross-study comparisons should be made cautiously, but it is pertinent to note that both the

absolute and relative  $\dot{V}O_2$  slow component amplitude (0.28 L·min<sup>-1</sup> and 13%) found on test occasion 2 was similar to that previously reported by Özeyner et al. (2001) in adults during heavy-intensity cycling exercise (0.22 L·min<sup>-1</sup> and 12 %).

The magnitude of the  $\dot{V}O_2$  slow component found on test occasion 2 was similar to that reported by Lai et al. (2008) in teenage boys. However, in contrast to the double-exponential model used in previous studies, we opted to fit a single exponential model in order to resolve the phase II  $\tau$  within a predetermined fitting window that excluded the  $\dot{V}O_2$  slow component, with an ‘excess’  $\dot{V}O_2$  computed as the difference between the primary amplitude and the  $\dot{V}O_2$  at end-exercise. It is of interest to note that when applying a double-exponential model to the current data on test occasion 2, the mean 95% confidence intervals for the phase II  $\tau$  increased from  $\pm 4.8$  to  $\pm 8.2$  s and resulted in 13 participants obtaining confidence intervals greater than  $\pm 5$  s. This not only identifies the limitations of cross study comparisons but also reinforces concerns Fawkner & Armstrong (2008a) have examined empirically in terms of combining exponential terms within a single model when attempting to estimate phase II  $\dot{V}O_2$  kinetics at the onset of heavy-intensity exercise in young people.

In combination with the previous investigation from Fawkner & Armstrong (2004a), the present findings indicate an age-related increase in the magnitude of the  $\dot{V}O_2$  slow component between the ages of 11 to 16 yrs. It is widely accepted that a reduction in exercise efficiency which manifests itself as the  $\dot{V}O_2$  slow component originates from processes occurring within contracting muscle (Poole, et al., 1991), and is temporally

correlated with an increased muscle PCr cost of exercise (Rossiter, Ward, Howe, et al., 2002). Previous studies have demonstrated the emergence of the  $\dot{V}O_2$  slow component to coincide both temporally and in magnitude with markers of muscle activity (Endo, et al., 2007; Saunders, et al., 2000; Shinohara & Moritani, 1992), supporting the notion that putative changes in motor unit recruitment might contribute to the development of the  $\dot{V}O_2$  slow component. A significant correlation between the percentage of type II muscle fibres inferred from biopsy data and amplitude of the  $\dot{V}O_2$  slow component lends further support to this hypothesis (Barstow, et al., 1996; Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003). Likewise, the selective glycogen depletion of type I fibres elicits a  $\dot{V}O_2$  slow component amplitude in the moderate-intensity domain under conditions in which the recruitment of type II fibres is isolated (Krustrup, Soderlund, Mohr, et al., 2004a).

Given the above, it is conceivable that developmental changes in the expression of muscle fibre types at rest and/or their recruitment pattern during exercise might be linked to age-related increases in the  $\dot{V}O_2$  slow component during heavy-intensity exercise. In a comprehensive review, Jansson (1996) concluded that the percentage of type I fibres decreased from ~ 58 % to ~ 48 % from the ages of 10 to circa 20 yr in male humans. Furthermore, an increased  $P_i$ /PCr response and fall in pH during incremental forearm exercise has been correlated with a reduced expression of type I muscle fibres in adults (Mizuno, et al., 1994). From this perspective, it is pertinent to note that children have been characterised by a lower  $P_i$ /PCr response and attenuated reduction in pH during high-intensity exercise compared to adults (Zanconato, et al., 1993). A logical hypothesis could therefore be that during high-intensity exercise younger children achieve the required power output through recruiting more highly oxidative type I muscle fibres thereby

attenuating the accumulation of fatigue inducing metabolites ( $P_i$  and  $H^+$ ) within the contracting muscle and reducing the need to recruit less efficient type II motor units (Armstrong & Barker, 2009).

Data from animal models has demonstrated an impaired capacity in type II myocytes to sustain the microvascular  $O_2$  pressure head requisite for capillary-to-muscle  $O_2$  diffusion at the onset of contractions (Behnke, et al., 2003; McDonough, et al., 2005) thereby presumably obligating a greater reliance on substrate-level phosphorylation in these fibres. Type II muscle fibres are also purported to have a have an increased ATP consumption per unit of tension development compared to type I fibres in small mammals (Crow & Kushmerick, 1982). The additional recruitment of higher-order type II muscle fibres during heavy-intensity exercise might therefore be expected to increase the overall phosphate cost of force production and elevate the eventual  $\dot{V}O_2$  gain (as shown in figure 4.2 for test occasion 2 compared to the first test occasion). Therefore, these data, coupled with previous evidence of an age-related decline in the expression of type I muscle fibres (Crow & Kushmerick, 1982; Glenmark, et al., 1992; Jansson, 1996; Lexell, et al., 1992), might explain the increased  $\dot{V}O_2$  slow component and elevated total  $\dot{V}O_2$  gain found over the 2-yr interval in the present investigation. However, this hypothesis requires further confirmation using non-invasive interventions such as work-to-work exercise transitions (Brittain, et al., 2001; Wilkerson & Jones, 2007) and extreme pedal cadences (Barstow, et al., 1996; Pringle, Doust, Carter, Tolfrey, & Jones, 2003) in order to manipulate muscle fibre recruitment patterns in participants at different stages of maturation.

#### 4.5 Conclusion

In summary, this is the first investigation to examine longitudinal changes in  $\dot{V}O_2$  kinetics during heavy-intensity exercise in teenage boys. We found a significantly slower phase II  $\tau$  and greater  $\dot{V}O_2$  slow component over a 2-yr time period. It is proposed these results might be linked to an age dependent change in putative phosphate linked controller(s) of mitochondrial oxidative phosphorylation, and/or differences in muscle fibre recruitment patterns.

Note: the data reported here were collected by N. Armstrong, S.G. Fawkner and J.R. Welsman but extracted from the CHERC database, analysed and interpreted by B.C. Breese.



## Chapter Five

### **THE INFLUENCE OF AGE ON PULMONARY O<sub>2</sub> UPTAKE AND MUSCLE DEOXYGENATION KINETICS DURING VERY HEAVY-INTENSITY EXERCISE TRANSITIONS INITIATED FROM AN ELEVATED BASELINE WORK RATE**

#### **5.1 Introduction**

During step exercise transitions to a higher metabolic rate, pulmonary O<sub>2</sub> uptake ( $\dot{V}O_2$ ) rises exponentially with similar kinetics (denoted by the phase II  $\tau$ ) to that of muscle O<sub>2</sub> consumption ( $m\dot{V}O_2$ ) after the muscle-to-lung transit delay has been accounted for (Grassi, et al., 1996; Krstrup, et al., 2009). Therefore, exploration of the factor(s) limiting  $\dot{V}O_2$  kinetics during exercise in youth can provide useful information on developmental aspects of muscle oxidative metabolism.

Cross-sectional studies have revealed more rapid  $\dot{V}O_2$  kinetics during step exercise above the gas exchange threshold (GET) in pre-pubertal children compared to adult counterparts (Armon, et al., 1991; Williams, et al., 2001). Longitudinal studies in healthy boys aged 11-17 yrs have demonstrated that  $\dot{V}O_2$  kinetics are modulated from childhood into adolescence with an estimated ~ 76% increase in the phase II  $\dot{V}O_2$   $\tau$  (Breese, et al., 2010; Fawcner & Armstrong, 2004a). Although the mechanism(s) that underpin developmental changes in  $\dot{V}O_2$  kinetics remain uncertain [see Armstrong & Barker (2009) for a recent review], a negative correlation between the phase II  $\tau$  and the proportion of type I muscle fibres in adult *m. vastus lateralis* (Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003) might suggest that age-dependent alterations in phase II  $\dot{V}O_2$  dynamics could be linked to

differences in muscle fibre recruitment during high-intensity exercise. Given the purported decline in type I fibre expression with age (Glenmark, et al., 1992; Lexell, et al., 1992), it is therefore conceivable that a shorter phase II  $\dot{V}O_2$   $\tau$  in children might reside in the recruitment of fewer type II motor units compared to adults.

It has been proposed that initiating step exercise transitions from an elevated baseline work rate (i.e. work-to-work exercise) might be a useful approach to non-invasively explore muscle fibre specific influences on  $\dot{V}O_2$  kinetics (DiMenna, et al., 2009a; DiMenna, et al., 2008). This supposition is based on the size principle that posits an orderly recruitment of motor units in relation to motoneurone size and the intensity of muscle contractile activity (Henneman & Mendell, 1981). Smaller motoneurons that innervate fewer muscle fibres with a greater oxidative capacity (i.e. type I motor units) are recruited first at low force outputs (i.e. during moderate exercise). Conversely, larger motor units (i.e. type IIa and IIx) are recruited as the requirement for muscle force production is increased. Slower phase II  $\dot{V}O_2$  kinetics and a greater  $\dot{V}O_2$  gain during work-to-work exercise compared to transitions initiated from unloaded pedalling has therefore been suggested to reflect the metabolic properties of higher-order motor units that are unveiled on the  $\dot{V}O_2$  response in this condition (Brittain, et al., 2001; DiMenna, Bailey, Vanhatalo, Chidnok, & Jones, 2010; DiMenna, et al., 2008; Wilkerson & Jones, 2006, 2007). Reports of an increased  $O_2$  cost and slower  $\dot{V}O_2$  kinetics during submaximal exercise following selective glycogen depletion and neural blockade of type I muscle fibres in humans is consistent with this proposal (Krustrup, et al., 2008; Krustrup, Soderlund, Mohr, et al., 2004a).

Previous demonstrations of an unchanged HR  $\tau$  [and by extension cardiac output ( $\dot{Q}$ ) dynamics] despite slower phase II  $\dot{V}O_2$  kinetics when step transitions above the GET are initiated from an elevated baseline work rate compared to unloaded pedalling have been interpreted to suggest that bulk  $O_2$  transport is not rate limiting at the onset of work-to-work exercise (DiMenna, Bailey, et al., 2010; DiMenna, et al., 2008; Wilkerson & Jones, 2006). Likewise, a pronounced time delay ( $\sim 8s$ ) prior to increases in deoxygenated (HHb) hemoglobin within muscle regions interrogated by near-infrared spectroscopy (NIRS) has been interpreted to indicate that blood flow delivery is matched to the rate of muscle  $O_2$  utilisation ( $\dot{Q}O_2/\dot{V}O_2$ ) at the onset of upright exercise in adults (Grassi, et al., 2003). In contrast, a faster reduction in microvascular  $PO_2$  (analogues to an increased [HHb] response) following the onset of contractions in fast-twitch compared to slow-twitch animal muscle (Behnke, et al., 2003; McDonough, et al., 2005) might suggest a fibre-type dependency on the  $\dot{Q}O_2/\dot{V}O_2$  response toward a greater reliance on fractional  $O_2$  extraction in type II motor units.

The primary purpose of this study was to utilise the work-to-work exercise model in order to explore the putative influence of muscle fibre recruitment on pulmonary  $\dot{V}O_2$  and muscle [HHb] kinetics in relation to age. It was hypothesised that very heavy-intensity exercise transitions initiated from an elevated baseline work rate (M $\rightarrow$ VH) would: 1) speed primary [HHb] kinetics (TD +  $\tau$ ) following the onset of exercise; 2) lengthen the phase II  $\dot{V}O_2$   $\tau$ ; 3) increase the muscle [HHb] per unit increment in  $\dot{V}O_2$  during the primary phase, and; 4) increase the total  $O_2$  cost or ‘gain’ per unit increment in work rate above baseline pedalling ( $\Delta \dot{V}O_2/\Delta WR$ ) compared to step exercise elicited from unloaded pedalling (U $\rightarrow$ M

and U→VH) in teenagers (T) and men (M). It was also hypothesised that the same  $\dot{V}O_2$  kinetic and muscle [HHb] parameters would not be altered in M→VH compared to U→M and U→VH exercise in younger boys (B).

## 5.2 Methods

### 5.2.1 Participants

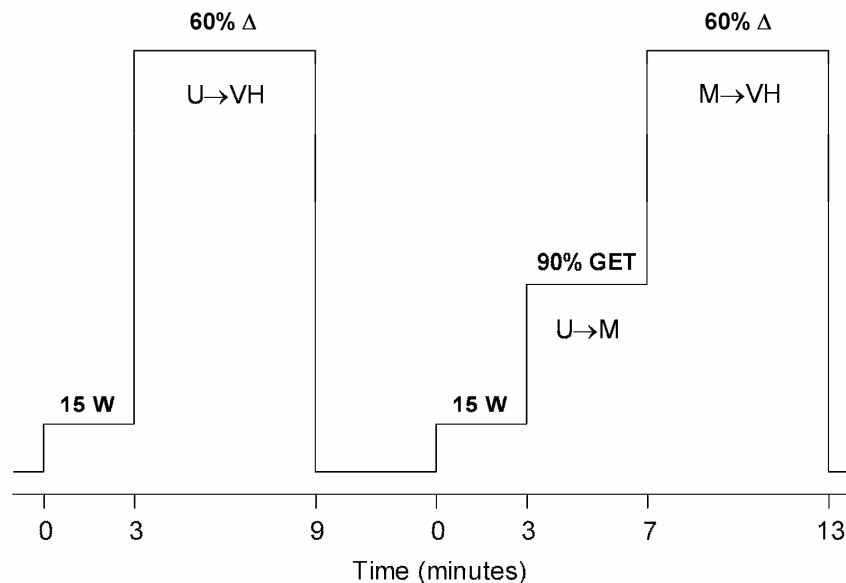
Twenty-six male subjects (8 boys [B], 9 teenagers [T], and 9 men [M]) volunteered to take part in this study after written informed consent had been obtained from each individual and the children's parents/guardians. In order to provide an estimate of biological maturity in youth participants', sex-specific regression algorithms were used to determine a maturity offset score from the age at peak height velocity (PHV) using anthropometric measurements (Mirwald, et al., 2002). Each individual youth subject (aged 11-16 yr) reported regular exercise training including representative sports participation at local school and/or club level. All procedures employed in this study were approved by the institutional ethics committee at the University of Exeter.

### 5.2.2 Experimental procedures

Participants completed a total of nine, seven, and five exercise tests in B, T, and M, respectively over a 4-wk period at approximately the same time of day ( $\pm 2$  hrs). Each was requested to arrive in a rested and well hydrated state having also abstained from food or caffeine in the preceding 3 hr. On the first visit, participants completed a ramp incremental exercise test to voluntary exhaustion for determination of  $\dot{V}O_{2\text{ peak}}$  and the gas exchange threshold (GET). After 3-min baseline cycling at 15 W, the work rate increased continuously by 15-30 W·min<sup>-1</sup> to attain a test duration of 8-12 min in each individual.

Participants were instructed to select a preferred pedal rate of between 70-80 rev·min<sup>-1</sup> and maintain this cadence throughout the test. The  $\dot{V}O_{2\text{peak}}$  was taken as the highest recorded 10-s stationary average value during the incremental test which has been shown to reflect a maximum  $\dot{V}O_2$  in 93% of young people performing ramp exercise (Barker, Williams, Jones, & Armstrong, 2009). The GET was determined as the first disproportionate increase in CO<sub>2</sub> output ( $\dot{V}CO_2$ ) relative to the increase in  $\dot{V}O_2$  [i.e. the V-slope method (Beaver, et al., 1986)] and subsequently verified from visual inspection of the increase in the ventilatory equivalent for  $\dot{V}O_2$  ( $\dot{V}_E / \dot{V}O_2$ ) with no increase in  $\dot{V}_E / \dot{V}CO_2$ .

The power outputs that would require 90% of the GET (moderate-intensity exercise) and 60% of the difference ( $\Delta$ ) between the GET and  $\dot{V}O_{2\text{peak}}$  (very heavy-intensity exercise, 60%  $\Delta$ ) were estimated for each participant. Each participant then returned to the laboratory



**Figure 5.1** Schematic illustration of the experimental protocol including each exercise condition (U→M, U→VH, and M→VH).

to perform one of two step exercise protocols: 1) 3-min of cycling at 15 W, followed by 6-min of very heavy-intensity cycling (U→VH); and 2) 3-min of cycling at 15 W, followed by 4-min of moderate-intensity cycling (U→M), followed by 6-min of very heavy-intensity cycling (M→VH) (see figure 5.1). Four, three, and two repetitions of protocols 1) and 2) were completed in each child, teenager and adult subject, respectively. Each protocol was presented to participants in random order with each laboratory visit separated by  $\geq 48$  hr.

### 5.2.3 *Experimental measures*

All exercise tests were performed on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) with the seat height, handlebar height, and crank length adapted to each subject and subsequently maintained throughout the testing period. Pulmonary gas exchange and ventilation were measured and displayed breath-by-breath (Metalyser 3B Cortex, Biophysik, Leipzig, Germany) during each exercise trial. Gas fractions of O<sub>2</sub> and CO<sub>2</sub> were drawn continuously from a face mask-turbine assembly following calibration with gases of known concentration. Expired volume was measured using a DVT turbine digital transducer which was manually calibrated using a 3-L syringe (Hans Rudolph, Kansas City, MO) before each test. All calibration procedures were repeated before each experimental test. Heart rate was recorded every breath during all exercise tests using short-range radiotelemetry (Polar S610, Polar Electro Oy, Kempele, Finland) and subsequently exported as a separate data column into a single spreadsheet file that included all other measured ventilatory and gas exchange variables.

Muscle oxygenation in the microcirculation of the *m. vastus lateralis* was monitored using a commercially available near-infrared system (Portamon, Artnis Medical Systems, The Netherlands). The system consists of an emission probe which has three light sources and

emits two 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 relative changes relative to baseline in oxygenated and deoxygenated haemoglobin concentrations ( $[\text{HbO}_2]$  and  $[\text{HHb}]$  respectively). The  $[\text{HHb}]$  signal is considered to reflect the dynamic (im)balance between muscle  $\text{O}_2$  supply and  $\text{O}_2$  utilisation and is therefore considered an indicator of muscle fractional  $\text{O}_2$  extraction within the field of interrogation (DeLorey, et al., 2003; Grassi, et al., 2003). Since the contribution of myoglobin to the NIRS signal is currently unresolved, the  $[\text{HHb}]$  signal described herein should be considered to reflect the combined concentration of both deoxygenated haemoglobin and myoglobin. The area was initially cleaned and the portable probe secured to the skin surface at the midway point between the greater trochanter and lateral epicondyle of the left leg using physiotherapists tape (Kinesio Tex Gold). Pen marks were made over the skin to identify the margins of the device in order to check for any downward displacement of the probe and for accurate probe repositioning. 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 leg enclosing the probe.

Neuromuscular activity of the right leg *m. vastus lateralis* was measured using bipolar surface EMG. The leg was initially abraded and cleaned with alcohol to enhance the muscle electrical signal and graphite snap electrodes (Unilect 40713, Unomedical, Stonehouse, UK) were adhered to the skin surface in a bipolar arrangement (interelectrode distance: 40 mm) positioned at the midway point between the greater trochanter and lateral epicondyle of the right leg. A ground electrode was also placed on the *m. rectus femoris* equidistant from the active electrodes with an elastic bandage wrapped around the participant's leg to prevent displacement of the electrodes during cycling. The sites of electrode placement

were chosen to most accurately reflect the muscle force output of the quadriceps region based on previous EMG measurements performed during multi-joint exercise (Alkner, Tesch, & Berg, 2000). To secure electrodes and wires in place and minimize movement during cycling, an elastic bandage was wrapped around the subject's leg. Pen marks were made around the electrodes to enable reproduction of the placement in subsequent tests. The EMG signal was recorded using a ME3000PB Muscle Tester (Mega Electronics).

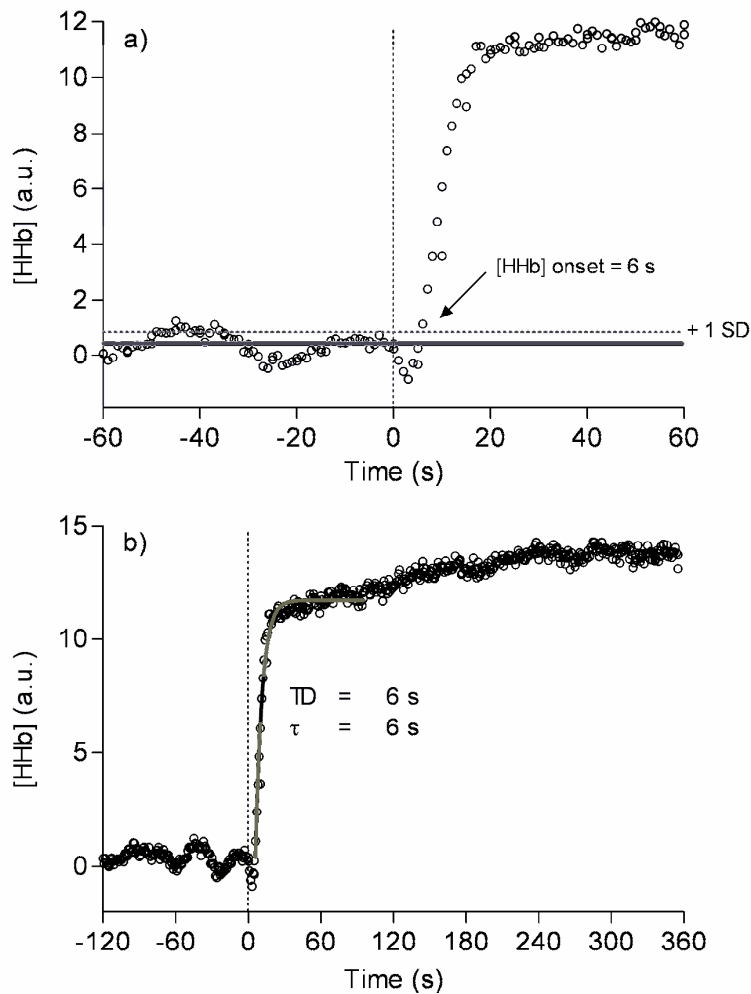
EMG measurements at a sampling frequency of 1000 Hz were recorded throughout all exercise tests. The bipolar signal was amplified (amplifier input impedance > 1 M $\Omega$ ), and data were collected online in raw form and stored on a personal computer using MegaWin software (Mega Electronics). The raw EMG data were subsequently exported as an ASCII file and digitally filtered using Labview 8.2 (National Instruments, Newbury, UK). Initially, the signals were filtered with a 20-Hz high-pass, second-order Butterworth filter to remove contamination from movement artefacts. The signal was then rectified and low-pass filtered at a frequency of 50 Hz to produce a linear envelope. The average iEMG was calculated for 15-s intervals throughout exercise, with these values normalized to the average measured during 15-180 s of unloaded cycling before the initial transition. Therefore, all iEMG data are presented as a percentage of the initial unloaded cycling phase.

#### *5.2.4 Data analysis*

All procedures stated in section 3.3.1 on the treatment and modelling of breath-by-breath  $\dot{V}O_2$  data in response to step exercise were completed.



The muscle [HHb] response derived from NIRS was averaged into 1-s time intervals, time aligned to the onset of exercise, and ensemble averaged to yield a single response in each participant. Since the muscle [HHb] signal increases following a short delay in response to step exercise (DeLorey, et al., 2003; Grassi, et al., 2003), the time onset for the exponential-like rise in [HHb] was therefore defined as a 1 SD increase in [HHb] above the mean value measured from 15-180 s during baseline pedaling (figure 5.2). The model in *equation 3.1*.



**Figure 5.2** Modelling of the muscle [HHb] response during VH exercise in a single child participant. The solid grey line denotes the mean baseline [HHb] value in (a) where the  $x$ -axis has been expanded to identify the time point at which [HHb] increased 1 SD above the mean (dashed grey line) following the onset of exercise. In this example, modeling the [HHb] signal within a fitting window commencing from  $t = 6$  s and excluding the [HHb] slow component yields an overall MRT ( $TD + \tau$ ) of 12 s (b).

was then used to resolve the [HHb]  $\tau$  and TD after omitting data points preceding the exponential-like increase. For exercise work rates above the GET, the model fitting window was constrained to the onset of the [HHb] slow component determined using the iterative curve fitting procedure as described for  $\dot{V}_{O_2}$  in section 3.3.1. The primary [HHb] amplitude was divided by the phase II  $\dot{V}_{O_2}$  asymptote in order to determine the  $[\Delta\text{HHb}]/\Delta\dot{V}_{O_2}$  ratio as an index of the change in fractional muscle  $O_2$  extraction required to elicit a given  $\Delta\dot{V}_{O_2}$  above baseline during the primary phase.

HR kinetics were also modelled for each exercise condition using similar procedures but with the TD parameter in *equation 3.1* constrained to  $t = 0$  s (i.e. monoexponential model with no delay). As described for  $\dot{V}_{O_2}$  data, an iterative curve fitting procedure was used to resolve the HR  $\tau$  within a fitting window that excluded the HR slow component during U $\rightarrow$ VH and M $\rightarrow$ VH exercise.

#### 5.2.5 Statistical analyses

One-way ANOVA was used to compare anthropometric measures and physiological responses to ramp exercise between age groups. Mean differences in  $\dot{V}_{O_2}$ , iEMG, and HR parameters were examined using a mixed model ANOVA with age (B vs. T vs. M) and exercise condition (U $\rightarrow$ M vs. U $\rightarrow$ VH vs. M $\rightarrow$ VH) as the model factors. If a significant condition  $\times$  age interaction was observed, the main effect results were not reported. Mean differences were followed up using paired and independent samples  $t$ -tests as appropriate to locate statistically significant differences with the alpha value adjusted using the Bonferroni procedure. Pearson product-moment correlation coefficients were used to investigate

relationships between the parameters for  $\dot{V}O_2$  and HR. All results are presented as means  $\pm$  SD with rejection of the null hypotheses accepted at an alpha level of 0.05.

### 5.3 Results

The participants' anthropometric characteristics and physiological responses to ramp exercise are presented in Table 5.1.

**Table 5.1** Participant descriptive characteristics and incremental exercise responses

Variable	ANOVA	B ( <i>n</i> = 8)	T ( <i>n</i> = 9)	M ( <i>n</i> = 9)
Age (yr)	<i>P</i> < 0.001	12.5 $\pm$ 0.5	14.9 $\pm$ 0.5*	26.0 $\pm$ 2.9*#
Body mass (kg)	<i>P</i> < 0.001	38.9 $\pm$ 4.1	61.2 $\pm$ 11.1*	72.1 $\pm$ 8.6*##
Stature (m)	<i>P</i> < 0.001	1.49 $\pm$ 0.06	1.73 $\pm$ 0.09*	1.77 $\pm$ 0.09*
Estimated years from PHV (yr)	-	-2.3 $\pm$ 0.5	+0.5 $\pm$ 1.1*	-
$\dot{V}O_{2\text{ peak}}$ (L·min <sup>-1</sup> )	<i>P</i> = 0.001	1.94 $\pm$ 0.14	2.89 $\pm$ 0.81*	3.25 $\pm$ 0.62*
$\dot{V}O_{2\text{ peak}}$ (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	<i>P</i> = 0.37	50 $\pm$ 3	47 $\pm$ 8	45 $\pm$ 8
HR <sub>peak</sub> (b·min <sup>-1</sup> )	<i>P</i> = 0.44	190 $\pm$ 10	191 $\pm$ 8	186 $\pm$ 6
GET (L·min <sup>-1</sup> )	<i>P</i> = 0.001	1.03 $\pm$ 0.15	1.44 $\pm$ 0.40**	1.65 $\pm$ 0.29*
GET (% $\dot{V}O_{2\text{ peak}}$ )	<i>P</i> = 0.59	53 $\pm$ 7	50 $\pm$ 5	51 $\pm$ 6

Data are presented as means  $\pm$  SD. ANOVA, one-way ANOVA results. Bonferroni adjusted paired comparisons: Significantly different from B; \**P* < 0.01, \*\**P* < 0.05. Significantly different from T; #*P* < 0.01, ##*P* < 0.05. PHV, peak height velocity; GET, gas exchange threshold.

The boys had a significantly greater maturity offset score from age at peak height velocity compared with teenagers (range -1.6 to -2.9 vs. -0.4 to 1.7, *P* < 0.001). There were no age differences in  $\dot{V}O_{2\text{ peak}}$  normalised per body mass with the GET occurring at a similar fraction (%) of  $\dot{V}O_{2\text{ peak}}$  across age groups. The work rates calculated for moderate- and

very heavy-intensity exercise were  $42 \pm 4$  and  $115 \pm 7$  W in B,  $79 \pm 18$  and  $188 \pm 31$  W in T, and  $101 \pm 19$  and  $230 \pm 28$  W in M, respectively.

### 5.3.1 Step exercise responses

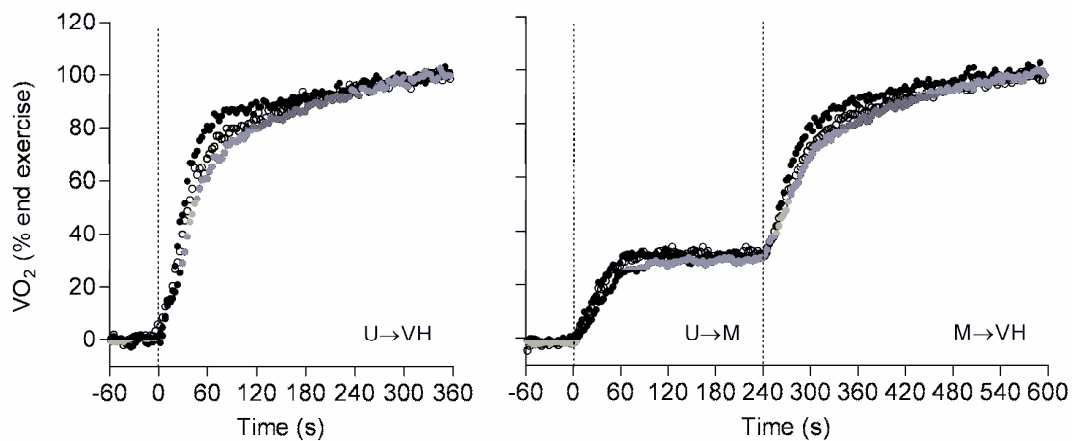
Step exercise transitions resulted in a similar fractional (%) utilisation of  $\dot{V}O_{2\text{ peak}}$  at end-exercise between B, T and M in U→M ( $47 \pm 3$  vs.  $50 \pm 4$  vs.  $48 \pm 8\%$ ), U→VH ( $89 \pm 3$  vs.  $93 \pm 10$  vs.  $93 \pm 7\%$ ) and M→VH ( $86 \pm 5$  vs.  $93 \pm 9$  vs.  $95 \pm 9\%$ ). Table 5.2 presents the  $\dot{V}O_2$  kinetic parameters in each exercise condition. Group mean responses for U→VH, U→M, and M→VH transitions are shown in figure 5.3. Step exercise was initiated from an elevated baseline  $\dot{V}O_2$  in M→VH compared to U→M and U→VH exercise in B, T, and M ( $P < 0.001$ ). The phase II  $\dot{V}O_2$   $\tau$  was significantly slowed during work-to-work exercise compared to step transitions elicited from unloaded pedalling within each age group ( $P < 0.009$ ). Phase II  $\dot{V}O_2$  kinetics were not different between U→M and U→VH within each group ( $P > 0.06$ ). The phase II  $\tau$  was significantly faster in B compared to M in each condition ( $P < 0.002$ ) and compared to T during U→VH and M→VH exercise ( $P = 0.021$  and  $0.011$ ). Figure 5.4 presents phase II  $\tau$  values for each condition within age groups.

In T and M, the primary  $\dot{V}O_2$  gain increased and the  $\dot{V}O_2$  slow component amplitude was reduced in M→VH compared to U→VH exercise ( $P < 0.038$ ) but both parameters were similar between VH conditions in B ( $P > 0.14$ ). The primary  $\dot{V}O_2$  gain fell significantly from U→M to U→VH exercise in B ( $P = 0.013$ ) but was elevated in the latter condition compared to T and M ( $P < 0.032$ ). There were no differences in the relative  $\dot{V}O_2$  slow component amplitude between age groups in either U→VH and M→VH exercise. The total

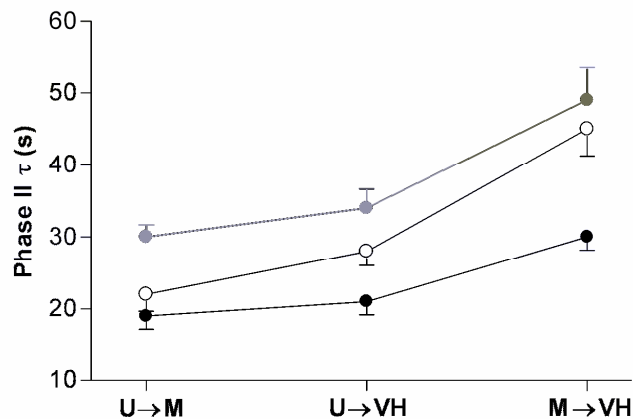
**Table 5.2** Pulmonary O<sub>2</sub> uptake kinetics during step transitions in relation to age.

Variable	AVOVA	Boys (n = 8)			Teenage boys (n = 9)			Men (n = 9)		
		U→M	U→VH	M→VH	U→M	U→VH	M→VH	U→M	U→VH	M→VH
$\dot{V}_{O_{2\text{ bl}}} \text{ (L}\cdot\text{min}^{-1}\text{)}$	<sup>c</sup> $P < 0.001$	$0.57 \pm 0.05^{\text{fg}}$	$0.59 \pm 0.07^{\text{fg}}$	$0.91 \pm 0.08^{\text{defg}}$	$0.79 \pm 0.23$	$0.82 \pm 0.23$	$1.43 \pm 0.39^{\text{de}}$	$0.83 \pm 0.12$	$0.82 \pm 0.12$	$1.54 \pm 0.25^{\text{de}}$
TD <sub>1</sub> (s)	<sup>a</sup> $P = 0.006$	$12 \pm 3$	$11 \pm 3$	$9 \pm 2$	$14 \pm 4$	$10 \pm 3$	$10 \pm 6$	$12 \pm 6$	$12 \pm 6$	$8 \pm 6^{\text{e}}$
$\tau_1$ (s)	<sup>ab</sup> $P < 0.001$	$19 \pm 5^{\text{f}}$	$21 \pm 5^{\text{f}}$	$30 \pm 5^{\text{defg}}$	$22 \pm 7^{\text{f}}$	$28 \pm 6$	$45 \pm 11^{\text{de}}$	$30 \pm 5$	$34 \pm 8$	$49 \pm 14^{\text{de}}$
A <sub>1</sub> (L·min <sup>-1</sup> )	<sup>c</sup> $P < 0.001$	$0.34 \pm 0.04^{\text{fg}}$	$0.99 \pm 0.11^{\text{dfg}}$	$0.64 \pm 0.07^{\text{defg}}$	$0.64 \pm 0.17$	$1.53 \pm 0.31^{\text{d}}$	$1.07 \pm 0.27^{\text{de}}$	$0.70 \pm 0.23$	$1.83 \pm 0.28^{\text{d}}$	$1.28 \pm 0.27^{\text{de}}$
G <sub>p</sub> (ml·min <sup>-1</sup> ·W <sup>-1</sup> )	<sup>c</sup> $P = 0.006$	$10.6 \pm 1.1^{\text{f}}$	$9.5 \pm 1.0^{\text{dg}}$	$9.1 \pm 0.8^{\text{d}}$	$9.3 \pm 1.3$	$8.6 \pm 0.8$	$9.7 \pm 1.3^{\text{e}}$	$8.5 \pm 1.4$	$8.7 \pm 0.4$	$9.9 \pm 1.4$
TD <sub>2</sub> (s)	<sup>c</sup> $P = 0.043$	-	$172 \pm 26$	$161 \pm 44$	-	$149 \pm 32$	$188 \pm 26$	-	$155 \pm 22$	$175 \pm 30$
A' <sub>2</sub> (L·min <sup>-1</sup> )	$P = 0.002$ $P < 0.001$	-	$0.12 \pm 0.05^{\text{fg}}$	$0.12 \pm 0.06^{\text{f}}$	-	$0.30 \pm 0.15$	$0.15 \pm 0.06^{\text{f}}$	-	$0.36 \pm 0.12$	$0.24 \pm 0.07$
Rel. A' <sub>2</sub> (%)	NS	-	$11 \pm 4$	$15 \pm 7$	-	$16 \pm 6$	$13 \pm 6$	-	$16 \pm 3$	$16 \pm 6$
$\dot{V}_{O_{2\text{ tot}}} \text{ (L}\cdot\text{min}^{-1}\text{)}$	<sup>c</sup> $P < 0.001$	$0.91 \pm 0.08^{\text{fg}}$	$1.70 \pm 0.12^{\text{dfg}}$	$1.67 \pm 0.13^{\text{dfg}}$	$1.43 \pm 0.38$	$2.64 \pm 0.61^{\text{d}}$	$2.66 \pm 0.62^{\text{d}}$	$1.53 \pm 0.26$	$3.01 \pm 0.43^{\text{d}}$	$3.06 \pm 0.44^{\text{d}}$
G <sub>tot</sub> (ml·min <sup>-1</sup> ·W <sup>-1</sup> )	<sup>c</sup> $P = 0.003$	$10.6 \pm 1.1^{\text{f}}$	$10.7 \pm 1.1$	$10.7 \pm 1.2$	$9.3 \pm 1.3$	$10.2 \pm 1.1^{\text{d}}$	$11.2 \pm 1.0^{\text{de}}$	$8.5 \pm 1.4$	$10.4 \pm 0.6^{\text{d}}$	$11.8 \pm 1.0^{\text{de}}$
MRT	<sup>ab</sup> $P < 0.001$	$31 \pm 9^{\text{f}}$	$40 \pm 9^{\text{df}}$	$54 \pm 9^{\text{defg}}$	$36 \pm 9$	$54 \pm 8^{\text{d}}$	$73 \pm 10^{\text{de}}$	$46 \pm 9$	$66 \pm 15^{\text{d}}$	$83 \pm 17^{\text{de}}$

Data are presented as mean  $\pm$  SD. AVOVA, 3  $\times$  3 mixed model ANOVA results: <sup>a</sup>significant main effect for condition; <sup>b</sup>significant main effect for age; <sup>c</sup>significant age  $\times$  condition interaction; NS, no significant differences found ( $P > 0.05$ ). Bonferroni adjusted paired comparisons: <sup>d</sup>significantly different from U→M condition, <sup>e</sup>significantly different from U→VH condition, <sup>f</sup>significantly different from men within-condition, <sup>g</sup>significantly different from teenage boy's within-condition (all  $P < 0.05$ ).  $\dot{V}_{O_{2\text{ bl}}}$ , mean  $\dot{V}_{O_2}$  during baseline pedalling;  $\tau_1$ , TD<sub>1</sub>, A<sub>1</sub>, time constant, time delay and asymptotic amplitude of the phase II  $\dot{V}_{O_2}$  response; TD<sub>2</sub>, A'<sub>2</sub>, time delay and amplitude of the  $\dot{V}_{O_2}$  slow component; G<sub>p</sub>, G<sub>tot</sub>, gain ( $\Delta \dot{V}_{O_2} / \Delta \text{WR}$ ) of the primary response and at end-exercise; MRT, mean response time.



**Figure 5.3** Group mean  $\dot{V}O_2$  responses in B (●), T (○), and M (●) during step exercise in each condition. The  $\dot{V}O_2$  data are normalised relative to the end-exercise amplitude above 15W pedalling. The onset of step exercise is indicated by the vertical dotted line.

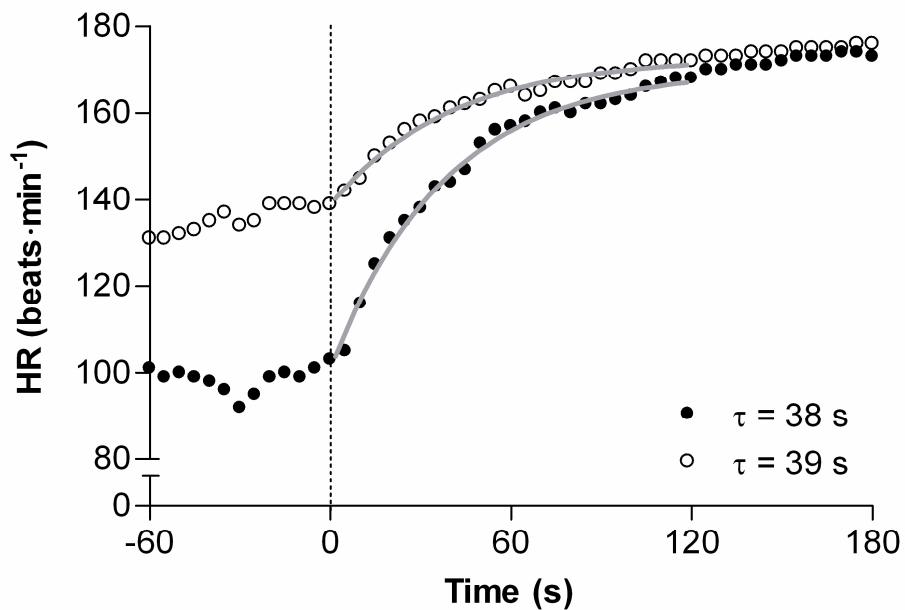


**Figure 5.4** Group mean  $\pm$  (S.E.) time constant ( $\tau$ ) for the phase II  $\dot{V}O_2$  response in B (●), T (○), and M (●) during step exercise in each condition. Note the greater increase in the  $\tau$  from U→M to M→VH in T compared to B. See Table 5.2 for statistical information on differences between and within age groups.

$\dot{V}O_2$  gain progressively increased from U→M to M→VH in T and M ( $P < 0.011$ ) but was similar across exercise conditions in B ( $P > 0.78$ ). The overall MRT was significantly slowed in M→VH compared to U→M and U→VH within each group ( $P < 0.006$ ) but was significantly faster in B compared to T and M during work-to-work exercise ( $P < 0.008$ ).

### 5.3.2 HR kinetics

A representative HR response in a child subject during VH exercise is shown in figure 5.5. Baseline pedalling significantly increased HR prior to the onset of M→VH exercise in each group (B:  $122 \pm 8$  b·min<sup>-1</sup>, T:  $116 \pm 12$  b·min<sup>-1</sup>, M:  $110 \pm 12$  b·min<sup>-1</sup>) compared to other exercise conditions ( $P < 0.003$ ). However, there were no significant differences in the HR  $\tau$  between U→VH and M→VH exercise in B ( $35 \pm 8$  s vs.  $40 \pm 11$  s,  $P = 0.17$ ), T ( $46 \pm 18$  s vs.  $52 \pm 17$  s,  $P = 0.26$ ), or M ( $39 \pm 12$  s vs.  $46 \pm 16$  s,  $P = 0.21$ ). Relative (%) changes in the phase II  $\dot{V}O_2$   $\tau$  were not correlated with alterations in HR kinetics across U→VH and M→VH conditions in B ( $r = 0.55$ ,  $P = 0.16$ ), T ( $r = 0.05$ ,  $P = 0.91$ ), or M ( $r = -0.56$ ,  $P = 0.19$ ).



**Figure 5.5** Heart rate response in an 11-yr-old boy following the onset of step exercise in U→VH (●) and M→VH (○). Note the unchanged HR  $\tau$  despite a 32% increase in the phase II  $\dot{V}O_2$   $\tau$  during work-to-work exercise in this particular subject.

### 5.3.3 Muscle [HHb] kinetics

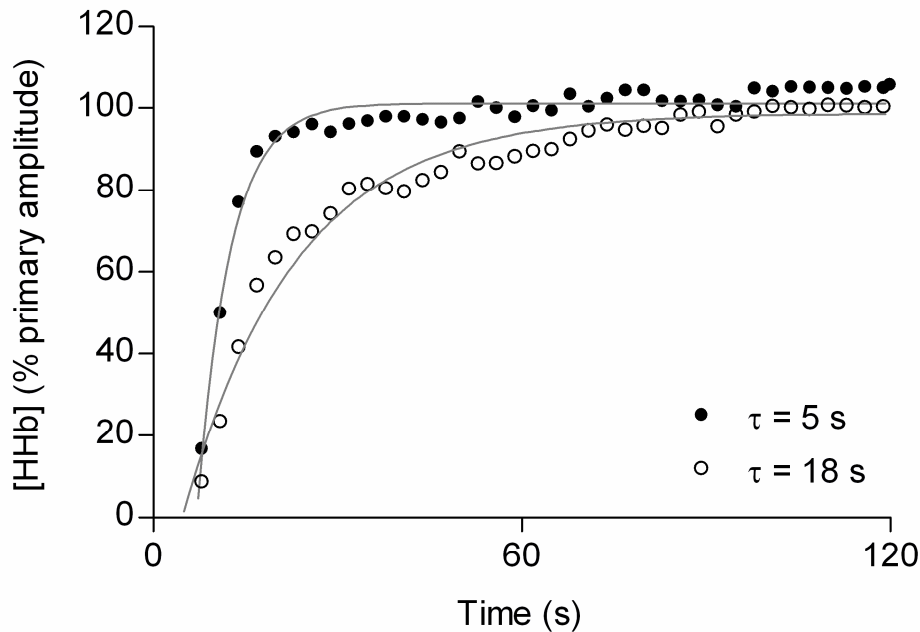
Table 5.3 presents the mean [HHb] kinetic parameters. In each condition, muscle [HHb] increased in an exponential manner after an initial TD following the onset of step exercise. Compared to U→M, the [HHb] TD was reduced during exercise above the GET within each age group ( $P < 0.001$ ) and in M→VH compared to U→VH in B and T ( $P = 0.029$  and  $0.003$ ). The [HHb]  $\tau$  was not different between U→M and U→VH exercise but was significantly slower in M→VH compared to U→VH in both groups ( $P < 0.002$ ). This yielded significantly faster primary [HHb] kinetics (TD +  $\tau$ ) in U→VH compared to U→M in B and T ( $P = 0.001$ ) but a slower [HHb] MRT in M→VH compared to U→VH in each age group ( $P < 0.007$ ). Boys demonstrated an enhanced primary  $\Delta[\text{HHb}]/\Delta \dot{V}\text{O}_2$  response in M→VH compared to U→M ( $P = 0.038$ ) but there were no differences in fractional  $\text{O}_2$  extraction per increment in  $\dot{V}\text{O}_2$  between conditions within T ( $P > 0.18$ ). Muscle [HHb] responses in a representative child subject during VH exercise are shown in figure 5.6.



**Table 5.3** NIRS derived muscle deoxygenation (HHb) kinetics during step exercise in youth participants

Variable	ANOVA	Boys ( <i>n</i> = 8)			Teenage boys ( <i>n</i> = 9)		
		U→M	U→VH	M→VH	U→M	U→VH	M→VH
Baseline [HbO <sub>2</sub> ] (AU)	NS	0.8 ± 1.8	0.0 ± 1.4	0.2 ± 1.6	-0.2 ± 2.0	-0.3 ± 1.9	-1.5 ± 2.5
End-exercise [HbO <sub>2</sub> ] (AU)	<sup>a</sup> <i>P</i> < 0.001	0.6 ± 1.7	-5.9 ± 2.4 <sup>c</sup>	-3.7 ± 2.4 <sup>c</sup>	-0.2 ± 2.0	-5.3 ± 2.8 <sup>c</sup>	-4.3 ± 3.2 <sup>c</sup>
Primary [HHb] TD (s)	<sup>a</sup> <i>P</i> < 0.001	15 ± 3	8 ± 3 <sup>c</sup>	5 ± 3 <sup>c</sup>	12 ± 3	7 ± 1 <sup>c</sup>	3 ± 3 <sup>cd</sup>
Primary [HHb] τ (s)	<sup>a</sup> <i>P</i> < 0.001	8 ± 5	7 ± 3	16 ± 4 <sup>cd</sup>	11 ± 6	6 ± 1	19 ± 7 <sup>d</sup>
Primary [HHb] MRT (s)	<sup>a</sup> <i>P</i> < 0.001	23 ± 3	15 ± 3 <sup>c</sup>	21 ± 3 <sup>d</sup>	23 ± 6	13 ± 1 <sup>c</sup>	22 ± 6 <sup>d</sup>
Primary [HHb] amplitude (AU)	<sup>a</sup> <i>P</i> < 0.001	2.1 ± 1.1	7.1 ± 2.4 <sup>c</sup>	4.9 ± 1.9 <sup>cd</sup>	3.9 ± 1.9	9.0 ± 3.1 <sup>c</sup>	5.7 ± 2.2 <sup>d</sup>
Primary Δ[HHb]/Δ $\dot{V}O_2$ (AU/L·min <sup>-1</sup> )	<sup>b</sup> <i>P</i> = 0.045	6.5 ± 3.8	7.3 ± 3.2	7.8 ± 3.1	6.3 ± 2.7	6.1 ± 1.8	5.4 ± 1.7
[HHb] slow component amplitude (AU)	<sup>b</sup> <i>P</i> = 0.006	-	1.1 ± 0.7 <sup>e</sup>	1.1 ± 0.5	-	2.9 ± 1.2	1.6 ± 1.3 <sup>d</sup>
Total Δ[HHb]/Δ $\dot{V}O_2$ (AU/L·min <sup>-1</sup> )	NS	6.5 ± 3.8	7.5 ± 3.1	8.0 ± 3.0	6.3 ± 2.7	6.8 ± 1.9	5.9 ± 1.9

Data are presented as mean ± SD. AVOVA, 2 × 3 mixed model ANOVA results: <sup>a</sup>significant main effect for condition; <sup>b</sup>significant age × condition interaction; NS, no significant differences found (*P* > 0.05). Bonferroni adjusted paired comparisons: <sup>c</sup>significantly different from U→M condition, <sup>d</sup>significantly different from U→VH condition, <sup>e</sup>significant within-condition age difference (all *P* < 0.05).



**Figure 5.6** Muscle deoxyhaemoglobin/myoglobin response in a representative 13-yr old boy at the onset of U→VH (●) and M→VH (○) exercise. The [HHb] data are normalised relative to the primary amplitude with the  $x$  axis expanded to illustrate differences in muscle [HHb] dynamics between conditions. Note the reduced [HHb] TD prior to a slower exponential rise in muscle deoxygenation following the onset of ‘work-to-work’ exercise.

#### 5.3.4 Muscle iEMG activity

Baseline pedalling in M→VH significantly increased iEMG activity in B, T, and M ( $192 \pm 69$  vs.  $253 \pm 70$  vs.  $237 \pm 64\%$ , respectively) compared to other exercise conditions ( $P < 0.007$ ). The iEMG of the *m. vastus lateralis* was significantly ( $P < 0.001$ ) elevated at *minute 2* during U→VH and M→VH compared to U→M exercise in B ( $412 \pm 88$  vs.  $389 \pm 159$  vs.  $192 \pm 69\%$ ), T ( $446 \pm 159$  vs.  $475 \pm 208$  vs.  $253 \pm 70\%$ ) and M ( $419 \pm 151$  vs.  $429 \pm 134$  vs.  $237 \pm 64\%$ ). There were no differences in iEMG at *minute 2* between VH exercise conditions ( $P > 0.52$ ).

## 5.4 Discussion

This study employed a non-invasive exercise intervention in order to manipulate muscle fibre recruitment patterns and explore the physiological factors involved in modulating pulmonary  $\dot{V}O_2$  kinetics with increasing age. In line with our experimental hypothesis, very heavy-intensity transitions initiated from an elevated baseline work rate (i.e. work-to-work exercise) lengthened the phase II  $\dot{V}O_2$   $\tau$  and increased the  $\dot{V}O_2$  gain in teenagers and men compared to transitions initiated from unloaded pedalling. In contrast to our experimental hypothesis, similar work-to-work exercise influences on phase II  $\dot{V}O_2$  kinetics were also manifest in younger children during step transitions at the same relative exercise intensity. These  $\dot{V}O_2$  responses coincided with a slower rate of fractional muscle  $O_2$  extraction in the microcirculation (using NIRS interrogation) during M→VH compared to U→VH exercise in B and T.

Step transitions below and above the GET (U→M and U→VH) resulted in similar phase II  $\dot{V}O_2$  kinetics in B and T. These results are consistent with previous reports in youth during treadmill running (Williams, et al., 2001) and cycling exercise (Lai, et al., 2008) and therefore indicate that the phase II  $\dot{V}O_2$   $\tau$  is independent of exercise intensity (at least for work rates spanning the moderate-to-very heavy-intensity domains) in young people. In contrast, M→VH exercise lengthened the phase II  $\dot{V}O_2$   $\tau$  in B and T, with similar work-to-work exercise influences also reported herein in men and from previous studies in adults (DiMenna, Bailey, et al., 2010; DiMenna, et al., 2008; Wilkerson & Jones, 2007). These data demonstrate that slower phase II  $\dot{V}O_2$  kinetics when step exercise transitions are

initiated from an elevated baseline work rate are not confined to adult populations and is independent of age.

Slower phase II  $\dot{V}O_2$  kinetics was manifest when baseline HR was elevated at the onset of M→VH compared to U→VH exercise. It has been intimated that a shift in the regulation of HR from parasympathetic removal to sympathetic activation under these circumstances might result in slower  $\dot{Q}$  dynamics and therefore exacerbate an  $O_2$  supply limitation over the phase II  $\dot{V}O_2$  transient at the onset of exercise (Hughson & Morrissey, 1982; MacPhee, Shoemaker, Paterson, & Kowalchuk, 2005). In our study, there were no differences in HR kinetics between U→VH and M→VH exercise within either age group (despite a lengthened phase II  $\tau$  in the latter condition). Furthermore, an increased phase II  $\tau$  from U→VH to M→VH was not associated with any concomitant alterations in HR kinetics. It is therefore unlikely that phase II  $\dot{V}O_2$  kinetics were restricted by a slower adaptation in cardiac output ( $\dot{Q}$ ) and hence bulk  $O_2$  delivery across the M→VH transient in B or T.

Slower muscle PCr kinetics during moderate-to-high-intensity knee extension exercise compared to transitions initiated from rest have been interpreted to suggest that intracellular metabolic factors might principally slow  $\dot{V}O_2$  kinetics during work-to-work exercise (Dimenna, Fulford, et al., 2010; Jones, Wilkerson, & Fulford, 2008). It is therefore pertinent that an increased iEMG of the *m. vastus lateralis* at the onset of M→VH exercise coincided with a slower rate of oxidative energy transfer in B and T (as indicated by the lengthened  $\tau$  in this condition) compared to transitions elicited from unloaded pedalling. The size principle (Henneman & Mendell, 1981) would predict that muscle fibres with a greater oxidative capacity were initially recruited during baseline pedalling in M→VH.

Further increases in iEMG from baseline to *minute 2* might therefore be expected to predominately involve the activation of higher-order (type II) motor units to augment force production over the work-to-work transient. Type II muscle have been demonstrated to possess a lower microvascular O<sub>2</sub> pressure head, reduced oxidative capacity, and slower  $\dot{V}O_2$  kinetics compared to type I fibres in small mammals (Behnke, et al., 2003; Crow & Kushmerick, 1982; McDonough, et al., 2005; Willis & Jackman, 1994). Notwithstanding that our iEMG data preclude any direct inferences pertaining to muscle fibre-specific activation, it is conceivable that M→VH exercise revealed the oxidative properties of muscle fibres positioned higher in the recruitment hierarchy in youth subjects based on established principles of motor unit recruitment.

Krustrup et al. (2004b) have demonstrated substrate utilisation exclusively in type I fibres during low-intensity cycling (50%  $\dot{V}O_{2\text{ peak}}$ ), with further reductions in intramuscular glycogen and [PCr] in single type I and II fibres close to the onset of high-intensity exercise (80%  $\dot{V}O_{2\text{ peak}}$ ). The phase II  $\dot{V}O_2$   $\tau$  during unloaded-to-heavy/very heavy exercise transitions has therefore been suggested to summate the oxidative response characteristics in muscle fibres (with varying individual  $\tau$  values) recruited over the primary exponential component (Dimenna, et al., 2009a; Jones, et al., 2005b). It is therefore interesting that dividing a full U→VH bout into two discrete step bouts (i.e. U→M and M→VH) yielded faster phase II  $\dot{V}O_2$  kinetics in B compared to T in the upper but not lower step. These results might be interpreted to suggest an age-dependent modulation in the oxidative capacity of higher-order type II muscle fibres and/or differences in muscle fibre type distribution compared to younger children. Moreover, an overall ~ 105% slowing in phase II  $\dot{V}O_2$  kinetics from U→M to M→VH in T (compared to ~ 58% in B) could explain, in

part, a progressive lengthening in the phase II  $\tau$  observed with increasing age (Breese, et al., 2010; Fawcner & Armstrong, 2004a) if the predominant recruitment of type II muscle fibres were to contribute to the external power output (and hence pulmonary  $\dot{V}O_2$  signal) from the onset of step exercise above the GET [as demonstrated in Krstrup et al. (2004b)].

During U→M exercise in B and T, there was an initial TD (~ 12-15 s) before muscle [HHb] increased therefore reflecting matched delivery of blood flow to the rate of muscle  $O_2$  utilisation at the onset of exercise. In contrast, the muscle [HHb] TD was shortened to augment the rise in  $\dot{V}O_2$  during U→VH and M→VH exercise hence indicative of a lower  $\dot{Q}O_2/\dot{V}O_2$  response in the interrogated NIRS region. Such response characteristics are consistent with the reported fibre-type dependency on reductions in microvascular  $PO_2$  demonstrated in animal muscle (Behnke, et al., 2003; McDonough, et al., 2005) and the requirement to recruit type II motor units at higher cycling power outputs in humans (Gollnick, et al., 1974; Krstrup, Soderlund, Mohr, et al., 2004b; Vollestad & Blom, 1985). Primary [HHb] kinetics (TD +  $\tau$ ) were speeded thereafter in U→VH compared to U→M which coincided with an unchanged phase II  $\dot{V}O_2$   $\tau$  between these conditions in both B and T. In contrast, muscle  $O_2$  extraction could not be increased sufficiently during M→VH exercise (as indicated by the lengthened [HHb] MRT compared to U→VH) and  $\dot{V}O_2$  kinetics were slowed at the onset of exercise. The latter might be anticipated if a fraction of the available muscle fibre pool with a reduced oxidative capacity were recruited over the work-to-work exercise transient in youth.

## 5.5 Conclusion

This study presented evidence linking slower pulmonary  $\dot{V}O_2$  and muscle deoxygenation kinetics with differences in muscle iEMG activity at the onset of very heavy-intensity exercise initiated from an elevated baseline work rate in children and teenagers. Furthermore, slower  $\dot{V}O_2$  kinetics in the absence of changes in HR dynamics suggests that intracellular factors are principally limiting oxidative metabolism during work-to-work exercise in youth. Work-to-work transitions also accentuated a greater lengthening in the phase II  $\tau$  relative to moderate exercise in teenage males compared to boys aged 11-12 yr. Based on established patterns of motor unit recruitment, it is proposed that slower phase II  $\dot{V}O_2$  (and by extension  $m\dot{V}O_2$ ) kinetics with increasing age might be linked to the activation of higher-order motor units during intense submaximal exercise.

## Chapter Six

### **THE EFFECT OF PEDAL RATE ON PULMONARY O<sub>2</sub> UPTAKE KINETICS DURING VERY HEAVY INTENSITY EXERCISE IN TRAINED AND UNTRAINED TEENAGE BOYS**

#### **6.1 Introduction**

The  $\dot{V}_{O_2}$  kinetic response following the onset of exercise is well documented in youth with previous investigations demonstrating an age-related modulation in the control of muscle oxidative metabolism [see Armstrong and Barker (2009) for a review]. A recent longitudinal study revealed that teenage participants demonstrated slower phase II  $\dot{V}_{O_2}$  kinetics and consumed a reduced proportion of the total  $\dot{V}_{O_2}$  during the initial exponential phase compared to younger counterparts, resulting in a greater  $\dot{V}_{O_2}$  slow component during heavy-intensity exercise (Breese, et al., 2010). The physiological factors that underpin developmental changes in  $\dot{V}_{O_2}$  kinetics are currently unresolved.

In response to a step change in metabolic rate, the Fick principle dictates that muscle O<sub>2</sub> consumption ( $m\dot{V}_{O_2}$ ) is the product of O<sub>2</sub> delivery ( $\dot{Q}_{O_2}$ ) and muscle O<sub>2</sub> extraction. It has been demonstrated that this relationship is fibre-type dependent in animal muscle (Behnke, et al., 2003; McDonough, et al., 2005). For a given change in  $\dot{V}_{O_2}$ , there is a greater fractional O<sub>2</sub> extraction in type II fibres to compensate for a blunted  $\dot{Q}_{O_2}$  response that compromises O<sub>2</sub> flux between the capillary and muscle (Behnke, et al., 2003; McDonough, et al., 2005). This might, in part, explain the link between slower  $\dot{V}_{O_2}$  kinetics and increased type II muscle fibre recruitment in exercising humans (Barstow, et al., 1996;



Krustrup, Soderlund, Mohr, et al., 2004a; Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003; Wilkerson & Jones, 2006, 2007). Type II fibres are known to possess a higher total creatine content and reduced oxidative enzyme activity (Crow & Kushmerick, 1982; Soderlund & Hultman, 1991; Willis & Jackman, 1994), which would predispose these muscle fibres to slow  $\dot{V}_{O_2}$  kinetics as predicted by models of respiratory control (Glancy, Barstow, & Willis, 2008; Meyer, 1988; Paganini, Foley, & Meyer, 1997). Furthermore, muscle  $O_2$  consumption is demonstrated to increase *in vivo* following neural blockade of slow-twitch fibres suggesting a lower contractile efficiency (higher  $O_2$  cost) in higher-order motor units (Krustrup, et al., 2008).

Given the potential for muscle fibre type to impact on  $\dot{V}_{O_2}$  kinetics during exercise, a non-invasive intervention that could amplify the oxidative response characteristics of fibre pools at the same relative intensity would be a useful model to explore the determinants of  $\dot{V}_{O_2}$  kinetics in youth. It has been suggested that muscle fibre recruitment is influenced by contraction velocity such that a shift in pedal rate from low to high frequencies might be expected to increase the contribution of higher-order fibres (i.e. type IIa and IIx) to force production (Ferguson, et al., 2001; MacIntosh, Neptune, & Horton, 2000; Sargeant, 1999). Consistent with this proposal are previous studies that have reported markedly slower  $\dot{V}_{O_2}$  kinetics (i.e. a lengthened phase II  $\tau$  and/or increased  $\dot{V}_{O_2}$  slow component amplitude) during pedalling at high ( $\sim 115 \text{ rev}\cdot\text{min}^{-1}$ ) compared to low ( $\sim 35 \text{ rev}\cdot\text{min}^{-1}$ ) pedal rates in adults (Dimenna, et al., 2009a, 2009b; Pringle, Doust, Carter, Tolfrey, & Jones, 2003; Vercruyssen, et al., 2009). Such response characteristics might be predicted if muscle fibres with an inherently lower oxidative capacity and increased  $O_2$  cost of force production were predominately recruited at faster cadences.

Utilising the muscle biopsy technique, the proportion of type I muscle fibres in the vastus lateralis muscle has been shown to correlate negatively with the phase II  $\tau$  (Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003) and magnitude of the  $\dot{V}_{O_2}$  slow component (Barstow, et al., 1996; Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003). It is therefore conceivable that a lengthened phase II  $\tau$  and increased  $\dot{V}_{O_2}$  slow component observed longitudinally from pre-puberty through adolescence (Breese, et al., 2010; Fawkner & Armstrong, 2004a) could be related, in part, to altered muscle fibre recruitment in older children. Conversely, faster phase II  $\dot{V}_{O_2}$  kinetics reported in teenage swimmers (McNarry, Welsman, & Jones, 2010) and youth soccer players (Marwood, Roche, Rowland, Garrard, & Unnithan, 2010) allied to a greater type I muscle fibre expression in aerobically trained children (Dahlstrom, Esbjornsson Liljedahl, Gierup, Kaijser, & Jansson, 1997; Mero, Jaakkola, & Komi, 1991) might infer that training and/or genetic influences could mitigate a reduction in the muscle oxidative capacity with increasing age. Therefore, exploring the interaction between pedal rate and trained status could provide further mechanistic insight into muscle fibre-specific influences on  $\dot{V}_{O_2}$  kinetics in youth.

The purpose of this study was to examine the influence of pedal cadence on  $\dot{V}_{O_2}$  kinetics during very heavy-intensity exercise in trained and untrained teenage boys. We hypothesised that pedalling at 115 rev·min<sup>-1</sup> would: 1) slow phase II  $\dot{V}_{O_2}$  kinetics; 2) reduce the phase II  $\dot{V}_{O_2}$  gain, and; 3) increase the  $\dot{V}_{O_2}$  slow component, compared to 50 rev·min<sup>-1</sup> pedalling in untrained (UT) teenagers. We also hypothesised that the same  $\dot{V}_{O_2}$  kinetic parameters would be unaltered by pedal rate in a group of trained (T) junior cyclists.

## **6.2 Methods**

### *6.2.1 Participants*

Eight UT teenage boys and seven T junior cyclists volunteered to participate in this study. The trained cyclists had all competed in national junior competition (40 km time trials) over the preceding 12 months and had a minimum two years training experience in endurance cycling. Somatic maturity was estimated using regression algorithms to determine a maturity offset score from age at peak height velocity (PHV) using anthropometric measurements (Mirwald, et al., 2002). Written, informed consent was obtained from each participant and their parents prior to the commencement of the study after verbal and written explanations of the study's aims, risks, and procedures were given. The procedures employed in this study were approved by the Institutional Research Ethics Committee. Participants were instructed to visit the laboratory on a minimum of four occasions over a 2-wk period, and testing took place at approximately the same time of day ( $\pm 2$  hrs).

### *6.2.2 Experimental procedures*

All exercise tests were performed on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) with the seat height, handlebar height, and crank length adapted to each subject and subsequently maintained throughout the testing period. During all exercise tests, breath by breath changes in gas exchange and ventilation were measured using a standard algorithm (Beaver, et al., 1973) and displayed using an on-line computer system. Gas fractions of O<sub>2</sub> and CO<sub>2</sub> were drawn continuously from a low dead space (90 mL) mouthpiece-turbine assembly and determined by mass spectroscopy following calibration with gases of known concentration (EX671, Morgan Medical, Kent, UK). Expired volume was measured using a turbine flowmeter (Interface Associates) which was manually calibrated using a 3-L syringe (Hans Rudolph, Kansas City, MO) over a

range of flow speeds. The sum of the gas-transport and analyzer-response delay terms was determined, and appropriate adjustments were made in the software. All calibration procedures were repeated before each experimental test.

On each of the first two visits subjects completed a ramp incremental test to voluntary exhaustion at a pedal rate of either  $50 \text{ rev}\cdot\text{min}^{-1}$  or  $115 \text{ rev}\cdot\text{min}^{-1}$  for determination of the cadence-specific peak  $\dot{V}_{O_2}$  and the gas exchange threshold (GET). After 3-min baseline cycling at 20 W, the resistance increased continuously by  $30 \text{ W}\cdot\text{min}^{-1}$  to attain a test  $\sim 8 - 12$  min in duration. Subjects were asked to maintain the prescribed pedal rate and verbal instruction was given if/when they deviated by more than  $\pm 5 \text{ rev}\cdot\text{min}^{-1}$ . The peak  $\dot{V}_{O_2}$  was taken as the highest recorded 10-s stationary average value during the maximal exercise test. The GET was determined non-invasively as the first disproportionate increase in  $\text{CO}_2$  production ( $\dot{V}_{\text{CO}_2}$ ) relative to the increase in  $\dot{V}_{O_2}$  [the V-slope method, Beaver et al. (1986)], and subsequently verified from visual inspection of the increase in the ventilatory equivalent for  $\dot{V}_{O_2}$  ( $\dot{V}_E / \dot{V}_{O_2}$ ) with no increase in  $\dot{V}_E / \dot{V}_{\text{CO}_2}$ .

The power outputs that would require 70% of the difference ( $\Delta$ ) between the cadence-specific GET and peak  $\dot{V}_{O_2}$  were estimated for each pedal rate. The averaged  $\dot{V}_{O_2}$  over the last 2 min of baseline pedalling for each ramp test was taken to represent the  $\text{O}_2$  cost of cycling at 20 W at both respective pedal rates. The added power output required to elicit a similar baseline  $\dot{V}_{O_2}$  when cycling at  $50 \text{ rev}\cdot\text{min}^{-1}$  was then estimated based on extrapolation of the  $\Delta \dot{V}_{O_2} / \Delta \text{WR}$  relationship established from the sub-GET region of the corresponding ramp test. Since adding the estimated  $\Delta 70\%$  work rate to the adjusted baseline power output resulted in a prescribed exercise intensity above that attained during

the 50 rev·min<sup>-1</sup> ramp test, the work rate correction was applied during baseline pedalling only in the lower cadence condition.

On each of the subsequent two visits participants performed two square-wave bouts of very heavy-intensity exercise. A single laboratory visit consisted of 4-min baseline pedalling followed by 6-min of very heavy- intensity exercise (70%Δ) at either 50 rev·min<sup>-1</sup> or 115 rev·min<sup>-1</sup>; this sequence was repeated after 60-min recovery with the very heavy bout performed at an alternate pedal rate. This recovery duration was selected to ensure that muscle blood flow and  $\dot{V}_{O_2}$  had returned to resting levels before commencement of the subsequent exercise bout (Burnley, Doust, & Jones, 2006; Krstrup, Hellsten, & Bangsbo, 2004). On each occasion, the pedal rate conditions were presented to participants in random order, and laboratory visits were separated by  $\geq 48$  hr.

### *6.2.3 Data analysis procedures*

All procedures stated in section 3.3.1 on the treatment and modelling of breath-by-breath  $\dot{V}_{O_2}$  data in response to step exercise were completed.

### *6.2.4 Statistics*

Mean differences in  $\dot{V}_{O_2}$  kinetic parameters were examined using a mixed model ANOVA with training status (UT vs. T) and pedal rate (50 rev·min<sup>-1</sup> vs. 115 rev·min<sup>-1</sup>) as the model factors. Mean differences were followed up using either Tukey's Honestly Significant Difference (HSD) test or a modified Tukey test, depending upon whether the ANOVA main effect or interaction factors were significant respectively (Stevens, 1996). All results

are presented as means  $\pm$  SD with rejection of the null hypotheses accepted at an alpha level of 0.05.

### 6.3 Results

The participant descriptive statistics are presented in Table 6.1.

**Table 6.1** Physical characteristics of participants.

	UT ( $n = 8$ )	T ( $n = 7$ )
Age (yr)	15.6 $\pm$ 0.5	16.7 $\pm$ 1.0
Stature (m)	1.81 $\pm$ 0.42	1.81 $\pm$ 0.07
Mass (kg)	71.4 $\pm$ 9.0	68.5 $\pm$ 4.0
Estimated years post PHV (yr)	+2.1 $\pm$ 0.8	+2.9 $\pm$ 0.9

Values are mean  $\pm$  SD. PHV, peak height velocity. No significant differences were present.

#### 6.3.1 Ramp exercise

Peak  $\dot{V}_{O_2}$  relative to body mass was significantly ( $P < 0.01$ ) greater in T compared to UT during incremental exercise at 50  $\text{rev}\cdot\text{min}^{-1}$  (68  $\pm$  6 vs. 50  $\pm$  7  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) and 115  $\text{rev}\cdot\text{min}^{-1}$  (69  $\pm$  7 vs. 51  $\pm$  5  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) respectively. There was no significant difference in peak  $\dot{V}_{O_2}$  (absolute or relative to body mass) between pedal rates in each group ( $P > 0.05$ ). The GET occurred at a significantly ( $P < 0.05$ ) lower absolute  $\dot{V}_{O_2}$  in UT (1.92  $\pm$  0.25 vs. 2.31  $\pm$  0.39  $\text{L}\cdot\text{min}^{-1}$ ) and T (2.92  $\pm$  0.33 vs. 3.40  $\pm$  0.36  $\text{L}\cdot\text{min}^{-1}$ ) at 50  $\text{rev}\cdot\text{min}^{-1}$  compared to 115  $\text{rev}\cdot\text{min}^{-1}$  respectively. The GET also occurred at a significantly ( $P < 0.01$ ) greater percentage peak  $\dot{V}_{O_2}$  in T compared to UT at 50  $\text{rev}\cdot\text{min}^{-1}$  (63  $\pm$  7 vs. 54  $\pm$  7 %) and 115  $\text{rev}\cdot\text{min}^{-1}$  (71  $\pm$  5 vs. 63  $\pm$  8 %) respectively.

### 6.3.2 Step exercise

There were no significant ( $P>0.05$ ) differences in pre-exercise  $\dot{V}_{O_2}$  values between *bout 1* and *bout 2* in UT ( $0.46 \pm 0.11$  vs.  $0.55 \pm 0.23$  L $\cdot$ min $^{-1}$ ) or T ( $0.42 \pm 0.12$  vs.  $0.49 \pm 0.11$  L $\cdot$ min $^{-1}$ ). The 60-min resting period therefore enabled complete recovery of  $\dot{V}_{O_2}$  between step exercise transitions in a single laboratory visit. Table 6.2 presents the mean  $\dot{V}_{O_2}$  kinetic parameters for both UT and T at each pedal rate. The  $\dot{V}_{O_2}$  kinetic responses of a representative UT and T participant are presented in figure 6.1. The group mean  $\dot{V}_{O_2}$  responses at each pedal rate are illustrated in figure 6.2. The baseline power output during 50 rev $\cdot$ min $^{-1}$  pedalling was adjusted to  $79 \pm 4$  and  $79 \pm 2$  W in UT and T groups respectively. There were no differences in baseline  $\dot{V}_{O_2}$  between pedal rate conditions in either group ( $P>0.05$ ). The mean exercise power outputs were significantly ( $P<0.01$ ) greater in T compared to UT at 50 rev $\cdot$ min $^{-1}$  ( $317 \pm 9$  vs.  $234 \pm 42$  W) and 115 rev $\cdot$ min $^{-1}$  ( $300 \pm 26$  vs.  $216 \pm 21$  W) respectively.

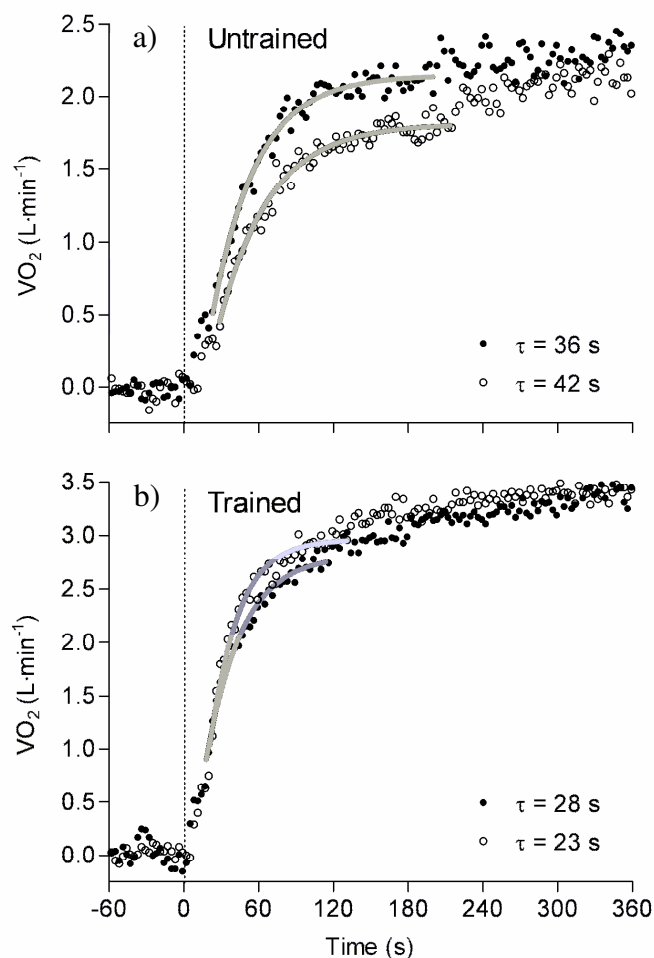
The phase II  $\dot{V}_{O_2}$  time delay and amplitude were unaffected by pedal rate in UT and T ( $P>0.05$ ). The phase II  $\dot{V}_{O_2}$   $\tau$  was significantly greater in UT during 115 rev $\cdot$ min $^{-1}$  pedalling ( $P<0.01$ ) but was unaltered by pedal rate in T ( $P>0.05$ ). The phase II  $\dot{V}_{O_2}$   $\tau$  was significantly faster in T compared to UT during 50 rev $\cdot$ min $^{-1}$  ( $P<0.05$ ) and 115 rev $\cdot$ min $^{-1}$  pedalling ( $P<0.01$ ). The phase II  $\dot{V}_{O_2}$  gain was significantly reduced in UT and T at the higher pedal rate ( $P<0.01$ ) but was significantly greater in T during 115 rev $\cdot$ min $^{-1}$  pedalling compared to UT ( $P<0.01$ ). No significant differences were found for the phase II  $\dot{V}_{O_2}$  gain between UT and T during 50 rev $\cdot$ min $^{-1}$  pedalling ( $P>0.05$ ).

**Table 6.2** Oxygen uptake kinetic responses during very heavy-intensity exercise at disparate pedal rates.

Variable	AVOVA	UT ( <i>n</i> = 8)		T ( <i>n</i> = 7)	
		50 rev·min <sup>-1</sup>	115 rev·min <sup>-1</sup>	50 rev·min <sup>-1</sup>	115 rev·min <sup>-1</sup>
$\dot{V}_{O_2 \text{ bl}}$ (L·min <sup>-1</sup> )	NS	1.37 ± 0.11	1.59 ± 0.34	1.42 ± 0.06	1.35 ± 0.19
TD <sub>1</sub> (s)	NS	12 ± 5	8 ± 9	10 ± 3	11 ± 2
A <sub>1</sub> (L·min <sup>-1</sup> )	<sup>b</sup> <i>P</i> < 0.001	1.73 ± 0.42	1.57 ± 0.24	2.55 ± 0.24 <sup>##</sup>	2.67 ± 0.36 <sup>##</sup>
τ <sub>1</sub> (s)	<sup>c</sup> <i>P</i> = 0.022	32 ± 5	42 ± 11 <sup>**</sup>	26 ± 4 <sup>#</sup>	22 ± 6 <sup>##</sup>
G <sub>p</sub> (mL·min <sup>-1</sup> ·W <sup>-1</sup> )	<sup>c</sup> <i>P</i> = 0.011	10.7 ± 0.7	8.0 ± 1.3 <sup>**</sup>	10.6 ± 0.7	9.2 ± 0.8 <sup>***#</sup>
TD <sub>2</sub> (s)	<sup>a</sup> <i>P</i> = 0.023, <sup>b</sup> <i>P</i> = 0.010	171 ± 28	160 ± 32	137 ± 18 <sup>#</sup>	120 ± 20 <sup>#</sup>
A' <sub>2</sub> (L·min <sup>-1</sup> )	<sup>a</sup> <i>P</i> = 0.015, <sup>b</sup> <i>P</i> < 0.001	0.20 ± 0.07	0.30 ± 0.09 <sup>*</sup>	0.41 ± 0.16 <sup>##</sup>	0.54 ± 0.13 <sup>##</sup>
Rel. A' <sub>2</sub> (%)	<sup>a</sup> <i>P</i> = 0.005	10 ± 3	16 ± 5 <sup>*</sup>	14 ± 5	17 ± 3
$\dot{V}_{O_2 \text{ tot}}$ (L·min <sup>-1</sup> )	<sup>a</sup> <i>P</i> = 0.017, <sup>b</sup> <i>P</i> < 0.001	3.31 ± 0.52	3.45 ± 0.46	4.38 ± 0.28 <sup>##</sup>	4.56 ± 0.29 <sup>##</sup>
G <sub>tot</sub> (mL·min <sup>-1</sup> ·W <sup>-1</sup> )	<sup>c</sup> <i>P</i> = 0.021	11.9 ± 0.8	9.6 ± 1.2 <sup>**</sup>	12.3 ± 0.8	11.1 ± 0.9 <sup>***#</sup>
$\dot{V}_{O_2}$ MRT (s)	<sup>c</sup> <i>P</i> = 0.016	54 ± 9	68 ± 15 <sup>**</sup>	48 ± 5 <sup>#</sup>	49 ± 8 <sup>##</sup>

Data are presented as mean ± SD. Two-way mixed model AVOVA results (*P* < 0.05): <sup>a</sup>significant main effect for pedal rate; <sup>b</sup>significant main effect for training status; <sup>c</sup>significant pedal rate by training status interaction. NS, no significant differences found (*P* > 0.05). Significant difference between pedal rates in UT or T groups (<sup>\*</sup>*P* < 0.05, <sup>\*\*</sup>*P* < 0.01), significant difference between UT and T pedalling at 50 rev·min<sup>-1</sup> or 115 rev·min<sup>-1</sup> (<sup>#</sup>*P* < 0.05, <sup>##</sup>*P* < 0.01).  $\dot{V}_{O_2 \text{ bl}}$ , mean  $\dot{V}_{O_2}$  during baseline pedalling; τ<sub>1</sub>, TD<sub>1</sub>, A<sub>1</sub>, time constant, time delay and asymptotic amplitude of the phase II  $\dot{V}_{O_2}$  response; TD<sub>2</sub>, A'<sub>2</sub>, time delay and amplitude of the  $\dot{V}_{O_2}$  slow component; G<sub>p</sub>, G<sub>tot</sub>, gain (Δ $\dot{V}_{O_2}$  / ΔWR) of the primary response and at end-exercise; MRT, mean response time.

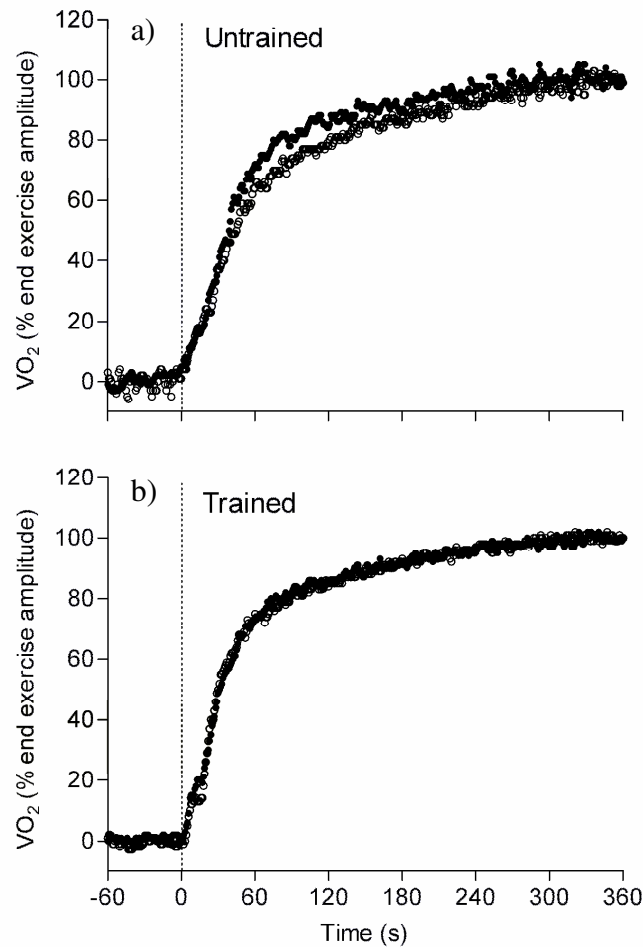




**Figure 6.1** Pulmonary  $\dot{V}_{O_2}$  response to very heavy-intensity during 50  $\text{rev}\cdot\text{min}^{-1}$  (●) and 115  $\text{rev}\cdot\text{min}^{-1}$  (○) pedalling in a representative UT (a) and T (b) participant.  $\dot{V}_{O_2}$  values are presented above baseline with the onset of exercise illustrated by the vertical dashed line.

The  $\dot{V}_{O_2}$  slow component onset was significantly reduced in T compared to UT within each pedal rate condition ( $P < 0.05$ ). The absolute and relative  $\dot{V}_{O_2}$  slow component amplitudes were significantly greater at 115  $\text{rev}\cdot\text{min}^{-1}$  compared to 50  $\text{rev}\cdot\text{min}^{-1}$  in UT ( $P < 0.05$ ) but were not different between pedal rates in T ( $P > 0.05$ ). There were no differences in the end-exercise  $\dot{V}_{O_2}$  between 50  $\text{rev}\cdot\text{min}^{-1}$  and 115  $\text{rev}\cdot\text{min}^{-1}$  when expressed relative to the cadence-specific peak  $\dot{V}_{O_2}$  in UT ( $92 \pm 7$  vs.  $95 \pm 6$  %) and T (95

$\pm 5$  vs.  $95 \pm 5$  %), respectively ( $P > 0.05$ ). The overall  $\dot{V}_{O_2}$  kinetics (denoted by the MRT) were significantly slower in UT during  $115 \text{ rev}\cdot\text{min}^{-1}$  pedalling ( $P < 0.01$ ) but were unaltered by pedal rate in T ( $P > 0.05$ ).



**Figure 6.2** Mean  $\dot{V}_{O_2}$  responses to very heavy-intensity exercise during  $50 \text{ rev}\cdot\text{min}^{-1}$  (●) and  $115 \text{ rev}\cdot\text{min}^{-1}$  (○) pedalling in UT (a) and T (b) participants. Data are presented above baseline and normalised relative to the end-exercise amplitude. The vertical dashed line represents the onset of exercise.

#### 6.4 Discussion

The principal finding of this investigation was that manipulation of pedal rate altered  $\dot{V}_{O_2}$  kinetics in UT teenage boys but not in T cyclists of a similar age. Consistent with our hypotheses,  $115 \text{ rev}\cdot\text{min}^{-1}$  pedalling resulted in slower  $\dot{V}_{O_2}$  kinetics (lengthened phase II

$\dot{V}_{O_2}$   $\tau$  and increased  $\dot{V}_{O_2}$  slow component amplitude) compared to 50 rev·min<sup>-1</sup> pedalling in UT, but there were no differences in these  $\dot{V}_{O_2}$  parameters between pedal cadences in T. These data, therefore, indicate that influences on  $\dot{V}_{O_2}$  kinetics in response to manipulating pedal rate (and by inference muscle fibre recruitment patterns) are confined to UT populations in youth.

Similar to previous reports in adults (Dimenna, et al., 2009a, 2009b; Pringle, Doust, Carter, Tolfrey, & Jones, 2003; Vercruyssen, et al., 2009), the phase II  $\dot{V}_{O_2}$   $\tau$  was markedly slower (~31%) during 115 rev·min<sup>-1</sup> compared to 50 rev·min<sup>-1</sup> pedalling in UT participants. It has been suggested that increasing muscle contraction frequency might restrict blood flow ( $\dot{Q}_M$ ) owing to a shorter muscle relaxation phase between contractions (Hoelting, Scheuermann, & Barstow, 2001). However, Ferreira et al. recently reported that muscle fractional O<sub>2</sub> extraction [estimated using near-infrared spectroscopy (NIRS)] was not different between 60 rev·min<sup>-1</sup> and 100 rev·min<sup>-1</sup> pedalling during ramp exercise (Ferreira, Lutjemeier, Townsend, & Barstow, 2006). This would not be expected if a lower  $\dot{Q}_M/\dot{V}_{O_2}$  response were manifest in the higher pedal rate condition and hence might suggest that  $\dot{V}_{O_2}$  kinetics were unlikely limited by bulk O<sub>2</sub> delivery at 115 rev·min<sup>-1</sup>. However, the possibility that spatial differences in  $\dot{Q}_M/\dot{V}_{O_2}$  influenced  $\dot{V}_{O_2}$  kinetics at disparate pedal cadences cannot be excluded from the present study.

An alternative explanation is that increasing pedal rate might have reduced the capacity of recruited muscle fibres to utilise O<sub>2</sub> at the onset of exercise. For example, glycogen depletion rates are greater in type II fibre pools during submaximal exercise (~90% peak

$\dot{V}_{O_2}$ ) when pedalling at 120 rev·min<sup>-1</sup> compared to 60 rev·min<sup>-1</sup> (Beelen, et al., 1993). There is evidence that  $\dot{V}_{O_2}$  kinetics are slower in type II muscle fibres in small mammals (Crow & Kushmerick, 1982), and in exercising humans during work-to-work transitions when the size principle (Henneman & Mendell, 1981) would predict a greater contribution from higher-order fibres to muscle force production (Brittain, et al., 2001; Dimenna, et al., 2009a; DiMenna, et al., 2008; Wilkerson & Jones, 2006, 2007). Slower phase II  $\dot{V}_{O_2}$  kinetics during 115 rev·min<sup>-1</sup> pedalling in UT might therefore be interpreted to suggest that muscle fibre pools with a lower oxidative capacity were predominately recruited at the onset of exercise in this condition.

A novel finding from the present study is that despite slower phase II  $\dot{V}_{O_2}$  kinetics in UT pedalling at 115 rev·min<sup>-1</sup>, the phase II  $\dot{V}_{O_2}$   $\tau$  was unaltered by pedal rate in T participants. Endurance training increases mitochondrial volume and oxidative enzyme activities in adults (Holloszy & Coyle, 1984), with similar improvements in muscle oxidative capacity also reported in youth (Eriksson, et al., 1973; Fournier, et al., 1982). Interestingly, the T participants in the present study reported selecting higher pedal rates (~ 90-95 rev·min<sup>-1</sup>) during training and competition, similar to previous reports in trained adult cyclists (Marsh & Martin, 1993, 1997). It is therefore plausible that training-induced adaptations specifically at higher pedal rates might have increased the mitochondrial O<sub>2</sub> utilisation potential in higher-order muscle fibres and thus rendered  $\dot{V}_{O_2}$  kinetics insensitive to altering pedal cadence in T participants.

Previous analyses of muscle biopsy specimens have revealed a greater type I muscle fibre distribution in aerobically trained children and adolescents (Dahlstrom, et al., 1997; Mero,

et al., 1991). Furthermore, studies in adults have indicated a close relationship between aerobic fitness and type I fibre proportion (Barstow, et al., 1996; Costill, et al., 1976). The high peak  $\dot{V}_{O_2}$  values reported in our junior cyclists (range 59 – 76 mL·kg<sup>-1</sup>·min<sup>-1</sup>) might therefore have predisposed these individuals to faster  $\dot{V}_{O_2}$  kinetics irrespective of the pedal rate employed. Indeed, the range of values for the phase II  $\dot{V}_{O_2}$   $\tau$  during 115 rev·min<sup>-1</sup> pedalling in UT (from 21 to 58 s) compared to T (from 19 to 29 s) participants is consistent, albeit indirectly, with the recruitment of muscle fibres with similar metabolic properties (i.e. faster  $\dot{V}_{O_2}$  kinetics) in cyclists at the higher pedal rate.

In UT, pedalling at 115 rev·min<sup>-1</sup> reduced the phase II  $\dot{V}_{O_2}$  gain and resulted in a ~50% increase in the  $\dot{V}_{O_2}$  slow component amplitude compared to 50 rev·min<sup>-1</sup> cycling. Similar  $\dot{V}_{O_2}$  responses have also been reported in adults with an increased type II muscle fibre proportion in quadriceps muscle (Barstow, et al., 1996; Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003) and during exercise following prior glycogen depletion of type I fibre pools (Krustrup et al., 2004b). It is therefore conceivable that increasing pedal rate (from 50 rev·min<sup>-1</sup> to 115 rev·min<sup>-1</sup>) might have resulted in a greater reduction in the primary  $\dot{V}_{O_2}$  gain and increased the  $\dot{V}_{O_2}$  slow component in teenage boys by altering muscle fibre recruitment at the onset of exercise and/or progressively throughout the bout. However, the extent to which delayed muscle fibre activation contributed to a greater  $\dot{V}_{O_2}$  slow component [purported to reflect a continual rise in ATP turnover (Krustrup et al., 2003; Rossiter et al., 2002)] at 115 rev·min<sup>-1</sup> is unclear. From this perspective, it is pertinent to note that an excess  $\dot{V}_{O_2}$  during constant work rate exercise has been temporally linked to

markers of muscle activity (Burnley et al., 2002; Endo et al., 2007) and additional recruitment of both type I and II fibres (Krustrup et al., 2004c).

In T, the proportional contribution of the  $\dot{V}_{O_2}$  slow component to the total increase in  $\dot{V}_{O_2}$  above baseline was unaltered by pedal rate and the  $\dot{V}_{O_2}$  MRT was similar between 50 rev·min<sup>-1</sup> and 115 rev·min<sup>-1</sup> pedalling. It has been suggested that cyclists might adopt higher pedal cadences in order to reduce intramuscular tension per crank revolution and hence augment blood flow during exercise (Takaishi et al., 2002). If present in our T cyclists, this could have negated the requirement to recruit additional muscle fibres over time resulting in an unchanged  $\dot{V}_{O_2}$  slow component between pedal rate conditions. It might therefore be inferred that cyclists adopt faster pedal cadences in order to reduce muscle activation (MacIntosh, et al., 2000; Takaishi, Yasuda, Ono, & Moritani, 1996) without compromising the  $\dot{V}_{O_2}$  kinetic response. This might be considered especially ergogenic since restraining the  $\dot{V}_{O_2}$  slow component at higher pedal rates would enable the tolerable duration of exercise to be extended below the cyclists' peak  $\dot{V}_{O_2}$  during competitive race performances (Burnley and Jones, 2007).

## 6.5 Conclusion

This is the first study to examine the influence of manipulated pedal cadence on  $\dot{V}_{O_2}$  kinetics during very heavy-intensity exercise in youth. Pedalling at fast pedal rates markedly slowed the phase II  $\dot{V}_{O_2}$   $\tau$ , reduced the phase II  $\dot{V}_{O_2}$  gain, and resulted in a greater  $\dot{V}_{O_2}$  slow component response in UT teenage boys. Conversely, manipulation of pedal rate did not alter either phase II  $\dot{V}_{O_2}$  kinetics or the  $\dot{V}_{O_2}$  slow component in trained

junior cyclists. It is proposed that alterations in muscle fibre recruitment and/or enhancement in the oxidative capacity of recruited muscle fibres, either due to genetic or training influences, might account for differences in  $\dot{V}_{O_2}$  kinetics between T and UT participants at disparate pedal rates.

## Chapter Seven

# THE INFLUENCE OF AGE ON ELECTROMYOGRAM ACTIVITY AND THE SLOW COMPONENT OF PULMONARY O<sub>2</sub> UPTAKE DURING VERY HEAVY-INTENSITY EXERCISE IN HUMANS

## 7.1 Introduction

Following the onset of step exercise above the gas exchange threshold (GET), the primary phase  $\dot{V}_{O_2}$  amplitude is supplemented by a slow rise in  $\dot{V}_{O_2}$  that extends the O<sub>2</sub> cost of exercise above the predicted  $\Delta \dot{V}_{O_2} / \Delta WR$  requirement for the external power output (Poole, et al., 1994; Whipp & Wasserman, 1972). The so called  $\dot{V}_{O_2}$  slow component expressed at the lung originates predominantly from within recruited skeletal muscle (Poole, et al., 1991) and is temporally linked to a fall in muscle [PCr] during high-intensity exercise (Rossiter, Ward, Howe, et al., 2002) thereby signalling a progressive reduction in muscle contractile efficiency over time.

Although a  $\dot{V}_{O_2}$  slow component is discernible in most children cycling in the heavy-intensity domain (Fawkner & Armstrong, 2004b), previous studies have shown that younger children demonstrate a greater primary  $\dot{V}_{O_2}$  gain and reduced  $\dot{V}_{O_2}$  slow component compared to older counterparts (Armon, et al., 1991; Breese, et al., 2010; Fawkner & Armstrong, 2004a; Williams, et al., 2001). It has been proposed, at least in adults, that alterations in motor unit recruitment might play an important role in modulating the  $\dot{V}_{O_2}$  slow component amplitude during exercise above the GET (Jones, et al., 2005b). For example, an increase in  $\dot{V}_{O_2}$  from the 3<sup>rd</sup> to 6<sup>th</sup> minute of constant work rate (CWR) exercise has been shown to temporally coincide with markers of muscle activity (Endo, et



al., 2007; Saunders, et al., 2000) and additional muscle fibre recruitment (Krustrup, Soderlund, Mohr, et al., 2004b). It is therefore pertinent that an elevated primary  $\dot{V}O_2$  gain and subsequent reduction in the  $\dot{V}O_2$  slow component amplitude following prior exercise is reported to coincide with reciprocal changes in the measured profile of the integrated electromyogram (iEMG) in quadriceps muscle (Bailey, Vanhatalo, et al., 2009; Burnley, et al., 2002; c.f. Scheuermann, et al., 2001). However, to our knowledge, no previous study has quantified whether similar alterations in motor unit recruitment might contribute to the observed differences in the primary and slow component amplitudes between adults and children.

In this study, which provides a re-analysis of data obtained within the U→VH condition from chapter five, it was hypothesised that constant work rate exercise would increase electromyogram (EMG) activity of the *m. vastus lateralis* from *minute 2* to *minute 6* and elicit a greater  $\dot{V}O_2$  slow component in teenagers (T) and men (M) compared to younger boys (B).

## 7.2 Methods

The participants' descriptive characteristics and maturity status are included within Table 5.1. Each individual completed multiple step exercise transitions initiated from 15 W pedalling to a work rate corresponding to  $\Delta 60\%$  (U→VH). The amplitude of the  $\dot{V}O_2$  slow component was calculated as the difference between the mean of the last 30 s of exercise and the primary phase asymptote and expressed as a percentage of the total increase in  $\dot{V}O_2$  above baseline pedalling in order to facilitate comparison between age groups. The average iEMG was calculated at 15-s intervals throughout exercise, with these values normalized to

the average measured during 15-180 s of unloaded cycling prior to the initiation of step exercise. Therefore, all iEMG data are presented as a percentage of the initial unloaded cycling phase. Data from repeat trials were averaged, and the  $\Delta iEMG_{6-2}$  was defined as the difference between the average iEMG over the last 15 s of exercise and the average from 105–120 s.

### 7.2.1 Statistical analyses

One-way ANOVA was used to compare  $\dot{V}_{O_2}$  kinetic parameters and  $\Delta iEMG_{6-2}$  during VH exercise with follow-up Fishers LSD tests used as appropriate to locate statistically significant differences between groups. Two-way ANOVA was used to explore mean differences in iEMG with age group (B vs. T vs. M) and time (*minute 2* vs. *minute 6*) as the model factors. Pearson product-moment correlation coefficients were employed to investigate the relationship between the relative  $\dot{V}_{O_2}$  slow component amplitude and the  $\Delta iEMG_{6-2}$  response. All results are presented as means  $\pm$  SD unless otherwise stated with rejection of the null hypotheses accepted at an alpha level of 0.05.

## 7.3 Results

Table 7.1 presents the mean  $\dot{V}_{O_2}$  slow component and iEMG data within each group. Group mean  $\dot{V}_{O_2}$  responses and iEMG activity during VH exercise are shown in figures 7.1 and 7.2.

### 7.3.1 Step exercise $\dot{V}_{O_2}$ response

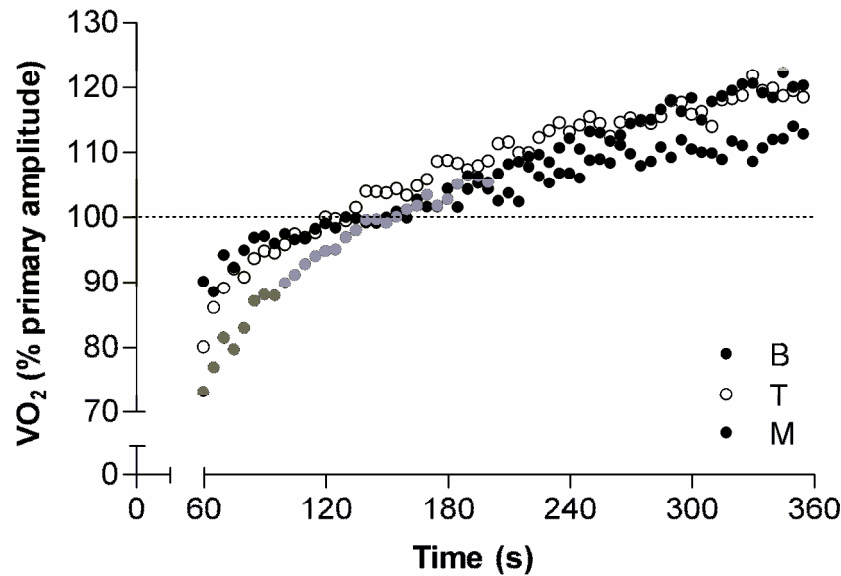
One-way ANOVA revealed a significant main effect on the primary  $\dot{V}_{O_2}$  parameters (gain and  $\tau$ ) and the relative  $\dot{V}_{O_2}$  slow component amplitude ( $P < 0.04$ ). The phase II  $\tau$  was

significantly faster in B compared to T and M ( $21 \pm 5$  vs.  $28 \pm 6$  vs.  $34 \pm 8$  s,  $P < 0.022$ ) and the primary  $\dot{V}_{O_2}$  gain was increased in younger compared to older counterparts over the initial exponential phase (B:  $9.5 \pm 1.0$  vs. T:  $8.6 \pm 0.8$  vs. M:  $8.7 \pm 0.4$  mL·min<sup>-1</sup>·W<sup>-1</sup>,  $P < 0.033$ ). This coincided with a reduced contribution from the  $\dot{V}_{O_2}$  slow component to the overall change in  $\dot{V}_{O_2}$  above baseline pedalling in B compared to T and M ( $P = 0.034$  and  $P = 0.015$ ). The  $\Delta\dot{V}_{O_2}/\Delta WR$  response above the primary  $\dot{V}_{O_2}$  amplitude (i.e. the  $\dot{V}_{O_2}$  slow component gain) was significantly greater in T and M compared to B ( $P < 0.043$ ). The relative  $\dot{V}_{O_2}$  slow component amplitude tended to correlate with age from peak height velocity in youth subjects ( $r = 0.48$ ,  $P = 0.051$ ). No differences were reported for the total  $\dot{V}_{O_2}$  gain above baseline exercise between age groups.

**Table 7.1** Pulmonary O<sub>2</sub> uptake and integrated electromyogram (iEMG) response during very heavy-intensity constant work rate exercise

	B ( $n = 8$ )	T ( $n = 9$ )	M ( $n = 9$ )
<i><math>\dot{V}_{O_2}</math> slow component parameters:</i>			
Time delay (s)	$172 \pm 26$	$149 \pm 32$	$155 \pm 22$
Amplitude (L·min <sup>-1</sup> )	$0.12 \pm 0.05$	$0.30 \pm 0.15^*$	$0.36 \pm 0.12^*$
Relative amplitude (%)	$11 \pm 4$	$16 \pm 6^{**}$	$16 \pm 3^{**}$
Gain (mL·min <sup>-1</sup> ·W <sup>-1</sup> )	$1.2 \pm 0.5$	$1.6 \pm 0.7^{**}$	$1.7 \pm 0.4^{**}$
<i>Average iEMG:</i>			
iEMG at 120 s (% baseline)	$406 \pm 92$	$425 \pm 159$	$405 \pm 149$
iEMG at 360 s (% baseline)	$413 \pm 83$	$446 \pm 177$	$454 \pm 145$
$\Delta iEMG_{6-2}$ (% baseline)	$7 \pm 25$	$21 \pm 75$	$49 \pm 48^{**}$

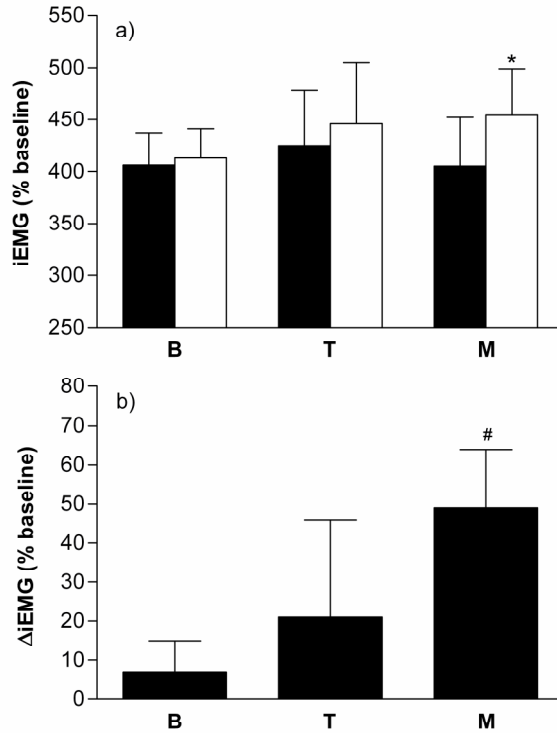
Data are presented as means  $\pm$  SD. Significantly different from B; \*  $P < 0.01$ , \*\*  $P < 0.05$ .  $\Delta iEMG_{6-2}$ , change in ( $\Delta iEMG$ ) between *minute 2* and *minute 6* of VH exercise.



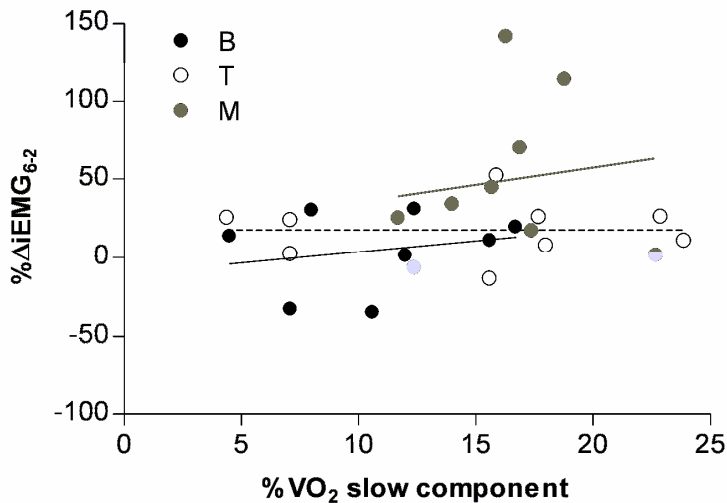
**Figure 7.1** Group mean  $\dot{V}_{O_2}$  responses in relation to age during VH constant work rate exercise.  $\dot{V}_{O_2}$  data are normalised relative to the primary amplitude (horizontal dotted line) and presented  $>60$  s to facilitate comparison of the  $\dot{V}_{O_2}$  slow component between groups.

### 7.3.2 iEMG activity

Two-way ANOVA revealed a significant main effect for time ( $P = 0.045$ ) on iEMG activity during constant work rate exercise. Follow-up paired comparisons demonstrated significant increases in iEMG from *minute 2* to *minute 6* in M ( $P = 0.010$ ) but not in B or T ( $P > 0.42$ ). This resulted in a significantly greater  $\Delta iEMG_{6-2}$  in M compared to B ( $P = 0.030$ ) but there were no differences in T compared to other groups for this variable ( $P > 0.25$ ). The  $\% \Delta iEMG_{6-2}$  was not correlated with the relative  $\dot{V}_{O_2}$  slow component amplitude in either B ( $r = 0.23$ ,  $P = 0.58$ ), T ( $r = 0.02$ ,  $P = 0.96$ ) or M ( $r = 0.15$ ,  $P = 0.71$ ) (figure 7.3).



**Figure 7.2** Group mean  $\pm$  (S.E.) integrated electromyogram activity (iEMG) of the *m. vastus lateralis* during VH constant work rate exercise. The percentage of baseline iEMG measured at *minute 2* (closed bars) and at *minute 6* (open bars) are shown in (a) with % increases in iEMG over the same time interval presented in (b). \*Significant difference between *minute 2* and *minute 6* within group ( $P < 0.05$ ). #Significantly different from B ( $P < 0.05$ ).



**Figure 7.3** Relationship between the relative  $\dot{V}O_2$  slow component amplitude and percent increases in iEMG of the *m. vastus lateralis* in each group. No significant correlations were reported ( $P > 0.05$ ).

## 7.4 Discussion

This study investigated the influence of motor unit recruitment on the  $\dot{V}_{O_2}$  slow component in relation to age during very heavy-intensity cycling exercise. In line with our experimental hypothesis, an elevated relative  $\dot{V}_{O_2}$  slow component amplitude in men coincided with an increased integrated electromyogram (iEMG) of the *m. vastus lateralis* over time compared to 11-12 yr old children. An unchanged iEMG activity in the presence of an extended  $\dot{V}_{O_2}$  above the primary amplitude in boys and teenagers is consistent with the notion that the development of the  $\dot{V}_{O_2}$  slow component might not obligate alterations in muscle activity as CWR exercise proceeds in youth.

Iterative curve fitting procedures revealed a similar time onset for the  $\dot{V}_{O_2}$  slow component between B and T but there was considerable inter-subject variability in the relative  $\dot{V}_{O_2}$  slow component amplitude ranging from 5 % to 23 % in males aged 11 - 16 yr. Moreover, the proportional contribution from the  $\dot{V}_{O_2}$  slow component to the total increase in  $\dot{V}_{O_2}$  above baseline pedalling was linearly related (albeit non-significantly) to the subjects' maturity offset score from age at peak height velocity. These results are consistent with the development of a  $\dot{V}_{O_2}$  slow component in children during cycling exercise above the GET; the magnitude of which is modulated throughout growth and maturation (Breese, et al., 2010; Fawcner & Armstrong, 2004a, 2004b).

The demonstration of a greater relative  $\dot{V}_{O_2}$  slow component in T compared to B despite an unchanged  $\Delta iEMG_{6-2}$  might be interpreted to suggest an increased  $O_2$  cost of the external power output in muscle fibres already recruited from the onset of exercise in older children. For example, a reduced muscle contractile efficiency within fatiguing motor units

has been implicated in increasing muscle ATP turnover indiscriminately from alterations in muscle fibre recruitment in order to maintain the target work rate (Hepple, Howlett, Kindig, Strydom, & Hogan, 2010; Zoladz, et al., 2008). Our findings in youth are therefore consistent with the notion that additional muscle fibre activation is not requisite to elicit a  $\dot{V}_{O_2}$  slow component during voluntary exercise in humans (Vanhatalo, et al., 2011). However, the possibility that interrogation of superficial electrical activity rendered the iEMG signal insensitive to locating spatial differences in muscle activation patterns between B and T cannot be discounted.

Alternatively, acceptance that the  $\dot{V}_{O_2}$  slow component is elicited after an independent time delay (Barstow & Mole, 1991) is consistent with the notion that the delayed activation of muscle fibres might be principally linked to increasing the  $O_2$  cost of the external power output as exercise proceeds (Endo, et al., 2007; Krusturup, Soderlund, Mohr, et al., 2004b; Whipp, 1994). Therefore, a novel finding from the present investigation was that a lower relative  $\dot{V}_{O_2}$  slow component amplitude coincided with a reduced  $\Delta iEMG_{6-2}$  of the *m. vastus lateralis* in boys compared to men during U→VH exercise. These results might be interpreted to suggest that a lower rate of fatigue development in youth subjects negated the requirement for either increased motor unit firing frequency and/or recruitment of previously inactive muscle fibres in order to sustain constant work rate exercise. However, inferences with regard to causality should be made with caution since the  $\% \Delta iEMG_{6-2}$  was not correlated with the relative  $\dot{V}_{O_2}$  slow component amplitude in either age group. Previous reports that markers of muscle activity are associated in magnitude with the  $\dot{V}_{O_2}$  slow component (Borroni, et al., 2001; Endo, et al., 2007; Saunders, et al., 2000; Shinohara & Moritani, 1992) were therefore not corroborated by the present study.

## 7.5 Conclusions

This study presented evidence linking differences in the  $\dot{V}_{O_2}$  slow component to alterations in motor unit recruitment between boys and men during CWR exercise above the GET. In contrast, an elevated  $\dot{V}_{O_2}$  slow component response in teenagers compared to boys occurred independently from any changes in iEMG of the *m. vastus lateralis*. In the latter case, increased metabolic demands within muscle fibres recruited close to the onset of U→VH exercise might be involved in extending the  $O_2$  cost of exercise above the primary  $\dot{V}_{O_2}$  amplitude in older compared to younger children.



## Chapter Eight

# THE INFLUENCE OF THIGH MUSCLE RECRUITMENT ON THE $\dot{V}_{O_2}$ SLOW COMPONENT DURING VERY HEAVY-INTENSITY EXERCISE IN BOYS AND MEN

### 8.1 Introduction

Cycling exercise above the gas exchange threshold (GET) elicits a slow rise in pulmonary  $O_2$  uptake ( $\dot{V}_{O_2}$ ) above the primary phase amplitude thereby extending to the  $O_2$  cost or 'gain' above the value predicted for the work rate (Barstow & Mole, 1991; Whipp & Wasserman, 1972). Previous studies have revealed an age-dependent modulation in relation to the  $\dot{V}_{O_2}$  slow component during heavy-intensity exercise [see Armstrong & Barker (2009) for a review]. Specifically, younger children tend to demonstrate an increased  $\dot{V}_{O_2}$  gain over the primary exponential phase with a concomitant reduction in the relative  $\dot{V}_{O_2}$  slow component amplitude compared to older counterparts (Fawkner & Armstrong, 2004a; Williams, et al., 2001). The mechanism(s) that underpin child-adult differences in the  $\dot{V}_{O_2}$  response profile are presently unknown.

It has been demonstrated that the  $\dot{V}_{O_2}$  slow component predominantly originates from contracting skeletal muscle (Krustrup, et al., 2009; Poole, et al., 1991) and is temporally linked to an increased muscle phosphocreatine (PCr) cost of exercise (Rossiter, Ward, Howe, et al., 2002). Moreover, the development of the  $\dot{V}_{O_2}$  slow component has been reported to coincide with the recruitment of additional muscle fibres (Krustrup, Soderlund, Mohr, et al., 2004b) and is increased in magnitude in subjects with a higher proportion of

type II muscle fibres in the *m. vastus lateralis* (Barstow, et al., 1996; Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003). Collectively, these findings suggest that alterations in muscle recruitment patterns might be linked to increasing the ATP (and hence  $\dot{V}_{O_2}$ ) cost of the external power output as heavy/very heavy exercise proceeds in adults. In contrast, the potential for similar factors to contribute to the etiology of the  $\dot{V}_{O_2}$  slow component in children has not been previously investigated. This is perhaps unsurprising given ethical restrictions in measuring single fibre substrate utilization in young people. The application of non-invasive markers of muscle recruitment might therefore provide an important advance in attempting to elucidate the physiological factors linked to age differences in the  $\dot{V}_{O_2}$  slow component during intense submaximal exercise.

Interrogation of muscle activity using integrated electromyography (iEMG) has yielded conflicting results with either increases in  $\dot{V}_{O_2}$  and iEMG activity shown over time (Burnley, et al., 2002; Shinohara & Moritani, 1992) or no association reported between the  $\dot{V}_{O_2}$  slow component and markers of muscle recruitment (c.f. Scheuermann, et al., 2001). It is conceivable that methodological difficulties in interpreting iEMG might be linked to previous inconsistent results; for example, the low spatial resolution confined to recording surface electrical activity and the potential for an increased firing frequency (i.e. rate coding) of motor units to influence the iEMG signal thereby reducing the fidelity in which the recruitment of ‘additional’ muscle fibres can be revealed from such measurements [see De Luca (1997) for review].

An exercise-induced increase in the  $^1\text{H}$  transverse relaxation time ( $T_2$ ) of muscle water enhances tissue contrast within the MR image that remains elevated following the cessation

of muscular work in humans (Meyer & Prior, 2000). Whilst this technique cannot directly infer single fibre type activation, it has been used to locate spatial differences in muscle recruitment across the exercising limb (Richardson, Frank, et al., 1998) and to provide an index of the intensity of contractile activity within individual muscle(s) engaged in the exercise task (Jenner, et al., 1994). Using functional MRI, previous studies have shown a linear relationship between increases in  $\dot{V}_{O_2}$  from the 3<sup>rd</sup> to 6<sup>th</sup> minute of exercise and the rise in quadriceps  $T_2$  during high-intensity cycling (Endo, et al., 2007; Saunders, et al., 2000). This study therefore utilised  $T_2$ -weighted MR imaging in order to test the hypothesis that a lower  $\dot{V}_{O_2}$  slow component in boys would be associated with a reduced change in  $T_2$  over time during very heavy-intensity exercise compared to men. If present, these findings would be consistent with the notion that an age-linked modulation of the  $\dot{V}_{O_2}$  slow component is associated with differences in muscle activation in order to sustain a constant external power output.

## **8.2 Methods**

### *8.2.1 Participants*

Eight boys and eight men volunteered to participate in exercise testing after written, informed consent had been obtained from each participant and the children's parent(s) / guardian(s) prior to the commencement of the study. The participants mean age, body mass and stature was  $11.4 \pm 0.4$  yr,  $38.0 \pm 5.2$  kg and  $1.46 \pm 0.06$  m in boys, and  $25.4 \pm 3.3$  yr,  $72.5 \pm 9.1$  kg and  $1.76 \pm 0.09$  m in men. Estimates for somatic maturity yielded a mean age from peak height velocity of  $-2.2 \pm 0.4$  yr in boys using sex-specific algorithms to determine a maturity offset score based on anthropometric measurements (Mirwald, et al., 2002). The procedures employed in this study were approved by the institutional research

ethics committee at the University of Exeter. Participants were requested to arrive at the laboratory in a rested and well hydrated state having also abstained from food or caffeine in the preceding 3 hr. Each participant completed a total of six exercise protocols over a 2-wk period at approximately the same time of day ( $\pm 2$  hr).

### 8.2.2 *Exercise protocols*

On the first visit, participants performed a ramp incremental exercise test to the limit of tolerance on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) for determination of  $\dot{V}_{O_2 \text{ peak}}$  and the gas exchange threshold (GET). After 3-min baseline cycling at 15 W, the work rate increased continuously by 15 W $\cdot$ min<sup>-1</sup> in boys and 30 W $\cdot$ min<sup>-1</sup> in men to attain a test duration of 8-12 min in each individual. Participants were instructed to select a preferred pedal rate of between 70-80 rev $\cdot$ min<sup>-1</sup> and maintain this cadence throughout the test. The  $\dot{V}_{O_2 \text{ peak}}$  was taken as the highest recorded 10-s stationary average value during the maximal exercise test. The GET was determined as the first disproportionate increase in CO<sub>2</sub> output ( $\dot{V}_{CO_2}$ ) relative to the increase in  $\dot{V}_{O_2}$  [i.e. the V-slope method (Beaver, et al., 1986)] and was subsequently verified from visual inspection of the increase in the ventilatory equivalent for  $\dot{V}_{O_2}$  ( $\dot{V}_E/\dot{V}_{O_2}$ ) with no increase in  $\dot{V}_E/\dot{V}_{CO_2}$ .

The power outputs that would require 60% of the difference ( $\Delta$ ) between the  $\dot{V}_{O_2}$  at the GET and  $\dot{V}_{O_2 \text{ peak}}$  [very heavy-intensity exercise (VH),  $\Delta 60\%$ ] were estimated for each individual. Each participant then returned to the laboratory to perform three identical step transitions of 3-min of 15W pedalling followed by 6-min of VH exercise. Each step exercise bout was completed on a different day with each laboratory visit separated by  $\geq 24$

hr. The averaged  $\dot{V}_{O_2}$  data were subsequently modelled (as described in section 3.1.1) in order to determine the  $\dot{V}_{O_2}$  slow component time delay ( $SC_{td}$ ) for each individual. Participants were then asked to perform two additional step exercise bouts at  $\Delta 60\%$  in a separate laboratory adjacent to the bore of a 1.5 T super-conducting magnet including; 1) 3-min of 15 W pedalling followed by  $x$  number of minutes cycling at  $\Delta 60\%$  dependent on the predetermined  $SC_{td}$  for each individual, and; 2) 3-min of 15 W pedalling followed by 6-min of  $\Delta 60\%$  exercise. Each protocol was separated by 60-min of recovery in order to obtain MRI images of the upper thigh at each time point (rest,  $SC_{td}$ , minute 6) within a single laboratory visit.

### 8.2.3 *Cardio-respiratory measures*

Pulmonary gas exchange and ventilation were measured and displayed breath-by-breath (Metalyser 3B Cortex, Biophysik, Leipzig, Germany) during each exercise trial. Gas fractions of  $O_2$  and  $CO_2$  were drawn continuously from a face mask-turbine assembly following calibration with gases of known concentration. Expired volume was measured using a DVT turbine digital transducer which was manually calibrated using a 3-L syringe (Hans Rudolph, Kansas City, MO) before each test. All calibration procedures were repeated before each experimental test. Heart rate was recorded at 5-s time intervals throughout exercise using short-range radiotelemetry (Polar S610, Polar Electro Oy, Kempele, Finland).

### 8.2.4 *Determination of $T_2$ using MRI*

The mid point of the thigh where all analysis was undertaken was identified by measuring the distance halfway between the greater trochanter and the lateral epicondyle with this

point identified by drawing on the skin's surface with a marker pen. During scanning a cod liver capsule was placed on the marked point so as to allow identification within the MRI images and hence ensure position repeatability between scans. Subjects' lay horizontal in the supine position within the bore of a 1.5-T superconducting magnet (Achieva, Philips Medical System, Best, The Netherlands) in order to acquire an MRI image of the upper thigh region at rest. After the initial pre-exercise scan, subjects then moved into an adjacent room and performed the required duration of exercise at  $\Delta 60\%$  on an electronically-braked cycle ergometer immediately after which they were repositioned back within the bore of the magnet in order to obtain post-exercise scan images. The time taken from the cessation of exercise to the commencement of the scan was kept consistent at 120 s for each subject.

All imaging was undertaken using a body coil with slices acquired in the axial plane and centered on the cod liver oil marker. Initially, 5 contiguous  $T_1$ -weighted turbo spin echo slices were acquired to obtain structural muscle information and allow identification of each separate muscle group during the  $T_2$  calculation. In-plane resolution was  $1.04 \times 1.04$  mm and the slice thickness was 5mm with an echo time (TE) of 15 ms and a repetition time (TR) of 160 ms. Subsequently, a multishot spin-echo, echo-planar imaging (EPI) sequence (SE-GraSE) was utilized to calculate  $T_2$  values (EPI factor 5). In plane resolution was  $3.13 \times 3.17$  mm with a slice thickness of 10 mm and a TR of 145 ms. 10 echoes were obtained with the shortest TE of 5 ms with a 5 ms increment. 20 averages were summed to improve signal to noise resulting in a total scanning time of 30.6 s.

In order to calculate  $T_2$ , a region of interest (ROI) was manually drawn for each of 8 muscle groups; rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), vastus intermedius (VI), sartorius (Sar), gracilis (Gr), adductor magnus (AM), semitendinosus

(semi), using the Philips scanner software and based upon the structural scans obtained. The signal intensity (S) for each echo time, for each muscle group was then recorded and the value of T<sub>2</sub> calculated based on the relationship:

$$S=S_0 \exp^{(-TE/T_2)} \quad \text{Equation 8.1}$$

Where S<sub>0</sub> is a constant and the signal S is corrected for background electronic noise, based upon the recorded signal from an ROI placed on an area in the image in which no tissue is present. If the natural log of S is plotted against TE for all TE values, the gradient of the best fit line is then given by 1/T<sub>2</sub>. Muscle T<sub>2</sub> values from the RF, VL, VM, and VI were also averaged to provide an index of quadriceps recruitment at each time point in all subjects. All T<sub>2</sub> calculations were performed by the same investigator with an error of 6% coefficient of variation (CV).

#### 8.2.5 *Data analysis procedures*

All procedures stated in section 3.3.1 on the treatment and modelling of breath-by-breath  $\dot{V}_{O_2}$  data in response to step exercise were completed.

#### 8.2.6 *Statistical analysis*

Independent-samples *t* tests were used to compare  $\dot{V}_{O_2}$  kinetic parameters during VH exercise between boys and men. Mean differences in T<sub>2</sub> values were examined using a mixed model ANOVA with age (boys vs. men) and time (rest vs. SC<sub>td</sub> vs. minute 6) as the model factors. If a significant main effect for time was observed, follow-up paired *t*-tests were performed on planned comparisons with the alpha level adjusted using the bonferroni

procedure in order to locate statistically significant differences within boys and men for each muscle. Pearson product-moment correlation coefficients were used to investigate the relationship between the relative  $\dot{V}_{O_2}$  slow component amplitude and percent changes in  $T_2$  from the  $SC_{td}$  to minute 6 of exercise ( $\Delta T_{2_{6-SC_{td}}}$ ). All results are presented as means  $\pm$  SD with rejection of the null hypotheses accepted at an alpha level of 0.05.

### 8.3 Results

The peak HR and exercise power output at the termination of the ramp test was  $194 \pm 9$  b $\cdot$ min $^{-1}$  and  $154 \pm 25$  W and  $186 \pm 7$  b $\cdot$ min $^{-1}$  and  $329 \pm 30$  W in boys and men, respectively. The GET occurred at a similar fraction of  $\dot{V}_{O_2_{peak}}$  in both groups (Boys:  $51 \pm 6\%$  vs. Men:  $51 \pm 6\%$ ,  $P = 0.898$ ). Based on the ramp exercise responses, the work rates calculated to require  $\Delta 60\%$  were  $108 \pm 17$  W in boys and  $226 \pm 26$  W in men.

The  $\dot{V}_{O_2}$  kinetic parameters during step exercise are presented in Table 8.1. Representative  $\dot{V}_{O_2}$  plots and mean profiles in children and adults are shown in figure 8.1. The exponential rise in phase II  $\dot{V}_{O_2}$  was initiated after a similar TD but the  $\tau$  was significantly faster in boys compared to men. The primary  $\dot{V}_{O_2}$  gain was greater in boys compared to men where it remained elevated in the former until the end of exercise. There were no age differences in the  $\dot{V}_{O_2}$  slow component amplitude when normalized as a percentage of the total  $\Delta \dot{V}_{O_2}$  above baseline pedalling. Likewise, the  $\% \dot{V}_{O_2_{peak}}$  attained at end-exercise was similar between boys and men ( $94 \pm 9$  vs.  $97 \pm 5\%$ ,  $P = 0.56$ ). Modeling the entire response from  $t = 0$  s to the end of exercise yielded a faster overall  $\dot{V}_{O_2}$  MRT in boys compared to men.



**Table 8.1** Pulmonary O<sub>2</sub> uptake kinetics during VH intensity exercise in boys and men

Variable	Boys ( <i>n</i> = 8)	Men ( <i>n</i> = 8)	<i>P</i> value
Baseline $\dot{V}_{O_2}$ (L·min <sup>-1</sup> )	0.62 ± 0.07	0.88 ± 0.13	<0.001
Phase II TD (s)	11 ± 4	12 ± 6	0.684
Phase II $\tau$ (s)	23 ± 3	35 ± 8	0.004
Primary $\dot{V}_{O_2}$ amplitude (L·min <sup>-1</sup> )	1.00 ± 0.10	1.71 ± 0.22	<0.001
Primary gain (mL·min <sup>-1</sup> ·W <sup>-1</sup> )	10.8 ± 0.9	8.1 ± 0.4	<0.001
$\dot{V}_{O_2}$ SC onset (s)	152 ± 32	145 ± 15	0.588
$\dot{V}_{O_2}$ SC amplitude (L·min <sup>-1</sup> )	0.18 ± 0.10	0.41 ± 0.16	0.003
$\dot{V}_{O_2}$ SC relative amplitude (%)	15 ± 7	19 ± 4	0.145
End-exercise $\dot{V}_{O_2}$ (L·min <sup>-1</sup> )	1.79 ± 0.18	3.00 ± 0.46	<0.001
Overall $\dot{V}_{O_2}$ gain (mL·min <sup>-1</sup> ·W <sup>-1</sup> )	12.7 ± 0.7	10.1 ± 0.5	<0.001
$\dot{V}_{O_2}$ mean response time (s)	47 ± 9	71 ± 16	0.002

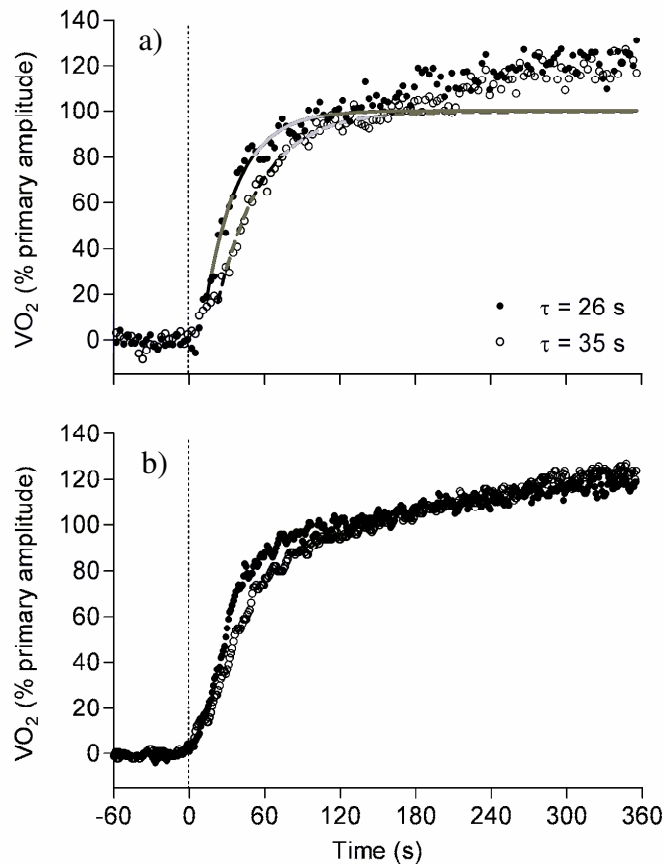
Data are presented as means ± SD. TD, time delay;  $\tau$ , time constant of the response; SC, slow component.

**Table 8.2** Resting T<sub>2</sub> values for consecutive exercise bouts interspersed with 60-min recovery

Muscle	Boys		Men	
	<i>Bout 1</i>	<i>Bout 2</i>	<i>Bout 1</i>	<i>Bout 2</i>
Rectus femoris	33.1 ± 5.2	32.8 ± 8.8	36.3 ± 3.5	36.1 ± 3.8
Vastus lateralis	39.7 ± 3.4	39.8 ± 2.8	37.7 ± 2.6	38.6 ± 3.1
Vastus medialis	38 ± 4.2	38.1 ± 2.8	36.3 ± 2.1	36.8 ± 2.2
Vastus intermedius	36.7 ± 3.1	37.7 ± 1.6	38.4 ± 2.2	38.6 ± 2.7
Sartorius	45.8 ± 7.1	48.4 ± 9	43.9 ± 4.3	43.4 ± 3.2
Gracilis	45.2 ± 8.3	45.6 ± 9.9	40.8 ± 5.1	40.7 ± 5.2
Adductor magnus	42.0 ± 3.1	43.0 ± 4.0	39.8 ± 4.5	39.7 ± 4.0
Semitendinosus	38.8 ± 4.2	40.7 ± 6.6	40.7 ± 8.1	39.5 ± 7.3

Data are presented as means ± SD. No significant group differences were found.

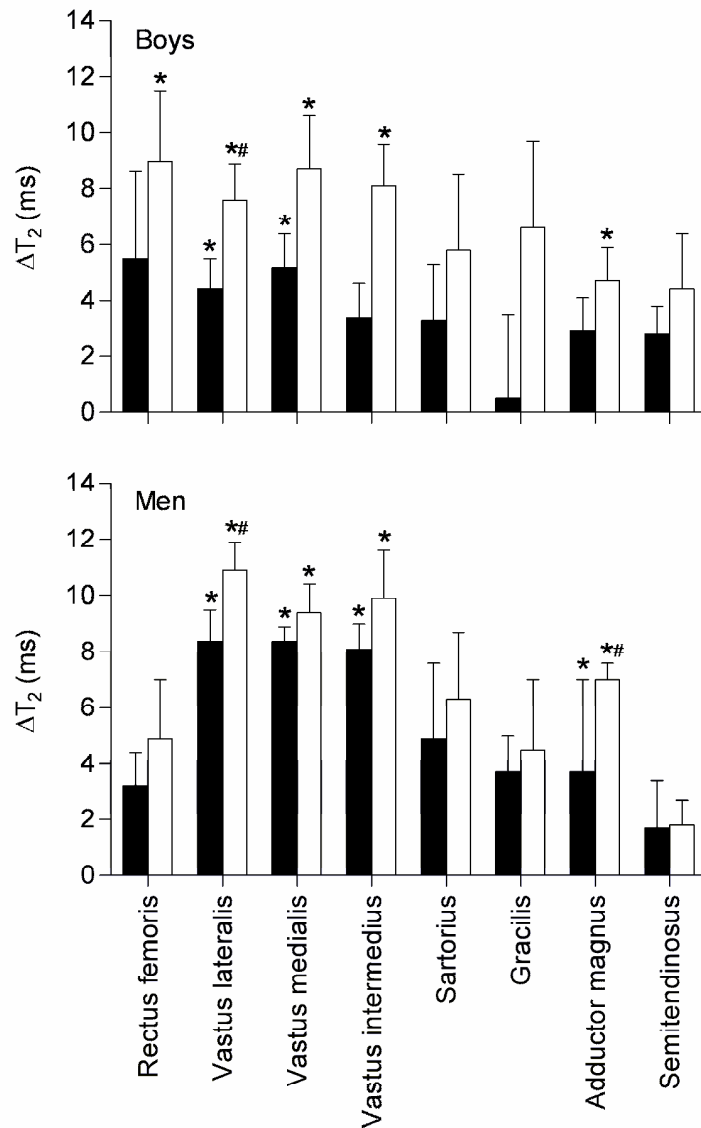
Table 8.2 presents  $T_2$  values across the upper thigh at rest in boys and men. There were no significant ( $P > 0.05$ ) differences in pre-exercise  $T_2$  values for each muscle between repeated exercise trials completed within a single laboratory session; the 60-min rest period



**Figure 8.1** Representative  $\dot{V}_{O_2}$  responses in a child ( $\bullet$ ) and adult ( $\circ$ ) subject during VH exercise in (a) with group mean  $\dot{V}_{O_2}$  profiles shown in (b). The  $\dot{V}_{O_2}$  data are normalized relative to the primary amplitude. In (a), the phase II model fit has been extended to illustrate the magnitude of the  $\dot{V}_{O_2}$  slow component.

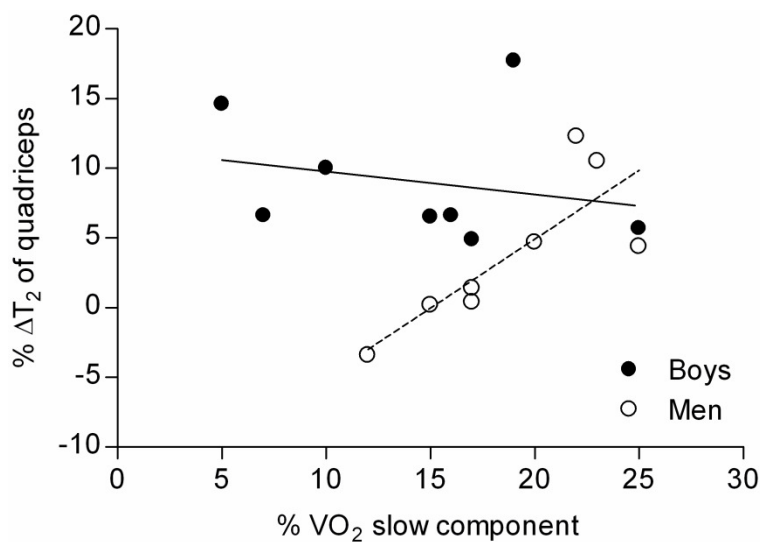
therefore enabled the recovery of muscle  $T_2$  in boys and men. Figure 8.2 illustrates that  $T_2$  progressively increased over time during VH exercise for each muscle in both groups.  $T_2$  significantly increased from rest to the  $SC_{td}$  in the vastus muscles (VL, VM, and VI) and AM in men, with increases in the VL and VM also reported in boys ( $P < 0.005$ ). The  $\Delta T_2$  response above rest over the primary  $\dot{V}_{O_2}$  phase was significantly ( $P < 0.026$ ) greater

within the vastus muscles (VL, VM, and VI) in men compared to boys but there were no group differences for this parameter extended to minute 6 for either of the muscles studied. Muscle  $T_2$  significantly increased from the  $SC_{td}$  to minute 6 of exercise in the VL in both groups ( $P < 0.046$ ) with  $T_2$  increases over the same period reported in the AM in men only ( $P = 0.006$ ). There was also a tendency for  $T_2$  to increase in the VI over the duration of the



**Figure 8.2** Group mean ( $\pm$  SE) change in  $T_2$  above rest in each muscle of the upper thigh at the  $\dot{V}_{O_2}$  slow component time delay ( $SC_{td}$ , closed bars) and at minute 6 (open bars). \*Significant increase above rest, #Significant increase from  $TD_{sc}$  to minute 6,  $P < 0.05$ .

$\dot{V}_{O_2}$  slow component in boys ( $P = 0.096$ ). Within men, there was a significant correlation between the relative  $\dot{V}_{O_2}$  slow component amplitude and the  $\Delta T_{2_{6-SC_{td}}}$  in the VM ( $r = 0.72$ ,  $P = 0.044$ ) and VI ( $r = 0.77$ ,  $P = 0.027$ ), but the same parameters were not related across each muscle of the upper thigh in boys. Likewise, the averaged quadriceps  $\Delta T_{2_{6-SC_{td}}}$  was significantly correlated to the relative  $\dot{V}_{O_2}$  slow component in men ( $r = 0.81$ ,  $P = 0.015$ ) but not in boys ( $r = -0.23$ ,  $P = 0.58$ ) during VH exercise (figure 8.3).



**Figure 8.3** Relationship between the relative  $\dot{V}_{O_2}$  slow component amplitude and percent increases in  $T_2$  of the *m. quadriceps femoris* in boys ( $r = -0.23$ ,  $P = 0.58$ ) and men ( $r = 0.81$ ,  $P = 0.015$ ) during VH exercise.

#### 8.4 Discussion

This study used functional MRI in order to investigate the influence of  $T_2$  changes as a marker of muscle recruitment on  $\dot{V}_{O_2}$  kinetics in children compared adults. As opposed to the stated experimental hypothesis, age differences in the relative  $\dot{V}_{O_2}$  slow component amplitude were not statistically significant with  $T_2$  increases of the upper thigh muscles reported in boys and men over time. In boys compared to men, a reduced  $T_2$  change of the

vastus muscles following the onset of VH exercise is consistent with the notion that differences in muscle fibre recruitment might be linked to a shortened phase II  $\tau$  and increased primary  $\dot{V}_{O_2}$  gain in youth.

Although the physiological factors responsible for exercise-induced  $T_2$  changes are controversial, the uptake and/or redistribution of fluid within muscle linked to the accumulation of metabolites (e.g.  $P_i$  and  $H^+$ ) has received empirical support [see Meyer & Prior (2000) for review]. For example,  $T_2$  increases are more pronounced during exercise involving a greater fall in muscle pH (Cheng, et al., 1995), and following electrical stimulation in regions comprising a higher proportion of fast-twitch (glycolytic) fibres compared to slow-twitch (oxidative) muscle (Prior, Ploutz-Snyder, Cooper, & Meyer, 2001). These findings, as noted by Reid et al. (2001), underscore the importance of normalizing exercise intensity in relation to the maximal aerobic capacity in order to provide meaningful cross-sectional comparisons in terms of muscle recruitment during exercise. From this perspective, it is important to note that the development of the  $\dot{V}_{O_2}$  slow component precluded the establishment of a  $\dot{V}_{O_2}$  steady-state and resulted in the near attainment of  $\dot{V}_{O_2 \text{ peak}}$  at the termination of exercise in boys and men. Such responses would be expected if the imposed external work rate were above the individuals' critical power (Poole, et al., 1988; Williams, et al., 2008) and would therefore suggest that employing the 'Δ' concept resulted in both groups exercising within the VH intensity domain.

Consistent with previous studies (Fawcner & Armstrong, 2004a; Williams, et al., 2001), step exercise transitions above the GET elicited a shorter phase II  $\tau$  and elevated the primary  $\dot{V}_{O_2}$  gain in younger compared to older counterparts. Given the purported

influence of muscle fibre recruitment on the primary  $\dot{V}_{O_2}$  parameters (Barstow, et al., 1996; Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003), the present results might be interpreted to suggest age differences in the muscle fibre oxidative capacity following the onset of exercise. In boys and men, initial  $T_2$  changes across the lower limb would suggest that metabolic activity within the quadriceps region (with the exception of the RF in men) predominantly supported the external power output over the primary  $\dot{V}_{O_2}$  phase (figure 8.2). However,  $T_2$  increases were accentuated in the vastus muscles from rest to the  $SC_{td}$  in men compared to boys. Given that  $T_2$  changes principally reflect type II fibre activation (Prior, et al., 2001), it is conceivable that the metabolic properties of type II muscle fibres therefore influenced the pulmonary  $\dot{V}_{O_2}$  response earlier into the exercise transition in men compared to boys. Type II fibres are reported to have a reduced microvascular  $O_2$  pressure head (Behnke, et al., 2003; McDonough, et al., 2005) and slower  $\dot{V}_{O_2}$  kinetics (Crow & Kushmerick, 1982; Krstrup, et al., 2008) compared to type I fibres and would therefore be likely to lengthen the phase II  $\tau$  and restrict the primary  $\dot{V}_{O_2}$  gain if recruited following the onset of exercise. However, the potential for alterations in muscle fibre type distribution between children and adults to impact on  $T_2$  changes following the cessation of exercise cannot be discounted from the present study.

Although  $T_2$  increased in all muscles following the onset of exercise, only changes in the VL and VM attained statistical significance in boys. In contrast,  $T_2$  increases above rest were reported in five out of eight muscles at minute 6 in boys with a significant  $\Delta T_2$  reported in the VL over the duration of the  $\dot{V}_{O_2}$  slow component in both groups. These results suggest that the progressive recruitment of muscle fibres might have contributed to extending the  $O_2$  cost of exercise above the primary amplitude independent of age. In line

with previous studies (Endo, et al., 2007; Saunders, et al., 2000), the demonstration of a positive linear slope relating the  $\dot{V}_{O_2}$  slow component to percent increases in quadriceps  $T_2$  is consistent with this proposal in our adult subjects (figure 8.3). Conversely, the lack of association between these two variables in boys might suggest that other factors aside from the recruitment of additional muscle fibres might be involved in young people. For example, the protracted response of type II muscle fibres with slow  $\dot{V}_{O_2}$  kinetics and/or increases in the metabolic requirement as these fibres are initially recruited has been implicated in the development of the  $\dot{V}_{O_2}$  slow component (Vanhatalo, et al., 2011; Zoladz, et al., 2008). However, an earlier contribution from higher-order (type II) motor units to the external power output would be expected to enhance  $T_2$  changes over the primary phase (Prior, et al., 2001) which did not occur in boys relative to men in the present study.

## **8.5 Conclusion**

This investigation provided novel insight into the physiological factors linked to muscle recruitment influencing  $\dot{V}_{O_2}$  kinetics during very heavy-intensity exercise in youth. An overall speeding of the  $\dot{V}_{O_2}$  mean response time in boys compared to men was principally linked to a shorter phase II  $\tau$  in the former as there were no significant age differences in the relative  $\dot{V}_{O_2}$  slow component amplitude. Moreover, an excess  $\dot{V}_{O_2}$  above the primary amplitude temporally coincided with increases in quadriceps  $T_2$  (a marker of muscle recruitment) in boys during constant work rate exercise. It is proposed that fatigue linked to the delayed onset of muscle activation might, at least in part, contribute to the development of the  $\dot{V}_{O_2}$  slow component in children.

## Chapter Nine

### SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Given the ethical restrictions in obtaining muscle biopsies in youth, the primary objective of this thesis was to utilise alternative non-invasive techniques in order to explore the potential involvement of alterations in muscle fibre recruitment on age-dependent differences in pulmonary  $\dot{V}O_2$  kinetics during exercise. Specifically, the studies outlined in this thesis were designed to address the following:

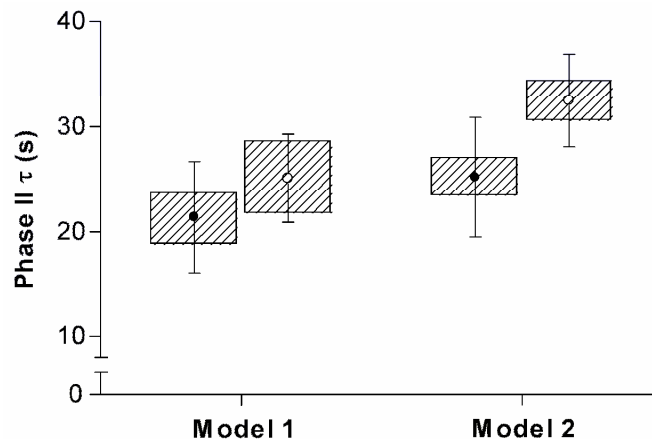
1. To establish whether longitudinal changes in  $\dot{V}O_2$  kinetics observed through early childhood during heavy-intensity exercise persist into the teenage years characterised by continual growth and physical development (Chapter 4).
2. To investigate the influence of alterations in muscle fibre recruitment on  $\dot{V}O_2$  kinetics in relation to age through initiating step exercise transitions from an elevated baseline metabolic rate (Chapter 5).
3. To explore the interaction between manipulated pedal cadence and trained status on  $\dot{V}O_2$  kinetics during very-heavy intensity exercise in teenage boys (Chapter 6).
4. To examine the temporal association between integrated electromyogram (iEMG) activity of the *m. vastus lateralis* and the  $\dot{V}O_2$  slow component during very heavy-intensity exercise in youth compared to men (Chapter 7).
5. To investigate the influence of quadriceps muscle activation on  $\dot{V}O_2$  kinetics using functional MRI during very heavy-intensity exercise in boys and men (Chapter 8).



## 9.1 Summary of experimental chapters

### 9.1.1 Chapter 4

The purpose of the first experimental investigation was to extend the earlier longitudinal findings outlined by Fawkner & Armstrong (2004a) with reference to developmental changes in  $\dot{V}O_2$  kinetics over a 2-yr time interval in fourteen teenage boys (aged 14.1 [0.2] yr) during heavy-intensity exercise. Mathematical modelling revealed a superior fit of the pulmonary  $\dot{V}O_2$  response using a double-exponential model (*equation 2.3*) thereby confirming the development of a  $\dot{V}O_2$  slow component in older children. Importantly, however, subject's  $\dot{V}O_2$  responses when fitted using a double-exponential model resulted in considerable overlap between the upper and lower 95 % confidence intervals (CI) for the resolved phase II  $\tau$  on test occasions 1 and 2, respectively (figure 9.1).



**Figure 9.1** Mean  $\pm$  (SD) phase II  $\tau$  values during heavy-intensity exercise on test occasion 1 (●) and 2 (○) resolved using a double-exponential function (*model 1*) and a single-exponential model within a fitting window that excluded the  $\dot{V}O_2$  slow component (*model 2*). *Hashed areas* illustrate the mean 95% confidence intervals (CI) for parameter estimation.

Figure 9.1 illustrates that the application of a double-exponential model would have led to the erroneous conclusion that phase II  $\dot{V}O_2$  [and by extension  $m\dot{V}O_2$ ] (Grassi, et al., 1996;

Krustrup, et al., 2009)] kinetics was not altered longitudinally during adolescence. In contrast, modelling the phase II  $\dot{V}O_2$  response independently reduced the mean 95% CI on test occasions 1 and 2 (4 [1] vs. 5 [1] s) and revealed a significant (~20%) slowing of phase II  $\tau$  in boys over the 2-y study. Furthermore, the overall  $O_2$  cost of exercise was extended due to a greater relative  $\dot{V}O_2$  slow component amplitude on the second test occasion. Taken collectively, these data indicated a progressive reduction in muscle oxidative capacity and metabolic efficiency during heavy-intensity exercise with increasing age.

### 9.1.2 Chapter 5

With the demonstration that longitudinal changes in  $\dot{V}O_2$  kinetics were extended into adolescence, the second investigation applied well established principles of motor unit recruitment in order to explore the influence of alterations in muscle fibre activation on  $\dot{V}O_2$  responses in boys (B), teenagers (T), and men (M). Specifically, cycling transitions from unloaded pedalling to very heavy-intensity exercise (U→VH) were divided into two step exercise bouts (U→M to M→VH) in order drive an orderly sequence of motor unit recruitment and amplify the metabolic response properties of the lower- and higher-order muscle fibres on the pulmonary  $\dot{V}O_2$  signal. In T and M, an increased surface iEMG of the *m. vastus lateralis* prior to the onset of work-to-work exercise resulted in slower overall  $\dot{V}O_2$  kinetics and extended the total  $O_2$  cost of exercise due principally to a lengthened  $\tau$  and increased  $\dot{V}O_2$  gain during the primary phase (i.e. the  $\dot{V}O_2$  slow component amplitude was reduced in M→VH). Furthermore, markedly slower phase II  $\dot{V}O_2$  kinetics were observed during the 2<sup>nd</sup> but not 1<sup>st</sup> step bout of work-to-work exercise in T compared to B with the latter evincing an invariant  $\dot{V}O_2$  gain between U→M and M→VH conditions.

These results are consistent with the notion that age-dependent differences in  $\dot{V}O_2$  kinetics during very heavy-intensity exercise might reside in the recruitment of higher-order (type II) muscle fibres. The potential for longitudinal transformations in muscle fibre type to impact on  $\dot{V}O_2$  kinetics during work-to-work transitions cannot be excluded and awaits further investigation.

### 9.1.3 Chapter 6

The third study tested the hypothesis that increasing pedal cadence would lengthen the phase II  $\tau$  and increase the  $\dot{V}O_2$  slow component during very heavy-intensity cycling in untrained (UT) but not trained (T) boys aged 14-17 yr. In UT subjects, pedalling at 115 rev·min<sup>-1</sup> slowed phase II  $\dot{V}O_2$  kinetics and increased the relative  $\dot{V}O_2$  slow component amplitude compared to 50 rev·min<sup>-1</sup> pedalling with no pedal rate influences on the  $\dot{V}O_2$  kinetic parameters reported in T youth cyclists. These data therefore corroborated earlier findings during work-to-work transitions in teenage boys using an alternative experimental model purported to elevate type II muscle fibre recruitment during exercise (Ferguson, et al., 2001; Sargeant, 1999). Importantly in this study, the predictable changes in  $\dot{V}O_2$  kinetics at higher pedal cadences were not manifest in teenage boys with high aerobic fitness ( $\dot{V}O_{2\text{ peak}}: \sim 70 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) therefore consistent with the notion that genetic and/or training influences could mitigate a reduction in the muscle oxidative capacity with increasing age.

#### 9.1.4 Chapter 7

The objective of the fourth study was to investigate the involvement of alterations in neuromuscular activity on the development of the  $\dot{V}O_2$  slow component during very heavy-intensity exercise in youth. Using surface iEMG interrogation, an increased electrical activity within superficial regions of the *m. vastus lateralis* coincided with a greater relative  $\dot{V}O_2$  slow component amplitude in M compared to B. These findings suggested that a lower  $O_2$  cost of the external work rate per unit of time might have been linked to the recruitment of fewer motor units in children from the 2<sup>nd</sup> minute of very heavy-intensity exercise compared to adults. In contrast, an increased  $\dot{V}O_2$  slow component in T compared to B occurred indiscriminately from alterations in surface iEMG activity during constant work rate exercise. In the latter case, the protracted response of initially recruited muscle fibres with slow  $\dot{V}O_2$  kinetics and/or reduced contractile efficiency might have contributed to extending the  $\dot{V}O_2$  gain above the primary amplitude in older compared to younger children.

#### 9.1.5 Chapter 8

In a follow-up investigation, the objective of the final experimental chapter was to examine the influence of muscle recruitment on  $\dot{V}O_2$  kinetics using higher resolution T2-weighted imaging in boys and men. Repeated step exercise transitions were time interpolated and averaged in order to enhance the  $\dot{V}O_2$  signal-to-noise response and importantly identify the  $\dot{V}O_2$  slow component time delay ( $TD_{sc}$ ) in each child and adult subject. Subsequent exercise trials were performed close to the bore of a 1.5 T superconducting magnet with scan images of the upper thigh obtained at separate time intervals (e.g. at rest,  $TD_{sc}$ , and

*minute* 6) in order to track changes in quadriceps muscle recruitment during the primary  $\dot{V}O_2$  phase and  $\dot{V}O_2$  slow component. The T2 signal intensity increased within the *m. vastus lateralis* and *m. vastus intermedius* from the  $TD_{sc}$  to *minute* 6 during very heavy-intensity exercise in boys. Although muscle fibre-specific influences could not be elucidated, the reported dependence of T2 changes on metabolite accumulation (Prior, et al., 2001) is consistent with the notion that initial fatigue linked to the recruitment of additional muscle fibres (likely to reside higher in the recruitment hierarchy with lower metabolic efficiency) might have contributed to the development of the  $\dot{V}O_2$  slow component in children. These findings therefore indicated for the first time that changes in muscle fibre recruitment during CWR exercise might be involved in the etiology of the  $\dot{V}O_2$  slow component independent of age.

## **9.2 Advances on current perspectives in paediatric exercise physiology**

Since  $\dot{V}O_2$  kinetics under non steady-state exercise conditions reflects the integrated capacity of the pulmonary, cardiovascular, and metabolic systems to couple  $O_2$  transport to the rate of muscle  $O_2$  utilisation, then developmental changes in either components of the ‘ $O_2$  delivery cascade’ and/or peripheral metabolic factors would be expected to influence the rapidity at which oxidative metabolism can adapt to a step increase in exercise work rate. From this perspective, the lack of experimental studies that have elicited a speeding of phase II  $\dot{V}O_2$  kinetics in younger adults following interventions purported to enhance muscle  $O_2$  availability has been interpreted to suggest that slower  $\dot{V}O_2$  kinetics with increasing age might be principally linked to an intracellular oxidative inertia which presides over the  $\dot{V}O_2$  kinetic response at higher exercise intensities. However, the physiological factors that underpin age differences in metabolic responses to exercise have

not been studied empirically with few experimental data available on the factors regulating oxidative metabolism in youth.

### *9.2.1 Age-linked modulation of $\dot{V}O_2$ kinetics: Dependence on muscle fibre recruitment*

It has been suggested that a greater reliance on oxidative ATP turnover and/or lower substrate level phosphorylation during exercise in children might be linked to an augmented recruitment of type I muscle fibres in younger compared to older counterparts (Barker & Armstrong, 2010). This supposition is supported on several fronts: 1) increases in muscle fibre size are accompanied by alterations in muscle fibre distribution with a reduced type I fibre expression reported during growth (Glenmark, et al., 1992; Lexell, et al., 1992); 2) children demonstrate lower fatigability during sustained maximal voluntary contractions (MVC) and repeated ‘all-out’ sprint exercise (Falk & Dotan, 2006; Halin, et al., 2003; Ratel, Duche, & Williams, 2006); 3) children’s metabolic responses to high-intensity exercise are characterised by an attenuated muscle PCr breakdown and blunted rise in muscle pH as work rate is incremented above the LT (Barker, Welsman, et al., 2010; Zanconato, et al., 1993); and 4) step exercise transitions above the GET elicit a similar  $\dot{V}O_2$  response profile in younger children (Fawkner & Armstrong, 2004a; Williams, et al., 2001) compared to adults with a greater proportion of type I muscle fibres (Barstow, et al., 1996; Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003).

Given the aforementioned muscle fibre type and/or recruitment and metabolic response characteristics, it was therefore hypothesized that work-to-work exercise influences on  $\dot{V}O_2$  kinetics would be dependent on age with an invariant phase II  $\tau$  predicted in younger

children. This did not manifest since the phase II  $\tau$  lengthened by ~58% during M→VH compared to U→M exercise in boys aged 11-12 yr. However, slower phase II  $\dot{V}O_2$  kinetics from U→M to M→VH exercise were accentuated in T (~105%) with no group differences reported between B and T in the former condition. These findings might therefore be interpreted to explain, at least in part, the shorter phase II  $\tau$  demonstrated in younger children compared to older counterparts during step exercise above the GET (Fawcner & Armstrong, 2004a; Williams, et al., 2001). For example, if a large proportion of the available muscle mass (including type I and II fibre pools) were recruited close to the onset of intense exercise (Krustrup, Soderlund, Mohr, et al., 2004b; Krustrup, Soderlund, Mohr, Gonzalez-Alonso, et al., 2004), then the oxidative response kinetics within higher-order (type II) muscle fibres might be expected to elicit a greater net slowing of the pulmonary  $\dot{V}O_2$  signal in T and M compared to B. An increased lengthening of the phase II  $\tau$  from U→M to U→VH exercise in T compared B (11 vs. 27 %, respectively) would be consistent with this proposal.

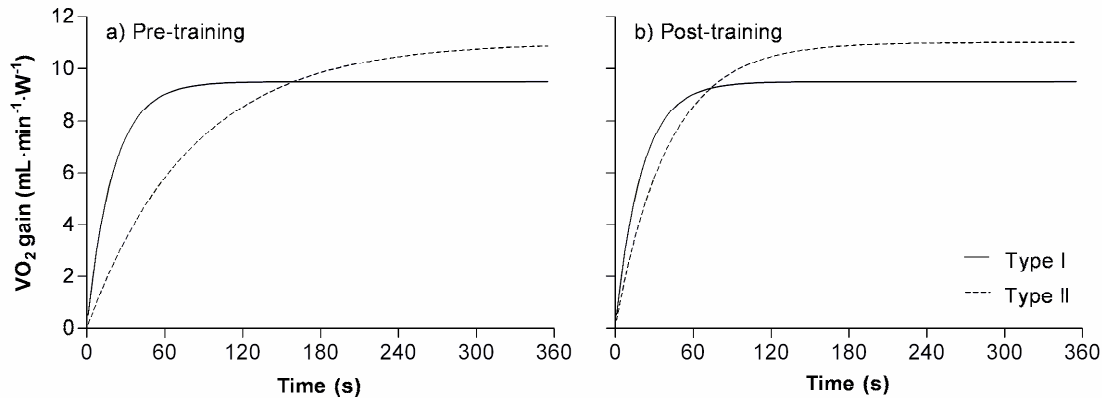
An important theoretical question is whether faster  $\dot{V}O_2$  kinetics during work-to-work exercise in B compared to T and M reflected an enhanced oxidative capacity in type II muscle fibres and/or was principally linked to age-related differences in muscle fibre distribution. Although the present experimental procedures cannot discriminate between these physiological mediators, further consideration of how differences in muscle fibre recruitment might impact upon the principal factors proposed to regulate  $m\dot{V}O_2$  kinetics during exercise might shed some light on this issue. For example, an increased cellular [Cr] at rest and/or reduced oxidative enzyme activity reported in type II compared to type I muscle fibres (Bottinelli & Reggiani, 2000) would be expected to the slow the kinetics of

muscle PCr breakdown [and by extension the rise in  $m\dot{V}O_2$  (Mahler, 1985)] at the onset of exercise in accord with current models of respiratory control (Glancy, et al., 2008; Meyer, 1988; Paganini, et al., 1997). It is therefore conceivable, given an elevated type II muscle fibre expression with increasing age (Jansson, 1996), that work-to-work exercise transitions amplified an increased intracellular oxidative inertia residing within muscle fibres positioned higher in the recruitment hierarchy in older compared to younger counterparts. The present findings therefore support the contention that age differences in oxidative metabolism are dependent upon an interaction between exercise intensity and muscle fibre recruitment (Armstrong & Barker, 2009; Barker & Armstrong, 2010; Barker, Welsman, et al., 2010).

Whether repeated exposure of the type II muscle fibre population to exercise training during adolescence attenuates a slowing of  $\dot{V}O_2$  kinetics in response to work-to-work exercise is unclear. For example, previous studies have demonstrated that faster  $\dot{V}O_2$  kinetics in response to repeated sprint training [likely to recruit type II fibres at higher muscle shortening velocities (Sargeant, 1999)] is accompanied by an increased fractional muscle  $O_2$  extraction following the onset of intense exercise in adults (Bailey, Wilkerson, Dimenna, & Jones, 2009; Krstrup, Hellsten, et al., 2004). It might therefore be hypothesised that similar training-induced adaptations linked specifically to enhancing  $O_2$  utilisation within type II muscle fibres would return phase II  $\dot{V}O_2$  kinetics in teenagers toward values obtained in younger children. This might further explain, at least in part, an ‘insensitivity’ of the phase II  $\tau$  to manipulations in pedal rate (and by inference muscle fibre recruitment) manifest in trained youth cyclists (chapter 6). The higher pedal cadences adopted during training and competition in these subjects ( $\sim 85\text{-}95 \text{ rev}\cdot\text{min}^{-1}$ ) could have



ameliorated a reduction in muscle fibre oxidative capacity with increasing age. However, given the cross-sectional study design, the influence of exercise training influences *per se*



**Figure 9.2** Hypothetical model illustrating the influence of exercise training on  $\dot{V}O_2$  kinetics within specific segments of the muscle fibre pool during transitions above the GET. Note that training-induced enhancements in oxidative function within type II fibres would be expected to elicit a net speeding of pulmonary  $\dot{V}O_2$  at the onset of exercise in (b) compared to (a).

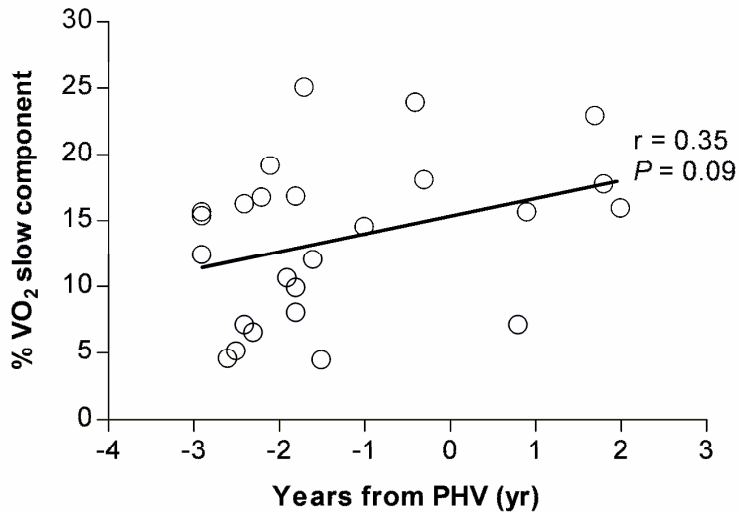
on  $\dot{V}O_2$  kinetics could not be revealed. This reinforces the potential for within-subject training studies in conjunction with work-to-work exercise transitions in order to explore the influence of muscle fibre-type specific adaptations on  $\dot{V}O_2$  kinetics in youth.

### 9.2.2 $\dot{V}O_2$ slow component: Mechanistic basis and future applications

In chapter 7, younger B demonstrated an increased  $O_2$  cost of exercise over the initial exponential phase (i.e. greater primary  $\dot{V}O_2$  gain) with a concomitant reduction in the proportional contribution from the  $\dot{V}O_2$  slow component to the end-exercise  $\dot{V}O_2$  compared to T and M. Similar findings have been linked to differences in muscle fibre recruitment and/or improved matching of  $O_2$  delivery to the rate of muscle  $O_2$  utilisation ( $\dot{Q}O_2/\dot{V}O_2$ ) at the onset of exercise in younger compared to older counterparts (Fawcner &

Armstrong, 2004a). For example, experimental perturbations that presumably restrict muscle  $O_2$  delivery and/or increase type II fibre recruitment during exercise tend to reduce the primary  $\dot{V}O_2$  gain and increase the  $\dot{V}O_2$  slow component amplitude (Engelen, et al., 1996; Koga, et al., 1999; Pringle, Doust, Carter, Tolfrey, & Jones, 2003). It might therefore be hypothesised that an elevated type II muscle fibre expression (Jansson, 1996) and/or reduced blood flow per unit of quadriceps muscle mass (Koch, 1984) with increasing age might have contributed to an increase in the  $\dot{V}O_2$  slow component amplitude from early childhood into adolescence. However, this impacted neither on the primary [HHb] kinetics ( $TD + \tau$ ) at the onset of U→VH exercise or on alterations in motor unit recruitment over time in T compared to B (i.e. surface iEMG activity did not significantly increase from *minute 2* to *minute 6* of VH exercise in either group). The extent to which restricting muscle  $O_2$  availability impacts on the initial  $\dot{Q}O_2/\dot{V}O_2$  response and therefore mandates delayed motor unit activation in order to sustain CWR exercise is unclear in youth and therefore warrants further investigation.

Chapter 8 presented evidence that challenged the notion of an age-dependent difference in the  $\dot{V}O_2$  slow component response during high-intensity exercise. Importantly, this investigation also revealed for the first time a temporal link between increases in muscle recruitment using MRI interrogation of the quadriceps and an excess  $\dot{V}O_2$  during intense CWR exercise in children. However, it is conceivable that large inter-subject variability in the relative  $\dot{V}O_2$  slow component amplitude coupled with a small sample size in boys may have precluded statistical significance. Figure 9.3 therefore presents data pooled across chapters 7 and 8 ( $n = 25$ ) with values for the relative  $\dot{V}O_2$  slow component amplitude



**Figure 9.3** Relationship between the relative  $\dot{V}O_2$  slow component amplitude and years from peak height velocity (PHV) in twenty-five youth subjects during very-heavy intensity cycling exercise.

plotted against youth participants' level of somatic maturity. This analysis revealed no significant relationship between physical maturation and the  $\dot{V}O_2$  slow component during very heavy-intensity exercise in 11-16 yr old boys. This raises an important theoretical question as to what physiological mechanisms might contribute to the development of the  $\dot{V}O_2$  slow component in young people that, in some children aged 11 yr, accounted for up to ~ 25 % of the total  $\dot{V}O_2$  response.

The demonstration in chapter 8 that muscle  $T_2$  changes attended the development of the  $\dot{V}O_2$  slow component in boys might be interpreted to suggest that progressive recruitment of 'less efficient' type II muscle fibres contributed to increasing the O<sub>2</sub> cost of the external power output as exercise proceeded. However, whilst simultaneous measurement of pulmonary and limb  $\dot{V}O_2$  have confirmed that the majority of the  $\dot{V}O_2$  slow component originates from contracting skeletal muscle in adults (Krustrup, et al., 2009; Poole, et al.,

1991), resolution of this issue awaits further investigative scrutiny in children before other potential mediators can be discounted. For example, fatigue of the respiratory muscles during high-intensity exercise increases the  $\dot{V}_{O_2}$  requirement at a given  $\dot{V}_E$  and has therefore been suggested to provide a minor contribution to the  $\dot{V}_{O_2}$  slow component amplitude (Bailey, et al., 2010; Carra, et al., 2003; Cross, Sabapathy, Schneider, & Haseler, 2010). Therefore, the possibility that metabolic processes external to the recruited muscle may have accounted for a small fraction of the  $\dot{V}_{O_2}$  slow component evinced in young people cannot be discounted from the present thesis. From this perspective, it is pertinent to note that a recent investigation reported a pronounced fall in quadriceps [PCr] below the primary phase asymptote established using conventional curve fitting procedures in youth subjects (mean age =  $13 \pm 1$  y) during heavy knee-extensor exercise (Willcocks, et al., 2010). Collectively, these findings could be interpreted to suggest that alterations in muscle recruitment linked to an increased ATP cost of force production might explain, at least in part, the development of the  $\dot{V}_{O_2}$  slow component in children during constant work rate exercise.

### **9.3 Future study recommendations**

The Fick principle dictates that  $m\dot{V}_{O_2}$  kinetics is influenced by the rate of  $O_2$  delivery to mitochondria and peripheral factors linked to muscle  $O_2$  utilization at the onset of step exercise. Therefore, alterations in muscle fibre recruitment (and associated metabolic properties) would be expected to contribute to the regulation of  $\dot{V}_{O_2}$  kinetics under conditions in which the rate of  $O_2$  supply exceeds or is equal to metabolic demand. In the present thesis, step exercise transitions were performed in the upright body position where  $O_2$  availability is reported not to be rate limiting in young adults (Burnley, et al., 2000;

Wilkerson, et al., 2005) and more recently in 9-13 yr old boys (Barker, Jones, & Armstrong, 2010). It was therefore suggested that a markedly lengthened phase II  $\tau$  during work-to-work transitions and pedaling at fast cadences in boys and teenagers might have been principally linked to intrinsic metabolic factors (e.g. an elevated recruitment of muscle fibres with lower oxidative capacity). However, confirmation of this proposal would require that phase II  $\dot{V}O_2$  kinetics are shown to be unaltered despite enhancing muscle  $O_2$  supply in the experimental condition in order for  $O_2$  delivery to be refuted as a candidate mechanism. For example, if the driving pressure for capillary-to-myocyte  $O_2$  diffusion were restricted in higher-order type II fibres [as has been indicated in animal models (Behnke, et al., 2003; McDonough, et al., 2005)] then providing supplemental  $O_2$  might be expected to speed phase II  $\dot{V}O_2$  kinetics during work-to-work exercise in young people. From this perspective, it is pertinent to note that DiMenna and colleagues (2008) have reported an unchanged phase II  $\tau$  during work-to-work transitions following prior exercise therefore consistent with the notion that muscle fibers recruited under these circumstances are principally restricted by a limited capacity to utilise  $O_2$  rather than  $O_2$  delivery *per se*. However, the same authors demonstrated a priming-induced speeding of phase II  $\dot{V}O_2$  kinetics during work-to-work exercise in the supine body position in which muscle  $O_2$  perfusion was likely compromised (DiMenna, Wilkerson, et al., 2010). There is therefore future scope to combine interventions purported to influence muscle  $O_2$  delivery with work-to-work and extreme pedal cadence models in order to explore the influence of differences in muscle fibre recruitment on the ‘tipping point’ hypothesis (see figure 2.9) in young people.

In order to limit the potential confounding influence of mixed sex groups, the data presented within the experimental chapters were obtained exclusively in male subjects. This was especially important given that Fawkner & Armstrong (2004c) have demonstrated significant sex differences in the phase II  $\tau$  and relative  $\dot{V}O_2$  slow component amplitude between 10-11 yr old boys and girls exercising in the heavy-intensity domain. The mechanistic basis for sex differences in  $\dot{V}O_2$  kinetics is unclear, although regional alterations in matching of  $O_2$  delivery to the rate of  $O_2$  utilization and/or muscle fibre recruitment patterns have been implicated (Fawkner & Armstrong, 2008b). Since phase II  $\dot{V}O_2$  kinetics are independent of sex during moderate-intensity (80% GET) cycling, it is conceivable that differences between boys and girls manifest during cycling exercise at  $\Delta 40\%$  might be linked to the recruitment of type II muscle fibres as work rate is increased. The demonstration that the phase II  $\tau$  is negatively correlated to the percentage of type I muscle fibres during step exercise above but not below the GET in adults is consistent with this proposal (Pringle, Doust, Carter, Tolfrey, Campbell, et al., 2003). It would therefore be of interest to compare the influence of manipulated pedal cadence and work-to-work transitions on  $\dot{V}O_2$  kinetics between boys and girls of similar maturational status. Likewise, if delayed onset muscle fibre activation were to contribute to the development of the  $\dot{V}O_2$  slow component in youth, then an extended  $O_2$  cost of exercise above the primary amplitude in girls compared to boys might be hypothesized to coincide with a greater increase in markers of muscle activity (e.g. iEMG and  $T_2$ ) in the former sex group. The experimental procedures and model interventions developed from the present thesis might therefore be applied to further investigate the physiological factors linked to sex-dependent differences in  $\dot{V}O_2$  kinetics during heavy/very heavy exercise.

## References

- Adams, G. R., Duvoisin, M. R., & Dudley, G. A. (1992). Magnetic resonance imaging and electromyography as indexes of muscle function. *J Appl Physiol*, 73(4), 1578-1583.
- Alkner, B. A., Tesch, P. A., & Berg, H. E. (2000). Quadriceps EMG/force relationship in knee extension and leg press. *Med Sci Sports Exerc*, 32(2), 459-463.
- Armon, Y., Cooper, D. M., Flores, R., Zanconato, S., & Barstow, T. J. (1991). Oxygen uptake dynamics during high-intensity exercise in children and adults. *J Appl Physiol*, 70(2), 841-848.
- Armstrong, N., & Barker, A. R. (2009). Oxygen uptake kinetics in children and adolescents: a review. *Pediatr Exerc Sci*, 21(2), 130-147.
- Armstrong, N., & Welsman, J. R. (2001). Peak oxygen uptake in relation to growth and maturation in 11- to 17-year-old humans. *Eur J Appl Physiol*, 85(6), 546-551.
- Bailey, S. J., Romer, L. M., Kelly, J., Wilkerson, D. P., DiMenna, F. J., & Jones, A. M. (2010). Inspiratory muscle training enhances pulmonary O<sub>2</sub> uptake kinetics and high-intensity exercise tolerance in humans. *J Appl Physiol*, 109(2), 457-468.
- Bailey, S. J., Vanhatalo, A., Wilkerson, D. P., Dimenna, F. J., & Jones, A. M. (2009). Optimizing the "priming" effect: influence of prior exercise intensity and recovery duration on O<sub>2</sub> uptake kinetics and severe-intensity exercise tolerance. *J Appl Physiol*, 107(6), 1743-1756.
- Bailey, S. J., Wilkerson, D. P., Dimenna, F. J., & Jones, A. M. (2009). Influence of repeated sprint training on pulmonary O<sub>2</sub> uptake and muscle deoxygenation kinetics in humans. *J Appl Physiol*, 106(6), 1875-1887.

- Barker, A. R., & Armstrong, N. (2010). Insights into developmental muscle metabolism through the use of  $^{31}\text{P}$ -magnetic resonance spectroscopy: a review. *Pediatr Exerc Sci*, 22(3), 350-368.
- Barker, A. R., Bond, B., Toman, C., Williams, C. A., & Armstrong, N. Critical power in adolescents: physiological bases and assessment using all-out exercise. *Eur J Appl Physiol*, in press.
- Barker, A. R., Jones, A. M., & Armstrong, N. (2010). The influence of priming exercise on oxygen uptake, cardiac output, and muscle oxygenation kinetics during very heavy-intensity exercise in 9- to 13-yr-old boys. *J Appl Physiol*, 109(2), 491-500.
- Barker, A. R., Welsman, J. R., Fulford, J., Welford, D., & Armstrong, N. (2008). Muscle phosphocreatine kinetics in children and adults at the onset and offset of moderate-intensity exercise. *J Appl Physiol*, 105(2), 446-456.
- Barker, A. R., Welsman, J. R., Fulford, J., Welford, D., & Armstrong, N. (2010). Quadriceps muscle energetics during incremental exercise in children and adults. *Med Sci Sports Exerc*, 42(7), 1303-1313.
- Barker, A. R., Welsman, J. R., Fulford, J., Welford, D., Williams, C. A., & Armstrong, N. (2008). Muscle phosphocreatine and pulmonary oxygen uptake kinetics in children at the onset and offset of moderate intensity exercise. *Eur J Appl Physiol*, 102(6), 727-738.
- Barker, A. R., Williams, C. A., Jones, A. M., & Armstrong, N. (2009). Establishing maximal oxygen uptake in young people during a ramp cycle test to exhaustion. *Br J Sports Med*, 45(6), 498-503.
- Barstow, T. J. (1994). Characterization of  $\dot{V}\text{O}_2$  kinetics during heavy exercise. *Med Sci Sports Exerc*, 26(11), 1327-1334.



- Barstow, T. J., Jones, A. M., Nguyen, P. H., & Casaburi, R. (1996). Influence of muscle fiber type and pedal frequency on oxygen uptake kinetics of heavy exercise. *J Appl Physiol*, *81*(4), 1642-1650.
- Barstow, T. J., & Mole, P. A. (1991). Linear and nonlinear characteristics of oxygen uptake kinetics during heavy exercise. *J Appl Physiol*, *71*(6), 2099-2106.
- Beaver, W. L., Wasserman, K., & Whipp, B. J. (1973). On-line computer analysis and breath-by-breath graphical display of exercise function tests. *J Appl Physiol*, *34*(1), 128-132.
- Beaver, W. L., Wasserman, K., & Whipp, B. J. (1986). A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol*, *60*(6), 2020-2027.
- Beelen, A., Sargeant, A. J., Lind, A., de Han, A., Kernell, D., & van Mechelen, W. (1993). Effect of contraction velocity on the pattern of glycogen depletion in human muscle fibre types. In A. J. Sargeant & D. Kernell (Eds.), *Neuromuscular Fatigue* (pp. 93-95): North Holland, Amsterdam.
- Behnke, B. J., Barstow, T. J., Kindig, C. A., McDonough, P., Musch, T. I., & Poole, D. C. (2002). Dynamics of oxygen uptake following exercise onset in rat skeletal muscle. *Respir Physiol Neurobiol*, *133*(3), 229-239.
- Behnke, B. J., McDonough, P., Padilla, D. J., Musch, T. I., & Poole, D. C. (2003). Oxygen exchange profile in rat muscles of contrasting fibre types. *J Physiol*, *549*(Pt 2), 597-605.
- Bell, R. D., MacDougall, J. D., Billeter, R., & Howald, H. (1980). Muscle fiber types and morphometric analysis of skeletal muscle in six-year-old children. *Med Sci Sports Exerc*, *12*(1), 28-31.
- Berg, A., Kim, S. S., & Keul, J. (1986). Skeletal muscle enzyme activities in healthy young subjects. *Int J Sports Med*, *7*(4), 236-239.

- Bessman, S. P., & Geiger, P. J. (1981). Transport of energy in muscle: the phosphorylcreatine shuttle. *Science*, *211*(4481), 448-452.
- Bigland-Ritchie, B., Donovan, E. F., & Roussos, C. S. (1981). Conduction velocity and EMG power spectrum changes in fatigue of sustained maximal efforts. *J Appl Physiol*, *51*(5), 1300-1305.
- Blimkie, C. J. (1989). Age-, and sex-associated variation in strength during childhood: anthropometric, morphologic, neurologic, biomechanic, endocrinologic, genetic and physical activity correlates. In C. V. Gisolfi & D. R. Lamb (Eds.), *Perspectives in Exercise Science and Sport Medicine: Youth, Exercise and Sport* (Vol. 2, pp. 99-163). Indianapolis, IN: Benchmark Press.
- Borrani, F., Candau, R., Millet, G. Y., Perrey, S., Fuchslocher, J., & Rouillon, J. D. (2001). Is the  $\dot{V}O_2$  slow component dependent on progressive recruitment of fast-twitch fibers in trained runners? *J Appl Physiol*, *90*(6), 2212-2220.
- Bottinelli, R., & Reggiani, C. (2000). Human skeletal muscle fibres: molecular and functional diversity. *Prog Biophys Mol Biol*, *73*(2-4), 195-262.
- Bouckaert, J., Jones, A. M., & Koppo, K. (2004). Effect of glycogen depletion on the oxygen uptake slow component in humans. *Int J Sports Med*, *25*(5), 351-356.
- Breese, B. C., Williams, C. A., Barker, A. R., Welsman, J. R., Fawcner, S. G., & Armstrong, N. (2010). Longitudinal changes in the oxygen uptake kinetic response to heavy-intensity exercise in 14- to 16-year-old boys. *Pediatr Exerc Sci*, *22*(1), 69-80.
- Brittain, C. J., Rossiter, H. B., Kowalchuk, J. M., & Whipp, B. J. (2001). Effect of prior metabolic rate on the kinetics of oxygen uptake during moderate-intensity exercise. *Eur J Appl Physiol*, *86*(2), 125-134.

- Brody, L. R., Pollock, M. T., Roy, S. H., De Luca, C. J., & Celli, B. (1991). pH-induced effects on median frequency and conduction velocity of the myoelectric signal. *J Appl Physiol*, *71*(5), 1878-1885.
- Brown, G. C. (2000). Nitric oxide as a competitive inhibitor of oxygen consumption in the mitochondrial respiratory chain. *Acta Physiol Scand*, *168*(4), 667-674.
- Burnley, M., Doust, J. H., Ball, D., & Jones, A. M. (2002). Effects of prior heavy exercise on  $\dot{V}O_2$  kinetics during heavy exercise are related to changes in muscle activity. *J Appl Physiol*, *93*(1), 167-174.
- Burnley, M., Doust, J. H., & Jones, A. M. (2006). Time required for the restoration of normal heavy exercise  $\dot{V}O_2$  kinetics following prior heavy exercise. *J Appl Physiol*, *101*(5), 1320-1327.
- Burnley, M., Jones, A. M., Carter, H., & Doust, J. H. (2000). Effects of prior heavy exercise on phase II pulmonary oxygen uptake kinetics during heavy exercise. *J Appl Physiol*, *89*(4), 1387-1396.
- Carra, J., Candau, R., Keslacy, S., Giolbas, F., Borrani, F., Millet, G. P., et al. (2003). Addition of inspiratory resistance increases the amplitude of the slow component of  $O_2$  uptake kinetics. *J Appl Physiol*, *94*(6), 2448-2455.
- Carter, H., Jones, A. M., Barstow, T. J., Burnley, M., Williams, C. A., & Doust, J. H. (2000). Oxygen uptake kinetics in treadmill running and cycle ergometry: a comparison. *J Appl Physiol*, *89*(3), 899-907.
- Carter, H., Pringle, J. S., Boobis, L., Jones, A. M., & Doust, J. H. (2004). Muscle glycogen depletion alters oxygen uptake kinetics during heavy exercise. *Med Sci Sports Exerc*, *36*(6), 965-972.
- Casaburi, R., Barstow, T. J., Robinson, T., & Wasserman, K. (1989). Influence of work rate on ventilatory and gas exchange kinetics. *J Appl Physiol*, *67*(2), 547-555.

- Cheng, H. A., Robergs, R. A., Letellier, J. P., Caprihan, A., Icenogle, M. V., & Haseler, L. J. (1995). Changes in muscle proton transverse relaxation times and acidosis during exercise and recovery. *J Appl Physiol*, 79(4), 1370-1378.
- Cleuziou, C., Lecoq, A. M., Candau, R., Courteix, D., Guenon, P., & Obert, P. (2002). Kinetics of oxygen uptake at the onset of moderate and heavy exercise in trained and untrained prepubertal children. [Journal article]. *Science and Sports* 17, 291-296.
- Colling-Saltin, A.-S. (1980). Skeletal muscle development in the human fetus and during childhood. In K. Berg & B. O. Eriksson (Eds.), *Children and Exercise IX* (pp. 193-207). Baltimore: University Park Press.
- Cooper, D. M., Berry, C., Lamarra, N., & Wasserman, K. (1985). Kinetics of oxygen uptake and heart rate at onset of exercise in children. *J Appl Physiol*, 59(1), 211-217.
- Cooper, D. M., Weiler-Ravell, D., Whipp, B. J., & Wasserman, K. (1984). Aerobic parameters of exercise as a function of body size during growth in children. *J Appl Physiol*, 56(3), 628-634.
- Costill, D. L., Daniels, J., Evans, W., Fink, W., Krahenbuhl, G., & Saltin, B. (1976). Skeletal muscle enzymes and fiber composition in male and female track athletes. *J Appl Physiol*, 40(2), 149-154.
- Coyle, E. F., Sidossis, L. S., Horowitz, J. F., & Beltz, J. D. (1992). Cycling efficiency is related to the percentage of type I muscle fibers. *Med Sci Sports Exerc*, 24(7), 782-788.
- Cross, T. J., Sabapathy, S., Schneider, D. A., & Haseler, L. J. (2010). Breathing He-O<sub>2</sub> attenuates the slow component of O<sub>2</sub> uptake kinetics during exercise performed above the respiratory compensation threshold. *Exp Physiol*, 95(1), 172-183.

- Crow, M. T., & Kushmerick, M. J. (1982). Chemical energetics of slow- and fast-twitch muscles of the mouse. *J Gen Physiol*, 79(1), 147-166.
- Dahlstrom, M., Esbjornsson Liljedahl, M., Gierup, J., Kaijser, L., & Jansson, E. (1997). High proportion of type I fibres in thigh muscle of young dancers. *Acta Physiol Scand*, 160(1), 49-55.
- De Luca, C. J. (1997). The use of surface electromyography in biomechanics. *Journal of Applied Biomechanics*, 13, 135-163.
- DeLorey, D. S., Kowalchuk, J. M., & Paterson, D. H. (2003). Relationship between pulmonary O<sub>2</sub> uptake kinetics and muscle deoxygenation during moderate-intensity exercise. *J Appl Physiol*, 95(1), 113-120.
- DeLorey, D. S., Kowalchuk, J. M., & Paterson, D. H. (2004). Effects of prior heavy-intensity exercise on pulmonary O<sub>2</sub> uptake and muscle deoxygenation kinetics in young and older adult humans. *J Appl Physiol*, 97(3), 998-1005.
- DiMenna, F. J., Bailey, S. J., Vanhatalo, A., Chidnok, W., & Jones, A. M. (2010). Elevated baseline  $\dot{V}O_2$  per se does not slow O<sub>2</sub> uptake kinetics during work-to-work exercise transitions. *J Appl Physiol*, 109(4), 1148-1154.
- Dimenna, F. J., Fulford, J., Bailey, S. J., Vanhatalo, A., Wilkerson, D. P., & Jones, A. M. (2010). Influence of priming exercise on muscle [PCr] and pulmonary O<sub>2</sub> uptake dynamics during 'work-to-work' knee-extension exercise. *Respir Physiol Neurobiol*, 172(1-2), 15-23.
- Dimenna, F. J., Wilkerson, D. P., Burnley, M., Bailey, S. J., & Jones, A. M. (2009a). Influence of extreme pedal rates on pulmonary O<sub>2</sub> uptake kinetics during transitions to high-intensity exercise from an elevated baseline. *Respir Physiol Neurobiol*, 169(1), 16-23.

- DiMenna, F. J., Wilkerson, D. P., Burnley, M., Bailey, S. J., & Jones, A. M. (2009b). Influence of priming exercise on pulmonary O<sub>2</sub> uptake kinetics during transitions to high-intensity exercise at extreme pedal rates. *J Appl Physiol*, *106*(2), 432-442.
- DiMenna, F. J., Wilkerson, D. P., Burnley, M., Bailey, S. J., & Jones, A. M. (2010). Priming exercise speeds pulmonary O<sub>2</sub> uptake kinetics during supine "work-to-work" high-intensity cycle exercise. *J Appl Physiol*, *108*(2), 283-292.
- DiMenna, F. J., Wilkerson, D. P., Burnley, M., & Jones, A. M. (2008). Influence of priming exercise on pulmonary O<sub>2</sub> uptake kinetics during transitions to high-intensity exercise from an elevated baseline. *J Appl Physiol*, *105*(2), 538-546.
- Endo, M. Y., Kobayakawa, M., Kinugasa, R., Kuno, S., Akima, H., Rossiter, H. B., et al. (2007). Thigh muscle activation distribution and pulmonary  $\dot{V}O_2$  kinetics during moderate, heavy, and very heavy intensity cycling exercise in humans. *Am J Physiol Regul Integr Comp Physiol*, *293*(2), R812-820.
- Engelen, M., Porszasz, J., Riley, M., Wasserman, K., Maehara, K., & Barstow, T. J. (1996). Effects of hypoxic hypoxia on O<sub>2</sub> uptake and heart rate kinetics during heavy exercise. *J Appl Physiol*, *81*(6), 2500-2508.
- Eriksson, B. O. (1980). Muscle metabolism in children--a review. *Acta Paediatr Scand Suppl*, *283*, 20-28.
- Eriksson, B. O., Gollnick, P. D., & Saltin, B. (1973). Muscle metabolism and enzyme activities after training in boys 11-13 years old. *Acta Physiol Scand*, *87*(4), 485-497.
- Eriksson, B. O., & Saltin, B. (1974). Muscle metabolism during exercise in boys aged 11 to 16 years compared to adults *Acta Paediatr Belg*, *28*, 257-265.
- Falk, B., & Dotan, R. (2006). Child-adult differences in the recovery from high-intensity exercise. *Exerc Sport Sci Rev*, *34*(3), 107-112.

- Falk, B., Usselman, C., Dotan, R., Brunton, L., Klentrou, P., Shaw, J., et al. (2009). Child-adult differences in muscle strength and activation pattern during isometric elbow flexion and extension. *Appl Physiol Nutr Metab*, 34(4), 609-615.
- Fawkner, S. G., & Armstrong, N. (2002). Assessment of critical power with children. *Pediatric Exercise Science*, 14, 259-268.
- Fawkner, S. G., & Armstrong, N. (2003). The Slow-component Response of  $\dot{V}O_2$  to Heavy Intensity Exercise in Children In T. Reilly & M. Marfell-Jones (Eds.), *Kinanthropometry VIII* (pp. 105-113). London: Routledge.
- Fawkner, S. G., & Armstrong, N. (2004a). Longitudinal changes in the kinetic response to heavy-intensity exercise in children. *J Appl Physiol*, 97(2), 460-466.
- Fawkner, S. G., & Armstrong, N. (2004b). Modelling the  $\dot{V}O_2$  kinetic response to heavy intensity exercise in children. *Ergonomics*, 47(14), 1517-1527.
- Fawkner, S. G., & Armstrong, N. (2004c). Sex differences in the oxygen uptake kinetic response to heavy-intensity exercise in prepubertal children. *Eur J Appl Physiol*, 93(1-2), 210-216.
- Fawkner, S. G., & Armstrong, N. (2008). Oxygen uptake kinetics. In N. Armstrong & W. Van Mechelen (Eds.), *Paediatric Exercise Science and Medicine* (pp. 297-307): Oxford University Press.
- Fawkner, S. G., Armstrong, N., Childs, D. J., & Welsman, J. (2002). Reliability of the visually identified ventilatory threshold and V-slope in children. *Pediatric Exercise Science*, 14, 181-193.
- Fawkner, S. G., Armstrong, N., Potter, C. R., & Welsman, J. R. (2002). Oxygen uptake kinetics in children and adults after the onset of moderate-intensity exercise. *J Sports Sci*, 20(4), 319-326.

- Ferguson, R. A., Ball, D., Krstrup, P., Aagaard, P., Kjaer, M., Sargeant, A. J., et al. (2001). Muscle oxygen uptake and energy turnover during dynamic exercise at different contraction frequencies in humans. *J Physiol*, 536(Pt 1), 261-271.
- Ferreira, L. F., Lutjemeier, B. J., Townsend, D. K., & Barstow, T. J. (2006). Effects of pedal frequency on estimated muscle microvascular O<sub>2</sub> extraction. *Eur J Appl Physiol*, 96(5), 558-563.
- Fisher, M. J., Meyer, R. A., Adams, G. R., Foley, J. M., & Potchen, E. J. (1990). Direct relationship between proton T<sub>2</sub> and exercise intensity in skeletal muscle MR images. *Invest Radiol*, 25(5), 480-485.
- Fournier, M., Ricci, J., Taylor, A. W., Ferguson, R. J., Montpetit, R. R., & Chaitman, B. R. (1982). Skeletal muscle adaptation in adolescent boys: sprint and endurance training and detraining. *Med Sci Sports Exerc*, 14(6), 453-456.
- Gaesser, G. A., & Poole, D. C. (1996). The slow component of oxygen uptake kinetics in humans. *Exerc Sport Sci Rev*, 24, 35-71.
- Gerbino, A., Ward, S. A., & Whipp, B. J. (1996). Effects of prior exercise on pulmonary gas-exchange kinetics during high-intensity exercise in humans. *J Appl Physiol*, 80(1), 99-107.
- Glancy, B., Barstow, T., & Willis, W. T. (2008). Linear relation between time constant of oxygen uptake kinetics, total creatine, and mitochondrial content in vitro. *Am J Physiol Cell Physiol*, 294(1), C79-87.
- Glenmark, B., Hedberg, G., & Jansson, E. (1992). Changes in muscle fibre type from adolescence to adulthood in women and men. *Acta Physiol Scand*, 146(2), 251-259.
- Gollnick, P. D., Piehl, K., & Saltin, B. (1974). Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. *J Physiol*, 241(1), 45-57.



- Grassi, B. (2005). Limitations of skeletal muscle  $\dot{V}O_2$  kinetics by inertia of cellular respiration. In A. M. Jones & D. C. Poole (Eds.), *Oxygen Uptake Kinetics in Sport, Exercise, and Medicine* (pp. 212-229). London and New York: Routledge.
- Grassi, B., Gladden, L. B., Samaja, M., Sary, C. M., & Hogan, M. C. (1998). Faster adjustment of  $O_2$  delivery does not affect  $\dot{V}O_2$  on-kinetics in isolated in situ canine muscle. *J Appl Physiol*, 85(4), 1394-1403.
- Grassi, B., Gladden, L. B., Sary, C. M., Wagner, P. D., & Hogan, M. C. (1998). Peripheral  $O_2$  diffusion does not affect  $\dot{V}O_2$  on-kinetics in isolated insitu canine muscle. *J Appl Physiol*, 85(4), 1404-1412.
- Grassi, B., Hogan, M. C., Greenhaff, P. L., Hamann, J. J., Kelley, K. M., Aschenbach, W. G., et al. (2002). Oxygen uptake on-kinetics in dog gastrocnemius in situ following activation of pyruvate dehydrogenase by dichloroacetate. *J Physiol*, 538(Pt 1), 195-207.
- Grassi, B., Hogan, M. C., Kelley, K. M., Aschenbach, W. G., Hamann, J. J., Evans, R. K., et al. (2000). Role of convective  $O_2$  delivery in determining  $\dot{V}O_2$  on-kinetics in canine muscle contracting at peak  $\dot{V}O_2$ . *J Appl Physiol*, 89(4), 1293-1301.
- Grassi, B., Hogan, M. C., Kelley, K. M., Howlett, R. A., & Gladden, L. B. (2005). Effects of nitric oxide synthase inhibition by L-NAME on oxygen uptake kinetics in isolated canine muscle in situ. *J Physiol*, 568(Pt 3), 1021-1033.
- Grassi, B., Pogliaghi, S., Rampichini, S., Quaresima, V., Ferrari, M., Marconi, C., et al. (2003). Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on-transitions in humans. *J Appl Physiol*, 95(1), 149-158.
- Grassi, B., Poole, D. C., Richardson, R. S., Knight, D. R., Erickson, B. K., & Wagner, P. D. (1996). Muscle  $O_2$  uptake kinetics in humans: implications for metabolic control. *J Appl Physiol*, 80(3), 988-998.

- Gurd, B. J., Peters, S. J., Heigenhauser, G. J., LeBlanc, P. J., Doherty, T. J., Paterson, D. H., et al. (2009). Prior heavy exercise elevates pyruvate dehydrogenase activity and muscle oxygenation and speeds O<sub>2</sub> uptake kinetics during moderate exercise in older adults. *Am J Physiol Regul Integr Comp Physiol*, 297(3), R877-884.
- Halin, R., Germain, P., Bercier, S., Kapitaniak, B., & Buttelli, O. (2003). Neuromuscular response of young boys versus men during sustained maximal contraction. *Med Sci Sports Exerc*, 35(6), 1042-1048.
- Han, Y. S., Geiger, P. C., Cody, M. J., Macken, R. L., & Sieck, G. C. (2003). ATP consumption rate per cross bridge depends on myosin heavy chain isoform. *J Appl Physiol*, 94(6), 2188-2196.
- Haralambie, G. (1982). Enzyme activities in skeletal muscle of 13-15 years old adolescents. *Bull Eur Physiopathol Respir*, 18(1), 65-74.
- Haseler, L. J., Kindig, C. A., Richardson, R. S., & Hogan, M. C. (2004). The role of oxygen in determining phosphocreatine onset kinetics in exercising humans. *J Physiol*, 558(Pt 3), 985-992.
- Haseler, L. J., Richardson, R. S., Videen, J. S., & Hogan, M. C. (1998). Phosphocreatine hydrolysis during submaximal exercise: the effect of FiO<sub>2</sub>. *J Appl Physiol*, 85(4), 1457-1463.
- He, Z. H., Bottinelli, R., Pellegrino, M. A., Ferenczi, M. A., & Reggiani, C. (2000). ATP consumption and efficiency of human single muscle fibers with different myosin isoform composition. *Biophys J*, 79(2), 945-961.
- Hebestreit, H., Kriemler, S., Hughson, R. L., & Bar-Or, O. (1998). Kinetics of oxygen uptake at the onset of exercise in boys and men. *J Appl Physiol*, 85(5), 1833-1841.

- Henneman, E., & Mendell, L. M. (1981). Functional Organisation of a Motorneuron Pool and its Inputs. In V. B. Brooks (Ed.), *Handbook of Physiology I, Vol. II, Part 1* (pp. 423-507): American Physiological Society, Bethesda.
- Hepple, R. T., Howlett, R. A., Kindig, C. A., Stary, C. M., & Hogan, M. C. (2010). The O<sub>2</sub> cost of the tension-time integral in isolated single myocytes during fatigue. *Am J Physiol Regul Integr Comp Physiol*, 298(4), R983-988.
- Hoelting, B. D., Scheuermann, B. W., & Barstow, T. J. (2001). Effect of contraction frequency on leg blood flow during knee extension exercise in humans. *J Appl Physiol*, 91(2), 671-679.
- Holloszy, J. O., & Coyle, E. F. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol*, 56(4), 831-838.
- Howald, H., Hoppeler, H., Claassen, H., Mathieu, O., & Straub, R. (1985). Influences of endurance training on the ultrastructural composition of the different muscle fiber types in humans. *Pflugers Arch*, 403(4), 369-376.
- Hughson, R. L., & Morrissey, M. (1982). Delayed kinetics of respiratory gas exchange in the transition from prior exercise. *J Appl Physiol*, 52(4), 921-929.
- Hughson, R. L., Tschakovsky, M. E., & Houston, M. E. (2001). Regulation of oxygen consumption at the onset of exercise. *Exerc Sport Sci Rev*, 29(3), 129-133.
- Jansson, E. (1996). Age-related fiber type changes in human skeletal muscle. In R. Maughan & S. Shirreffs (Eds.), *Biochemistry of Exercise IX* (pp. 297-307). Champaign, IL: Human Kinetics.
- Jenner, G., Foley, J. M., Cooper, T. G., Potchen, E. J., & Meyer, R. A. (1994). Changes in magnetic resonance images of muscle depend on exercise intensity and duration, not work. *J Appl Physiol*, 76(5), 2119-2124.

- Jones, A. M., Berger, N. J., Wilkerson, D. P., & Roberts, C. L. (2006). Effects of "priming" exercise on pulmonary O<sub>2</sub> uptake and muscle deoxygenation kinetics during heavy-intensity cycle exercise in the supine and upright positions. *J Appl Physiol*, *101*(5), 1432-1441.
- Jones, A. M., Fulford, J., & Wilkerson, D. P. (2008). Influence of prior exercise on muscle [phosphorylcreatine] and deoxygenation kinetics during high-intensity exercise in men. *Exp Physiol*, *93*(4), 468-478.
- Jones, A. M., Koppo, K., & Burnley, M. (2003). Effects of prior exercise on metabolic and gas exchange responses to exercise. *Sports Med*, *33*(13), 949-971.
- Jones, A. M., Koppo, K., Wilkerson, D. P., Wilmschurst, S., & Campbell, I. T. (2004). Dichloroacetate does not speed phase II pulmonary  $\dot{V}O_2$  kinetics following the onset of heavy intensity cycle exercise. *Pflugers Arch*, *447*(6), 867-874.
- Jones, A. M., Pringle, J. S., & Carter, H. (2005). Influence of muscle fibre type and motor unit recruitment on  $\dot{V}O_2$  kinetics. In A. M. Jones & D. C. Poole (Eds.), *Oxygen Uptake Kinetics in Sport, Exercise, and Medicine* (pp. 261-293): Routledge, London and New York.
- Jones, A. M., Wilkerson, D. P., & Fulford, J. (2008). Muscle [phosphocreatine] dynamics following the onset of exercise in humans: the influence of baseline work-rate. *J Physiol*, *586*(3), 889-898.
- Jones, A. M., Wilkerson, D. P., Koppo, K., Wilmschurst, S., & Campbell, I. T. (2003). Inhibition of nitric oxide synthase by L-NAME speeds phase II pulmonary  $\dot{V}O_2$  kinetics in the transition to moderate-intensity exercise in man. *J Physiol*, *552*(Pt 1), 265-272.
- Jones, A. M., Wilkerson, D. P., Wilmschurst, S., & Campbell, I. T. (2004). Influence of L-NAME on pulmonary O<sub>2</sub> uptake kinetics during heavy-intensity cycle exercise. *J Appl Physiol*, *96*(3), 1033-1038.

- Kaczor, J. J., Ziolkowski, W., Popinigis, J., & Tarnopolsky, M. A. (2005). Anaerobic and aerobic enzyme activities in human skeletal muscle from children and adults. *Pediatr Res*, 57(3), 331-335.
- Karlsson, H., Lindborg, B., & Linnarsson, D. (1975). Time courses of pulmonary gas exchange and heart rate changes in supine exercise. *Acta Physiol Scand*, 95(3), 329-340.
- Kindig, C. A., Howlett, R. A., Stary, C. M., Walsh, B., & Hogan, M. C. (2005). Effects of acute creatine kinase inhibition on metabolism and tension development in isolated single myocytes. *J Appl Physiol*, 98(2), 541-549.
- Kindig, C. A., McDonough, P., Erickson, H. H., & Poole, D. C. (2001). Effect of L-NAME on oxygen uptake kinetics during heavy-intensity exercise in the horse. *J Appl Physiol*, 91(2), 891-896.
- Kobzik, L., Reid, M. B., Brecht, D. S., & Stamler, J. S. (1994). Nitric oxide in skeletal muscle. *Nature*, 372(6506), 546-548.
- Koch, G. (1984). Maximal Oxygen Transport Capacity in Adolescents Aged 12 to 17 Years. In N. Bachl, L. Prokop & R. Suckert (Eds.), *Current Topics in Sports Medicine* (pp. 479-497). Wien: Urban and Schwarzenberg.
- Koga, S., Poole, D. C., Shiojiri, T., Kondo, N., Fukuba, Y., Miura, A., et al. (2005). Comparison of oxygen uptake kinetics during knee extension and cycle exercise. *Am J Physiol Regul Integr Comp Physiol*, 288(1), R212-220.
- Koga, S., Shiojiri, T., Shibasaki, M., Kondo, N., Fukuba, Y., & Barstow, T. J. (1999). Kinetics of oxygen uptake during supine and upright heavy exercise. *J Appl Physiol*, 87(1), 253-260.

- Koppo, K., Bouckaert, J., & Jones, A. M. (2002). Oxygen uptake kinetics during high-intensity arm and leg exercise. *Respir Physiol Neurobiol*, 133(3), 241-250.
- Krustrup, P., Hellsten, Y., & Bangsbo, J. (2004). Intense interval training enhances human skeletal muscle oxygen uptake in the initial phase of dynamic exercise at high but not at low intensities. *J Physiol*, 559(Pt 1), 335-345.
- Krustrup, P., Jones, A. M., Wilkerson, D. P., Calbet, J. A., & Bangsbo, J. (2009). Muscular and pulmonary O<sub>2</sub> uptake kinetics during moderate- and high-intensity sub-maximal knee-extensor exercise in humans. *J Physiol*, 587(Pt 8), 1843-1856.
- Krustrup, P., Secher, N. H., Relu, M. U., Hellsten, Y., Soderlund, K., & Bangsbo, J. (2008). Neuromuscular blockade of slow twitch muscle fibres elevates muscle oxygen uptake and energy turnover during submaximal exercise in humans. *J Physiol*, 586(Pt 24), 6037-6048.
- Krustrup, P., Soderlund, K., Mohr, M., & Bangsbo, J. (2004a). Slow-twitch fiber glycogen depletion elevates moderate-exercise fast-twitch fiber activity and O<sub>2</sub> uptake. *Med Sci Sports Exerc*, 36(6), 973-982.
- Krustrup, P., Soderlund, K., Mohr, M., & Bangsbo, J. (2004b). The slow component of oxygen uptake during intense, sub-maximal exercise in man is associated with additional fibre recruitment. *Pflugers Arch*, 447(6), 855-866.
- Krustrup, P., Soderlund, K., Mohr, M., Gonzalez-Alonso, J., & Bangsbo, J. (2004c). Recruitment of fibre types and quadriceps muscle portions during repeated, intense knee-extensor exercise in humans. *Pflugers Arch*, 449(1), 56-65.
- Lai, N., Nasca, M. M., Silva, M. A., Silva, F. T., Whipp, B. J., & Cabrera, M. E. (2008). Influence of exercise intensity on pulmonary oxygen uptake kinetics at the onset of exercise and recovery in male adolescents. *Appl Physiol Nutr Metab*, 33(1), 107-117.

- Lamarra, N., Whipp, B. J., Ward, S. A., & Wasserman, K. (1987). Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. *J Appl Physiol*, 62(5), 2003-2012.
- Lexell, J., Sjoström, M., Nordlund, A. S., & Taylor, C. C. (1992). Growth and development of human muscle: a quantitative morphological study of whole vastus lateralis from childhood to adult age. *Muscle Nerve*, 15(3), 404-409.
- MacDonald, M., Pedersen, P. K., & Hughson, R. L. (1997). Acceleration of  $\dot{V}O_2$  kinetics in heavy submaximal exercise by hyperoxia and prior high-intensity exercise. *J Appl Physiol*, 83(4), 1318-1325.
- MacDonald, M. J., Shoemaker, J. K., Tschakovsky, M. E., & Hughson, R. L. (1998). Alveolar oxygen uptake and femoral artery blood flow dynamics in upright and supine leg exercise in humans. *J Appl Physiol*, 85(5), 1622-1628.
- MacIntosh, B. R., Neptune, R. R., & Horton, J. F. (2000). Cadence, power, and muscle activation in cycle ergometry. *Med Sci Sports Exerc*, 32(7), 1281-1287.
- MacPhee, S. L., Shoemaker, J. K., Paterson, D. H., & Kowalchuk, J. M. (2005). Kinetics of  $O_2$  uptake, leg blood flow, and muscle deoxygenation are slowed in the upper compared with lower region of the moderate-intensity exercise domain. *J Appl Physiol*, 99(5), 1822-1834.
- Mahler, M. (1985). First-order kinetics of muscle oxygen consumption, and an equivalent proportionality between  $QO_2$  and phosphorylcreatine level. Implications for the control of respiration. *J Gen Physiol*, 86(1), 135-165.
- Marsh, A. P., & Martin, P. E. (1993). The association between cycling experience and preferred and most economical cadences. *Med Sci Sports Exerc*, 25(11), 1269-1274.

- Marsh, A. P., & Martin, P. E. (1997). Effect of cycling experience, aerobic power, and power output on preferred and most economical cycling cadences. *Med Sci Sports Exerc*, 29(9), 1225-1232.
- Marwood, S., Roche, D., Rowland, T., Garrard, M., & Unnithan, V. B. (2010). Faster pulmonary oxygen uptake kinetics in trained versus untrained male adolescents. *Med Sci Sports Exerc*, 42(1), 127-134.
- McDonough, P., Behnke, B. J., Padilla, D. J., Musch, T. I., & Poole, D. C. (2005). Control of microvascular oxygen pressures in rat muscles comprised of different fibre types. *J Physiol*, 563(Pt 3), 903-913.
- McNarry, M. A., Welsman, J. R., & Jones, A. M. (2010). Influence of training status and exercise modality on pulmonary O<sub>2</sub> uptake kinetics in pubertal girls. *Eur J Appl Physiol*, 111(4), 621-631.
- Mero, A., Jaakkola, L., & Komi, P. V. (1991). Relationships between muscle fibre characteristics and physical performance capacity in trained athletic boys. *J Sports Sci*, 9(2), 161-171.
- Meyer, R. A. (1988). A linear model of muscle respiration explains monoexponential phosphocreatine changes. *Am J Physiol*, 254(4 Pt 1), C548-553.
- Meyer, R. A., & Prior, B. M. (2000). Functional magnetic resonance imaging of muscle. *Exerc Sport Sci Rev*, 28(2), 89-92.
- Mirwald, R. L., Baxter-Jones, A. D., Bailey, D. A., & Beunen, G. P. (2002). An assessment of maturity from anthropometric measurements. *Med Sci Sports Exerc*, 34(4), 689-694.
- Mizuno, M., Secher, N. H., & Quistorff, B. (1994). <sup>31</sup>P-NMR spectroscopy, rsEMG, and histochemical fiber types of human wrist flexor muscles. *J Appl Physiol*, 76(2), 531-538.



- Obert, P., Cleuziou, C., Candau, R., Courteix, D., Lecoq, A. M., & Guenon, P. (2000). The slow component of O<sub>2</sub> uptake kinetics during high-intensity exercise in trained and untrained prepubertal children. *Int J Sports Med*, 21(1), 31-36.
- Ozyener, F., Rossiter, H. B., Ward, S. A., & Whipp, B. J. (2001). Influence of exercise intensity on the on- and off-transient kinetics of pulmonary oxygen uptake in humans. *J Physiol*, 533(Pt 3), 891-902.
- Paasuke, M., Ereline, J., & Gapeyeva, H. (2000). Twitch contraction properties of plantar flexor muscles in pre- and post-pubertal boys and men. *Eur J Appl Physiol*, 82(5-6), 459-464.
- Paganini, A. T., Foley, J. M., & Meyer, R. A. (1997). Linear dependence of muscle phosphocreatine kinetics on oxidative capacity. *Am J Physiol*, 272(2 Pt 1), C501-510.
- Paterson, D. H., & Whipp, B. J. (1991). Asymmetries of oxygen uptake transients at the on- and offset of heavy exercise in humans. *J Physiol*, 443, 575-586.
- Perrey, S., Betik, A., Candau, R., Rouillon, J. D., & Hughson, R. L. (2001). Comparison of oxygen uptake kinetics during concentric and eccentric cycle exercise. *J Appl Physiol*, 91(5), 2135-2142.
- Perrey, S., Candau, R., Rouillon, J. D., & Hughson, R. L. (2003). The effect of prolonged submaximal exercise on gas exchange kinetics and ventilation during heavy exercise in humans. *Eur J Appl Physiol*, 89(6), 587-594.
- Poole, D. C., Barstow, T. J., Gaesser, G. A., Willis, W. T., & Whipp, B. J. (1994).  $\dot{V}O_2$  slow component: physiological and functional significance. *Med Sci Sports Exerc*, 26(11), 1354-1358.

- Poole, D. C., Barstow, T. J., McDonough, P., & Jones, A. M. (2008). Control of oxygen uptake during exercise. *Med Sci Sports Exerc*, *40*(3), 462-474.
- Poole, D. C., & Jones, A. M. (2005). Towards an understanding of the mechanistic bases of  $\dot{V}O_2$  kinetics. In A. M. Jones & D. C. Poole (Eds.), *Oxygen Uptake Kinetics in Sport, Exercise and Medicine*. (pp. 294-328). London and New York: Routledge
- Poole, D. C., Schaffartzik, W., Knight, D. R., Derion, T., Kennedy, B., Guy, H. J., et al. (1991). Contribution of exercising legs to the slow component of oxygen uptake kinetics in humans. *J Appl Physiol*, *71*(4), 1245-1260.
- Poole, D. C., Ward, S. A., Gardner, G. W., & Whipp, B. J. (1988). Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics*, *31*(9), 1265-1279.
- Potter, C. R., Childs, D. J., Houghton, W., & Armstrong, N. (1999). Breath-to-breath "noise" in the ventilatory and gas exchange responses of children to exercise. *Eur J Appl Physiol Occup Physiol*, *80*(2), 118-124.
- Pringle, J. S., Doust, J. H., Carter, H., Tolfrey, K., Campbell, I. T., Sakkas, G. K., et al. (2003). Oxygen uptake kinetics during moderate, heavy and severe intensity "submaximal" exercise in humans: the influence of muscle fibre type and capillarisation. *Eur J Appl Physiol*, *89*(3-4), 289-300.
- Pringle, J. S., Doust, J. H., Carter, H., Tolfrey, K., & Jones, A. M. (2003). Effect of pedal rate on primary and slow-component oxygen uptake responses during heavy-cycle exercise. *J Appl Physiol*, *94*(4), 1501-1507.
- Prior, B. M., Ploutz-Snyder, L. L., Cooper, T. G., & Meyer, R. A. (2001). Fiber type and metabolic dependence of  $T_2$  increases in stimulated rat muscles. *J Appl Physiol*, *90*(2), 615-623.

- Ratel, S., Duche, P., & Williams, C. A. (2006). Muscle fatigue during high-intensity exercise in children. *Sports Med*, 36(12), 1031-1065.
- Ray, C. A., & Dudley, G. A. (1998). Muscle use during dynamic knee extension: implication for perfusion and metabolism. *J Appl Physiol*, 85(3), 1194-1197.
- Reid, R. W., Foley, J. M., Jayaraman, R. C., Prior, B. M., & Meyer, R. A. (2001). Effect of aerobic capacity on the T<sub>2</sub> increase in exercised skeletal muscle. *J Appl Physiol*, 90(3), 897-902.
- Reybrouck, T., Weymans, M., Stijns, H., Knops, J., & van der Hauwaert, L. (1985). Ventilatory anaerobic threshold in healthy children. Age and sex differences. *Eur J Appl Physiol Occup Physiol*, 54(3), 278-284.
- Richardson, R. S., Duteil, S., Wary, C., Wray, D. W., Hoff, J., & Carlier, P. G. (2006). Human skeletal muscle intracellular oxygenation: the impact of ambient oxygen availability. *J Physiol*, 571(Pt 2), 415-424.
- Richardson, R. S., Frank, L. R., & Haseler, L. J. (1998). Dynamic knee-extensor and cycle exercise: functional MRI of muscular activity. *Int J Sports Med*, 19(3), 182-187.
- Richardson, R. S., Noyszewski, E. A., Kendrick, K. F., Leigh, J. S., & Wagner, P. D. (1995). Myoglobin O<sub>2</sub> desaturation during exercise. Evidence of limited O<sub>2</sub> transport. *J Clin Invest*, 96(4), 1916-1926.
- Richardson, R. S., Tagore, K., Haseler, L. J., Jordan, M., & Wagner, P. D. (1998). Increased  $\dot{V}O_2$  max with right-shifted Hb-O<sub>2</sub> dissociation curve at a constant O<sub>2</sub> delivery in dog muscle in situ. *J Appl Physiol*, 84(3), 995-1002.
- Rossiter, H. B., Howe, F. A., & Ward, S. A. (2005). Intramuscular phosphate and pulmonary  $\dot{V}O_2$  kinetics during exercise. In A. M. Jones & D. C. Poole (Eds.), *Oxygen Uptake Kinetics in Sport, Exercise, and Medicine* (pp. 154-184). London and New York: Routledge

- Rossiter, H. B., Ward, S. A., Doyle, V. L., Howe, F. A., Griffiths, J. R., & Whipp, B. J. (1999). Inferences from pulmonary O<sub>2</sub> uptake with respect to intramuscular [phosphocreatine] kinetics during moderate exercise in humans. *J Physiol*, 518 ( Pt 3), 921-932.
- Rossiter, H. B., Ward, S. A., Howe, F. A., Kowalchuk, J. M., Griffiths, J. R., & Whipp, B. J. (2002). Dynamics of intramuscular <sup>31</sup>P-MRS P<sub>i</sub> peak splitting and the slow components of PCr and O<sub>2</sub> uptake during exercise. *J Appl Physiol*, 93(6), 2059-2069.
- Rossiter, H. B., Ward, S. A., Howe, F. A., Wood, D. M., Kowalchuk, J. M., Griffiths, J. R., et al. (2003). Effects of dichloroacetate on  $\dot{V}O_2$  and intramuscular <sup>31</sup>P metabolite kinetics during high-intensity exercise in humans. *J Appl Physiol*, 95(3), 1105-1115.
- Rossiter, H. B., Ward, S. A., Kowalchuk, J. M., Howe, F. A., Griffiths, J. R., & Whipp, B. J. (2001). Effects of prior exercise on oxygen uptake and phosphocreatine kinetics during high-intensity knee-extension exercise in humans. *J Physiol*, 537(Pt 1), 291-303.
- Rossiter, H. B., Ward, S. A., Kowalchuk, J. M., Howe, F. A., Griffiths, J. R., & Whipp, B. J. (2002). Dynamic asymmetry of phosphocreatine concentration and O<sub>2</sub> uptake between the on- and off-transients of moderate- and high-intensity exercise in humans. *J Physiol*, 541(Pt 3), 991-1002.
- Sahlin, K., Soderlund, K., Tonkonogi, M., & Hiraoka, K. (1997). Phosphocreatine content in single fibers of human muscle after sustained submaximal exercise. *Am J Physiol*, 273(1 Pt 1), C172-178.
- Sargeant, A. J. (1994). Human power output and muscle fatigue. *Int J Sports Med*, 15(3), 116-121.

- Sargeant, A. J. (1999). Neuromuscular determinants of human performance. In B. J. Whipp & A. J. Sargeant (Eds.), *Physiological Determinants of Exercise Tolerance in Humans*. (pp. 13-28). London: Portland Press.
- Saunders, M. J., Evans, E. M., Arngrimsson, S. A., Allison, J. D., Warren, G. L., & Cureton, K. J. (2000). Muscle activation and the slow component rise in oxygen uptake during cycling. *Med Sci Sports Exerc*, 32(12), 2040-2045.
- Scheuermann, B. W., Hoelting, B. D., Noble, M. L., & Barstow, T. J. (2001). The slow component of O<sub>2</sub> uptake is not accompanied by changes in muscle EMG during repeated bouts of heavy exercise in humans. *J Physiol*, 531(Pt 1), 245-256.
- Shinohara, M., & Moritani, T. (1992). Increase in neuromuscular activity and oxygen uptake during heavy exercise. *Ann Physiol Anthropol*, 11(3), 257-262.
- Sjogaard, G. (1978). Force velocity curve for bicycle work In A. Asmussen & K. Jorgensen (Eds.), *Biomechanics VI-A* (pp. 93-99). Baltimore: University Park Press.
- Soderlund, K., & Hultman, E. (1991). ATP and phosphocreatine changes in single human muscle fibers after intense electrical stimulation. *Am J Physiol*, 261(6 Pt 1), E737-741.
- Springer, C., Barstow, T. J., Wasserman, K., & Cooper, D. M. (1991). Oxygen uptake and heart rate responses during hypoxic exercise in children and adults. *Med Sci Sports Exerc*, 23(1), 71-79.
- Stevens, J. (1996). *Applied Multivariate Statistics for the Social Sciences* (3<sup>rd</sup> ed.): New Jersey: Lawrence Erlbaum Associates
- Takaishi, T., Yasuda, Y., Ono, T., & Moritani, T. (1996). Optimal pedaling rate estimated from neuromuscular fatigue for cyclists. *Med Sci Sports Exerc*, 28(12), 1492-1497.

- Timmons, J. A., Gustafsson, T., Sundberg, C. J., Jansson, E., & Greenhaff, P. L. (1998). Muscle acetyl group availability is a major determinant of oxygen deficit in humans during submaximal exercise. *Am J Physiol*, 274(2 Pt 1), E377-380.
- Tonson, A., Ratel, S., Le Fur, Y., Vilmen, C., Cozzone, P. J., & Bendahan, D. (2010). Muscle energetics changes throughout maturation: a quantitative  $^{31}\text{P}$ -MRS analysis. *J Appl Physiol*, 109(6), 1769-1778.
- Tordi, N., Perrey, S., Harvey, A., & Hughson, R. L. (2003). Oxygen uptake kinetics during two bouts of heavy cycling separated by fatiguing sprint exercise in humans. *J Appl Physiol*, 94(2), 533-541.
- Tschakovsky, M. E., & Hughson, R. L. (1999). Interaction of factors determining oxygen uptake at the onset of exercise. *J Appl Physiol*, 86(4), 1101-1113.
- Vanhatalo, A., Poole, D. C., DiMenna, F. J., Bailey, S. J., & Jones, A. M. (2011). Muscle fiber recruitment and the slow component of  $\text{O}_2$  uptake: constant work rate vs. all-out sprint exercise. *Am J Physiol Regul Integr Comp Physiol*, 300(3), R700-707.
- Vercruyssen, F., Missenard, O., & Brisswalter, J. (2009). Relationship between oxygen uptake slow component and surface EMG during heavy exercise in humans: Influence of pedal rate. *J Electromyogr Kinesiol*, 19, 676-684.
- Vincent, W.J. (1999). *Statistics in Kinesiology* (2<sup>nd</sup> ed.): Champaign (IL): Human Kinetics.
- Vollestad, N. K., & Blom, P. C. (1985). Effect of varying exercise intensity on glycogen depletion in human muscle fibres. *Acta Physiol Scand*, 125(3), 395-405.
- Wagner, P. D. (2000). New ideas on limitations to  $\dot{V}\text{O}_2\text{max}$ . *Exerc Sport Sci Rev*, 28(1), 10-14.

- Walsh, B., Tonkonogi, M., Soderlund, K., Hultman, E., Saks, V., & Sahlin, K. (2001). The role of phosphorylcreatine and creatine in the regulation of mitochondrial respiration in human skeletal muscle. *J Physiol*, 537(Pt 3), 971-978.
- Weiler-Ravell, D., Cooper, D. M., Whipp, B. J., & Wasserman, K. (1983). Control of breathing at the start of exercise as influenced by posture. *J Appl Physiol*, 55(5), 1460-1466.
- Whipp, B. J. (1994). The slow component of O<sub>2</sub> uptake kinetics during heavy exercise. *Med Sci Sports Exerc*, 26(11), 1319-1326.
- Whipp, B. J., & Rossiter, H. B. (2005). The kinetics of oxygen uptake: physiological inferences from the parameters. In A. M. Jones & D. C. Poole (Eds.), *Oxygen Uptake Kinetics in Sport, Exercise, and Medicine* (pp. 62-94). London and New York: Routledge.
- Whipp, B. J., & Ward, S. A. (1990). Physiological determinants of pulmonary gas exchange kinetics during exercise. *Med Sci Sports Exerc*, 22(1), 62-71.
- Whipp, B. J., Ward, S. A., Lamarra, N., Davis, J. A., & Wasserman, K. (1982). Parameters of ventilatory and gas exchange dynamics during exercise. *J Appl Physiol*, 52(6), 1506-1513.
- Whipp, B. J., & Wasserman, K. (1972). Oxygen uptake kinetics for various intensities of constant-load work. *J Appl Physiol*, 33(3), 351-356.
- Wilkerson, D. P., Berger, N. J., & Jones, A. M. (2006). Influence of hyperoxia on pulmonary O<sub>2</sub> uptake kinetics following the onset of exercise in humans. *Respir Physiol Neurobiol*, 153(1), 92-106.
- Wilkerson, D. P., Campbell, I. T., & Jones, A. M. (2004). Influence of nitric oxide synthase inhibition on pulmonary O<sub>2</sub> uptake kinetics during supra-maximal exercise in humans. *J Physiol*, 561(Pt 2), 623-635.

- Wilkerson, D. P., & Jones, A. M. (2006). Influence of initial metabolic rate on pulmonary O<sub>2</sub> uptake on-kinetics during severe intensity exercise. *Respir Physiol Neurobiol*, 152(2), 204-219.
- Wilkerson, D. P., & Jones, A. M. (2007). Effects of baseline metabolic rate on pulmonary O<sub>2</sub> uptake on-kinetics during heavy-intensity exercise in humans. *Respir Physiol Neurobiol*, 156(2), 203-211.
- Wilkerson, D. P., Rittweger, J., Berger, N. J., Naish, P. F., & Jones, A. M. (2005). Influence of recombinant human erythropoietin treatment on pulmonary O<sub>2</sub> uptake kinetics during exercise in humans. *J Physiol*, 568(Pt 2), 639-652.
- Willcocks, R. J., Williams, C. A., Barker, A. R., Fulford, J., & Armstrong, N. (2010). Age- and sex-related differences in muscle phosphocreatine and oxygenation kinetics during high-intensity exercise in adolescents and adults. *NMR Biomed*, 23(6), 569-577.
- Williams, C. A., Carter, H., Jones, A. M., & Doust, J. H. (2001). Oxygen uptake kinetics during treadmill running in boys and men. *J Appl Physiol*, 90(5), 1700-1706.
- Williams, C. A., Dekerle, J., McGawley, K., Berthoin, S., & Carter, H. (2008). Critical power in adolescent boys and girls-an exploratory study. *Appl Physiol Nutr Metab*, 33(6), 1105-1111.
- Willis, W. T., & Jackman, M. R. (1994). Mitochondrial function during heavy exercise. *Med Sci Sports Exerc*, 26(11), 1347-1353.
- Wilson, D. F. (1994). Factors affecting the rate and energetics of mitochondrial oxidative phosphorylation. *Med Sci Sports Exerc*, 26(1), 37-43.



- Winlove, M. A., Jones, A. M., & Welsman, J. R. (2009). Influence of training status and exercise modality on pulmonary O<sub>2</sub> uptake kinetics in pre-pubertal girls. *Eur J Appl Physiol*, 108(6), 1169-1179.
- Wretling, M. L., Gerdle, B., & Henriksson-Larsen, K. (1987). EMG: a non-invasive method for determination of fibre type proportion. *Acta Physiol Scand*, 131(4), 627-628.
- Zanconato, S., Buchthal, S., Barstow, T. J., & Cooper, D. M. (1993). <sup>31</sup>P-magnetic resonance spectroscopy of leg muscle metabolism during exercise in children and adults. *J Appl Physiol*, 74(5), 2214-2218.
- Zoladz, J. A., Gladden, L. B., Hogan, M. C., Nieckarz, Z., & Grassi, B. (2008). Progressive recruitment of muscle fibers is not necessary for the slow component of  $\dot{V}O_2$  kinetics. *J Appl Physiol*, 105(2), 575-580.

**Appendix 1** – Worked calculations of sample size (n)

*Chapter 5: work-to-work exercise*

$$*N = \frac{2(15)^2(1.96 + 0.84)^2}{21^2} = \underline{\underline{8}}$$

\*Based on values for the phase II  $\tau$  of  $27 \pm 4$  s in U→H and  $48 \pm 11$  s in M→H cited in Wilkerson & Jones (2007).

*Chapter 6: disparate pedal rates*

$$\#N = \frac{2(4.9)^2(1.96 + 0.84)^2}{6.4^2} = \underline{\underline{9}}$$

#Based on values for the phase II  $\tau$  of  $23.8 \pm 1.8$  s and  $30.2 \pm 3.1$  s during 35 and 115  $\text{rev}\cdot\text{min}^{-1}$  pedalling cited in Pringle et al. (2003).

*Chapters 7 and 8: child-adult differences in the  $\dot{V}_{O_2}$  slow component*

$$\dagger N = \frac{2(10)^2(1.96 + 0.84)^2}{13^2} = \underline{\underline{9}}$$

†Based on values for the relative  $\dot{V}_{O_2}$  slow component amplitude of  $9 \pm 3\%$  in boys (Barker, Jones, et al., 2010) and  $22 \pm 7\%$  in men (Dimenna, Bailey, et al., 2010) during cycling exercise at  $\Delta 60\%$ .

## Appendix 2 – Ethics approval certificates



SCHOOL OF SPORT  
AND HEALTH SCIENCES

St Luke's Campus  
Heavitree Road  
Exeter  
UK EX1 2LU

Telephone +44 (0)1392 262896/262807  
Fax +44 (0)1392 264726  
Email sshs-school-office@exeter.ac.uk  
Web www.exeter.ac.uk/sshs

### Certificate of Ethical Approval

#### Proposal 4 (7/5/08)

Title: The effect of baseline metabolic rate on pulmonary O<sub>2</sub> uptake kinetics during severe exercise in children and adolescents

Applicant: Associate Professor Craig Williams with Mr Brynmor Breese (Research Student) and Dr James Mottram (qualified medic currently studying sports medicine at the University of Bath)

The proposal (circulated previously) was discussed by the Committee. The Committee advised that the application should be amended as follows:

- i. The application form be signed by Associate Professor Craig Williams (the applicant must be a University staff member).
- ii. Clarify where recruitment of participants is going to take place.
- iii. Clarify on the Consent Form how many visits are required and for how long (the Parent/Guardian form suggests 5 visits and assent form at least 5).
- iv. Explain references to coach on the Information Sheet and Consent Form.
- v. Include a title on the Information Sheet to indicate that it is an Information Sheet and who it is for (participants, parents/helpers, etc). Amend 'used' to 'use' in the title of the project. Amend the first sentence under 'What tests will I be doing?' to 'we would like' rather than 'we need'.
- vi. Specify how long the results will be stored on the Consent Form and Information Sheet.

*Decision: the Committee AGREED to provisionally approve the proposal until February 2009, but required the amendments outlined above (i-vi). The amendments need to be returned to JW prior to the commencement of the study.*

***Decision: The amendments (as outlined above) were received by JW and were deemed satisfactory and the proposal was approved.***

**School Ethics Committee Reference Number: 7/5/08#4**

Signature: *Joanne R Welsman* Date: *9/6/08*

Name/Title of Chair: Dr J Welsman

*Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.*

**Certificate of Ethical Approval**

Proposal 6 (27/2/08)

Title: Effect of pedal rate on oxygen uptake kinetics during severe exercise in young trained cyclists

Applicants: Associate Professor Craig Williams with Mr Brynmor Breese (Research Student) and Dr James Mottram (sports medicine student at Bath University)

The proposal (circulated previously) was discussed by the Committee. The Committee advised that the application should be amended as follows:

- i. Provide sufficient detail to replicate the power calculations.
- ii. Revise the Information Sheet so that the title is included and it is written in a more user-friendly way for a lay audience. In addition, please change 'you' to 'we' in the sub-title 'How are you going to find these things out', remove the statement 'Is it safe', and explain why the study is being undertaken.
- iii. Clarify how many times the child has to visit and ensure that it is consistent throughout the paperwork.

*Decision: the Committee AGREED to provisionally approve the proposal until September 2008, but required the amendments outlined above (i-iii). The amendments need to be returned to JW prior to the commencement of the study.*

***Decision: The amendments (as outlined above) were received by JW and were deemed satisfactory and the proposal was approved.***

From: April 2008  
To: September 2008

**School Ethics Committee Reference Number: 27/2/08#6**

Signature: *Joanne Reveloman* Date: *22/4/08*

Name/Title of Chair: Dr J Welsman

*Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.*

**Certificate of Ethical Approval**

Proposal 4 (24/02/10)

Title:  $^1\text{H}$  Transverse relaxation times ( $T_2$ ) derived indices of muscle activation during exercise in children and adults

Applicant: Assoc Prof Craig Williams (Staff) with Dr Jon Fulford (staff) and Brynmor Breese (PG Research Student)

The proposal was reviewed by the Ethics Committee on 24/02/2010 and following amendments has now been approved until August 2010.

**Decision: The proposal was approved from March 2010 to August 2010.**

Signature: 

Date: 25/03/2010

Name/Title of Ethics Committee Reviewer: Prof. Adrian Taylor

*Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.*