

# **Pulmonary Oxygen Uptake and Muscle Oxygenation Responses to Exercise in Well- Trained Young and Middle-Aged Cyclists**

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by

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# ABSTRACT

This thesis details four consecutive research investigations which were designed to examine the effect of age on the pulmonary oxygen uptake ( $\dot{V}O_2$ ) and muscle oxygenation (mOxy) responses to exercise in well-trained cyclists.

*Study One: Physiological, histochemical, enzymatic and performance characteristics in well-trained young and middle-aged cyclists.*

The aim of study one was to examine the effect of age on a range of physiological and performance characteristics of well-trained cyclists. Seven well-trained young (Y) (age=  $19.6 \pm 1.7$  y;  $\dot{V}O_{2\max} = 55.2 \pm 7.0$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ) and seven middle-aged (MA) (age=  $44.8 \pm 2.7$  y;  $\dot{V}O_{2\max} = 50.2 \pm 6.4$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ) male cyclists were recruited for the series of investigations. Following pre-exercise screening and familiarisation, both age-groups completed a ramp test on an electromagnetically-braked cycle ergometer until maximal aerobic power ( $\dot{V}O_{2\max}$ ) was attained. Throughout the ramp test, heart rate,  $\dot{V}O_2$  and muscle mOxy were continuously monitored. Independent sample *t*-tests revealed no significant effect of age in the cyclist's ventilatory threshold (VT) (Y:  $71.4 \pm 5.1$  % $\dot{V}O_{2\max}$ ; MA:  $70.2 \pm 4.0$  % $\dot{V}O_{2\max}$ ;  $p > 0.05$ ) or  $\dot{V}O_{2\max}$  (Y:  $3.8 \pm 0.5$  L $\cdot$ min $^{-1}$ , MA:  $3.9 \pm 0.6$  L $\cdot$ min $^{-1}$ ,  $p > 0.05$ ). On a separate occasion, duplicate resting muscle biopsies were taken from the vastus lateralis (VL) of each cyclist for histochemical and biochemical analyses. No significant differences were observed in the muscle fibre composition ( $p > 0.05$ ), cross-sectional area ( $p > 0.05$ ) or capillarisation ( $p > 0.05$ ) of the VL between age-groups. Maximal specific activities of both glycolytic (PFK and LDH;  $p > 0.05$ ) and oxidative (CS,  $\beta$ -HAD and 2-OGDH;  $p > 0.05$ ) enzymes were similar between age-groups. Each cyclist then completed a 30 minute time trial (30TT)

to provide a measure of cycling performance. No significant differences were observed between age groups in the mean relative power output (RPO) sustained across the 30TT (Y:  $3.1 \pm 0.5 \text{ W}\cdot\text{kg BM}^{-1}$ ; MA:  $3.3 \pm 0.5 \text{ W}\cdot\text{kg BM}^{-1}$ ;  $p>0.05$ ). In conclusion, the results of the first study suggest that the physiological and performance characteristics of the well-trained young and middle-aged cyclists were similar on the tests and variables chosen.

*Study Two: On-transient  $\dot{V}O_2$  and mOxy responses to moderate, heavy and severe-intensity exercise in well-trained young and middle-aged cyclists.*

Study two examined the effect of age on the on-transient  $\dot{V}O_2$  and mOxy kinetic responses to moderate (80% VT), heavy (50% $\Delta$  VT- $\dot{V}O_{2\text{max}}$ ) and severe- (80% $\Delta$  VT- $\dot{V}O_{2\text{max}}$ ) intensity square wave transitions (SWT) in well-trained cyclists. Secondary aims of this study were to relate these responses to the changes in a number of hematological parameters (blood pH,  $pO_2$ ,  $[HCO_3^-]$  and  $[BLa^-]$ ) across the three SWT intensities, as well as to the histochemical and biochemical characteristics described within Study One. All cyclists completed three separate six minute SWT at each exercise intensity previously determined from the ramp test in Study One. Each SWT was preceded and followed by three minutes of 'unloaded' cycling at 20 W. Both the  $\dot{V}O_2$  and mOxy responses were modelled using a single or double exponential function to quantify the on-transient responses. For the purpose of Study two, only the modelling parameters fitting the primary component of the  $\dot{V}O_2$  and mOxy responses were of interest. Repeated Measures Analysis of Variance (RMANOVA) revealed no significant ( $p>0.05$ ) effect of age in either the primary amplitude ( $A_p$ ), time delay ( $TD_p$ ) or time constant ( $\tau_p$ ) of the  $\dot{V}O_2$  or mOxy on-transient responses across the three exercise intensities. In the on-transient

$\dot{V}O_2$  response, both the  $A_p$  and  $TD_p$  demonstrated a significant ( $p < 0.05$ ) effect of intensity, whereas the  $\dot{V}O_2 \tau_p$  remained stable across exercise intensities. In the mOxy on-transient response, only the  $A_p$  demonstrated a significant ( $p < 0.05$ ) effect of intensity in the young and middle-aged cyclists. The speed of the on-transient  $\dot{V}O_2$  and mOxy responses were significantly ( $p < 0.05$ ) related across both the moderate and heavy-intensity SWT in the young cyclists. The mOxy  $\tau_p$  and  $\dot{V}O_2 \tau_p$  were significantly ( $p < 0.05$ ) related to changes in blood pH and  $[BLa^-]$  in the young and middle-aged cyclists, respectively. In the young cyclists, the speed of the moderate and heavy-intensity  $\dot{V}O_2$  responses was significantly ( $p < 0.05$ ) related to muscle fibre composition and capillary to fibre ratio. In the middle-aged cyclists, the moderate and severe-intensity  $\dot{V}O_2 \tau_p$  were significantly ( $p < 0.05$ ) related to muscle fibre composition and capillary contacts per fibre area, respectively. In conclusion, no significant effect of age was observed in the on-transient  $\dot{V}O_2$  and mOxy responses in well-trained cyclists matched for  $\dot{V}O_{2max}$  and peripheral muscle characteristics.

*Study Three:  $\dot{V}O_2$  and mOxy slow components determined during heavy and severe-intensity exercise in well-trained young and middle-aged cyclists.*

The third study examined the effect of age on the nature of the  $\dot{V}O_2$  and mOxy slow components across constant-load heavy and severe-intensity SWT. A secondary aim was to relate the  $\dot{V}O_2$  and mOxy slow components to changes in hematological parameters, and the peripheral muscle characteristics of the well-trained young and middle-aged cyclists as described within earlier studies. A further purpose of Study Three was to investigate the relationship between the development of the  $\dot{V}O_2$  and mOxy slow components and any changes in muscle activity and fibre recruitment patterns of the VL and vastus medialis

(VM) throughout the high-intensity SWT using surface electromyography (sEMG). The data for Study Three were obtained using the methods outlined in Study Two. The  $\dot{V}O_2$  and mOxy slow components were fitted using a second exponential component, starting after the completion of the primary component. RMANOVA revealed no significant ( $p>0.05$ ) effect of age or age x intensity interactions in the amplitude or speed of the  $\dot{V}O_2$  or mOxy slow components in the cyclists. However, a significant ( $p<0.05$ ) effect of intensity was observed in the  $\dot{V}O_2$  slow component  $\tau$  ( $\tau_s$ ) in the young cyclists, with the heavy-intensity  $\tau_s$  being significantly ( $p<0.05$ ) longer than that of the severe-intensity SWT. No significant ( $p>0.05$ ) effect of intensity was observed in any mOxy slow component parameters in either age group. The heavy-intensity  $\dot{V}O_2$  slow component was only significantly ( $p<0.05$ ) related to maximal CS activity in the young cyclists, and no further relationships were observed between the  $\dot{V}O_2$  and mOxy slow component kinetics and peripheral muscle characteristics. No significant ( $p>0.05$ ) relationships were observed between the  $\dot{V}O_2$  and mOxy slow components and changes in blood pH,  $pO_2$ ,  $[HCO_3^-]$  or  $[BLa^-]$  during the two high-intensity SWT. Finally, non-significant ( $p>0.05$ ) trends across time were observed in both the integrated EMG and mean power frequency responses of the VL and VM during the heavy and severe-intensity SWT. No significant ( $p>0.05$ ) relationships were observed with the sEMG responses and either the  $\dot{V}O_2$  or mOxy slow component for either age group. In summary, no effect of age was observed in the  $\dot{V}O_2$  and mOxy slow components in the well-trained cyclists, which may reflect the similar peripheral muscle characteristics and recruitment patterns of the working muscles of the two age groups.

*Study Four:  $\dot{V}O_2$  and mOxy responses following moderate, heavy and severe-intensity exercise in well-trained young and middle-aged cyclists.*

Lastly, the fourth and final study examined the effect of age on the off-transient  $\dot{V}O_2$  and mOxy responses following moderate, heavy and severe-intensity exercise in well-trained cyclists. This study reported the relationship between the off-transient  $\dot{V}O_2$  and mOxy responses to the concentration of the hematological parameters at SWT completion and the peripheral muscle characteristics reported earlier. The  $\dot{V}O_2$  and mOxy off-transient responses were recorded during three minutes of 'unloaded' pedalling at 20 W following the completion of the moderate, heavy and severe-intensity SWT as detailed in Study Two. The off-transient responses were modelled using either a single or double-exponential function in order to quantify their speed and amplitude. RMANOVA revealed no significant ( $p>0.05$ ) effect of age or age x intensity interaction for any off-transient  $\dot{V}O_2$  or mOxy parameter. However, significant ( $p<0.05$ ) main effects of intensity were observed for the off-transient  $\dot{V}O_2$  and mOxy amplitudes ( $A_f$ ). The  $\dot{V}O_2$  off-transient  $\tau$  ( $\tau_f$ ) was also observed to significantly ( $p<0.05$ ) lengthen following the severe-intensity SWT compared to the moderate and heavy-intensity SWT. No such effect of intensity was observed in the mOxy response. Following the moderate-intensity SWT, the off-transient  $\dot{V}O_2$   $\tau_f$  and  $MRT_f$  were significantly ( $p<0.05$ ) related to the  $[HCO_3^-]$  and  $[BLa^-]$  responses of the young cyclists. The off-transient moderate and heavy-intensity  $\dot{V}O_2$  responses of the middle-aged cyclists were significantly ( $p<0.05$ ) related to changes in  $[HCO_3^-]$ . In conclusion, no effect of age was observed in the off-transient  $\dot{V}O_2$  and mOxy responses following moderate-, heavy- or severe-intensity constant load cycling in the matched well-trained cyclists.

In summary, no significant effect of age was observed in the on- or off-transient responses of  $\dot{V}O_2$  or mOxy in well-trained cyclists matched for  $\dot{V}O_{2\max}$  and peripheral muscle characteristics. However, the present data support previous findings of a significant effect of exercise intensity on several amplitude and speed measures of the  $\dot{V}O_2$  and mOxy kinetic responses. The absence of an effect of age is most likely due to the similar physiological and peripheral muscle characteristics reported within Study One. The results of the present series of studies suggest that physical training can maintain the rate of metabolic adaptation to exercise compared to a similarly trained younger cohort. Therefore, physical training may help to reduce the slowing of metabolic adaptation previously reported with sedentary aging.

# DECLARATION

This thesis describes the original work of the author except where acknowledged in the text. I hereby declare that I have not submitted this material either in whole or part for a degree at this or any other academic institution.

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Benjamin James Dascombe

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# LIST OF EQUATIONS

**Eq<sup>n</sup> 1:**  $\dot{V}O_2(t) = \dot{V}O_2(b) + A_p \cdot [1 - e^{-(t-TD_p)/\tau_p}]$

**Eq<sup>n</sup> 2:**  $\dot{V}O_2(t) = \dot{V}O_2(b) + A_p \cdot [1 - e^{-(t-TD_p)/\tau_p}] + A_s \cdot [1 - e^{-(t-TD_s)/\tau_s}]$

**Eq<sup>n</sup> 3:**  $MRT(s) = [A_p/(A_p + A_s)] \cdot (TD_p + \tau_p) + [A_s/(A_p + A_s)] \cdot (TD_s + \tau_s)$

**Eq<sup>n</sup> 4:**  $\dot{V}O_2(t) = EE\dot{V}O_2 - A_f \cdot [1 - e^{-(t-TD_f)/\tau_f}] \cdot u_1$

**Eq<sup>n</sup> 5:**  $A = \varepsilon \cdot [c] \cdot L \cdot B + G$

**Eq<sup>n</sup> 6:**  $mOxy(t) = mOxy(b) - A_p \cdot [1 - e^{-(t-TD_p)/\tau_p}]$

**Eq<sup>n</sup> 7:**  $mOxy(t) = mOxy(b) - A_p \cdot [1 - e^{-(t-TD_p)/\tau_p}] - A_s \cdot [1 - e^{-(t-TD_s)/\tau_s}]$

**Eq<sup>n</sup> 8:**  $mOxy(t) = EEmOxy + A_f \cdot [1 - e^{-(t-TD_f)/\tau_f}] \cdot u_1$

**Eq<sup>n</sup> 9:**  $mOxy(t) = EEmOxy + A_f \cdot [1 - e^{-(t-TD_f)/\tau_f}] \cdot u_1 + A_{fs} \cdot [1 - e^{-(t-TD_{fs})/\tau_{fs}}] \cdot u_1$

**Eq<sup>n</sup> 10:**  $MPF = LS \cdot \text{time} + IMF$

**Eq<sup>n</sup> 11:**  $DD_{\text{mean}} = \left[ \frac{0.207 + 0.232}{\text{C:F Ratio}} \right] \times \sqrt{\text{average fibre cross-sectional area}}$

**Eq<sup>n</sup> 12:**  $DD_{\text{max}} = \left[ \frac{0.415 + 0.477}{\text{C:F Ratio}} \right] \times \sqrt{\text{average fibre cross-sectional area}}$

**Eq<sup>n</sup> 13:**

$PFK \text{ Activity } (\mu\text{mol/g/min}) = \left( \frac{F_{\text{sample}}}{F_{\text{standard}}} \right) \times (mM_{\text{standard}} \times mL_{\text{standard}}) \div g_{\text{wet muscle}} \div \text{min}$

**Eq<sup>n</sup> 14:**

$LDH \text{ Activity } (\mu\text{mol/g/min}) = \left( \frac{F_{\text{sample}}}{F_{\text{standard}}} \right) \times (mM_{\text{standard}} \times mL_{\text{standard}}) \div g_{\text{wet muscle}} \div \text{min}$

**Eq<sup>n</sup> 15:**

$CS \text{ Activity } (\mu\text{mol/g/min}) = \frac{\Delta\text{Abs/min} \times \text{Total volume}}{\text{Sample volume} \times 13.6} \times \text{Dilution factor}$

**Eq<sup>n</sup> 16:**

$\beta\text{-HAD Activity } (\mu\text{mol/g/min}) = \frac{\Delta\text{Abs/min} \times \text{Total volume}}{\text{Sample volume} \times 6.22} \times \text{Dilution factor}$

**Eq<sup>n</sup> 17:**

$2\text{-OGDH Activity } (\mu\text{mol/g/min}) = \frac{\Delta\text{Abs/min} \times \text{Total volume}}{\text{Sample volume} \times 6.22} \times \text{Dilution factor}$

# LIST OF ABBREVIATIONS AND NOMENCLATURE

2-OGDH	2-Oxoglutarate dehydrogenase
20TT	Twenty kilometre time trial
30TT	Thirty minute time trial
$\Delta\text{Abs}\cdot\text{min}^{-1}$	Change in absorbance per minute
A	Optical density
A/D	Analogue to digital conversion
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
$A_f$	Off-transient amplitude
$A_{fs}$	Slow off-transient amplitude
$A_p$	Primary component amplitude
$A_s$	Slow component amplitude
a-vO <sub>2</sub> diff	Arterio-venous oxygen difference
B	Path length of the scattering light
$b\cdot\text{min}^{-1}$	Beats per minute
BLa <sup>-</sup>	Blood lactate
[BLa <sup>-</sup> ]	Blood lactate concentration
$\beta$ -HAD	Beta-hydroxyacyl-CoA dehydrogenase
BSA	Bovine serum albumin
C	Chromophore concentration
$\text{cap}\cdot\text{mm}^{-2}$	Capillaries per millimetre squared
C/F	Capillary to fibre ratio
CC/F	Capillary contacts per fibre

$CC \cdot \mu m^{-2}$	Capillary contact per fibre area
CI	Confidence interval
cm	Centimetre
CO <sub>2</sub>	Carbon dioxide
CoA	Co-enzyme A
CS	Citrate synthase
CSA	Cross-sectional area
CV%	Percentage of Co-variance
°	Degree
°C	Degrees Celsius
$\Delta$	Difference between VT and VO <sub>2</sub> max
DD <sub>mean</sub>	Mean diffusion distance
DD <sub>max</sub>	Maximum diffusion distance
DTNB	3,3'-dithiobis(6-nitrobenzoic acid)
$\epsilon$	Chromophore extinction coefficient
EDTA	Ethylenediaminetetraacetate
EE $\dot{V}O_2$	End-exercise oxygen consumption
EE <sub>m</sub> Oxy	End-exercise muscle oxygenation
EPOC	Excess post-exercise oxygen consumption
Eq <sup>n</sup>	Equation
$\eta^2$	Partial eta squared
F <sub>sample</sub>	Fluorescence of sample
F <sub>std</sub>	Fluorescence of standard
F <sub>I</sub> O <sub>2</sub>	Fraction of inspired oxygen
g	Gram
G	Geometry coefficient

$g_{wt \text{ muscle}}$	Wet mass of tissue sample
$G_p$	Primary component gain
$G_s$	Slow component gain
$G_o$	Overall (primary + slow component) gain
$h$	Hour
$Hb$	Hemoglobin
$HbO_2$	Oxyhemoglobin
$HCl$	Hydrochloric acid
$[HCO_3^-]$	Blood bicarbonate concentration
$HR$	Heart rate
$HR_{max}$	Maximum heart rate
$\%HR_{max}$	Percentage of maximum heart rate
$Hz$	Hertz
$iEMG$	Integrated electromyography
$KCl$	Potassium chloride
$K_2HPO_4$	Dipotassium hydrogen phosphate
$kg$	Kilogram
$k\Omega$	Kilo-ohms
$L$	Litres
$l$	Distance between light source and detectors
$L \cdot min^{-1}$	Litres per minute
$LDH$	Lactate dehydrogenase
$LSD$	Least significant difference
$LT$	Lactate threshold
$m$	Metre
$M$	Mole

$M^{-1} \cdot cm^{-1}$	Mole per centimetre
Mb	Myoglobin
MbO <sub>2</sub>	Oxymyoglobin
$\bar{X}$	Mean
MgCl <sub>2</sub>	Magnesium chloride
MHC	Myosin heavy chain
min	Minute
mL	Millilitre
mL <sub>vol std</sub>	Volume of standard
m-ATPase	Myosin ATPase
mmHg	Millimetres of mercury
mg	Milligram
$mL \cdot kg^{-1} \cdot min^{-1}$	Millilitre per kilogram per minute
$mL \cdot min^{-1}$	Millilitre per minute
$mL \cdot min^{-1} \cdot 100g^{-1}$	Millilitre per minute per 100 grams
$mL \cdot min^{-1} \cdot W^{-1}$	Millilitre per minute per watt
$mL \cdot W^{-1}$	Millilitre per watt
mm	Millimetre
mm <sup>2</sup>	Millimetre squared
mM	Millimolar
mmol $\cdot$ L <sup>-1</sup>	Millimole per litre
mmol $\cdot$ kg w.w. <sup>-1</sup>	Millimole per kilogram wet weight
mOxy	Muscle oxygenation
MPEG	Megapixels
MPF	Mean power frequency
MRI	Magnetic resonance imaging

ms	Millisecond
mV	Millivolts
n	Sample size
N <sub>2</sub>	Nitrogen molecule
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced)
NaOH	Sodium hydroxide
NIRS	Near infrared spectroscopy
nm	Nanometre
O <sub>2</sub>	Oxygen molecule
<i>p</i>	Alpha
PAS	Periodic acid-schiff
PCr	Phosphocreatine
%	Percent
%•W <sup>-1</sup>	Percent per watt
PFK	Phosphofructokinase
PO	Power output
<i>p</i> O <sub>2</sub>	Partial pressure of oxygen
<i>p</i> CO <sub>2</sub>	Partial pressure of carbon dioxide
$\dot{Q}$	Cardiac output
<i>r</i>	Correlation co-efficient
RF	Rectus femoris
RH	Relative humidity
RMANOVA	Repeated measures analysis of variance
RMS	Root mean square
RPM	Revolutions per minute

RPO	Relative power output
s	Second
SD	Standard deviation
SDH	Succinate dehydrogenase
SEM	Standard error of measurement
sEMG	Surface electromyography
$\sqrt{\phantom{x}}$	Square root
SWT	Square wave transition
SV	Stroke volume
$\Sigma$	Sum
$\tau$	Time constant
$\tau^{1/2}$	Half-life
$\tau_f$	Off-transient time constant
$\tau_{fs}$	Slow off-transient time constant
$\tau_p$	Primary component time constant
$\tau_s$	Slow component time constant
$TD_f$	Off-transient time delay
$TD_{fs}$	Slow off-transient time delay
$TD_p$	Primary component time delay
$TD_s$	Slow component time delay
TEM	Technical error of measurement
TEM%	Technical error of measurement percentage
TT	Time trial
$\mu\text{g}\cdot\mu\text{L}^{-1}$	Microgram per microlitre
$\mu\text{L}$	Microlitre
$\text{U}\cdot\text{mL}^{-1}$	Units per millilitre

$\mu\text{m}$	Micrometre
$\mu\text{m}^2$	Micrometre squared
$\mu\text{mol}$	Micromole
$\mu\text{mol}\cdot\text{g}_{\text{w.w.}}^{-1}\cdot\text{min}^{-1}$	Micromole per gram wet weight per minute
$\mu\text{mol}\cdot\text{g}_{\text{protein}}^{-1}\cdot\text{min}^{-1}$	Micromole per gram of protein per minute
$\mu\text{mol}\cdot\text{L}^{-1}$	Micromole per litre
$\mu\text{mol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$	Micromole per litre per minute
vs.	Versus
$\dot{V}\text{O}_2\text{max}$	Velocity at maximal oxygen consumption
$\text{VCO}_2$	Volume of carbon dioxide produced
$\text{VE}$	Volume of expired air
$\text{VL}$	Vastus lateralis
$\text{VM}$	Vastus medialis
$\dot{V}\text{O}_2$	Volume of oxygen consumed
$\dot{V}\text{O}_2$ (b)	Baseline oxygen consumption
$\dot{V}\text{O}_2\text{max}$	Maximal oxygen consumption
$\%\dot{V}\text{O}_2\text{max}$	Percentage of maximal oxygen consumption
$\text{VT}$	Ventilatory threshold
$\text{W}$	Watt
$\text{W}\cdot\text{kg BM}^{-1}$	Watts per kilogram body mass
$\text{wMRT}$	Weighted mean response time
$\text{wMRT}_f$	Off-transient weighted mean response time
$y$	Year

# CHAPTER 1

## INTRODUCTION

### **Background Information**

The investigation of physiological responses to exercise originated over a half a century ago (Hill and Lupton 1923; Margaria, Edwards and Dill 1933; Whipp and Wasserman 1972) and has become an important method in both clinical and exercise physiology research (Jones and Poole 2005a). Historically, the measurement of pulmonary oxygen consumption ( $\dot{V}O_2$ ) in response to changes in work intensity has been of great interest to researchers as it provides a systemic measure of cardiorespiratory and metabolic functioning (Whipp and Wasserman 1972; Carter, Pringle, Jones and Doust 2002; Pringle, Doust, Carter, Tolfrey, Campbell and Jones 2003b). The use of such research also provides information as to systemic functioning of metabolic adaptations to exercise, but it has been proposed that the mechanisms regulating these responses lie within the working muscle and changes in oxygen ( $O_2$ ) delivery and utilisation (Grassi 2005).

More recently, the instantaneous measurement of changes in the oxygen content or oxygenation (mOxy) within the working muscle has been made possible through the introduction of Near-Infrared Spectroscopy (NIRS) to exercise physiology research (Chance, Dait, Zhang, Hamaoka and Hagerman 1992; Belardinelli, Barstow, Porszasz and Wasserman 1995a; Turner, Cathcart, Parker, Butterworth, Wilson and Ward 2006). NIRS uses changes in specific physiological chromophores to monitor changes in the

metabolic environment of the working muscle in response to bouts of exercise (Bae, Tyasukochi, Kan, Sasaki, Koseki, Hamaoka, Iwane and Haga 1996; Mancini 1997; Ding, Wang, Lei, Wang, Huang, Xia and Wu 2001; Bhambhani 2004). The recent development of NIRS technology has limited the quantity of research that has reported on concurrent  $\dot{V}O_2$  and mOxy responses to exercise (Belardinelli, Barstow, Porszasz and Wasserman 1995b; Bhambhani, Buckley and Susaki 1999; Demarie, Quaresima, Ferrari, Sardella, Billat and Faina 2001; DeLorey, Kowalchuck and Paterson 2004a; Turner et al. 2006).

### **Introduction to Metabolic Adaptation**

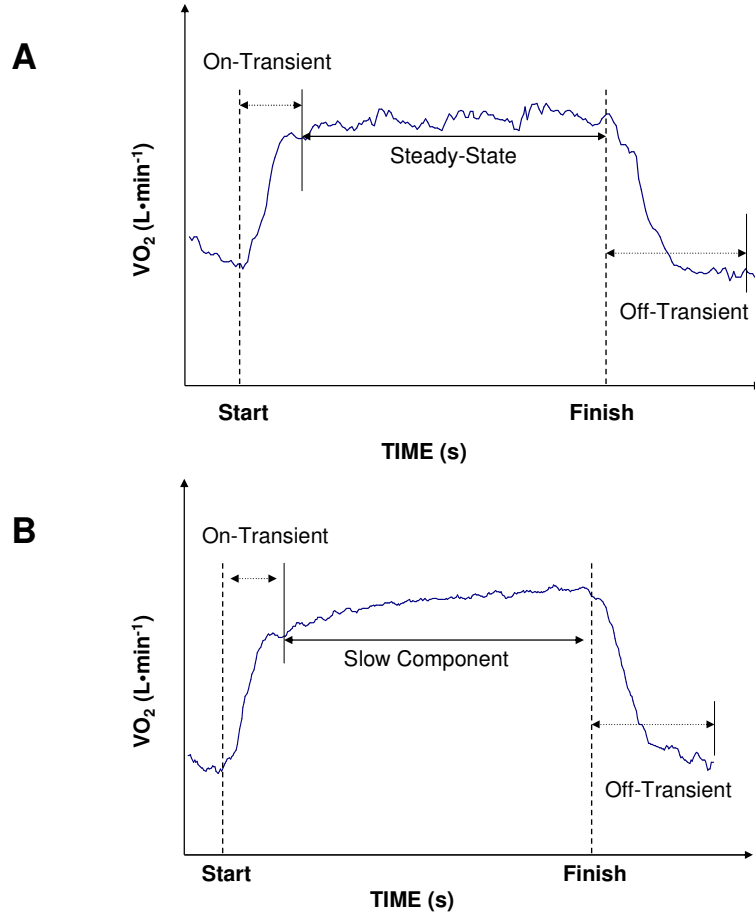
Whipp and Wasserman (1972) originally reported that the  $\dot{V}O_2$  response to moderate-intensity exercise could be fitted to a monoexponential function in order to quantify its speed and amplitude. Since this observation, numerous investigations have used similar modelling techniques to report upon metabolic adaptations in a number of conditions which may either enhance or retard cardiorespiratory adaptation to exercise (Xu and Rhodes 1999; Jones and Poole 2005a; 2005b). This broad research area has become known as  $\dot{V}O_2$  kinetics and primarily focuses upon the speed and amplitude of  $\dot{V}O_2$  adjustment in response to changes in work intensity. In their original investigation, Whipp and Wasserman (1972) identified that the metabolic and  $\dot{V}O_2$  response consisted of three important phases of transition to a bout of constant-load exercise. These identified phases include:

### *1. On-Transient Response (Primary Component)*

The on-transient response comprises two phases. Phase I encompasses the cardiodynamic phase where deoxygenated blood is returned to the lungs and a sharp linear increase in  $\dot{V}O_2$  is observed. Phase II is the rapid exponential increase in  $\dot{V}O_2$  or decrease in mOxy observed following the original Phase delay. This increase in  $\dot{V}O_2$  continues until the energy requirements of the exercise are completely met through aerobic metabolism. The on-transient response demonstrates the Primary Component (Phase I and Phase II) of the overall  $\dot{V}O_2$  response to a moderate (A) and heavy (B) intensity SWT as shown in Figure 1.1 over the page.

### *2. Steady-State or Slow Component Responses*

During moderate-intensity (< Ventilatory Threshold (VT)) exercise and after the initial primary component, a steady-state in  $\dot{V}O_2$  and mOxy is observed where aerobic energy production is equal to the energy requirements of the exercise intensity. During high-intensity (>VT) exercise, no steady-state in  $\dot{V}O_2$  or mOxy is developed, and a decrease in muscular and metabolic efficiency is observed. The gradual increase in  $\dot{V}O_2$  across high-intensity exercise has been termed the  $\dot{V}O_2$  slow component. The corresponding decrease in mOxy within the muscle has been defined as the mOxy slow component. An example of the  $\dot{V}O_2$  slow component is shown in Figure 1.1B.



**Figure 1.1:** Typical  $\dot{V}O_2$  responses to moderate (A) and heavy-intensity (B) exercise bouts

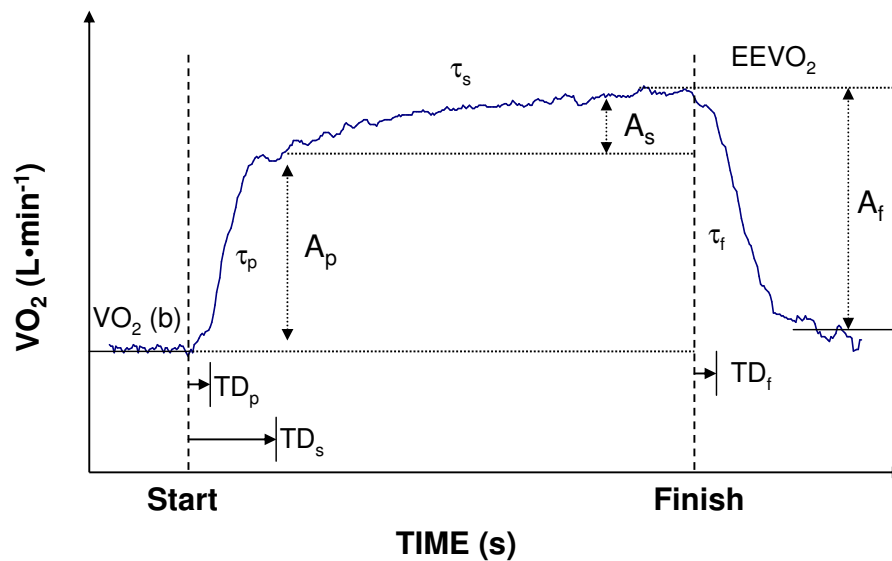
### 3. Off-Transient Response

The off-transient response is the recovery of  $\dot{V}O_2$  and mOxy measures to resting baselines following the completion of an exercise bout. The nature of the off-transient response is influenced by both the intensity and duration of the exercise bout. Examples of the off-transient  $\dot{V}O_2$  response to moderate (A) and high-intensity (B) exercise are shown above in Figure 1.1.

Previous investigations have reported that the magnitude and nature of these  $\dot{V}O_2$  and mOxy responses vary significantly with differences in laboratories and measurement systems, aerobic capacity, disease states, training status, gender or other physiological factors, making the nature of these responses somewhat difficult to compare and contrast (Koga, Shiojiri and Kondo 2005; Whipp, Ward and Rossiter 2005). As such, the fitting of the metabolic response to exponential equations allows the quantification of speed and amplitude measures for each individual response (Koga et al. 2005; Whipp and Rossiter 2005). However, the methods used to model these kinetic responses rely ultimately on the intensity of the exercise bout. Moderate intensity ( $<V_T$ ) exercise is able to be modelled using a single function exponential component, whereas high-intensity ( $>V_T$ ) exercise requires a double exponential model, due to the development of the  $\dot{V}O_2$  or mOxy slow component (Bearden and Moffat 2001). Each exponential component is fitted using a number of kinetic parameters to describe both the amplitude and speed of the metabolic responses. The exponential functions used to model the physiological responses consist of the following kinetic parameters, and are schematically represented in Figure 1.2.

- Amplitude (A): the absolute amplitude ( $\dot{V}O_2$ :  $L \cdot min^{-1}$ ; mOxy: %) of each physiological phase ( $A_p$ : Primary component amplitude;  $A_s$ : Slow component amplitude;  $A_f$ : Off-transient amplitude);
- Time delay (TD): time taken (s) from SWT commencement or completion to beginning of exponential phase of the on- or off-transient response ( $TD_p$ : Primary component time delay;  $TD_s$ : Slow component time delay;  $TD_f$ : Off-transient time delay);

- Time constant ( $\tau$ ): time taken (s) to reach 63% of each individual exponential component ( $\tau_p$ : Primary component time constant;  $\tau_s$ : Slow component time constant;  $\tau_f$ : Off-transient time constant); and,
- Weighted mean response time (wMRT): time taken (s) to reach 63% of the overall on- or off-transient response across multiple exponential components, and is weighted on both the TD and  $\tau$  of each function (wMRT: On-transient weighted mean response time; wMRT<sub>f</sub>: Off-transient weight mean response time).



**Figure 1.2:** The kinetic markers used within the exponential functions to quantify the speed and amplitude of the on-transient Phase II and slow components as well as the off-transient response of  $VO_2$  adaptation to an exercise bout (see above for key).

In summary, the capacity to accurately describe and quantify the nature of the  $\dot{V}O_2$  and mOxy responses across a constant-load exercise bout allows researchers to identify causal or influencing factors which may limit the rate of  $\dot{V}O_2$  or mOxy adjustment in response to changes in work. The examination and quantification of  $\dot{V}O_2$  and mOxy kinetics has applications within both clinical and athletic populations given the wide variety of conditions which affect the metabolic response and the proposed clinical and performance benefits of a speeded response.

### **Effects of Aging on Metabolic Adaptation**

To date, research has observed that sedentary aging is related to significant declines in several physiological characteristics, such as VT and LT (Allen, Seals, Hurley, Ehsani and Hagberg 1985; Masse-Biron, Mercier, Collomp, Hardy and Prefaut 1992),  $\dot{V}O_{2\max}$  (Babcock, Paterson and Cunningham 1992; Tanaka, Desouza, Jones, Stevenson, Davy and Seals 1997; Katzel, Sorkin and Fleg 2001; Tanaka and Seals 2006), muscle fibre composition and capillarisation (Frontera, Hughes, Krivickas and Roubenoff 2001; Andersen 2003; Deschenes 2004) and glycolytic or oxidative enzyme activities (Coggan, Spina, Rogers, King, Brown, Nemeth and Holloszy 1992; Russ and Kent-Braun 2004). Similarly, sedentary aging has also been shown to significantly attenuate the amplitude and speed of the  $\dot{V}O_2$  on-transient kinetic response to various exercise intensities and incremental exercise tests (Babcock, Paterson, Cunningham and Dickinson 1994b; Chilibeck, Paterson, Petrella and Cunningham 1996a; DeLorey et al. 2004a). Sedentary aging has also been observed to improve the mOxy response and the relationship to the  $\dot{V}O_2$  responses during moderate and heavy-intensity exercise (Stathokostas,

DeLorey, Kowalchuk and Paterson 2003; DeLorey, Kowalchuck and Paterson 2005). DeLorey et al. (2004a) reported that the on-transient response to moderate-intensity exercise was slower but demonstrated greater total O<sub>2</sub> extraction within the working muscle in older sedentary subjects compared to a similar young cohort. More recently, DeLorey, Kowalchuck and Paterson (2005) showed that in response to heavy-intensity exercise, older sedentary subjects demonstrated slower  $\dot{V}O_2$  kinetics but faster mOxy kinetics than younger subjects. The investigators hypothesised that this may be suggestive of a slower adaptation of local muscle blood flow in the elderly subjects. However, to date no such data are available on trained older subjects.

Furthermore, a number of previous investigations have reported that the  $\dot{V}O_2$  slow component is reduced with sedentary aging either as a result of a decrease in a number of factors including  $\dot{V}O_{2max}$ , changes in the peripheral muscle characteristics, or alterations in muscle fibre recruitment patterns (Rossiter, Ward, Kowalchuk, Howe, Griffiths and Whipp 2001; Sabapathy, Schneider, Comadira, Johnston and Morris 2004). DeLorey et al. (2005) observed the effect of age on the initial  $\dot{V}O_2$  and mOxy primary components in untrained subjects in response to heavy-intensity exercise, but did not report upon any changes with relation to the development of the slow component.

To date, no research has investigated the effect of aging on the mOxy slow component during high-intensity exercise. The majority of the slow component has been identified to occur within the working muscle (Poole 1994) and therefore changes in the histochemical and enzymatic environment within the muscle are likely to influence its development. Such changes have been

reported to occur with sedentary aging (Frontera and Hughes 2000). While the  $\dot{V}O_2$  and mOxy slow components have been shown to be significantly related to each other during high-intensity exercise in young trained individuals (Miura, Araki, Matoba and Kitagawa 1999; Demarie et al. 2001), no research has yet investigated this relationship in middle-aged individuals.

Lastly, the effect of aging on the off-transient  $\dot{V}O_2$  and mOxy responses has yet to be fully investigated. It has been suggested that the off-transient  $\dot{V}O_2$  response is significantly slowed with sedentary aging (Chick, Cagle, Vegas, Poliner and Murata 1991; Chilibeck, Paterson, Cunningham, Taylor and Noble 1997; Chilibeck, Paterson, McCreary, Marsh, Cunningham and Thompson 1998). No data exist on the age-related changes in the off-transient mOxy response in well-trained older subjects.

### **Statement of the Research Problem**

At present, a small number of recent investigations have examined the nature of both the  $\dot{V}O_2$  and mOxy kinetics during bouts of exercise at various intensities (Miura et al. 1999; Demarie et al. 2001; Grassi, Pogliaghi, Rampichini, Quaresima, Ferrari, Marconi and Cerretelli 2003). The effect of age on the  $\dot{V}O_2$  and mOxy responses to both moderate and high-intensity cycling exercise in sedentary subjects has previously been investigated and described (DeLorey et al. 2004a; 2005). No data to date have described the effect of age on these responses in well-trained individuals. Given that a number of physiological capacities that influence metabolic adaptation can be maintained into older age (i.e. LT,  $\dot{V}O_{2\max}$ , muscle fibre composition) with physical training (Pollock, Foster, Knapp, Rod and Schmidt 1987; Tanaka and Seals 2003), it is

of interest as to whether there is an effect of age on  $\dot{V}O_2$  and mOxy responses in well-trained older athletes. Finally, while it appears that the relationship between the  $\dot{V}O_2$  and mOxy responses is significantly changed with sedentary aging, no research has identified age-related mechanisms responsible for this effect of age.

### **Purpose of the Thesis**

Therefore, the current series of research investigations has a number of purposes:

1. To investigate and describe the effect of age on physiological and peripheral muscle characteristics in well-trained cyclists.
2. To examine the effect of age on the on-transient  $\dot{V}O_2$  and mOxy responses to moderate-, heavy- and severe-intensity SWT in well-trained cyclists.
3. To examine the effect of age on the development of the  $\dot{V}O_2$  and mOxy slow components during heavy- and severe-intensity SWT in well-trained cyclists.
4. To investigate the relationships between the  $\dot{V}O_2$  and mOxy responses and changes in hematological parameters across moderate-, heavy- and severe-intensity SWT in well-trained young and middle-aged cyclists.

5. To investigate the relationships between the  $\dot{V}O_2$ , mOxy kinetics across moderate-, heavy- and severe-intensity SWT and the peripheral muscle histochemical and enzymatic characteristics of well-trained young and middle-aged cyclists.
6. To investigate the role of changes in muscle electromyographic activity and fibre recruitment in the development of the  $\dot{V}O_2$  and mOxy slow components during heavy- and severe-intensity SWT in well-trained young and middle-aged cyclists.
7. To examine the effect of age on the off-transient  $\dot{V}O_2$  and mOxy responses following moderate- heavy- and severe-intensity exercise in well-trained cyclists.

### **Limitations and Assumptions**

The following limitations and assumptions may apply to the present study:

1. *Specificity of the results*

The data collected during the present study were obtained from well-trained young and middle-aged cyclists who were competitive during the twelve months prior to the study. The results may not be valid for all athletes of similar physiological capacities or different training modalities or performance levels.

2. *Maintenance of physiological capacities*

All physiological testing detailed within the subsequent studies was completed within four weeks for each individual subject. It is assumed

that the research subjects maintained their physiological capacities throughout all testing.

### *3. External lifestyle and genetic influences*

Many of the physical and physiological characteristics reported within the present study may be influenced by lifestyle and genetic factors which cannot be controlled. As with most aging research, a large variation within the results is likely given the pronounced effect of long-term environmental and lifestyle influences on such a cohort (Shephard 2002). However, by setting specific inclusion criteria for participation, these influences were likely to be minimised.

### *4. Representative nature of the muscle biopsy results*

The muscle fibre composition, fibre cross-sectional area, capillarisation and enzyme activity data from the present study is representative of the whole VL at the standardised sampling site. It is known that such factors may change with sampling position and depth of the sample location within such peripheral muscles (Lexell, Taylor and Sjostrom 1985; Dwyer, Browning and Weinstein 1999; Porter, Koolage and Lexell 2002).

### *5. Representative nature of the NIRS and sEMG results*

The NIRS and surface Electromyography (sEMG) measures taken from the VL are representative of changes in the entire muscle. Both apparatus were placed over a motor point of the VL and therefore would be expected to represent whole muscle changes in metabolic and neuromuscular activity (Kendall, McCreary and Provance 1993).

#### 6. *Subject compliance*

All subjects completed all sessions within the present investigation. It was assumed that subjects followed standardised dietary and prior exercise guidelines for the 24 h prior to the commencement of each testing session.

#### 7. Human ethics constraints

The initial research design had proposed the inclusion of an untrained middle-aged group to allow the effect of training into middle-age to be examined. However, the CQU Human Research Ethics Committee reached the conclusion that the required exercise testing would not be safe in such a population and therefore the group was excluded from the present study.

#### 8. Recovery between heavy and severe-intensity exercise

In the current study, set criteria of a resting  $\dot{V}O_2$  of  $3.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  was used to determine when each subject had adequately recovered from the heavy-intensity exercise bout. At the time of the research design, no data was available detailing the recovery duration required to restore normal on-transient responses to high-intensity exercises. Recently, Burnley, Doust and Jones (2006) produced data suggesting that 45 min of recovery is required to restore normal  $\dot{V}O_2$  kinetics. Therefore, the methods of the current investigation do not meet these criteria and the data from the severe-intensity SWT may have been influenced by the lack of recovery following the heavy-intensity SWT.

## **Delimitations**

The following delimitations may apply to the present study:

1. *Limited sample size*

Due to the time-demanding and invasive nature of the experiments conducted in this thesis, only a small number of well trained young (n=7) and middle-aged (n=7) cyclists were recruited. However, the sample size is typical of that seen in previous reports investigating kinetic responses to repeated exercise bouts and invasive needle biopsies (Barstow, Jones, Nguyen and Casaburi 1996; Pringle, Doust, Carter, Campbell, Tolfrey and Jones 2002; DeLorey et al. 2004a; DeLorey et al. 2005).

2. *Gender restriction of subjects*

The subjects recruited within the present series of studies were restricted to males to avoid any effect of gender or hormonal changes due to menstruation or menopause in either age-group.

3. *Sport restriction of the subjects*

The subjects recruited within the present study were restricted to competitive triathletes and cyclists currently in training. The results of the present study may therefore not apply to older athletes who are competitive in different sports or activities.

4. *Age restriction of subjects*

The age of the well-trained cyclists recruited for the present series of studies were limited to young (18-25 y) and middle-aged (45-55 y) athletes.

#### 5. *Sample Representation*

The data contained within the present series of studies are based from a specific sample of subjects, and therefore might not be representative of similar populations.

#### 6. *Dietary Guidelines*

The cyclists were only given food and fluid intake instructions for the 12 h period prior to any exercise testing session. No attempt was made to restrict or monitor dietary intake outside of this time period.

#### 7. *Representative nature of the NIRS and sEMG results*

The NIRS and sEMG results are representative of the VL and VM, and other proximal muscles. During a crank cycle, all thigh and shank muscles are activated periodically at particular activation ranges (Jorge and Hull 1986; Raymond, Joseph and Gabriel 2005; Chapman, Vicenzino, Blanch, Knox and Hodges 2006). Therefore, VL and VM activity have been recorded as an indicator of changes in muscle activity and fibre recruitment within the thigh and shank.

## CHAPTER 2

# REVIEW OF LITERATURE

To date, it is not clear whether the reported age-related slowing of the pulmonary oxygen consumption ( $\dot{V}O_2$ ) and muscle oxygenation (mOxy) responses is the result of a prolonged sedentary lifestyle or a significant effect of aging *per se*. From what evidence is available, it appears that previous 'age-related' adaptations are attenuated in older, well-trained individuals compared to age matched sedentary controls. The majority of research detailing the metabolic responses to changes in work intensity has provided information about aged sedentary individuals. However, little is known as to the benefits of physical training into middle-age on these responses. Therefore, this review has several purposes:

- To review the available literature describing the effect of aging on the  $\dot{V}O_2$  and mOxy responses to changes in work intensity;
- To discuss the nature of the on- and off-transient  $\dot{V}O_2$  and mOxy kinetic responses across increasing exercise intensities;
- To identify and discuss any possible causal or contributing mechanisms of the  $\dot{V}O_2$  and mOxy slow component during high-intensity exercise;
- To discuss physiological factors that may influence the nature of the on- and off-transient  $\dot{V}O_2$  and mOxy responses; and,
- To review the literature describing the effects of physical training on the  $\dot{V}O_2$  and mOxy responses across increasing exercise intensities.

## BACKGROUND INFORMATION

Historically, an important area of exercise physiology research has focused upon the adaptation of  $\dot{V}O_2$ , heart rate (HR) and mOxy to adapt to changes in work intensity (Zoladz, Duda and Majerczak 1998; Tschakovsky and Hughson 1999; Xu and Rhodes 1999; Bangsbo 2000; Borsheim and Bahr 2003). Within this area of research, a topic of much debate relates to the factors associated with the observed exponential increases in  $\dot{V}O_2$  and mOxy with changes in work rate, and whether the mechanisms underpinning these responses are limited by  $O_2$  utilisation or delivery (Richardson, Grassi, Gavin, Haseler, Tagore, Roca and Wagner 1999; Grassi 2000; Grassi 2001; Grassi 2005; Jones and Poole 2005b). Similar debate has focused upon the off-transient  $\dot{V}O_2$  and mOxy responses. However, fewer studies have reported on the nature of the off-transient  $\dot{V}O_2$  and mOxy responses (Chilibeck, Paterson and Cunningham 1995; Puente-Maestu, Tena, Trascasa, Perez-Parra, Godoy, Garcia and Stringer 2003; duManoir, Delorey, Heenan, Kowalchuk and Paterson 2005).

Substantial research has also reported upon a gradual drift in  $\dot{V}O_2$  during high-intensity constant-load exercise which has been termed the  $\dot{V}O_2$  slow component (Poole, Schaffartzik, Knight, Derion, Kennedy, Guy, Prediletto and Wagner 1991; Barstow 1994; Poole, Barstow, Gaesser, Willis and Whipp 1994). Such a phenomenon has recently been observed in the mOxy responses to similar exercise intensities (Miur et al. 1999; Demarie et al. 2001). To date, no definitive causal mechanisms have been identified to explain the  $\dot{V}O_2$  and mOxy slow components, although a number have been proposed, including increases in muscle temperature, decreases in muscle pH, and/or

shifts in muscle fibre recruitment patterns (Poole 1994; Poole et al. 1994; Whipp 1994; Xu and Rhodes 1999; Borrani, Candau, Millet, Perrey, Fuchslocher and Rouillon 2001; Zoladz and Korzeniewski 2001; Garland, Newham and Turner 2004).

Given that the  $\dot{V}O_2$  response to exercise is related to both the extraction and consumption of  $O_2$  within the muscle, more recent and novel research using Near Infrared Spectroscopy (NIRS) has enabled the investigation of changes in mOxy during periods of exercise transition (Mancini 1997; Neary 2004). These studies have provided data to help understand the relationship between pulmonary  $\dot{V}O_2$  responses and real-time changes in  $O_2$  content within the working muscle (Bhambhani 2004; Neary 2004). However, despite the recent application of NIRS, limited studies have investigated the relationships between concurrent  $\dot{V}O_2$  and mOxy kinetics, particularly within trained and/or aged populations (Babcock et al. 1992; 1994a; 1994b; DeLorey, Kowalchuk and Paterson 2003a; 2003b; Grassi et al. 2003; Stathokostas et al. 2003). Therefore, this review of literature aims to primarily discuss the nature of metabolic adaptation throughout exercise transitions, and secondly, to discuss the effects of physical training and age on these metabolic responses.

### **Measurement of Oxygen Uptake Responses**

The quantification of  $\dot{V}O_2$  kinetics is often utilised as a measure of an individual's ability to metabolically adapt to changes in work intensities (Jones and Poole 2005a). Changes in the utilisation and delivery of  $O_2$  are required to allow increased aerobic energy metabolism and maintenance of the cellular homeostatic environment (Tschakovsky and Hughson 1999; Xu and Rhodes

1999; Bangsbo 2000). Measurement of  $\dot{V}O_2$  kinetics has historically been performed using mass spectrometers or automated gas analysis systems for over thirty years (Whipp and Wasserman 1972; Xu and Rhodes 1999), and is based upon the measurement of  $O_2$  and  $CO_2$  concentrations of the expired air from the lungs.

At exercise onset, the  $\dot{V}O_2$  response follows an exponential path until it reaches a steady-state matched to the required rate of aerobic ATP production of the exercise intensity (Tschakovsky and Hughson 1999; Bangsbo 2000). However,  $\dot{V}O_2$  does not instantaneously increase to the amplitude required to fulfil the aerobic metabolism demands (Di Prampero, Boutellier and Pietsch 1983; Barstow, Casaburi and Wasserman 1993; Chilibeck et al. 1998). There is a lagging of the  $\dot{V}O_2$  response that has been related to a number of physiological mechanisms including the delay in the return of venous mixed  $O_2$ -content blood, muscle phosphocreatine (PCr) kinetics,  $O_2$  delivery to the working muscle and the overcoming of metabolic inertia (Tschakovsky and Hughson 1999; Bangsbo 2000). The magnitude of the mismatch between  $\dot{V}O_2$  demand and actual  $\dot{V}O_2$  response is termed the  $O_2$  deficit (Linnarsson, Karlsson, Fagraeus and Saltin 1974; Bearden and Moffatt 2000). The nature of the exponential  $\dot{V}O_2$  increase at exercise onset is commonly quantified by exponential equations which provide a number of amplitude and speed parameters that can be used for analysis of the on-transient response (Morton 1985; Swanson and Hughson 1988; Barstow 1994; Rossiter, Ward, Doyle, Howe, Griffiths and Whipp 1999; Carter et al. 2002).

The measurement and interpretation of the  $\text{VO}_2$  kinetic response helps to provide a great indication and assessment of cardiorespiratory function (Xu and Rhodes 1999). Through these measures, adaptations in metabolic function and cardiorespiratory capacity can be interpreted and quantified (Whipp and Rossiter 2005). However, the use of  $\text{VO}_2$  kinetics does not provide detailed information on the rate at which metabolic adaptations occur within the working muscle but simply provides an overall indication of systemic adaptation and efficiency (Behnke, Barstow and Poole 2005; Whipp and Rossiter 2005).

The monitoring of mOxy helps to describe changes in both muscle  $\text{O}_2$  extraction in response to imposed work bouts and changes in the working muscles capacity for  $\text{O}_2$  utilisation (Maikala and Bhambhani 1999; Boushel and Piantadosi 2000; Quaresima and Ferrari 2002a). The concurrent measurement of both  $\text{VO}_2$  and mOxy helps to provide measures of both systemic and peripheral responses to exercise. The on-transient  $\text{VO}_2$  response is multifactorial, and relies upon the capacity to rapidly change the delivery and utilisation of  $\text{O}_2$  within the working muscle. However, while the measurement of  $\text{VO}_2$  kinetics provides an adequate measure of such capacity, the monitoring of  $\text{O}_2$  utilisation through the use of NIRS within the working muscle may help to identify peripheral limitations to this metabolic adaptation (Mancini 1997; Ding et al. 2001).

### **Measurement of Muscle Oxygenation Responses**

While the description of pulmonary  $\text{VO}_2$  kinetics provides a valid and useful indication of cardiorespiratory function, it does not allow the measurement of changes in the  $\text{O}_2$  content of the working muscle. The

monitoring of changes in mOxy during the adaptation to work bouts may provide useful information on both the utilisation of  $O_2$  within the working muscle and peripheral oxygen utilisation limitations (Belardinelli et al. 1995a; 1995b). Such observations may contribute to the  $O_2$  utilisation or delivery limitation debate and provide data to explain the development of the  $\dot{V}O_2$  slow component. Changes in mOxy following exercise bouts may also give useful information as to the rate of muscle reoxygenation and recovery (Puentes-Maestu et al. 2003; duManoir et al. 2005). Thus, the measurement and quantification of mOxy helps to provide an indirect non-invasive assessment of the rate of  $O_2$  extraction within the working muscle, allowing greater insight into intra-muscular  $\dot{V}O_2$  kinetics (Quaresima and Ferrari 2002b; Quaresima, Lepanto and Ferrari 2003; Neary 2004).

NIRS is a relatively recent research technology which allows the real time quantification of mOxy within working muscle during exercise (Quaresima and Ferrari 2002a; Quaresima et al. 2003; Neary 2004). NIRS measures changes in the optical density of light shone into the muscle to quantify changes in mOxy throughout exercise. NIRS systems monitor the optical density of the light reflecting out of the muscle at 760 nm and 850 nm, which correspond to concentrations of Hb/Mb and  $HbO_2/MbO_2$ , respectively (Chance et al. 1992). Relative changes in mOxy status are commonly interpreted as the difference between the optical density at the two wavelengths ( $\Delta 760-850$  nm) (Chance et al. 1992; van Beekvelt, Colier, Wevers and van Engelen 2001). The theoretical basis of NIRS relies upon the Beer-Lambert law modified for scattering media (Schmidt 1999; Boushel and Piantadosi 2000; Quaresima et al. 2003).

The modified Beer-Lambert law states that the amount of light recovered from an illuminated tissue depends upon the intensity of incident light on the tissue, the physical separation of the diodes and photodetectors, the degree of light scattered by tissue, and the amount of tissue absorbency due to chromophore concentration within the tissue (Maikala and Bhambhani 1999). The intensity of the light returning to the photodetectors is dependent upon how saturated the localised muscle and surrounding microvascular structures are with Hb and HbO<sub>2</sub> (i.e. arterioles, capillaries and venules) (DeLorey et al. 2003b).

The valid monitoring of changes in mOxy is subject to a number of methodological limitations which may impact upon the interpretation of the trends in mOxy. Firstly, given that the absorption spectra of myoglobin (Mb) and oxymyoglobin (MbO<sub>2</sub>) overlap that of Hb and HbO<sub>2</sub>, and therefore the specific concentrations of the two chromophores can not be separated. As such, changes within mOxy are interpreted as changes in the oxygenation state of both HbO<sub>2</sub> and MbO<sub>2</sub> stores (Chance et al. 1992). There is debate as to the contribution of Mb to changes in mOxy, with some researchers suggesting it may contribute as much as 25-35% (Chance et al. 1992). Secondly, the differential path length of the NIR light cannot be measured, and as a result the changes in absolute concentrations in Hb/Mb and HbO<sub>2</sub>/MbO<sub>2</sub> are not quantifiable (Bhambhani, Maikala, Jeon and Bell 1998). The majority of investigations report relative changes in mOxy determined through the application of cuff ischemia of the thigh. This technique provides a nadir value of complete tissue deoxygenation (0%) and a hyperaemic response interpreted

as complete reoxygenation (100%) (van Beekvelt et al. 2001; Quaresima and Ferrari 2002a; 2002b).

The thickness of subcutaneous fat below the NIRS probe location may also affect the quality of the NIRS signal within the active muscle (Homma, Fukunaga and Kagaya 1996; Lin, Niwayama, Shiga, Kudo, Takahashi and Yamamoto 2000; Hiroyuki, Hamaoka, Sako, Nishio, Kime, Murakami and Katsumura 2002). Despite reporting this, Homma et al. (1996) observed that NIR light penetrates shallow portions (2-4 cm) of the muscle despite an adipose tissue thickness of up to 15 mm. Hiroyuki et al. (2002) suggested that this limitation of varying subcutaneous fat thickness may be minimised by normalising individual NIRS signals through use of cuff ischemia as discussed above, reporting localised subcutaneous fat measurements, and ensuring homogenous subject characteristics.

Given the small area of muscle monitored by the NIRS probe, the changes observed in mOxy are accepted as being reflective of the whole muscle. Historically, the NIRS probe is positioned over the belly of the VL muscle, 14 cm superior to the patella (Chance et al. 1992; Sahlin 1992; Belardinelli et al. 1995a; Bhambhani, Buckley and Susaki 1997; Costes, Prieur, Feasson, Geyssant, Barthelemy and Denis 2001; Neary, McKenzie and Bhambhani 2002; Grassi et al. 2003). As this point is reported to be a motor point of the VL, it should therefore reflect all recruitment and metabolic activities of the muscle (Kendall et al. 1993). A third limitation of the technology, is that the scattering of light within the muscle may not be consistent within or between

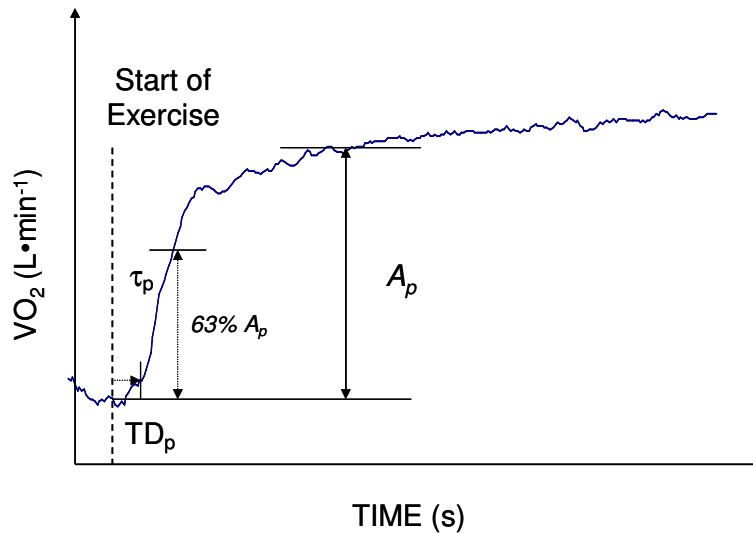
an individual and therefore the use of a constant scattering coefficient may overestimate changes in NIRS variables during exercise (Ferreira et al. 2007).

Despite its limitations, the use of NIRS technology allows the continuous *in vivo* measurement of mOxy during exercise transitions (Chance et al. 1992; Quaresima et al. 2003). Changes in mOxy during exercise reflect the balance between O<sub>2</sub> delivery and utilisation within the localised working muscle, and published data strongly suggest that the use of NIRS is both valid and reliable (Bhambhani et al. 1998). It is suggested that the monitoring of mOxy during exercise transitions provides much more specific and valid measures of changes in the muscle  $\dot{V}O_2$ , than the systemic  $\dot{V}O_2$  measured at the mouth (Maikala and Bhambhani 1999; Boushel and Piantadosi 2000; Neary 2004). However, the concurrent monitoring of both  $\dot{V}O_2$  and mOxy kinetics during exercise transitions may allow a greater understanding of the rate at which O<sub>2</sub> is extracted within the working muscle (Quaresima et al. 2003; Neary 2004). The comparison of such systemic and peripheral measures of O<sub>2</sub> consumption or extraction is likely to provide useful information regarding the metabolic adaptation during both the on- and off-transients to an exercise bout.

## **ON-TRANSIENT KINETIC RESPONSES**

The  $\dot{V}O_2$  and mOxy responses to SWT are most commonly fitted using either a single or double-exponential function to quantify the magnitude and speed of the response. Whipp and Wasserman (1972) were the first to report upon changes in  $\dot{V}O_2$  kinetics in terms of fitting the on-transient response to a single component exponential function. These investigators were the first to use such techniques in order to quantify the nature of such metabolic responses.

Historically, the on-transient  $\dot{V}O_2$  response has been reported to comprise three separate components as described below (Whipp and Wasserman 1972; Xu and Rhodes 1999). A typical  $\dot{V}O_2$  response to moderate-intensity exercise is presented as Figure 2.1.



**Figure 2.1:** Schematic representation of the on-transient  $\dot{V}O_2$  response to moderate-intensity steady-state exercise ( $A_p$  = Primary component amplitude;  $TD_p$  = Primary component time delay;  $\tau_p$  = Primary component time constant).

1. *Phase I (initial 20 s):* is termed the *cardiodynamic phase* and represents an initial linear rise in  $\dot{V}O_2$  that typically represents the increased ventilation and cardiac responses required of the exercise bout (Jones and Poole 2005).
2. *Phase II:* consists of a rapid exponential increase in  $\dot{V}O_2$  in response to the muscle  $\dot{V}O_2$  requirements of the working muscle. This increase reflects the influence of the metabolic and  $O_2$  content change within the muscle (Xu and Rhodes 1999). It has also been reported that this phase is reflective of both PCr and muscle  $O_2$  utilisation kinetics (Barstow 1994;

Barstow, Buchthal, Zanconato and Cooper 1994). Phase I and II are often grouped together and comprise the *primary component*.

3. *Phase III*: at intensities  $<V_T$  it is represented by a steady state  $\dot{V}O_2$  matched to the required  $\dot{V}O_2$  for maintenance of aerobic ATP production. At exercise intensities  $>V_T$ , this phase is observed as a decrease in metabolic efficiency or the  $\dot{V}O_2$  slow component.

The majority of previous investigations have recommended that the first 20 s of  $\dot{V}O_2$  adjustment are removed for analysis as to remove the Phase I dynamics which may influence the fitting of the subsequent functions (Rossiter et al. 1999; Koppo, Bouckaert and Jones 2004). The intensity of the exercise bout determines the nature of the modelling equation. Moderate-intensity ( $<V_T$ ) exercise is universally fitted using a single component function, whereas heavy or severe-intensity exercise ( $>V_T$ ) is fitted using a double component function, assuming a visible slow component is observed (Bearden and Moffat 2001). The use of a double exponential function in order to quantify the  $\dot{V}O_2$  slow component has been shown to be a much more valid and accurate quantification method compared to the calculation of the absolute difference between the third and sixth minute of a high-intensity SWT (Bearden and Moffat 2001).

In regards to the adaptation of mOxy at the onset of exercise, the adaptation to the required steady-state values is significantly faster than that of the  $\dot{V}O_2$  response (Kawaguchi, Tabusadani, Sekikawa, Hayashi and Onari

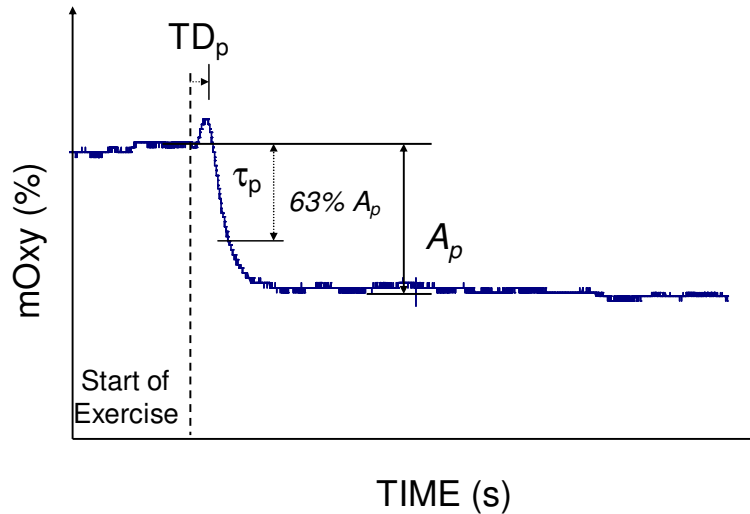
2001; DeLorey, Kowalchuk and Paterson 2002; Grassi et al. 2003; DeLorey et al. 2003b; 2004a; 2005).

Research from Chance and others (1992) and Belardinelli and colleagues (1995b) has previously proposed that the on-transient mOxy response to exercise comprises a three phase response (similar to  $\dot{V}O_2$ ):

1. *Phase I*: is represented by an immediate increase in mOxy in comparison to resting baseline values. This is most likely due to an increase in limb blood flow, and HbO<sub>2</sub> concentration (Belardinelli, Barstow et al. 1995b).
2. *Phase II*: is represented by an exponential decline in mOxy from the hyperaemic value which decreased below resting values (in response to a load) until the O<sub>2</sub> utilisation meets the aerobic metabolism demands. This phase coincides with the rapid exponential increase witnessed in  $\dot{V}O_2$  measured at the mouth.
3. *Phase III*: is characterised during moderate-intensity constant load exercise by a plateau in mOxy that is equal to the O<sub>2</sub> consumption required for the energy intensity. During high-intensity exercise (>VT), the gradual decrease in mOxy is thought to represent the development of the slow component within the working muscle (Demarie et al. 2001).

The on-transient mOxy response is quantified using similar modelling techniques to those described above for the  $\dot{V}O_2$  response. A number of previous investigations have demonstrated that mOxy responses during both

exercise on- and off-transition periods could be fitted to either single or double-exponential component functions (Bhambhani et al. 1999; Grassi et al. 2003; Shibuya, Tanaka and Ogaki 2004).



**Figure 2.2:** Schematic representation of the primary mOxy on-transient response to moderate-intensity exercise ( $A_p$  = Primary component amplitude;  $TD_p$  = Primary component time delay;  $\tau_p$  = Primary component time constant).

Another commonly used kinetic parameter to describe the overall response of both the combined primary and slow components is the weighted mean response time (wMRT). The wMRT is representative of the time taken to reach 63% of the total  $\dot{V}O_2$  or mOxy amplitude to a SWT, rather than an individual component. This value is of interest to researchers given it is equal to a time constant of the total response, which is a method of quantifying speed, and five time constants should attain ~99% of the total amplitude response (Whipp and Rossiter, 2005). Therefore, quantify the time taken to reach 63% of the response amplitude provides a standardise measure of the speed of adaptation. The wMRT is likely to vary between the  $\dot{V}O_2$  and mOxy measures, given the quicker response time in changes of mOxy (DeLorey et al. 2002;

DeLorey et al. 2003b; Grassi et al. 2003). Normal wMRT observed for  $\dot{V}O_2$  and mOxy responses throughout the on-transient period range from 30-65 s and 15-60 s, respectively (Stathokostas et al. 2003; DeLorey et al. 2004a; DeLorey et al. 2005; duManoir et al. 2005).

The modelling of both the on-transient  $\dot{V}O_2$  and mOxy responses is important to help provide a standardised quantitative measurement of the metabolic adaptation to exercise transitions (Walsh and Lee 1998). These kinetic parameters help researchers to both quantify the rate of metabolic adjustment and investigate methods to manipulate the speed of this adjustment (Whipp and Rossiter 2005). Similarly, the amplitude of the  $\dot{V}O_2$  and mOxy responses may help to assess both the muscular energetic and  $O_2$  cost of SWT intensities. These measures may also be useful to track changes in cardiorespiratory function with age, physical training or across intensity domains in normal, clinical and athletic populations. Previously, both the amplitude of the  $\dot{V}O_2$  and mOxy primary and slow components has been reported to be influenced by a wide range of factors. For the purpose of the current investigation, these primarily relate to aging (Babcock et al. 1992; Chilibeck et al. 1995; Chilibeck et al. 1998), physical training (Casaburi, Storer, Bendov and Wasserman 1987; Gaesser 1994; Edge, Bishop and Duffield 2003; Saunders, Evans, Arngrimsson, Allison and Cureton 2003; Koppo, Bouckaert et al. 2004; Berger, Rittweger, Kwiet, Michaelis, Williams, Tolfrey and Jones 2006; Berger, Tolfrey, Williams and Jones 2006), and/or exercise intensity (Draper, Wood and Fallowfield 2003; Pringle et al. 2003b; Koppo et al. 2004).

With regards to the speed of the metabolic adaptation, researchers commonly report the time delay (TD) and time constant ( $\tau$ ) of each separate component as indices of the rate of this adaptation. At present, the majority of literature describing  $\dot{V}O_2$  kinetics has excluded the Phase I component from the modelled  $\dot{V}O_2$  response as a result of the lack of  $\dot{V}O_2$  adjustment within this 20 s period (Rossiter et al. 1999; Koppo et al. 2004). Zoladz et al. (1998) have suggested that the omission of this first 20 s also removes any physiological changes which may be incurred during the physical response of adaptation to the work load. The primary component TD ( $TD_p$ ) is defined as the time taken for  $\dot{V}O_2$  to initiate its exponential increase following exercise transition. The slow component time delay ( $TD_s$ ) is the time taken for the slow component to originate after initial exercise onset (Koga et al. 2005).

Normal  $\dot{V}O_2$   $TD_p$  and  $TD_s$  range between 10-25 s and 100-150 s for the primary and slow  $\dot{V}O_2$  components, respectively (Paterson and Whipp 1991; Pringle et al. 2003b; Koppo et al. 2004). Both the  $\dot{V}O_2$   $TD_p$  and  $TD_s$  appear to be stable across exercise intensities (Pringle et al. 2003b). The fitting of the mOxy response has previously been shown to display a shorter TD than the  $\dot{V}O_2$  response, with mOxy  $TD_p$  and  $TD_s$  ranging from between 3-8 s, and between 70-80 s for the primary and slow components, respectively (Miura et al. 1999; Demarie et al. 2001). This difference is most likely due to the required transport time of blood from the working muscle where it is deoxygenated to the lungs where the  $\dot{V}O_2$  is measured at the mouth. Changes in mOxy within the working muscle are instantaneous and are not subject to such transit delays (Grassi et al. 2003; DeLorey et al. 2002; 2003b; 2005).

The majority of literature examining  $\dot{V}O_2$  kinetics has focused upon the rapid exponential increase in the on-transient  $\dot{V}O_2$  response (Rossiter et al. 1999; Burnley, Jones, Carter and Doust 2000a; Burnley, Jones, Carter and Doust 2000b; Lucia, Hoyos, Santalla, Perez and Chicharro 2002; Koppo et al. 2004). It is unclear as to whether the primary component on-transient  $\dot{V}O_2$  kinetics are limited by the delivery of  $O_2$  to the working muscle, or the ability of the muscle to utilise  $O_2$  during exercise transients (Richardson et al. 1999; Grassi 2000; Grassi 2001). Whilst the majority of empirical evidence suggests that the utilisation of  $O_2$  controls  $\dot{V}O_2$  adjustment during the initial adaptation, no definitive explanation has been put forward to support this suggestion (Grassi 2005; Kalliokoski, Knuuti and Nuutila 2005). The speed of the rapid exponential  $\dot{V}O_2$  increase (Phase II) is most commonly represented through the primary time constant ( $\tau_p$ ), which represents the time taken to reach 63% of the primary component amplitude (Koga et al. 2005). Normal  $\dot{V}O_2$   $\tau_p$  values reported within the literature range from 15-30 s for SWT adaptation, or between 30-45 s for ramp test in young healthy subjects (Paterson and Whipp 1991; Pringle et al. 2003b; Koppo et al. 2004). The  $\dot{V}O_2$   $\tau_p$  values may lengthen with factors such as sedentary aging (Babcock et al. 1992; 1994a; 1994b; DeLorey et al. 2004a; 2005), reductions in  $O_2$  delivery or utilisation (Tschakovsky and Hughson 1999; Xu and Rhodes 1999; Bangsbo 2000; Grassi 2005) or disease states (Bauer, Regensteiner, Brass and Hiatt 1999; Pouliou, Nanas, Papamichalopoulos, Kyprianou, Perpati, Mavrou and Roussos 2001; Puente-Maestu et al. 2003). Normal mOxy  $\tau_p$  have been reported to be much faster than the  $\dot{V}O_2$   $\tau_p$ , and are typically between 8-16 s in healthy younger and older subjects (DeLorey et al. 2004a; 2005).

The reporting and comparison of these  $\dot{V}O_2$  and  $mO_2$  amplitude and speed parameters discussed above help to provide a greater understanding of the metabolic responses to bouts of exercise. To date, the influence of exercise intensity on  $\dot{V}O_2 \tau_p$  remains equivocal. Previous research suggests the  $\dot{V}O_2 \tau_p$  either remains constant (Barstow and Mole 1991; Barstow et al. 1993; Carter, Jones, Barstow, Burnley, Williams and Doust 2000a; Ozyener, Rossiter, Ward and Whipp 2001) or lengthens with increasing exercise intensity (Casaburi, Barstow, Robinson and Wasserman 1989; Paterson and Whipp 1991; Phillips, Green, Macdonald and Hughson 1995; Engelen, Porszasz, Riley, Wasserman, Maehara and Barstow 1996; Jones, Carter, Pringle and Campbell 2002; Koppo et al. 2004). The lengthening of the  $\dot{V}O_2 \tau_p$  has been reported in both trained (Koppo et al. 2004) and untrained (Paterson and Whipp 1991) subjects and is thought to represent an increased delay in the utilisation or delivery of  $O_2$  within the muscle in response to the increased exercise intensity (Koppo et al. 2004). In contrast, the suggestion of a stable  $\dot{V}O_2 \tau_p$  is supportive of  $O_2$  utilisation limitations controlling the speed of  $\dot{V}O_2$  adjustment regardless of intensity (Carter et al. 2000; Ozyener et al. 2001).

For the on-transient response, the major limiting factors responsible for the lagging metabolic response remain unknown (Grassi 2000; 2001). However, the on-transient response appears to be limited by either the delayed utilisation or delivery of  $O_2$  within the working muscle (Grassi 2005). In order to investigate these factors limiting the on-transient responses, a number of research investigations have attempted to manipulate the delivery or utilisation of  $O_2$  during adaptations to various exercise intensities or modality, lactate and catecholamine concentrations, muscle temperature, histochemical and

biochemical characteristics, training status and age (Poole et al. 1994; Tschakovsky and Hughson 1999; Bangsbo 2000; Zoladz and Korzeniewski 2001; Borsheim and Bahr 2003). To date, these investigations have failed to identify the major limiting factors for metabolic adaptation to an exercise bout.

As discussed above, the relationship between the  $\dot{V}O_2$  and mOxy responses is comparable and appears to be influenced by similar factors. Given the recent introduction of NIRS technology, there is limited research literature which has concurrently examined the concurrent  $\dot{V}O_2$  and mOxy responses to an exercise bout (Babcock et al. 1992; 1994a; 1994b; Grassi et al. 2003; Stathokostas et al. 2003; DeLorey et al. 2003a; 2003b; 2004a; 2005; duManoir et al. 2005). To date, no research has fully examined the effect of aging and/or exercise intensity on the on-transient mOxy responses.

DeLorey et al. (2003b) investigated the concurrent  $\dot{V}O_2$  and mOxy responses in young ( $26 \pm 3$  y) healthy subjects in response to moderate-intensity SWT. In this study, the observed  $\dot{V}O_2$   $\tau_p$  was  $30 \pm 8$  s, whereas the  $\tau_p$  of the mOxy response was much faster at  $10 \pm 3$  s, suggesting that the extraction of  $O_2$  within the working muscle is more rapid than that observed at the mouth. The wMRT of the mOxy response ( $23 \pm 4$  s) was also significantly faster than the pulmonary  $\dot{V}O_2$  wMRT ( $30 \pm 8$  s). Later work by DeLorey and colleagues (2004a; 2005) investigated the concurrent  $\dot{V}O_2$  and mOxy responses across moderate and heavy-intensity exercise in young ( $24 \pm 4$  y) and old ( $68 \pm 3$  y) sedentary subjects. The investigators reported that the mOxy kinetics were also significantly faster than the  $\dot{V}O_2$  response in both groups.

While the elderly cohort demonstrated a significantly slower  $\dot{V}O_2$  response than the young subjects, no difference was observed in the mOxy response.

The difference between the  $\dot{V}O_2$  and mOxy responses may be due to the factors associated with the utilisation of  $O_2$  within external processes such as the contraction of stabilising and synergistic muscles, intra-muscular buffering reactions, and unrelated metabolic processes (Tschakovsky and Hughson 1999). These suggestions are supported by the previous work of Bangsbo et al. (2000) who observed an initial delay followed by a rapid increase in  $O_2$  extraction within the quadriceps during high-intensity leg-extension exercise which would support the observations observed in mOxy within the data of DeLorey et al. (2003b; 2004a; 2005) and Grassi et al. (2003). Taken together, the findings of these investigators suggest that the decreases within mOxy occur earlier and at a faster rate than the reported exponential increase in pulmonary  $\dot{V}O_2$ .

Thus, the metabolic adaptation at the onset of an exercise bout is met with rapid exponential increases in  $\dot{V}O_2$  and mOxy until the aerobic demands for ATP production are met. While researchers have investigated the  $\dot{V}O_2$  response for a number of decades, the introduction of NIRS to monitor changes in  $O_2$  content within the muscle has been of great significance. The reporting of changes in mOxy helps the understanding of the nature of aerobic metabolic adaptation within the working muscle and may be more valid than measures of pulmonary  $\dot{V}O_2$  which are open to a number of external influences. However, this NIRS research still does not conclusively suggest that the utilisation of  $O_2$  within the muscle is the only limiting factor at exercise onset. A number of other

mechanisms have been observed to influence the  $\dot{V}O_2$  on-transient response (Tschakovsky and Hughson 1999; Xu and Rhodes 1999; Bangsbo 2000).

### **Factors Influencing the On-Transient Responses**

Previous literature has revealed a large number of factors which may influence the on-transient  $\dot{V}O_2$  and  $mO_2$  responses (Grassi, Poole, Richardson, Knight, Erickson and Wagner 1996; Bangsbo et al. 2000; Grassi 2000; Grassi 2001; Grassi 2005; Jones and Poole 2005b; Whipp et al. 2005). These potential factors may alter either the delivery (i.e. gas concentration, body posture, muscle capillarisation) or utilisation (i.e. catecholamines, [BLA], muscle fibre composition and enzyme activity, prior exercise) of  $O_2$  within the working muscle at the onset of exercise. Valid arguments have supported both the  $O_2$  delivery and utilisation hypotheses, and it appears that exercise intensity may influence the limitations which may influence the on-transient metabolic response.

The delivery of  $O_2$  to the working muscle has been suggested to limit the rate of increase in the  $\dot{V}O_2$  response to sudden increases in exercise intensity (Hughson and Smyth 1983; Hughson, Xing, Borkhoff and Butler 1991; Wagner 1995; Tschakovsky and Hughson 1999; Whipp et al. 2005). Tschakovsky and Hughson (1999) suggested that  $O_2$  delivery limitations reflect the resting inertia of transporting the  $O_2$  from the lungs in the blood to the mitochondria within the working muscle. That is, oxidative metabolism and  $O_2$  utilisation can only increase if a higher cellular  $pO_2$  is sustained during exercise on-transitions, suggesting that the  $pO_2$  within the mitochondria is not saturated within all working muscle fibres during the adaptation. This model suggests that an

increased delivery of  $O_2$  to the working muscle would allow sustained utilisation of  $O_2$  through oxidative metabolism through maintaining mitochondrial  $pO_2$  across the metabolic adaptation. In their review, Wagner (1995) identified several factors affecting the delivery of  $O_2$  that may limit the rate of  $\dot{V}O_2$  adjustment at exercise onset. These include a reduced inspired  $pO_2$ , disease, a reduced cardiac output and muscle blood flow, a reduced Hb concentration, or an impaired diffusion of  $O_2$  between red blood cells and the mitochondria. Whilst these are valid factors, it is unlikely that they will be of significant influence to changes in exercise intensity in healthy athletic populations.

In summary, the on-transient  $\dot{V}O_2$  response has been proposed to be limited by the rate of adaptation of the delivery or utilisation of  $O_2$  within the working muscle immediately after load application. While the interaction between these limitations is not fully understood at present, a great deal of research has investigated methods to isolate their effects to improve the on-transient  $\dot{V}O_2$  adaptation (Xu and Rhodes 1999; Grassi 2001; 2005; Jones and Poole 2005a). The following section will discuss empirical research that has examined mechanisms that may limit the on-transient  $\dot{V}O_2$  response through either  $O_2$  utilisation or delivery limitations.

## **$O_2$ Delivery Limitations**

### *Catecholamines*

Research has investigated the influence of catecholamines on the  $O_2$  utilisation and delivery limitations previously discussed (Hughson and Morrissey 1983; Hughson and Smyth 1983; Hughson et al. 1991; Tschakovsky and

Hughson 1999). However, such research has not proven any such effect of catecholamine secretion on the on-transient metabolic response.

To date, a slowed  $\dot{V}O_2$  kinetics response has been observed through the infusion of  $\beta$ -blockers to slow the HR response and reduce  $O_2$  delivery (Hughson and Morrissey 1983). Hughson and Morrissey (1983) investigated the effect of  $\beta$ -blockade on the on-transient  $\dot{V}O_2$  response to submaximal exercise (80% VT) in 17 male subjects ( $21 \pm 1$  y;  $3.84 \pm 0.15$  L $\cdot$ min $^{-1}$ ). While no differences were observed in  $\dot{V}O_2$  amplitude or [BLa], the speed of the  $\dot{V}O_2$  response was significantly slowed with  $\beta$ -blockade. The  $O_2$  deficit was ~200 mL larger and cardiac output significantly reduced in the  $\beta$ -blockade condition. These results suggest that reducing  $O_2$  delivery through slowing the HR responses has a significant slowing effect on the on-transient  $\dot{V}O_2$  response.

In their later review, Tschakovsky and Hughson (1999) suggested that the influence of catecholamines on  $\dot{V}O_2$  adaptation may be the result of the control of the sympathetic nervous system on factors such as HR and stroke volume (SV) during rest or low-intensity exercise ( $<100$  b $\cdot$ min $^{-1}$ ). This may be due to the sympathetic nervous system's role as a slow-acting mediator in cardiac adaptation during low intensity exercise transitions (Åstrand, Rodahl, Dahl and Stromme 2003). Further, it may be possible that administration of adrenaline may also be responsible for an increase in  $O_2$  utilisation within the working muscle, given previous observations showed an increased glycolytic capacity and acetyl group availability within muscle infused with adrenaline (Watt, Howlett, Febbraio, Spriet and Hargreaves 2001). At present, no research is available to validate this suggestion. However, no evidence has reported a

speeded  $\dot{V}O_2$  response across exercise transitions with adrenaline infusion (Gaesser 1994). In summary, the infusion of  $\beta$ -blockade agents has been reported to retard the on-transient  $\dot{V}O_2$  response. This finding suggests other  $O_2$  delivery controlling mechanisms.

### *Inspired Gas Concentrations*

Other research has focused upon the influence of modifying gas concentrations on  $\dot{V}O_2$  kinetics during exercise on-transients (Linnarsson, Karlsson et al. 1974; Hughson and Kowalchuk 1995; MacDonald, Pedersen and Hughson 1997; Bell, Paterson, Kowalchuk and Cunningham 1999; Evans, Savasi, Heigenhauser and Spriet 2001; Peltonen, Tikkanen and Rusko 2001). These studies have consistently shown that the breathing of either hypoxic or hyperoxic gas significantly influences the nature of the on-transient  $\dot{V}O_2$  response.

Hughson and Kowalchuk (1995) investigated the on-transient  $\dot{V}O_2$  kinetics in six healthy volunteers ( $30.3 \pm 3.3$  y) during moderate-intensity (<VT) cycling exercise in hypoxic ( $F_{IO_2} = 0.14$ ), normoxic ( $F_{IO_2} = 0.21$ ) and hyperoxic ( $F_{IO_2} = 0.30$ ) conditions. The on-transient  $\dot{V}O_2$  speed ( $\tau_p$ ; wMRT) were significantly slowed by hypoxia ( $26.6 \pm 2.9$  s;  $35.9 \pm 1.7$  s) compared to the normoxic ( $16.5 \pm 2.8$  s;  $29.5 \pm 1.9$  s) or hyperoxic ( $15.7 \pm 2.1$  s;  $28.6 \pm 1.8$  s) conditions. Furthermore, the  $O_2$  deficit calculated for the hypoxic conditions ( $525 \pm 24$  mL) was significantly larger than that observed for either normoxia ( $420 \pm 29$  mL) or hyperoxia ( $414 \pm 25$  mL) conditions. Therefore, the proposed increased delivery of  $O_2$  through breathing of a hyperoxic gas mixture appears to have no significant influence on the  $\dot{V}O_2$  response during adaptation to

moderate-intensity exercise. In contrast, breathing a hypoxic gas to reduce  $O_2$  delivery to the working muscle was observed to slow the  $\dot{V}O_2$  response.

In a later study, MacDonald et al. (1997) examined the effects of breathing normoxic ( $F_{IO_2} = 0.21$ ) and hyperoxic ( $F_{IO_2} = 0.70$ ) gases during adaptation to both moderate ( $<VT$ ) and heavy-intensity ( $>VT$ ) SWT. The  $\dot{V}O_2$  wMRT was not significantly improved during the moderate-intensity SWT between the hyperoxic ( $31.4 \pm 1.4$  s) and normoxic ( $31.3 \pm 1.3$  s) conditions. Interestingly, the  $\dot{V}O_2$  MRT was significantly faster in the hyperoxic conditions ( $44.1 \pm 5.2$  s) compared to the normoxic ( $53.9 \pm 6.2$  s) for the heavy-intensity SWT. No difference was observed in the magnitude of the  $O_2$  deficit at exercise onset. The results of MacDonald et al. (1997) further support the absence of a significant effect of hyperoxia during transitions to moderate-intensity exercise, and may suggest possible  $O_2$  delivery limitations during high-intensity transitions in normoxic conditions.

In summary, the breathing of hypoxic gas has been observed to slow the on-transient  $\dot{V}O_2$  response as a result of a reduced  $O_2$  delivery within the muscle. In contrast, the inspiration of hyperoxic gas has been shown to improve the on-transient  $\dot{V}O_2$  responses to high-intensity exercise, but not with moderate-intensity exercise. Therefore, these results suggest that the delivery of  $O_2$  to the working muscle may not be an issue at moderate-intensity exercise but may play a role in metabolic adaptations to higher-intensity exercise.

### *Body Posture*

Due to the effects of gravity on the blood flow responses, researchers have investigated alterations in the delivery of  $O_2$  to the working muscle through changing the posture of subjects to augment these gravitational effects (Hughson et al. 1991; MacDonald, Shoemaker, Tschakovsky and Hughson 1998; Sirna, Paterson, Kowalchuk and Cunningham 1998; MacDonald, Naylor, Tschakovsky and Hughson 2001).

Originally, Hughson et al. (1991) investigated the kinetics of ventilation and gas exchange variables during supine and upright cycling in 12 healthy young men during moderate-intensity exercise. A significant increase was noted in the speed ( $\tau_p$ ; wMRT) of the  $\dot{V}O_2$  response of the upright ( $26.3 \pm 1.9$  s;  $31.6 \pm 1.3$  s) compared to the supine ( $35.1 \pm 3.8$  s;  $40.3 \pm 2.3$  s) position. Hughson et al. (1991) suggested that these results supported the hypothesis that a reduction in the delivery of  $O_2$  to working muscles is due to a gravitational reduction in blood flow when in the supine position.

MacDonald et al. (1998) later investigated alveolar  $\dot{V}O_2$  and femoral artery blood flow during upright and supine leg extensor exercise in seven young healthy volunteers during six minutes of knee extension exercise at 40W. The speed ( $\tau_p$ ; wMRT) of the primary  $\dot{V}O_2$  adaptation was significantly slower during the supine ( $38.7 \pm 5.1$  s;  $39.7 \pm 3.8$  s) as opposed to the upright ( $23.1 \pm 4.1$  s;  $29.3 \pm 3.0$  s) condition. This slowing of the  $\dot{V}O_2$  response during supine exercise was matched by a non-significant increase in the wMRT of leg blood flow ( $27.6 \pm 3.9$  s) compared to the upright condition ( $17.3 \pm 4.0$  s). The wMRT for  $\dot{V}O_2$  and leg blood flow were reduced by 35% and 60%, respectively,

during supine versus upright exercise as a result of exercise position. Taken together, these findings support the earlier observation of reductions in  $O_2$  delivery influencing the on-transient  $\dot{V}O_2$  time constant. However, these results were contrasted by Sirna et al. (1998) who showed no difference between the supine and upright cycling positions in either healthy sedentary young ( $n = 8$ , 20-35 y) and old ( $n = 8$ , 60-80 y) subjects.

In summary, it appears that reductions in blood flow and  $O_2$  delivery to the working muscle due to changes in body posture may have a significant influence on the on-transient  $\dot{V}O_2$  response. The work of MacDonald et al. (1998) supports that the slower on-transient  $\dot{V}O_2$  response is related to reductions in blood flow to the working muscle.

#### *Heart Rate Kinetics*

The rate of  $O_2$  delivery to the working muscle also has the capacity to significantly influence the on-transient  $\dot{V}O_2$  response, particularly to high-intensity exercise. At exercise onset, it appears that HR increases exponentially before reaching a steady state adequate to match the metabolic demands for the work intensity, and the nature of the HR and  $\dot{V}O_2$  responses would be somewhat related (Hughson and Morrissey 1983; Kay, Ashar, Bubien and Dailey 1995).

At present, it appears that the HR wMRT is similar to that of  $\dot{V}O_2$  for moderate-intensity exercise, but longer for high-intensity exercise (Sietsema, Daly and Wasserman 1989). Sietsema et al. (1989) investigated the influence of work rate on the early dynamics of both  $\dot{V}O_2$  and HR in ten healthy young

male subjects (29-42 y;  $47 \pm 14 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) across a number of various-intensity six minute SWT (25 W, 50 W, 100 W and 150 W). The researchers suggested that the early dynamics of HR and  $\dot{V}\text{O}_2$  were dependent on both the exercise intensity and the individual's aerobic fitness level. This observation may be the result of the use of absolute rather than relative work loads in this investigation. Later research by Chilibeck et al. (1996b) reported upon the cardiorespiratory kinetics during bouts of plantar flexion and cycling in young (Y) ( $26.3 \pm 2.5 \text{ y}$ ) and old (O) ( $66.7 \pm 6.7 \text{ y}$ ) groups at varying exercise intensities. The younger cohort demonstrated a significantly shorter on-transient HR  $\tau$  than the elderly cohort for the cycling exercise (Y:  $46.4 \pm 22.3 \text{ s}$ ; O:  $73.1 \pm 36.4 \text{ s}$ ) but not the plantar flexion exercise (Y:  $35.3 \pm 28.0 \text{ s}$ ; O:  $32.6 \pm 29.3 \text{ s}$ ) which was also observed in the  $\dot{V}\text{O}_2$  response. It is likely that this is a result of the cycling exercise being performed at 90% VT, whereas the plantar flexion was performed at 45% peak work rate, suggesting significantly different exercise intensities. The HR  $\tau$  was strongly correlated to the  $\dot{V}\text{O}_2 \tau_p$  during both the cycling and plantar flexion exercise for the young group and the cycling modality for the elderly group (Chilibeck et al. 1996a). The researchers suggested that this may reflect a relationship between the  $\dot{V}\text{O}_2$  response and the adaptation of  $\text{O}_2$  delivery at the onset of exercise.

In summary, these results provide equivocal findings suggesting that HR kinetics influence the on-transient  $\dot{V}\text{O}_2$  response. A slowed HR response would result in a reduction in the speed of  $\text{O}_2$  delivery across exercise intensities. The above evidence suggests  $\text{O}_2$  delivery limitations control the speed of metabolic adaptation at exercise onset, in particular to high-intensity constant load exercise.

## *Lactate*

Lactate is a common by-product and widely used measure of anaerobic metabolism, together with decreases in muscle and blood pH (Åstrand et al. 2003). Previous research has observed that  $[BLa^-]$  is correlated with a number of speed and amplitude parameters from the  $\dot{V}O_2$  and mOxy responses (Roston, Whipp, Davis, Cunningham, Effros and Wasserman 1987; Barstow et al. 1993; Demarie et al. 2001; Jones, Koppo and Burnley 2003). A rightward shift noted in the  $HbO_2$  dissociation curve due to a decrease in muscle and blood pH may help to improve  $O_2$  delivery during exercise transients and act to accelerate the  $\dot{V}O_2$  and mOxy responses (Roston et al. 1987; Gaesser and Poole 1996; Grassi et al. 1999; Demarie et al. 2001).

Edge et al. (2003) recently investigated the  $\dot{V}O_2$  response at the onset of high intensity exercise and the accumulation of metabolites in the blood and muscle in 17 active young females. A significant correlation was observed between the  $\dot{V}O_2 \tau_p$  and the change in  $[BLa^-]$  across the high-intensity exercise bout ( $r = 0.60$ ,  $p < 0.05$ ). The  $O_2$  deficit was significantly related to changes in both muscle ATP stores ( $r = 0.64$ ,  $p < 0.05$ ) and blood pH ( $r = 0.51$ ,  $p < 0.05$ ), but not  $[BLa^-]$ . The results of Edge et al. (2003) contradicted previous findings that observed no relationship between the primary  $\dot{V}O_2$  response and  $[BLa^-]$  accumulation. More recently, Endo, Usui, Fukuoka, Miura, Rossiter and Fukuba (2004) reported that the elevation of  $[BLa^-]$  by prior supra-threshold intensity was not significantly related to the on-transient  $\dot{V}O_2 \tau_p$ . These investigators did observe a significant inverse relationship ( $r = -0.41$ ,  $p < 0.05$ ) between the  $\dot{V}O_2 \tau_p$  across a wide range of  $[BLa^-]$ . Similar results were previously presented by

Burnley, Doust and Jones (2002b) throughout bouts of heavy-intensity and maximal sprint exercise.

Researchers have also suggested that increased  $[BLa^-]$  and decreased muscle and blood pH resulting from high-intensity exercise may facilitate a rightward shift in the  $HbO_2$  dissociation curve to increase  $O_2$  delivery to within the muscle cell (Stringer, Wasserman, Casaburi, Porszasz, Maehara and French 1994). Given that the mOxy is calculated as the difference between total Hb and  $HbO_2$  (Chance et al. 1992), any factor which allows greater release of  $O_2$  from Hb will allow greater muscle deoxygenation. To date, no data have demonstrated significant relationships between the on-transient mOxy responses and changes in muscle or blood pH during exercise.

In summary, recent research suggests that the concentration of  $[BLa^-]$  either influences or is related to the on-transient  $VO_2$  and mOxy responses to exercise. The effect of an increased  $[BLa^-]$  *per se* on these metabolic responses is unknown, given the additional changes in muscle metabolism that result from prior exercise.

### *Muscle Temperature*

It has been widely suggested that changes in muscle temperature may influence the on-transient  $VO_2$  response through either the  $Q_{10}$  effect, vasoconstriction or vasodilation responses, or the Bohr effect (Beelen and Sargeant 1991; Koga, Shiojiri, Kondo and Barstow 1997; Shiojiri, Shibasaki, Aoki, Kondo and Koga 1997; Binzoni and Delpy 2001; Ferguson, Ball and Sargeant 2002). In further support of this suggestion, previous research has

shown that  $\dot{V}O_2$  kinetics are slowed with reduced muscle temperature (Ishii, Ferretti and Cerretelli 1992; Ferretti, Binzoni, Hulo, Kayser, Thomet and Cerretelli 1995). However, no evidence has shown that increases in muscle temperature through hot water convection (Koga et al. 1997) or prior exercise (Koppo, Jones, Vanden Bossche and Bouckaert 2002) speeds the on-transient  $\dot{V}O_2$  response.

Ishii et al. (1992) investigated  $\dot{V}O_2$  kinetics in response to cycling at 75 W and 125 W following a reduction in muscle temperature from  $35.5 \pm 1.0$  °C to  $28.0 \pm 1.6$  °C due to cold water immersion in six untrained men ( $31 \pm 8$  y). The results showed that the  $O_2$  deficit for the cold condition was slightly increased compared to normal muscle temperature at both 75 W (803 vs. 714 mL) and 125 W (1360 vs. 1283 mL). The  $\dot{V}O_2$   $\tau_p$  was slightly longer following the cold-water immersion ( $41.4 \pm 10.0$  vs.  $36.2 \pm 6.7$  s @ 75 W;  $43.8 \pm 14.0$  vs.  $41.6 \pm 8.6$  s @ 125 W). In support of these findings, Ferretti et al. (1995) later reported that the half-time ( $\tau_{1/2}$ ) of the  $\dot{V}O_2$  response ( $43.4 \pm 8.6$ ;  $33.3 \pm 5.0$  s) and  $O_2$  deficit ( $3.05 \pm 1.12$ ;  $2.30 \pm 0.68$  L) were significantly longer and greater in cold ( $27.5 \pm 1.8$  °C) versus normal temperature ( $34.5 \pm 1.2$  °C) conditions during heavy-intensity cycling. The proposed mechanism responsible was a leftward shift in the  $HbO_2$  dissociation curve as a result of a decreased muscle and blood temperature which may have decreased the  $pO_2$  within the working muscle.

Of greater interest is whether the  $\dot{V}O_2$  and mOxy kinetic responses are improved through the elevation of muscle temperature. Koga et al. (1997) elevated the temperature of the thigh muscle to  $\sim 39^\circ\text{C}$  in young male subjects

( $25.7 \pm 9.2$  y;  $44.5 \pm 9.8$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ) using hot water pants prior to completing repeat SWT at intensities of 50W and 50% of the difference between VT and  $\dot{V}O_{2\text{max}}$  (50% $\Delta$ ). The results revealed no significant differences in the  $\dot{V}O_2$   $\tau_p$ , amplitude or economy of exercise between muscle temperature conditions. More recently, Koppo et al. (2002) suggested that the speeding of the on-transient  $\dot{V}O_2$  response observed with higher muscle temperatures was through the alleviation of any controlling  $O_2$  delivery or utilisation limitations, despite not supporting this with results. This mechanism was supported by Burnley et al. (2002c) who reported no significant improvement in  $\dot{V}O_2$  kinetics through the passive warming of muscle. Therefore, it appears as though the heating of muscle prior to exercise does not facilitate a sufficient shift in the Hb $O_2$  dissociation curve to speed the on-transient metabolic responses.

In summary, a decrease in muscle temperature have been shown to slow the on-transient  $\dot{V}O_2$  response, while a rise in muscle temperature appears to have no impact on the  $\dot{V}O_2$  response in the studies discussed above. Specifically, the reduction in muscle temperature and decreased  $O_2$  availability has been related to a slowed  $\dot{V}O_2$  response, most likely as a result of a leftward shift in the Hb $O_2$  dissociation curve. In contrast, the on-transient  $\dot{V}O_2$  response is not significantly speeded after prior warming of the recruited muscles.

In conclusion, a number of research investigations have suggested that the on-transient  $\dot{V}O_2$  response is limited by  $O_2$  delivery factors (Hughson and Smyth 1983; Hughson et al. 1991; Wagner 1995; Tschakovsky and Hughson

1999; Whipp et al. 2005). The significant effect of these O<sub>2</sub> delivery influences have only been observed during adaptation to high-intensity exercise (MacDonald et al. 1997). Alternatively, the majority of contemporary literature has refuted that the on-transient VO<sub>2</sub> response is limited by the delivery and availability, but rather suggest that the capacity to utilise O<sub>2</sub> within the working muscle controls the speed of adaptation (Grassi et al. 1996; DeLorey et al. 2004a; 2005; Grassi 2005). Factors affecting the utilisation of O<sub>2</sub> within the muscle which may be related to the on-transient responses are discussed below.

## **O<sub>2</sub> Utilisation Limitations**

### *Prior Exercise*

Many studies have supported a faster exponential increase in the VO<sub>2</sub> response following prior exercise (Gerbino, Ward and Whipp 1996; MacDonald et al. 1997; Bearden and Moffatt 2000; Koppo and Bouckaert 2002; Endo et al. 2004). The majority of literature has shown that prior exercise below VT has no speeding effect on VO<sub>2</sub> kinetics, whereas a faster on-transient VO<sub>2</sub> response has been observed following prior high-intensity (>VT) exercise (Gerbino, Ward et al. 1996; MacDonald et al. 1997; Burnley, Doust, Carter and Jones 2001; Koppo and Bouckaert 2001; 2002; Koppo et al. 2002; Jones et al. 2003).

The most likely explanation for the speeded VO<sub>2</sub> response observed following prior exercise is the reduction of the metabolic inertia required to be overcome prior to the metabolic adaptation (Gerbino et al. 1996; MacDonald et al. 1997; Burnley et al. 2001; Rossiter et al. 2001). This may be due to increases in the activities of several inter- and intra-cellular metabolites and

oxidative enzyme related to muscle  $\dot{V}O_2$  (Bangsbo 2000). Previously, a number of investigations noted that oxidative enzyme activities are increased with prior exercise (Tokonogi, Harris and Sahlin 1997; Burnley et al. 2001; Leek, Mudaliar, Henry, Mathieu-Costello and Richardson 2001; Burnley, Doust, Ball and Jones 2002a). Burnley et al. (2002a) highlighted that improved  $\dot{V}O_2$  kinetics through prior exercise has only been noted for exercise transients when preceded by exercise of sufficient intensity to decrease blood pH which may increase  $O_2$  availability via the Bohr effect and metabolic vasodilation. Furthermore, Timmons et al. (1998a) hypothesised that the recovery kinetics of other influential muscle hormones (adrenaline, noradrenaline) and metabolites ( $K^+$ ) may be too quick to influence the  $\dot{V}O_2$  response following prior exercise. This suggests that the prolonged increased activities of oxidative enzymes may play a role in the faster adaptation of  $O_2$  utilisation.

To date, only one study by DeLorey, Kowalchuck and Paterson (2004b) has reported upon the effect of prior exercise on the concurrent  $\dot{V}O_2$  and mOxy kinetics in response to a moderate-intensity SWT test in both young ( $25 \pm 3$  y) and old ( $68 \pm 3$  y) healthy male subjects. The researchers reported that following a prior heavy-intensity warm-up (HWU) the  $\dot{V}O_2 \tau_p$  was significantly shorter than no warm-up (NWU) in the older subjects (NWU:  $38 \pm 5$  s; HWU:  $30 \pm 7$  s) but not in the young subjects (NWU:  $26 \pm 7$  s; HWU:  $25 \pm 5$  s). Further, a significant effect of age was observed between the  $\dot{V}O_2 \tau_p$  in the no warm up condition, but was not present in the warm-up intervention. In terms of the mOxy response, the  $TD_p$  was significantly shorter in both the young (NWU:  $12 \pm 2$  s; HWU:  $10 \pm 2$  s) and old (NWU:  $11 \pm 2$  s; HWU:  $8 \pm 2$  s) age-groups following the high-intensity warm up. Interestingly, the mOxy  $\tau_p$  significantly

lengthened following the high-intensity warm-up in both age-groups (Y: NWU:  $11 \pm 10$  s; HWU:  $14 \pm 4$  s; O: NWU:  $9 \pm 3$  s; HWU:  $32 \pm 17$  s). It is likely that the high-intensity warm-up was responsible for an increase in both skin and muscle temperature, which have recently been shown to significantly affect the mOxy response during exercise due to vasodilation responses (Davis, Fadel, Cui, Thomas and Crandall 2006). The high-intensity warm-up may have reduced the metabolic inertia required to be overcome at exercise onset, allowing faster adaptation of muscle O<sub>2</sub> extraction and consumption.

In summary, it is likely that prior exercise may help to alleviate any O<sub>2</sub> delivery or utilisation constraints which limit the  $\dot{V}O_2$  and mOxy adjustment to exercise. The speeding of  $\dot{V}O_2$  kinetics as a result of prior exercise is most likely facilitated by increased blood flow, enzyme activities, and increased HbO<sub>2</sub> dissociation within the blood (Jones et al. 2003). However, there would appear to be a number of enzymes or reactions which may be responsible for limiting the rate of O<sub>2</sub> extraction and consumption at the onset of exercise by controlling the rate of change in oxidative phosphorylation. The overcoming of the metabolic inertia at exercise onset due to the prior exercise is most likely due to changes in the muscle energetics within the working muscle (Grassi 2005).

### *Muscle Energetics*

It is well established that the utilisation of O<sub>2</sub> and flux of energy pathways is dependent upon the activity of oxidative enzymes within the mitochondria (Sahlin, Ren and Broberg 1988; Greenhaff and Timmons 1998; Timmons et al. 1998a; Timmons, Gustafsson, Sundberg, Jansson, Hultman, Kaijser, Chwalbinska-Moneta, Constantin-Teodosiu, Macdonald and Greenhaff 1998b;

Bangsbo 2000; Bell, Paterson, Kowalchuk, Moy, Thorp, Noble, Taylor and Cunningham 2001; Russ and Kent-Braun 2004). Furthermore, it appears that the initial improvements in muscle energetics are responsible for stimulating ATP production and  $O_2$  utilisation within the muscle which control or influence the  $\dot{V}O_2$  and mOxy responses (Barstow et al. 1994; Grassi 2005).

Sahlin et al. (1988) suggested an alternative biochemical explanation for the development of the  $O_2$  deficit at the onset of exercise. They suggested that the  $O_2$  deficit is dependent upon the regulation of cellular changes in muscle metabolites such as Adenosine Diphosphate (ADP), Inorganic Phosphate ( $P_i$ ), and Nicotinamide Adenine Dinucleotide (NADH). Sahlin, Ren et al. (1988) further suggested that the initial breakdown of ATP at exercise onset stimulates ATP production and PCr breakdown, releasing free  $P_i$  to stimulate glycogenolysis and glycolysis due to the increase in the concentration of low level phosphates. They also suggest that this increased anaerobic energy turnover may reduce tissue  $\dot{V}O_2$  demands and be evidenced by a decreased or stable  $\dot{V}O_2$  at exercise onset. However, when a steady state of ADP and  $P_i$  is attained, mitochondrial respiration and  $\dot{V}O_2$  will also remain constant at sub-maximal workloads.

Similarly, the oxidative capacity of muscle may also affect the rate at which  $\dot{V}O_2$  is able to be utilised within the muscle cell (Grassi 2000; 2005). In particular, this may be reflected by the maximal activities of several oxidative enzymes [pyruvate dehydrogenase (PDH), citrate synthase (CS), and 2-oxoglutarate dehydrogenase (2-OGDH)] which have been proposed to limit the

rate of Tricarboxylic Acid (TCA) cycle flux and muscle  $\dot{V}O_2$  (Russ and Kent-Braun 2004).

### *Pyruvate Dehydrogenase*

PDH is considered the rate limiting enzyme for the degradation of pyruvate in skeletal muscle and has been closely linked to muscle  $\dot{V}O_2$  (Bangsbo, Gibala, Krstrup, Gonzalez-Alonso and Saltin 2002). The activity of PDH during exercise transients has been suggested to limit the speed of  $\dot{V}O_2$  adjustment as it provides acetyl groups to the necessary energy pathways (Greenhaff and Timmons 1998; Bangsbo 2000; Jones et al. 2003).

Originally, Greenhaff and Timmons (1998) suggested that  $O_2$  utilisation is limited during exercise on-transients due to an insufficient production of acetyl-CoA for the TCA cycle. This inadequate supply of acetyl-CoA is most likely the result of the delayed activation of PDH (Greenhaff and Timmons 1998). In support of this suggestion, Parolin et al. (2000) demonstrated that at the onset of exercise ~86% of PDH is activated after 15 s, which is approximately the same length as Phase I of the  $\dot{V}O_2$  response, suggesting the kinetics of PDH activation may be closely linked to the exponential increase in  $\dot{V}O_2$ .

To test this hypothesis, a number of researchers stimulated PDH prior to performing an exercise bout through the infusion of dichloroacetate (DCA) in dogs and humans (Timmons et al. 1998a; 1998b; Howlett, Heigenhauser, Hultman, Hollidge-Horvat and Spriet 1999). The infusion of DCA was linked to a significantly reduced level of cellular level phosphorylation, which is most likely

due to a reduced  $O_2$  deficit as a result of the faster rate of adaptation of muscle  $\dot{V}O_2$  at exercise onset (Timmons et al. 1998a; 1998b). In contrast, recent evidence from Bangsbo et al. (2002) reported that enhanced PDH activity does not improve the changes in muscle  $\dot{V}O_2$  at the onset of one legged knee-extensor exercise at an work rate of  $\sim 110\%$  of peak thigh  $\dot{V}O_2$ . These researchers elevated PDH activity through DCA administration prior to a 15 s severe-intensity exercise bout and reported that PDH elevation did not significantly increase  $\dot{V}O_2$  or  $a\text{-}\dot{v}O_2\text{diff}$  from the thigh musculature. This finding suggests that PDH may not be related to the on-transient  $\dot{V}O_2$  response to high-intensity exercise. This lack of an increase in  $\dot{V}O_2$  or  $O_2$  extraction may be the result of the 'anaerobic' exercise intensity employed by Bangsbo et al. (2002). However, limited research is available on the relationship between PDH activity and  $\dot{V}O_2$  measured at the mouth in response to more aerobic and submaximal exercise intensities.

In summary, while recent research has produced equivocal results as to the role of PDH on the rate of  $\dot{V}O_2$  adjustment at exercise onset, PDH has been linked to the production of the substrates required to maintain the TCA cycle flux which in may play a role as a controlling factor of the  $\dot{V}O_2$  response. While some previous research has found such a relationship, the published observations of the PDH effect on the  $\dot{V}O_2$  response remains unclear.

### *Citrate Synthase*

Another important mitochondrial respiratory enzyme is CS which is responsible for the combination of acetyl-CoA and oxaloacetate to produce citrate, an important intermediate within the TCA cycle. It has previously been

suggested that CS activity within the muscle is related to aerobic fitness as well as endurance training and performance (Bell et al. 2001; Carter, Rennie, Hamilton and Tarnopolsky 2001; Short, Vittone, Bidelow, Proctor, Rizza, Coenen-Schimke and Nair 2003). A paucity of literature has examined the influence of CS activity or its enhancement following endurance training on the on-transient  $\dot{V}O_2$  response to exercise (Bell et al. 2001).

Bell and colleagues (2001) investigated the effects of nine weeks of single leg leg-extension training at an intensity of 75-85%  $\dot{V}O_{2\max}$  on the relationship between CS activity and  $\dot{V}O_2$  kinetics in five elderly male subjects ( $77 \pm 7$  y). They observed that the on-transient  $\dot{V}O_2$   $\tau_p$  was significantly decreased in the trained limb post-training compared to pre-training ( $92 \pm 44$  vs.  $48 \pm 22$  s) but not the untrained limb ( $104 \pm 43$  vs.  $126 \pm 35$  s). It was also reported that CS activity of the VL significantly increased in the trained leg from  $6.7 \pm 2.0$  to  $11.4 \pm 3.6 \mu\text{mol}\cdot\text{g}_{\text{w.w.}}^{-1}\cdot\text{min}^{-1}$ , but not in the untrained leg ( $5.9 \pm 0.5$  to  $7.9 \pm 1.9 \mu\text{mol}\cdot\text{g}_{\text{w.w.}}^{-1}\cdot\text{min}^{-1}$ ). Furthermore, Bell et al. (2001) observed no improvement in the kinetics of mean blood velocity within the femoral artery. This suggests that the improvement observed in the on-transient  $\dot{V}O_2$  response was most likely due to an increased  $O_2$  extraction. The relationship between the improved  $\dot{V}O_2$  on-transient responses and increased CS activity was not reported by the researchers. At present, no data exists on the relationship between enzyme activities and trends in mOxy in response to exercise bouts in any population.

In summary, limited research has examined the relationship between CS activity and the on-transient  $\dot{V}O_2$  response. While it appears that an increased

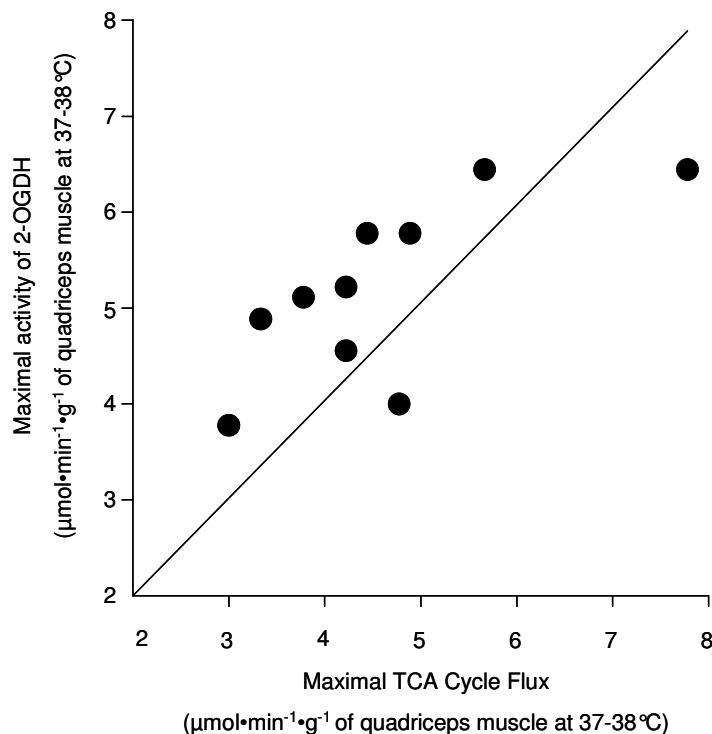
CS activity is related to improved muscle  $\dot{V}O_2$  during exercise, further research is required to validate this observation. Nevertheless, the present data are supportive of muscle utilisation limitations controlling the on-transient  $\dot{V}O_2$  response to exercise onset.

### *2-Oxoglutarate Dehydrogenase*

Whilst CS and SDH activities are commonly used measures of muscle oxidative capacity, it appears they are not closely related to the TCA cycle flux *in vitro* (Blomstrand, Radegran and Saltin 1997). 2-OGDH is a key regulatory enzyme within the TCA cycle responsible for the conversion of 2-oxoglutarate and coenzyme A into succinyl-CoA and  $CO_2$ , allowing NADH to be generated from NAD (Blomstrand, Challiss, Cooney and Newsholme 1983). Blomstrand et al. (1997) have suggested that the maximal flux of the TCA cycle is best related to 2-OGDH activity. Limited evidence has been presented which relates 2-OGDH activity to  $\dot{V}O_2$  adjustment in response to imposed workloads (Blomstrand et al. 1997).

Blomstrand et al. (1997) determined the relationship between the maximal activities of a number of oxidative enzymes and the maximal  $\dot{V}O_2$  for the quadriceps muscle during incremental leg extension exercise. The researchers observed that the maximum leg  $\dot{V}O_2$  was  $845 \pm 100 \text{ mL} \cdot \text{min}^{-1}$ , or  $353 \pm 33 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for the quadriceps muscle, and was significantly correlated to the maximal activities of CS ( $r = 0.79$ ,  $p < 0.05$ ) and 2-OGDH ( $r = 0.72$ ,  $p < 0.05$ ). The mean exercise  $\dot{V}O_2$  corresponded to a TCA cycle flux of  $4.6 \pm 0.4 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ , which was similar to that noted for 2-OGDH ( $5.1 \pm 0.3 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ), but not CS ( $48 \pm 1.5 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ). The maximal activity

of CS appeared to be 940% higher than the estimated TCA cycle flux. The relationship between the maximal 2-OGDH activity and TCA cycle flux is shown in Figure 2.3. The importance of 2-OGDH activity upon the TCA cycle flux has been identified within several other investigations using animal muscle (Cooney, Taegtmeyer and Newsholme 1981; Blomstrand et al. 1983). However, it has been reported that 2-OGDH activity is not significantly related to exercise performance characteristics (i.e. LT,  $\dot{V}O_{2\max}$ ) of endurance athletes, whereas other oxidative enzymes such as CS and SDH have been related to such performance measures (Bishop, Jenkins, McEniery and Carey 2000). However, limited evidence is available on this relationship at present.



**Figure 2.3:** Relationship between maximal activity of 2-OGDH and TCA cycle flux within the vastus lateralis during one-legged knee extension exercise at 37-38°C (Adapted from Blomstrand et al. 1997).

In summary, the activity of 2-OGDH appears to be related to the TCA cycle flux across exercise intensities, and as such may be a more valid oxidative measure than CS or SDH activities (Blomstrand et al. 1997). It is likely that the utilisation of O<sub>2</sub> at exercise onset is linked to the activity of such oxidative enzymes. Therefore, any O<sub>2</sub> utilisation limitations are likely to be representative of the oxidative capacity of the muscle (Grassi 2005). The research discussed above suggests that the rate of adaptation in the metabolic transition at exercise onset is controlled by factors related to O<sub>2</sub> utilisation within the working muscle (Tschakovsky and Hughson 1999; Bangsbo et al. 2000; Grassi 2005). Other factors which may be related to the on-transient VO<sub>2</sub> response may include histochemical parameters, physical training and aging. These factors may be influential through either changing O<sub>2</sub> utilisation or delivery limitations within the working muscle.

### **Influence of Histochemical Parameters on the On-Transient Responses**

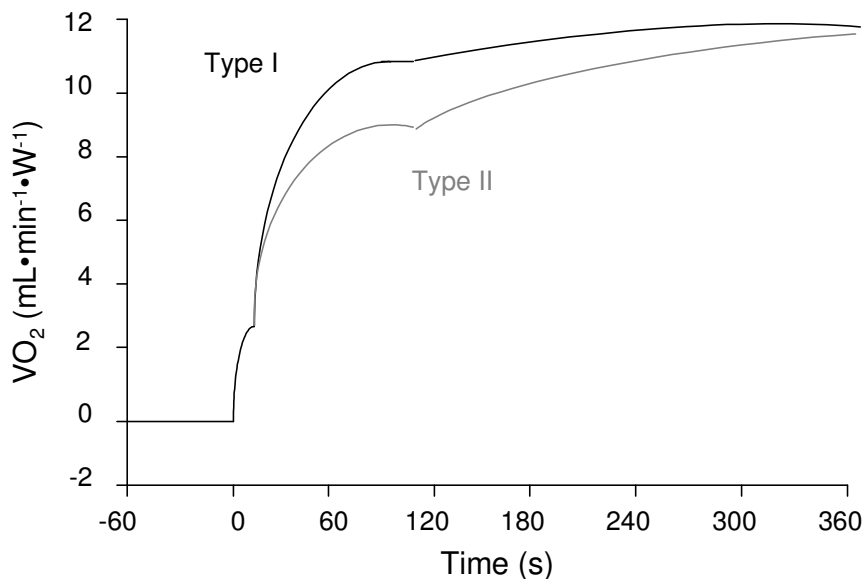
The speed of the VO<sub>2</sub> response at exercise onset is controlled through either the delivery or utilisation of O<sub>2</sub> within the active muscle cell (Xu and Rhodes 1999; Grassi 2000; 2001). However, both the delivery and utilisation of O<sub>2</sub> within the working muscle is related to muscle fibre type composition and thus muscle capillarisation, enzyme activity and bioenergetics (Bottinelli and Reggiani 2000; He, Bottinelli, Pellegrino, Ferenczi and Reggiani 2000). This supports the earlier hypothesis of Poole (1994) suggesting that the major influencing factors lie within the working muscle.

Previous research has identified that muscle fibre composition has a significant effect on the VO<sub>2</sub>-Work relationship during exercise (Barstow et al.

1996; Barstow, Jones, Nguyen and Casaburi 2000; Pringle et al. 2002; 2003b; Jones, Campbell and Pringle 2004). The metabolic gain of the primary component ( $G_p$ ), calculated as  $\Delta\dot{V}O_2/\Delta W$ , has been shown to be an important parameter in describing on-transient  $\dot{V}O_2$  response efficiency.

Barstow et al. (2000), Pringle et al. (2002; 2003b) have all suggested that the  $\dot{V}O_2$ -Work relationship is significantly related to the population of both Type I and II fibre types within the working muscle. Barstow et al. (2000) reported upon the  $\dot{V}O_2$ -Work relationship for both sub-VT and supra-VT intensity exercise in a group of nine healthy young ( $31 \pm 8y$ ;  $3.4 \pm 0.5 \text{ L}\cdot\text{min}^{-1}$ ) male subjects. Both Jones et al. (2004) and Mallory et al. (2002) reported no significant relationship between Type I fibre population of the VL and the  $\Delta\dot{V}O_2/\Delta W$  for sub-VT exercise. They observed no significant relationship between the percentage of Type I fibres and the  $\dot{V}O_2 \tau_p$  for heavy-intensity exercise across a wide range of cadences (45, 60, 75 and 90 RPM). However, they did observe significant ( $p < 0.05$ ) correlations between fibre type composition and the  $\Delta\dot{V}O_2/\Delta W$  for exercise intensities both below and above VT. Specifically, they reported that the  $\Delta\dot{V}O_2/\Delta W$  for sub-VT exercise was  $\sim 9 \text{ mL}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$  for individuals with a low percentage of Type I fibres, whereas a high percentage of Type I fibres demonstrated a gain of  $\sim 11 \text{ mL}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$  as shown below in Figure 2.4. This difference may be the result of the  $\sim 18\%$  lower mitochondrial  $P_i/O_2$  ratio, greater  $\text{Ca}^{2+}$  ATPase activity and lower energy efficiency previously noted for Type II fibres (Bottinelli and Reggiani 2000; He et al. 2000).

In agreement with Barstow and colleagues (2000), Pringle et al. (2003b) reported that the proportion of Type I muscle fibres and the  $\Delta\dot{V}O_2/\Delta W$  were significantly related across moderate ( $r= 0.65$ ,  $p<0.05$ ), heavy ( $r= 0.57$ ,  $p<0.05$ ) and severe-intensity ( $r= 0.57$ ,  $p<0.05$ ) exercise in 14 young ( $25 \pm 4$  y;  $47.9 \pm 2.3 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) male and female subjects. The  $\dot{V}O_2 G_p$  was also significantly related to the capillary to fibre ratio (C:F ratio) at both heavy ( $r= 0.65$ ,  $p<0.05$ ) and severe-intensity ( $r= 0.63$ ,  $p<0.05$ ) SWT, and  $\dot{V}O_{2\text{max}}$  at heavy exercise ( $r= 0.67$ ,  $p<0.05$ ). The  $\dot{V}O_2 \tau_p$  was found to be similar across all three intensities, and significantly related to the percentage of Type I fibres only for heavy-intensity exercise ( $r= -0.68$ ,  $p<0.05$ ). The Type IIx population was significantly related to the  $\dot{V}O_2 \tau_p$  for both heavy ( $r= 0.69$ ,  $p<0.01$ ) and severe-intensity ( $r= 0.56$ ,  $p<0.05$ ) SWT. Importantly, subjects with a low percentage of Type I fibres exhibited a slower  $\dot{V}O_2 \tau_p$  for the heavy and severe-intensity SWT.



**Figure 2.4:** The gain for the primary component versus time for moderate, heavy and severe-intensity exercise for subjects with high percentages of Type I and Type II fibres (Adapted from Pringle et al. 2003b).

Pringle et al. (2003b) suggested that the reported relationship between  $\dot{V}O_2 \tau_p$  and percentage of Type I fibres is related to the enhancement of  $O_2$  delivery and oxidative enzymes in comparison to Type II fibres. The increased capillarisation of Type I muscle is also likely to reduce any heterogeneity of blood flow at exercise transients, allowing improved utilisation of  $O_2$  within muscle by reducing the  $O_2$  diffusion distance (Richardson et al. 1999). However, muscle capillarisation does not appear to influence the rate of  $\dot{V}O_2$  adjustment during on-transient periods (Chilibeck et al. 1997; Pringle et al. 2003b). Thus, it appears that on-transient  $\dot{V}O_2$  responses are not limited by the delivery of  $O_2$  to the cell but rather the actual utilisation of  $O_2$  within the muscle cell. Therefore, the precise mechanism responsible for a faster  $\dot{V}O_2 \tau_p$  across supra-VT exercise on-transients in subjects with a high proportion of Type I fibres is yet to be determined. Possible causal factors might include the increased oxidative enzyme activities, mitochondrial density or capillarisation (Essen-Gustavsson and Borges 1986; Coggan et al. 1992).

The effect of histochemical characteristics on the speed and amplitude of the mOxy response at exercise onset has received little attention. To date, only one investigation has reported the effect of histochemical characteristics of the working muscle on the mOxy responses with exercise (Hamaoka, Mizuno, Katsumura, Osada, Shimomitsu and Quistroff 1998). In this study, the kinetics of the mOxy on-transient response were significantly related to the composition of Type I fibres from the VL. The suggestion that Type I fibres are related to greater and faster decreases in mOxy at exercise onset is most likely due to the greater capacity for blood to be delivered and consumed within the muscle cell. This increased potential for  $O_2$  consumption is due to Type I fibres possessing

greater oxidative enzyme activities, myoglobin stores and capillarisation (Bottinelli and Reggiani 2000). This greater oxidative capacity and capillarisation and aerobic enzyme of Type I fibres would facilitate greater muscle deoxygenation through greater desaturation of HbO<sub>2</sub> and MbO<sub>2</sub> stores.

In summary, it appears that muscle fibre composition and related enzymatic characteristics are significantly related to the speed and amplitude of the on-transient  $\dot{V}O_2$  response. The majority of previous literature has suggested that muscle fibre type has a significant influence on the speed ( $\tau_p$ ; wMRT) at which  $\dot{V}O_2$  and mOxy adjust to sub-VT and supra-VT intensity exercise intensities (Borroni et al. 2001; Krstrup, Soderlund, Mohr and Bangsbo 2004b). However, limited research has examined the relationship between the on-transient mOxy responses and the histochemical characteristics of muscle. Therefore, a purpose of the present study is to further investigate the relationship between the on-transient  $\dot{V}O_2$  and mOxy responses and peripheral muscle histochemical and enzymatic characteristics in well-trained cyclists.

### **Influence of Physical Training on the On-Transient Responses**

Previous investigations have reported significant improvements in  $\dot{V}O_{2\max}$  (Denis, Dormois and Lacour 1984; Pollock et al. 1987; Katzel et al. 2001), BLa<sup>-</sup> thresholds (Sjodin, Jacobs and Karlsson 1981; Masse-Biron et al. 1992) and economy (Millet, Jaouen, Borroni and Candau 2002) with endurance training. The O<sub>2</sub> delivery or utilisation limitations within the working muscle which have previously been discussed may be reduced with physical training of

sufficient intensity and duration given the observed peripheral muscle adaptations (Babcock et al. 1994a; Carter et al. 2000a; Koppo et al. 2004).

Past training studies have observed positive changes in muscle blood flow, capillarisation and HR kinetics with physical training in sedentary subjects, to suggest that improvements in  $O_2$  delivery to the working muscle occur (Denis, Chatard, Dormois, Linossier, Geyssant and Lacour 1986; Grassi 2001). The activity of oxidative enzymes and mitochondrial density related to  $O_2$  utilisation are also increased with endurance training (Green, Thomson, Daub, Houston and Ranney 1979; Gollnick and Saltin 1982; Dawson, Fitzsimons, Green, Goodman, Carey and Cole 1998). It is likely that these peripheral training adaptations are in part responsible for the improved  $\dot{V}O_2$  response to exercise (Carter et al. 2000a; Billat, Mille-Hamard, Demarle and Koralsztejn 2002).

Billat et al. (2002) observed the effects of a four week endurance-training program on  $\dot{V}O_2$  kinetics in seven young ( $25 \pm 1$  y;  $56.0 \pm 6.8 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) male physical education students. They observed significant speeding in the on-transient  $\dot{V}O_2$  response after as little as four days of endurance training, and prior to any significant improvement in  $\dot{V}O_{2\text{max}}$ . They noted that the  $\dot{V}O_2 \tau_p$  for treadmill runs at 90% and 95%  $v\dot{V}O_{2\text{max}}$  significantly decreased from  $28 \pm 5$  to  $22 \pm 4$  s (90%  $v\dot{V}O_{2\text{max}}$ ) and  $28 \pm 5$  to  $20 \pm 7$  s (95%  $v\dot{V}O_{2\text{max}}$ ) after the four week training intervention. Similarly, Carter et al. (2000a) observed significant improvements in both LT and  $\dot{V}O_{2\text{max}}$  in 23 healthy male subjects after a six-week continuous and interval running training program. However, no adaptations were noted in either the amplitude or the  $\dot{V}O_2 \tau_p$  for the moderate

or heavy-intensity treadmill running, despite a significant reduction in the wMRT of heavy-intensity exercise response.

Recently, Fukuoka et al. (2002) investigated the effects of cycling endurance training on both the on- and off-transient  $\dot{V}O_2$  responses in a group of middle-aged ( $51 \pm 3$  y) healthy male subjects during repeat SWT at 50%  $\dot{V}O_{2\max}$ . Subjects performed a 90 day endurance-training program consisting of 30 min of cycling at 50% HR reserve. Fukuoka et al. (2002) observed non-significant improvements in both  $\dot{V}O_{2\max}$  and peak  $[BLa^-]$  during the first 30 days, but significant improvements after both 60 and 90 days of endurance training. The on-transient  $\dot{V}O_2 \tau_p$  improved significantly after seven days ( $38.1 \pm 14.2$  s) of training, as well as after 15 days ( $34.4 \pm 12.6$  s) of training in comparison to the pre-training values ( $46.9 \pm 18.3$  s). No further improvements were observed in the  $\dot{V}O_2 \tau_p$  after 30 ( $28.8 \pm 6.8$  s), 60 ( $30.2 \pm 8.0$  s) and 90 ( $30.4 \pm 12.4$  s) days of training, respectively. Following 90 days of training, the on-transient  $\dot{V}O_2 \tau_p$  of the middle-aged subjects were comparable to the matched younger group ( $21.6 \pm 0.5$  y;  $29.2 \pm 5.3$  s) undertaking the same training. However, neither the on-transient  $\dot{V}O_2$  amplitude nor  $TD_p$  was significantly affected by endurance training in either group. In addition, Fukuoka et al. (2002) showed that the HR  $\tau_{1/2}$  significantly improved after 15 days of training in the middle aged group, but no further improvements were noted after this time. This suggests that the initial speeding of the  $\dot{V}O_2$  kinetics response may be due to an enhanced  $O_2$  delivery, and further improvements may be due to peripheral muscle adaptations.

The effect of physical training on changes in mOxy during exercise has only recently been investigated, and limited data are available (Costes et al. 2001; Neary et al. 2002; Puente-Maestu et al. 2003). The limited research suggests that physical training allows greater decreases in mOxy during exercise, most likely as a result of peripheral adaptations in the capacity of muscle to utilise O<sub>2</sub> (Costes et al. 2001; Neary et al. 2002; Puente-Maestu et al. 2003). Costes et al. (2001) investigated the influence of endurance training on mOxy responses during submaximal cycling exercise in seven healthy young ( $20 \pm 2$  y) volunteers. The researchers employed a four-week training program consisting of cycling at between 70% and 80% HR<sub>max</sub> consecutively for two hours per day, six days a week. They investigated the cardiorespiratory, [BLa] and mOxy responses to two 15 min submaximal tests at 50% and 80% VO<sub>2</sub>max. The training program was not observed to alter either the amplitude or pattern of the mOxy responses at 50% VO<sub>2</sub>max, but greater muscle deoxygenation was observed across the 80% VO<sub>2</sub>max SWT. Costes et al. (2001) also reported a weak but significant relationship ( $r = 0.42$ ,  $p < 0.05$ ) between the greater mOxy amplitude and lowered [BLa]. However, the change in mOxy was significantly related to changes in measures of capillarisation of the VL suggesting an influence on O<sub>2</sub> delivery mechanisms. As a result of the training, the activities of CS and  $\beta$ -HAD were significantly increased, but no significant relationships were observed with the changes in the on-transient mOxy response to a moderate-intensity exercise bout. Therefore, it may be possible that the observed changes in mOxy during exercise are dependent upon the peripheral histochemical and enzymatic adaptations which influence O<sub>2</sub> delivery and utilisation.

In a more recent study, Neary et al. (2002) investigated the effect of short-term endurance training on mOxy responses in a group of eight experienced and well-trained male cyclists ( $23 \pm 5$  y,  $4.39 \pm 0.66$  L $\cdot$ min $^{-1}$ ). The researchers employed a training program of cycling at an intensity of 85-90%  $\dot{V}O_{2\max}$  for 1 h a day, four days a week for three weeks. Following the training program, the researchers investigated the cardiorespiratory and mOxy responses during a graded exercise test to exhaustion and a simulated 20 km time trial (20TT). The researchers reported no significant difference in the mOxy observed at  $\dot{V}O_{2\max}$  and across the 20TT pre- and post-training. At the completion of the 20TT, mean mOxy was significantly decreased following the training, falling from  $-550 \pm 292$  to  $-707 \pm 227$  mV. Maximal deoxygenation also non-significantly decreased ( $-807 \pm 344$  to  $-1009 \pm 331$  mV). The researchers suggested that the cyclists who showed the greatest improvement in  $\dot{V}O_{2\max}$  demonstrated the greatest improvement in 20TT performance and muscle deoxygenation following the three weeks of cycling training. The improvement in 20TT performance was significantly related to the capacity for greater muscle deoxygenation ( $r = -0.75$ ,  $p < 0.05$ ). In order to quantify these changes, Neary et al. (2002) used the maximum values of mOxy from the  $\dot{V}O_{2\max}$  test, rather than the previously validated and favourably employed cuff ischemia method (Sahlin 1992; Bhambhani et al. 1999; Costes et al. 1999; Foster, Rundell, Snyder, Stray-Gunersen, Kemkers, Thometz, Broker and Knapp 1999; Boushel and Piantadosi 2000). It might be suggested that this relationship is a result of adaptations within the peripheral muscle, allowing an increased O<sub>2</sub> extraction and utilisation which allow greater fibre recruitment and force production.

In summary, it appears that endurance training significantly improves both the  $\dot{V}O_2$  and mOxy on-transient responses (Costes et al. 2001; Neary et al. 2002). The mechanisms associated with these improvements in the metabolic adaptation are hypothesised to occur within the peripheral muscle histochemical and biochemical parameters. Endurance training has also been observed to improve important physiological characteristics related to the on-transient metabolic responses such as VT,  $\dot{V}O_{2\max}$  and  $BLa^-$  responses (Midgley, McNaughton and Wilkinson 2006). Importantly, sufficient physical training helps to alleviate the  $O_2$  delivery or utilisation limitations previously discussed to influence the on-transient metabolic responses. Aging has also been shown to alter the histochemical characteristics of peripheral muscle, as well as other physiological responses that may influence the metabolic adaptation at exercise onset.

### **Influence of Aging on the On-Transient Responses**

The speed of the on-transient  $\dot{V}O_2$  and mOxy response appears to be slowed with sedentary aging, which most likely reflects either a reduced capacity to deliver or utilise  $O_2$  (Babcock et al. 1994a; Chilibeck et al. 1995; 1998; Bell et al. 1999; Stathokostas et al. 2003; DeLorey et al. 2003a; 2004a; 2005). Age-related changes have been observed in a number of physiological responses that may reduce the capacity to deliver or utilise  $O_2$ . These include changes to the initial HR response, muscle capillarisation, changes in muscle fibre composition, and oxidative enzyme activities (Babcock et al. 1994a; 1994b; DeLorey et al. 2003a).

In their classic investigation, Babcock et al. (1994a) investigated the exercise on-transient  $\dot{V}O_2$  responses in 46 male subjects aged between 30 and 80 y. Each subject performed repeated six min bouts of cycling at a moderate-intensity (80% VT). The researchers observed that the on-transient  $\dot{V}O_2 \tau_p$  was significantly longer with increasing age, averaging  $38.8 \pm 9.5$  s,  $48.6 \pm 11.2$  s and  $60.8 \pm 17.6$  s for young (30-44 y), middle aged (45-59 y) and elderly (65-80 y) age-groups, respectively. It was also reported that the  $\dot{V}O_2 \tau_p$  was significantly correlated with age ( $r = 0.64$ ,  $p < 0.05$ ). The data suggested that  $\dot{V}O_2 \tau_p$  slowed by 0.7 s per year across the age range included in the study. The  $\dot{V}O_2 \tau_p$  was also found to be significantly related to the  $\dot{V}CO_2 \tau_p$  ( $r = 0.86$ ,  $p < 0.05$ ), and the  $\dot{V}E \tau_p$  ( $r = 0.58$ ,  $p < 0.05$ ), as well as  $\dot{V}O_{2\max}$  ( $r = -0.51$ ,  $p < 0.05$ ), but not HR  $\tau_p$  ( $r = 0.21$ ,  $p > 0.05$ ). The researchers hypothesised that the slowing of the  $\dot{V}O_2 \tau_p$  may be the result of a reduced arterial  $pO_2$ , poorer vascular conductance or an age-related increase of 'non-muscle' tissue in the active musculature. Orlander and Aniansson (1980) supported these findings and suggested that an age-related increase in the  $\dot{V}O_2 \tau_p$  may be the result of a decreased  $O_2$  utilisation capacity through decreases in mitochondrial density or enzyme activities within the peripheral muscles.

Babcock et al. (1994b) later demonstrated that aerobic endurance training in older men ( $72 \pm 4.4$  y:  $1.77 \pm 0.19$  L $\cdot$ min $^{-1}$ ) significantly improved on-transient  $\dot{V}O_2$  kinetic responses to moderate-intensity (80% VT) cycling. Each of the subjects completed a 24 week endurance-training program consisting of three 40 min cycling sessions per week at an intensity of 50% $\Delta$ . The  $\dot{V}O_2 \tau_p$  was significantly reduced from  $62.2 \pm 15.5$  to  $31.9 \pm 7$  s following the training program in the aged sedentary males. An age-matched control group showed a

significantly longer  $\dot{V}O_2 \tau_p$  in the post-testing session ( $53.7 \pm 9.9$  s), which demonstrates that  $\dot{V}O_2 \tau_p$  is significantly shortened with endurance training. Babcock et al. (1994b) suggested that this improvement may have been the result of increases in mitochondrial density or other histochemical parameters, although these parameters were not measured.

Previous investigations have also reported that the on-transient  $\dot{V}O_2$  response is slowed in older subjects due to a delayed  $O_2$  transport from the lungs to the mitochondria (Babcock et al. 1992; Chilibeck et al. 1995; 1996a). Chilibeck and others (1995) reported a significant correlation between the accelerated  $\dot{V}O_2 \tau_p$  and HR  $\tau$  ( $r = 0.78$ ,  $p < 0.05$ ) following endurance-training, suggesting that the faster on-transient  $\dot{V}O_2$  response is partially the result of an improved HR response. The speeded HR kinetics, both at heavy submaximal and maximal exercise intensities appear to be explained by an age-related reduction in the adrenergic response together with a reduced sensitivity of cardiac tissue to catecholamines (McCully, Fielding, Evans, Leigh Jr. and Posner 1993; Houmard, Weidner, Gavigan, Tyndall, Hickey and Alshami 1998).

A number of investigations have observed that the oxidative capacity of muscle is maintained in muscles which are used in activities of daily living (e.g. gastrocnemius), as opposed to muscle groups involved in sport-specific activities (e.g. VL) (Chilibeck et al. 1995; 1996b; Russ and Kent-Braun 2004). In a recent review, Russ and Kent-Braun (2004) stated that oxidative capacity decreases with aging, but can be maintained at comparable levels of younger sedentary populations despite advancing age if physical training is maintained.

Limited research has examined the peripheral delivery and utilisation of  $O_2$  in aging populations (Costes et al. 1999; Kutsuzawa, Shioya, Kurita, Haida and Yamabayashi 2001; DeLorey et al. 2003a; Grassi et al. 2003; Stathokostas et al. 2003). Recent data from Grassi and others (2003) suggest that NIRS may be used to detect delayed adjustment of oxidative metabolism *in vivo* throughout exercise transients. This is of great interest, particularly with the alterations in the bioenergetics of skeletal muscle with aging or a prolonged sedentary lifestyle (Hansford 1983). To date, only a handful of empirical studies have investigated the age-associated changes in mOxy responses to exercise (Costes et al. 1999; Kutsuzawa et al. 2001; Stathokostas et al. 2003; DeLorey et al. 2003a; 2004a; DeLorey et al. 2005). Given the age-related changes in  $\dot{V}O_2$  kinetics and muscle histochemical and biochemical characteristics (Proctor, Sinning, Walro, Sieck and Lemon 1995; Chilibeck et al. 1997; Houmard et al. 1998), the on-transient mOxy responses may also show a significant effect of age.

Research by Costes et al. (1999) investigated the age-related decrease in cardiovascular function and changes in mOxy during incremental exercise in young ( $27 \pm 4$  y) and elderly ( $67 \pm 5$  y) healthy subjects. Resting mOxy was significantly lower in the older group ( $55.0 \pm 16.7\%$ ) compared to that of the younger group ( $80.6 \pm 20.0\%$ ,  $p < 0.01$ ). Furthermore, mOxy at  $\dot{V}O_{2\max}$  was found to be significantly lower in the older ( $27.7 \pm 24.8\%$ ) compared to the younger ( $51.1 \pm 21.1\%$ ,  $p < 0.01$ ) subjects. No significant difference was noted in the amplitude of change in mOxy between the old and young groups ( $27.3 \pm 16.7\%$  vs.  $24.3 \pm 12.9\%$ ). The mOxy  $\tau_{1/2}$  was observed to be similar for both the young ( $33.5 \pm 17.5$  s) and old ( $28.2 \pm 10.5$  s) groups. Costes et al. (1999)

suggested that impaired O<sub>2</sub> utilisation in the older subjects appeared to be unlikely given the significantly greater desaturation observed at maximal intensity exercise. The researchers suggested that any effect of aging on the metabolic response to exercise is more likely the result of a reduced delivery of O<sub>2</sub> to the working muscle.

More specific to the present thesis is the research of Stathokostas et al. (2003) and DeLorey et al. (2003a; 2004a) who reported upon the mOxy response during the exercise on-transients in aged populations. Firstly, Stathokostas et al. (2003) examined the effect of age on the  $\dot{V}O_2$  and mOxy relationship during a ramp cycling test to fatigue in five young ( $26 \pm 3$  y) and old ( $68 \pm 3$  y) males. They reported a lower  $\dot{V}O_{2\max}$  in the older subjects, but observed similar changes in Hb across the incremental test between the young ( $28 \pm 12$   $\mu\text{M}$ ) and elderly ( $22 \pm 7$   $\mu\text{M}$ ) cohorts. The researchers reported that for a given absolute submaximal  $\dot{V}O_2$  ( $1.5 \text{ L}\cdot\text{min}^{-1}$ ), the amplitude of muscle deoxygenation was significantly higher in the old ( $64 \pm 19\%$ ) compared to the younger ( $27 \pm 6\%$ ) subjects. This may reflect the higher relative intensity performed by the older subjects at this constant  $\dot{V}O_2$ .

DeLorey and others (2003a; 2004a; 2005) described the  $\dot{V}O_2$  and mOxy responses to moderate (80% VT) and heavy-intensity (50% $\Delta$ ) cycling in young ( $25 \pm 3$  y) and old ( $68 \pm 3$  y) healthy subjects. In response to the moderate-intensity SWT, the  $\dot{V}O_2$   $\tau_p$  was significantly slower in the older cohort (O) ( $42 \pm 9$  s;  $11 \pm 1$  s; ( $\tau_p$ ; TD)) compared to the young (Y) ( $26 \pm 7$  s;  $12 \pm 2$  s). In contrast, both the TD (Y:  $12 \pm 2$  s; O:  $11 \pm 1$  s) and  $\tau_p$  (Y:  $13 \pm 10$  s; O:  $9 \pm 3$  s) of the mOxy response were not different between the age-groups.

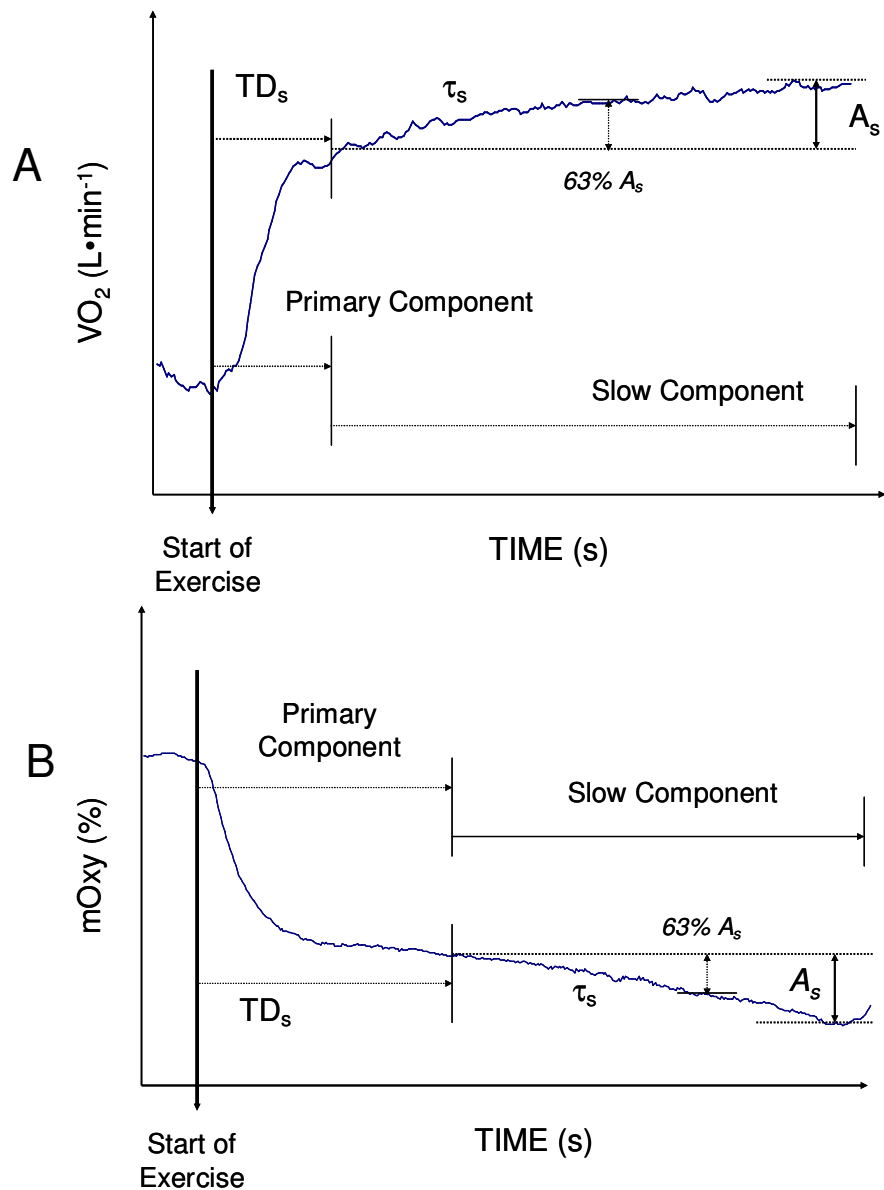
However, DeLorey et al. (2003a) reported that the older population had a significant increase in mOxy ( $13 \pm 4 \mu\text{M}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$ ) for the relative work rate compared to the younger cohort ( $7 \pm 2 \mu\text{M}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$ ). In response to the heavy-intensity SWT, DeLorey et al. (2005) reported that the  $\dot{V}\text{O}_2 \tau_p$  was significantly slower in the old ( $49 \pm 8 \text{ s}$ ) compared to the young ( $29 \pm 4 \text{ s}$ ) research group. While the on-transient mOxy  $\text{TD}_p$  was similar between age-groups (Y:  $7 \pm 1 \text{ s}$ ; O:  $8 \pm 3 \text{ s}$ ), the mOxy  $\tau_p$  was significantly faster in the older ( $8 \pm 2 \text{ s}$ ) compared to the young ( $14 \pm 2 \text{ s}$ ).

Taken together, these results suggest that the speed of the metabolic adaptation to moderate-intensity exercise is limited by the delivery of  $\text{O}_2$  to the muscle, given the similar rate of adjustment in mOxy (DeLorey et al. 2004b; 2005), despite the slowed HR and  $\dot{V}\text{O}_2$  kinetic responses in the older exercisers. The researchers highlighted that these results suggest that the capacity for elderly muscle to extract and consume  $\text{O}_2$  is maintained with age, and that the slowed  $\dot{V}\text{O}_2$  response is the result of a decrease in vascular conduction and matching of  $\text{O}_2$  delivery to work intensities. However, DeLorey et al. (2004b) concluded that the fundamental limitation to  $\dot{V}\text{O}_2$  adaptation to moderate-intensity exercise is most likely related to other intra-muscular factors than  $\text{O}_2$  delivery. The results from both these previous studies suggest that despite a slowing in the  $\dot{V}\text{O}_2$  response in older sedentary subjects (Babcock et al. 1994b; DeLorey et al. 2004a), the rate of mOxy adaptation is speeded with aging. This suggests that the capacity to extract and utilise  $\text{O}_2$  is maintained or improved within elderly muscle. As such,  $\text{O}_2$  delivery limitations may control the speed of the on-transient metabolic adaptation in older individuals.

In summary, the available research suggests that the age-related slowing of  $\dot{V}O_2$  kinetics is due to a decreased capacity to match  $O_2$  delivery to the metabolic requirements of the exercise intensity. While a number of hypotheses have been suggested to explain the slowed  $\dot{V}O_2$  kinetic response, the monitoring of changes in mOxy during SWT suggests that the capacity to extract and utilise  $O_2$  within the cell is maintained with age (Costes et al. 1999; DeLorey et al. 2004a; 2005). However, the mechanisms associated with the slowed  $\dot{V}O_2$  response may not be solely identified through the reported changes in mOxy within the working muscle. Thus it may be possible that this 'age-related' decline is more the consequence of a prolonged sedentary lifestyle rather than aging *per se*. Thus, a purpose of Study Two of the present series of investigations was to examine the effect of age on the on-transient  $\dot{V}O_2$  and mOxy responses in well-trained cyclists.

## **SLOW COMPONENT DEVELOPMENT**

The concept of a metabolic steady-state being attained during constant intensity exercise is only applicable to exercise intensities below VT (Barstow 1994; Poole 1994; Poole et al. 1994; Whipp 1994). Previous research has identified a gradual decrease in the mechanical and/or metabolic efficiency during high-intensity ( $>VT$ ) exercise, which is observed as an 'additional' rise in  $\dot{V}O_2$  visible ~80-180 s after the onset of exercise (Poole 1994; Poole et al. 1994; Xu and Rhodes 1999; Zoladz and Korzeniewski 2001). This gradual increase in  $\dot{V}O_2$  is termed the  $\dot{V}O_2$  slow component, and is demonstrated in Figure 2.6.



**Figure 2.5:** Schematic representation of the  $\text{VO}_2$  (A) and mOxy (B) slow component and kinetic parameters during heavy-intensity submaximal exercise ( $A_s$ ; Slow component amplitude;  $\text{TD}_s$ ; Slow component time delay;  $\tau_s$ ; Slow component time constant).

It is thought that this  $\text{VO}_2$  slow component represents a decrease in efficiency with the energy cost at such exercise intensities appearing to be greater than that predicted from the linear  $\text{VO}_2$ –Work relationship observed

during moderate-intensity ( $<V_T$ ) exercise (Zoladz, Rademaker and Sargeant 1995). During heavy- or severe-intensity exercise ( $>V_T$ ), the  $\dot{V}O_2$  slow component may demonstrate amplitudes of more than  $1 \text{ L}\cdot\text{min}^{-1}$ , or alternatively may continue to rise until  $\dot{V}O_{2\text{max}}$  is attained and subsequent fatigue occurs (Poole 1994; Poole et al. 1994). Previous research has suggested a wide range of possible causal mechanisms, but as yet, no definitive primary causal mechanism has been identified. The  $\dot{V}O_2$  slow component has been suggested to occur as a result of a decrease in metabolic and/or mechanical efficiency arising from changes in biochemical or neurological factors only facilitated through high-intensity exercise (Xu and Rhodes 1999; Demarie et al. 2001; Zoladz and Korzeniewski 2001).

The  $\dot{V}O_2$  slow component was originally measured as the difference in  $\dot{V}O_2$  between the 6<sup>th</sup> and 3<sup>rd</sup> min of a high-intensity constant load exercise bout (Xu and Rhodes 1999). More recent research has demonstrated that the  $\dot{V}O_2$  slow component begins considerably earlier than three minutes after exercise onset ( $\sim 90\text{-}120 \text{ s}$ ) (Bearden, Henning, Bearden and Moffat 2002; Koppo and Bouckaert 2002). It has more recently become preferred that the slow component is modelled as an additional exponential component to compensate for differences in its TD and amplitude (Bearden and Moffat 2001). Koppo et al. (2002) have also suggested that the classical method of quantification may underestimate the actual magnitude of the  $\dot{V}O_2$  slow component during high-intensity exercise.

While the mechanisms responsible for the  $\dot{V}O_2$  slow component are not completely understood, it has been suggested that  $\sim 86\%$  of the  $\dot{V}O_2$  slow

component is developed within the working muscle itself (Poole et al. 1991; Poole 1994). This is supported by Demarie et al. (2001) who reported a gradual decrease in mOxy during high-intensity running. The amplitudes of the mOxy and  $\dot{V}O_2$  slow components were significantly correlated in this investigation. Similarly, the work of Belardinelli et al. (1995a) and Grassi et al. (2003) has supported the observation of concurrent  $\dot{V}O_2$  and mOxy slow components during high-intensity constant-load exercise. However, the evidence from Grassi et al. (2003) provided no correlation between the respective amplitudes of the  $\dot{V}O_2$  and mOxy slow components. Thus, the available evidence suggests that the  $\dot{V}O_2$  slow component originates within the working muscle. This suggestion is supported by a small amount of early research also identifying a gradual decrease in mOxy across high-intensity submaximal workloads (Belardinelli et al. 1995a; 1995b; Bhambhani et al. 1997).

### **Factors Influencing the Slow Component Development**

Previous research has linked the  $\dot{V}O_2$  slow component to a number of mediating factors including muscle temperature, lactate accumulation, prior exercise, histochemical characteristics, and the change in muscle fibre recruitment patterns (Poole et al. 1994; Saunders, Evans, Arngrimsson, Allison, Warren and Cureton 2000; Koppo and Bouckaert 2002; Koppo et al. 2002). More recent research has suggested that the most likely contributor to the  $\dot{V}O_2$  slow component may be the gradual recruitment of Type II muscle fibres in response to the fatigue of Type I fibres (Borrani et al. 2001; Krstrup et al. 2004b), despite contrasting results (Scheuermann, Hoelting, Noble and Barstow 2001).

It would appear that regardless of the causal mechanisms of the  $\dot{V}O_2$  slow component, changes within a number of physiological measures have been shown to decrease the magnitude of the  $\dot{V}O_2$  slow component through both endurance training (Womack, Davis, Blumer, Barrett, Weltman and Gaesser 1995; Carter et al. 2000a; Edge et al. 2003; Ocel, Miller, Pierson, Wooten, Hawkins, Myers and Herbert 2003; Saunders et al. 2003) and sedentary aging (Chick et al. 1991; Scheuermann, Bell, Paterson, Barstow and Kowalchuk 2002; Sabapathy et al. 2004). A great deal of literature has suggested that development of the  $\dot{V}O_2$  slow component is multifactorial in nature (Poole 1994; Poole et al. 1994; Poole, Gladden, Kurdak and Hogan 1995; Bauer et al. 1999; Billat, Morton, Blondel, Berthoin, Bocquet, Koralsztejn and Barstow 2000; Carter, Jones, Barstow, Burnley, Williams and Doust 2000b; Koga, Barstow, Shiojiri, Takaishi, Fukuba, Kondo, Shibasaki and Poole 2001; Perrey, Betik, Candau, Rouillon and Hughson 2001; Pringle, Carter, Doust and Jones 2002; Hill, Halcomb and Stevens 2003; Jones et al. 2003; Pringle, Doust, Carter, Tolfrey and Jones 2003a; Pringle et al. 2003b; Tordi, Perrey, Harvey and Hughson 2003).

### *Muscle Temperature*

Muscle temperature has been shown to be related to a number of physiological factors which may affect the  $\dot{V}O_2$  slow component during high-intensity constant-load exercise (Koga et al. 1997; 2002). These include an influence of the  $Q_{10}$  effect which increases the rate of metabolic reactions, the mechanical efficiency of muscles, or a temperature-regulated rightward shift in the  $HbO_2$  dissociation curve via the Bohr Effect (Poole 1994; Poole et al. 1995).

However, the direct effect of increased muscle temperature on the  $\dot{V}O_2$  slow component has not been supported with sufficient research evidence.

In a previous investigation, Koga et al. (1997) elevated thigh muscle temperature to  $\sim 39^\circ\text{C}$  using hot water pants and performed a number of repeated rest to work transitions of moderate- (50W) and high-intensity (50% $\Delta$ ) exercise in seven untrained healthy young male volunteers ( $25.7 \pm 9.2$  y;  $44.5 \pm 9.8$  mL $\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). The investigators reported that the  $\dot{V}O_2$  slow component was significantly smaller for the heated condition ( $138 \pm 66$  mL $\cdot\text{min}^{-1}$ ) than the normal condition ( $205 \pm 70$  mL $\cdot\text{min}^{-1}$ ). They stated that this result was inconsistent with the suggestion that increased muscle temperature plays a role in the development of the  $\dot{V}O_2$  slow component during heavy-intensity exercise. Similarly, Koppo et al. (2002) investigated the elevation of muscle temperature through prior exercise on  $\dot{V}O_2$  kinetics. Koppo et al. (2002) successfully raised the temperature of the VL ( $35.3 \pm 1.0$   $^\circ\text{C}$ ) through both prior exercise ( $37.3 \pm 0.6$   $^\circ\text{C}$ ) and passive heating ( $37.2 \pm 0.3$   $^\circ\text{C}$ ) prior to the completion of a high-intensity (90%  $\dot{V}O_{2\text{max}}$ ) SWT. The researchers observed that beyond the second minute of high-intensity exercise, the  $\dot{V}O_2$  response was elevated by prior exercise, and that the  $\dot{V}O_2$  slow component amplitude was reduced from  $556 \pm 72$  mL $\cdot\text{min}^{-1}$  for the control condition to  $241 \pm 162$  mL $\cdot\text{min}^{-1}$  and  $461 \pm 148$  mL $\cdot\text{min}^{-1}$  for the prior exercise and passive leg heating interventions, respectively. It was suggested that the elevation of muscle temperature results in an increased metabolic efficiency and smaller  $\dot{V}O_2$  slow component rather than actually causing a decrease in either the metabolic or mechanical efficiency. The mechanism responsible for this increase in metabolic efficiency with an increased muscle temperature is not yet fully understood.

To date, three hypotheses have been suggested to explain the above findings of Koga et al. (1997; 2002) and Koppo et al. (2002). Firstly, Brooks et al. (1971) hypothesised that an increase in muscle temperature is associated with an elevated transient and steady-state  $\dot{V}O_2$  due to the  $Q_{10}$  effect on the metabolism and phosphorylation efficiency (ADP/ $O_2$  molecule) and this may increase metabolic efficiency. It is unlikely that the oxidative phosphorylation within mitochondria becomes uncoupled, causing a decrease in metabolic efficiency, as this only occurs at higher muscle temperatures than those employed within the discussed investigations ( $\leq 40^\circ\text{C}$ ) (Gaesser and Poole 1996; Xu and Rhodes 1999; Demarie et al. 2001). Secondly, an increase in muscle temperature is linked to an increase in the mechanical efficiency of the muscle which would decrease the  $\dot{V}O_2$  required at any exercise intensity as a result of the lowered viscous resistance of the contractile component of the muscle (Binzoni and Delpy 2001). Finally, increases in muscle temperature may produce a rightward shift in the  $\text{HbO}_2$  dissociation curve to allow greater  $O_2$  to be uncoupled from  $\text{HbO}_2$  stores, thus increasing the  $O_2$  delivery to the cell (Koga et al. 1997; Koppo et al. 2002). However, this mechanism would only be of benefit if the metabolic efficiency was reduced during the constant-load high-intensity exercise.

In summary, no research to date has definitively identified an elevated muscle temperature as a possible facilitator in the development in the  $\dot{V}O_2$  slow component during high-intensity exercise, despite muscle temperature having been shown to change the mechanical and metabolic efficiency of muscle tissue during exercise. Possible casual or influential mechanisms worthy of

discussion may include lactate accumulation or the recruitment of less efficient Type II muscle fibres.

### *Lactate*

The observation that the  $\dot{V}O_2$  slow component is only observed at work intensities above VT strongly suggests that the appearance of lactate within the muscle and blood may facilitate the development of the  $\dot{V}O_2$  slow and mOxy component. A number of studies have suggested hypotheses for this relationship, including an increased HbO<sub>2</sub> dissociation, an increased  $\dot{V}O_2$  cost of lactate oxidation through gluconeogenesis, as well as a decrease in mechanical efficiency due to the interference of anaerobic by-products such as [H<sup>+</sup>] with muscle contraction (Roston et al. 1987; Stringer et al. 1994; Gaesser and Poole 1996; Xu and Rhodes 1999; Demarie et al. 2001; Zoladz and Korzeniewski 2001).

Roston et al. (1987) were one of the first to examine the effects of lactate concentration on  $\dot{V}O_2$  kinetics during cycling in six healthy young men during repeat six minute SWT at intensities ranging from 80% LT to 80%Δ. The investigators reported that the  $\dot{V}O_2$  slow component was significantly correlated ( $r = 0.855$ ,  $p < 0.05$ ) with the rise in [BLa<sup>-</sup>] from rest to 6 min during the high-intensity cycling. These researchers suggested that the increase in  $\dot{V}O_2$  during exercise may be due to the oxidation of lactate, and/or gluconeogenesis of lactate in both the liver and skeletal muscle.

Other researchers have also noted a relationship between the appearance of BLa<sup>-</sup> and the onset of the  $\dot{V}O_2$  slow component (Casaburi et al.

1989; Whipp and Ward 1990). However, it may be possible that the correlation between increases in  $\dot{V}O_2$  and  $[BLa^-]$  during heavy-intensity exercise may be coincidental rather than causal. This observation may be more related to the exercise intensity and subsequent mechanical properties of the peripheral muscle rather than  $[BLa^-]$  *per se* (Stringer et al. 1994). Poole et al. (1995) have suggested the decrease in blood pH associated with the increases in  $[BLa^-]$  causes an increased  $O_2$  delivery via the Bohr effect, which may account for up to ~62% of the  $\dot{V}O_2$  slow component amplitude. The rightward shift noted in the  $HbO_2$  dissociation curve from metabolic acidosis may therefore be a primary contributor to the development of the  $\dot{V}O_2$  slow component (Stringer et al. 1994).

Other investigators have hypothesised that lactate may be responsible for the  $\dot{V}O_2$  slow component due to the extra  $O_2$  requirement for its oxidation (Barstow 1994; Gaesser and Poole 1996). Gaesser and Poole (1996) observed that ~70% of lactate formed during exercise above VT is oxidised within active musculature, while the balance is removed through hepatic gluconeogenesis. Despite the majority of the  $\dot{V}O_2$  slow component being developed within the working muscle, (Poole et al. 1994; Poole 1994), it is most likely the increased  $\dot{V}O_2$  required to oxidise lactate within skeletal muscle and liver is not a significant contributor to the  $\dot{V}O_2$  slow component. Poole (1994) infused isolated canine gastrocnemius with lactate for two 60 min periods during which the muscle was electrically stimulated as to not change blood or muscle pH. The researchers observed a significant decrease in muscle  $\dot{V}O_2$ , from  $5.1 \pm 0.3$  to  $4.1 \pm 0.2 \text{ mL} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  across the 60 min in comparison to control values, suggesting that lactate does not facilitate the development of the  $\dot{V}O_2$  slow

component. Thus, it may appear that the proposed influence of lactate on  $\dot{V}O_2$  kinetics observed in the literature may be due to the influence of other metabolic factors, such as a decrease in pH or recruitment of less efficient Type II muscle fibres during high-intensity exercise (Stringer et al. 1994; Borrani et al. 2001). In summary, it appears that the development and magnitude of the  $\dot{V}O_2$  slow component is related to mechanisms observed concurrently with lactate production, however it appears not to be a major causal factor.

More recently, Demarie et al. (2001) reported that the magnitude of the  $\dot{V}O_2$  slow component appears related to deoxygenation of the VL (as measured by NIRS) and the increase in  $[BLa^-]$  during high-intensity running in eleven young male amateur soccer players ( $29 \pm 2$  y;  $58.0 \pm 4.6$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ). Both  $\dot{V}O_2$  and  $[BLa^-]$  significantly increased, and mOxy decreased between the third and sixth minutes of high-intensity treadmill exercise. There was a significant but weak negative relationship ( $r = -0.38$ ,  $p < 0.05$ ) observed between changes in mOxy and  $[BLa^-]$  between the third and sixth minutes of exercise. The relationship between changes in  $\dot{V}O_2$  and  $[BLa^-]$  during the same time period was reported to be non-significant ( $r = 0.30$ ,  $p = 0.127$ ). These findings support Poole (1994) who suggested that the majority of the  $\dot{V}O_2$  slow component is developed within the muscle, observed as a gradual deoxygenation of the working muscle.

In conclusion, it appears that the relationship between increases in  $[BLa^-]$  and the  $\dot{V}O_2$  slow component is equivocal. The secondary effects on the metabolic efficiency of the body suggest that  $La^-$  may play a minor role in the development of the  $\dot{V}O_2$  slow component. However, the majority of the  $\dot{V}O_2$

slow component during high-intensity constant-load endurance exercise is developed from peripheral mechanisms. Since the  $\dot{V}O_2$  slow component is only developed at intensities above VT, this suggests that its origin lies within other physiological mechanisms encountered during high-intensity exercise requiring anaerobic metabolism and  $BLa^-$  production.

### **Influence of Histochemical Parameters on the Slow Components**

It is well established that Type II fibres have different energetic properties, consume a higher  $O_2$  cost per unit of energy output and higher lactate output than Type I muscle fibres (Bottinelli and Reggiani 2000). At present, equivocal evidence has suggested that the development of the  $\dot{V}O_2$  slow component is due to in part an increased recruitment of less efficient Type II muscle fibres during high-intensity exercise (Saunders et al. 2000).

It has been well established that the development and magnitude of the  $\dot{V}O_2$  slow component is related to the percentage of Type II fibres within the recruited musculature (Shinohara and Moritani 1992; He et al. 2000; Saunders et al. 2000; Borrani et al. 2001; Pringle et al. 2003b). For example, Pringle et al. (2003b) observed that the  $\dot{V}O_2$  slow component amplitude was inversely correlated with the percentage of Type I fibres for both heavy ( $r = -0.74$ ,  $p < 0.01$ ) and severe-intensity ( $r = -0.64$ ,  $p < 0.05$ ) exercise. Of greater importance, the  $\dot{V}O_2$  slow component amplitude was significantly related to the percentage of Type II fibres for heavy-intensity exercise ( $r = 0.60$ ,  $p < 0.05$ ), and Type IIx fibres for both the heavy ( $r = 0.60$ ,  $p < 0.05$ ) and severe ( $r = 0.62$ ,  $p < 0.05$ ) intensity SWT. Further relationships between the  $\dot{V}O_2$  slow component amplitude and the combined muscle fibre cross-sectional area to capillary contact ratio for the

heavy ( $r = 0.63$ ,  $p < 0.05$ ) and severe-intensity ( $r = 0.74$ ,  $p < 0.01$ ) SWT were observed. Similarly, Russell et al. (2002) observed significant relationships between  $\dot{V}O_2$  slow component amplitude and both Type I ( $r = -0.57$ ,  $p < 0.05$ ) and Type IIa ( $r = 0.60$ ,  $p < 0.05$ ) composition. These researchers also reported that the amplitude of the  $\dot{V}O_2$  slow component was inversely related to  $\dot{V}O_{2\max}$  ( $r = -0.73$ ,  $p < 0.01$ ), and maximal CS activity within the VL ( $r = -0.71$ ,  $p < 0.01$ ), suggesting that aerobically fit subjects will exhibit an attenuated slow component amplitude. Taken together, these studies demonstrate that the decrease in efficiency associated with the slow component is related to muscle fibre histochemical, and perhaps biochemical characteristics. However, it is the recruitment of these fibres which is proposed to be the primary factor responsible for the slow component (Saunders et al. 2000; Borrani et al. 2001; Krstrup et al. 2004b).

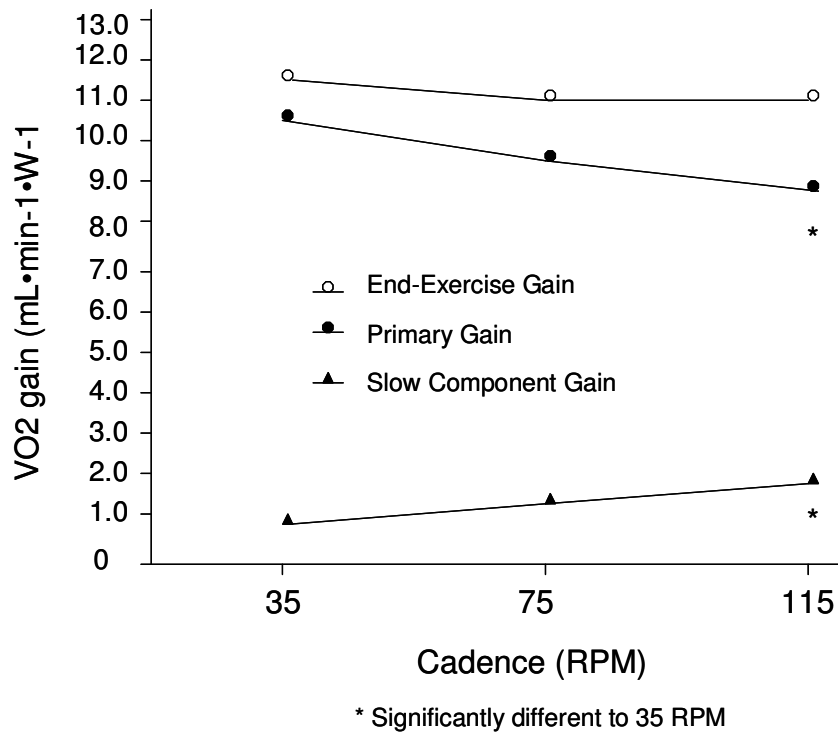
While the composition of Type II fibres are related to the  $\dot{V}O_2$  slow component amplitude during high-intensity endurance exercise, it is more likely the neuromuscular recruitment of these fibres during the high-intensity submaximal exercise which is responsible for the gradual rise in  $\dot{V}O_2$  (Saunders et al. 2000; Borrani et al. 2001; Krstrup et al. 2004b). In a clinical trial, Saunders et al. (2000) investigated the surface electromyographic (sEMG) and magnetic resonance imaging (MRI) changes of the VL and rectus femoris (RF) after 3 and 15 min of moderate (85% VT) and heavy-intensity (60% $\Delta$ ) cycling. The investigators observed no  $\dot{V}O_2$  slow component for the sub-VT work intensity, with no changes in [BLa], ventilation or muscle fibre recruitment at that sub-VT intensity. In contrast, high-intensity cycling produced a significant  $\dot{V}O_2$  slow component, which was accompanied by significant ( $p < 0.05$ )

increases in both the mean power frequency and root mean square observed from the VL between 3 ( $72.1 \pm 6.9$  mV;  $0.19 \pm 0.08$  mV) and 15 min ( $76.3 \pm 9.0$  mV;  $0.21 \pm 0.07$  mV) of cycling. These findings support the suggestion of an increased recruitment of Type II muscle fibres during sustained high-intensity exercise. Other research supporting the Type II fibre hypothesis have suggested that the fatigue Type II fibres may continue to utilise  $O_2$  for recovery ( $Na^+-K^+$  pumping etc.) despite no contribution towards force production (Rossiter et al. 1999; Pringle et al. 2003). This may also contribute to the commonly observed decrease in efficiency.

Further justification of this mechanism was evident through significant increases in muscle activity (as measured by MRI muscle relaxation times) between the 3<sup>rd</sup> and 15<sup>th</sup> min for both the VL ( $33.1 \pm 1.6$  vs.  $36.0 \pm 2.9$  ms) and RF ( $29.8 \pm 1.8$  vs.  $30.6 \pm 0.8$  ms) (Saunders et al. 2000). These increased muscle relaxation times for the recruited muscles were significantly related to the rise in  $VO_2$  between the third and fifteenth minutes of cycling ( $r = 0.63$ ,  $p < 0.05$ ). These findings are supported by a number of sEMG investigations (Shinohara and Moritani 1992; Borrani et al. 2001; Krstrup, Soderlund, Mohr and Bangsbo 2004a; Krstrup et al. 2004b; Sabapathy, Schneider and Morris 2005) but not all (Scheuermann et al. 2001; Cleuziou, Perrey, Borrani, Lecoq, Courteix, Germain and Obert 2004). For example, Cleuziou et al. (2004) reported upon the development of the  $VO_2$  slow component and changes in fibre recruitment patterns for both moderate- (80% VT) and heavy-intensity (50% $\Delta$ ) SWT. Cleuziou et al. (2004) reported that the  $VO_2$  slow component was only observed across the heavy-intensity SWT, despite the changes in EMG activity of the VL being similar between the

different SWT intensities. This finding may suggest that the  $\dot{V}O_2$  slow component is related to other intra-muscular mechanisms than changes in fibre recruitment patterns.

The above findings reporting that the increased recruitment of Type II fibres are significantly related to the amplitude of the  $\dot{V}O_2$  slow component support the work of Rossiter et al. (2001). These investigators suggested that the  $\dot{V}O_2$  slow component is related to a higher phosphate cost with sustained force production at high-intensity submaximal exercise, which can only be maintained through the recruitment of Type II fibres. To test this hypothesis, Pringle et al. (2003a) investigated the  $\dot{V}O_2$  slow component across different cadences (35, 75 and 115 RPM) during a high-intensity SWT (50% $\Delta$ ) in a group of young ( $26 \pm 4$  y;  $3.58 \pm 0.18$  L $\cdot$ min $^{-1}$ ) well-trained cyclists. They hypothesised that the higher cadences would facilitate an increased recruitment of Type II fibres, given the faster contraction speed required. Such changes in the recruitment pattern would most likely require an additional  $\dot{V}O_2$  given the changes in fibre-specific efficiencies across increasing cadences. The researchers reported that the high cadence condition (115 RPM) produced a significantly larger  $\dot{V}O_2$  slow component, supporting an increased recruitment of the inefficient Type II fibres (Figure 2.6).



**Figure 2.6:** The effect of cadence on the primary and end-exercise VO<sub>2</sub> gains, and VO<sub>2</sub> slow component magnitude (Adapted from Pringle et al. 2003).

It may also have been possible that the Type II fibres recruited during the faster cadences may have been subject to fatigue, which may have then required Type I fibres to be recruited. Previous evidence has shown that the efficiency of different fibre types to be similar with their preferred recruitment intensities and contractile speed (He et al. 2000). Therefore, the Type I fibres recruited during the high cadence condition may have been equally inefficient and helped to facilitate the development of the VO<sub>2</sub> slow component. However, the investigation did not employ sEMG techniques to monitor fibre recruitment, and therefore these results suggesting an increased Type II fibre recruitment are speculative.

In order to further test the hypothesis of increased Type II fibre recruitment, recent investigations have attempted to specifically deplete glycogen levels in Type I fibres in order to increase the reliance on Type II recruitment (Bouckaert, Jones and Koppo 2004; Carter, Pringle, Boobis, Jones and Doust 2004; Krstrup et al. 2004a). Carter et al. (2004) employed both low and high-intensity exercise protocols to specifically deplete muscle glycogen stores in Type I and II fibres, prior to completing a six minute high-intensity (50%Δ) SWT in active young men ( $25.5 \pm 4.5$  y;  $2.95 \pm 0.5$  L•min<sup>-1</sup>). No significant differences were observed in either the amplitude or time parameters of the  $\dot{V}O_2$  slow component between the control ( $0.24 \pm 0.04$  L•min<sup>-1</sup>;  $110.9 \pm 6.9$  s) or low-intensity ( $0.27 \pm 0.05$  L•min<sup>-1</sup>;  $104.8 \pm 5.4$  s) depletion protocols. However,  $\dot{V}O_2$  kinetics following the high-intensity depletion protocol demonstrated a lower amplitude ( $0.18 \pm 0.03$  L•min<sup>-1</sup>) and longer time of onset ( $127.4 \pm 7.9$  s) of the  $\dot{V}O_2$  slow component than the other two conditions. Therefore, the depletion of glycogen specifically within Type II fibres significantly influenced the nature of the  $\dot{V}O_2$  slow component, and supports the hypothesis of altered recruitment patterns being responsible for the decrease in efficiency.

In summary, the most likely mechanism responsible for the development of the  $\dot{V}O_2$  slow component lies with the increased recruitment of Type II muscle fibres which are required to sustain power output after Type I fibres fatigue during sustained high-intensity SWT. The magnitude of the  $\dot{V}O_2$  slow component has been related to the muscle fibre composition across exercise intensities, which have also been shown to be related to and influenced by both physical training and aging.

## **Influence of Physical Training on the Slow Components**

The mechanisms responsible for the development of the  $\dot{V}O_2$  slow component have been shown to lie predominately within the working musculature (Poole 1994). A reduction in the  $\dot{V}O_2$  slow component amplitude has strong implications for athletes who compete in constant-load high-intensity endurance events, theoretically through a reduction in metabolic fatigue (Xu and Rhodes 1999). However, the actual effects of physical training on the mechanisms responsible for the  $\dot{V}O_2$  slow component remain to be fully evaluated, and it is difficult to suggest what training-related adaptations may affect the  $\dot{V}O_2$  slow component.

Carter et al. (2000a) investigated the effects of a six-week combined continuous and interval running training in 23 untrained ( $22 \pm 3$  y;  $54.9 \pm 2.1$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ) healthy volunteers. Each subject performed repeat six minute moderate (80% VT) and heavy-intensity (50% $\Delta$ ) SWT. The researchers observed a significant reduction in the development of the  $\dot{V}O_2$  slow component (pre:  $321.1 \pm 32$  mL $\cdot$ min $^{-1}$ ; post:  $217 \pm 23$  mL $\cdot$ min $^{-1}$ ) following the training intervention. This decrease in the  $\dot{V}O_2$  slow component was significantly related to the reduction in ventilation ( $r = 0.46$ ,  $p < 0.05$ ), but not to the reduction in end-exercise [BLa] ( $r = 0.39$ ,  $p > 0.05$ ).

The observations of Carter et al. (2000a) have been supported by a number of research investigations which have reported similar changes in the  $\dot{V}O_2$  slow component with endurance training (Womack et al. 1995; Edge et al. 2003; Ocel et al. 2003; Saunders et al. 2003). For example, Womack et al. (1995) observed the effect of endurance training on the  $\dot{V}O_2$  slow component in

a group of untrained males ( $n = 7$ ,  $25.6 \pm 1.5$  y;  $3.20 \pm 0.19$  L $\cdot$ min $^{-1}$ ) during bouts of high-intensity (50% $\Delta$ ) cycling. As a result of a six week endurance training protocol consisting of four 40-min cycling sessions per week, the researchers reported a significant improvement in  $\dot{V}O_{2\max}$  ( $1.57 \pm 0.12$  vs.  $1.97 \pm 0.09$  L $\cdot$ min $^{-1}$ ) and power output at LT ( $103 \pm 11$  vs.  $132 \pm 9$  W). They also observed that the amplitude of the  $\dot{V}O_2$  slow component was significantly reduced from pre-training levels of  $0.42 \pm 0.06$  L $\cdot$ min $^{-1}$  to  $0.20 \pm 0.04$  L $\cdot$ min $^{-1}$  after two weeks, and was further reduced at the completion of the six-week endurance-training program to  $0.15 \pm 0.04$  L $\cdot$ min $^{-1}$ . This reduction in the magnitude of the  $\dot{V}O_2$  slow component was not significantly related to decreases in either end-exercise [BLa],  $\dot{V}E$ , plasma adrenaline or plasma noradrenaline. However, the researchers did not specify whether the absolute power output at post-testing was adjusted for training, or was identical to the pre-testing workload. If the workload was not adjusted for the effects of the training intervention, the decreased  $\dot{V}O_2$  slow component amplitude may be due to the lower relative exercise intensity.

Additional evidence to support the effect of physical training on the  $\dot{V}O_2$  slow component was put forward by Ocel et al. (2003) who examined the effects of six weeks of cycling endurance training on 18 apparently healthy young ( $23 \pm 1$  y;  $3.18 \pm 0.18$  L $\cdot$ min $^{-1}$ ) men, consisting of moderate (<VT) or high (>VT) intensity exercise. They observed that the magnitude of the  $\dot{V}O_2$  slow component was reduced by 44% after one week of high-intensity training, which was significantly higher than the 20% and 12% reductions noted for the moderate-intensity training and control groups, respectively. No significant difference was observed for the reduction in the  $\dot{V}O_2$  slow component in the

training group between the moderate- or high-intensity exercise training groups following the six-week training program. However, the  $\dot{V}O_2$  slow component amplitude was significantly reduced in comparison to the control group. Ocel et al. (2003) observed that this attenuation of the  $\dot{V}O_2$  slow component with moderate and high-intensity training was strongly related to the training-related decreases in both end-exercise  $[BLa^-]$  ( $r = 0.76$ ,  $p < 0.01$ ) and  $\dot{V}E$  ( $r = 0.59$ ,  $p < 0.05$ ). Neither  $[BLa^-]$  or  $\dot{V}E$  have been shown to have a primary role in the development of the  $\dot{V}O_2$  slow component (Poole 1994), which suggests that the smaller amplitudes of the  $\dot{V}O_2$  slow component were most likely due to increases in aerobic fitness and oxidative capacity of the working muscle.

Thus, it appears that prolonged moderate and high-intensity physical training can significantly alter the development of the  $\dot{V}O_2$  slow component (Carter et al. 2000a; Ocel et al. 2003). However, the mechanisms responsible for the decrease in the  $\dot{V}O_2$  slow component with training are yet to be elucidated but most likely occur within the peripheral muscle recruited during training and exercise (Poole 1994). It is more likely that the major mechanism for the training-related decrease in the  $\dot{V}O_2$  slow component is the more efficient muscle recruitment patterns and improved fatigue resistance of muscle fibres. However, no research investigation to date has fully utilised sEMG to determine whether training-related changes in muscle recruitment patterns are responsible for this improved efficiency across high-intensity exercise. Similarly, no research has reported the training-related effects in mOxy on the concurrent development of the slow component within the working muscle.

In summary, the reduction in the  $\dot{V}O_2$  slow component with physical training has wide implications for high-intensity endurance sports performance (Xu and Rhodes 1999; Carter et al. 2000b). While a number of physiological factors may contribute to this training-facilitated attenuation of the  $\dot{V}O_2$  slow component, the most likely physiological mechanism may be the changes in the recruitment patterns and efficiency of Type II muscle fibres. However, other factors such as aging and its influence on the innervation and composition of Type II fibres have also been observed to alter the  $\dot{V}O_2$  slow component development.

### **Influence of Aging on the Slow Components**

To date, little evidence exists examining the effects of aging on the  $\dot{V}O_2$  slow component (Chick et al. 1991; Xu and Rhodes 1999; Scheuermann et al. 2002; Sabapathy et al. 2004). However, sedentary aging has been shown to somewhat influence the peripheral muscle characteristics where the  $\dot{V}O_2$  slow component is developed, and therefore the  $\dot{V}O_2$  slow component may be subject to such aging effects. Previous data has reported that the  $\dot{V}O_2$  slow component is reduced with sedentary aging, (Chick et al. 1991; Scheuermann et al. 2002; Sabapathy et al. 2004), but no investigation has reported on this effect of aging in older trained subjects. It might be suggested that the  $\dot{V}O_2$  slow component may be significantly reduced in such a population, given the significant decreases previously discussed to occur with both physical training and aging.

In sedentary aging individuals, Scheuermann et al. (2002) investigated the  $\dot{V}O_2$  response to high-intensity ( $>V_T$ ) exercise in eight young ( $25 \pm 3$  y), and

nine elderly ( $71 \pm 5$  y) healthy volunteers. They observed that the  $\dot{V}O_2$  slow component amplitude was not significantly different between the young ( $175 \pm 92 \text{ mL}\cdot\text{min}^{-1}$ ) and elderly ( $102 \pm 70 \text{ mL}\cdot\text{min}^{-1}$ ) cohorts. They also observed that the end-exercise  $[\text{BLa}^-]$  was significantly reduced in the elderly ( $5 \pm 1 \text{ mmol}\cdot\text{L}^{-1}$ ) group compared to the young ( $9 \pm 2 \text{ mmol}\cdot\text{L}^{-1}$ ). No significant relationship was observed between this difference in  $[\text{BLa}^-]$  and the reduction in the  $\dot{V}O_2$  slow component. In contrast, the observation of an age-related decline in the  $\dot{V}O_2$  slow component was previously reported by Chick, Cagle, Vegas, Poliner and Murata (1991). These researchers reported that elderly individuals ( $68 \pm 7.5$  y) demonstrated a significantly attenuated  $\dot{V}O_2$  slow component amplitude ( $111 \pm 78 \text{ mL}\cdot\text{min}^{-1}$ ) compared to a younger ( $29.5 \pm 6.4$  y;  $406 \pm 172 \text{ mL}\cdot\text{min}^{-1}$ ) cohort. They suggested that the reduced  $\dot{V}O_2$  slow component observed in the older population was the result of either a reduced adrenergic response to exercise or the reduced population of Type II muscle fibres widely observed within aging populations (Lexell 1995; Porter, Vandervoort and Lexell 1995; Andersen, Terzis and Kryger 1999). These age-related changes may reduce the glycogenolytic rate during exercise and  $\text{La}^-$  production which may attenuate the  $\dot{V}O_2$  slow component.

It is more likely that changes within the histochemical and neuromuscular systems are responsible for changes in the age-related changes in the  $\dot{V}O_2$  slow component. A large number of previous investigations have reported histochemical changes within aged sedentary individuals, with the majority of research reporting atrophy of Type II fibres (Larsson 1983; McCarter 1990; Chilibeck et al. 1995; Lexell 1995; Porter et al. 1995; Proctor et al. 1995; Kirkendall and Garrett Jr 1996; Houmard et al. 1998; Conley, Jubrias

and Esselman 2000; Frontera, Hughes, Fielding, Fiatarone, Evans and Roubenoff 2000; Frontera et al. 2001; Trappe, Lindquist and Carrithers 2001; Thompson 2002; Andersen 2003). Previous research has shown that muscle fibres move towards age-related co-expression of myosin-heavy chain (MHC) contents, suggesting that the muscle fibres are losing their high power and anaerobic capacity, allowing an increased oxidative capacity (Andersen et al. 1999). This co-expression of MHC content has been shown to be reduced through undertaking activity which allows the continued recruitment of Type II fibres through moderate-to-high-intensity resistance training (Williamson, Godard, Porter, Costill and Trappe 2000). An age-related shift towards more oxidative muscle fibres may be the most likely reason that the magnitude of the  $\dot{V}O_2$  slow component decreases due to a reduced number and size of the Type II fibres that can be recruited to help maintain power output after Type I fibres fatigue. In contrast, Type II fibres, as well as their force and power characteristics, appear to be maintained in masters athletes who continue high-intensity physical training (Coggan, Spina, Rogers, King, Brown, Nemeth and Holloszy 1990). Therefore, the role of histochemical adaptations with aging on the development of the  $\dot{V}O_2$  slow component is not yet fully understood, and future research should attempt to identify differences between trained and untrained older individuals.

Recently, Sabapathy et al. (2004) investigated the nature of the  $\dot{V}O_2$  slow component and changes in muscle fibre recruitment patterns in young ( $21.2 \pm 0.9$  y;  $3.71 \pm 0.21$  L $\cdot$ min $^{-1}$ ) and elderly ( $71.6 \pm 0.8$  y;  $2.12 \pm 0.11$  L $\cdot$ min $^{-1}$ ) subjects during heavy-intensity (50% $\Delta$ ) cycling. The investigators reported that the amplitude and  $TD_s$  of the  $\dot{V}O_2$  slow component were

significantly higher and faster in the young ( $595 \pm 65 \text{ mL} \cdot \text{min}^{-1}$ ;  $118 \pm 10 \text{ s}$ ) than the elderly ( $223 \pm 28 \text{ mL} \cdot \text{min}^{-1}$ ;  $178 \pm 14 \text{ s}$ ) subjects, respectively. These discrepancies are not surprising given the significant differences in age and maximal aerobic capacities. Interestingly, when the amplitude of the  $\dot{V}\text{O}_2$  slow component was made relative to the change in  $\dot{V}\text{O}_2$  per unit of time, no significant difference was observed between the groups. Sabapathy et al. (2004) also observed that the changes in the mean power frequency (MPF) across the high-intensity SWT were similar regardless of age, with both the young ( $6.4 \pm 1.0\%$ ) and elderly ( $5.4 \pm 0.7\%$ ) showing significant increases. As such, the researchers suggested that the causal nature of the  $\dot{V}\text{O}_2$  slow component may not change with age, but rather that the age-related decrease in  $\dot{V}\text{O}_{2\text{max}}$  may be responsible for the significantly lower absolute slow component amplitude. Therefore, the maintenance of  $\dot{V}\text{O}_{2\text{max}}$  with aging through physical training may allow similar  $\dot{V}\text{O}_2$  slow component amplitudes to be observed in vastly different age groups.

In summary, limited research to date has described the effects of aging on the development of the  $\dot{V}\text{O}_2$  slow component during high-intensity exercise (Chick et al. 1991; Scheuermann et al. 2002). From the limited evidence available, aging appears to reduce the  $\dot{V}\text{O}_2$  slow component amplitude, which is most likely due to an age-related denervation and atrophy of low-efficiency high-force Type II muscle fibres noted in sedentary aging individuals (Coggan et al. 1992; Lexell 1995; Porter et al. 1995; Andersen et al. 1999). Therefore, while it is observed that the peripheral muscle characteristics and  $\dot{V}\text{O}_{2\text{max}}$  are significantly changed with aging, the maintenance of such characteristics

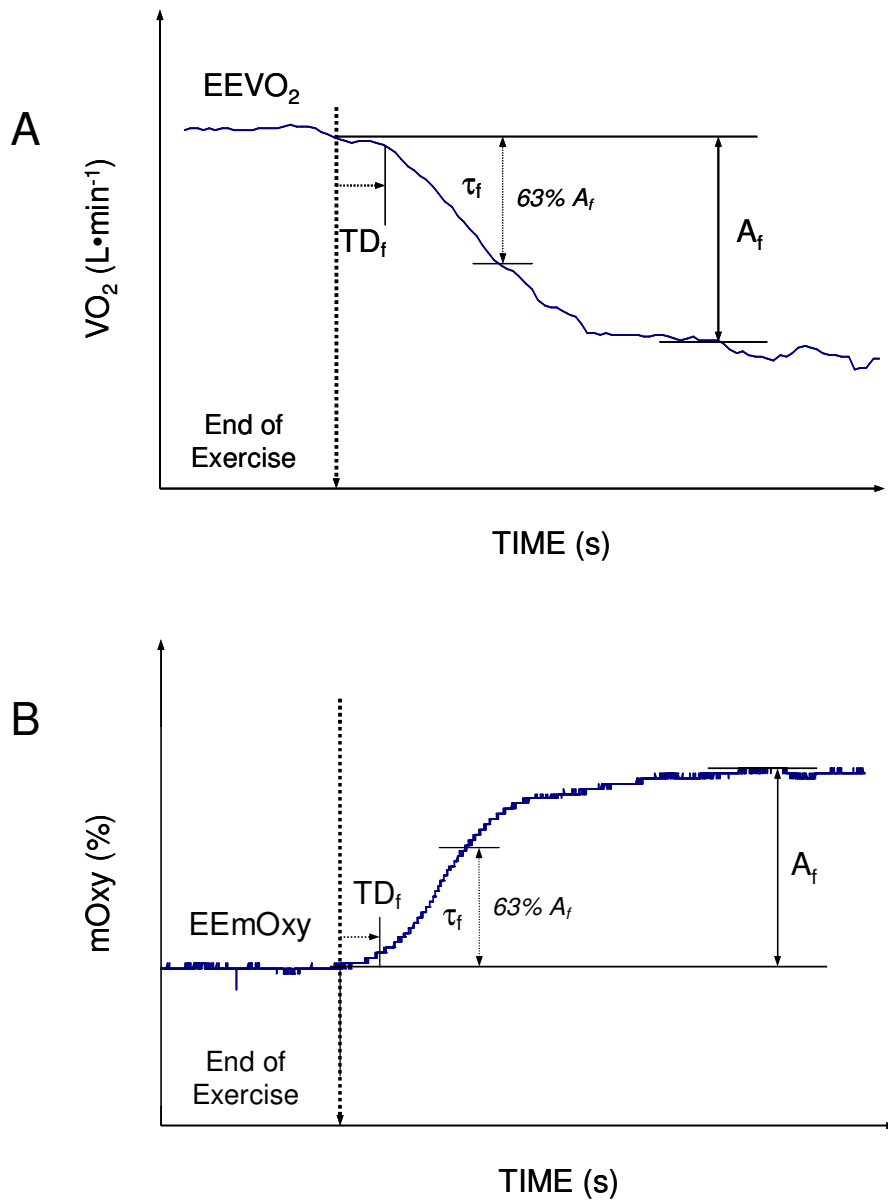
through physical training may negate any such effect of aging effect *per se* on the development of the  $\text{VO}_2$  slow component.

In conclusion, the  $\text{VO}_2$  slow component is proposed to represent an 'additional' volume of  $\text{O}_2$  consumed within the working muscle during high-intensity exercise (Poole 1994; Poole et al. 1994; 1995; Whipp 1994; Womack et al. 1995; Billat, Richard, Binsse, Koralsztein and Haouzi 1998; Scheuermann, Kowalchuk and Barstow 1999; Lucia, Hoyos and Chicharro 2000; Borrani et al. 2001; Demarie et al. 2001; Demarie, Sardella, Billat, Magini and Faina 2001; Koppo and Bouckaert 2002; Koppo et al. 2002; Borrani, Candau, Perrey, Millet, Millet and Rouillon 2003; Fernandes, Cardoso, Soares, Cascensao, Colaco and Vilas-Boas 2003; Santalla, Perez, Montilla, Vicente, Davison, Earnest and Lucia 2003; Deley, Millet, Borrani, Lattier and Brondel 2005; Sabapathy et al. 2005). It has been suggested that the majority of the decrease in efficiency and appearance of the  $\text{VO}_2$  slow component occurs within the working muscle (Poole 1994). Whilst the majority of available evidence is supportive of this hypothesis, the use of NIRS to monitor changes in mOxy within the working muscle has received minimal attention (Demarie et al. 2001). Such research may help to identify the extent as to which the slow component is developed within active musculature, and other factors influencing a decrease in muscular efficiency within aged and trained populations. Therefore, the purpose of Study Three in the present series of investigations was to examine the effect of age on the development of the  $\text{VO}_2$  and mOxy slow components in well-trained cyclists.

## OFF-TRANSIENT KINETIC RESPONSES

The third and final metabolic phase is the off-transient response which details the return of  $\dot{V}O_2$  from end-exercise values to resting baseline (Hill and Lupton 1923; Bahr and Sejersted 1991; Bahr 1992; Borsheim and Bahr 2003). As discussed earlier, a large volume of literature has described the on-transient metabolic responses across exercise intensities. In contrast, a limited amount of research has investigated the metabolic recovery following the completion of exercise (Gaesser and Brooks 1984; Bahr and Sejersted 1991; Bahr 1992; Borsheim and Bahr 2003).

The rate at which  $\dot{V}O_2$  recovers to baseline is of practical significance for a wide range of populations, especially those who suffer from chronic disease or a prolonged sedentary lifestyle as they may endure longer periods of metabolic recovery from activities of daily living (Palange, Galassetti, Mannix, Farber, Manfredi, Serra and Carlone 1995; Nanas, Nanas, Kassiotis, Alexopoulos, Samakovli, Kanakakis, Tsolakis and Roussos 1999; Myers, Gianrossi, Schwitter, Wagner and Dubach 2001; Pouliou et al. 2001; Arena, Humphrey, Peberdy and Madigan 2002). For recreational or competitive athletes, the off-transient  $\dot{V}O_2$  response is particularly important for individuals whose sport requires repeated bouts of exercise as seen during interval training or prolonged high-intensity intermittent sports (Gaesser and Brooks 1984; Bahr and Sejersted 1991; Bahr 1992; Borsheim and Bahr 2003). Therefore, an understanding of the controlling mechanisms of the off-transient  $\dot{V}O_2$  response is important in order to attempt to maximise recovery responses.



**Figure 2.7:** Schematic representation of the  $\dot{V}O_2$  (A) and  $mOxy$  (B) off-transient response and kinetic parameters following heavy-intensity submaximal exercise (EE: End-exercise;  $A_f$ : Off-transient amplitude;  $TD_f$ : Off-transient time delay;  $\tau_f$ : Off-transient time constant).

Following the completion of an exercise bout, it is widely accepted that  $\dot{V}O_2$  decreases exponentially toward a resting baseline level (Figure 2.7) (Borsheim and Bahr 2003). This elevated  $\dot{V}O_2$  following exercise completion

was originally termed O<sub>2</sub> debt (Hill and Lupton 1923; Margaria et al. 1933) but has more recently been labelled excess post-exercise O<sub>2</sub> consumption (EPOC) (Gaesser and Brooks 1984; Bangsbo, Gollnick and Graham 1990; Bahr and Sejersted 1991; Bahr 1992). The off-transient response has been reported to follow either a single or double component path until a resting baseline is reached (Borsheim and Bahr 2003). Previously, Bahr (1992) showed that for heavy-intensity exercise (>VT), the off-transient  $\dot{V}O_2$  response could be fitted with a mono-exponential function, despite a double-exponential model being required to fit the on-transient  $\dot{V}O_2$  response.

While the  $\dot{V}O_2$  off-transient response has been reported in numerous previous investigations (Bahr and Sejersted 1991; Paterson and Whipp 1991; Chilibeck et al. 1995; Chilibeck et al. 1996b, 1997; Borsheim and Bahr 2003), the nature of the mOxy recovery is less documented (Chance, Dait et al. 1992; DeLorey et al. 2003b; Puente-Maestu et al. 2003; duManoir et al. 2005). duManoir, Delorey, Heenan, Kowlachuk and Paterson (2005) investigated the adaptation of the  $\dot{V}O_2$ , mOxy and leg blood flow responses following moderate-intensity knee-extensor exercise in seven ( $27 \pm 5$  y) healthy adults. The investigators reported that the speed of the off-transient  $\dot{V}O_2$   $\tau_f$  ( $32 \pm 5$  s) and leg blood flow  $\tau_f$  ( $25 \pm 5$  s) were significantly quicker than the mOxy  $\tau_f$  ( $91 \pm 26$  s). This observation suggests that the decrease in blood flow to the leg following exercise was greater than the utilisation of O<sub>2</sub> within the muscle, which resulted in a slow increase in mOxy following exercise (duManoir et al. 2005). However, the speed of the  $\dot{V}O_2$  and blood flow recovery responses was similar which suggests that the nature of the two responses may be related. The observation of a significantly slower mOxy  $\tau_f$  compared to either the  $\dot{V}O_2$  or

blood flow responses suggests that the reoxygenation of intra-muscular  $\text{HbO}_2$  and  $\text{MbO}_2$  stores, as well as lactate oxidation control the metabolic recovery response. The findings of Chance et al. (1992) support this hypothesis by suggesting that the  $\text{mOxy}$  recovery responses of the working muscle may be dependent upon the muscle fibre type and biochemical environment. These intra-muscular parameters are likely to change with exercise intensity and duration, training status and aging and may influence the kinetics of muscle reoxygenation following constant-load exercise bouts.

In summary, the off-transient response is of both clinical and practical significance to a wide range of populations. However, to date, the off-transient  $\text{VO}_2$  response has received limited scientific interest compared to either the on-transient or slow component responses (Borsheim and Bahr 2003). From the available research, the data suggesting that the off-transient  $\text{VO}_2$  response is dependent upon exercise intensity are unequivocal (Borsheim and Bahr 2003). Similar to the on-transient and slow component  $\text{VO}_2$  responses, the off-transient  $\text{VO}_2$  response is influenced by a number of physiological factors and adaptations that will be discussed below.

### **Factors Influencing the Off-Transient Responses**

The nature of metabolic recovery from a bout of exercise has been suggested to depend upon intra-muscular factors (Bahr 1992). These factors that may influence the off-transient metabolic response include lactate oxidation, exercise intensity and duration, as well as physical training and aging.

### *Lactate Oxidation*

The oxidation of lactate has been suggested as a primary mechanism responsible for the exponential decrease in  $\dot{V}O_2$  following exercise (Brooks 1986; Bahr 1992). As observed by Brooks, Hittelman and colleagues (1971) and Bahr and Sejersted (1991) the magnitude of EPOC exhibits a curvilinear relationship with exercise intensity which is most likely related to an elevated  $[BLa^-]$ . For example, Brooks (1986) observed that ~70% of lactate accumulated during exercise is oxidised within active muscles, whilst the balance of lactate is converted to glycogen in the liver through the  $O_2$  mediated glycogenesis. It may be possible that high-intensity exercise results in an increased amplitude of the off-transient  $\dot{V}O_2$  response, due to the increased amount of  $O_2$  required to either oxidise lactate and/or aid in glycogen and pyruvate resynthesis (Bahr 1992).

Bahr and Sejersted (1991) investigated the effect of exercise intensity and duration on the magnitude of the off-transient  $\dot{V}O_2$  response in six young ( $23 \pm 0.6$  y;  $49.9 \pm 1.4$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ) physically-active male subjects. Each subject completed three 80 min bouts of cycling exercise at 29%, 50% and 75%  $\dot{V}O_{2max}$  at a cadence of 75 RPM. The resulting increase in lactate was small, with  $[BLa^-]$  concentrations of  $0.87 \pm 0.11$  mmol $\cdot$ L $^{-1}$ ,  $1.10 \pm 0.07$  mmol $\cdot$ L $^{-1}$  and  $3.83 \pm 0.39$  mmol $\cdot$ L $^{-1}$ , respectively. The investigation revealed a non-significant relationship ( $r= 0.65$ ,  $p<0.10$ ) between the off-transient  $\dot{V}O_2$  amplitude and  $[BLa^-]$ . No data was provided on the rate at which  $\dot{V}O_2$  returned to a baseline condition following the completion of the 80 min exercise bouts. The observation of a non-significant relationship may also be due to the relatively

low [BLa<sup>-</sup>] levels observed by the researchers, given that none were above normal anaerobic threshold levels ( $\sim 4 \text{ mmol} \cdot \text{L}^{-1}$ ).

More recently, Billat et al. (2002) investigated the  $\dot{V}\text{O}_2$  off-transient responses to a series of treadmill runs to exhaustion which elicited intensities of 25% $\Delta$ , 50% $\Delta$ , 75% $\Delta$  and  $\dot{V}\text{O}_{2\text{max}}$  in nine young healthy endurance-trained males ( $25 \pm 1 \text{ y}$ ;  $56.0 \pm 6.8 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). These investigators reported that  $\dot{V}\text{O}_2 \tau_f$  was not significantly related to either the running velocity, or end-exercise [BLa<sup>-</sup>] at any exercise intensity. Although the majority of the current literature suggests that the  $\dot{V}\text{O}_2$  off-transient response is lengthened with elevated [BLa<sup>-</sup>], it appears that only a small number of research investigations have adequately investigated this relationship. To date, no causal association has been observed between [BLa<sup>-</sup>] and the off-transient  $\dot{V}\text{O}_2$  response (Brooks et al. 1971; Bahr and Sejersted 1991; Bahr 1992; Billat et al. 2002).

In summary, following high-intensity exercise requires an elevated  $\dot{V}\text{O}_2$  for the oxidation of lactate, although conflicting evidence has questioned this relationship (Gaesser and Brooks 1984; Billat et al. 2002). The link between the off-transient  $\dot{V}\text{O}_2$  response and [BLa<sup>-</sup>] may be dependent upon both the intensity and duration of the exercise, as these factors have been shown to play a significant role in the metabolic recovery responses.

#### *Exercise Intensity and Duration*

During on-transient adjustment at the commencement of exercise, the exponential paths of  $\dot{V}\text{O}_2$  and mOxy continue to meet the oxidative requirements of the energy pathways of the muscle which is dependent upon

the intensity of the prior exercise bout (Bahr and Sejersted 1991; Bahr 1992). Similarly, the duration of exercise is also likely to affect the recovery  $O_2$  response due to factors such as the gradual desaturation of  $HbO_2$  and  $MbO_2$  stores and increased carbohydrate depletion observed with lengthening exercise bouts (Bahr and Sejersted 1991; Bahr 1992; Scheuermann et al. 1999; Borsheim and Bahr 2003).

Previous research has suggested the existence of an exponential relationship between the off-transient  $\dot{V}O_2$  response and exercise intensity and a linear relationship with exercise duration, provided that the exercise is performed at intensities of  $>50\%$   $\dot{V}O_{2max}$  (Borsheim and Bahr 2003). In their recent review, Borsheim and Bahr (2003) suggested that the intensity of exercise accounted for  $\sim 46\%$  of the off-transient  $\dot{V}O_2$  response, with the duration of exercise, and the interaction between exercise intensity and duration accounting for 8.9% and 7.7%, respectively. Borsheim and Bahr (2003) also suggested that exercise intensity influenced both the amplitude and speed of the off-transient response. Furthermore, these researchers suggested that exercise duration only had the potential to influence the speed of the off-transient response which supports the previous work of Bahr and Sejersted (1991).

Earlier, Billat et al. (2002) investigated the influence of the duration of exercise on the off-transient  $\dot{V}O_2$  response during treadmill running to exhaustion in nine endurance-trained males ( $38 \pm 7$  y;  $57.9 \pm 5.6$   $mL \cdot kg^{-1} \cdot min^{-1}$ ) across a range of high intensities ( $25\% \Delta$ ,  $50\% \Delta$ ,  $75\% \Delta$ ; and  $\dot{V}O_{2max}$ ). The  $EE\dot{V}O_2$  and the off-transient  $\dot{V}O_2$  amplitude were both

significantly increased across the exercise intensities. The  $\dot{V}O_2$   $TD_f$  was significantly decreased with increasing exercise intensity. The researchers also observed that the  $\dot{V}O_2$   $\tau_f$  did not significantly lengthen with increasing intensity treadmill running across the 25% $\Delta$  ( $44 \pm 15$  s); 50% $\Delta$  ( $47 \pm 8$  s); 75% $\Delta$  ( $46 \pm 10$  s); and 100% $\Delta$  ( $53 \pm 14$  s) SWT. These results support the curvilinear relationship between the  $\dot{V}O_2$  response and exercise intensity previously suggested by Bahr (1992). Furthermore, Billat et al. (2002) reported that the off-transient  $\dot{V}O_2$  response was best fitted by a mono-exponential function throughout the range of exercise intensities between  $V_T$  and  $\dot{V}O_{2max}$ .

It may also be possible that the  $\dot{V}O_2$  slow component may affect the  $\dot{V}O_2$  off-transient response following high-intensity exercise bouts, given that it may significantly increase both the  $EE\dot{V}O_2$ , and off-transient  $\dot{V}O_2$  amplitude. For example, Scheuermann et al. (1999) investigated whether the magnitude of the  $\dot{V}O_2$  slow component influenced the off-transient  $\dot{V}O_2$  response in seven young ( $25 \pm 5$  y) male subjects during ramp tests. The researchers reported that as a result of either a fast ( $65 \text{ W}\cdot\text{min}^{-1}$ ) or slow ( $8 \text{ W}\cdot\text{min}^{-1}$ ) ramp cycling test that neither the  $\dot{V}O_2$   $\tau_f$  ( $29.5 \pm 3.5$  s vs.  $32.1 \pm 8.7$  s) nor the amplitude ( $2478 \pm 641 \text{ mL}\cdot\text{min}^{-1}$  vs.  $2691 \pm 619 \text{ mL}\cdot\text{min}^{-1}$ ) were significantly different between the two ramp protocols. However, the researchers did not quantify any differences in the slow component amplitude between testing protocols.

At present, few studies have examined the effect of exercise intensity on the nature of the mOxy recovery response following various intensity constant-load exercise bouts (Chance et al. 1992; DeLorey et al. 2003b; Puente-Maestu et al. 2003; duManoir et al. 2005). Puente-Maestu et al. (2003) investigated the

effect of intensity on the reoxygenation of muscle in patients with chronic obstructive pulmonary disease. The results from this investigation revealed that the off-transient mOxy  $\tau_f$  lengthened as exercise intensity increased. The mOxy  $\tau_f$  lengthened with increasing exercise intensities from 80% LT ( $63.0 \pm 15.0$  s), 45% $\Delta$  ( $77.0 \pm 26.0$  s) and 65% $\Delta$  ( $74.0 \pm 15.0$  s) prior to a six-week endurance-training program. A similar trend was observed in the post-training mOxy off-transient  $\tau_f$  across the same exercise intensities ( $46.0 \pm 27.0$  s;  $57.0 \pm 10.0$  s;  $62.0 \pm 26.0$  s). The observation of a lengthening mOxy  $\tau_f$  with increasing exercise intensity suggests an  $O_2$  utilisation mechanism within the working muscle controlling the speed of  $\dot{V}O_2$  recovery. From this research, it appears as though exercise intensity has a significant effect on the off-transient mOxy response, while no research has reported the effect of exercise duration.

In conclusion, both the magnitude and speed of the off-transient  $\dot{V}O_2$  and mOxy responses is related to both the intensity and duration of the prior exercise bout. The relationship between the off-transient  $\dot{V}O_2$  response and exercise intensity appears to be curvilinear across increasing intensities, whereas exercise duration appears to share a linear relationship at moderate to high work intensities ( $>50\% \dot{V}O_{2max}$ ) with the off-transient  $\dot{V}O_2$  response (Borsheim and Bahr 2003). The kinetics for the  $\dot{V}O_2$  off-transients appear unrelated to either the on-transient response or the appearance of the slow component. However, the off-transient response has been shown to be influenced by both physical training and aging.

## **Influence of Physical Training on the Off-Transient Responses**

It appears that the nature of the off-transient metabolic response is controlled through intra-muscular mechanisms, which may suggest that the off-transient metabolic response is influenced by physical training (Chilibeck et al. 1997; Myers et al. 2001; Billat et al. 2002; Puente-Maestu et al. 2003). Similar to the literature discussed earlier on on-transient  $\dot{V}O_2$  kinetics, the speed of  $\dot{V}O_2$  off-kinetics is increased by physical training, although evidence elucidating the mechanisms to explain this effect is lacking (Short and Sedlock 1997; Carter et al. 2000a; Billat et al. 2002).

Billat et al. (2002) reported that the off-transient  $\dot{V}O_2$   $\tau_f$  following runs at 90% ( $62 \pm 19$  to  $44 \pm 11$  s) and 95%  $v\dot{V}O_{2\max}$  ( $63 \pm 22$  to  $51 \pm 9$  s) in young males ( $25 \pm 1$  ;  $56.0 \pm 6.8$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ) was significantly reduced following four weeks of interval running training. This acceleration of the  $\dot{V}O_2$  off-transient response following training was shown to be related to the  $[BLa^-]$  at 95%  $v\dot{V}O_{2\max}$  but not at 90%  $v\dot{V}O_{2\max}$ . Despite this relationship, no difference was observed in the  $[BLa^-]$  and  $EE\dot{V}O_2$  between the two exercise intensities. Importantly, the total EPOC during the first three minutes of recovery was not significantly affected by training [(90%  $v\dot{V}O_{2\max}$ :  $4.8 \pm 0.5$  vs.  $4.4 \pm 0.6$  L)( 95%  $v\dot{V}O_{2\max}$ :  $4.7 \pm 0.7$  vs.  $4.6 \pm 0.7$  L)] for pre- and post-training tests, respectively. Additionally, given that the oxidation of lactate may be a major contributor to the post-exercise  $\dot{V}O_2$  response, it is possible that the reduced  $[BLa^-]$  due to the decreased relative intensity may have explained the improved  $\dot{V}O_2$  off-transient kinetics, (Gaesser and Brooks 1984; Bahr and Sejersted 1991; Bahr 1992).

Training adaptations in the peripheral muscle fibre composition and capillarisation may also influence the off-transient metabolic response (Denis et al. 1986; Baba, Kawamura, Shibata, Sohirad and Kamiya 1995; Chilibeck et al. 1997). Chilibeck et al. (1997) examined the relationship between the off-transient  $\dot{V}O_2$  response following moderate-intensity plantar flexion exercise and measures of capillarisation in both young ( $25.9 \pm 2.1$  y) and older ( $66.0 \pm 6.3$  y) healthy individuals. The researchers hypothesised that increased muscle capillarisation would allow greater  $O_2$  delivery, and therefore speed the off-transient  $\dot{V}O_2$  response following exercise. However, they observed no effect of age or difference in capillarisation between the two age groups. Moreover, Chilibeck et al. (1997) observed that the off-transient  $\dot{V}O_2 \tau$  was significantly related to the capillary density ( $r = -0.68$ ,  $p < 0.05$ ), capillary contacts per fibre area ( $r = -0.83$ ,  $p < 0.05$ ), as well as the maximal diffusion distance ( $r = 0.68$ ,  $p < 0.05$ ) for the two combined groups, but not for either cohort. Moreover, the capillary density and capillary contacts per fibre area measures were significantly related to the off-transient  $\dot{V}O_2 \tau$  when the data were pooled. The results from Chilibeck et al. (1997) suggest that the  $\dot{V}O_2 \tau_f$  was decreased by a training-induced increased capillarisation increasing  $O_2$  delivery to within the muscle cell.

To date, Puente-Maestu et al. (2003) are the only investigators to describe the effect of physical training on the off-transient mOxy responses to both moderate- (80% VT) and high-intensity (45% $\Delta$ ; 65% $\Delta$ ) exercise. This study examined the effects of six weeks of cycling at 70%  $\dot{V}O_{2max}$  on changes in muscle oxidative capacity and reoxygenation kinetics in patients ( $63 \pm 10$  y;  $1.33 \pm 0.31$  L $\cdot$ min $^{-1}$ ) with chronic obstructive pulmonary disease. Puente-

Maestu et al. (2003) reported that the off-transient mOxy  $\tau_f$  significantly improved between pre- and post-SWT testing time points at 80% LT ( $63.0 \pm 15$  s vs.  $46.0 \pm 27.0$  s), 45% $\Delta$  ( $77.0 \pm 26.0$  s vs.  $57.0 \pm 10.0$  s) and 65% $\Delta$  ( $74 \pm 15$  s vs.  $69.0 \pm 11.0$  s). In addition, the researchers reported significant improvements in the oxidative capacity of the VL as evidenced by increased CS activity ( $20.2 \pm 9.9$  vs.  $29.6 \pm 13.1$   $\mu\text{mol}\cdot\text{g}_{\text{w.w.}}^{-1}\cdot\text{min}^{-1}$ ), but not  $\beta$ -HAD activity ( $2.6 \pm 0.6$  vs.  $2.9 \pm 0.9$   $\mu\text{mol}\cdot\text{g}_{\text{w.w.}}^{-1}\cdot\text{min}^{-1}$ ) as a result of the training regime. Puente-Maestu et al. (2003) reported that the improvement in the time course of muscle reoxygenation was significantly correlated to the change in CS activity following both the 45% $\Delta$  and 65% $\Delta$  SWT. Therefore, the data presented by Puente-Maestu et al. (2003) suggest that the reoxygenation response after high-intensity submaximal exercise is dependent upon the utilisation of O<sub>2</sub> within the muscle. It may also be possible that the improvement in mOxy recovery kinetics is due to other peripheral training adaptations which were not measured by Puente-Maestu et al. (2003).

In summary, physical training appears to improve the speed but not the amplitude of the off-transient  $\dot{V}\text{O}_2$  response. It is likely that this improvement in the off-transient  $\dot{V}\text{O}_2$  response is the result of an increased O<sub>2</sub> delivery to the muscle cell by various physiological and histochemical adaptations which facilitate faster lactate oxidation and gluconeogenesis. It may also be possible that sedentary aging may lead to the slowing of the off-transient  $\dot{V}\text{O}_2$  response due to the detraining effects that accompany the aging process (Chick et al. 1991; Chilibeck et al. 1995; 1997).

## **Influence of Aging on the Off-Transient Responses**

Finally, it has been suggested that the aging process may influence the off-transient metabolic recovery responses (Chick et al. 1991; Chilibeck et al. 1995; 1997). As previously discussed, sedentary aging has been shown to have an influence in both histochemical and biochemical properties of peripheral muscle fibres (Campbell, McComas and Petito 1973; Houmard et al. 1998; Conley et al. 2000; Frontera et al. 2001). Therefore, changes such as those in lactate responses, capillarisation and fibre composition have the potential to influence the metabolic responses of recovery from constant-load exercise.

Only a few studies have investigated the effects of aging on the off-transient  $\dot{V}O_2$  and mOxy responses following constant-load exercise bouts (Chick et al. 1991; Chilibeck et al. 1995; 1997; DeLorey et al. 2003b). For example, Chilibeck et al. (1997) studied nine elderly ( $66 \pm 6.3$  y) and eleven young ( $25.9 \pm 2.1$  y) volunteers during calf plantar flexion at 45% of peak work rate. The researchers reported that the off-transient  $\dot{V}O_2 \tau$  ( $44.1 \pm 18.8$  s) and wMRT ( $47.0 \pm 22.7$  s) was significantly slowed in the aged cohort compared to the younger population ( $36.8 \pm 19.0$  s;  $33.1 \pm 16.6$  s). They observed weak to moderate significant correlations between the off-transient  $\dot{V}O_2 \tau$  and capillary density ( $r = -0.48$ ,  $p < 0.05$ ), and capillary contacts per muscle fibre CSA ( $r = -0.59$ ,  $p < 0.05$ ) for the younger group, but not the aged subjects. The researchers proposed that these significant relationships may indicate that the delivery of  $O_2$  to the muscle is of physiological significance during the  $\dot{V}O_2$  off-transient response in the young subjects.

An earlier study by the same research group also investigated the  $\dot{V}O_2$  kinetics during both cycling and plantar flexion exercise modalities in young ( $26.9 \pm 2.5$  y) and old ( $66 \pm 7.7$  y) sedentary individuals (Chilibeck et al. 1995). They reported that the older group had a significantly longer  $\dot{V}O_2 \tau_f$  in comparison to the young group ( $49.7 \pm 14.1$  s vs.  $34.9 \pm 5.9$  s) following heavy-intensity (90%  $\dot{V}O_{2max}$ ) cycling exercise. However, following plantar flexion exercise, this significance difference disappeared between the age groups ( $35.2 \pm 12.1$  vs.  $32.4 \pm 13.6$  s). Taken together, these findings suggest that the  $\dot{V}O_2$  recovery following exercise may also be influenced by the local metabolic activity or histochemical characteristics of the active muscles, and any such local adaptations acquired from physical training or repeated activities of daily living. Similar results were observed by Chick et al. (1991) who reported that sedentary aging demonstrated a slowing effect on the off-transient  $\dot{V}O_2$  response, regardless of fitness level. Chick and colleagues (1991) suggested that the delayed transfer of metabolites such as  $[H^+]$  and  $CO_2$  from the muscle to the blood and the delayed elimination of  $CO_2$  in respired air due to an age-related reduced ventilatory  $CO_2$  chemosensitivity was responsible for the slowed recovery responses.

More recently, DeLorey et al. (2003a) investigated the relationship between the off-transients for  $\dot{V}O_2$  and muscle deoxygenation in sedentary young ( $25 \pm 3$  y) and elderly ( $68 \pm 3$  y) groups following a moderate-intensity (80% VT) cycling SWT. In this study, DeLorey et al. (2003a) reported that the  $\dot{V}O_2 \tau_f$  was significantly longer in the old ( $44 \pm 9$  s) when compared to the young ( $30 \pm 5$  s) cohort, despite the mOxy recovery kinetics being non-significantly faster in the older than the younger group ( $35 \pm 24$  vs.  $51 \pm 16$  s). These results

suggest that the greater capacity to utilize  $O_2$  within elderly muscle previously discussed may influence the off-transient metabolic response and that age-related changes in the  $\dot{V}O_2$  response appear to be related to  $O_2$  transport limitations. The dissociation between the off-transient  $\dot{V}O_2$  and mOxy responses suggests a lack of a physiological relationship between these two factors. This lack of a relationship may be due to unrelated  $O_2$  costs within non-working muscle to remove  $BLa^-$  and 'repay'  $HbO_2$  stores deoxygenated at exercise onset to help maintain sufficient  $pO_2$  level at the mitochondrial level (Bahr 1992).

In summary, the mechanisms responsible for the age-related slowing of the recovery  $\dot{V}O_2$  kinetics remain unknown, but appear to be related to both the utilisation and delivery of  $O_2$  within the muscle cell. The available results suggests that the maintenance of the off-transient  $\dot{V}O_2$  responses following activities of daily living (plantar/dorsi flexion), but significant slowing of the off-transient response following unfamiliar exercise such as cycling (Chilibeck et al. 1995). It is presently unknown which aging mechanism is associated with the slowing of the off-transient metabolic responses. However, it appears as though the delivery of  $O_2$  to within the muscle plays a vital role, given that the mOxy recovery kinetics have been shown to be unrelated to the  $\dot{V}O_2$  off-transient response. Limited literature has described the concurrent effect of training and aging on the off-transient metabolic responses. Thus, the purpose of Study Four from the present series of investigations is to examine the effect of age on the off-transient  $\dot{V}O_2$  and mOxy responses following moderate-, heavy- and severe-intensity SWT in well-trained cyclists.

## SUMMARY OF LITERATURE

The present review of literature has attempted to discuss and synthesise the available research regarding the effect of training and aging on the metabolic responses during exercise transitions. Present research has strongly focused upon  $\text{VO}_2$  kinetics, with a large emphasis on the on-transient metabolic adaptation (Whipp and Wasserman 1972; Babcock et al. 1994b; Grassi et al. 1996; Hebestreit, Kriemler, Hughson and Bar-Or 1998; Xu and Rhodes 1999; Bangsbo et al. 2000; Bearden and Moffatt 2000; Grassi 2000; Hughson, O'Leary, Betik and Hebestreit 2000; Grassi 2001; Billat et al. 2002; Carter, Grice, Dekerle, Brickley, Hammond and Pringle 2005; DeLorey et al. 2005; Kilding, Challis, Winter and Fysh 2005). This large body of research has suggested that either  $\text{O}_2$  utilisation or delivery limitations are responsible for controlling the rate of metabolic adaptation at the start of exercise (Whipp and Wasserman 1972; Xu and Rhodes 1999; Bangsbo et al. 2000; Grassi 2001; Grassi 2005; Jones and Poole 2005b). Such research has also suggested that the rate of adaptation in response to exercise bouts is influenced by peripheral muscle characteristics (Whipp and Wasserman 1972; Xu and Rhodes 1999; Bangsbo et al. 2000; Grassi 2001; 2005; Jones and Poole 2005b), physical training (Babcock et al. 1994a; Carter et al. 2000a; Koppo et al. 2004) and aging (Babcock et al. 1994a; Chilibeck et al. 1995; 1998; Bell et al. 1999; DeLorey et al. 2003a; 2003b; 2004a; 2005). To date, the consensus is that the on-transient  $\text{VO}_2$  and  $\text{mOxy}$  responses are slowed with sedentary aging, but improved with physical training. Limited data are available as to whether concurrent physical training with aging has any such effect on these responses (Babcock et al. 1994a).

Secondly, a large body of literature has investigated the  $\dot{V}O_2$  slow component observed during exercise of varying intensities (Poole 1994; Poole et al. 1994; Xu and Rhodes 1999; Zoladz and Korzeniewski 2001). In contrast, limited studies have examined the mOxy slow component during high-intensity exercise (Miura et al. 1999; Demarie et al. 2001). The slow component is thought to represent a decrease in either metabolic or mechanical efficiency as observed by a gradual increase in the  $\dot{V}O_2$  requirements of the working muscle. From the available data, it appears that the majority of the slow component is developed within the working muscle (Poole 1994; Gaesser and Poole 1996; Demarie et al. 2001). This intra-muscular development has been related to either muscle fibre composition or altered recruitment patterns with sustained high-intensity exercise. While the development of the slow component has been related to a number of other physiological mechanisms, the identified relationships appear to be more coincidental rather than causal. Similarly, the  $\dot{V}O_2$  and mOxy slow components have also been observed to be benefited by physical training (Poole 1994; Gaesser and Poole 1996; Carter et al. 2000a; Demarie et al. 2001) and aging (Poole 1994; Gaesser and Poole 1996), but limited evidence has been presented detailing the effects of concurrent aging and physical training on these responses.

Lastly, limited research has investigated the off-transient responses of the  $\dot{V}O_2$  and mOxy measures following the completion of various intensity exercise bouts (Bahr and Sejersted 1991; Paterson and Whipp 1991; Chilibeck et al. 1995; Chilibeck et al. 1996b; 1997; Borsheim and Bahr 2003). The available evidence suggests that the elevated aerobic metabolism following exercise is due to the restoration of the working muscle to homeostasis, and

oxidation/removal of intra-muscular metabolites. The nature of the recovery metabolic responses has also been reported to be influenced by both exercise intensity and duration (Bahr and Sejersted 1991; Bahr 1992; Borsheim and Bahr 2003). As with the on-transient and slow component responses, sedentary aging and physical training have been reported to lengthen the off-transient kinetic responses, but no data have reported the effects of concurrent physical training and aging on the off-transient metabolic responses.

In conclusion, while the available evidence suggests that the capacity to adapt metabolically is slowed with aging, this capacity appears more apparent in the  $\dot{V}O_2$  kinetics compared to the mOxy kinetics. Physical training has been shown to improve the rate of metabolic adaptation in response to exercise transitions in young subjects, but limited literature has reported the beneficial effects of concurrent training and aging on the  $\dot{V}O_2$  and mOxy responses. Therefore, the present study aims to contribute to this small body of literature, and examine the  $\dot{V}O_2$  and mOxy responses of young and middle-aged well-trained cyclists across a range of moderate to severe exercise intensities.

## CHAPTER 3

# METHODS

### APPROACH TO THE PROBLEM

Limited evidence is available describing the effect of age on the  $\dot{V}O_2$  and mOxy responses to various exercise intensities. Even fewer data are available reporting the effect of age on  $\dot{V}O_2$  and mOxy responses in trained individuals. The present series of studies were designed to identify a possible aging effect on the  $\dot{V}O_2$  and mOxy kinetic responses in trained cyclists. Previous research investigating the effect of age on these responses have used aged groups unmatched for  $\dot{V}O_{2\max}$  and training status, both of which have been shown to influence the metabolic response to exercise.

In the present series of studies, Study One was designed to match the young and middle-aged cyclists on physiological capacities and peripheral muscle characteristics. The three subsequent studies (2-4) were designed to investigate the effects of age on the  $\dot{V}O_2$  and mOxy on-transient, slow component and off-transient responses, respectively. Further aims of this series of studies were to relate the  $\dot{V}O_2$  and mOxy responses to changes in a number of hematological parameters measured across a series of SWT of increasing intensity. The current series of studies aimed to relate these responses to the peripheral muscle characteristics described within Study One. Lastly, Study Three aimed to relate the  $\dot{V}O_2$  and mOxy slow components to changes in muscle activation and fibre recruitment using novel methodological techniques which have previously not been utilised.

## **SUBJECTS**

Young (18-25 y; n=7) and middle-aged (45-55 y; n=7) cyclists were recruited from the local cycling and triathlon community to participate in the current series of studies. Criteria for inclusion were that they had been consistently training for competitive cycling or triathlon over the previous 12 months. All subjects gave verbal and written informed consent after a full explanation of the requirements and risks of all testing procedures (Appendix 1). Prior to participation, all cyclists were screened for cardiovascular risk factors using a revised Physical Activity Readiness Questionnaire (r-PARQ) which was based upon the criteria established by the American College of Sports Medicine (Appendix 2). All experimental procedures and consent mechanisms were granted approval by the Central Queensland University Human Research Ethics Committee (Appendix 3).

### **Anthropometry**

Prior to exercise testing, a restricted anthropometric profile was performed on each cyclist by a trained anthropometrist using the procedures described by Norton and Olds (1996). Stature was measured to the nearest 0.1 cm with a fixed *Blaydon* stadiometer (Lugarna, NSW, Australia) and body mass to the nearest 0.1 kg using previously-calibrated electronic scales (Tanita Corporation, Tokyo, Japan). The sum of nine skinfolds ( $\sum 9$  SF) was measured with *Harpender* skinfold calipers (John Bull Instruments, West Sussex, UK) to the nearest 0.1 mm. The nine skinfold sites included triceps, biceps, subscapular, supraspinale, mid-axilla, iliocristale, abdominal, medial thigh and calf. Girth measurements included arm (relaxed), arm (flexed), hip, waist and maximal calf and were measured to the nearest 0.1 mm with a *Lufkin Executive*

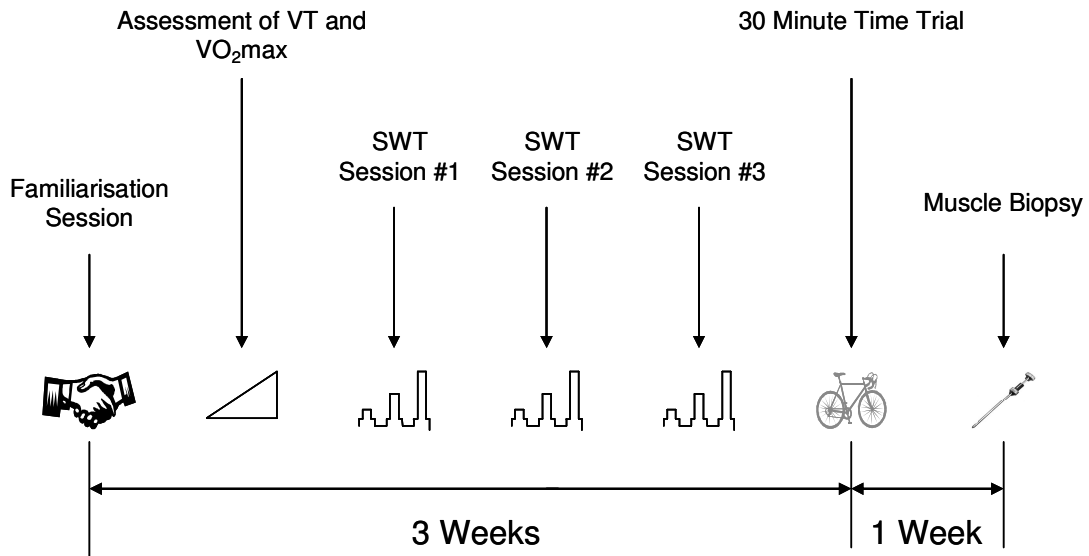
tape (CooperTools, Apex, NC, USA). Humeral and femoral bone breadths were measured to the nearest 0.1 cm using *Rosscraft Campbell* bone calipers (Rosscraft Campbell, Canada). All anthropometric calculations ( $\Sigma$ 9 SF, % body fat and lean body mass) were performed using the *Lifesize* software (Human Kinetics, Lower Mitcham, SA, Australia). The intra-tester reliability of the anthropometrist was acceptable for skinfold measurement (TEM: 0.9 mm; TEM%: 0.25%).

## EXERCISE PROTOCOLS

Each subject completed six visits to the School of Health and Human Performance Laboratory within a three-week period, and a seventh visit to the local hospital. The six laboratory sessions included familiarisation, assessment of ventilatory threshold (VT) and maximal aerobic power ( $\text{VO}_{2\text{max}}$ ), three repeat square wave transition (SWT) sessions and a 30 minute time trial (30TT). The testing sequence is presented as Figure 3.1.

All testing sessions were performed at the same time of day in order to avoid any circadian influence. Prior to each testing session, subjects were instructed to eat a carbohydrate-rich meal within 12 h prior to testing; abstain from caffeine and alcohol for 4 h prior to testing and to refrain from physical training for at least 24 h prior to testing. All exercise testing was conducted on an electromagnetically-braked cycle ergometer (Excalibur, Lode, Groningen, The Netherlands) which was interfaced to a programmable workload control box. Each cyclist used their own personal cycling shoes and cleats. Subjects were instructed to maintain a cadence of 90 RPM, as this closely matches the preferred cadence of trained cyclists (Marsh and Martin 1997). The

standardisation of cadence also allowed neuromuscular responses to be comparable across all exercise tests. All laboratory testing conditions were performed in a standardised environment at  $22 \pm 2$  °C and  $< 70\%$  relative humidity.



**Figure 3.1:** Schematic representation of the testing sequence throughout the series of studies.

During the first visit, informed consent, pre-exercise health screening and anthropometric assessment were completed. During this visit, the height and position of the seat and handlebars of the cycle ergometer were matched to each subject's personal bike. This position was recorded and remained consistent throughout subsequent testing sessions. The cyclists were also familiarised with all exercise procedures and equipment used in subsequent visits.

## **Assessment of Ventilatory Threshold and Maximal Aerobic Power**

At the second visit to the laboratory, each cyclist completed a ramp test to exhaustion for the determination of their VT and  $\dot{V}O_{2\max}$ . The ramp test was increased 5 W every 12 s, after an initial free wheeling period of 20 W for 3 min. The ramp test was terminated either when the cyclist could no longer maintain the required cadence or  $\dot{V}O_{2\max}$  had been attained.

VT was determined by two independent researchers using the methods of Beaver, Wasserman and Whipp (1986), where the threshold lies at the point of either:

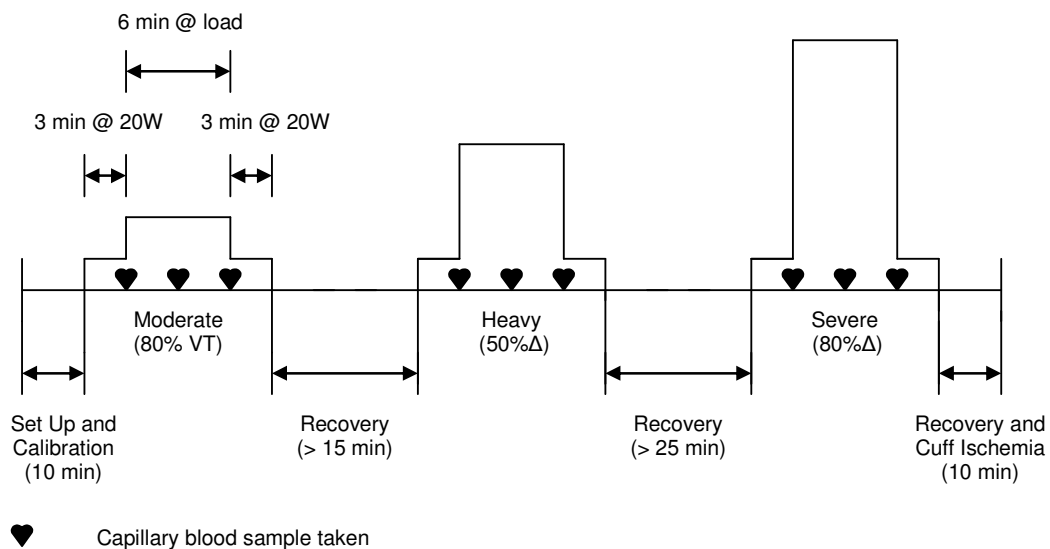
- 1) A non-linear increase in the volume of carbon dioxide production ( $\dot{V}CO_2$ ) against  $\dot{V}E$ ; or,
- 2) An increase in expired ventilation ( $\dot{V}E$ ) with respect to  $\dot{V}O_2$  with no increase in  $\dot{V}O_2/\dot{V}CO_2$ .

$\dot{V}O_{2\max}$  was defined as the highest 30 s rolling average of the data recorded during the ramp test.  $\dot{V}O_{2\max}$  was accepted when the subjects displayed any two of the criteria of Howley, Bassett Jnr and Welch (1995) which included:

- 1) Volitional exhaustion;
- 2) Age-predicted maximal heart rate ( $220 - \text{age}$ ) ( $\pm 10 \text{ b} \cdot \text{min}^{-1}$ );
- 3) Respiratory Exchange Ratio value  $\geq 1.15$ ; or,
- 4) Plateau in oxygen consumption (increase  $< 2 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) with an increase in work rate.

## Square Wave Transitions

During each of the next three testing sessions, the cyclists were required to complete three sequential SWT at moderate (80% VT), heavy [50% of the difference between VT and  $\dot{V}O_{2\max}$  (50% $\Delta$ )] and severe [80% of the difference between VT and  $\dot{V}O_{2\max}$  (80% $\Delta$ )] exercise intensities (Pringle, Doust et al. 2003b). The power output for each of the three SWT intensities was calculated through the linear regression of  $\dot{V}O_2$  versus power output from the initial ramp test performed in Visit 2. The sequence of the SWT within a testing session is shown in Figure 3.2. Three repeat SWT were performed at each exercise intensity on separate days to increase the signal-to-noise ratio and ensure adequate reliability for each intensity transition (Lamarra, Whipp, Ward and Wasserman 1987; Markovitz, Sayre, Storer and Cooper 2004).



**Figure 3.2:** Schematic representation of the order of SWT within a testing session

Cyclists were given no indication of when the load was to be applied, and the load was applied instantaneously. At least 15 min rest was given

between moderate- and heavy-intensity SWT, with 25 min between heavy and severe-intensity SWT to ensure full metabolic recovery as evidenced by a resting  $\dot{V}O_2$  less than  $3.5 \pm 2.0 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ .

### **30 Minute Time Trial**

During the sixth and final visit to the laboratory, all cyclists completed a 30 min time trial (30TT) to provide an index of their endurance cycling performance.

Prior to commencing the 30TT, all cyclists completed a five min warm up at 100 W and then a two min variable warm up, where the subjects self-selected their power output. Initially, all cyclists commenced the 30TT at the power output equivalent to their VT as determined during the initial ramp test (Visit 2). Throughout the 30TT, cyclists were able to freely manipulate their power output using an electric switch positioned on the handle bar of the ergometer integrated to the workload control box. Power output was manually recorded at the end of each min and the average absolute power output (W) and relative power output ( $\text{W}\cdot\text{kg BM}^{-1}$ ) were recorded as measures of cycling performance. Subjects were instructed to maintain a cadence of 90 RPM and received standardised verbal encouragement throughout the 30TT. The use of such time trial protocols has been previously shown to be reliable and display small day-to-day variation in performance of well-trained cyclists (Jeukendrup, Saris, Brouns and Kester 1996).

## PHYSIOLOGICAL MEASURES

### Expired Gas Analysis

Expired gas analysis was conducted throughout the ramp test, repeat SWT and 30TT using a *Medgraphics CPX/D* system (Medgraphics<sup>®</sup>, St Paul, MN, USA). Subjects wore a mouthpiece with saliva trap and a nose clip throughout all testing (Medgraphics<sup>®</sup>, St Paul, MN, USA). Breath-by-breath expired gas was collected using a *preVent<sup>TM</sup>* pneumotach (Medgraphics<sup>®</sup>, St Paul, MN, USA) and carried through sample lines, where O<sub>2</sub> and CO<sub>2</sub> concentration was measured by high-response analysers [O<sub>2</sub>: Zirconia (<80 ms;  $\pm 0.03\%$  O<sub>2</sub>); CO<sub>2</sub>: infrared absorption (<130 ms;  $\pm 0.05\%$  CO<sub>2</sub>)]. Prior to each test, the gas analysis system was calibrated with gases of known concentrations (Reference:  $21 \pm 0.2\%$  O<sub>2</sub>; Calibration:  $12.1 \pm 0.2\%$  O<sub>2</sub>,  $5.05 \pm 0.10\%$  CO<sub>2</sub>) as per the manufacturer's instructions. The *preVent<sup>TM</sup>* pneumotach has a low dead space volume (39 mL) and was calibrated before each test using a three litre syringe (Medgraphics<sup>®</sup>, St Paul, MN, USA) according to the manufacturer's instructions. Real time display of gas concentration and flow measures for each test was displayed using a personal computer. The reliability of expired gas measurements at  $\dot{V}O_{2\max}$  (TEM:  $0.21 \text{ L}\cdot\text{min}^{-1}$ ; TEM%: 5.25%) within this laboratory was acceptable according to Gore (2000).

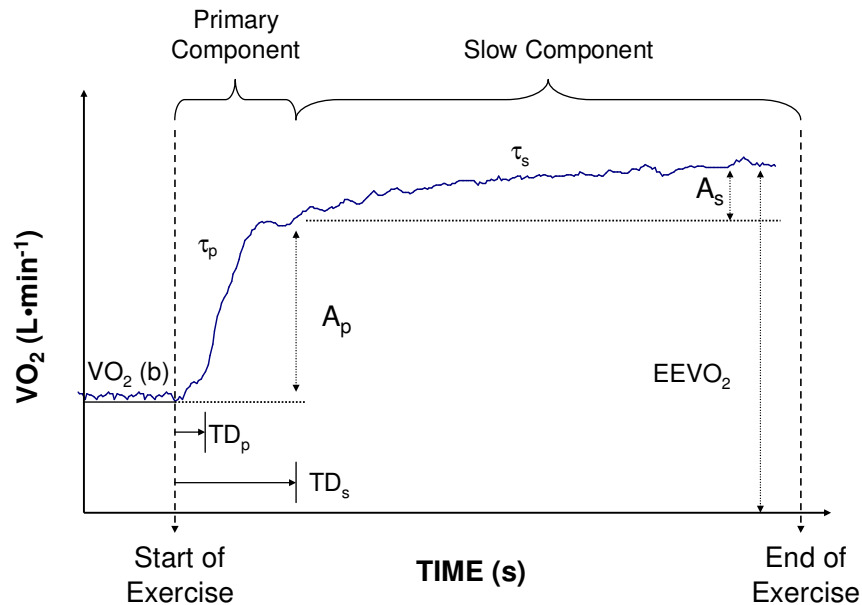
### Modelling of VO<sub>2</sub> Kinetics Data

#### *On-Transient Response*

The VO<sub>2</sub> data were manually examined to identify and exclude any erroneous data caused by coughing, swallowing, or breath holding. Data which were found to lie more than four standard deviations away from the mean response were deleted. The breath-by-breath data was linearly interpolated to

second-by-second values. The three repeat  $\dot{V}O_2$  responses for each of the SWT intensities (80% VT, 50% $\Delta$  and 80% $\Delta$ ) were time aligned and averaged to enhance the response characteristics which increases the signal-to-noise ratio by a factor of  $\sqrt{n}$  (Linnarsson 1974).

The non-linear least-squares regression technique was used to model the time course of the  $\dot{V}O_2$  response after the onset of exercise using either a single (Eq<sup>n</sup> 1) or double- (Eq<sup>n</sup> 2) exponential component equation as detailed below et al. 2004). Schematic representation of the modelling parameters is displayed in Figure 3.3. The initial 20 s of the  $\dot{V}O_2$  response was not included within the analysis in order to ignore the influence of the initial cardiodynamic component (Phase I) (Koppo et al. 2004).



**Figure 3.3:** Schematic representation of the exponential parameters involved in modelling the on-transient  $\dot{V}O_2$  response.

*Moderate (80% VT) Intensity*

$$(Eq^n 1) \quad \dot{V}O_2(t) = \dot{V}O_2(b) + A_p \cdot [1 - e^{-(t-TD_p)/\tau_p}]$$

*Heavy (50%Δ) and Severe (80%Δ) Intensity*

$$(Eq^n 2) \quad \dot{V}O_2(t) = \dot{V}O_2(b) + A_p \cdot [1 - e^{-(t-TD_p)/\tau_p}] + A_s \cdot [1 - e^{-(t-TD_s)/\tau_s}]$$

In these equations,  $\dot{V}O_2(t)$  is the  $\dot{V}O_2$  at a given time;  $\dot{V}O_2(b)$  is the baseline value across the last 2 min of 'unloaded' cycling at 20 W;  $A_p$  and  $A_s$  are the asymptotic amplitudes for the primary and slow component;  $\tau_p$  and  $\tau_s$  are the time constant for each component; and  $TD_p$  and  $TD_s$  are the time delay for each component.

The use of exponential modelling to quantify the  $\dot{V}O_2$  slow component response has been reported to be a more accurate description than the outdated method of reporting the change in  $\dot{V}O_2$  between the third min of exercise and SWT completion (Bearden and Moffat 2001). The computation of best-fit parameters was performed using the 'Solver' function within Microsoft *Excel* (Microsoft Corporation™, Redmond, Washington, USA).

The overall time course of the  $\dot{V}O_2$  response to the exercise transition was determined from the weighted mean response time (wMRT) and was calculated using the methods of MacDonald, Pedersen and Hughson (1997). The wMRT was defined as the time taken to reach ~63% of the total amplitude of the response from pre-exercise  $\dot{V}O_2$  baseline to the final  $\dot{V}O_2$  plateau. The wMRT

was calculated as a weighted sum of the TD and  $\tau$  for each component, as shown below.

(Eq<sup>n</sup> 3)

$$wMRT (s) = [A_p/(A_p + A_s)] \cdot (TD_p + \tau_p) + [A_s/(A_p + A_s)] \cdot (TD_s + \tau_s)$$

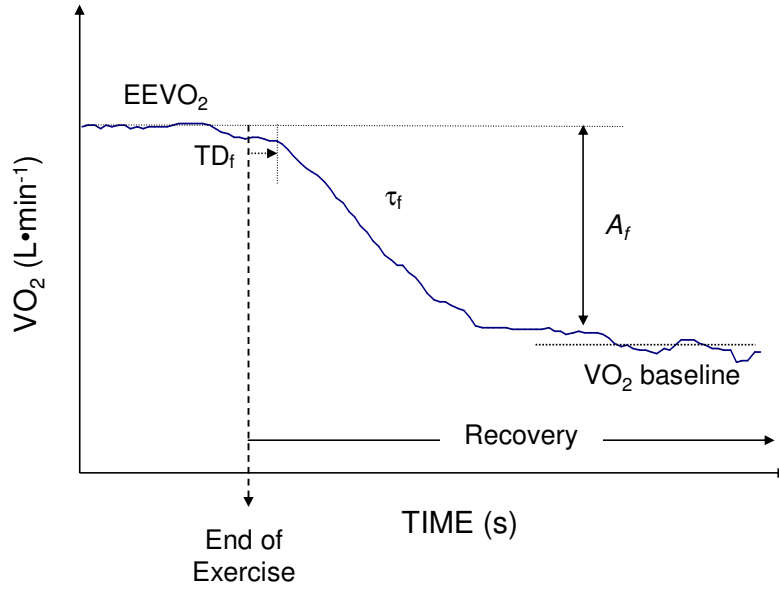
In this equation, wMRT is the weighted mean response time (s);  $A_p$  and  $A_s$  are the asymptotic amplitudes for the primary and slow components component;  $\tau_p$  and  $\tau_s$  are the time constants for each component; and  $TD_p$  and  $TD_s$  are the time delays for each component.

The metabolic efficiency ( $\Delta\dot{V}O_2/\Delta W$ ) for the three SWT intensities was also examined. The  $\Delta\dot{V}O_2/\Delta W$  was calculated for the primary and slow components, as well as the total on-transient response across the three SWT intensities (Pringle et al. 2003b).

1. Primary gain ( $G_p$ ): from the start of exercise to the end of Phase II;
2. Slow gain ( $G_s$ ): from the start of the  $\dot{V}O_2$  slow component until exercise cessation;
3. Total gain ( $G_o$ ): from the start of exercise until exercise cessation.

### *Off-Transient Response*

The off-transient response was modelled for three minutes post exercise using a modified single-component exponential function as proposed by Engelen, Porszasz, Riley, Wasserman, Maehara and Barstow (1996). The off-transient modelling parameters are displayed in Figure 3.4 over the page



**Figure 3.4:** Schematic representation of the exponential parameters involved in modelling the off-transient  $\dot{V}O_2$  response.

$$(Eq^n 4) \quad \dot{V}O_2(t) = EE\dot{V}O_2 - A_f \cdot [1 - e^{-(t-TD_f)/\tau_f}] \cdot u_1$$

In this equation,  $\dot{V}O_2(t)$  is the  $\dot{V}O_2$  at a given time;  $EE\dot{V}O_2$  is the end-exercise  $\dot{V}O_2$  value;  $A_f$  is the asymptotic amplitude;  $\tau_f$  is the time constant and  $TD_f$  is the time delay; and  $u_1 = 0$  for  $t < TD$ ,  $u_1 = 1$  for  $t \geq TD$ .

### Near-Infrared Spectroscopy

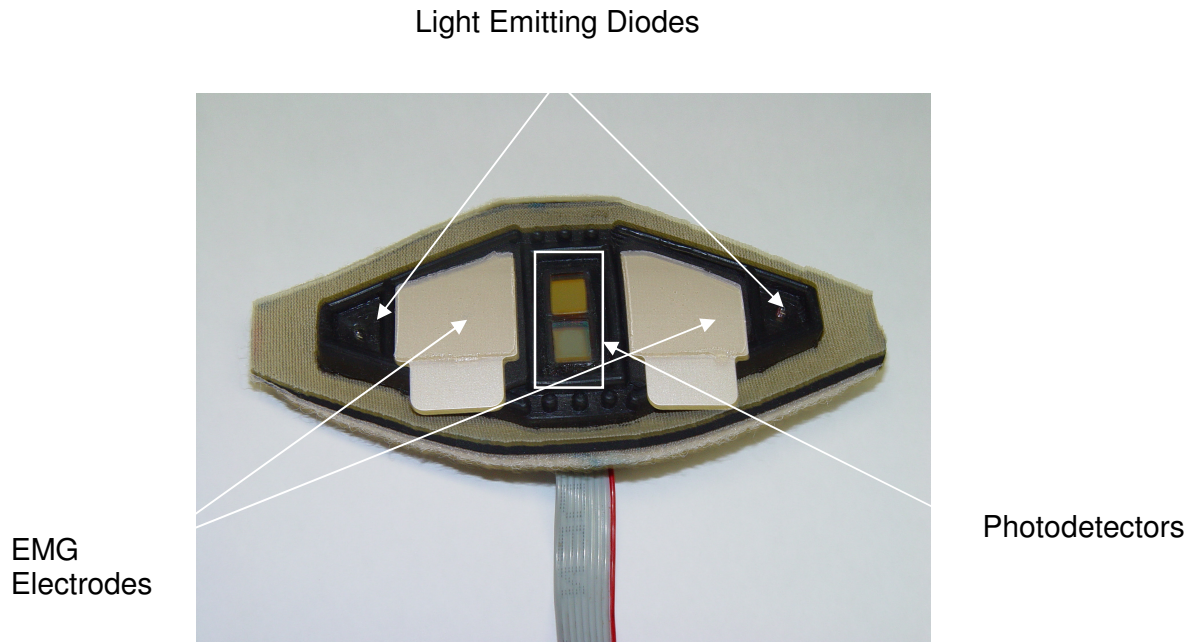
During all testing, changes in the level of muscle oxygenation (mOxy) were monitored in the vastus lateralis (VL) using a continuous wave near infrared spectroscopy (NIRS) device (*Runman®* CWS-2000; RunMan, NIM, Philadelphia, Pa., USA) as originally described by Chance et al. (1992). A probe consisting of two light emitting diodes (760 and 850 nm) and two photodetectors is used to determine changes in the levels of oxyhemoglobin ( $HbO_2$ ) and oxymyoglobin ( $MbO_2$ ), as well as deoxygenated hemoglobin (Hb)

and myoglobin (Mb) during exercise (Figure 3.5). The distance between the light source and photodetectors remained constant at 4 cm. The scattering and absorption of the light at these wavelengths calculates the relative levels of HbO<sub>2</sub>/MbO<sub>2</sub> and Hb/Mb within the muscle tissue (Chance et al. 1992), in accordance with the modified Beer-Lambert law (Eq<sup>n</sup> 5) below.

$$(Eq^n 5) \quad A = \epsilon \cdot [c] \cdot l \cdot B + G$$

In Eq<sup>n</sup> 5, A is the absorption of light expressed as optical density;  $\epsilon$  is the extinction coefficient of chromophore for specific wavelength ( $\mu\text{M}/\text{cm}$ ); c = chromophore concentration ( $\mu\text{M}$ ); l = distance between point of light entry and exit (cm); B = pathlength factor of light through the tissue accounting for scattering (cm); G = geometrical correction factor to account for the geometry of tissue and optode positioning.

This calculation is dependent upon the known absorption properties of Hb, as well as the extinction coefficients of the two chromophores (Hb/Mb and HbO<sub>2</sub>/MbO<sub>2</sub>) at each wavelength. The difference between the two signals ( $\Delta 760\text{--}850\text{ nm}$ ) was used to indicate changes in mOxy.



**Figure 3.5:** Modified NIRS probe for the Runman unit to allow concurrent EMG and NIRS analysis.

The modified NIRS probe was positioned 14 cm from the centre of the knee joint along the vertical axis of the thigh and over the belly of the VL in accordance with previous investigations (Chance et al. 1992; Belardinelli et al. 1995a). This position has been shown to represent a motor point within the VL and therefore reflects whole muscle activity and fibre recruitment (Kendall et al. 1993). Prior to the application of the probe, the skinfold thickness over the site was measured using *Harpender* skinfold calipers (John Bull Instruments, UK) to the nearest 0.1 mm to ensure that the signal was not affected by excessive and varying skinfold thickness (Homma et al. 1996). No significant difference was observed in the thigh skinfold thickness between the young ( $12.5 \pm 3.0$  mm) and middle-aged ( $16.2 \pm 3.6$  mm) cyclists. The probe application site was carefully shaved and a clear *Opsite*<sup>TM</sup> dressing (Smith & Nephew, London, UK) positioned over the photodetectors to prevent distortion caused by sweat

accumulation. The probe was securely bandaged to the leg using black cloth to prevent movement and to ensure that no visible light was detectable by the photodetectors. The NIRS probe was modified to incorporate the placement of two bipolar sEMG electrodes within the spacing between the light-emitting diodes and photodetectors (Figure 3.5). Pilot work within our laboratory showed that the placement of these electrodes had no effect on the NIRS signal recorded from the VL during cycling such as that used in this series of studies.

The NIRS system was interfaced with a *Labview* CB-68-LP A/D card (National Instruments, Austin, Texas, USA) and recorded at 20 Hz. Custom written *Labview* software (National Instruments, Austin, Texas, USA) was written to display and record both the 760 and 850 nm signals during testing. Prior to each exercise test, the NIRS unit was calibrated at both 760 and 850 nm wavelengths according to the manufacturer's instructions. During calibration, subjects remained in a seated position with their right leg at the bottom of the crank cycle. The  $\Delta 760\text{--}850$  nm NIRS signal was manually adjusted to a baseline value ( $0 \pm 10$  mV) so that all changes in mOxy were relative to this point. After this baseline was met, the gain was adjusted to ensure adequate signal amplification at both 760 (+500 mV) and 850 nm (-500 mV) wavelengths. The difference between these two signals was calibrated to be <10%. The NIRS signal was required to stabilise for 30 s at each calibration setting prior to acceptance.

Changes in mOxy can be semi-quantified to create normalised results compared to maximal deoxygenation and oxygenation of muscle through the application of cuff ischemia during recovery (Sahlin 1992; Miura et al. 1999;

Costes et al. 2001; Hiroyuki et al. 2002; Quaresima and Ferrari 2002a; Grassi et al. 2003). After the completion of each exercise test (i.e. ramp, SWT sequence and 30TT), a thigh cuff (Calibrated V-Lok® baunmanometer, W.A. Baum Co Inc., Copiague, New York, USA) was positioned around the superior thigh. The cuff was rapidly inflated to 250 mmHg to ensure suprasystolic pressure to overcome arterial pressure and that minimal venous blood volume was trapped in the leg musculature during arterial occlusion. The cuff pressure was maintained until the mOxy signal had reached a nadir, whereupon the cuff was quickly released to allow a hyperaemic response within the thigh. The nadir of the mOxy signal was recorded as 0% oxygenation whereas the peak mOxy observed during the hyperaemic response was recorded as 100% oxygenation. Exercise mOxy values were normalised within this scale with previous research indicating that even maximal intensity exercise values still existed within the 0-100% occlusion-hyperaemia scale (van Beekvelt et al. 2001; Quaresima and Ferrari 2002a; 2002b).

The practice of using cuff ischemia to quantify changes in mOxy has been shown previously to be both safe and reliable (Bhambhani et al. 1998). Subject's subjective pain was recorded on a ten-point Leichhardt scale throughout the cuff ischemia to monitor discomfort and pain as a safety precaution. The pain scale results of the cuff ischemia are shown in Appendix 4. The reliability of the mOxy measures at  $\text{VO}_{2\text{max}}$  was acceptable within this laboratory (TEM: 3.8 %; TEM%: 10.8%).

## Modelling of mOxy Kinetics Data

### *On-Transient Response*

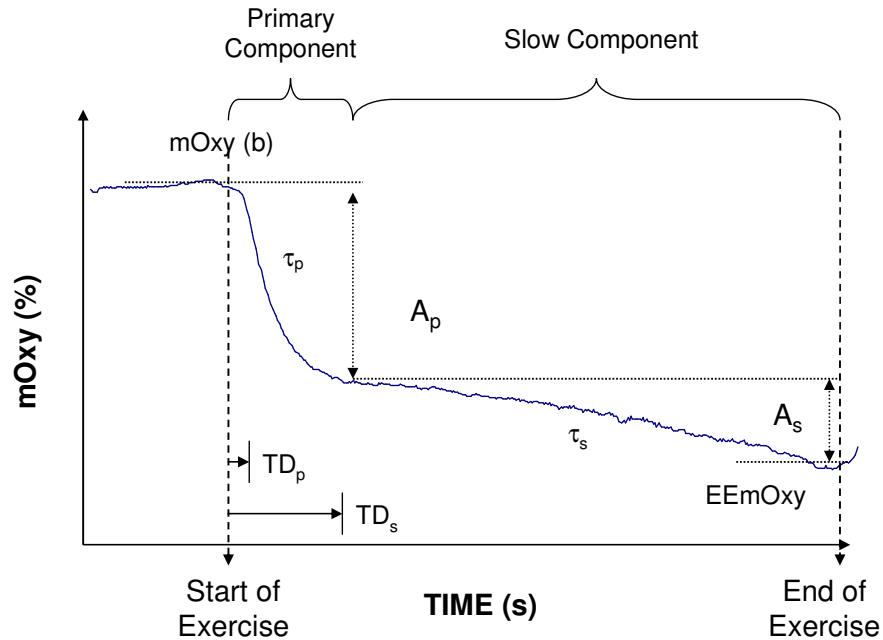
The changes in mOxy were linearly interpolated to provide second-by-second values and exponential modelled to quantify the amplitude and speed of the response. The three repeat mOxy responses were time aligned and averaged to increase the signal-to-noise ratio by a factor of  $\sqrt{n}$  (Linnarsson 1974). The non-linear least-squares regression technique was used to model the time course of the on-transient mOxy responses. The on-transient mOxy responses were fitted to either a single (Eq<sup>n</sup> 6) or double exponential function (Eq<sup>n</sup> 7) for each of the three SWT intensities (80% VT, 50%Δ and 80%Δ) depending upon the observation of a slow component. The modelling parameters are schematically presented in Figure 3.6. Any data that were found to lie more than four standard deviations away from the mean response were removed. For the purposes of the present study, the curve fit was only performed if the NIRS signal fell below baseline values. The computation of best-fit parameters was performed using the Solver function within Microsoft *Excel* (Microsoft Corporation™, Redmond, Washington, USA).

$$\text{(Eq}^n \text{ 6)} \quad \text{mOxy (t)} = \text{mOxy (b)} - A_p \cdot [1 - e^{-(t-TD_p)/\tau_p}]$$

$$\text{(Eq}^n \text{ 7)} \quad \text{mOxy (t)} = \text{mOxy (b)} - A_p \cdot [1 - e^{-(t-TD_p)/\tau_p}] - A_s \cdot [1 - e^{-(t-TD_s)/\tau_s}]$$

In these equations, mOxy (t) is the mOxy at a given time; mOxy (b) is the baseline value across the last two minutes of ‘unloaded’ cycling at 20 W;  $A_p$  and  $A_s$  are the asymptotic amplitudes for the primary and slow components;  $\tau_p$

and  $\tau_s$  are the time constants for each component; and  $TD_p$  and  $TD_s$  are the time delays for each component.



**Figure 3.6:** Schematic representation of the exponential parameters involved in modelling the on-transient mOxy response.

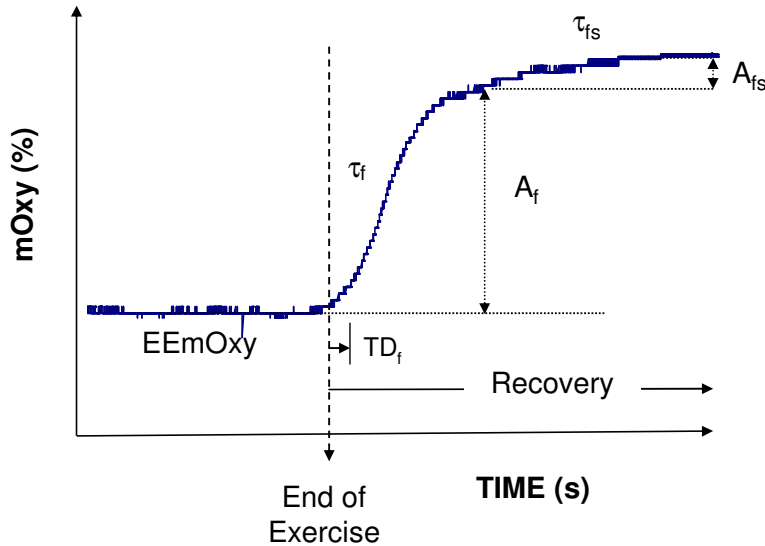
#### *Off-Transient Response*

The off-transient mOxy response was modelled using a single (Eq<sup>n</sup> 8) or double component (Eq<sup>n</sup> 9) exponential model with a common TD (Engelen et al. 1996). The mathematical parameters used to model the off-transient mOxy response are shown below in Figure 3.7. The function used to model the off-transient mOxy response was similar to that proposed to model the off-transient  $\dot{V}O_2$  response. The mOxy off-transient models are shown below:

$$(Eq^n 8) \quad mOxy(t) = EEmOxy + A_f \cdot [1 - e^{-(t-TD)/\tau_f}] \cdot u_1$$

$$(Eq^n 9) \quad mOxy(t) = EEmOxy + A_f \cdot [1 - e^{-(t-TD)/\tau_f}] \cdot u_1 + A_{fs} \cdot [1 - e^{-(t-TD)/\tau_{fs}}] \cdot u_1$$

In Eq<sup>n</sup> 8 and 9, the  $mOxy(t)$  is the  $mOxy$  at a given time;  $EEmOxy$  is the end-exercise  $mOxy$ ;  $A_f$  and  $A_{fs}$  are the asymptotic amplitudes of the off-transient primary and slow components;  $\tau_f$  and  $\tau_{fs}$  are the time constants;  $TD$  is the common time delay; and  $u_1 = 0$  for  $t < TD$ ,  $u_1 = 1$  for  $t \geq TD$ .



**Figure 3.7:** Schematic representation of the exponential parameters involved in modelling the off-transient  $mOxy$  response.

### Heart Rate Measurement

Heart rate was recorded telemetrically using a *Polar s610i* heart rate monitor (Polar Electro Oy, Kempele, Finland), and later downloaded to a personal computer using *Polar Advantage Software*<sup>TM</sup> version 4.0 (Polar, Electro OY, Kempele, Finland).

## Hematological Measures

### *Capillary Blood Sampling*

Capillary blood samples were drawn from a hyperaemic fingertip and collected into duplicate 100  $\mu$ L heparinised capillary tubes (Bacto Laboratories, Liverpool, NSW). Duplicate samples were drawn 30 s prior to SWT load application (0 min); mid-SWT (3 min) and immediately following SWT completion (6 min) of each SWT. During the 30TT, capillary samples were drawn every 10 min of cycling. Prior to sampling, the puncture site was cleansed with alcohol, dried and the first drop post-puncture was excluded from the sample. Both capillary tubes were filled simultaneously. The first sample was expelled from the capillary tube into the sample well of an *i-STAT* CG<sub>4</sub><sup>+</sup> cartridge (*i-STAT* Corporation, New Jersey, USA) and all air bubbles were removed from the sample prior to the cartridge being closed. The duplicate sample was stored on ice as a reserve until the first sample had been analysed. Both samples were discarded after one capillary tube had been successfully analysed.

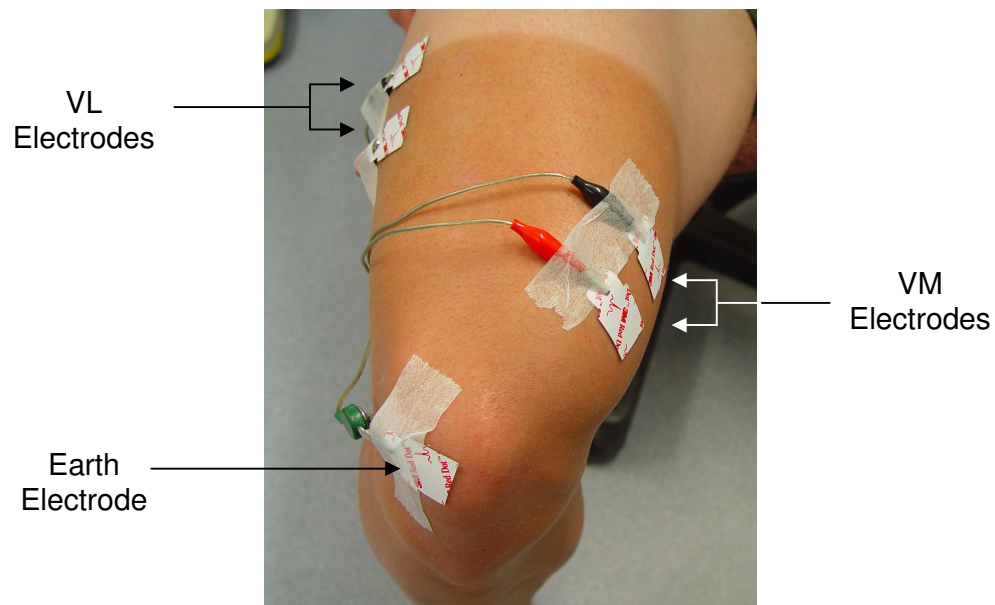
### *Hematological Analysis*

All capillary blood samples were analysed for pH,  $pO_2$ ,  $[HCO_3^-]$  and  $[BLa^-]$  using *i-STAT* CG<sub>4</sub><sup>+</sup> cartridges and an *i-STAT* clinical analyser (*i-STAT* Corporation, New Jersey, NJ, USA). Prior to each testing session, the *i-STAT* analyser was self-calibrated using a routine electronic stimulation. Level 2 *i-STAT* control solution (*i-STAT* Corporation, New Jersey, NJ, USA) was also analysed following every 50 samples across testing to ensure accuracy. The *i-STAT* cartridges were stored prior to use as per manufacturer's instructions (2-8 °C), and were brought to room temperature approximately 5 min prior to

use. The *i-STAT* clinical analyser and  $\text{CG}_4^+$  cartridges have recently been shown to be reliable across the exercise intensities used in the present series of studies (Dascombe, Reaburn, Sirotic and Coutts, 2007). Results obtained using the *i-STAT*  $\text{CG}_4^+$  cartridges and analyser have shown to be reliable within our laboratory following the completion of an incremental  $\text{VO}_{2\text{max}}$  step test [blood pH (0.02: 0.24% (TEM: TEM%));  $p\text{O}_2$  (3.15 mmHg: 3.8%);  $[\text{HCO}_3^-]$  (0.87  $\text{mmol}\cdot\text{L}^{-1}$ : 6.49%); and  $[\text{BLa}^-]$  (0.5  $\text{mmol}\cdot\text{L}^{-1}$ : 3.12%)].

### **Surface Electromyography**

Surface electromyography (sEMG) of the VL and vastus medialis (VM) was monitored to observe changes in fibre recruitment and activity across each SWT (Saunders et al. 2000; Borrani et al. 2001). sEMG data were collected from the VL and VM on the right thigh of each subject at standardised locations (Cram, Kasman and Holtz 1998). Two self adhesive Ag/AgCl electrodes (Red Dot No. 2258, 3M Medical-Surgical Division, St Paul, USA) were placed in a bipolar configuration over the belly of the VL and VM, as displayed in Figure 3.8 below. The electrodes for the VL were incorporated into the modified NIRS probe as detailed earlier in Figure 3.5 (Page 126) to ensure that the placement (14 cm superior of the patella) and inter-electrode distance of 4 cm remained consistent across testing sessions. Electrodes were aligned parallel to the direction of the muscle fibres.



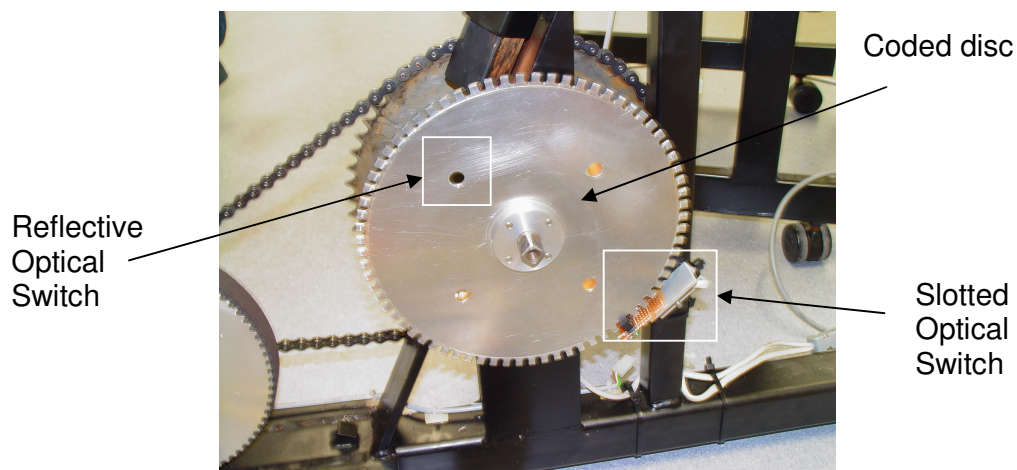
**Figure 3.8:** Illustration of the electrode placement for the VL and VM sEMG measurement.

Prior to the placement of the electrodes, the site was carefully shaved, abraded to remove the top layer of skin cells, and wiped with an alcohol swab to remove oils and salts to reduce subcutaneous impedance to less than 10 k $\Omega$ . All leads were taped down to reduce movement artefact. A single electrode was placed upon the bony surface of the patella to act as an earth contact.

The raw sEMG signal was collected during the last 10 s of each minute during both the ramp test and 30TT. During the SWT sequence testing, sEMG was recorded during the last 10 s of each 30 s across each SWT, or until volitional exhaustion during the severe-intensity SWT. The sEMG signal was recorded by an AMLAB computer system (AMLAB, Peak Performance Technologies Inc., Englewood, USA). The raw signal was amplified by a gain of 1000 and passed through an analogue to digital (A/D) card (AMLAB, Peak

Performance Technologies Inc., Englewood, USA). The signal was captured on a personal computer at 1000 Hz and processed through a 4 pole Butterworth bandpass filter with corner frequencies of 10 Hz and 450 Hz.

Crank angle was recorded simultaneously with sEMG using a custom-built coded disc attached to the crank of the Lode ergometer as shown in Figure 3.9. The coded disc had slots continually around its outer perimeter to provide an angular resolution of  $5^\circ$ . A slotted optical switch (Model 304-560, RS Components, Corby, UK) was used to monitor crank position in  $5^\circ$  increments. A separate reflective optical switch (Model 307-913, RS Components, Corby, UK) was used to recognise when the crank angle returned to  $0^\circ$ . The sEMG signals were only analysed for segments of the cycle stroke which had previously been shown to exhibit the greatest activity in cycling for both the VL ( $315\text{-}110^\circ$ ) and VM ( $305\text{-}135^\circ$ ) according to Jorge and Hull (1986).



**Figure 3.9:** Configuration of the angular displacement system for the Lode Excalibur Cycle.

The sEMG activity for both VL and VM throughout this range was subsequently analysed in custom-written Labview software to determine changes in both the integrated EMG (iEMG) signal and Median Power Frequency (MPF) of each cycle stroke recorded across the 10 s collection period.

Analysis of the raw sEMG signal was performed using a Fast Fourier Transform (FFT) with a Hanning window and zero padding. iEMG was calculated by converting all amplitudes into absolute parameters, and calculating the integral for this signal across time. MPF was determined using the methods of Sparto, Parnianpour, Reinsel and Simon (1997) which utilised simple linear regression according to Eq<sup>n</sup> 10:

$$\text{(Eq}^n \text{ 10)} \quad \text{MPF} = \text{LS} * \text{time} + \text{IMF}$$

In Eq<sup>n</sup> 10, MPF is the Median Power Frequency; LS is the linear slope (Hz/% total time); and, IMF is the Initial Median Frequency (Hz). An R<sup>2</sup> value of the linear regression equation was also calculated.

## **MUSCLE HISTOCHEMICAL AND ENZYMATICAL CHARACTERISTICS**

### **Muscle Biopsies**

On a separate day but within a week of completion of the last laboratory visit, two resting muscle biopsies were taken by an Orthopaedic surgeon from the mid-portion (i.e. 12-16 cm above the patella) of the right VL under local anaesthetic (0.5% *Xylocaine*) at a local medical facility. After the local anaesthetic had taken effect, the sample site was cleaned with a disinfectant

(Betadine™, Mayne Consumer Products, Baulkham Hills, NSW, Australia). A small skin incision (2-3 cm) was made through the skin and muscle fascia using a number 12 scalpel blade. A 5 mm Bergstrom biopsy needle (Stille, Sweden) was then used to obtain two muscle samples according to the method of Bergstrom (1962). To maximise muscle biopsy sample size, a length of surgical tubing connected to a 60 mL injection syringe was inserted into the proximal end of the biopsy cannula and suction applied by a co-investigator immediately prior to sampling (Evans, Pinney and Young 1982). Every effort was made to biopsy at a standardised depth of 3 cm within the muscle belly since significant differences in fibre composition have been observed between the deep and superficial fibres of the VL (Lexell et al. 1985). Immediately following the biopsy, 1-2 stitches were inserted into the incision and the thigh was wrapped with a pressure bandage. The subject was provided with post-operative instructions on wound care to minimise soreness and risk of infection. Subjects were monitored for pain and care of the biopsy site for 48 h following sampling and the results are included within Appendix 4.

The first muscle biopsy sample was removed from the biopsy cannula and orientated longitudinally under a microscope (Olympus CH-2™, Olympus Corporation, Tokyo, Japan.) in *OCT* embedding medium (TissueTek, Thuringowa, Queensland, Australia) on a cork disc and then frozen in 2-methylbutane (Sigma Number: 320404, Sigma-Aldrich Corporation, St Louis, Missouri, USA) cooled to its freezing point by liquid nitrogen (N<sub>2</sub>) for later histochemical analysis. The second biopsy sample was removed from the biopsy cannula and immediately frozen in liquid N<sub>2</sub> for later biochemical analysis. Both samples were stored in separate polypropylene cryovials (Nalge

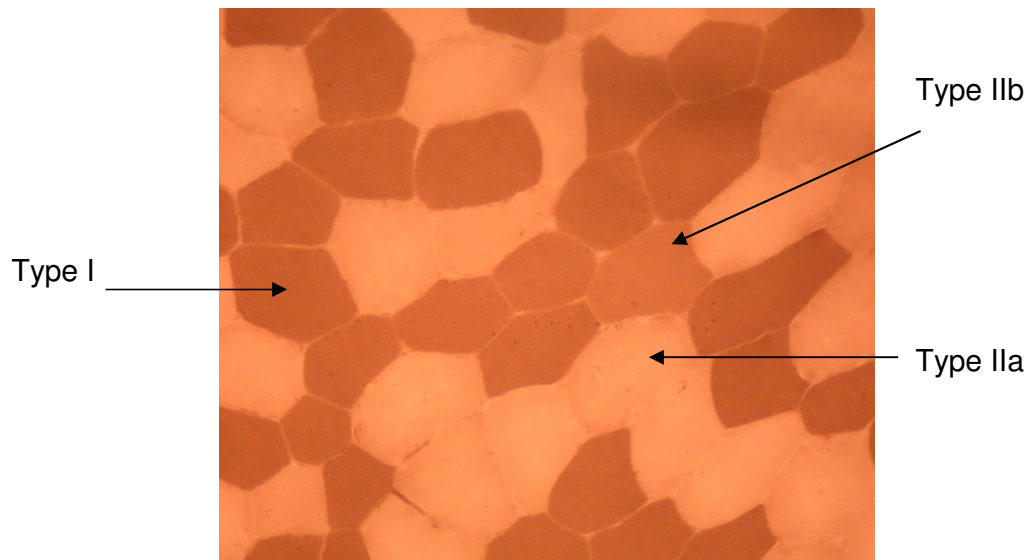
Nunc International, Rochester, New York, USA) at -80° C until subsequent analysis.

### **Histochemistry**

Histochemical analysis of the muscle biopsies was performed at the Tissue Pathology Laboratory at the Royal Brisbane Hospital, Brisbane, Queensland, Australia. All samples were transported from Rockhampton to Brisbane in a sealed container surrounded by frozen CO<sub>2</sub> [dry ice] at -79.5° C. All histochemical staining was performed by the same qualified tissue pathologist.

### *Fibre Composition and Morphology*

Each biopsy sample was cut into serial 10 µm thick cross-sections using a Leica CM1850 cryostat (Leica Microsystems GmbH, Wetzlar, Germany) at -25° C. Fibre composition was determined by staining sections for myofibrillar adenosine triphosphatase (m-ATPase) after pre-incubation at a pH of 4.5, to allow staining of Type I, IIa and IIb muscle fibres according to the methods of Brooke and Kaiser (1970). As shown below in Figure 3.10, digital photographs (3.34 MPEG) were taken of the serial sections using a Nikon Coolpix 995 (Nikon Photo Products Inc., Tokyo, Japan) attached to a Nikon Eclipse E600 microscope (Nikon Instruments Inc., Kanagawa, Japan) at a magnification of x 20.



**Figure 3.10:** A typical cross-section of m-ATPase stained (pH 4.5) muscle demonstrating Type I, IIa and IIb fibres.

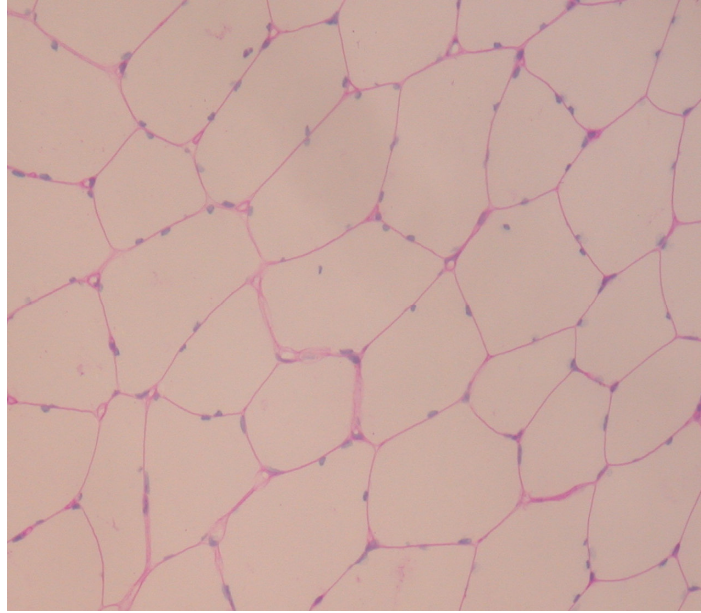
Percentage fibre composition was calculated using at least 200 fibres, or all countable fibres if there were fewer than 200 fibres using the public domain *Image J* program (version 1.32, U.S. National Institutes of Health, Bethesda, Maryland, USA) as counted by one operator (Blomstrand and Ekblom 1982). Two operators independently performed repeat measurements of fibre composition and demonstrated acceptable levels of intra- (TEM: 2.9%; TEM%: 5.8%) and inter-reliability (TEM: 3.9%; TEM%: 7.8%).

The cross-sectional area of at least 40 (or as many as possible if 40 were not visible) of the Type I, IIa and IIb fibres were analysed using the *Image J* software. The perimeter of the fibre was outlined and the area within the border calculated according to a constant pixel density. Prior to each fibre's measurement, the scale of measurement was calibrated using a photograph of

a 1 mm objective micrometer (Graticules Ltd, Tonbridge, Kent, UK). Only fibres without artefacts, distinct cell borders, and no tendency towards longitudinal cuts were included in the analysis (Blomstrand and Ekblom 1982). Slides were analysed in a randomized sequence and subject identity not disclosed until consensus values were obtained for all specimens. Two operators independently performed repeat measurements of morphometry and produced acceptable levels of intra- (TEM: 374.3  $\mu\text{m}^2$ ; TEM%: 4.1%) and inter-reliability (TEM: 732.7  $\mu\text{m}^2$ ; TEM%: 8.1%).

### *Capillarisation*

Additional 10  $\mu\text{m}$  cross-sections of each biopsy sample were stained using the periodic acid-Schiff (PAS) stain to visualise capillaries according to the method described by Fink and Costill (1990). Detailed photographs (3.34 MPEG) were taken of each stained section using a Nikon Coolpix 995 attached to a Nikon Eclipse E600 microscope. Capillarisation measures were performed using the *Image J* software. Analysis of capillarisation was taken directly from numerous artefact-free regions of sections until 2.0  $\text{mm}^2$  of muscle had been analysed. An example of a PAS capillarisation stain is shown below in Figure 3.11.



**Figure 3.11:** A typical cross-section of muscle stained for measures of capillarisation.

Capillary density ( $\text{cap} \cdot \text{mm}^{-2}$ ) was used to determine any differences in supply of capillaries to the VL within the young and middle-aged cyclists. This measure was calculated by manually counting capillaries within a randomly selected 1.0 mm square using the *Image J* software. Capillaries in contact with the borders of the grid were included within the capillary count. The capillary-to-fibre ratio (C/F) was calculated by using the grid described above and counting both the number of fibres and capillaries within the area. Capillary contacts per fibre (CC/F) was calculated by manually counting the number of capillaries in contact with individual fibres within the grid and then averaging the number per fibre. Such methods for determination of muscle capillarisation have been used in similar investigations (Chilibeck et al. 1997; Pringle et al. 2003b). Two operators independently performed repeat measurements of capillary density

and produced acceptable levels of intra- (TEM: 14.4 cap•mm<sup>-2</sup>; TEM%: 6.3%) and inter-reliability (TEM: 21.3 cap•mm<sup>-2</sup>; TEM%: 9.7%).

In order to examine the potential effects of O<sub>2</sub> diffusion capacity, both the average (DD<sub>mean</sub>) (Eq<sup>n</sup> 11) and maximum (DD<sub>max</sub>) (Eq<sup>n</sup> 12) diffusion distances were estimated using the equations developed by Snyder (1987) for capillaries distributed in random arrays.

$$(Eq^n 11) \quad DD_{mean} = \left[ \frac{0.207 + 0.232}{C:F \text{ Ratio}} \right] \times \sqrt{\text{average fibre cross-sectional area}}$$

$$(Eq^n 12) \quad DD_{max} = \left[ \frac{0.415 + 0.477}{C:F \text{ Ratio}} \right] \times \sqrt{\text{average fibre cross-sectional area}}$$

These estimates are based upon the cumulative frequency of the area of each fibre within a measured distance from a capillary. DD<sub>mean</sub> is defined as the distance to where 50% of the fibre area is served by a capillary, where DD<sub>max</sub> refers to 95% of this area. The equations employed for capillarisation were reliant upon the random distribution of capillaries throughout muscle, and based upon the C/F ratio and CC data of each fibre (Plyley and Groom 1975).

## Enzyme Analysis

Biochemical analysis of the muscle biopsy samples was conducted at the School of Health Sciences, Deakin University, Melbourne, Australia by the current PhD Candidate (BD). All muscle samples were transported from Rockhampton to Melbourne in a sealed container surrounded by frozen carbon dioxide (CO<sub>2</sub>) [dry ice] at -79.5° C. Upon arrival, the samples were stored at

-80° C at Deakin University. Several 5-10 mg portions of muscle were dissected and weighed on previously-calibrated Denver Instrument DI-100 electronic scales (Denver Instrument, Denver, Colorado, USA) and then stored at -80° C until homogenisation.

### *Homogenisation*

One 5-10 mg portion was removed and homogenised for the measurement of PFK and LDH activity. The sample was removed from -80° C storage and allowed to thaw on ice (0° C). A 1:50 homogenate of each muscle sample was prepared in a homogenising medium (50 mM Tris; 10mM K<sub>2</sub>HPO<sub>4</sub>; 5 mM 2-mercaptoethanol; 0.5 mM EDTA; 0.02% BSA) at pH 8.1 using a glass pestle homogeniser kept on ice. The sample was homogenised by performing 15 passes of the plunger. The homogenate was stored on ice and immediately analysed for PFK and LDH activity using the methods detailed below.

For measurement of CS and  $\beta$ -HAD activity, a separate 5-10 mg muscle sample was homogenised in a 1:50 buffer containing 0.175 M KCl and 2 mM EDTA at pH 7.4 using a *Polytron* PT 1200 homogeniser (Scientific Exchange, Manotick, Ontario, Canada). The homogenate was then freeze-thawed twice, and centrifuged at 10,000 RPM for 1 min. The supernatant of the homogenate was transferred to a 1.5 mL Eppendorf tube and stored at -80° C until subsequent analysis of CS and  $\beta$ -HAD.

A 10-15 mg sample of each muscle sample was dissected and homogenised for the measurement of 2-OGDH activity. A 1:10 homogenate was made on ice using a 50 mM Tris-HCl; 5 mM MgCl<sub>2</sub>; 1 mM EDTA

homogenising buffer at pH 8.2 using the glass pestle method described earlier. The homogenate was analysed immediately for 2-OGDH activity.

Total protein content of each homogenate was analysed using a BCA Pierce Protein Assay (Rockford, Illinois, USA). A 10  $\mu\text{L}$  aliquot of the 2-OGDH sample was diluted in 200  $\mu\text{L}$  of mixed reagent and incubated for 30 min at 37 $^{\circ}$  C, and then let stand at room temperature for a further 15 min. Total protein was measured using a *Labsystems* Multiskan RC plate reader (Labsystems, Stockholm, Sweden) and *Genesis* software v4.3 (Labsystems, Stockholm, Sweden) at 550 nm. The repeatability of the BCA Pierce Protein assay was acceptable (TEM:  $1.51 \times 10^{-3}$   $\mu\text{g} \cdot \mu\text{L}^{-1}$ ; TEM%: 3.48%; CV%: 6.3%).

#### *Phosphofructokinase (PFK)*

The activity of PFK in the muscle homogenate was determined by the method of Chi et al. (1983). The homogenate was further diluted to 1:1500 in the homogenising medium containing 50 mM Tris; 10 mM  $\text{K}_2\text{HPO}_4$ ; 5 mM 2-Mercaptoethanol; 0.5 mM EDTA; 0.02% BSA at pH 8.1. A 5  $\mu\text{L}$  aliquot of the 1:1500 homogenate was added to a 0.1 mL reagent mixture (50 mM Tris, pH 8.1; 25 mM HCl; 1 mM fructose-6-phosphate; 1 mM ATP; 10 mM  $\text{K}_2\text{HPO}_4$ ; 2 mM  $\text{MgCl}_2$ ; 1 mM 2-mercaptoethanol; 0.02% BSA; 0.1 U/mL aldolase; 20  $\mu\text{M}$  NADH; 1 U/mL TPI/ $\alpha$ -GPDH) in separate borosilicate glass culture tubes (Kimble, Vineland, New Jersey, USA). Each tube was immediately mixed by vortexing (Ratek VM1 Vortex<sup>TM</sup>, Ratek Instruments Pty. LTd., Boronia, Victoria, Australia) and then incubated at room temperature for 1 h. Next, 10  $\mu\text{L}$  of 0.75M HCL was added to each tube, briefly mixed by vortexing and incubated for a further 10 min at room temperature. After this incubation, 1 mL of a 6 M

NaOH-10 mM Imidazole solution was added to each tube, and mixed thoroughly by vortexing. The tubes were then placed in a *Grant Y6* water bath (Grant Instruments Cambridge Ltd., Herts, UK) set at 60° C for 20 min. Following this, the tubes were transferred to a water bath at room temperature for a further 10 min. Each tube was then dried. The absorbance of NADH was read (at 340 nm excitation and 465 nm emission) on an *Optical Technology Devices Inc. Ratio 2* filter fluorometer (Optical Technology Devices, Valhalla, NY, USA).

PFK activity was determined through NADH reduction to NAD, and was calculated through the equation below (Eq<sup>n</sup> 13). Blanks and working fructose-1,6-biphospate standard solutions of 1 µM, 2.5 µM and 3.5 µM were also measured to provide a standard curve and allow quantification of the assay. All samples were measured in triplicate and the mean taken for data analysis. The absolute (µmol•g<sup>-1</sup>•min<sup>-1</sup>) and specific (µmol•g<sub>protein</sub><sup>-1</sup>•min<sup>-1</sup>) activity of PFK was calculated for each muscle sample. The repeatability of the PFK assay was acceptable (TEM: 0.86 µmol•g<sup>-1</sup>•min<sup>-1</sup>; TEM%: 2.46%; CV%: 2.6%).

(Eq<sup>n</sup> 13)

$$\text{PFK Activity (}\mu\text{mol/g/min)} = \left( \frac{F_{\text{sample}}}{F_{\text{standard}}} \right) \times (\text{mM}_{\text{standard}} \times \text{mL}_{\text{standard}}) \div \text{g}_{\text{wet muscle}} \div \text{min}$$

Where:  $F_{\text{sample}}$  = fluorescence of sample;  $F_{\text{standard}}$  = fluorescence of standard;  $\text{mM}_{\text{std}}$  = concentration of standard;  $\text{mL}_{\text{standard}}$  = volume of standard;  $\text{g}_{\text{wet muscle}}$  = wet mass of tissue within sample; min = time of incubation prior to addition of HCl.

### *Lactate Dehydrogenase (LDH)*

LDH activity was measured using the method of Chi et al. (1983). Immediately after homogenisation, the sample was centrifuged at 3000 g for 15 min at 4°C. A 40 µL aliquot of homogenate supernatant was added to 1 mL of a 200 mM imidazole-0.1% BSA buffer at pH 7.0 (1:1250). A 100 µL volume of reagent mixture (0.1 M imidazole; 1 M HCl; 2 mM pyruvate; 0.05% BSA; 0.3 mM NADH at pH 7.0) was added to triplicate Kimble tubes (Kimble, Vineland, New Jersey, USA). The reaction was started by adding 1 µL of the muscle homogenate supernatant to each Kimble tube, and immediately mixed by vortexing. The samples were then incubated at room temperature for 1 h. 10 µL of 1 M HCl was then added to the mixture and the assay was let stand at room temperature for a further 10 min. After this, 1 mL of 6 M NaOH-10mM imidazole mixture was then added to each Kimble tube and immediately mixed by vortexing. Samples were then placed in the *Grant Y6* water bath set at 60°C for 20 min. Following this, the tubes were transferred to a water bath at room temperature for a further 10 min. Each tube was then dried. The absorbance of NADH was read (at 340 nm excitation and 465 nm emission) on an *Optical Technology Devices Inc. Ratio 2* filter fluorometer.

LDH activity was measured through the change in fluorescence as a result of NADH reduction to NAD, using the formula below (Eq<sup>n</sup> 14). Blanks and working NAD standards of 25 µM, 50 µM and 75 µM were measured to provide a standard curve and allow quantification of the assay. All samples were measured in triplicate and the mean taken for data analysis. The absolute ( $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ) and specific ( $\mu\text{mol}\cdot\text{g}_{\text{protein}}^{-1}\cdot\text{min}^{-1}$ ) activity of LDH was calculated

for each muscle sample. The repeatability of the LDH assay was acceptable (TEM: 4.14  $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ; TEM%: 2.74%; CV%: 3.0%).

(Eq<sup>n</sup> 14)

$$\text{LDH Activity } (\mu\text{mol/g/min}) = \left( \frac{F_{\text{sample}}}{F_{\text{standard}}} \right) \times (\text{mM}_{\text{standard}} \times \text{mL}_{\text{standard}}) \div \text{g}_{\text{wet muscle}} \div \text{min}$$

Where:  $F_{\text{sample}}$  = fluorescence of sample;  $F_{\text{standard}}$  = fluorescence of standard;  $\text{mM}_{\text{std}}$  = concentration of standard;  $\text{mL}_{\text{standard}}$  = volume of standard;  $\text{g}_{\text{wet muscle}}$  = wet mass of tissue within sample; min = time of incubation prior to addition of HCl.

#### *Citrate Synthase (CS)*

The activity of CS was measured using the method reported by Chi et al. (1983). The reagent mixture consisted of 100 mM Tris (pH 8.3), 50  $\mu\text{L}$  of 1 mM DTNB, and 80  $\mu\text{L}$  of 3 mM acetyl-CoA. A 330  $\mu\text{L}$  volume of reagent mixture and 10  $\mu\text{L}$  of muscle homogenate were added together in 10 mL quartz cuvettes (Starna Pty Ltd, Baulkham Hills, NSW, Australia). The cuvettes were then inserted in a *Helios Unicam* spectrophotometer (Unicam Spectrometry, Cambridge, UK) set to 412 nm set at a temperature of 25° C. All cuvettes were allowed to pre-incubate at 25°C for 5 min. To commence the reaction, 30  $\mu\text{L}$  of 10mM oxalacetate was added into each cuvette, and mixed by gentle inversion. Each cuvette was then placed back into the spectrophotometer, zeroed and then the change in DTNB absorbance was recorded at 15 s intervals for 3 min. For the measurement of the blank sample, the identical procedure was performed as for the reagent mixture and muscle homogenate, but no

oxalacetate was added. The activity of CS was then calculated using the equation below (Eq<sup>n</sup> 15), based upon the changes within absorbance of DTNB as measured by the spectrophotometer. All samples were measured in duplicate and the mean value used for data analysis. The absolute ( $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ) and specific ( $\mu\text{mol}\cdot\text{g}_{\text{protein}}^{-1}\cdot\text{min}^{-1}$ ) activity of CS was calculated for each muscle sample. The reliability of the CS assay was acceptable (TEM:  $1.69 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ; TEM%: 8.39%; CV%: 8.4%).

(Eq<sup>n</sup> 15)

$$\text{CS Activity } (\mu\text{mol/g/min}) = \frac{\Delta\text{Abs/min} \times \text{Total volume}}{\text{Sample volume} \times 13.6} \times \text{Dilution factor}$$

Where:  $\Delta\text{Abs/min}$  = average change in absorbance per minute; Total volume = total volume of cuvette; Sample volume = volume of sample in cuvette; Dilution factor = magnitude of sample dilution;  $13.6 \text{ M}^{-1}\cdot\text{cm}^{-1}$  = the molar extinction coefficient of DTNB at 412 nm.

#### *$\beta$ -Hydroxyacyl-CoA Dehydrogenase ( $\beta$ -HAD)*

$\beta$ -HAD activity was determined using the method of Chi et al. (1983). A 430  $\mu\text{L}$  volume of reagent mixture (1 M Tris-HCl (pH 7.0); 200 mM EDTA; 5 mM NADH) and 10  $\mu\text{L}$  of a 10% Triton X-100 solution (0.5 mL Triton x 100; 2 mL Ethanol; 2.5 mL  $\text{H}_2\text{O}$ ) were added to 10 mL quartz cuvettes (Starna Pty Ltd, Baulkham Hills, NSW, Australia). Next a 50  $\mu\text{L}$  volume of muscle homogenate was also added to the cuvette, which was then placed and pre-incubated at 30° C for 5 min. The decrease in NADH absorbance was measured by a *Helios Unicam* spectrophotometer set at 340 nm at 30°C. Prior to the reaction being

triggered, the changes in NADH production were recorded for 2 min to enable each cuvette to act as its own control. To start the reaction, 10  $\mu\text{L}$  of acetoacetyl-CoA was added to each cuvette and mixed through gentle inversion. The reduction in NADH was recorded in 15 s intervals across 4 min.  $\beta$ -HAD activity was calculated using the equation below (Eq<sup>n</sup> 16). All samples were measured in triplicate and the mean taken for analysis. The absolute ( $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ) and specific ( $\mu\text{mol}\cdot\text{g}_{\text{protein}}^{-1}\cdot\text{min}^{-1}$ ) activity of  $\beta$ -HAD was calculated for each muscle sample. The reliability of the  $\beta$ -HAD assay was found to be acceptable (TEM:  $0.58 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ; TEM%: 9.35%; CV%: 8.5%).

(Eq<sup>n</sup> 16)

$$\beta\text{-HAD Activity } (\mu\text{mol/g/min}) = \frac{\Delta\text{Abs/min} \times \text{Total volume}}{\text{Sample volume} \times 6.22} \times \text{Dilution factor}$$

Where:  $\Delta\text{Abs/min}$  = average change in absorbance per minute; Total volume = total volume of cuvette; Sample volume = volume of sample in cuvette; Dilution factor = magnitude of sample dilution;  $6.22 \text{ M}^{-1}\cdot\text{cm}^{-1}$  = the molar extinction coefficient of NADH at 340 nm.

### *2-Oxoglutarate Dehydrogenase (2-OGDH)*

The activity of 2-OGDH within the muscle samples was measured using the method of Blomstrand, Radegran and Saltin (1997). Firstly, 675  $\mu\text{L}$  of a reaction mixture containing 50 mM triethanolamine; 0.05% Triton; 10  $\mu\text{M}$  Rotenone at pH 7.4 and further volumes of 50  $\mu\text{L}$  of 40 mM NAD, 50  $\mu\text{L}$  of 40 mM 2-oxoglutarate and 25  $\mu\text{L}$  of 16 mM CoA were added to a 10 mL quartz cuvette. The cuvette was allowed to pre-incubate for 5 min in the

spectrophotometer at 25° C. Next, 25 µL of muscle homogenate was added to the cuvette to start the reaction. 2-OGDH activity was measured through the production of NADH measured using a *Helios Unicam* spectrophotometer set at 340 nm at 25° C. Changes in NADH production were monitored in 15 s intervals across 3 min. 2-OGDH activity was calculated using the equation detailed below (Eq<sup>n</sup> 17). Prior to the addition of muscle homogenate, the changes in NADH production were recorded for 2 min to enable each cuvette to act as its own control. All samples were measured in triplicate and the mean value taken for later data analysis. The absolute ( $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ) and specific ( $\mu\text{mol}\cdot\text{g}_{\text{protein}}^{-1}\cdot\text{min}^{-1}$ ) activity of 2-OGDH was calculated for each muscle sample. The reliability of the 2-OGDH assay was acceptable (TEM: 0.11  $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ; TEM%: 7.62% CV%: 9.1%).

(Eq<sup>n</sup> 17)

$$\text{2-OGDH Activity } (\mu\text{mol/g/min}) = \frac{\Delta\text{Abs/min} \times \text{Total volume}}{\text{Sample volume} \times 6.22} \times \text{Dilution factor}$$

Where:  $\Delta\text{Abs/min}$  = average change in absorbance per minute; Total volume = total volume of cuvette; Sample volume = volume of sample in cuvette; Dilution factor = magnitude of sample dilution;  $6.22 \text{ M}^{-1}\cdot\text{cm}^{-1}$  = the molar extinction coefficient of NADH at 340 nm.

## STATISTICAL ANALYSIS

Means and standard deviations ( $\bar{X} \pm \text{SD}$ ) were determined for all parameters of interest. In order to determine the likelihood of a Type I error, a Greenhouse-Geisser adjustment was performed to ensure the sphericity of all

measures. The continuous variables were assessed using a Kolmogorov-Smirnov test of homogeneity to ensure all data were normally distributed.

Independent sample *t*-tests were used to detect any between-group differences (e.g. young and middle-aged cyclists) in the anthropometric, physiological, histochemical and biochemical characteristics. Data variance was assessed using a Levene's Test. The observed differences were supported using Cohen's D effect size comparisons and 95% confidence intervals (CI) were calculated for all data. The magnitude of the observed differences was quantified using effect size statistics ( $\eta^2$ ) as described by Cohen (1992), where the values of 0.6, 0.8, 1.0 and >1.0 were representative of small, medium, large and very large effect sizes, respectively.

Significant differences in  $\dot{V}O_2$  and mOxy kinetic parameters between groups (young and middle-aged) and across intensities (moderate, heavy and severe) were identified using 2 x 3 Repeated Measures Analysis of Variance (RMANOVA). A 2 x 2 RMANOVA was used to determine differences in the kinetic parameters between groups (age) and physiological measures ( $\dot{V}O_2$  and mOxy) for each SWT intensity. 2 x 2 RMANOVA were also used to detect significant changes within kinetic parameters across the on- and off-transient responses at each SWT intensity. Significant changes within the hematological parameters and blood gases were also examined using 2 x 3 RMANOVA to report upon the effect of both age and time. Similarly, 2 x 3 RMANOVA's were used to examine the effect of age and time within a number of physiological variables measured across the 30TT.

For all multivariate tests, the mean F statistic was used to identify the level of significance between group effects of age, and within-group effects of intensity adjusted by the Greenhouse-Geisser epsilon values in the event of violation of the sphericity assumption. Following each RMANOVA, a Least Significant Differences (LSD) *post-hoc* comparison was utilised to detect the location of any significant differences observed across intensities. The magnitude of the observed differences across groups and intensities was quantified using effect size statistics ( $\eta^2$ ) as described by Cohen (1992), where the values of 0.6, 0.8, 1.0 and >1.0 were representative of small, medium, large and very large effect sizes, respectively.

Multiple Pearson product-moment correlations ( $r$ ) were used to investigate the relationships between anthropometric, physiological, histochemical and enzymatic parameters,  $\text{VO}_2$  and mOxy kinetic parameters, hematological parameters and 30TT performance measures within each age group.

The measures of reliability [Technical Error of Measurement (TEM); Technical Error of Measurement Percentage (TEM%) and Co-efficient of Variation (CV%)] used to assess the methods outlined in this chapter were calculated in accordance with the method of Norton and Olds (1996). Methodological procedures were deemed reliable if both the TEM% and CV% were less than 10%. All statistical calculations were performed using Statistical Package for Social Statistical software (version 11, SPSS Inc., Chicago, Illinois, USA). Statistical significance was accepted using an alpha ( $p$ ) level of 0.05.

## CHAPTER 4

### STUDY 1

# **Physiological, histochemical, enzymatic and performance characteristics in well-trained young and middle-aged cyclists**

## **OVERVIEW**

The purpose of Study One was to investigate and describe the effect of age on physiological and peripheral muscle characteristics in well-trained young and middle-aged cyclists. The results of Study One demonstrated no significant effect of age on the VT or  $\dot{V}O_{2\max}$  in the well-trained cyclists. Similarly, there was no significant effect of age on muscle fibre composition, muscle fibre CSA or capillarisation of the VL of the well-trained cyclists. Moreover, the maximal specific activities of several glycolytic (PFK and LDH) and oxidative (CS,  $\beta$ -HAD and 2-OGDH) enzymes of the VL were similar between the two age groups. Finally, no significant effect of age was observed in the 30TT cycling performance in the present study.

The present findings suggest that several physiological capacities previously reported to be influenced through sedentary aging are maintained into middle-age with physical training. Further, the young and middle-aged cohorts recruited within the present study were matched for several physiological characteristics that may significantly influence the on-transient  $\dot{V}O_2$  and mOxy responses to exercise bouts of varying intensity. Thus, these

results suggest that any significant effects of age observed within the subsequent studies (2-4) are most likely not influenced by  $\dot{V}O_2\text{max}$ , peripheral muscle histochemical and enzymatic characteristics, or performance characteristics.

## RESULTS

### Physical Characteristics

The physical characteristics of the young and middle-aged cyclists are shown in Table 4.1.

**Table 4.1:** Mean ( $\pm$  SD) physical characteristics of the young and middle-aged cyclists

Characteristic	Young	Middle-aged	Cohens D (95% CI)
Age (y)	19.6 $\pm$ 1.7	47.9 $\pm$ 2.7 *	1.91 (25.6 - 30.9)
Height (cm)	176.8 $\pm$ 3.9	178.3 $\pm$ 5.7	0.33 (-4.1 - 7.3)
Mass (kg)	69.6 $\pm$ 8.0	79.3 $\pm$ 15.7	0.85 (-2.6 - 21.9)
$\Sigma$ 9 SF (mm)	93.6 $\pm$ 19.8	121.7 $\pm$ 30.0	0.98 (-1.5 - 57.6)
$\dot{V}O_2\text{max}$ (L $\cdot$ min <sup>-1</sup> )	3.82 $\pm$ 0.46	3.95 $\pm$ 0.60	0.25 (-0.5 - 0.8)
$\dot{V}O_2\text{max}$ (mL $\cdot$ kg <sup>-1</sup> $\cdot$ min <sup>-1</sup> )	55.2 $\pm$ 7.0	50.2 $\pm$ 6.4	0.71 (-12.8 - 2.8)
VT (L $\cdot$ min <sup>-1</sup> )	2.68 $\pm$ 0.41	2.81 $\pm$ 0.40	0.36 (-0.3 - 0.6)
VT (% $\dot{V}O_2\text{max}$ )	71.4 $\pm$ 5.1	70.2 $\pm$ 4.0	0.26 (-0.31 - 0.57)
HR <sub>max</sub> (b $\cdot$ min <sup>-1</sup> )	194.4 $\pm$ 10.0	180.7 $\pm$ 12.1 *	1.0 (-26.6 - 0.7)
Training history (y)	9.7 $\pm$ 5.9	4.7 $\pm$ 2.3	1.23 (-0.2 - 10.2)

\* significant difference between age-groups (p<0.05)

## Muscle Histochemical and Enzymatical Characteristics

### *Muscle Fibre Composition and Morphology*

The histochemical characteristics of the VL from young and middle-aged well-trained cyclists are summarised in Table 4.2 below. No significant age differences were observed in any measure of muscle fibre composition (%) or muscle fibre CSA ( $\mu\text{m}^2$ ).

**Table 4.2:** Mean ( $\pm$  SD) histochemical characteristics of the vastus lateralis in the young and middle-aged cyclists.

<b>Histochemical Characteristic</b>	<b>Young</b>	<b>Middle-Aged</b>	<b>Cohens D (95% CI)</b>
Type I (%)	58.3 $\pm$ 9.6	58.7 $\pm$ 16.6	0.03 (-15.7 - 16.6)
Type IIa (%)	33.7 $\pm$ 7.7	34.8 $\pm$ 12.9	0.11 (-11.5 - 13.9)
Type IIb (%)	8.0 $\pm$ 3.0	6.4 $\pm$ 4.1	0.45 (-5.9 - 2.8)
Type I CSA ( $\mu\text{m}^2$ )	5599 $\pm$ 1478	6759 $\pm$ 1060	0.83 (-437 - 2757)
Type IIa CSA ( $\mu\text{m}^2$ )	6844 $\pm$ 1993	7026 $\pm$ 1456	0.10 (-1984 - 2348)
Type IIb CSA ( $\mu\text{m}^2$ )	6082 $\pm$ 2534	5985 $\pm$ 3170	0.03 (-3375 - 3382)

### *Muscle Capillarisation*

Table 4.3 shows the muscle capillarisation characteristics of the VL muscle from the young and middle-aged cyclists. No significant effect of age was observed in any measure of muscle capillarisation.

**Table 4.3:** Mean ( $\pm$  SD) capillarisation measures of the vastus lateralis in the young and middle-aged cyclists.

Capillarisation Measure	Young	Middle Aged	Cohens D (95% CI)
Capillary Density ( $\text{cap}\cdot\text{mm}^{-2}$ )	$301.0 \pm 57.4$	$287.0 \pm 40.0$	0.31 (-72.6 - 42.6)
Capillary to Fibre Ratio (C/F)	$2.3 \pm 0.8$	$2.6 \pm 0.4$	0.54 (-0.4 - 1.0)
Capillary Contacts per Fibre (CC/F)	$4.6 \pm 1.0$	$5.1 \pm 0.3$	0.66 (-0.7 - 1.4)
Capillary Contacts per Fibre Area ( $\text{CC}/\mu\text{m}^2$ )	$7.8 \pm 2.2 (10^{-4})$	$7.6 \pm 1.2 (10^{-4})$	0.16 (-2.2 - 1.7 ( $10^{-4}$ ))
Maximum Diffusion Distance ( $\mu\text{m}$ )	$14.5 \pm 7.1$	$18.9 \pm 3.8$	0.75 (-2.2 - 11.1)
Average Diffusion Distance ( $\mu\text{m}$ )	$24.6 \pm 3.8$	$24.5 \pm 1.4$	0.01 (-3.4 - 3.3)

### *Enzyme Activities*

Table 4.4 shows the maximal specific activities ( $\mu\text{mol}\cdot\text{g}_{\text{protein}}^{-1}\cdot\text{min}^{-1}$ ) of glycolytic (PFK and LDH) and oxidative (CS,  $\beta$ -HAD and 2-OGDH) enzymes from the VL of the young and middle-aged cyclists. No significant effect of age was observed in any of the maximal specific enzyme activities examined.

**Table 4.4:** Mean ( $\pm$  SD) maximal specific glycolytic and oxidative enzyme activities ( $\mu\text{mol}\cdot\text{g}_{\text{protein}}^{-1}\cdot\text{min}^{-1}$ ) of the vastus lateralis in the young and middle-aged cyclists.

Enzyme Activity	Young	Middle-Aged	Cohens D (95% CI)
PFK	$47.3 \pm 5.0$	$41.0 \pm 8.3$	0.85 (-14..3 - 1.7)
LDH	$242.3 \pm 36.6$	$202.1 \pm 86.1$	0.60 (-117.2 - 36.8)
CS	$17.0 \pm 4.8$	$15.3 \pm 5.5$	0.32 (-7.6 - 4.4)
$\beta$ -HAD	$1.0 \pm 0.1$	$1.0 \pm 0.3$	0.37 (-0.2 - 0.4)
2-OGDH	$0.10 \pm 0.03$	$0.10 \pm 0.04$	0.85 (-0.007 - 0.7)

### 30 Minute Time Trial Results

The mean ( $\pm$  SD) results for the 30TT performance variables are shown in Table 4.5 over the page.

Significant main effects of age were observed for both mean 30TT PO ( $F(2,12)=6.257$ ,  $p=0.028$ ,  $\eta^2=0.343$ ), 30TT  $\dot{V}O_2$  ( $F(2,12)=6.697$ ,  $p=0.024$ ,  $\eta^2=0.358$ ) and mean 30TT % $\dot{V}O_{2max}$  ( $F(1,12)=6.259$ ,  $p<0.001$ ,  $\eta^2=0.343$ ). A significant main effect of time was observed for the 30TT HR ( $F(2,24)=50.536$ ,  $p<0.001$ ,  $\eta^2=0.808$ ), 30TT %HR<sub>max</sub> ( $F(2,24)=52.528$ ,  $p<0.001$ ,  $\eta^2=0.814$ ) and 30TT % $\dot{V}O_{2max}$  ( $F(2,24)=4.429$ ,  $p=0.023$ ,  $\eta^2=0.270$ ). No significant age x time interactions were observed in any physiological or performance parameter during the 30TT.

Significant main effects of time were observed in a number of physiological measures across the 30TT in both the young and middle-aged cyclists. In the young cyclists, a significant effect of time was observed for HR ( $F(2,12)=14.549$ ,  $p<0.001$ ,  $\eta^2=0.708$ ), %HR<sub>max</sub> ( $F(2,12)=13.804$ ,  $p=0.001$ ,  $\eta^2=0.697$ ) and mOxy ( $F(2,9)=0.538$ ,  $p=0.049$ ,  $\eta^2=0.453$ ) across the 30TT. The middle-aged cyclists demonstrated a significant effect of time in HR ( $F(2,12)=74.445$ ,  $p<0.001$ ,  $\eta^2=0.925$ ), %HR<sub>max</sub> ( $F(2,12)=94.258$ ,  $p<0.001$ ,  $\eta^2=0.940$ ) and RPO ( $F(2,12)=3.943$ ,  $p=0.048$ ,  $\eta^2=0.397$ ) across the 30TT.

**Table 4.5:** Mean ( $\pm$  SD) performance and physiological characteristics of the young and middle-aged cyclists across the thirty minute time trial.

	Young			Middle-Aged		
	10 min	20 min	30 min	10 min	20 min	30 min
<b>PO (W)</b>	213.0 $\pm$ 37.9	206.7 $\pm$ 38.1	220.5 $\pm$ 41.1	253.7 $\pm$ 28.8 <sup>#</sup>	254.3 $\pm$ 30.0 <sup>#</sup>	263.0 $\pm$ 30.3 <sup>#</sup>
<b>RPO (W·kg BM<sup>-1</sup>)</b>	3.1 $\pm$ 0.5	3.0 $\pm$ 0.5	3.2 $\pm$ 0.6	3.2 $\pm$ 0.4	3.3 $\pm$ 0.5	3.4 $\pm$ 0.5 <sup>¥</sup>
<b>HR (b·min<sup>-1</sup>)</b>	158.8 $\pm$ 18.0	169.8 $\pm$ 17.3 <sup>¥</sup>	176.2 $\pm$ 10.2 <sup>¥</sup>	148.2 $\pm$ 8.2	160.6 $\pm$ 9.0 <sup>¥</sup>	166.4 $\pm$ 9.9 <sup>¥</sup>
<b>%HR<sub>max</sub> (%)</b>	81.7 $\pm$ 8.8	87.4 $\pm$ 8.7 <sup>¥</sup>	90.7 $\pm$ 5.0 <sup>¥</sup>	82.2 $\pm$ 4.9	89.0 $\pm$ 4.4 <sup>¥</sup>	92.2 $\pm$ 3.6 <sup>¥ §</sup>
<b><math>\dot{V}O_2</math> (mL·min<sup>-1</sup>)</b>	2712.5 $\pm$ 418.3	2840.9 $\pm$ 372.2	2844.1 $\pm$ 290.3	3233.1 $\pm$ 329.4 <sup>#</sup>	3430.0 $\pm$ 452.2 <sup>#</sup>	3422.1 $\pm$ 629.3 <sup>#</sup>
<b><math>\dot{V}O_2</math> (mL·kg<sup>-1</sup>·min<sup>-1</sup>)</b>	39.1 $\pm$ 4.7	40.9 $\pm$ 3.0	41.0 $\pm$ 2.5	41.4 $\pm$ 5.6	43.9 $\pm$ 7.4	43.7 $\pm$ 8.6
<b>%<math>\dot{V}O_{2max}</math> (%)</b>	71.3 $\pm$ 8.5	74.7 $\pm$ 8.1	75.0 $\pm$ 7.4	82.6 $\pm$ 8.6 <sup>#</sup>	87.6 $\pm$ 11.0 <sup>#</sup>	87.0 $\pm$ 13.2 <sup>#</sup>
<b>mOxy (%)</b>	69.9 $\pm$ 26.6	64.7 $\pm$ 23.9	50.5 $\pm$ 18.1	67.9 $\pm$ 18.3	66.5 $\pm$ 16.5	55.7 $\pm$ 18.9

PO = Power Output

HR= Heart Rate

% $\dot{V}O_{2max}$  = % of Maximum Oxygen Consumption

RPO =

Relative Power Output

$\dot{V}O_2$ =

Oxygen Consumption

mOxy =

Muscle Oxygenation

%HR<sub>max</sub> =

% of Maximum Heart Rate

<sup>#</sup> significantly different to the young cyclists (p<0.05); <sup>¥</sup> significantly different to 10 min (p<0.05); <sup>§</sup> significantly different to 20 min (p<0.05).

The mean ( $\pm$  SD) results for the hematological parameters measured across the 30TT are displayed in Table 4.6 over the page. No significant main effects of age or age x time interactions were observed in any hematological parameters measured across the 30TT. Significant main effects of time were observed for all hematological measures including blood pH ( $F(3,36)=35.921$ ,  $p<0.001$ ,  $\eta^2=0.750$ );  $pO_2$  ( $F(3,36)=39.671$ ,  $p<0.001$ ,  $\eta^2=0.768$ );  $[HCO_3^-]$  ( $F(3,36)=73.275$ ,  $p<0.001$ ,  $\eta^2=0.859$ ) and  $[BLa^-]$  ( $F(3,36)=71.483$ ,  $p<0.001$ ,  $\eta^2=0.867$ ).

The young cyclists demonstrated significant main effects of time for blood pH ( $F(2,24)=20.506$ ,  $p<0.001$ ,  $\eta^2=0.631$ ),  $[HCO_3^-]$  ( $F(2,12)=5.381$ ,  $p=0.015$ ,  $\eta^2=0.473$ ) and  $[BLa^-]$  ( $F(2,12)=10.313$ ,  $p=0.002$ ,  $\eta^2=0.632$ ) across the 30TT. In comparison, the middle-aged cyclists showed significant main effects of time for all the hematological parameters [blood pH ( $F(2,12)=31.689$ ,  $p<0.001$ ,  $\eta^2=0.864$ ), blood  $pO_2$  ( $F(2,12)=21.144$ ,  $p<0.001$ ,  $\eta^2=0.778$ ),  $[HCO_3^-]$  ( $F(2,12)=90.133$ ,  $p<0.001$ ,  $\eta^2=0.938$ ) and  $[BLa^-]$  ( $F(2,10)=31.689$ ,  $p<0.001$ ,  $\eta^2=0.864$ )] across the 30TT.

**Table 4.6:** Mean ( $\pm$  SD) hematological measures of the young and middle-aged cyclists across the thirty minute time trial.

	Young			Middle-Aged		
	10 min	20 min	30 min	10 min	20 min	30 min
<b>pH (AU)</b>	7.338 $\pm$ 0.058	7.334 $\pm$ 0.056	7.287 $\pm$ 0.044 <sup>¥</sup> <sup>\$</sup>	7.336 $\pm$ 0.037	7.332 $\pm$ 0.047	7.276 $\pm$ 0.040 <sup>¥</sup> <sup>\$</sup>
<b>pO<sub>2</sub> (mmHg)</b>	37.8 $\pm$ 2.6	36.3 $\pm$ 3.3	35.6 $\pm$ 3.8	35.5 $\pm$ 3.7	32.3 $\pm$ 3.9 <sup>#</sup>	31.5 $\pm$ 4.7 <sup>¥</sup>
<b>[HCO<sub>3</sub><sup>-</sup>] (mmol·L<sup>-1</sup>)</b>	20.4 $\pm$ 2.6	19.4 $\pm$ 3.2	17.1 $\pm$ 2.1 <sup>¥</sup> <sup>\$</sup>	19.3 $\pm$ 2.9	17.3 $\pm$ 3.5 <sup>#</sup>	15.0 $\pm$ 3.5 <sup>¥</sup> <sup>\$</sup>
<b>[BLa<sup>-</sup>] (mmol·L<sup>-1</sup>)</b>	8.7 $\pm$ 4.0	9.5 $\pm$ 4.3	12.6 $\pm$ 1.9 <sup>¥</sup> <sup>\$</sup>	9.5 $\pm$ 3.3	10.5 $\pm$ 3.8	13.2 $\pm$ 3.5 <sup>¥</sup> <sup>\$</sup>

<sup>#</sup> significantly different to the young cyclists (p<0.05); <sup>¥</sup> significantly different to 10 min (p<0.05); <sup>\$</sup> significantly different to 20 min (p<0.05).

## DISCUSSION

The purpose of Study One was to investigate and describe the effect of age on physiological and peripheral muscle characteristics in well-trained young and middle-aged cyclists. Previous research suggests that physiological and performance capacities are reduced with aging, despite either a prolonged sedentary lifestyle or continued physical training (Pollock et al. 1987; Rogers, Hagber, Martin, Ehsani and Holloszy 1990; Deruelle, Noury, Mucci, Bart, Grosbois, Lensel and Fabre 2005). However, the rate of decline in these capacities appears to be much slower in aging populations that continue high-intensity physical training into older age (Going, Williams and Lohman 1995; Galloway and Joki 1996; Hawkins and Wiswell 2003).

In Study One, no significant effect of age in the physiological (VT,  $\dot{V}O_{2\max}$ ) or peripheral muscle histochemical (fibre composition, morphology and capillarisation) and enzymatic (PFK, LDH, CS,  $\beta$ -HAD and 2-OGDH) characteristics were observed. Similarly, the two age groups appeared matched on cycling performance. This was demonstrated by the similarity of the average relative power output ( $W \cdot kg \text{ BM}^{-1}$ ) maintained during the 30TT. This matching of the two age groups on these physical characteristics and performance responses may help to isolate the actual effect of aging on the adaptation of  $\dot{V}O_2$  and mOxy in response to changes in work intensity that will be examined in subsequent studies as part of this thesis. The findings from this study suggest that the main difference between the two groups is age (~30 y) and that they possess similar physiological, histochemical, enzymatical and performance characteristics.

## Physical and Physiological Characteristics

The present results demonstrated that the young and middle-aged cyclists were matched on several physiological, histochemical and biochemical characteristics commonly reported within exercise and aging research. These observed similarities suggest that physical training helps to maintain these physiological characteristics into middle-age.

The two groups of cyclists in the present study possessed similar anthropometric characteristics, despite previous investigations demonstrating that aging is related to significant changes in body composition (Going et al. 1995; Guo, Zeller, Chumlea and Siervogel 1999). The young cyclists were observed to possess a non-significantly lower body mass (Y:  $69.6 \pm 8.0$  kg; MA:  $79.3 \pm 15.7$  kg) and lower  $\Sigma 9$  skinfolds (Y:  $93.6 \pm 19.8$  mm; MA:  $121.7 \pm 30.0$  mm) than the middle-aged cyclists. Both age groups demonstrated substantially higher body mass and  $\Sigma 9$  skinfolds than reported for young elite Australian cyclists (Craig, Walsh, Martin, Woolford, Bourdon, Stanef, Barnes and Savage 2000). Such increases in body mass and percent body fat have previously been reported with aging in both older sedentary (Going et al. 1995; Guo et al. 1999) and physically-trained populations (Pollock et al. 1987; Tanaka et al. 1997; Maharam, Bauman, Kalman, Skolnik and Perle 1999).

Importantly for the present series of investigations, Study One demonstrated that the cyclists were matched on both their VT and  $\text{VO}_{2\text{max}}$  values, despite the significant age difference between the two groups. The VT of the young and middle-aged cyclists was similar in both absolute (Y:  $2.68 \pm 0.41$  L $\cdot\text{min}^{-1}$ ; MA:  $2.81 \pm 0.40$  L $\cdot\text{min}^{-1}$ ) and as a relative percentage of  $\text{VO}_{2\text{max}}$  (Y:  $70.2 \pm 4.0$  % $\text{VO}_{2\text{max}}$ ;

MA:  $71.4 \pm 5.1$  % $\dot{V}O_{2\max}$ ). The  $\dot{V}O_{2\max}$  values of the well-trained cyclists were also similar in both relative and absolute units between the young ( $55.2 \pm 7.0$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ;  $3.82 \pm 0.46$  L $\cdot$ min $^{-1}$ ) and middle-aged ( $50.2 \pm 6.4$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ;  $3.95 \pm 0.60$  L $\cdot$ min $^{-1}$ ) cohorts. These observed  $\dot{V}O_{2\max}$  values are considerably lower than that reported for high-performance or elite endurance cyclists (Craig et al. 2000). However, they are considerably higher than the mean  $\dot{V}O_{2\max}$  values reported for sedentary Australian populations for the corresponding age groups (Y:  $45.5 \pm 10.6$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ; MA:  $37.9 \pm 8.5$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ) (Gore and Edwards 1992).

Given the proposed influence of  $\dot{V}O_{2\max}$  on the metabolic and energetic responses during exercise (Ebfield, Hoffman and Stegemann 1987; Carter et al. 2000a), the matching of the well-trained cyclists on their  $\dot{V}O_{2\max}$  is of particular importance to the present series of studies. The primary purpose of the subsequent series of present investigations is to examine the effect of age on the  $\dot{V}O_2$  and mOxy responses to changes in work intensity. To achieve this, the subsequent studies aim to isolate the effect of aging by matching the young and middle-aged cyclists on physiological factors that may influence the  $\dot{V}O_2$  and mOxy responses, including  $\dot{V}O_{2\max}$  (Ebfield et al. 1987; Carter et al. 2000a) and muscle histochemical characteristics (Barker, Hopkins, Kellogg, Olfert, Brutsaert, Gavin, Entin, Rice and Wagner 1999; Barstow et al. 2000; Pringle et al. 2003b). This approach allowed us to identify any factors other than  $\dot{V}O_{2\max}$  and muscle histochemistry that influenced the  $\dot{V}O_2$  and mOxy responses to changes in exercise intensities. Therefore, any significant effect of age observed in these metabolic responses to changes in work intensity will be suggestive of other factors related to the aging process.

## Muscle Histochemical Characteristics

The muscle histochemical characteristics (muscle fibre composition, muscle fibre CSA and capillarisation) of the well-trained cyclists in the present study were observed to be similar in both age groups. Previous research has reported upon the effect of aging on such muscle histochemical characteristics in much older age groups than the middle-aged cohort examined in the present study (Coggan et al. 1992; Andersen 2003; Chelly, Chamari, Verney and Denis 2006). Moreover, the effect of aging on these muscle histochemical characteristics has been well researched in both sedentary and physically-trained aged populations (Essen-Gustavsson and Borges 1986; Coggan et al. 1990; Houmard et al. 1998). However, despite a large body of research examining this area, the true effect of aging on changes in the muscle histochemical characteristics is still not well understood (Deschenes 2004).

### *Muscle Fibre Composition*

In the present study, no significant effect of age was observed in the muscle fibre composition of the VL between the well-trained cyclists. Furthermore, both age groups displayed muscle fibre compositions similar to those previously reported for endurance-trained athletes (Coggan et al. 1990; Coggan et al. 1992; Proctor et al. 1995; Houmard et al. 1998; Pringle et al. 2002). Both the young and middle-aged cyclists possessed a high percentage of Type I fibres (55-60%) and subsequent lower percentages of both Type IIa (30-35%) and Type IIb (5-10%) muscle fibres. The muscle fibre composition measured in this study is different to that reported for sedentary older populations which often demonstrate a reduced percentage of Type IIa or IIb fibres (Thompson 2002; Andersen 2003). The muscle fibre composition of the young and middle-aged cyclists in the present study are similar to that reported

for young ( $26 \pm 3$  y;  $3.96 \pm 0.36$  L $\cdot$ min $^{-1}$ ; Type I:  $60.3 \pm 9.6\%$ ; Type IIa:  $33.4 \pm 10.3\%$ ; Type IIb:  $6.3 \pm 7.1\%$ ) and masters ( $63 \pm 6$  y;  $3.36 \pm 0.04$  L $\cdot$ min $^{-1}$ ;  $59.6 \pm 13.6\%$ ;  $38.1 \pm 14.7$ ;  $2.3 \pm 1.4\%$ ) endurance-trained runners (Coggan et al. 1990). Therefore, it appears that physical training can maintain muscle fibre composition in aged individuals to younger counterparts, particularly if matched on athletic performance.

Separately, previous research has suggested that the percentage of Type I fibres in VL is not reduced with aging but is either maintained or slightly increased (Frontera et al. 2000; Andersen 2003; Harris 2005). This is proposed to occur due to an age-related atrophy and denervation of Type II fibres, specifically Type IIb fibres within aged sedentary populations. The similar muscle fibre compositions between the two age groups is of importance to this present series of investigations as previous research has demonstrated that muscle fibre composition strongly influences the  $\dot{V}O_2$ -Work relationship across exercise intensities (Barstow et al. 1996; Pedersen, Sorensen, Jensen, Johansen and Levin 2002; Pringle et al. 2002; Russell et al. 2002; Pringle et al. 2003b). These previous investigations have also suggested that muscle fibre composition and CSA significantly influences the nature of the  $\dot{V}O_2$  response across various intensity exercise transitions. In addition, muscle fibre composition has been reported to be related to changes in mOxy during exercise, which may reflect the greater myoglobin content and capillarisation possessed by Type I fibres (Hamaoka et al. 1998). To date, no research has reported a significant relationship between the mOxy responses to changes in work intensity and muscle histochemical characteristics. While muscle histochemical characteristics appear to be significantly related to the nature of the  $\dot{V}O_2$  response to changes in work intensity, to date no research has reported upon this relationship with the mOxy response. Therefore, the influence of the muscle histochemical

characteristics on the amplitude, speed and efficiency of the  $\dot{V}O_2$  and mOxy responses is proposed to be similar between the two age groups.

### *Muscle Fibre Morphology*

Within the present study, no significant effect of age was observed in the muscle fibre CSA for any of the three muscle fibre types examined in the two groups of well-trained cyclists. This finding may further support the suggestion that the two groups were matched on physiological capacity and peripheral muscle characteristics. The muscle fibre CSA of each fibre type measured in this study was comparable to that reported for other physically-trained populations from previous studies (Coggan et al. 1990; 1992; Proctor et al. 1995; Houmard et al. 1998; Pringle et al. 2002).

In agreement with previous researchers (Deschenes 2004), the Type IIa and IIb fibre CSA suggests there was no age-related fibre-specific atrophy in the well-trained middle-aged cyclists in the present study, a finding previously reported in older sedentary subjects. Therefore, it seems that the well-trained middle-aged cyclists in the present study maintained the size of their Type I and II fibres as a result of their continued high-intensity physical training. However, it may be possible that the middle-aged cyclists in the present study ( $44.8 \pm 2.7$  y) were not of sufficient age to exhibit the reported muscle fibre specific atrophy previously reported within the literature (Deschenes 2004). Other investigations have suggested that such fibre-specific atrophy may not occur until after 50 years of age (Deschenes 2004).

Previous investigations have supported the age-related Type II fibre-specific atrophy hypothesis and suggested it is the result of a decreased quantity of high-

intensity activities with aging (Chilibeck et al. 1995; 1997; Deschenes 2004). This hypothesis is based on the premise that Type II fibres are recruited at higher stimulation frequencies than Type I fibres, and if Type II fibre recruitment is not frequently maintained then these fibres may either atrophy or take on more oxidative characteristics (Komi and Tesch 1979; Gamet, Duchene, Garapon-Bar and Goubel 1990; Loscher, Cresswell and Thorstensson 1994; Wakeling 2004). In contrast, Type I fibres are preferentially recruited for tasks of low to moderate intensity or long duration such as postural tone, locomotion and activities of daily living, which help to maintain their population, CSA and innervation characteristics (Chilibeck et al. 1997). Therefore it is likely that the maintenance of muscle fibre composition and morphology observed in the present study is related to the continued physical training into middle-age. The absence of an age effect may reflect that the training of the middle-aged cyclists is of sufficient intensity to continue to recruit Type II fibres, rather than be of moderate-intensity and predominately recruit Type I fibres and suffer the loss of Type II fibres as reported elsewhere. However, without a detail training history and record of training intensities this remains hypothetical.

### *Muscle Capillarisation*

No significant differences were observed in the capillarisation of the VL between the young and middle-aged cyclists in the present investigation. Additionally, the capillary density, C/F and CC/F ratios were comparable to that reported in similarly-trained populations (Coggan et al. 1990; Chilibeck et al. 1997) with the exception of  $DD_{max}$  and  $DD_{mean}$  which were lower than previously reported (Chilibeck et al. 1997). The current results support the maintenance of muscle capillarisation with sustained physical training into middle-age as previously reported by other investigations (Coggan et al. 1990; Proctor et al. 1995).

Previous investigations have suggested that continued high-intensity endurance training counteracts the age-related decline observed in muscle capillarisation with sedentary aging (Coggan et al. 1990; Proctor et al. 1995; Harris 2005). For example, Coggan et al. (1990) reported no significant differences in capillary density between performance and fitness-matched young ( $26 \pm 3$  y,  $n = 8$ ) and masters ( $63 \pm 6$  y,  $n = 8$ ) runners. Within the same study, a separate sub-sample of young highly-competitive runners ( $28 \pm 3$  y,  $n = 8$ ) (significantly higher  $\text{VO}_2\text{max}$  and performance) was observed to have significantly higher capillarisation in the VL than the groups of performance-matched young and masters runners. This suggests that muscle capillarisation reflects aerobic fitness, which may in turn be related to the metabolic adaptations to exercise that are of interest within the subsequent studies. Other investigations have reported a similar maintenance of muscle capillarisation in highly-trained older athletes (Chilibeck et al. 1995; Proctor et al. 1995). Such muscle capillarisation characteristics have been significantly related to the  $\text{O}_2$  delivery and rate of  $\text{VO}_2$  adjustment in response to changes in work intensity (Bell et al. 2001). This suggests that muscle capillarisation reflect aerobic fitness, which may in turn be related to the metabolic adaptations to exercise that are of interest within the subsequent studies.

In the present study, the similarities in muscle fibre composition, fibre size and capillarisation of the VL is of interest given their proposed influence on the  $\text{VO}_2$ -Work relationship (Barstow et al. 1996; Pedersen et al. 2002; Pringle et al. 2002; Russell et al. 2002; Pringle et al. 2003b). The influence of histochemical characteristics on the  $\text{VO}_2$  kinetic responses is most likely due to the varying fibre-specific functional characteristics such as metabolic efficiency, preferable contraction speeds, innervation frequencies, oxidative capacity and mitochondrial density (Bottinelli and

Reggiani 2000; He et al. 2000). Moreover, while muscle histochemical characteristics have been significantly related to changes in mOxy during sustained exercise (Hamaoka et al. 1998), their relationship with the mOxy kinetic responses has yet to be examined. Therefore, it appears that the specific metabolic characteristics of the various muscle fibre types will influence the adaptation of the  $\dot{V}O_2$  or mOxy responses to and during exercise. Such an effect of muscle fibre composition may help to suggest that increases in  $O_2$  are responsible for controlling the speed of the metabolic adaptation during exercise bouts. As such, the muscle enzymatic characteristics of the working muscle may provide information on  $O_2$  utilisation mechanisms which help control such metabolic responses.

### **Muscle Enzymatic Characteristics**

While it is acknowledged that muscle histochemical characteristics may influence the  $\dot{V}O_2$  and mOxy responses to exercise, the reporting of specific maximal activities of muscle enzymes may help identify muscle oxidative capacities and any  $O_2$  utilisation limitations within the working muscle (Bell et al. 2001; Puente-Maestu, et al. 2003). To date, the literature describing the effect of aging on both glycolytic and oxidative enzyme activities is equivocal (Kiessling, Pilstrom, Bylund, Saltin and Piehl 1974; Taylor, Noble, Cunningham, Paterson and Rechnitzer 1992; Houmard, Weidner et al. 1998; Dubouchaud, Butterfield, Wolfel, Bergman and Brooks 2000; Puente-Maestu et al. 2003). The combination of aging and physical training has been proposed to attenuate any sedentary age-related changes in muscle enzyme activities (Orlander and Aniansson 1980; Proctor et al. 1995). As such, the present results support this suggestion that physical training attenuates enzymatic adaptations with skeletal muscle reported with sedentary aging.

### *Glycolytic Enzymes*

The present data demonstrated no significant effect of age on the maximal specific activities of either LDH or PFK within the VL of the well-trained cyclists. This suggests no age-related decrease in the activities of glycolytic enzymes of the well-trained middle-aged cyclists. The maximal activities of these anaerobic enzymes have previously been reported to decline with age (20 - 60 y) in both sedentary subjects (Simoneau and Bouchard 1999; Pastoris, Boschi, Verri, Baiardi, Felzani, Vecchiet, Dossena and Catapano 2000) and competitive endurance-trained runners (Coggan et al. 1990;1992). It has been proposed that the changes in the maximal activities of these glycolytic enzymes are related to the age-related atrophy or denervation of the Type II fibres (Simoneau and Bouchard 1999; Pastoris et al. 2000). The maximal activities of these glycolytic enzymes has also previously been shown to be higher in Type II compared to Type I muscle fibres (Tikkanen, Naveri and Harkonen 1995). Therefore in the present study, the similar maximal glycolytic enzyme activities are most likely related to the matched muscle fibre composition of the VL in the two age groups. However, the influence of oxidative enzyme activities is of more practical importance to the present series of investigations given their influence on O<sub>2</sub> utilisation across changes in work intensity within the muscle.

### *Oxidative Enzymes*

In the present study, the activities of CS,  $\beta$ -HAD and 2-OGDH in the VL demonstrated no effect of age between the young and middle-aged cyclists. Significant age-related declines in the maximal activities of such oxidative enzymes have previously been reported to occur in sedentary subjects (Coggan et al. 1992; McCully, Fielding, Evans, Leigh Jr. and Posner 1993; Houmard et al. 1998; Pastoris et al. 2000). However, in older trained subjects, Coggan and colleagues (1990)

reported that competitive masters runners ( $63 \pm 6$  y) showed significantly higher maximal activities of both SDH and  $\beta$ -HAD than younger ( $26 \pm 3$  y) performance and fitness-matched runners. In contrast, the maximal activity of CS was not significantly different between the two age-groups. Furthermore, Coggan et al. (1990) also reported that a cohort of young competitive runners ( $26 \pm 3$  y) possessed significantly higher activities of these oxidative enzymes than both of the performance-matched age-groups. Therefore, these findings strongly suggest that the maximal activities of oxidative enzymes can be sustained or improved with concurrent aging and concurrent physical training. However, the data of Coggan et al. (1990) may also suggest that the maximal activities of oxidative enzymes can't be maintained to levels observed in high-performance younger athletes by middle-aged athletes. Given the decline in physical function with aging, it appears difficult to compare any characteristics between highly-trained young and older athletes of the same performance level. Regardless, the data of the present study suggests that the maximal activity of these oxidative enzymes can be maintained into middle-age in well-trained athletes.

However, the role of such maximal enzyme activities and muscle fibre composition on the metabolic adaptations to work intensity transitions is subject to a number of methodological limitations. Firstly, the absence of any significant effect of age in the reported muscle histochemical or biochemical characteristics may be due to the suggestion that the middle-aged cyclists were not old enough to exhibit any of the previously reported age-related changes for these characteristics (Coggan et al. 1992; Proctor et al. 1995; Kirkendall and Garrett Jr 1996; Houmard et al. 1998). Previous investigations have reported that any age-related changes in the oxidative metabolism do not occur until around 50 years of age, which is older than the

middle-aged cyclists ( $44.8 \pm 2.7$  y) recruited within the present study (Conley, Esselman, Jubrias, Cress, Inglin, Mogadam and Schoene 2000; Conley et al. 2000; Russ and Kent-Braun 2004). Therefore, this may be of practical importance if no significant effect of age is observed on the metabolic transients to exercise within the subsequent studies in this thesis.

In summary, the muscle histochemical and biochemical characteristics of the VL were similar in the young and middle-aged cyclists within the present study. As reported within the previous literature, both the muscle histochemical and enzymatic characteristics of the VL are related to the  $\dot{V}O_2$  and mOxy responses to changes in work intensity (Barstow et al. 1996; Pringle et al. 2003b). The absence of any significant effect of age in these muscle characteristics supports previous suggestions that physical training into older age can maintain such characteristics until the age of  $\sim 50$  years (Kießling et al. 1974; Taylor et al. 1992; Houmard et al. 1998). While the two groups appear matched on physiological and muscle histochemical and enzymatic characteristics, it was also important for the present series of investigations that the two age groups were matched on their performance across a 30TT.

### 30TT Performance Responses

No significant effect of age was observed on the 30TT performance in the young and middle-aged cyclists. The relative measures of  $\dot{V}O_2$  ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) or RPO ( $\text{W}\cdot\text{kg}^{-1}$  BM) across the 30TT were similar between groups. However, the absolute 30TT PO (Y:  $213.3 \pm 41.1$  W; MA:  $257.0 \pm 31.4$  W) and  $\dot{V}O_2$  (Y:  $2.80 \pm 0.44$   $\text{L}\cdot\text{min}^{-1}$ ; MA:  $3.36 \pm 0.55$   $\text{L}\cdot\text{min}^{-1}$ ) were significantly higher in the middle-aged cyclists, which may be related to their higher body mass. Since cycling performance

is related to an individual's power to weight ratio, the relative measures to body mass may provide a more sensitive and valid measure of cycling performance (Paton and Hopkins 2001). Therefore, the similar RPO (Y:  $3.04 \pm 0.59 \text{ W}\cdot\text{kg BM}^{-1}$ ; MA:  $3.28 \pm 0.49 \text{ W}\cdot\text{kg BM}^{-1}$ ) and  $\dot{V}\text{O}_2$  (Y:  $40.23 \pm 6.26 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ; MA:  $42.40 \pm 6.95 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) observed throughout the 30TT between the two age-groups of well-trained cyclists shows that they were matched on actual cycling performance.

The observed physiological intensities sustained in each age-group varied across the 30TT. Both groups of cyclists demonstrated similar relative exercise intensities as measured by  $\%\text{HR}_{\text{max}}$ , despite the younger subjects maintaining a higher absolute HR across the 30TT. Significant effects of time in both the HR and  $\%\text{HR}_{\text{max}}$  responses were also observed in both age groups which suggests a role of cardiovascular drift across the 30TT. Such a finding is widely observed across prolonged high-intensity constant-load exercise bouts (Coyle and Gonzalez-Alonso 2001). In terms of  $\%\text{VO}_{2\text{max}}$ , the middle-aged cyclists demonstrated a significantly higher relative intensity than the young cyclists across the 30TT. Despite this observation, the two age-groups were observed to sustain similar relative physiological intensities across the 30TT.

The changes in blood pH,  $p\text{O}_2$ ,  $[\text{HCO}_3^-]$  and  $[\text{BLa}^-]$  across the 30TT demonstrated no significant effect of age in the well-trained cyclists in the present study. This finding suggests that both age groups sustained similar physiological exercise intensities across the 30TT. All hematological parameters demonstrated significant effects of time which may be representative of the high-intensity maintained across the 30TT. The gradual changes in blood pH,  $[\text{HCO}_3^-]$  and  $[\text{BLa}^-]$  suggest an increased anaerobic metabolism across the 30TT. It has previously been

suggested that during such exercise bouts, cyclists work at intensities around or above their anaerobic thresholds, which require substantial and prolonged anaerobic energy metabolism (Bentley, McNaughton, Thompson, Vleck and Batterham 2001). The work intensities sustained across the 30TT by the young and middle-aged cyclists were slightly lower than those previously reported for similarly-trained populations for similar exercise bouts (Bentley et al. 2001; Bentley, Vleck and Millet 2005; Hajoglou, Foster, De Koning, Lucia, Kernozek and Porcari 2005).

The present results demonstrated no significant effect of age in 30TT cycling performance. The young subjects in this study were competitive cyclists and triathletes, but were not elite or high-performance cyclists. Importantly, this allowed the matching of the two cycling cohorts on important physiological and performance characteristics such as  $\dot{V}O_2\text{max}$  and 30TT performance. The recruitment of a younger higher-performance cohort of cyclists would not have assisted in the purpose of isolating the sole effect of aging on the  $\dot{V}O_2$  and mOxy responses to changes in work intensity.

## **SUMMARY**

Within the present investigation, no significant effect of age was observed in important physiological, muscle histochemical and enzymatic characteristics, and cycling performance responses in the well-trained young and middle-aged cyclists. The findings of Study One suggest that the two age cohorts of cyclists possessed similar muscle fibre composition, CSA and capillarisation of the VL. The maximal activities of a number of glycolytic and oxidative enzymes from the VL were also found to be comparable across the two age groups. The two groups of well-trained cyclists were also found to be matched on the performance responses during the

30TT. Therefore, these results support the suggestion that discussed age-related declines in physiological and performance measures are attenuated with physical training (Kiessling et al. 1974; Proctor et al. 1995; Short et al. 2003).

Importantly, previous research has identified that physiological characteristics such as  $\dot{V}O_2\text{max}$  (Ebfield et al. 1987; Carter et al. 2000a) and peripheral muscle histochemical and enzymatic characteristics (Barstow et al. 1996; 2000; Pedersen et al. 2002; Pringle et al. 2002; 2003b) significantly influence the  $\dot{V}O_2$  kinetic responses across changes in work intensities. While the mOxy responses are also of particular interest within the present series of investigations, limited data is available on the mOxy relationship with muscle histochemical or enzymatic characteristics.

The matching of the young and middle-aged cyclists in the present study will minimise the influence of the reported physiological characteristics on the  $\dot{V}O_2$  and mOxy responses detailed within studies two to four of the present investigation. Therefore, any observed significant effect of age in the adaptation of the  $\dot{V}O_2$  and mOxy responses may reflect the similar physical characteristics of the two age groups.

## CHAPTER 5

### STUDY 2

#### **On-transient $\dot{V}O_2$ and mOxy responses to moderate-, heavy- and severe-intensity exercise in well-trained young and middle-aged cyclists**

##### OVERVIEW

The purpose of Study Two was to examine the effect of age on the on-transient  $\dot{V}O_2$  and mOxy responses to moderate, heavy and severe-intensity SWT in well-trained cyclists. The results of Study Two demonstrated no significant effect of age on the amplitude ( $A_p$ ) or speed ( $TD_p$ ;  $\tau_p$ ) measures of the on-transient  $\dot{V}O_2$  or mOxy responses in the well-trained cyclists. However, the current data revealed a significant effect of exercise intensity on the  $\dot{V}O_2$  and mOxy  $A_p$ . In contrast, no significant effects of exercise intensity were observed for the on-transient  $\dot{V}O_2$  or mOxy  $TD_p$  or  $\tau_p$  in either age group. The stable  $\dot{V}O_2$  and mOxy  $\tau_p$  across SWT intensities suggests that adaptation of  $O_2$  utilisation may limit the metabolic responses to increases in work intensity.

A small number of significant relationships were observed in the speed of the on-transient  $\dot{V}O_2$  response and changes in a number of hematological parameters representative of anaerobic metabolism (blood pH and  $[BLa^-]$ ). Significant relationships were also observed between the speed of the on-transient  $\dot{V}O_2$  response, muscle fibre composition and maximal 2-OGDH activity in both age groups.

The results of Study Two suggest that the amplitude and speed of the on-transient  $\dot{V}O_2$  and mOxy responses are maintained with physical training into middle-age. This observation contrasts previous research investigations suggesting that the on-transient  $\dot{V}O_2$  response is slowed with sedentary aging (Babcock et al. 1994a; 1994b; DeLorey et al. 2004a; 2005). However, the present data supports the absence of a significant effect of age on the mOxy  $\tau_p$  in trained older individuals (DeLorey et al. 2004a). The absence of a significant effect of age in the present study is most likely due to the matching of the two cycling cohorts on their physiological attributes, specifically  $\dot{V}O_{2\max}$  and muscle histochemical and enzymatic characteristics. The present study is the first to suggest that the on-transient  $\dot{V}O_2$  and mOxy responses to exercise are not subject to a significant effect of age in well-trained athletes that are matched on physiological and performance characteristics at least up to the age of 50 years. After this, the effect of prolonged physical training on these characteristics remains unknown, and these parameters may decline.

## **RESULTS**

### **On-Transient $\dot{V}O_2$ Responses**

The time and amplitude values of the on-transient  $\dot{V}O_2$  response to each of the moderate, heavy and severe-intensity SWT are shown in Table 5.1 over the page.

**Table 5.1:** Mean ( $\pm$  SD) time and amplitude values of the on-transient  $\dot{V}O_2$  responses of the young and middle-aged cyclists across the three square wave transition intensities.

	Young			Middle-aged		
	Moderate	Heavy	Severe <sup>€</sup>	Moderate	Heavy	Severe <sup>€</sup>
<b>SWT Power (W)</b>	178.8 $\pm$ 29.5	281.3 $\pm$ 36.1	316.7 $\pm$ 37.1	197.8 $\pm$ 22.7	316.7 $\pm$ 32.5	358.4 $\pm$ 35.6
<b>Baseline <math>\dot{V}O_2</math> (mL<math>\cdot</math>min<sup>-1</sup>)</b>	1118 $\pm$ 141	1180 $\pm$ 136	1423 $\pm$ 258 <sup>\$</sup> <sup>¥</sup>	1293 $\pm$ 134	1386 $\pm$ 273	1560 $\pm$ 304 <sup>\$</sup>
<b>A<sub>p</sub> (mL<math>\cdot</math>min<sup>-1</sup>)</b>	13202 $\pm$ 239	1953 $\pm$ 319 <sup>#</sup>	1733 $\pm$ 285 <sup>\$</sup>	1336 $\pm$ 151	1829 $\pm$ 376 <sup>#</sup>	2054 $\pm$ 480 <sup>\$</sup>
<b>TD<sub>p</sub> (s)</b>	3.2 $\pm$ 2.1	3.9 $\pm$ 2.4	4.2 $\pm$ 1.5	5.9 $\pm$ 2.0	3.1 $\pm$ 1.4 <sup>#</sup>	3.1 $\pm$ 1.0 <sup>\$</sup>
<b><math>\tau_p</math> (s)</b>	23.2 $\pm$ 6.9	23.0 $\pm$ 4.6	26.0 $\pm$ 5.8	25.5 $\pm$ 7.0	25.5 $\pm$ 5.0	30.4 $\pm$ 3.2
<b>G<sub>p</sub> (mL<math>\cdot</math>min<sup>-1</sup><math>\cdot</math>W<sup>-1</sup>)</b>	7.4 $\pm$ 0.5	6.9 $\pm$ 1.4 <sup>#</sup>	5.5 $\pm$ 0.8 <sup>\$</sup> <sup>¥</sup>	7.5 $\pm$ 0.5	6.2 $\pm$ 1.1 <sup>#</sup>	6.1 $\pm$ 1.3 <sup>\$</sup>
<b>G<sub>o</sub> (mL<math>\cdot</math>min<sup>-1</sup><math>\cdot</math>W<sup>-1</sup>)</b>	7.4 $\pm$ 0.5	8.8 $\pm$ 1.2	7.7 $\pm$ 1.3	7.5 $\pm$ 0.5	8.3 $\pm$ 1.3	6.3 $\pm$ 1.5
<b>EE<math>\dot{V}O_2</math> (mL<math>\cdot</math>min<sup>-1</sup>)</b>	2469 $\pm$ 363	3487 $\pm$ 510 <sup>#</sup>	3405 $\pm$ 418 <sup>\$</sup>	2622 $\pm$ 239	3648 $\pm$ 345 <sup>#</sup>	3636 $\pm$ 363 <sup>\$</sup>
<b>wMRT (s)</b>	39.2 $\pm$ 5.9	54.4 $\pm$ 11.8 <sup>#</sup>	38.7 $\pm$ 11.7 <sup>¥</sup>	36.3 $\pm$ 3.5	51.2 $\pm$ 14.5 <sup>#</sup>	29.2 $\pm$ 7.5 <sup>¥</sup>

A<sub>p</sub>= Primary Amplitude  
G<sub>o</sub>= Overall Response Gain

TD<sub>p</sub>= Primary Component Time Delay  
EE $\dot{V}O_2$  = End-Exercise  $\dot{V}O_2$

$\tau_p$ = Primary Time Constant  
wMRT= Weighted Mean Response Time

G<sub>p</sub>= Primary Component Gain

<sup>#</sup> significant difference between moderate and heavy-intensities (p<0.05); <sup>\$</sup> significant difference between moderate and severe-intensities (p<0.05); <sup>¥</sup> significant difference between heavy and severe-intensities (p<0.05) <sup>€</sup> no subject completed the six minute severe-intensity SWT.

Firstly, no significant main effect of age or age x intensity interaction was observed in the baseline  $\dot{V}O_2$  prior to SWT commencement. However, the baseline  $\dot{V}O_2$  prior to each SWT was significantly elevated prior to the severe-intensity SWT in comparison to both the moderate- ( $F(2,12)=11.265$ ,  $p=0.007$ ,  $\eta^2=0.652$ ) and heavy-intensity ( $F(2,12)=11.265$ ,  $p=0.025$ ,  $\eta^2=0.652$ ) SWT in the young cyclists. This elevation of baseline  $\dot{V}O_2$  was only observed between the moderate and severe-intensity SWT ( $F(2,12)=2.326$ ,  $p=0.047$ ,  $\eta^2=0.279$ ) in the middle-aged cyclists.

There was no significant main effect of age or age x intensity interaction observed in the  $\dot{V}O_2 A_p$  across the three SWT intensities. In the young cyclists, the  $\dot{V}O_2 A_p$  to the moderate-intensity SWT was significantly lower than that observed for either the heavy- ( $F(2,12)=20.62$ ,  $p<0.001$ ,  $\eta^2=0.775$ ) or the severe-intensity ( $F(2,12)=20.62$ ,  $p=0.030$ ,  $\eta^2=0.775$ ) SWT. In the middle-aged cyclists, the moderate-intensity  $\dot{V}O_2 A_p$  was also significantly lower than either the heavy- ( $F(2,12)=6.986$ ,  $p=0.005$ ,  $\eta^2=0.538$ ) or severe-intensity ( $F(2,12)=6.986$ ,  $p=0.008$ ,  $\eta^2=0.538$ ) SWT.

No significant main effects of age or intensity were observed in the  $\dot{V}O_2$   $TD_p$  or  $\tau_p$  across the three exercise intensities. Significant main age x intensity interactions were observed for both the on-transient  $\dot{V}O_2$   $TD_p$  ( $F(2,24)=5.392$ ,  $p=0.012$ ,  $\eta^2=0.310$ ) and  $\tau_p$  ( $F(2,24)=3.569$ ,  $p=0.044$ ,  $\eta^2=0.229$ ). In the middle-aged cyclists, the moderate-intensity  $\dot{V}O_2$   $TD_p$  was significantly shorter than that of the heavy ( $F(2,12)=11.704$ ,  $p=0.002$ ,  $\eta^2=0.661$ ) and severe-intensity ( $F(2,12)=11.704$ ,  $p=0.011$ ,  $\eta^2=0.661$ ) SWT. No significant effect of intensity was observed in the  $\dot{V}O_2$   $\tau_p$  in either age group within the present study.

No significant effect of age or age x intensity interaction was observed in the  $\dot{V}O_2$  wMRT. However, a significant effect of intensity was observed ( $F(2,12)=15.311$ ,  $p<0.001$ ,  $\eta^2=0.561$ ). The heavy-intensity  $\dot{V}O_2$  wMRT was significantly longer than the moderate-intensity wMRT in both the young ( $F(2,12)=6.102$ ,  $p=0.011$ ,  $\eta^2=0.504$ ) and middle-aged ( $F(2,12)=9.775$ ,  $p=0.032$ ,  $\eta^2=0.620$ ) cyclists. In addition, the heavy-intensity wMRT was also significantly longer than the severe-intensity SWT in the young ( $F(2,12)=6.102$ ,  $p=0.045$ ,  $\eta^2=0.504$ ) and middle-aged ( $F(2,12)=9.775$ ,  $p=0.009$ ,  $\eta^2=0.620$ ) cyclists.

No significant main effect of age or age x intensity interaction was observed in the  $\dot{V}O_2$   $G_p$  or  $G_o$  across the three SWT intensities. The  $\dot{V}O_2$   $G_p$  was observed to significantly decrease between the moderate and heavy-intensity SWT in both the young ( $F(2,12)=14.999$ ,  $p=0.015$ ,  $\eta^2=0.714$ ) and middle-aged ( $F(2,12)=4.579$ ,  $p=0.009$ ,  $\eta^2=0.393$ ) cyclists. The  $\dot{V}O_2$   $G_p$  also significantly decreased between the moderate and severe-intensity SWT in the young ( $F(2,12)=14.999$ ,  $p=0.001$ ,  $\eta^2=0.714$ ) and middle-aged ( $F(2,12)=3.887$ ,  $p=0.050$ ,  $\eta^2=0.393$ ) cyclists. Lastly, the  $\dot{V}O_2$   $G_p$  was observed to significantly decrease between the heavy and severe-intensity SWT in the young cyclists ( $F(2,12)=14.999$ ,  $p=0.023$ ,  $\eta^2=0.714$ ), but not in the middle-aged cohort. A significant main effect of intensity was observed ( $F(2,24)=6.769$ ,  $p=0.005$ ,  $\eta^2=0.381$ ) in the  $G_o$ , with *post-hoc* analysis revealing a significant difference between the moderate and heavy-intensity ( $F(2,12)=3.236$ ,  $p=0.016$ ,  $\eta^2=0.393$ ) SWT in the young cyclists, and between the heavy and severe-intensity ( $F(2,12)=4.97$ ,  $p=0.027$ ,  $\eta^2=0.453$ ) SWT in the middle-aged cyclists.

## On-Transient mOxy Responses

The on-transient mOxy time and amplitude parameters across the three SWT intensities are shown in Table 5.2.

Similar to the  $\dot{V}O_2$  results, no significant main effect of age was observed in the mOxy baseline prior to SWT commencement. However, the mOxy baseline demonstrated a significant age x intensity interaction ( $F(2,22)=3.684$ ,  $p=0.042$ ,  $\eta^2=0.251$ ) and main effect of intensity ( $F(2,22)=10.854$ ,  $p=0.001$ ,  $\eta^2=0.497$ ). The mOxy baseline prior to the severe-intensity SWT was significantly lower than that prior to both the moderate ( $F(2,11)=6.360$ ,  $p=0.015$ ,  $\eta^2=0.560$ ) and heavy-intensity ( $F(2,11)=6.360$ ,  $p=0.006$ ,  $\eta^2=0.560$ ) SWT in the young cyclists. A similar difference was observed with the mOxy baseline between the severe and heavy-intensity SWT ( $F(2,11)=7.361$ ,  $p=0.003$ ,  $\eta^2=0.551$ ) in the middle-aged cyclists.

There was no significant main effect of age or age x intensity interaction observed in the mOxy  $A_p$  in the cyclists. The mOxy  $A_p$  demonstrated a significant main effect of intensity ( $F(2,22)=9.662$ ,  $p=0.001$ ,  $\eta^2=0.468$ ), which was observed in the middle-aged ( $F(2,11)=10.220$ ,  $p=0.003$ ,  $\eta^2=0.63$ ) but not the young cyclists. A significant increase in the mOxy  $A_p$  was observed in the middle-aged cyclists between moderate and heavy-intensity ( $F(2,11)=10.220$ ,  $p=0.028$ ,  $\eta^2=0.630$ ) SWT. No main effects of age or age x intensity interactions were observed for the mOxy  $TD_p$  or  $\tau_p$  in the present data. A significant main effect of intensity was observed for the mOxy  $TD_p$  ( $F(2,22)=11.231$ ,  $p<0.001$ ,  $\eta^2=0.505$ ). This significant effect of intensity was also observed in both the young ( $F(2,11)=4.568$ ,  $p=0.039$ ,  $\eta^2=0.477$ ) and middle-aged

**Table 5.2:** Mean ( $\pm$  SD) time and amplitude values of the on-transient mOxy responses of the young and middle-aged cyclists across the three square wave transition intensities.

	Young			Middle-Aged		
	Moderate	Heavy	Severe <sup>€</sup>	Moderate	Heavy	Severe <sup>€</sup>
<b>SWT Power (W)</b>	178.0 $\pm$ 29.5	281.0 $\pm$ 36.1	317.0 $\pm$ 37.1	198.0 $\pm$ 22.7	317.0 $\pm$ 32.5	358.0 $\pm$ 35.6
<b>Baseline mOxy (%)</b>	82.0 $\pm$ 8.9	78.4 $\pm$ 22.2	58.9 $\pm$ 14.1 <sup>\$¥</sup>	81.8 $\pm$ 10.3	89.3 $\pm$ 3.2	78.1 $\pm$ 6.0 <sup>¥</sup>
<b>A<sub>p</sub> (%)</b>	24.5 $\pm$ 8.5	35.2 $\pm$ 12.5	39.3 $\pm$ 14.3	32.3 $\pm$ 10.0	45.3 $\pm$ 12.4 <sup>#</sup>	48.9 $\pm$ 13.2
<b>TD<sub>p</sub> (s)</b>	5.9 $\pm$ 2.1	5.2 $\pm$ 1.9	2.9 $\pm$ 1.3 <sup>\$¥</sup>	5.2 $\pm$ 1.8	5.0 $\pm$ 1.7	2.4 $\pm$ 1.3 <sup>\$¥</sup>
<b><math>\tau_p</math> (s)</b>	12.5 $\pm$ 6.1	16.0 $\pm$ 10.6	12.1 $\pm$ 6.3	15.4 $\pm$ 5.5	13.2 $\pm$ 14.8	10.3 $\pm$ 7.1
<b>G<sub>p</sub> (%·W<sup>-1</sup>)</b>	0.15 $\pm$ 0.06	0.13 $\pm$ 0.06	0.13 $\pm$ 0.07	0.19 $\pm$ 0.65	0.16 $\pm$ 0.05	0.12 $\pm$ 0.05 <sup>\$¥</sup>
<b>G<sub>o</sub> (%·W<sup>-1</sup>)</b>	0.15 $\pm$ 0.06	0.18 $\pm$ 0.10	0.17 $\pm$ 0.07	0.26 $\pm$ 0.09	0.19 $\pm$ 0.05	0.16 $\pm$ 0.05 <sup>\$¥</sup>
<b>EEmOxy (%)</b>	59.7 $\pm$ 14.1	31.0 $\pm$ 19.9 <sup>#</sup>	7.8 $\pm$ 8.4 <sup>\$¥</sup>	66.5 $\pm$ 10.5	35.6 $\pm$ 12.2 <sup>#</sup>	32.3 $\pm$ 15.0 <sup>\$¥</sup>
<b>wMRT (s)</b>	33.3 $\pm$ 9.1	47.6 $\pm$ 20.2	37.6 $\pm$ 18.2	45.7 $\pm$ 20.7	64.4 $\pm$ 37.2	34.5 $\pm$ 12.3

A<sub>p</sub>= Primary Amplitude

TD<sub>p</sub>= Primary Time Delay

$\tau_p$  = Primary Time Constant

G<sub>p</sub>= Primary Gain

G<sub>o</sub>= Overall Gain

EEmOxy= End-Exercise mOxy

wMRT= Weighted Mean Response Time

<sup>#</sup> significant difference between moderate and heavy-intensities (p<0.05); <sup>\$</sup> significant difference between moderate and severe-intensities (p<0.05); <sup>¥</sup> significant difference between heavy and severe-intensities (p<0.05); <sup>€</sup> no subject completed the six minute severe-intensity SWT.

( $F(2,11)=6.990$ ,  $p=0.01$ ,  $\eta^2=0.538$ ) cyclists. The severe-intensity mOxy  $TD_p$  was significantly shorter than both the moderate- ( $F(2,11)=6.99$ ,  $p=0.028$ ,  $\eta^2=0.538$ ) and heavy-intensity ( $F(2,11)=6.99$ ,  $p=0.007$ ,  $\eta^2=0.538$ ) SWT in the middle-aged cyclists. The heavy-intensity mOxy  $TD_p$  was significantly longer than the  $VO_2$   $TD_p$  in the middle-aged cyclists ( $t= -2.636$ ,  $p<0.05$ ), and in the moderate-intensity SWT ( $t= -2.249$ ,  $p<0.05$ ) in the young cyclists.

No significant main effect of age, intensity or age x intensity interaction was observed for the mOxy  $\tau_p$ . The  $VO_2$   $\tau_p$  was significantly slower in both the young ( $t = 4.054$ ,  $p<0.01$ ) and middle-aged ( $t = 6.225$ ,  $p<0.001$ ) compared to the mOxy  $\tau_p$  in the moderate-intensity SWT. The mOxy wMRT demonstrated no significant effect of age or age x intensity interaction despite being significantly influenced by exercise intensity ( $F(2,22)=3.969$ ,  $p=0.034$ ,  $\eta^2=0.265$ ). However, this significant effect of intensity on the mOxy wMRT was not observed in either age group. The severe-intensity  $VO_2$  wMRT of the middle-aged cyclists was observed to be significantly ( $t= -3.784$ ,  $p<0.05$ ) slower than the mOxy response.

Finally, no significant effect of age or age x intensity interaction was present in the mOxy  $G_p$  or  $G_o$  across the three SWT intensities. However, the mOxy  $G_p$  was significantly influenced by intensity in the middle-aged cyclists ( $F(2,11)=9.646$ ,  $p<0.001$ ,  $\eta^2=0.745$ ). In this group, the moderate-intensity mOxy  $G_p$  was significantly higher than that of the heavy ( $F(2,11)=17.505$ ,  $p=0.043$ ,  $\eta^2=0.745$ ) or severe-intensity ( $F(2,11)=17.505$ ,  $p=0.002$ ,  $\eta^2=0.745$ ) SWT. The heavy-intensity mOxy  $G_p$  was also significantly higher than that of the severe-intensity ( $F(2,11)=17.505$ ,  $p=0.004$ ,  $\eta^2=0.745$ ) SWT in the middle-aged cyclists. *Post-hoc* analysis revealed that the mOxy  $G_o$  of the middle-aged cyclists was

significantly higher in the moderate-intensity SWT than either the heavy ( $F(2,11)=7.91$ ,  $p=0.004$ ,  $\eta^2=0.613$ ) and severe-intensity ( $F(2,11)=7.91$ ,  $p=0.028$ ,  $\eta^2=0.613$ ) SWT.

## **Hematological Responses**

### *Blood pH*

The mean ( $\pm$  SD) changes in blood pH during the three SWT intensities are shown in Table 5.3. No significant main effects of age or age  $\times$  time interactions were observed in blood pH during the moderate, heavy or severe-intensity SWT. However, a significant main effect of time was observed for blood pH across both the heavy ( $F(2,24)=94.658$ ,  $p<0.001$ ,  $\eta^2=0.887$ ) and severe-intensity ( $F(2,24)=168.672$ ,  $p<0.001$ ,  $\eta^2=0.934$ ) SWT in both age groups.

Both the young ( $F(2,12)=144.36$ ,  $p<0.001$ ,  $\eta^2=0.960$ ) and middle-aged ( $F(2,12)=27.29$ ,  $p<0.001$ ,  $\eta^2=0.820$ ) cyclists demonstrated significant effects of time in blood pH across the heavy-intensity SWT. The young cyclists demonstrated a significant decrease in blood pH between 0 and 3 min ( $F(2,12)=144.36$ ,  $p<0.001$ ,  $\eta^2=0.960$ ) and then between 3 and 6 min ( $F(2,12)=144.36$ ,  $p<0.001$ ,  $\eta^2=0.960$ ) during the heavy-intensity SWT. Similar results were observed in the middle-aged cyclists across the heavy-intensity SWT between 0 and 3 min ( $F(2,12)=27.29$ ,  $p=0.002$ ,  $\eta^2=0.820$ ) and 3 to 6 min ( $F(2,12)=27.29$ ,  $p=0.003$ ,  $\eta^2=0.820$ ).

**Table 5.3:** Mean ( $\pm$  SD) blood pH (AU) values during the moderate-, heavy- and severe-intensity square wave transitions in the young and middle-aged cyclists.

	Young			Middle-Aged		
	0 min	3 min	6 min	0 min	3 min	6 min
<b>Moderate</b>	7.404 $\pm$ 0.022	7.389 $\pm$ 0.024	7.387 $\pm$ 0.034	7.410 $\pm$ 0.026	7.403 $\pm$ 0.020	7.412 $\pm$ 0.018
<b>Heavy</b>	7.408 $\pm$ 0.015	7.320 $\pm$ 0.018 <sup>#</sup>	7.218 $\pm$ 0.032 <sup>§ ¥</sup>	7.418 $\pm$ 0.031	7.334 $\pm$ 0.021 <sup>#</sup>	7.237 $\pm$ 0.068 <sup>§ ¥</sup>
<b>Severe</b>	7.383 $\pm$ 0.026	7.199 $\pm$ 0.043 <sup>#</sup>		7.376 $\pm$ 0.022	7.229 $\pm$ 0.044 <sup>#</sup>	

<sup>#</sup> significant difference between 0 and 3 min; <sup>§</sup> significant difference between 3 and 6 min; <sup>¥</sup> significant difference between 0 and 6 min; no subject completed the severe-intensity SWT.

Significant main effects of time were observed in blood pH across the severe-intensity SWT in both the young ( $F(1,12)=76.856$ ,  $p<0.001$ ,  $\eta^2=0.928$ ) and middle-aged ( $F(1,12)=103.773$ ,  $p<0.001$ ,  $\eta^2=0.945$ ) cyclists. *Post-hoc* analysis revealed a significant decrease in blood pH between 0 and SWT exhaustion in both the young ( $F(2,12)=144.36$ ,  $p<0.001$ ,  $\eta^2=0.960$ ) and middle-aged ( $F(2,12)=103.773$ ,  $p<0.001$ ,  $\eta^2=0.945$ ) cyclists during the severe-intensity SWT.

#### Blood $pO_2$

The mean ( $\pm$  SD) changes in blood  $pO_2$  during the three intensity SWT for both age groups are shown below in Table 5.4. No significant main effect of age or age  $\times$  intensity interactions was observed for blood  $pO_2$  across the three SWT intensities. A significant main effect of time ( $F(2,24)=11.867$ ,  $p<0.001$ ,  $\eta^2=0.497$ ) was only observed in the heavy-intensity SWT for blood  $pO_2$ . In the middle-aged cyclists, the blood  $pO_2$  remained constant after 3 min of exercise during the

heavy-intensity SWT and had significantly decreased by 6 min compared to both the 0 ( $F(2,12)=5.946$ ,  $p=0.048$ ,  $\eta^2=0.498$ ) and 3 min ( $F(2,12)=5.946$ ,  $p=0.018$ ,  $\eta^2=0.498$ ) measures. A similar pattern was observed in the young cyclists during the heavy-intensity SWT, despite the difference between the 0 and 6 min values only approaching significance with a moderate effect ( $F(2,12)=6.398$ ,  $p=0.051$ ,  $\eta^2=0.516$ ), whereas the change in blood  $pO_2$  between 3 and 6 min was significant ( $F(2,12)=6.398$ ,  $p=0.027$ ,  $\eta^2=0.516$ ).

**Table 5.4:** Mean ( $\pm$  SD) blood  $pO_2$  (mmHg) values during the moderate-, heavy- and severe-intensity square wave transitions in the young and middle-aged cyclists.

	Young			Middle-Aged		
	0 min	3 min	6 min	0 min	3 min	6 min
<b>Moderate</b>	42.5 $\pm$ 2.5	41.8 $\pm$ 2.7	42.7 $\pm$ 7.8	41.7 $\pm$ 2.8	41.4 $\pm$ 1.6	40.9 $\pm$ 3.0
<b>Heavy</b>	41.2 $\pm$ 2.4	41.6 $\pm$ 2.7	36.9 $\pm$ 5.7 <sup>§</sup>	41.6 $\pm$ 2.9	40.2 $\pm$ 1.0	37.3 $\pm$ 2.4 <sup>§</sup> <sup>¥</sup>
<b>Severe</b>	38.3 $\pm$ 3.5	36.4 $\pm$ 4.3		37.7 $\pm$ 3.2	36.1 $\pm$ 2.8	

<sup>#</sup> significant difference between 0 and 3 min; <sup>§</sup> significant difference between 3 and 6 min; <sup>¥</sup> significant difference between 0 and 6 min; no subject completed the severe-intensity SWT.

#### *Blood Bicarbonate*

The mean ( $\pm$  SD) changes in blood  $[HCO_3^-]$  s during the three intensity SWT for both age groups are summarised in Table 5.5. No significant main effects of age or age x intensity interactions were observed for blood  $[HCO_3^-]$  across the three SWT intensities.

During the heavy-intensity SWT, a significant main effect of time ( $F(2,24)=82.867$ ,  $p<0.001$ ,  $\eta^2=0.874$ ) in blood  $[\text{HCO}_3^-]$  was observed. In the young cyclists, blood  $[\text{HCO}_3^-]$  decreased significantly during the 0 to 3 min period ( $F(2,12)=23.221$ ,  $p=0.019$ ,  $\eta^2=0.795$ ), and then further between the 3 and 6 min of exercise ( $F(2,12)=23.221$ ,  $p=0.046$ ,  $\eta^2=0.795$ ) across the heavy-intensity SWT.

**Table 5.5:** Mean ( $\pm$  SD) blood  $[\text{HCO}_3^-]$  ( $\text{mmol}\cdot\text{L}^{-1}$ ) values during the moderate-, heavy- and severe-intensity square wave transitions in the young and middle-aged cyclists.

	Young			Middle-Aged		
	0 min	3 min	6 min	0 min	3 min	6 min
<b>Moderate</b>	26.5 $\pm$ 1.2	25.4 $\pm$ 1.2	24.4 $\pm$ 1.8 <sup>§</sup>	26.5 $\pm$ 0.9	26.2 $\pm$ 1.3	26.1 $\pm$ 1.7
<b>Heavy</b>	26.1 $\pm$ 1.5	20.0 $\pm$ 4.6 <sup>#</sup>	15.3 $\pm$ 2.1 <sup>§¥</sup>	26.7 $\pm$ 1.1	21.4 $\pm$ 1.2 <sup>#</sup>	16.0 $\pm$ 1.8 <sup>§¥</sup>
<b>Severe</b>	22.8 $\pm$ 1.6	12.8 $\pm$ 3.0 <sup>#</sup>		22.8 $\pm$ 3.1	15.1 $\pm$ 1.3 <sup>#</sup>	

<sup>#</sup> significant difference between 0 and 3 min; <sup>§</sup> significant difference between 3 and 6 min; <sup>¥</sup> significant difference between 0 and 6 min; no subject completed the severe-intensity SWT.

The difference in the blood  $[\text{HCO}_3^-]$  between 0 and 6 min of the heavy-intensity SWT in the young cyclists was also significant ( $F(2,12)=23.221$ ,  $p<0.001$ ,  $\eta^2=0.795$ ). Similarly, a significant decrease in blood  $[\text{HCO}_3^-]$  in the middle-aged cyclists was observed between 0 and 3 min ( $F(2,12)=201.897$ ,  $p<0.001$ ,  $\eta^2=0.971$ ), as well as between 0 and 6 min ( $F(2,12)=201.897$ ,  $p<0.001$ ,  $\eta^2=0.971$ ), respectively. In the severe-intensity SWT,  $[\text{HCO}_3^-]$  significant decreased between 0 min and SWT exhaustion in both the young ( $F(2,12)=37.547$ ,  $p=0.001$ ,  $\eta^2=0.862$ ) and middle-aged ( $F(2,12)=40.433$ ,  $p=0.001$ ,  $\eta^2=0.871$ ) cyclists.

### *Blood Lactate*

The mean ( $\pm$  SD) changes in  $[BLa^-]$  during the three SWT intensities are shown in Table 5.6. No significant main effect of age or age  $\times$  time interaction was observed for changes in  $[BLa^-]$  across the three SWT intensities. However, significant increases in  $[BLa^-]$  were observed between 0 and 3 min [Y: ( $F(2,12)=80.772$ ,  $p<0.001$ ,  $\eta^2=0.971$ ); MA: ( $F(2,12)=80.772$ ,  $p=0.019$ ,  $\eta^2=0.498$ )] and 0 and 6 min [Y: ( $F(2,12)=80.772$ ,  $p<0.001$ ,  $\eta^2=0.971$ ); MA: ( $F(2,12)=80.772$ ,  $p=0.001$ ,  $\eta^2=0.498$ )] during moderate-intensity exercise in the young and middle-aged cyclists, respectively. No significant changes in  $[BLa^-]$  were observed between 3 and 6 min across the moderate-intensity SWT in either age group.

There was a significant increase in  $[BLa^-]$  during the heavy-intensity SWT between 0 and 3 min ( $F(2,12)=261.645$ ,  $p<0.001$ ,  $\eta^2=0.978$ ), and also between 3 and 6 min ( $F(2,12)=261.645$ ,  $p<0.001$ ,  $\eta^2=0.978$ ) in the young cyclists. A similar increase was also present during the heavy-intensity SWT between the 0 and 3 min ( $F(2,12)=161.5$ ,  $p<0.001$ ,  $\eta^2=0.964$ ) and 3 to 6 min ( $F(2,12)=161.5$ ,  $p<0.001$ ,  $\eta^2=0.964$ ) periods in the middle-aged cyclists. A significant increase in  $[BLa^-]$  was observed between 0 min and exhaustion of the severe-intensity SWT for both the young ( $F(2,12)=408.289$ ,  $p<0.001$ ,  $\eta^2=0.986$ ) and middle-aged ( $F(2,13)=86.124$ ,  $p<0.001$ ,  $\eta^2=0.935$ ) cyclists.  $[BLa^-]$  was significantly elevated at the start of the severe-intensity SWT compared to both the moderate- [Y:( $F(2,12)=10.026$ ,  $p=0.019$ ,  $\eta^2=0.626$ ); MA:( $F(2,12)=12.637$ ,  $p=0.012$ ,  $\eta^2=0.678$ ) and heavy-intensity [Y:( $F(2,12)=12.637$ ,  $p=0.019$ ,  $\eta^2=0.678$ ); MA:( $F(2,12)=12.637$ ,  $p=0.012$ ,  $\eta^2=0.678$ ) SWT.

**Table 5.6:** Mean ( $\pm$  SD) [BLa<sup>-</sup>] (mmol•L<sup>-1</sup>) values during the moderate-, heavy- and severe-intensity square wave transitions in the young and middle-aged cyclists.

	Young			Middle-Aged		
	0 min	3 min	6 min	0 min	3 min	6 min
<b>Moderate</b>	1.9 $\pm$	4.2 $\pm$	4.7 $\pm$	2.0 $\pm$	4.1 $\pm$	3.1 $\pm$
	0.7	0.3 <sup>#</sup>	0.9 <sup>¥</sup>	0.6	2.0 <sup>#</sup>	0.9 <sup>¥</sup>
<b>Heavy</b>	1.9 $\pm$	8.9 $\pm$	15.3 $\pm$	1.8	8.9	14.4 $\pm$
	0.7	1.1 <sup>#</sup>	1.9 <sup>§ ¥</sup>	$\pm$ 0.5	$\pm$ 1.3 <sup>#</sup>	2.4 <sup>§ ¥</sup>
<b>Severe</b>	4.7 $\pm$	16.4 $\pm$		5.3 $\pm$	15.2 $\pm$	
	1.9	0.7 <sup>§</sup>		2.6	1.0 <sup>§</sup>	

<sup>#</sup> significant difference between 0 and 3 min; <sup>§</sup> significant difference between 3 and 6 min; <sup>¥</sup> significant difference between 0 and 6 min; no subject completed the severe-intensity SWT.

### Correlations between $\dot{V}O_2$ and mOxy kinetics and hematological variables

The significant relationships between the  $\dot{V}O_2$  and mOxy kinetic parameters and hematological measures across the three SWT intensities for the young and middle-aged cyclists are listed in Tables 5.7a-c.

### Correlations between $\dot{V}O_2$ and mOxy kinetics and muscle histochemical and enzymatic characteristics

The significant correlations observed between the histochemical and enzymatic characteristics of the VL and the on-transient  $\dot{V}O_2$  response parameters of the young and middle-aged cyclists are presented below in Table 5.8a. The significant correlations between the reported histochemical and enzymatic characteristics and the on-transient mOxy response parameters are summarised in Table 5.8b.

**Table 5.7a:** Correlation coefficients (r) for the relationships between the amplitude and time parameters of the moderate-intensity  $\dot{V}O_2$  and mOxy kinetic responses and hematological variables in the young and middle-aged cyclists.

Young				Middle-Aged			
		r	p			r	p
$\dot{V}O_2 \tau_p$	mOxy $G_o$	0.98	0.001	$\dot{V}O_2 A_p$	mOxy MRT	0.81	0.027
	[BLa] @ 3 min	0.91	0.004	$\dot{V}O_2 TD_p$	[HCO <sub>3</sub> <sup>-</sup> ] @ 0 min	0.93	0.002
$\dot{V}O_2 wMRT$	mOxy MRT	0.77	0.044	$\dot{V}O_2 G_o$	[HCO <sub>3</sub> <sup>-</sup> ] $\Delta 0-6$ min	0.91	0.004
	mOxy $G_o$	0.81	0.049				
	[BLa] $\Delta 3-6$ min	0.86	0.013				

**Table 5.7b:** Correlation coefficients (r) for the relationships between the amplitude and time parameters of the heavy-intensity  $\dot{V}O_2$  and mOxy kinetic responses and hematological variables in the young and middle-aged cyclists.

Young				Middle-Aged			
		r	p			r	p
$\dot{V}O_2 A_p$	$\dot{V}O_2 A_f$	0.92	0.003	$\dot{V}O_2$ Baseline	mOxy Baseline	0.76	0.047
	$\dot{V}O_2 A_s$	0.86	0.013	$\dot{V}O_2 \tau_p$	mOxy $A_p$	-0.84	0.019
	pH $\Delta 0$ -3 min	-0.93	0.002		pH $\Delta 0$ -3 min	-0.82	0.024
$\dot{V}O_2 \tau_p$	mOxy $\tau_p$	0.94	0.005		$[HCO_3^-] \Delta 0$ -3 min	-0.80	0.033
$\dot{V}O_2 G_p$	$pO_2 @ 3$ min	-0.95	0.001	$\dot{V}O_2 G_o$	$[BLa^-] \Delta 3$ -6 min	-0.81	0.027
	$[HCO_3^-] \Delta 0$ -3 min	-0.76	0.047		mOxy $A_p$	0.77	0.043
	$[BLa^-] \Delta 0$ -6 min	0.94	0.001		$[BLa^-] @ 3$ min	0.78	0.038
$\dot{V}O_2 G_o$	$\dot{V}O_2 wMRT$	0.89	0.008		pH @ 6 min	-0.77	0.044
	$[BLa^-] @ 3$ min	0.77	0.044		$[BLa^-] \Delta 0$ -6 min	0.89	0.007
	$[HCO_3^-] \Delta 3$ -6 min	-0.79	0.033		$[HCO_3^-] \Delta 0$ -3 min	-0.78	0.039
	pH $\Delta 0$ -6 min	-0.76	0.049		$[HCO_3^-] \Delta 0$ -6 min	-0.81	0.027
	$[BLa^-] \Delta 0$ -6 min	0.81	0.029				

**Table 5.7c:** Correlation coefficients (r) for the relationships between the amplitude and time parameters of the severe-intensity  $\dot{V}O_2$  and mOxy kinetic responses and hematological variables in the young and middle-aged cyclists.

Young				Middle-Aged			
		r	p			r	p
$\dot{V}O_2 A_p$	$\dot{V}O_2 wMRT$	0.82	0.026	$\dot{V}O_2 A_p$	$\dot{V}O_2 G_p$	0.90	0.006
$\dot{V}O_2 \tau_p$	$\dot{V}O_2 G_p$	0.87	0.024		$\dot{V}O_2 G_o$	0.81	0.027
	$\dot{V}O_2 wMRT$	0.81	0.028		$[HCO_3^-] @ 3 \text{ min}$	0.77	0.043
$\dot{V}O_2 G_p$	$[BLa^-] @ 3 \text{ min}$	-0.94	0.002	$\dot{V}O_2 \tau_p$	$\dot{V}O_2 G_p$	0.80	0.031
$\dot{V}O_2 G_o$	$\dot{V}O_2 wMRT$	0.91	0.012		$\dot{V}O_2 wMRT$	0.96	0.001
	$\dot{V}O_2 \tau_p$	0.83	0.040	$\dot{V}O_2 G_p$	$[HCO_3^-] @ 3 \text{ min}$	-0.81	0.028
mOxy $\tau_p$	$[BLa^-] @ 3 \text{ min}$	-0.94	0.006		pH @ 3 min	0.81	0.027
				$\dot{V}O_2 wMRT$	$\dot{V}O_2 G_p$	0.76	0.049
					$\dot{V}O_2 G_o$	0.81	0.029
				EE $\dot{V}O_2$	$[HCO_3^-] @ 3 \text{ min}$	-0.87	0.012
				$\dot{V}O_2 TD_p$	$\dot{V}O_2 A_p$	0.77	0.043
					EE $\dot{V}O_2$	0.77	0.040

**Table 5.8a:** Correlation coefficients (r) for the relationships between the amplitude and time parameters of the  $\dot{V}O_2$  responses across the three square wave transition intensities and the peripheral muscle characteristics in the young and middle-aged cyclists.

Young				Middle-Aged			
Histochemical & Enzymatic Parameter	Kinetic Marker	r	p	Histochemical & Enzymatic Parameter	Kinetic Marker	r	p
Type II a %	Moderate $\tau$	0.81	0.028	Type I %	Moderate $\tau_p$	-0.89	0.019
	Moderate MRT	0.82	0.024		Severe $EE\dot{V}O_2$	0.84	0.032
Type IIb %	Severe $TD_p$	-0.80	0.031	Type I CSA	Severe wMRT	-0.90	0.015
Capillary Density	Heavy $A_p$	-0.80	0.033	Type IIa %	Moderate $\tau$	0.87	0.026
	Heavy $TD_p$	-0.84	0.019	Type IIa CSA	Severe $\tau_p$	-0.92	0.009
C:F Ratio	Moderate $\tau$	-0.78	0.041	Type IIb %	Severe wMRT	-0.92	0.009
	Moderate MRT	-0.85	0.015		Moderate $\tau$	0.85	0.034
CC/F	Moderate MRT	-0.81	0.028	CCFA	Severe $\tau_p$	0.77	0.044
CS activity	Moderate $A_p$	-0.78	0.037		Severe $\tau_p$	0.78	0.039
	Moderate $EE\dot{V}O_2$	-0.78	0.043				
	Heavy $A_p$	-0.89	0.039				
	Heavy $EE\dot{V}O_2$	-0.80	0.030				
	Heavy wMRT	-0.78	0.039				
	Severe $EE\dot{V}O_2$	0.87	0.011				
2-OGDH	Moderate MRT	-0.82	0.023				
	Heavy wMRT	-0.81	0.029				

**Table 5.8b:** Correlation coefficients (r) for the relationships between the amplitude and time parameters of the mOxy responses across the three square wave transition intensities and the peripheral muscle characteristics in the young and middle-aged cyclists.

Young				Middle-Aged			
Histochemical & Enzymatic Parameter	Kinetic Marker	r	p	Histochemical & Enzymatic Parameter	Kinetic Marker	r	p
Type IIa %	Moderate G <sub>o</sub>	0.88	0.021	Type IIb CSA	Heavy A <sub>s</sub>	0.96	0.011
Type IIb %	Moderate TD <sub>p</sub>	0.92	0.010	Capillary Density	Heavy TD <sub>p</sub>	-0.82	0.024
Capillary Density	Severe EEmOxy	-0.82	0.046	C:F Ratio	Severe TD <sub>p</sub>	0.83	0.008
C:F Ratio	Moderate A <sub>p</sub>	-0.91	0.013	CC/F	Heavy TD <sub>p</sub>	0.78	0.039
	Heavy A <sub>s</sub>	0.98	0.022		Heavy A <sub>s</sub>	0.96	0.010
CC/F	Heavy A <sub>s</sub>	0.96	0.045		Heavy wMRT	0.80	0.032
	Heavy G <sub>s</sub>	0.99	0.006		Severe TD <sub>p</sub>	0.83	0.020
DD <sub>max</sub>	Heavy $\tau_p$	0.89	0.016		Severe A <sub>s</sub>	0.93	0.007
	Heavy A <sub>s</sub>	0.99	0.010		Severe wMRT	0.80	0.032
2-OGDH Activity	Moderate $\tau_p$	0.85	0.034	CCFA	Heavy A <sub>s</sub>	-0.90	0.034
PFK Activity	Moderate TD <sub>p</sub>	-0.97	0.001		Severe TD <sub>p</sub>	0.76	0.047
LDH Activity	Heavy wMRT	0.90	0.016	DD <sub>max</sub>	Heavy A <sub>s</sub>	0.96	0.009
	Severe A <sub>p</sub>	0.84	0.035		Heavy TD <sub>p</sub>	0.79	0.034
				DD <sub>mean</sub>	Moderate wMRT	-0.85	0.033
					Heavy A <sub>s</sub>	0.96	0.009
				2-OGDH Activity	Moderate wMRT	-0.79	0.035
				PFK Activity	Heavy wMRT	-0.84	0.017

## DISCUSSION

The purpose of Study Two was to examine the effect of age on the on-transient  $\dot{V}O_2$  and mOxy responses to moderate-, heavy- and severe-intensity SWT in well-trained cyclists. The present study is the first to investigate the effect of age on the concurrent on-transient  $\dot{V}O_2$  and mOxy responses across increasing exercise intensities in well-trained cyclists. The results of Study Two demonstrated no significant effect of age in the on-transient  $\dot{V}O_2$  or mOxy responses across the three SWT intensities. This non-significant finding is most likely due to the similar aerobic powers and peripheral muscle characteristics reported for the young and middle-aged cyclists within Study One.

A significant main effect of intensity was observed in the  $\dot{V}O_2$  and mOxy  $A_p$  in both age groups. However, the  $\dot{V}O_2$  and mOxy responses demonstrated significant effects of intensity in their speed of adaptation ( $TD_p$ ;  $\tau_p$ ). Both the on-transient  $\dot{V}O_2$  and mOxy responses were significantly related to a small number of hematological parameters and peripheral muscle characteristics in the young and middle-aged cyclists. Collectively, these results suggest that physical training into middle-age reduces the previously reported effect of sedentary aging on the metabolic adaptation to exercise bouts of increasing intensity (Babcock et al. 1992; 1994b; DeLorey et al. 2004a; 2005).

The absence of a significant effect of age in the development of the  $\dot{V}O_2$  and mOxy slow components is most likely due to the similar physiological and muscle histochemical characteristics of the cyclists (as reported in Study One). Previous research has reported that the on-transient  $\dot{V}O_2$  response is strongly influenced by both both  $\dot{V}O_{2max}$  (Ebfield et al. 1987) and peripheral muscle histochemical

characteristics (Barstow et al. 1996; Pringle et al. 2003b) in younger populations. However, no such data were previously available on middle-aged cohorts such as that examined in the present study.

The present data suggest that previous age-related declines in metabolic responses to increases in work intensity (Babcock et al. 1992; 1994b; DeLorey et al. 2004a; 2005) may be due to the effect of a prolonged sedentary lifestyle or to physical detraining rather than the aging process *per se*. The present results further support the recent work of Berger, Rittweger, Kwiet, Michaelis, Williams, Tolfrey and Jones (2006) that reported no significant effect of age in the on-transient  $\dot{V}O_2$  response to moderate-intensity (80% VT) cycling in sprint and endurance-trained athletes between the ages of 45 to 85 y. On the basis of these results and the findings of the present study it appears that continued physical training helps to maintain both  $\dot{V}O_{2\max}$  and muscle metabolic characteristics into older age, which may help to attenuate age-related changes in the on-transient  $\dot{V}O_2$  and mOxy responses.

#### *On-Transient Amplitude Responses*

As presented in Tables 5.1 and 5.2 earlier, no significant effect of age was observed in the baseline or  $A_p$  measures of the  $\dot{V}O_2$  or mOxy responses to bouts of varying intensity exercise within the present study. The similar  $\dot{V}O_2$  and mOxy  $A_p$  measures in the young and middle-aged cyclists is most likely due to the matching of the two cycling cohorts on their  $\dot{V}O_{2\max}$  and muscle histochemical characteristics as observed in Study One. However, the on-transient  $\dot{V}O_2$  and mOxy baseline and  $A_p$  measures demonstrated a significant effect of intensity in both the young and middle-aged cyclists. This increasing effect of intensity was expected given the increasing

work rate across the three SWT intensities and greater  $\dot{V}O_2$  demands within the working muscle. The similar effects of intensity between the two age groups may again reflect the similar physiological capacities and muscle histochemical and enzymatic characteristics of the two age groups in the present study.

The  $\dot{V}O_2$  and mOxy baseline values measured prior to the initiation of the three SWT intensities were also not significantly influenced by age in the well-trained cyclists in the present study. However, the  $\dot{V}O_2$  and mOxy baseline values demonstrated significant effects of intensity in the young and middle-aged cyclists. This increasing effect of intensity suggests there may have been inadequate recovery between the heavy and severe-intensity SWT, despite the preset criteria for SWT commencement of resting  $\dot{V}O_2$  measures being consistently adhered to throughout the present study. The  $\dot{V}O_2$  and mOxy baselines observed in the present study are slightly higher than in previous investigations which most likely reflect methodological differences (Pringle et al. 2003b; Koppo et al. 2004). For example, the present study used cadences (90 RPM) reported to be preferred by trained cyclists that are higher than those favoured by untrained cyclists (60-70 RPM) (Marsh and Martin 1997; Lucia, Hoyos and Chicharro 2001; Nesi, Bosquet and Pelayo 2005) or used in similar metabolic investigations (Pringle et al. 2003b; Koppo et al. 2004). Therefore, the higher cadence employed within the present study may be responsible for the higher  $\dot{V}O_2$  and lower mOxy baseline measures. However, the higher cadences adopted in the present study allowed the well-trained cyclists to be familiar with the cycling activity, and ensure the specificity of exercise bout adaptations and metabolic efficiency.

The significantly higher  $\dot{V}O_2$  and lower mOxy baselines prior to the severe-intensity SWT may suggest increased cellular metabolism and lower metabolic inertia prior to the application of the SWT load. As such, the metabolic inertia required to be overcome at the severe-intensity SWT load application may have been decreased given the higher  $\dot{V}O_2$  and lower mOxy observed prior to the load application. The possibility of an increased delivery of  $O_2$  through an enhanced  $HbO_2$  dissociation via the Bohr effect (Stringer et al. 1994) being responsible for the observed differences in  $\dot{V}O_2$  and mOxy baselines is contrasted by no significant effect of intensity being observed in blood pH during the unloaded pedalling prior to SWT load application on either age group. However, despite this significant effect of intensity in the  $\dot{V}O_2$  and mOxy baseline measures, the practical influence of elevated baseline measures prior to each SWT would be similar between the two age groups given that no significant effect of age was reported in these parameters in the present study. However, future research should allow greater recovery time (> 45 min) between high-intensity cycling bouts used to determine  $\dot{V}O_2$  and mOxy kinetic responses to remove the effect of prior heavy-intensity exercise (Burnley et al. 2006).

In the present study, no significant effect of age was observed in the  $\dot{V}O_2$  or mOxy  $A_p$  across the three SWT intensities. Again, the most likely explanation for the absence of a significant effect of age in the  $\dot{V}O_2$   $A_p$  may be the result of the similar maximal aerobic capacities and peripheral muscle characteristics of the two groups examined in the present study. Both  $\dot{V}O_{2max}$  and muscle histochemical and of the on-transient  $\dot{V}O_2$  response (Ebfield et al. 1987; Babcock et al. 1994a; Barstow et al. 1996; Pringle et al. 2003b; Caputo and Denadai 2004).

The  $\dot{V}O_2 A_p$  values observed across the three SWT intensities in the present study are similar to those observed in previous investigations (Sirna et al. 1998; Pringle et al. 2003b; Koppo et al. 2004). Previous research investigations that have examined an effect of aging on the on-transient  $\dot{V}O_2$  response have recruited healthy sedentary young or elderly populations unmatched on  $\dot{V}O_{2max}$ , and have failed to report muscle histochemical or enzymatic characteristics (Babcock et al. 1994b; Stathokostas et al. 2003; DeLorey et al. 2004a; 2005; Berger et al. 2006). Age-related differences in these factors may contribute to previously reported reductions in the on-transient  $\dot{V}O_2 A_p$  reported in sedentary aged populations in these investigations. For example, DeLorey and colleagues (2004; 2005) examined the effect of age on the on-transient  $\dot{V}O_2$  and mOxy responses to moderate- and heavy-intensity exercise in young and elderly sedentary subjects. The young ( $26 \pm 3$  y) subjects demonstrated significantly higher maximal aerobic powers ( $3.8 \pm 0.4 \text{ L}\cdot\text{min}^{-1}$ ;  $49 \pm 6 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) than the elderly ( $68 \pm 3$ y;  $2.3 \pm 0.3 \text{ L}\cdot\text{min}^{-1}$ ;  $27 \pm 3 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) sedentary subjects. Therefore, the differences reported by these investigators in the metabolic adaptation to the moderate- and heavy-intensity bouts may be due to not only aging, but may also be the result of differences in  $\dot{V}O_{2max}$ .

The absence of a significant effect of age in the  $\dot{V}O_2 A_p$  in the present study is further supported by the comparable efficiency ( $\Delta\dot{V}O_2/\Delta W$ ) values indicated by the similar  $\dot{V}O_2 G_p$ ,  $G_o$  and SWT power output values between the two age groups. While no effect of intensity was observed in the  $\dot{V}O_2 G_p$  or  $G_o$ , the difference in power outputs would require different on-transient  $\dot{V}O_2 A_p$  to match the energy demands of the SWT intensities. The  $\dot{V}O_2 G_p$  and  $G_o$  ( $\sim 6\text{-}9 \text{ mL}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$ ) were considerably lower than those reported in previous literature ( $\sim 9\text{-}11 \text{ mL}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$ ) for

younger populations (Pringle et al. 2003b). This finding may be the result of the higher SWT power outputs but similar  $\dot{V}O_2 A_p$  of the well-trained cyclists in the present study compared to previous research (Pringle et al. 2003b), which may suggest greater mechanical efficiency in the subjects recruited in the present study.

The on-transient  $\dot{V}O_2$  response demonstrated no significant effect of age, but did demonstrate an effect of intensity in both age groups. Both the on-transient mOxy  $A_p$  and efficiency measures demonstrated similar effects of age and intensity to the  $\dot{V}O_2$  response. In summary, the above observations further support the suggestion that concurrent aging and physical training may reduce the reported decreases in physiological function observed in previous studies (Babcock et al. 1992; 1994b; DeLorey et al. 2004a; 2005).

In the present study, no significant effect of age was observed in the on-transient mOxy  $A_p$  across the three SWT intensities. Despite this finding, the middle-aged cyclists exhibited a consistent but non-significant but weak ( $\eta^2=0.125$ ) increase in mOxy  $A_p$  across the moderate- (8%), heavy- (10%) and severe-intensity (9%) SWT than the younger cohort. This possible clinically-significant finding supports the observations of DeLorey et al. (2004a; 2005) who reported similar and greater mOxy  $A_p$  in response to moderate and heavy-intensity SWT in older sedentary subjects compared to a younger cohort. Previous studies have suggested that aged muscle has an increased capacity to maintain oxidative potential via altered muscle fibre composition that may act to counteract the decrease in cardiovascular function observed with aging (Russ and Kent-Braun 2004). The present observations are supportive of such age-related adaptations with similar muscle histochemical and

enzymatic characteristics observed between the two age groups, despite the middle-aged cyclists possessing a significantly lower  $HR_{max}$ .

Both the on-transient  $\dot{V}O_2$  and mOxy  $A_p$  in the present study demonstrated significant increasing effects of intensity in both age groups. This finding supports previous research examining metabolic responses across exercise intensities (Carter et al. 2002; DeLorey et al. 2002; Pringle et al. 2003b; Koppo et al. 2004). The increased  $O_2$  cost observed demonstrated through the effect of exercise intensity most likely reflects the previously reported curvilinear relationships between work intensity and both  $\dot{V}O_2$  (Barstow et al. 1996; 2000; Pedersen et al. 2002; Pringle et al. 2002; 2003b) and mOxy (Belardinelli et al. 1995a; 1995b). Interestingly, in the present study, the  $\dot{V}O_2$  and mOxy  $A_p$  were similar between the heavy and severe-intensity SWT in both age groups. This similarity may reflect the higher  $\dot{V}O_2$  baseline, larger anaerobic contribution and decrease in metabolic efficiency at power outputs above 50%  $\dot{V}O_{2max}$  (Barstow et al. 1996; 2000; Pedersen et al. 2002; Pringle et al. 2002; 2003b). Past investigators have reported that during severe-intensity exercise subjects may not reach  $\dot{V}O_{2max}$  and exhibit submaximal  $\dot{V}O_2$  values despite reaching volitional fatigue, and therefore this observation is not novel (Jones and Poole, 2005).

In conclusion, it appears that the on-transient  $\dot{V}O_2$  and mOxy  $A_p$  observed in the well-trained cyclists in Study two demonstrated no significant effect of age. The two age groups of cyclists also demonstrated similar changes across increasing exercise intensities on the  $\dot{V}O_2$  and mOxy  $A_p$  and efficiency gains. These findings suggest that the similarities between the two age groups and across the three SWT intensities is due to the similar  $\dot{V}O_{2max}$  values and peripheral muscle characteristics

of the two age groups reported in Study One. These similar characteristics are also likely to be a major influence the speed of the on-transient  $\dot{V}O_2$  and mOxy responses across the three SWT intensities in the two age groups, given their influence on the utilisation and delivery of  $O_2$  within the working muscle.

### *On-Transient Speed Responses*

In the present study there was no significant effect of age in the speed values of the on-transient  $\dot{V}O_2$  or mOxy responses. As in the on-transient amplitude responses examined earlier, this most likely reflects the similar  $\dot{V}O_{2\max}$  values and peripheral muscle characteristics of the young and middle-aged well-trained cyclists.

Furthermore, no main effects of exercise intensity were observed for the  $\dot{V}O_2$  or mOxy  $TD_p$  in the current study. The  $TD_p$  of the on-transient responses helps to provide valuable information about any metabolic inertia which needs to be overcome for subsequent increases in aerobic metabolism and  $\dot{V}O_2$  adaptation (Koga et al. 2005). In the present study, the middle-aged cyclists demonstrated a significant shortening of the on-transient  $\dot{V}O_2$   $TD_p$  between the heavy and severe-intensity SWT that was not observed in the younger cyclists. As discussed earlier, the significantly higher baseline  $\dot{V}O_2$  and mOxy prior to the severe-intensity SWT may reflect an increased metabolic rate as a result of the prior high-intensity exercise. Such an effect may have provided less metabolic inertia to be overcome at the next load application, resulting in a shorter  $\dot{V}O_2$   $TD_p$ . However, given this intensity effect was not mirrored in the mOxy  $TD_p$ , it is possible that the shortened  $\dot{V}O_2$   $TD_p$  was due to accelerated cardiodynamics and enhanced blood flow through the working muscles (Koga et al. 2005).

Despite these effects of exercise intensity, the mOxy TD<sub>p</sub> was significantly faster than that observed in the  $\dot{V}O_2$  TD<sub>p</sub> in response to all SWT intensities of the present study. This observation may reflect the transit time of the deoxygenated blood returning to the lungs from the working muscle, which may result in a longer  $\dot{V}O_2$  TD<sub>p</sub>. The NIRS measurement of mOxy is more instantaneous and reflects changes in intra-muscular O<sub>2</sub> utilisation. The changes in O<sub>2</sub> utilisation is of great importance to metabolic adaptation across work intensities and any observed lengthening of the mOxy response across exercise intensities may suggest that changes in O<sub>2</sub> utilisation limit the metabolic adaptation to changes in work intensity.

Previously, the speed of the  $\dot{V}O_2$  response has been demonstrated to slow with sedentary aging, with no such effect being observed in the on-transient mOxy response (Babcock et al. 1992; 1994b; DeLorey et al. 2003b; 2004a; 2005). Originally, Babcock and colleagues (1992) reported that the  $\dot{V}O_2$   $\tau_p$  was slowed with aging in sedentary individuals, but later suggested that physical training may speed the  $\dot{V}O_2$  response in older populations (Babcock et al. 1994a). Recently, DeLorey and colleagues (2004a; 2005) reported that a sedentary older population (n= 6; 68 ± 3 y; 2.3 ± 0.3 L•min<sup>-1</sup>) demonstrated a slowed  $\dot{V}O_2$   $\tau_p$  in response to both moderate (Y: 26 ± 7 s; O: 42 ± 9 s) and heavy-intensity (Y: 29 ± 4 s; O: 49 ± 8 s) exercise compared to a younger (n= 5; 26 ± 3 y; 3.8 ± 0.4 L•min<sup>-1</sup>) cohort. More recently, concurrent training into older age has been shown to ameliorate the moderate-intensity  $\dot{V}O_2$   $\tau_p$  response in sprint and endurance-trained athletes between the ages of 45–85 y (Berger et al. 2006). Therefore, the current investigation supports the absence of a significant effect of age on the speed of the on-transient  $\dot{V}O_2$  responses to moderate-intensity exercise. Furthermore, the present study is the first to report no

effect of age on the speed of the  $\dot{V}O_2$  response to high-intensity exercise in well-trained athletes.

In contrast, the mOxy  $\tau_p$  has previously been suggested to remain stable or to be improved with aging across exercise transitions despite a slowed  $\dot{V}O_2$   $\tau_p$  response in sedentary aged subjects (DeLorey et al. 2004a; 2005). These investigators reported that the on-transient mOxy  $\tau_p$  was similar between young (Y) and old (O) sedentary populations in response to a moderate-intensity (Y:  $13 \pm 10$  s; O:  $9 \pm 3$  s) exercise bout. The same investigators reported that the sedentary elderly cohort ( $8 \pm 2$  s) demonstrated a significantly faster mOxy  $\tau_p$  than the younger subjects ( $14 \pm 2$  s) in response to heavy-intensity exercise. Other researchers have also suggested that the mOxy  $\tau_p$  response across both moderate and heavy-intensity SWT can be improved through physical training in older sedentary cohorts (Pogliaghi, Cevese and Schena 2004). However until the present study, no previous data were available examining the effect of age on the on-transient mOxy responses in well-trained athletes.

A major finding of Study Two was that neither the  $\dot{V}O_2$  or mOxy  $\tau_p$  demonstrated a significant effect of intensity in either age group. Thus, the present finding supports previous suggestions that the  $\dot{V}O_2$   $\tau_p$  is consistent across exercise intensities (Barstow and Mole 1991; Barstow et al. 1993; Carter et al. 2000a; Ozyener et al. 2001). However, in contrast to the present finding, other studies have reported a lengthened  $\dot{V}O_2$   $\tau_p$  with increasing exercise intensity (Casaburi et al. 1989; Paterson and Whipp 1991; Phillips et al. 1995; Engelen et al. 1996; Jones et al. 2002; Koppo et al. 2004). The observed stable  $\dot{V}O_2$   $\tau_p$  suggests that the speed of the exponential increase in  $\dot{V}O_2$  at exercise onset is limited by  $O_2$  utilisation (Carter et al.

2002; Grassi 2005). This control of  $O_2$  utilisation is further supported by recent studies that observed a significant speeding of both HR and leg blood flow kinetics with increases in exercise intensity with no subsequent benefits in the speed of metabolic adaptation (Koch, Newcomer and Proctor 2005; Tanaka, Shimizu, Ohmori, Muraoka, Kumagai, Yoshizawa and Kagaya 2006). Therefore, it appears that the physiological mechanisms limiting the on-transient metabolic response is controlled through changes in the utilisation of  $O_2$  within the working muscle.

#### *On-Transient Physiological Mechanisms*

The present data suggest the speed of the on-transient  $\dot{V}O_2$  response is most likely limited by the utilisation of  $O_2$  within the working muscle and supports a recent review of research examining the speed of the on-transient  $\dot{V}O_2$  responses (Grassi 2005). This suggestion of  $O_2$  utilisation limitations is further supported by the absence of a significant effect of intensity on the mOxy  $\tau_p$  in both groups in the present study, and is in strong agreement with the previous findings of Shibuya et al. (2004). These researchers reported that the mOxy  $\tau_p$  was not dependent upon exercise intensity in young healthy subjects (23-28 y) and hypothesised that  $O_2$  utilisation limitations are responsible for controlling metabolic adaptation at exercise onset.

Moreover, the present data suggest that the on-transient mOxy  $\tau_p$  was significantly faster than the  $\dot{V}O_2$   $\tau_p$  in both age groups during both the moderate and severe-intensity SWT. This observation is in agreement with DeLorey et al. (2004a; 2005) and Shibuya et al. (2004) who also observed that the speed of the mOxy responses are significantly faster than the  $\dot{V}O_2$  response during the on-transient metabolic adaptation. This difference is most likely due to the transit time of

deoxygenated blood to the lungs and the additional  $\dot{V}O_2$  requirements of several metabolic processes and stabiliser muscles that are not monitored through the sensitive NIRS measures of mOxy within the working muscle. Therefore,  $\dot{V}O_2$  measures may not adequately reflect the mechanisms responsible for controlling the metabolic adaptation at exercise onset. It might be suggested that any  $O_2$  utilisation limitations may be better identified through the mOxy response of the working muscle.

To date, a great deal of empirical research has attempted to identify the utilisation of  $O_2$  within muscle as the controlling mechanism of the metabolic responses at exercise onset (Xu and Rhodes 1999; Grassi 2000). In a recent review, Grassi (2005) reported that a number of previous investigations have attempted to change the metabolic environment within the working muscle to identify the actual  $O_2$  utilisation limitations. A strong influence of muscle fibre composition and CSA on the on-transient  $\dot{V}O_2$  response across work intensities has been reported by previous research (Barstow et al. 1996; Pringle et al. 2003b). Similarly, Hogan (2001) suggested that the lag in  $\dot{V}O_2$  at exercise onset might be related to redox state, phosphorylation potential and the kinetics of mitochondrial  $Ca^{2+}$  which have been shown to be muscle fibre-specific (Bottinelli and Reggiani 2000; He et al. 2000). Therefore, the most likely mechanism that limits  $\dot{V}O_2$  adaptation appears to lie within the utilisation of  $O_2$  within the muscle cell during periods of adjustment to an exercise bout.

The present results revealed significant relationships between the on-transient  $\dot{V}O_2 \tau_p$  in the moderate and severe-intensity SWT and the Type I and IIb fibre percentages, respectively, in the middle-aged cyclists. The heavy-intensity  $\dot{V}O_2 \tau_p$

was also significantly related to the Type IIa fibre composition in both age groups in the present study. These relationships agree with those originally presented by Pringle et al. (2003b) who observed significant relationships between the heavy-intensity  $\dot{V}O_2 \tau_p$  and Type IIa fibre composition. In addition, Pringle et al. (2003b) reported that the speed of the  $\dot{V}O_2$  response was significantly related to several muscle capillarisation characteristics in a young healthy cohort. However, in the present study, few significant correlations were observed between the on-transient  $\dot{V}O_2$  response and any of the capillarisation measures of the VL from either age group. Therefore, the current study suggests that the nature of the on-transient  $\dot{V}O_2$  response is related to muscle fibre composition and further supports the existence of  $O_2$  utilisation limitations within the working muscle.

In contrast to the on-transient  $\dot{V}O_2$  response, no significant relationships were observed between on-transient mOxy response measures and the muscle fibre composition, CSA or capillarisation within either cohort in the present study. However, the relationships observed between the on-transient  $\dot{V}O_2$  and mOxy responses and maximal enzyme activities may also provide novel data on  $O_2$  utilisation issues within the working muscles. Several key speed measures of the on-transient  $\dot{V}O_2$  response were also observed to be related to the muscle enzyme activities in the cohorts of the present study. Significant inverse relationships were observed between the moderate and heavy-intensity  $\dot{V}O_2 A_p$  and the maximal CS activity in the young cyclists. This relationship suggests that the maximal activity of CS may be significantly related to the efficiency and muscle  $\dot{V}O_2$  capacity of the working muscle. Interestingly, the present study also observed significant inverse relationships between the activity of 2-OGDH and the  $\dot{V}O_2$  wMRT of both the moderate and heavy-intensity SWT in the young cyclists. The present investigation is

the first to observe significant relationships between maximal 2-OGDH activity and the on-transient  $\dot{V}O_2$  response. This finding is supported by previous research that demonstrated that maximum 2-OGDH activity is the most closely-related enzyme to the maximal flux of the TCA cycle (Blomstrand et al. 1997). The current study may therefore suggest that 2-OGDH is a rate limiting enzyme within the TCA cycle, which may influence the speed of adaptation of the on-transient  $\dot{V}O_2$  response, particularly to moderate-intensity exercise. Further research is required to investigate the relationship between the on-transient  $\dot{V}O_2$  response and 2-OGDH activity across exercise intensities.

In order to fully investigate the effect of  $O_2$  utilisation during the on-transient  $\dot{V}O_2$  response, previous studies have increased the availability of the acetyl group through dichloroacetate (DCA) infusion which has been shown to reduce energy substrate degradation within the muscle during submaximal exercise, and allow faster  $\dot{V}O_2$  adaptation (Timmons et al. 1998a; Howlett et al. 1999). These improvements in muscular bioenergetics have not led to subsequent changes in the  $\dot{V}O_2$  kinetic response in either dogs (Grassi, Hogan, Greenhaff, Hamann, Kelle, Aschenbach, Constantin-Teodosiu and Gladden 2002) or humans (Bangsbo et al. 2002). Similar methods used to elevate the activity of the pyruvate dehydrogenase (PDH) complex have also been shown not to influence the  $\dot{V}O_2$  kinetic response (Evans et al. 2001). Additionally, the role of nitric oxide has been examined given its influence on the rate of oxidative metabolism through a number of energy pathways (Brown 2000). The inhibition of nitric oxide synthase through the use of  $N^\omega$ -nitro-L-arginine-methyl ester (L-NAME) has been shown to speed  $\dot{V}O_2$  kinetics in horses (Kindig, McDonough, Erickson and Poole 2001). However, similar research has shown no effect in humans (Frandsen, Bangsbo, Sander, Hoffner, Betak,

Saltin and Hellsten 2001). Therefore, while it is widely suggested that  $O_2$  utilisation mechanisms are responsible for controlling the speed of the on-transient metabolic responses, the exact metabolic factors responsible for controlling the on-transient metabolic response are not yet fully understood. In well-trained and aged subjects such as those examined in the present study, it may be suggested that the effect of any  $O_2$  utilisation limiting mechanism may be similar to those of a younger age group given the similar muscle histochemical and enzymatic characteristics of the two groups.

## **SUMMARY**

The present study is the first to demonstrate no significant effect of age on the on-transient  $\dot{V}O_2$  or mOxy responses in well-trained young and middle-aged cyclists. The current investigation is also the first to suggest that these metabolic responses are maintained through physical training to middle age and supports the recent data showing a similar effect in well-trained older athletes (45-85 y) (Berger et al. 2006). The present research contrasts previous studies detailing a slowed  $\dot{V}O_2$  and stable mOxy responses across moderate and heavy-intensity exercise with sedentary aging (DeLorey et al. 2004a; 2005). The absence of such a significant effect of age in the present study is most likely due to the similar  $\dot{V}O_{2\max}$  and peripheral muscle characteristics of the two age groups described in Study One.

The effect of intensity on the  $\dot{V}O_2$  and mOxy responses observed in the present study is consistent with that reported in literature for both sedentary and well-trained subjects (Xu and Rhodes 1999; Pringle et al. 2002; 2003b). The present study reported significant effects in the amplitude ( $A_p$ ) of the  $\dot{V}O_2$  and mOxy responses, but importantly not for the speed ( $TD_p$ ;  $\tau_p$ ) measures with increases in

exercise intensity (Carter et al. 2002; Shibuya et al. 2004). These results suggest that the utilisation of  $O_2$  within the working muscle is responsible for the lagging of the metabolic responses at the onset of exercise.

The present investigation also revealed significant relationships between the on-transient  $\dot{V}O_2$  and mOxy responses and several muscle histochemical and biochemical characteristics in the well-trained cyclists. These relationships suggest that limitations in the utilisation of  $O_2$  rather than  $O_2$  delivery are responsible for controlling the on-transient metabolic responses to an exercise bout. In summary, this study suggests that the on-transient  $\dot{V}O_2$  and mOxy responses can be maintained into middle-age through physical training of sufficient intensity and duration to maintain  $\dot{V}O_{2max}$  and muscle histochemical and enzymatic characteristics.

## CHAPTER 6

### STUDY 3

#### **$\dot{V}O_2$ and mOxy slow components determined during heavy- and severe-intensity exercise in well-trained young and middle-aged cyclists**

##### OVERVIEW

The purpose of Study Three was to examine the effect of age on the development of the  $\dot{V}O_2$  and mOxy slow components during heavy- and severe-intensity SWT in well-trained cyclists. The results suggest no significant effect of age in the  $\dot{V}O_2$  or mOxy slow components in either the heavy- or severe-intensity SWT in well-trained cyclists. However, both the  $\dot{V}O_2$  and mOxy slow components demonstrated significant main effects of intensity in the  $TD_s$  but not in the  $A_s$  or  $\tau_s$ . The  $\dot{V}O_2$  wMRT was significantly longer in the heavy-intensity SWT, whereas the mOxy wMRT was not significantly affected by SWT intensity in either age group.

No significant relationships were observed between the  $\dot{V}O_2$  or mOxy slow components with any of the hematological responses or histochemical and enzymatic characteristics of either the young or middle-aged cyclists. Additionally, the sEMG responses of the VL and VM demonstrated no significant effects of age in the well-trained cyclists during the heavy or severe-intensity SWT. However, several significant effects of time were observed for the sEMG measures in the VL and VM across both SWT intensities in both age groups. The changes observed in the

neuromuscular activity of the VL and VM were not significantly related to the development of the  $\dot{V}O_2$  or mOxy slow components in either age group.

In summary, the major finding of Study Three was the absence of any age-related differences in the  $\dot{V}O_2$  or mOxy slow components in well-trained cyclists or the proposed causal mechanisms across the high- and severe-intensity SWT.

## RESULTS

### $\dot{V}O_2$ Slow Component

The time and amplitude parameters of the  $\dot{V}O_2$  slow component measured during the heavy and severe-intensity SWT are shown in Table 6.1.

No significant main effects of age, intensity or age x intensity interactions were observed for the  $\dot{V}O_2$   $A_s$ . A strong and positive relationship was observed between the  $\dot{V}O_2$   $A_p$  and  $A_s$  during the heavy-intensity SWT ( $r = 0.86$ ,  $p = 0.01$ ) in the young cyclists but not the middle-aged cohort ( $r = 0.27$ ,  $p = 0.55$ ).

No significant main effects of age, intensity or age x intensity interactions were observed for the  $\dot{V}O_2$   $TD_s$  and  $\tau_s$ . However, *post-hoc* analysis revealed a significantly faster  $\dot{V}O_2$   $\tau_s$  in the young cyclists in the severe-intensity ( $F(1,5) = 7.497$ ,  $p = 0.041$ ,  $\eta^2 = 0.600$ ) compared to that of the heavy-intensity SWT.

**Table 6.1:** Mean ( $\pm$  SD) time and amplitude values of the  $\dot{V}O_2$  slow component of the young and middle-aged cyclists measured during the heavy- and severe-intensity square wave transition.

	Young		Middle-aged	
	Heavy	Severe	Heavy	Severe
<b><math>A_s</math> (mL<math>\cdot</math>min<math>^{-1}</math>)</b>	550 $\pm$ 264	708 $\pm$ 236	643 $\pm$ 253	350
<b><math>TD_s</math> (s)</b>	127.7 $\pm$ 23.8	85.7 $\pm$ 29.6	89.6 $\pm$ 23.9	96
<b><math>\tau_s</math> (s)</b>	105.8 $\pm$ 35.0	62.4 $\pm$ 21.8 <sup>¥</sup>	111.3 $\pm$ 42.1	62.3
<b><math>G_s</math> (mL<math>\cdot</math>min<math>^{-1}\cdot</math>W<math>^{-1}</math>)</b>	1.9 $\pm$ 0.8	2.2 $\pm$ 0.7	2.2 $\pm$ 0.7	1.2
<b><math>G_o</math> (mL<math>\cdot</math>min<math>^{-1}\cdot</math>W<math>^{-1}</math>)</b>	8.8 $\pm$ 1.2	7.7 $\pm$ 1.3	8.3 $\pm$ 1.3	6.3 $\pm$ 1.5
<b><math>EE\dot{V}O_2</math> (mL<math>\cdot</math>min<math>^{-1}</math>)</b>	3487 $\pm$ 510	3405 $\pm$ 418	3648 $\pm$ 345	3636 $\pm$ 363
<b>wMRT (s)</b>	54.4 $\pm$ 11.8	38.7 $\pm$ 11.7 <sup>¥</sup>	51.2 $\pm$ 14.5	29.2 $\pm$ 7.5 <sup>¥</sup>

$A_s$  = Slow Component Amplitude

$G_s$  = Slow Component Gain

wMRT= Weighted Mean Response Time

$TD_s$  = Slow Component Time Delay

$G_o$  = Overall Gain

$\tau_s$  = Slow Component Time Constant

$EE\dot{V}O_2$  = End-Exercise  $\dot{V}O_2$

<sup>¥</sup> significant difference between heavy and severe-intensities ( $p < 0.05$ ); <sup>€</sup> no subject completed the six minute severe-intensity SWT.

### **mOxy Slow Component**

The time and amplitude parameters of the mOxy slow component across the heavy and severe-intensity SWT are shown in Table 6.2.

No significant main effect of age or age x intensity interaction was observed in the mOxy  $A_s$ . However, the mOxy  $A_s$  demonstrated a significant main effect of intensity ( $F(2,22)=15.422$ ,  $p=0.002$ ,  $\eta^2=0.794$ ). This effect of intensity was not observed in the separated young or middle-aged cohorts.

No significant main effect of age, intensity or age x intensity interaction was observed for the mOxy  $TD_s$ . However, the mOxy  $TD_s$  was found to be significantly ( $t = -5.434$ ,  $p<0.001$ ) longer than the  $\dot{V}O_2$   $TD_s$  during the heavy-intensity SWT in the middle-aged cyclists, but not the young cyclists,.

No significant effect of age or age x intensity interaction was observed for the mOxy  $\tau_s$ . However, a significant main effect of intensity ( $F(2,8)=8.296$ ,  $p=0.011$ ,  $\eta^2=0.675$ ) was observed for the mOxy  $\tau_s$  in the well-trained cyclists. This significant effect of intensity was only observed in the middle-aged cyclists ( $F(2,6)=11.854$ ,  $p=0.008$ ,  $\eta^2=0.798$ ) between the heavy- and severe-intensity SWT.

**Table 6.2:** Mean ( $\pm$  SD) time and amplitude values of the mOxy slow component of the young and middle-aged cyclists measured during the heavy- and severe-intensity square wave transition.

	Young		Middle-Aged	
	Heavy	Severe <sup>€</sup>	Heavy	Severe <sup>€</sup>
<b>A<sub>s</sub> (%)</b>	18.5 $\pm$ 5.9	20.0 $\pm$ 10.2	17.4 $\pm$ 4.3	17.0 $\pm$ 7.8
<b>TD<sub>s</sub> (s)</b>	184.5 $\pm$ 67.3	92.5 $\pm$ 11.2	182.4 $\pm$ 61.59	75.1 $\pm$ 25.1
<b><math>\tau_s</math> (s)</b>	105.6 $\pm$ 31.5	70.2 $\pm$ 32.8	179.1 $\pm$ 56.9	55.9 $\pm$ 15.3 <sup>¥</sup>
<b>G<sub>s</sub> (%<math>\cdot</math>W<sup>-1</sup>)</b>	0.065 $\pm$ 0.024	0.052 $\pm$ 0.022	0.04 $\pm$ 0.04	0.04 $\pm$ 0.02
<b>G<sub>o</sub> (%<math>\cdot</math>W<sup>-1</sup>)</b>	0.18 $\pm$ 0.10	0.17 $\pm$ 0.07	0.19 $\pm$ 0.05	0.16 $\pm$ 0.05 <sup>¥</sup>
<b>EEmOxy (%)</b>	31.0 $\pm$ 19.9	7.8 $\pm$ 8.4 <sup>¥</sup>	35.6 $\pm$ 12.2	32.3 $\pm$ 15.0 <sup>¥</sup>
<b>wMRT (s)</b>	47.6 $\pm$ 20.2	37.6 $\pm$ 18.2	64.4 $\pm$ 37.2	34.5 $\pm$ 12.3

A<sub>s</sub> = Slow Component Amplitude

G<sub>s</sub> = Slow Component Gain

wMRT= Weighted Mean Response Time

TD<sub>s</sub> = Slow Component Time Delay

G<sub>o</sub> = Overall Gain

$\tau_s$  = Slow Component Time Constant

EEmOxy = End-Exercise mOxy

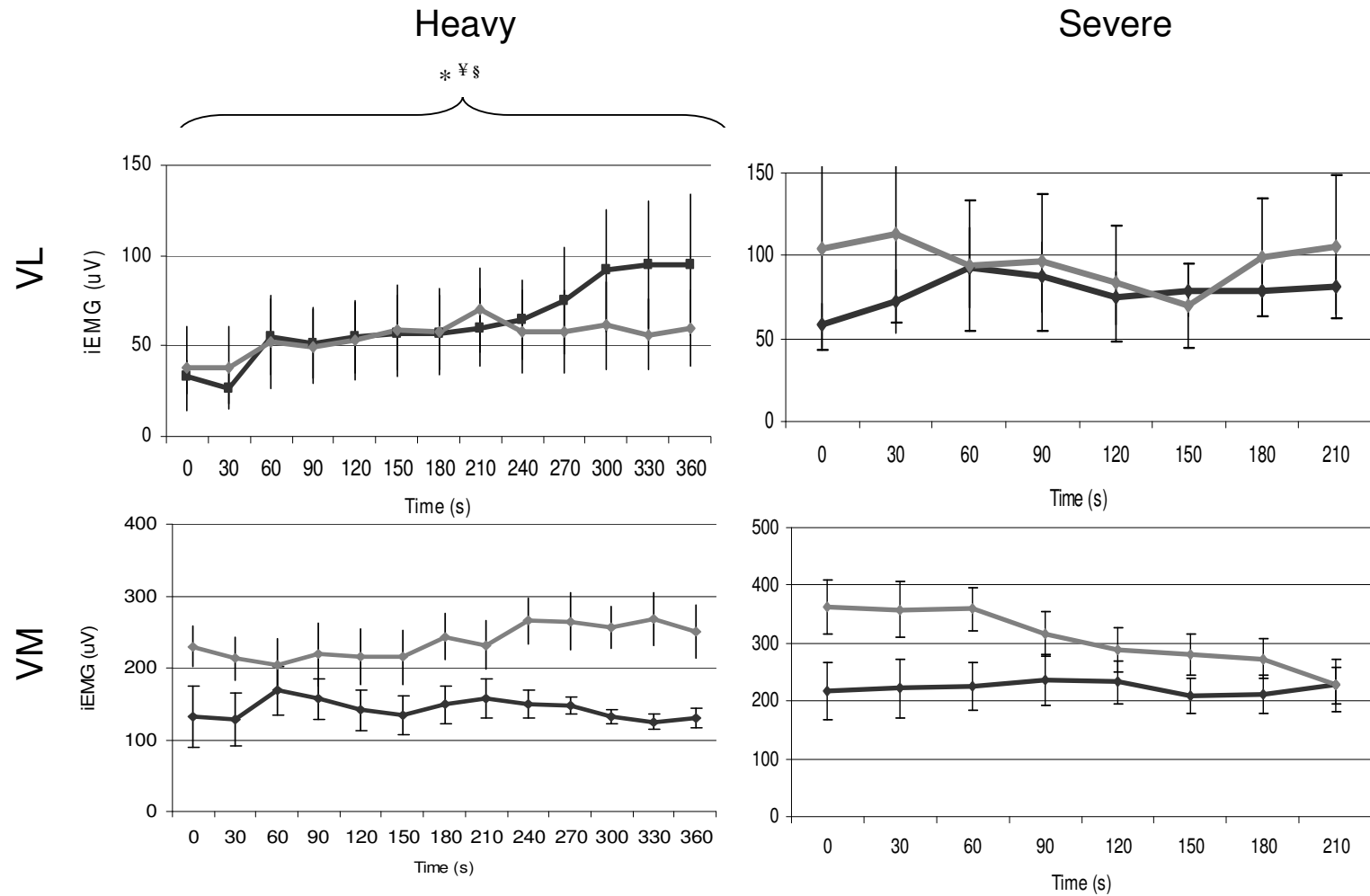
<sup>¥</sup> significant difference between heavy and severe-intensities ( $p < 0.05$ ); <sup>€</sup> no subject completed the six minute severe-intensity SWT.

## Electromyographic Responses

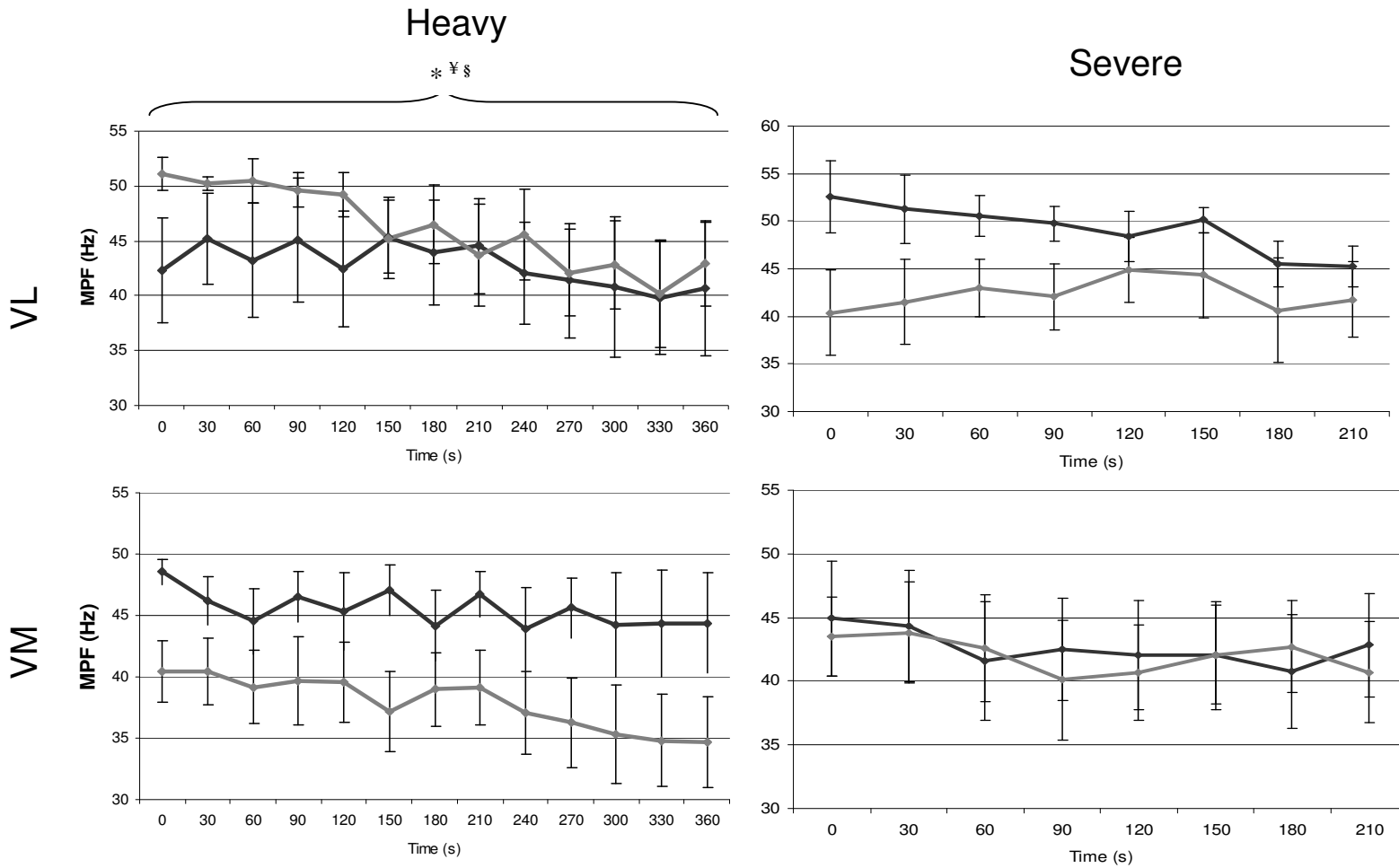
The mean ( $\pm$  SEM) changes in the iEMG responses on the VL and VM are shown in Figure 6.1. The mean ( $\pm$  SEM) changes in the MPF of the sEMG signal from the VL and VM across the two high-intensity SWT are shown in Figure 6.2.

The majority of sEMG parameters demonstrated no significant effect of age, with only the iEMG response of the VM during the heavy-intensity SWT being significantly different between the two age-groups ( $F(1,12)=5.464$ ,  $p=0.039$ ,  $\eta^2=0.332$ ). No significant age  $\times$  time interactions were observed in the iEMG or MPF response from the VL and VM. However, a significant main effect of time was observed for the changes in the increasing iEMG response of the VL during the heavy-intensity ( $F(12,144)=4.674$ ,  $p<0.001$ ,  $\eta^2=0.280$ ) SWT. Significant main effects of time were also observed in decreases in the MPF of the VL during the heavy-intensity SWT ( $F(12,144)=3.467$ ,  $p<0.001$ ,  $\eta^2=0.224$ ).

No significant changes were observed in the iEMG of the VM across either SWT intensity. The middle-aged cyclists demonstrated a significant increase across of time ( $F(12,72)=3.602$ ,  $p<0.001$ ,  $\eta^2=0.375$ ) in the iEMG of the VL during the heavy-intensity SWT. The young cyclists demonstrated a significant decrease across time in the MPF of VL during the heavy-intensity SWT ( $F(12,72)=3.832$ ,  $p<0.001$ ,  $\eta^2=0.390$ ).



**Figure 6.1:** Mean ( $\pm$  SEM) iEMG responses from the vastus lateralis (VL) (top) and vastus medialis (VM) (bottom) during the heavy- (left) and severe-intensity(right) SWT in the young (  $\circ$  ) and middle-aged (  $\bullet$  ) cyclists (\* significant effect of age ( $p < 0.05$ ); ¥ significant main effect of time in vastus lateralis ( $p < 0.05$ ); § significant increase across time in vastus lateralis of middle-aged cyclists ( $p < 0.05$ )).



**Figure 6.2:** Mean ( $\pm$  SEM) MPF responses from the vastus lateralis (VL) (top) and vastus medialis (VM) (bottom) during the heavy- (left) and severe-intensity (right) square wave transition in the young (—■—) and middle-aged (—■—) cyclists (\* significant effect of age ( $p < 0.05$ ); ¥ significant main effect of time in vastus lateralis ( $p < 0.05$ ); § significant decrease with time in vastus lateralis of young cyclists ( $p < 0.05$ )).

## DISCUSSION

The purpose of Study Three was to examine the effect of age on the development of the  $\dot{V}O_2$  and mOxy slow components observed across heavy- and severe-intensity SWT in well-trained cyclists. The present study is the first to investigate the effect of age on the development of these slow components during high-intensity exercise in well-trained cyclists.

The results of the present study demonstrated no significant effect of age or age x intensity interactions in either the  $\dot{V}O_2$  or mOxy slow components. Furthermore, the present results demonstrated no significant relationships between the  $\dot{V}O_2$  or mOxy slow components and changes in the hematological (blood pH,  $pO_2$ ,  $[HCO_3^-]$ ,  $[BLa^-]$ ) or neuromuscular (iEMG, MPF) parameters across the two high-intensity SWT. Finally, no significant relationships were observed between the development of the slow components and the muscle histochemical or enzymatic characteristics of the VL in either age group. Therefore, the present study suggests that the nature of the  $\dot{V}O_2$  and mOxy slow component responses and possible causal mechanisms are similar in matched young and middle-aged cyclists.

At present, the physiological mechanisms responsible for the development of the  $\dot{V}O_2$  slow component are not fully understood. A number of possible causal mechanisms have been hypothesised to be responsible for the observed decrease in mechanical or metabolic efficiency during prolonged high-intensity exercise. These factors may include increases in physiological factors such as muscle temperature, adrenaline, ventilation or  $[BLa^-]$ , or

changes in muscle fibre-specific recruitment patterns (Poole 1994; Poole et al. 1994; Whipp 1994; Gaesser and Poole 1996; Saunders et al. 2000).

Although the exact underlying mechanisms of the slow component remain to be elucidated, it is widely accepted that the majority of this decrease in metabolic efficiency and slow component development occurs within the working muscle (Poole 1994; Demarie et al. 2001). In support of this suggestion, previous evidence has reported that the nature of the  $\dot{V}O_2$  slow component is influenced by physiological characteristics such as  $\dot{V}O_{2\max}$  (Gaesser and Poole 1996; Carter et al. 2000a) and both muscle fibre composition and CSA (Barstow et al. 1996; Pringle et al. 2003b). To date, little research has examined these characteristics and their relationship to the development of the mOxy slow component. Further, no research has examined this relationship in older well-trained athletes. Therefore, it is plausible to suggest that the  $\dot{V}O_2$  and mOxy slow components are maintained in well-trained middle-aged cyclists to similar levels of a matched younger cohort. This is most likely a result of the similar physiological and performance characteristics between the two age groups.

#### *Slow Component Amplitude Responses*

In the present study, no significant effects of age, intensity or age x intensity interaction were demonstrated in the  $\dot{V}O_2$  or mOxy  $A_s$  across the heavy and severe-intensity SWT. This finding suggests that physical training into middle-age can maintain the  $\dot{V}O_2$  and mOxy  $A_s$ , and prevent the decline observed with sedentary aging (Chick et al. 1991; Scheuermann et al. 2002; Sabapathy et al. 2004). The  $\dot{V}O_2$   $A_s$  measured in the present study

(Y:  $\sim 550 \text{ mL} \cdot \text{min}^{-1}$ ; MA:  $\sim 650 \text{ mL} \cdot \text{min}^{-1}$ ) was similar to that observed in previous investigations reporting on both young (Scheuermann et al. 2001; Russell et al. 2002) and older (Sabapathy et al. 2004) populations. The mOxy  $A_s$  was difficult to compare to existing literature, given the different methods used to measure this parameter between investigations (e.g. difference between 6<sup>th</sup> and 3<sup>rd</sup> minutes vs. separate exponential function) (Demarie et al. 2001).

The absence of a significant effect of age in  $\dot{V}O_2$  and mOxy  $A_s$  is most likely due to the similar physiological and muscle histochemical characteristics of the well-trained cyclists reported in Study One of the present series of investigations. Despite the majority of previous literature suggesting that the muscle fibre composition of the working muscle has a primary influence on the development of the slow component (Barstow et al. 1996; Pringle et al. 2003b; Garland et al. 2004; Krstrup et al. 2004b), no such effect was observed in the present study.

The reported influence of muscle fibre composition on the slow component is thought to be related to the changes within the metabolic environment in the working muscle across high-intensity constant-load exercise (Poole 1994). The similar changes in blood pH measures across the high-intensity exercise in the young and middle-aged cyclists of the present study may suggest that the magnitude of anaerobic metabolism was comparable between age groups. These changes in blood pH may have resulted from an exhausted aerobic metabolism and an increase in the magnitude of anaerobic metabolic contribution required to match the energetic demands of the high-intensity exercise (Barstow et al. 1993).

Surprisingly, no significant correlations were observed between the  $\dot{V}O_2$  and mOxy slow components and the hematological responses in the present study. This finding contrasts those of previous investigations which have observed such relationships across similar exercise intensities as those used in the present study (Demarie et al. 2001). This difference between studies may be the result of the well-trained nature of the two cohorts in the current study. Therefore, the present data suggests that there is no significant relationship between the magnitude of anaerobic metabolism and the amplitude of the  $\dot{V}O_2$  and mOxy slow components. Such an absence of significant relationship was also observed with the speed of development of the two slow components.

#### *Slow Component Speed Responses*

Within the present study, the  $\dot{V}O_2$  and mOxy  $TD_s$  demonstrated no significant effect of age or age x intensity interaction. Previous research has reported a significant effect of age on the  $\dot{V}O_2$   $TD_s$  during heavy-intensity exercise in sedentary populations (Sabapathy et al. 2004). However, to date no such data are available on the effect of age on the mOxy slow component, or in older well-trained subjects. Both the young (Y) and middle-aged (MA) cyclists examined in the present study demonstrated similar  $\dot{V}O_2$   $TD_s$  (Y:  $127.7 \pm 23.8$  s; MA:  $85.7 \pm 29.6$  s) to that reported by Sabapathy and colleagues (2004) for young ( $21.7 \pm 0.9$  y) sedentary subjects ( $118 \pm 10$  s). However, the elderly ( $71.6 \pm 0.8$  y) subjects in this previous study demonstrated a considerably longer  $\dot{V}O_2$   $TD_s$  ( $178 \pm 14$  s) than the middle-aged cyclists in the present study (Sabapathy et al. 2004). This difference may be due to previously reported and age-related physiological changes of the elderly cohort (Sabapathy et al. 2004). This finding may suggest that if age-related changes in muscle fibre

composition are offset through physical training, then the development of the slow components may not be subject to a significant effect of aging.

In the present study, the  $\dot{V}O_2$  and mOxy  $\tau_s$  demonstrated no significant effect of age or age x intensity interaction. This finding further supports the origin of the slow component being within the working muscle during high-intensity constant-load exercise. Limited previous research has examined the  $\dot{V}O_2$  and mOxy  $\tau_s$  across high-intensity constant-load exercise in any population (Carter et al. 2002). Carter and colleagues (2002) reported that the average  $\dot{V}O_2$   $\tau_s$  ranged between 220-290 s for supra-threshold exercise intensities between 20-100% $\Delta$  in a young ( $27 \pm 7$  y) male population. In contrast, the present study is the first to examine the effect of exercise intensity on the mOxy  $\tau_s$  in any population. In the present study, the young and middle-aged cyclists demonstrated similar  $\dot{V}O_2$  and mOxy  $\tau_s$  across the heavy- (Y:  $105.8 \pm 35.0$  s; MA:  $111.3 \pm 42.1$  s) and severe-intensity (Y:  $62.4 \pm 21.8$  s; MA: 62.3 s) SWT. This may again reflect the similar physiological, muscle histochemical and enzymatic characteristics reported in the two age groups in Study One. These physiological and muscle characteristics have consistently been shown to be related to the observed changes in metabolic efficiency and the slow component across bouts of high-intensity constant-load exercise (Poole 1994; Poole et al. 1994; Whipp 1994; Gaesser and Poole 1996; Saunders et al. 2000).

The  $\dot{V}O_2$  and mOxy  $\tau_s$  responses both demonstrated effects of intensity in the well-trained young and middle-aged cyclists in the present study. The young cyclists demonstrated a significantly shorter  $\dot{V}O_2$   $\tau_s$  in the severe-

intensity compared to the heavy-intensity in the present study. However, this was not observed in the middle-aged cyclists. This observation should be taken with caution given that only one middle-aged cyclist demonstrated a  $\dot{V}O_2$  slow component during the severe-intensity SWT, which made statistical comparisons difficult. In contrast, the mOxy  $\tau_s$  demonstrated a significant effect of intensity between the heavy (Y:  $105.6 \pm 31.5$  s; MA:  $179.1 \pm 56.9$  s) and severe-intensity (Y:  $70.2 \pm 32.8$  s; MA:  $62.4 \pm 21.8$  s) SWT in the middle-aged cyclists. Therefore, it appears that the controlling mechanisms associated with the development of the slow components may be influenced by exercise intensity.

The different  $\dot{V}O_2$  and mOxy responses across the severe-intensity SWT are most likely due to the increased anaerobic energetic demands in the well-trained cyclists of the current study. This near-maximal SWT intensity may have facilitated greater disturbance in the metabolic environment and faster fibre-specific fatigue of the working muscle prior to fatigue. In comparison to pasta literature, the slower  $\dot{V}O_2$  and mOxy slow components observed in the present study may be a result of the difference in the SWT relative intensity, and the ability of the cyclists to complete the heavy-intensity SWT. During the heavy-intensity SWT, the proposed changes within the metabolic environment and recruitment patterns of the working muscle may not have occurred as quickly as that in the severe-intensity SWT. As such, it is likely that the magnitude of anaerobic metabolism and shifts in muscle fibre recruitment patterns may be augmented with increasing exercise intensity. This was supported by the changes in several hematological parameters in the present study. This difference in the energetic responses in response to the two work intensities

may be reflected as a significant effect of intensity in the development of the  $\dot{V}O_2$  and mOxy slow components. However, the present data contrast previous literature suggesting that the  $\dot{V}O_2 \tau_s$  remains stable across increasing high-intensity work rates (Carter et al. 2002). The present data are the first to report upon a significant effect of intensity on the mOxy slow component, with the severe-intensity mOxy slow component developing significantly faster than that observed during heavy-intensity slow component. However, this may reflect the elevated  $[BLa]$  prior to commencing the severe-intensity SWT in the present study, which may have helped  $O_2$  unloading within the muscle and a faster decrease in efficiency. Alternatively, it may suggest differences in the energy metabolism requirements of the working muscle between the heavy and severe intensities.

The results of Study Three suggested no significant effect of age in the  $\dot{V}O_2$  or mOxy slow components observed in the well-trained cyclists across the high-intensity exercise bouts (Sabapathy et al. 2004). Past investigations have suggested that the development of the  $\dot{V}O_2$  slow component during high-intensity constant-load exercise is slowed with sedentary aging, whereas the present investigation did not report such an effect in the well-trained middle-aged cyclists. The effect of intensity observed in the speeding of development of the  $\dot{V}O_2$  and mOxy slow components suggests that the causal mechanisms lie within gradual changes in the energetic processes within the actual working muscle. In summary, no significant relationships were observed between the cyclist's physical characteristics and the development of the slow components in the present study. This similar absence of relationships between the two age

groups most likely reflects their similar  $\dot{V}O_2$ max and muscle histochemical and enzymatic characteristics

### *Slow Component Physiological Mechanisms*

Previous investigations have suggested the slow components are developed due to increases in muscle temperature, ventilation, adrenaline or  $[BLa^-]$  (Poole et al. 1994; Gaesser and Poole 1996; Zoladz and Korzeniewski 2001). More recent studies have suggested that a decrease in efficiency of the working muscle may due in part to shifts in fibre-type recruitment during high-intensity constant-load exercise (Saunders et al. 2000; Borrani et al. 2001; Krustrup et al. 2004b; Sabapathy et al. 2005). However, in contrast to this suggestion, the present data observed no significant correlations between the  $\dot{V}O_2$  or mOxy slow components and any changes in the hematological parameters or muscle fibre composition and recruitment patterns across the high-intensity work bouts.

At present, the two most plausible physiological explanations for the  $\dot{V}O_2$  and mOxy slow components appear to be muscle fibre composition (Barstow et al. 1996; Pringle et al. 2003b; Krustrup et al. 2004b) and changes in fibre-specific recruitment patterns (Saunders et al. 2000; Borrani et al. 2001; Krustrup et al. 2004b; Sabapathy et al. 2005). As such, the VL muscle has been identified to be of practical importance for researchers examining the  $\dot{V}O_2$  slow component in cycling, given it is a prime mover of the cycle stroke and its activity is linearly related to cycling intensity (Jorge and Hull 1986; Akima, Kinugasa and Kuno 2005; Raymond et al. 2005). The development of the  $\dot{V}O_2$  slow component has been previously related to the muscle fibre composition

and recruitment patterns of the VL in numerous investigations (Barstow et al. 1996; Saunders et al. 2000; Borrani et al. 2001; Pringle et al. 2003b; Krstrup et al. 2004b; Sabapathy et al. 2005). The muscle fibre composition appears to influence the metabolic processes within the working muscle during the high-intensity exercise bouts.

The data from the present study did not support a relationship between muscle fibre composition and the development of the  $\dot{V}O_2$  and mOxy slow component. Previously, the  $\dot{V}O_2$  slow component has been associated with gradual increases in the phosphate cost of high-intensity exercise, which may occur as a result of decreases in muscle oxidative capacity and Type I fibres, or changes in the fibre-specific recruitment patterns during high-intensity exercise (Rossiter et al. 2001). No such relationships were observed between the development of the  $\dot{V}O_2$  and mOxy slow components and any muscle histochemical characteristics in the present study. This contrasts with previous investigations that have reported significant inverse correlations between the  $\dot{V}O_{2A_s}$  and Type I fibre composition of the VL during heavy- and severe-intensity cycling (Barstow et al. 1996; Pringle et al. 2003b). The Type I fibre composition of the working muscle may be representative of oxidative capacity of the muscle, given their preferable functional and metabolic characteristics for aerobic metabolism (He et al. 2000). Since Type I muscle fibres possess such higher oxidative capacities and enzyme activities, myoglobin concentrations and capillarisation, it is plausible that these previously reported effects of muscle fibre composition may be related to the development of both the  $\dot{V}O_2$  and mOxy slow components. Given that the current data did not lend itself to

this hypothesis, further research is required to support the relationship between muscle fibre composition and the development of the slow component.

The significant relationship previously reported between the  $\dot{V}O_2$  slow component development and muscle fibre composition (Barstow et al. 1996; Pringle et al. 2003b; Krstrup et al. 2004b) may suggest that individuals that possess lower oxidative potential (i.e. higher Type II fibre composition) within working muscles are likely to demonstrate an augmented  $\dot{V}O_2$  slow component. The significance of muscle fibre composition to the development of the  $\dot{V}O_2$  slow component may explain the previously observed effect of sedentary aging on the  $\dot{V}O_2 A_s$  and  $\tau_s$  (Sabapathy et al. 2004). While no definitive mechanism has been identified for this effect of age in the  $\dot{V}O_2$  slow component, it is possible that it is related to the muscle fibre-specific atrophy reported to occur in sedentary aged populations typically older than that recruited for the present investigation (Deschenes 2004).

Apart from the influence of muscle fibre composition, the consensus of previous research suggests that the  $\dot{V}O_2$  slow component is also developed as a result of Type I fibre fatigue and the subsequent gradual recruitment of the less aerobic and less efficient Type II fibres during high-intensity constant-load exercise (Saunders et al. 2000; Borrani et al. 2001; Krstrup et al. 2004a; 2004b). This change in the muscle fibre recruitment patterns is commonly reported through changes in both muscle activity (iEMG) and recruitment frequencies (MPF) of the working muscle (Miura et al. 1999; Saunders et al. 2000; Borrani et al. 2001; Krstrup et al. 2004a; 2004b). The iEMG and MPF responses of the VL have previously been shown to be significantly related to

both the  $\dot{V}O_2$  (Saunders et al. 2000; Borrani et al. 2001; Krstrup, et al. 2004a; Krstrup et al. 2004b) and mOxy slow components (Miura et al. 1999; Demarie et al. 2001). However, many investigations have not observed this relationship between changes in muscle fibre recruitment patterns as either iEMG or MPF measures and the development of the  $\dot{V}O_2$  slow component (Scheuermann et al. 2001; Cleuziou et al. 2004).

The present study did not show any significant effect of age in either the iEMG or MPF responses during heavy or severe-intensity SWT. Thus, the results of the present study contrast previous investigations that have reported significant relationships between the  $\dot{V}O_2$ , mOxy and EMG responses across high-intensity constant-load exercise in young healthy males (Miura et al. 1999; Saunders et al. 2000; Demarie et al. 2001). However, the present study observed significant effects of time in both the iEMG and MPF of the VL during the heavy-intensity SWT in both the young and middle-aged cyclists, respectively.

The observed changes in neuromuscular activity of the VL and VM muscles during the heavy-intensity SWT of the present study are suggestive of Type II fibre fatigue and a resultant increase in Type I fibre recruitment. The reported changes in fibre recruitment patterns contrasts that of previous investigations which have proposed that the  $\dot{V}O_2$  slow component is due to Type I fibre fatigue and increases in Type II fibre recruitment (Saunders et al. 2000; Borrani et al. 2001; Krstrup et al. 2004b). However, given the high relative power output of the heavy-intensity SWT (50% $\Delta$ ), it is possible that at the commencement of the high-intensity SWT, a large number of Type II fibres

are recruited to overcome this change in work rate and to maintain the constant power output required. As Type II fibres possess relatively lower oxidative capacities than Type I fibres, they may have fatigued more quickly, as demonstrated by increases in anaerobic metabolism and decreases in metabolic efficiency. The Type II fibre fatigue may have facilitated an increased dependence on Type I fibres to sustain the SWT power output (Bottinelli and Reggiani 2000). Due to their smaller force production capacities, a greater number of Type I fibres may have then been recruited in order to sustain the power output across the high-intensity SWT. These proposed changes in the fibre-specific recruitment patterns are represented as increases in iEMG and decreases in the MPF of the working muscles across the high-intensity SWT, both of which were observed in the present study across the two high-intensity SWT.

This alternative hypothesis of fibre-specific recruitment patterns is also supported by the current study's finding of a non-significant decrease in the MPF of the VL and VM across the high-intensity SWT. Type I fibres are innervated by smaller motor neurons and possess lower threshold frequencies than Type II fibres, as demonstrated through reductions in the MPF of the recruited muscles (De Luca 1985). While Type I muscle fibres possess greater metabolic efficiency and oxidative capacities, their continued recruitment may facilitate decreases in metabolic efficiency across sustained high-intensity exercise (He et al. 2000). He and others (2000) have reported that Type I and II muscle fibres possess similar thermodynamic efficiencies during repeated contractions, but their optimal efficiency occurs at significantly different power outputs and contraction speeds. Therefore, taking into account the high relative

intensities and cadences of the high-intensity SWT in the present study, it might be suggested that the Type I fibres were recruited outside their range of optimal efficiency. This may have been observed as a gradual increase in  $\dot{V}O_2$  and decrease in mOxy across the sustained high-intensity exercise bouts.

This physiological hypothesis may contribute to a greater understanding of the mechanisms underlying the  $\dot{V}O_2$  and mOxy slow components across such high-intensity work bouts. Therefore, while no observed effect of age was demonstrated in the iEMG or MPF responses in the present study, these results offer a novel alternative to explain the development of the  $\dot{V}O_2$  and mOxy slow components.

While the present study's sEMG responses differ to that reported within previous investigations (Saunders et al. 2000; Borrani et al. 2001; Krstrup et al. 2004b; Sabapathy et al. 2005), this may be the result of the novel method of sEMG analysis used within the present investigation. In the present study, the iEMG and MPF analysis of the raw sEMG signal was only performed through the angular range of the crank cycle where the VL (315-110°) and VM (305-135°) are proposed to be recruited (Jorge and Hull 1986). It may be suggested that frequency spectral analysis across such a dynamic task as cycling may be greatly influenced through the inclusion of larger periods of inactivity of the isolated muscles than previous EMG studies have used (Cram et al. 1998). Further, the reporting of the specific sEMG responses across the proposed recruitment range may also decrease the effect of muscle crosstalk and noise (Cram et al. 1998). The present iEMG and MPF responses from the VL and VM are therefore representative of the muscle activity through the narrow muscle-

specific angular recruitment range of the VL and VM muscles from the well-trained young and middle-aged cyclists. This angular-specific analysis technique provides novel and mechanistic information and thus adds to the body of literature attempting to identify the causal mechanisms of the  $\dot{V}O_2$  and mOxy slow components.

## **SUMMARY**

In conclusion, the results of Study Three demonstrated that the  $\dot{V}O_2$  and mOxy slow components were not significantly influenced by age in well-trained cyclists matched for  $\dot{V}O_{2\max}$  and muscle histochemical and enzymatic characteristics. To the researcher's knowledge, the present study is the first to demonstrate that concurrent aging and physical training maintains the  $\dot{V}O_2$  and mOxy slow components into middle-age compared to a similarly trained younger cohort.

The current results contrast with previous data that have reported a significant effect of sedentary aging on the  $\dot{V}O_2$  slow component (Sabapathy et al. 2004), and thus suggest that physical training into older age may maintain energy metabolism and potential slow component causal mechanisms within the peripheral muscle. The absence of a significant effect of age on the  $\dot{V}O_2$  and mOxy slow components may be the result of the similar physiological and muscle characteristics reported for the young and middle-aged cyclists. The differences in the speed of the  $\dot{V}O_2$  and mOxy slow components suggest that the causal mechanism of the slow component is related to exercise intensity, and changes with subsequent energetic metabolism within the working muscle.

Despite the proposed developmental role of the working muscle on the slow component, the present study did not observe any significant relationships between the slow component development and muscle histochemical characteristics. Previous investigations have suggested that the increased recruitment of Type II fibres may be responsible for the development of the slow component (Miura et al. 1999; Saunders et al. 2000; Demarie et al. 2001). The observed trends in the sEMG responses in the present study contrast previous findings, and suggest an increased recruitment of Type I fibres during prolonged high-intensity constant-load exercise in both age groups. The present study failed to identify any definitive mechanism responsible for the  $\dot{V}O_2$  and mOxy slow components. In summary, the development of the  $\dot{V}O_2$  and mOxy slow components appears to be maintained in well-trained young and middle-aged cyclists, matched for  $\dot{V}O_{2\max}$  and muscle histochemical and enzymatic characteristics.

## CHAPTER 7

### STUDY 4

#### **Off-transient $\dot{V}O_2$ and mOxy responses following moderate-, heavy- and severe-intensity exercise in well-trained young and middle-age cyclists**

##### OVERVIEW

The purpose of Study Four was to examine the effect of age on the off-transient  $\dot{V}O_2$  and mOxy responses following moderate-, heavy- and severe-intensity SWT in well-trained cyclists. The results of Study Four suggest no significant effect of age on the off-transient  $\dot{V}O_2$  or mOxy responses of well-trained cyclists. This absence of a significant effect of age may be explained by the similar relative SWT intensities,  $\dot{V}O_{2max}$  and/or muscle histochemical and enzymatic characteristics of the two age groups.

The results of Study Four demonstrated significant effects of intensity in the end-exercise amplitude ( $EE\dot{V}O_2$ ),  $A_f$ , and  $\tau_f$  of the  $\dot{V}O_2$  response. In addition, significant effects of intensity were also observed in the off-transient  $EE\text{mOxy}$  and mOxy  $A_f$ . Furthermore, a small number of significant relationships were observed between the off-transient  $\dot{V}O_2$  and mOxy responses and several hematological measures taken at the completion of the three SWT intensities. This study also observed several significant relationships between the off-transient  $\dot{V}O_2$  and mOxy responses and the muscle histochemical and

enzymatic characteristics of the well-trained cyclists. Therefore, the major finding of Study Four was the absence of a significant effect of age in the off-transient  $\dot{V}O_2$  or mOxy responses following the three SWT intensities in well-trained matched cyclists.

## RESULTS

### Off-Transient $\dot{V}O_2$ Responses

Table 7.1 over the page summarises the off-transient  $\dot{V}O_2$  kinetic parameters for each age group across the three SWT intensities.

No main effect of age or age x intensity interaction was observed for the EE $\dot{V}O_2$  in the present study. However, a significant increasing main effect of intensity ( $F(2,24)=187.613$ ,  $p<0.001$ ,  $\eta^2=0.940$ ) was observed on the EE $\dot{V}O_2$ . RMANOVA revealed a similar effect in the separated young ( $F(2,12)=106.62$ ,  $p<0.001$ ,  $\eta^2=0.947$ ) and middle-aged ( $F(2,12)=84.631$ ,  $p<0.001$ ,  $\eta^2=0.934$ ) cyclists. In the young cyclists, the EE $\dot{V}O_2$  for the heavy- ( $F(2,12)=106.62$ ,  $p<0.001$ ,  $\eta^2=0.947$ ) and severe-intensity ( $F(2,12)=106.62$ ,  $p<0.001$ ,  $\eta^2=0.947$ ) exercise was significantly higher than that following the moderate-intensity SWT. Similar findings were observed in the middle-aged cyclists between the EE $\dot{V}O_2$  of the moderate and heavy-intensity ( $F(2,12)=84.631$ ,  $p<0.001$ ,  $\eta^2=0.934$ ) SWT, as well as the severe-intensity ( $F(2,12)=84.631$ ,  $p<0.001$ ,  $\eta^2=0.934$ ) SWT.

**Table 7.1:** Mean ( $\pm$  SD) time and amplitude values of the off-transient  $\dot{V}O_2$  response of the young and middle-aged cyclists following the three square wave transition intensities.

	Young			Middle-Aged		
	Moderate	Heavy	Severe	Moderate	Heavy	Severe
<b><math>EE\dot{V}O_2</math> (<math>\text{mL}\cdot\text{min}^{-1}</math>)</b>	2469 $\pm$ 363	3487 $\pm$ 510 <sup>#</sup>	3405 $\pm$ 418 <sup>§</sup>	2623 $\pm$ 239	3648 $\pm$ 345 <sup>#</sup>	3636 $\pm$ 363 <sup>§</sup>
<b><math>A_f</math> (<math>\text{mL}\cdot\text{min}^{-1}</math>)</b>	1468 $\pm$ 287	2288 $\pm$ 375 <sup>#</sup>	2147 $\pm$ 305 <sup>§</sup>	1581 $\pm$ 254	2398 $\pm$ 308 <sup>#</sup>	2318 $\pm$ 370 <sup>§</sup>
<b><math>TD_f</math> (s)</b>	17.9 $\pm$ 4.8	20.3 $\pm$ 7.5	16.8 $\pm$ 6.5	14.5 $\pm$ 5.4	19.7 $\pm$ 3.5	15.4 $\pm$ 6.5
<b><math>\tau_f</math> (s)</b>	35.3 $\pm$ 5.3	41.2 $\pm$ 5.0	52.5 $\pm$ 11.9 <sup>§</sup>	35.0 $\pm$ 7.9	40.8 $\pm$ 6.7	56.7 $\pm$ 16.8 <sup>§</sup>
<b><math>wMRT_f</math> (s)</b>	53.2 $\pm$ 6.5	61.5 $\pm$ 10.9	69.3 $\pm$ 6.0 <sup>§</sup>	49.5 $\pm$ 9.3	60.5 $\pm$ 7.4	72.1 $\pm$ 18.2 <sup>§</sup>

$EE\dot{V}O_2$  = End-exercise  $\dot{V}O_2$   
 $\tau_f$  = Off-Transient Time Constant

$A_f$  = Off-Transient Amplitude  
 $wMRT_f$  = Off-Transient Weighted Mean Response Time

$TD_f$  = Off-Transient Time Delay

<sup>#</sup> significant difference between moderate and heavy-intensities ( $p < 0.05$ ); <sup>§</sup> significant difference between moderate and severe-intensities ( $p < 0.05$ ).

No significant effect of age or age x intensity interaction was observed on the  $\dot{V}O_2 A_f$  in the present study. However, a significant main effect of intensity ( $F(2,24)=81.906$ ,  $p<0.001$ ,  $\eta^2=0.872$ ) was observed for the  $\dot{V}O_2 A_f$ . The  $\dot{V}O_2 A_f$  was significantly different in both the young and middle-aged cyclists between the moderate and heavy-intensity SWT [Y: ( $F(2,12)=62.813$ ,  $p<0.001$ ,  $\eta^2=0.913$ ); MA: ( $F(2,12)=30.865$ ,  $p=0.001$ ,  $\eta^2=0.837$ )], as well as between the moderate and severe-intensity SWT [Y: ( $F(2,12)=62.813$ ,  $p<0.001$ ,  $\eta^2=0.913$ ); MA: ( $F(2,12)=30.865$ ,  $p<0.001$ ,  $\eta^2=0.837$ )]. Furthermore, the  $\dot{V}O_2 A_f$  was significantly correlated to the  $\dot{V}O_2 A_p$  of the heavy-intensity SWT ( $r= 0.92$ ,  $p= 0.03$ ) in the young cyclists, but not in the middle-aged cohort ( $r= -0.37$ ,  $p= 0.41$ ).

No significant main effects of age, intensity or age x intensity interaction were observed for the  $\dot{V}O_2 TD_f$  in the young and middle-aged cyclists. Moreover, no significant main effect of age or age x intensity interaction was observed in the  $\dot{V}O_2 \tau_f$ . However, a significant main effect of intensity ( $F(2,24)=12.406$ ,  $p<0.001$ ,  $\eta^2=0.508$ ) was observed for  $\dot{V}O_2 \tau_f$  which was demonstrated in the separated young ( $F(2,12)=6.794$ ,  $p=0.011$ ,  $\eta^2=0.556$ ) and middle-aged subjects ( $F(2,12)=6.058$ ,  $p=0.015$ ,  $\eta^2=0.561$ ). The moderate-intensity  $\dot{V}O_2 \tau_f$  was significantly shorter than the values measured during the severe-intensity SWT in both the young ( $F(2,12)=6.794$ ,  $p=0.027$ ,  $\eta^2=0.556$ ) and middle-aged ( $F(2,12)=6.058$ ,  $p=0.022$ ,  $\eta^2=0.561$ ) cyclists.

No significant effect of age or age x intensity interaction was observed for  $\dot{V}O_2 wMRT_f$ . However, a significant main effect of intensity ( $F(2,24)=7.223$ ,  $p=0.004$ ,  $\eta^2=0.376$ ) was observed for  $\dot{V}O_2 wMRT_f$ . A significant effect of

intensity ( $F(2,12)=4.736$ ,  $p=0.030$ ,  $\eta^2=0.441$ ) was only observed in the  $\dot{V}O_2$   $wMRT_f$  of the middle-aged cyclists. *Post-hoc* analysis revealed that the moderate- and severe-intensity  $\dot{V}O_2$   $wMRT_f$  were significantly different ( $F(2,12)=4.736$ ,  $p=0.037$ ,  $\eta^2=0.441$ ) in this cohort.

### **Off-Transient mOxy Responses**

The off-transient mOxy kinetics for all three SWT intensities are summarised in Table 7.2 over the page.

No significant main effect of age or age x intensity interaction was observed for the EEmOxy. However, a significant main effect of intensity in the EEmOxy was observed in both the young ( $F(2,11)=49.295$ ,  $p<0.001$ ,  $\eta^2=0.891$ ) and middle-aged ( $F(2,11)=31.295$ ,  $p<0.001$ ,  $\eta^2=0.862$ ) cyclists.

While no significant main effect of age was observed for mOxy  $A_f$ , a significant age x intensity interaction was observed for the mOxy  $A_f$  ( $F(2,22)=10.139$ ,  $p=0.001$ ,  $\eta^2=0.480$ ). A significant main effect of intensity ( $F(2,22)=10.139$ ,  $p=0.001$ ,  $\eta^2=0.480$ ) was observed for the mOxy  $A_f$ . *Post-hoc* analysis revealed significant differences between the moderate and severe-intensity exercise in the young cyclists ( $F(2,11)=4.062$ ,  $p=0.041$ ,  $\eta^2=0.448$ ), and the moderate- and heavy-intensity SWT in the middle-aged cyclists ( $F(2,11)=9.232$ ,  $p=0.001$ ,  $\eta^2=0.606$ ). Similar to the  $\dot{V}O_2$  response, the mOxy  $A_f$  was significantly correlated to the on-transient mOxy  $A_p$  in both the young ( $r= 0.92$ ,  $p= 0.01$ ) and the middle-aged ( $r= 0.91$ ,  $p= 0.004$ ) cyclists following the heavy-intensity SWT.

**Table 7.2:** Mean ( $\pm$  SD) time and amplitude values of the off-transient mOxy response of the young and middle-aged cyclists following the three square wave transition intensities.

	Young			Middle-Aged		
	Moderate	Heavy	Severe	Moderate	Heavy	Severe
<b>EEmOxy (%)</b>	59.7 $\pm$ 14.1	31.0 $\pm$ 19.9 <sup>#</sup>	7.8 $\pm$ 8.4 <sup>\$¥</sup>	66.5 $\pm$ 10.5	35.6 $\pm$ 12.2 <sup>#</sup>	23.6 $\pm$ 13.5 <sup>\$ ¥</sup>
<b>A<sub>f</sub> (%)</b>	30.6 $\pm$ 14.0	39.2 $\pm$ 17.8	41.5 $\pm$ 16.1 <sup>\$</sup>	25.0 $\pm$ 8.9	49.6 $\pm$ 10.7 <sup>#</sup>	32.3 $\pm$ 15.0
<b>TD<sub>f</sub> (s)</b>	5.2 $\pm$ 2.0	5.6 $\pm$ 2.0	5.1 $\pm$ 1.8	5.3 $\pm$ 1.0	6.4 $\pm$ 3.9	4.3 $\pm$ 1.5
<b><math>\tau_f</math> (s)</b>	39.5 $\pm$ 10.5	42.4 $\pm$ 14.2	43.5 $\pm$ 9.3	34.2 $\pm$ 26.3	38.7 $\pm$ 9.1	43.4 $\pm$ 3.7
<b>A<sub>fs</sub> (%)</b>			26.1			27.5 $\pm$ 1.4
<b>TD<sub>fs</sub> (s)</b>			62.8			67.5 $\pm$ 2.7
<b><math>\tau_{fs}</math> (s)</b>			18.6			20.3 $\pm$ 2.0
<b>wMRT<sub>f</sub> (s)</b>	44.7 $\pm$ 10.1	48.0 $\pm$ 14.1	46.9 $\pm$ 25.1	36.5 $\pm$ 26.2	39.5 $\pm$ 26.2	80.2 $\pm$ 42.2 <sup>\$</sup>

EEmOxy= End-exercise mOxy  
 $\tau_f$  = Off-transient Time Constant  
 $\tau_{fs}$  = Slow Off-transient Time

A<sub>f</sub>= Off-transient Amplitude  
A<sub>fs</sub>= Slow Off-transient Amplitude  
wMRT<sub>f</sub> = Off-Transient Weighted Mean Response Time

TD<sub>f</sub>= Off-transient Time Delay  
TD<sub>fs</sub> = Slow Off-transient Time Delay      Constant

<sup>#</sup> significant difference between moderate and heavy-intensities (p<0.05); <sup>\$</sup> significant difference between moderate and severe-intensities (p<0.05); <sup>¥</sup> significant difference between heavy and severe-intensities (p<0.05); N.B: No off-transient slow component was observed for the moderate or heavy-intensity SWT.

No significant main effect of age, intensity or age x intensity interaction was observed in the mOxy  $TD_f$ ,  $\tau_f$  or  $wMRT_f$  in the present study. However, the mOxy  $TD_f$  was found to be significantly shorter than the  $\dot{V}O_2$  response in both age groups following all three SWT intensities.

#### **Correlations between $\dot{V}O_2$ and mOxy kinetics and hematological variables**

Significant correlations between the  $\dot{V}O_2$  and mOxy off-transient kinetics and the hematological variables are shown in Table 7.3.

#### **Correlations between $\dot{V}O_2$ and mOxy kinetics and muscle histochemical and enzymatic characteristics**

Significant correlations between the muscle histochemical and enzymatic characteristics and the off-transient  $\dot{V}O_2$  kinetic parameters are shown in Table 7.4a. Significant correlations observed between all the muscle histochemical and enzymatic characteristics and the off-transient mOxy kinetic measures are listed in Table 7.4b.

**Table 7.3:** Correlation coefficients (r) for the relationships between the amplitude and time parameters of the off-transient  $\dot{V}O_2$  responses following the moderate-, heavy- and severe-intensity square wave transitions and changes in hematological parameters in the young and middle-aged cyclists.

Young				Middle-Aged			
		r	p			r	p
Moderate-Intensity							
$\dot{V}O_2 \tau_f$	[HCO <sub>3</sub> <sup>-</sup> ] @ 6 min	-0.91	0.005	$\dot{V}O_2 MRT_f$	$pO_2$ @ 6 min	-0.86	0.014
	[BLa <sup>-</sup> ] @ 6 min	0.79	0.033		[HCO <sub>3</sub> <sup>-</sup> ] @ 6 min	-0.73	0.003
	[BLa <sup>-</sup> ] Δ3-6 min	0.79	0.034				
$\dot{V}O_2 MRT_f$	[BLa <sup>-</sup> ] @ 6 min	0.77	0.041				
	[BLa <sup>-</sup> ] Δ3-6 min	0.86	0.013				
Heavy-Intensity							
				$\dot{V}O_2 A_f$	[HCO <sub>3</sub> <sup>-</sup> ] @ 6 min	0.77	0.043
				$\dot{V}O_2 \tau_f$	[HCO <sub>3</sub> <sup>-</sup> ] Δ3-6 min	0.76	0.049

**Table 7.4a:** Correlation coefficients (r) for the relationships between the amplitude and time parameters of the off-transient  $\dot{V}O_2$  responses following the moderate-, heavy- and severe-intensity square wave transitions and histochemical and enzymatic characteristics of the young and middle-aged cyclists.

Young				Middle-Aged			
		r	p			r	p
Type IIa %	Heavy $\tau_f$	0.78	0.037	Type I %	Severe $EE\dot{V}O_2$	0.84	0.032
Capillary Density	Heavy $EE\dot{V}O_2$	-0.76	0.048	Type I CSA	Heavy $MRT_f$	0.95	0.005
	Heavy $A_f$	-0.78	0.037	Type IIa %	Heavy $MRT_f$	0.84	0.036
CS activity	Moderate $EE\dot{V}O_2$	-0.78	0.043	Type IIb %	Heavy $A_f$	-0.96	0.002
	Heavy $EE\dot{V}O_2$	-0.80	0.030		Severe $A_f$	-0.81	0.049
	Heavy $A_f$	-0.77	0.045	Type IIb CSA	Heavy $MRT_f$	0.89	0.018
	Severe $EE\dot{V}O_2$	0.87	0.011	Capillary Density	Heavy $MRT_f$	-0.82	0.025
	Severe $A_f$	-0.84	0.017				

**Table 7.4b:** Correlation coefficients (r) for the relationships between the amplitude and time parameters of the off-transient mOxy responses following the moderate-, heavy- and severe-intensity square wave transitions and histochemical and enzymatic characteristics of the young and middle-aged cyclists.

Young		Middle-Aged					
		r	p			r	p
Type I %	Heavy $\tau_f$	0.82	0.044	PFK Activity	Moderate TD <sub>f</sub>	-0.85	0.016
Type IIb %	Heavy MRT <sub>f</sub>	-0.92	0.010				
	Severe MRT <sub>f</sub>	-0.82	0.009				
Capillary Density	Severe EEmOxy	-0.82	0.046				
C:F Ratio	Severe $\tau_f$	0.92	0.009				
CC/F	Severe $\tau_f$	0.93	0.008				
	Severe wMRT <sub>f</sub>	0.83	0.043				
DD <sub>max</sub>	Severe $\tau_f$	0.93	0.006				
	Severe MRT <sub>f</sub>	0.82	0.048				
CS Activity	Moderate $\tau_f$	0.84	0.034				
2-OGDH Activity	Moderate MRT <sub>f</sub>	0.81	0.049				

## DISCUSSION

The purpose of Study Four was to examine the effect of age on the off-transient  $\dot{V}O_2$  and mOxy kinetics following moderate-, heavy- and severe-intensity SWT in well-trained cyclists. No effect of age was observed on the off-transient  $\dot{V}O_2$  or mOxy responses in the well-trained cyclists in the present study.

Little data has been published examining the recovery kinetics of the concurrent off-transient  $\dot{V}O_2$  and mOxy responses following three different exercise intensities (Puente-Maestu et al. 2003; duManoir et al. 2005). Few studies have examined the effect of age on these physiological recovery responses. From the available research, the rate of metabolic recovery following an exercise bout appears to be slowed with sedentary aging (Chick et al. 1991; Chilibeck et al. 1997; Ichimura, Murase, Osada, Kime, Homma, Ueda, Nagasawa, Motobe, Hamaoka and Katsumura 2006). In contrast, it has recently been suggested that the off-transient  $\dot{V}O_2$  and mOxy responses are improved with physical activity, despite aging (Ichimura et al. 2006). Therefore, the current study is the first to provide data on the effect of concurrent aging and physical training on the off-transient  $\dot{V}O_2$  and mOxy responses following moderate-, heavy- and severe-intensity exercise.

The present study demonstrated no significant effect of age on the off-transient  $\dot{V}O_2$  or mOxy responses examined following the three SWT intensities in the well-trained cyclists. The absence of a significant effect of age on the off-transient responses is most likely the result of the matching of the two age groups on their physiological characteristics (e.g.  $\dot{V}O_{2max}$ , muscle

histochemical characteristics) as reported in Study One. Furthermore, the similar hematological responses and relative SWT intensities reported in the earlier studies of the present series of investigations may also help to explain the absence of an effect of age on these responses (Borsheim and Bahr 2003).

The present findings both support and contrast a range of previously reported effects of exercise intensity on the off-transient  $\dot{V}O_2$  and mOxy responses (Bahr and Sejersted 1991; Bahr 1992; Borsheim and Bahr 2003; Puente-Maestu et al. 2003). The current data demonstrated significant effects of intensity in both the end-exercise amplitude and  $A_f$  in both the  $\dot{V}O_2$  and mOxy responses within both age groups. Similarly, the  $\dot{V}O_2 \tau_f$  and  $wMRT_f$ , as well as the mOxy  $TD_f$  significantly lengthened across increasing exercise intensities. To date, few studies have reported upon the effect of age on the concurrent off-transient  $\dot{V}O_2$  and mOxy responses following various exercise intensities (Puente-Maestu et al. 2003; duManoir et al. 2005).

#### *Off-Transient Amplitude Responses*

The off-transient  $\dot{V}O_2$  and mOxy responses help to quantify and model the return of energy metabolism to baseline values within the working muscle cell following exercise (Bahr and Sejersted 1991; Bahr 1992). In the present study, no significant effects of age were observed in either the  $\dot{V}O_2$  or mOxy end-exercise amplitude ( $EE\dot{V}O_2$ ;  $EEmOxy$ ) or  $A_f$  for any of the three SWT intensities. The absence of any significant effect of age within the two groups may reflect the similar physiological and muscle characteristics observed in the two age groups examined in the present series of studies.

Given the curvilinear  $\dot{V}O_2$ -Work relationship observed across increasing exercise intensities (Barstow et al. 2000), the off-transient  $\dot{V}O_2$  and mOxy amplitude measures were expected to, and did demonstrate, a significant increase with exercise intensity in the present study. A similar effect was observed in both the  $\dot{V}O_2$  and mOxy on-transient responses in both age groups. This increase with higher exercise intensities on the off-transient  $\dot{V}O_2$  and mOxy amplitude measures reflects the increasing energetic demands of the three SWT and supports the suggestion that exercise intensity and duration are major influencing factors on the off-transient response (Borsheim and Bahr 2003). Furthermore, the matching of physiological and muscle histochemical and enzymatic characteristics of the present study's cohorts and relative SWT intensities may be responsible for the observed similarities in the off-transient  $A_f$  in the present study. The observation of similar increases in the off-transient  $\dot{V}O_2$  and mOxy  $A_f$  in each age group is suggestive of greater magnitude of recovery following each SWT intensity. It further suggests that the off-transient responses may be dependent upon either the physiological characteristics of the cyclists or the intensity or duration of the exercise task, and that physical training with aging attenuates any such effect of age on these responses.

#### *Off-Transient Speed Responses*

The speed of the off-transient response refers to the path that the  $\dot{V}O_2$  and mOxy measures take when returning to baseline levels following the completion of an exercise bout (Gaesser and Brooks 1984; Bahr 1992; Borsheim and Bahr 2003). The results of the present study revealed no significant effect of age on the off-transient speed measures ( $TD_f$ ;  $\tau_f$ ) of the  $\dot{V}O_2$  and mOxy responses in the well-trained cyclists. The absence of a significant

effect of age on these off-transient responses is in contrast to that previously reported for older sedentary populations (Chick et al. 1991; Ichimura et al. 2006).

The present data revealed no significant effect of age on the  $\dot{V}O_2$  or mOxy  $TD_f$  in the well-trained cyclists. Further, a stable  $\dot{V}O_2$   $TD_f$  across the three SWT intensities may suggest that the intra-muscular signalling time required for the decrease in energy metabolism is independent of exercise intensity (Hanada, Okita, Yonezawa, Ohtsubo, Kohya, Murakami, Nishijima, Tamura and Kitabatake 2000). The mOxy  $TD_f$  was significantly shorter than the  $\dot{V}O_2$   $TD_f$  in both the young and middle-aged cyclists across the three separate SWT intensities in the present study. This difference between the responses suggests that intra-muscular reoxygenation and recovery mOxy kinetics occur faster than proposed through the  $\dot{V}O_2$  measures observed at the mouth. This discrepancy between the two physiological measures may reflect the instantaneous measurement of mOxy, and the actual monitoring of the reoxygenation of the Hb and Mb stores within the working muscle by the NIRS technology. The  $\dot{V}O_2$   $TD_f$  may be lengthened due to the transit time of deoxygenated blood from within the muscle to the lungs, as well as the reoxygenation of Hb stores outside the working muscles.

No significant effect of age was observed for the  $\dot{V}O_2$  or mOxy  $\tau_f$  or  $wMRT_f$  in the present study. This absence of a significant effect of age in the  $\dot{V}O_2$  and mOxy  $\tau_f$  is consistent with the on-transient responses examined in Study Two of this series of investigations. Thus, the present study suggests that the off-transient  $\dot{V}O_2$  and mOxy responses are not slowed with concurrent

aging and physical training. This result may suggest that the nature of the off-transient  $\dot{V}O_2$  and mOxy responses is dependent upon either  $\dot{V}O_{2\max}$  or the histochemical and enzymatic characteristics within the working muscle. These characteristics may have a strong influence on the utilisation of  $O_2$  within the muscle following completion of an exercise bout. Interestingly, the two sedentary age groups described by Chilibeck et al. (1997) reported similar  $\dot{V}O_2 \tau_f$  (Y:  $33.1 \pm 16.6$  s; O:  $44.1 \pm 18.8$  s) to that reported in the present investigation for moderate-intensity exercise (Y:  $35.3 \pm 5.3$  s; MA:  $35.0 \pm 7.9$  s). However, in comparing the off-transient  $\dot{V}O_2$  and mOxy responses, previous research has reported that the  $\dot{V}O_2 \tau_f$  ( $32 \pm 5$  s) occurs significantly faster than the mOxy response ( $91 \pm 26$  s) in young ( $27 \pm 5$  y) healthy males following moderate-intensity leg extension exercise (duManoir et al. 2005). The results of the present study are in contrast to this observation and suggest that the  $\dot{V}O_2$  and mOxy  $\tau_f$  were similar across the three SWT intensities. This suggests that the off-transient  $\dot{V}O_2$  responses resemble that of the working muscle in terms of  $O_2$  extraction and energetic costs. This difference between the current study and the results of duManoir et al. (2005) may have resulted from the different modes of exercise or training status of the subjects as both have been reported to influence the incurred metabolic demands of exercise and the nature of the off-transient response.

The present data are the first to examine the off-transient mOxy responses in well-trained young and middle-aged athletes following exercise bouts of increasing intensity. No significant effect of age or intensity was observed in the off-transient mOxy  $\tau_f$  in the well-trained cyclists. The absence of a significant effect of age in the present study may suggest that the

mechanisms responsible for metabolic recovery are controlled by several important physiological or muscle histochemical characteristics. This is further supported by the absence of a significant effect of intensity on the mOxy  $\tau_f$  which again suggests that the controlling mechanisms lie within the working muscle and depend upon the capacity to utilise O<sub>2</sub> within the working muscle. Recently, Ichimura and colleagues (2006) reported that the mOxy  $\tau_{1/2}$  was significantly shorter in physically active, but not physically trained middle-aged ( $53 \pm 5$  y;  $22.5 \pm 3.3$  s) and elderly ( $67 \pm 5$  y;  $29.6 \pm 8.9$  s) subjects compared to a cohort of sedentary age-matched counterparts (MA:  $50 \pm 6$  y;  $35.7 \pm 9.0$  s; E:  $66 \pm 3$  y;  $45.7 \pm 13.6$  s). As such, the lack of difference between the experimental groups may be explained by the similar physiological capacities and muscle histochemical and enzymatic characteristics of the well-trained cyclists in the present study.

A significant lengthening effect of intensity was observed on the VO<sub>2</sub>  $\tau_f$  in the present investigation. In contrast, no such effect was demonstrated in the mOxy  $\tau_f$ . The observation of a lengthened VO<sub>2</sub>  $\tau_f$  with increasing exercising intensity in the present study most likely reflects the greater energy metabolism and associated recovery processes within and outside the muscle with increased exercise intensity. The VO<sub>2</sub>  $\tau_f$  values from the present study (33-55 s) are similar to those observed in younger populations performing similar exercise intensities (Paterson and Whipp 1991; Engelen et al. 1996; Ozyener et al. 2001; Perrey, Candau, Borrani, Millet and Rouillon 2002). The lengthened VO<sub>2</sub>  $\tau_f$  with increasing exercise intensities observed in the present study contrasts previous research findings reporting a stable VO<sub>2</sub>  $\tau_f$  across exercise intensities (Paterson and Whipp 1991; Cunningham, Croix, Paterson, Ozyener

and Whipp 2000; Ozyener et al. 2001), but agrees with others (Engelen et al. 1996; Billat et al. 2002) that have reported a slowed  $\dot{V}O_2 \tau_f$  with increasing exercise intensities. The lengthened  $\dot{V}O_2 \tau_f$  with increasing exercise intensities is suggestive of  $O_2$  consuming influences that do not necessarily occur within the working muscle. This suggestion is supported by the observation of no effect of intensity on the mOxy  $\tau_f$  in the present study. This significant lengthening of the  $\dot{V}O_2 \tau_f$  may reflect metabolic processes such as lactate oxidation within non-active muscles that occurs with increasing exercise intensities given the required  $O_2$  cost for conversion of lactate to pyruvate.

In contrast, the absence of a significant effect of intensity on the mOxy  $\tau_f$  suggest that the speed of reoxygenation of the working muscle following exercise is not influenced by prior exercise intensity. The mOxy  $\tau_f$  values reported in the present study across the three SWT intensities (35-45 s) appear faster than those reported previously by Puente-Maestu et al. (2003) for similar SWT intensities (~46-74 s) in chronic obstruction pulmonary disease patients. This difference in the mOxy  $\tau_f$  may be due to the diseased nature of the cohort reported upon by these previous investigators. Puente-Maestu and colleagues (2003) demonstrated that the mOxy  $\tau_f$  did not significantly lengthen with exercise intensity, either before or after a six week endurance-training regime. They did, however, report a significant improvement in the mOxy  $\tau_f$  across all SWT intensities as a result of the exercise training. This training effect suggests that the speed of the off-transient mOxy response is controlled through peripheral mechanisms that control the rate of  $O_2$  utilisation within the exercising muscle following an exercise bout.

The observed discrepancies in the off-transient  $\dot{V}O_2$  and mOxy  $\tau_f$  discussed above may be due to metabolic factors external to the working muscle associated with the measurement of changes in the  $\dot{V}O_2$  responses determined at the mouth. The present study is the first to concurrently examine the off-transient  $\dot{V}O_2$  and mOxy in a group of well-trained athletes across increasing exercise intensities. The results support the findings of Puente-Maestu and others (2003) of a significantly faster off-transient mOxy response compared to the  $\dot{V}O_2$  response across increasing exercise intensities. The previous work of Puente-Maestu et al. (2003) demonstrated that the off-transient  $\dot{V}O_2$   $\tau_f$  was much slower than that of the mOxy response following a constant-load exercise bout performed at 80% VT. It is proposed that this difference between the  $\dot{V}O_2$  and mOxy  $\tau_f$  may reflect the specific monitoring of metabolic recovery of the working muscle that requires  $O_2$  utilisation as measured through NIRS technology. In contrast, while the  $\dot{V}O_2$  response measured at the mouth is reflective of the recovery of the working muscle, it is also subject to influences from several metabolic processes outside the working muscle. These might include lactate oxidation within both active and non-active musculature, the metabolic cost of synergist and stabiliser muscles, and substrate oxidation (Bahr and Sejersted 1991; Bahr 1992). These factors and their effect on the off-transient responses will be discussed below.

#### *Off-Transient Physiological Mechanisms*

The present findings of a lengthening off-transient  $\dot{V}O_2$   $\tau_f$  compared to the mOxy  $\tau_f$  suggest that the physiological mechanisms that control the  $\dot{V}O_2$  and mOxy recovery responses are different. This finding supports the previous suggestion that the off-transient  $\dot{V}O_2$  response is slower than the mOxy

response following an exercise bout (Puente-Maestu et al. 2003). The off-transient mOxy response characterises the replenishment of O<sub>2</sub> content within the working muscle and may not reflect additional physiological mechanisms that may contribute to a slowed  $\dot{V}O_2$  recovery that occur outside the working muscle. The speed of the off-transient  $\dot{V}O_2$  and mOxy responses appears to be influenced by several physiological mechanisms, located within and external to the working muscle (Gaesser and Brooks 1984; Bahr 1992; Borsheim and Bahr 2003). These external mechanisms may include Hb reoxygenation, PCr resynthesis, lactate oxidation or the additional energy cost of synergistic or stabiliser muscles (Gaesser and Brooks 1984; Bahr 1992; Puente-Maestu et al. 2003).

The resynthesis of intra-muscular PCr stores has long been suggested as a contributor to the metabolic recovery of the working muscle following an exercise bout (Brooks et al. 1971; Gaesser and Brooks 1984; Rose, Hodgson, Kelso, McCutcheon, Reid, Bayley and Gollnick 1988; Langsetmo and Poole 1999; Borsheim and Bahr 2003). Previous investigations have matched the on-transient  $\dot{V}O_2$  response to the PCr kinetics at exercise onset (Barstow et al. 1994). Therefore, in order to metabolically recover, these PCr stores must be replenished following an exercise bout. This hypothesis is based on the suggestion that intra-cellular PCr reserves are consumed within the working muscle during the on-transient response until aerobic metabolism of ATP can match the requirements of the intensity of the exercise bout (Barstow et al. 1994). However, the resynthesis of PCr stores within the working muscle has been shown to account for only a small portion of the off-transient  $\dot{V}O_2$  amplitude in both humans (<10%) (Brooks et al. 1971) and horses (<1.5%)

(Rose et al. 1988). More recently, Langsetmo and Poole (1999) suggested that if PCr resynthesis was the sole mechanism responsible for the off-transient  $\dot{V}O_2$  response, then its recovery path would be identical to that of the on-transient response which has been reported to mimic PCr degradation kinetics (Rossiter et al. 1999). Therefore, it has been previously demonstrated that PCr resynthesis following moderate-, heavy- and severe-intensity exercise would have minimally contributed to the off-transient  $\dot{V}O_2$  and mOxy responses. The discussed metabolic processes primarily refer to the restoration of a homeostatic environment.

A second factor that may influence the off-transient  $\dot{V}O_2$  and mOxy responses is the reoxygenation of Hb and Mb stores originally deoxygenated at exercise onset (Grassi et al. 2003). It is proposed that the HbO<sub>2</sub> and MbO<sub>2</sub> stores within the working muscle act as a reserve of O<sub>2</sub> within the muscle to sustain aerobic metabolism until the delivery and utilisation of O<sub>2</sub> is increased to meet the energetic demands of the exercise bout (Mooren and Volker 2005). The HbO<sub>2</sub> and MbO<sub>2</sub> stores help to reduce the magnitude of O<sub>2</sub> deficit and anaerobic metabolism required at the onset of the exercise bout in order to meet its energetic demands. Therefore, following the completion of the exercise bout, the deoxygenated Hb and Mb stores are required to be reoxygenated, which is reflected through the TD<sub>f</sub> of the  $\dot{V}O_2$  and mOxy responses (Hanada et al. 2000). However, this reoxygenation occurs at the initial period of the off-transient response and therefore may not contribute to the overall speed of metabolic recovery within the muscle. The rate of  $\dot{V}O_2$  and mOxy recovery responses is most likely controlled through other metabolic processes within

and external to the working muscle such as lactate oxidation and PCr resynthesis (Borsheim and Bahr 2003).

Previous investigations have suggested that the off-transient response is strongly related to the concentration of several hematological parameters following exercise completion (Gaesser and Brooks 1984). This most likely reflects the role of  $O_2$  utilisation in returning the muscle cell to metabolic homeostasis. In the present study, the  $[HCO_3^-]$  was inversely related to both the  $\dot{V}O_2 \tau_f$  and the  $wMRT_f$  of the young and middle-aged cyclists, respectively. The importance of this relationship is reflected through the decreased  $[HCO_3^-]$  buffering potential being related to a lengthened off-transient  $\dot{V}O_2 \tau_f$  across the three SWT intensities. This observation suggests that a lengthened off-transient  $\dot{V}O_2$  response is related to the greater anaerobic metabolism and  $[H^+]$  accumulation within the working muscle during higher exercise intensities. Such processes have been suggested to play a role in the nature of the off-transient metabolic responses (Gaesser and Brooks 1984; Bahr 1992; Borsheim and Bahr 2003). The present data support this suggestion and also strongly links the nature of the off-transient  $\dot{V}O_2$  and mOxy responses to  $[BLa^-]$  at completion of the exercise bout.

Gaesser and Brooks (1984) have suggested that the oxidation of lactate within the muscle (active and non-active) and liver contributes to metabolic recovery following high-intensity exercise. It has been suggested that the fate of lactate following high-intensity exercise influences the nature of the off-transient  $\dot{V}O_2$  response (Gaesser and Brooks 1984; Borsheim and Bahr 2003). In the present data, the off-transient  $\dot{V}O_2 \tau_f$  was significantly related to the  $[BLa^-]$  at the

completion of the moderate-intensity SWT in the young cyclists, but not following the heavy or severe-intensity SWT in either age cohort. This is surprising, given the elevated  $[BLa^-]$  at the completion of the two high-intensity SWT in the present study. However, the absence of a significant relationship between the off-transient  $\dot{V}O_2$  and  $mOxy$  responses and the  $[BLa^-]$  at the end of the supra-threshold SWT may be due the large variance in the  $[BLa^-]$  response of the two cohorts. Therefore, it may be that the actual influence of lactate removal on the off-transient  $\dot{V}O_2$  and  $mOxy$  response is not yet fully understood.

Previous investigations have examined the fate of lactate following high-intensity exercise, with it being suggested that 75-90% of lactate is converted back to glycogen within working and non-working muscle following exercise (Hill and Lupton 1923; Gaesser and Brooks 1984). The oxidation of lactate within the muscle appears to be dependent upon the activity of gluconeogenic enzymes (e.g. Fructose-1, 6-diphosphatase) (Gaesser and Brooks 1984). The activity of this gluconeogenic enzyme is believed to vary between different muscle fibre types (Jobsis, Meijer and Vloedman 1976; Cutmore, Snow and Newsholme 1985; Tikkanen et al. 1995). Type II fibres are capable of intramuscular gluconeogenesis using lactate at physiologically significant rates due to a higher activity of the relevant enzymes (Donovan and Pagliassotti 2000). This suggestion may be supported by the present study's significant correlation observed between the Type IIb fibre composition and off-transient heavy and severe-intensity  $\dot{V}O_2$  and  $mOxy$  recovery responses in both the young and middle-aged cyclists. Given the various oxidative potential of the different muscle fibre types, it is likely that the influence of muscle histochemical

characteristics is the utilisation of  $O_2$  within the working muscle following an exercise bout.

The present data have suggested that the utilisation of  $O_2$  within the muscle cell is the most likely mechanism for controlling the speed of the metabolic recovery within the working muscle. Whilst the role of  $O_2$  utilisation during the off-transient  $\dot{V}O_2$  recovery phase has been highlighted, the delivery of  $O_2$  to the muscle has also been reported to influence the off-transient  $\dot{V}O_2$  response (Hughson et al. 1991; Chilibeck et al. 1997). Chilibeck and colleagues (1997) reported that the off-transient  $\dot{V}O_2 \tau_f$  following moderate-intensity plantar flexion exercise was significantly related to muscle capillarisation of the VL in young (~26 y) but not older (~66 y) sedentary subjects. These investigators suggested that the delivery of  $O_2$  may be of great importance, given the larger  $O_2$  gradients between blood and muscle following an exercise bout as compared to that during the on-transient  $\dot{V}O_2$  response. It is likely that the shorter  $O_2$  diffusion distances and greater streaming of  $O_2$  into the muscle cells associated with increased capillarisation allows an increased  $O_2$  utilisation within the mitochondria following exercise completion allowing faster recovery (Chilibeck et al. 1997). In the present study, significant correlations were observed between the capillary density and CS activity and the off-transient  $\dot{V}O_2 A_f$  in the young cyclists. The middle-aged cyclists only demonstrated a significant relationship between the capillary density and  $\dot{V}O_2 wMRT_f$  following the heavy-intensity SWT. Thus, the similar peripheral muscle characteristics of the present study's well-trained cyclists, together with the widely varied individual off-transient  $\dot{V}O_2$  and mOxy responses, make it difficult to identify the controlling factors of the off-transient responses in the present study.

In summary, the physiological basis of the off-transient response represents the metabolic recovery of the working muscle to baseline values following the completion of an exercise bout (Gaesser and Brooks 1984). The present data showed no significant effect of age in the off-transient  $\dot{V}O_2$  or mOxy responses. This may reflect the similar physiological capacities, muscle histochemical and enzymatic characteristics and relative exercise intensities observed in the two groups investigated. The findings of the current study also suggest that exercise intensity has a significant effect on the amplitude of the off-transient  $\dot{V}O_2$  and mOxy responses, as well as the speed of the off-transient  $\dot{V}O_2$  response. As no effect of intensity was observed in the off-transient mOxy response, this suggests that the off-transient  $\dot{V}O_2$  response is influenced by external metabolic processes following bouts of high-intensity exercise. The current consensus is that the elevated  $\dot{V}O_2$  requirements following a bout of exercise are due to several intra-muscular recovery processes such as PCr resynthesis, Hb and Mb reoxygenation, lactate oxidation and substrate metabolism (Gaesser and Brooks 1984; Bahr 1992; Borsheim and Bahr 2003). Such factors appear to be the casual mechanisms that control the utilisation of  $O_2$  within the working muscle, and determine the magnitude of metabolic recovery required after the completion of an exercise bout.

## **SUMMARY**

The purpose of Study Four was examine the effect of age on the off-transient  $\dot{V}O_2$  and mOxy responses following moderate, heavy and severe-intensity SWT in well-trained cyclists. No significant effects of age or age x intensity interactions were observed in the off-transient  $\dot{V}O_2$  or mOxy responses in the well-trained cyclists. The absence of a significant effect of age in the off-

transient responses examined within the present study reflects the similar physiological, muscle histochemical and enzymatic characteristics, as well as the similar relative exercise intensities used in the study by the two age groups.

In support of previous research findings, significant effects of intensity were observed in several off-transient  $\dot{V}O_2$  and mOxy amplitude and speed measures (Bahr and Sejersted 1991; Langetsmo, Weigle, Fedde, Erickson, Barstow and Poole 1997; Langsetmo and Poole 1999; Billat et al. 2002; Borsheim and Bahr 2003; Puente-Maestu et al. 2003). The present study did not identify the controlling mechanisms of the off-transient  $\dot{V}O_2$  or mOxy responses. However, the current study suggests that different mechanisms influence the nature of the off-transient  $\dot{V}O_2$  and mOxy responses, as evidenced by the conflicting effect of intensity on both the off-transient  $\dot{V}O_2$  and mOxy responses. This difference may suggest an influence of a number of  $O_2$  consuming processes outside the working muscle on the off-transient  $\dot{V}O_2$  response, but not in the off-transient mOxy response.

Thus, the results of the present study strongly suggest that the off-transient  $\dot{V}O_2$  and mOxy responses are maintained into middle-age through physical training. The present data also suggest that the off-transient  $\dot{V}O_2$  and mOxy responses are controlled through different physiological mechanisms. This was supported by several significant correlations between the off-transient  $\dot{V}O_2$  and mOxy responses and a number of hematological and histochemical characteristics in both age groups. These similar relationships further support the absence of a significant effect of age in the nature of the off-transient responses and controlling mechanisms in the present study.

## CHAPTER 8

# SUMMARY AND CONCLUSIONS

### SUMMARY

The current series of investigations examined the effect of age on the  $\dot{V}O_2$  and mOxy responses during and following moderate-, heavy- and severe-intensity SWT in well-trained cyclists.

*Study One: Physiological, histochemical, enzymatic and performance characteristics in well-trained young and middle-aged cyclist.*

The purpose of Study One was to examine the effect of age on a range of physiological and performance characteristics of well-trained cyclists. There was no significant effect of age on the VT or  $\dot{V}O_{2\max}$  in the well-trained cyclists. No significant differences were observed in the muscle fibre composition, fibre CSA or capillarisation of the VL between age groups. Maximal specific activities of both glycolytic (PFK and LDH) and oxidative (CS,  $\beta$ -HAD and 2-OGDH) enzymes in the VL were similar between age groups. Lastly, no significant difference was observed between the two age groups in the mean RPO sustained across a 30TT. In conclusion, the results of the first study support previous reports that physiological and performance characteristics can be maintained into middle-age through continued physical training.

*Study Two: On-transient  $\dot{V}O_2$  and mOxy kinetics during moderate-, heavy- and severe-intensity exercise in well-trained young and middle-aged cyclists.*

The purpose of Study Two was to examine the effect of age on the on-transient  $\dot{V}O_2$  and mOxy responses to moderate, heavy and severe-intensity SWT in well-trained cyclists. No significant effect of age was observed in the  $A_p$ ,  $TD_p$  or  $\tau_p$  of the  $\dot{V}O_2$  or mOxy on-transient responses across the three SWT intensities. In the on-transient  $\dot{V}O_2$  response, both the  $A_p$  and  $TD_p$  demonstrated a significant effect of intensity, whereas the  $\dot{V}O_2$   $\tau_p$  remained stable across the three SWT intensities. Only the mOxy  $A_p$  demonstrated a significant effect of intensity in the young and middle-aged cyclists. The speed of the  $\dot{V}O_2$  and mOxy on-transient responses was significantly related across the moderate and heavy-intensity SWT in the young cyclists. The speed of the mOxy  $\tau_p$  and  $\dot{V}O_2$   $\tau_p$  was significantly ( $p < 0.05$ ) related to changes in  $[BLa^-]$  and blood pH in the young and middle-aged cyclists, respectively. In the young cyclists, the speed of the moderate and heavy-intensity  $\dot{V}O_2$  responses was significantly ( $p < 0.05$ ) related to muscle fibre composition and C:F ratio. In the middle-aged cyclists, the moderate and severe-intensity  $\dot{V}O_2$   $\tau_p$  values were significantly ( $p < 0.05$ ) related to muscle fibre composition and capillary contacts per fibre area, respectively.

*Study Three:  $\dot{V}O_2$  and mOxy slow components determined during heavy- and severe-intensity exercise in well-trained young and middle-aged cyclists.*

The third study examined the effect of age on the development of the  $\dot{V}O_2$  and mOxy slow components across heavy- and severe-intensity SWT in well-trained cyclists. No significant effects of age were observed in the amplitude or speed of the  $\dot{V}O_2$  or mOxy slow components in the well-trained

cyclists. A significant effect of intensity was observed in the  $\dot{V}O_2 \tau_s$  in the young cyclists, with the heavy-intensity  $\tau_s$  being significantly longer than that of the severe-intensity SWT. No significant effect of intensity was observed in any mOxy slow component parameters in either age group. The heavy-intensity  $\dot{V}O_2$  slow component was significantly related to maximal CS activity in the young cyclists. No significant relationships were observed between the  $\dot{V}O_2$  and mOxy slow components and changes within blood pH,  $pO_2$ ,  $[HCO_3^-]$  or  $[BLa]$  during the high-intensity SWT. Lastly, non-significant trends across time were observed in both the iEMG and MPF responses of the VL and VM during the heavy and severe-intensity SWT. The available sEMG data suggests that Type II fibre fatigue occurs during high-intensity exercise, which then facilitates an increased recruitment of the number of Type I fibres required to sustain the power output.

*Study Four: Off-transient  $\dot{V}O_2$  and mOxy kinetics following moderate-, heavy- and severe-intensity exercise in well-trained young and middle-aged cyclists.*

Lastly, the fourth and final study examined the effect of age on the off-transient  $\dot{V}O_2$  and mOxy responses following moderate-, heavy- and severe-intensity-SWT in well-trained cyclists. No significant ( $p > 0.05$ ) effects of age or age x intensity interaction were observed for any off-transient  $\dot{V}O_2$  or mOxy parameter between the young and middle-aged cyclists. However, significant increasing effects of intensity were observed for the  $A_f$  of both the  $\dot{V}O_2$  and mOxy responses. The  $\dot{V}O_2 \tau_f$  was also significantly lengthened following the severe-intensity SWT compared to the moderate and heavy-intensity SWT, but no such observation was observed in the mOxy  $\tau_f$  response. Following the moderate-intensity SWT, the off-transient  $\dot{V}O_2 \tau_f$  and  $MRT_f$  were significantly

related to changes in  $[\text{HCO}_3^-]$  and  $[\text{BLa}^-]$  in the young cyclists. The off-transient moderate and heavy-intensity  $\text{VO}_2$  response of the middle-aged cyclists was significantly ( $p < 0.05$ ) related to changes in  $[\text{HCO}_3^-]$ .

## CONCLUSIONS

The current series of studies examined the effect of age on the  $\text{VO}_2$  and mOxy responses during and following bouts of moderate-, heavy- and severe-intensity exercise in well-trained cyclists, matched for physiological and performance capacities. Previous aging research has investigated older sedentary subjects, and as a result, very little data are available on the physiological capacities of well-trained older athletes. Therefore, the present research is original and provides novel insights to the areas of both aging and  $\text{VO}_2$  and mOxy kinetics. The following conclusions can be made from the present series of studies:

1. The physiological capacities ( $\text{VT}$  and  $\text{VO}_{2\text{max}}$ ) and peripheral muscle characteristics (fibre composition, fibre CSA, capillarisation and enzyme activities) can be maintained with physical training into middle-age at a level similar to that of a younger cohort of performance-matched cyclists.
2. The on-transient  $\text{VO}_2$  and mOxy responses are not influenced by age across exercise intensities in well-trained middle-aged cyclists. This observation is most likely due to the similarities in the physiological capacities and peripheral muscle characteristics between the two groups. Importantly, the stable  $\text{VO}_2$  and mOxy  $\tau_p$  across exercise

intensities is suggestive of  $O_2$  utilisation controlling the speed of the on-transient metabolic adaptation.

3. The development of the  $\dot{V}O_2$  and mOxy slow components during sustained heavy and severe-intensity exercise was not influenced by age in well-trained middle-aged cyclists. This may be the result of the similar physiological capacities, peripheral muscle characteristics and neuromuscular activity levels across the high-intensity SWT in the two age groups. The observed trends in sEMG analysis suggest an increase in Type I fibre recruitment during the high-intensity exercise SWT in both age groups.
4. The nature of the off-transient  $\dot{V}O_2$  and mOxy responses are not influenced by age following moderate, heavy and severe-intensity exercise in well-trained middle-aged cyclists. This similar off-transient response may also be due to the homogenous physiological capacities and peripheral muscle characteristics of the young and middle-aged cyclists. The similar  $\dot{V}O_2$  and mOxy  $\tau_f$  also suggest that  $O_2$  utilisation issues are involved in controlling the speed of the off-transient metabolic response.

## **FUTURE RESEARCH DIRECTIONS**

Based on the results and observations of this thesis, the follow directions of future research are suggested:

1. The current series of investigations should be repeated comparing the responses of the well-trained middle-aged cyclists to sedentary age-matched controls to help identify the influence of training and aerobic capacity on the  $\dot{V}O_2$  and mOxy kinetic responses. Originally, this was proposed to be included in the present investigation but was omitted due to ethical concerns by the University Ethics Committee.
2. The current series of investigations should also be repeated utilising older (60+ y) sedentary and well-trained subjects. While the middle-aged cyclists were significantly older than the young in the present study, they are not truly representative of an aging population. This may help to further delineate the effects of concurrent training and aging on the  $\dot{V}O_2$  and mOxy kinetic responses.
3. Future research also needs to investigate the relationship between  $\dot{V}O_2$  and mOxy kinetics and athletic performance. The use of performance tests such as a prolonged constant-load cycling time trials in order to relate the on- and off-transient  $\dot{V}O_2$  and mOxy kinetic responses to an individual's performance capacity is inappropriate and may hide the actual 'real-world' applications of such research.

4. Lastly, future research needs to thoroughly investigate the relationship between  $\dot{V}O_2$  and mOxy responses to a range of exercise intensities. The majority of  $\dot{V}O_2$  kinetics identifies influential mechanisms which are located within the working muscle, and changes in  $O_2$  utilisation in response to exercise has previously been difficult to quantify and measure. The introduction of NIRS technology has allowed the monitoring of  $O_2$  extraction to be performed non-invasively, and future investigations should aim to incorporate such technology to help identify important  $O_2$  delivery or utilisation limitations.

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## **APPENDICES**

## APPENDIX 1

### **Informed Consent and Subject Information Pack**



## INFORMED CONSENT

# **THE EFFECTS OF AGING AND TRAINING ON PULMONARY AND MUSCULAR OXYGEN KINETICS DURING MODERATE-, HEAVY- AND SEVERE-INTENSITY CYCLING EXERCISE**

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Dear Sir,

The purpose of this study is to examine the effects of training and aging on pulmonary and muscle oxygen uptake during different intensity cycling exercise. The following pages will provide you with information outlining the background of the study, the procedures you will undertake and list any possible risks or side effects associated with the study. The present study is being administrated through The School of Health and Human Performance, Central Queensland University.

### ***Introduction***

Gas analysis is a commonly performed measurement in sports and exercise, for both the monitoring of performance and metabolic purposes. The rate at which oxygen consumption changes with respect to work intensity has also been of interest as the quicker the metabolic transition, the improved performance at the start of exercise. Previously it has been unknown how well the changes in pulmonary oxygen consumption relate to changes in the oxygen capacity of the muscle.

The introduction of Near-Infrared spectroscopy (NIRS) has allowed the continual monitoring of muscle oxygenation and regional blood flow using non-invasive techniques, which has allowed the relationship between pulmonary and muscle oxygen consumption to be compared. To date, however, very few investigations have investigated this relationship using these technologies particularly with reference to muscle fibre type, age or training status. The implications for such research may be to help identify any limitations which occur with aging that slow the change in oxygen consumption after changes in work intensity. This has potential implications for aging individuals who struggle to complete daily tasks without suffering metabolic fatigue or athletes who wish to 'turn on' quicker at the start of a race.

### **Purpose of the Study**

The purpose of the proposed research project is to determine:

- The effect of aging and training status on pulmonary and muscle oxygenation kinetics during moderate, heavy and severe intensity exercise.
- The effect of aging and training on the relationship between oxygenation kinetics and muscle characteristics during moderate, heavy and severe intensity exercise.

## Significance of the Study

The present study is the first research investigation to describe the effects of aging muscle on its ability to consume oxygen. It is well established that with aging the composition of muscle changes, and that this can be changed further through physical training. In addition, limited research has described both the pulmonary and muscle oxygen kinetics in response to work transitions of various intensities in different populations. Therefore, the findings of the study have both clinical and exercise applications in order to describe what factors help to allow a quick transition on oxygen consumption between work intensities.

## Methods

### Session #1: Health Screening and Familiarisation (total time = 1 hour)

The initial visit to the Health and Human Performance laboratory will be used to screen you for any medical conditions that may prevent you doing heavy exercise. This will be performed through the answering of questionnaires and discussion with the chief investigator, Ben Dascombe. If you exhibit any medical conditions you will be asked to visit a general practitioner. A detailed explanation and demonstration of the testing techniques will also be given throughout this session. During this visit, the cycle ergometer will also be adjusted to your correct handlebar and seat positions to be used throughout the duration of the study. During this visit your body composition will also be assessed.

### Session #2: Ramp Test (total time = 1 hour)

The second visit to the lab will require you to perform a cycling test to exhaustion (lasts around 6-8 min). The purpose of the test is to determine your aerobic fitness and familiarise you with a number of the methods to be used later in the investigation.

During the exercise test, you will have a number of physiological measures being performed including:

1. *Heart Rate*: heart rate will be recorded by a Polar heart rate monitor attached to your chest throughout testing.
2. *Gas Analysis*: gas analysis will be performed using a Medgraphics CPX/D metabolic cart. Gas analysis is performed by analysing expired pulmonary gases which are collected through a mouthpiece and sampled each breath. A noseclip must also be worn throughout the test.
3. *Blood analysis*: the analysis of blood will consist of a collection of a capillary sample from the subject's fingertip. A 100µL capillary blood sample (4-5 drops) will be collected by a trained sports scientist into a capillary tube for storage and analysis both before and after the completion of the test. Blood samples will then be inserted into iSTAT CG<sub>4+</sub> cartridges which will then be analysed using the iSTAT clinical blood analyser.

4. *Surface EMG*: will also be recorded from the thigh to measure muscle electrical activity. EMG is performed through the placement of two electrodes being placed over the belly of the muscle whilst connected to a computer. The electrode area is to be shaven, and lightly sandpapered to ensure that adequate contact is made.
5. *Near Infrared Spectroscopy (NIRS)*: the testing procedure for NIRS comprises of the application of a device over the belly of the working thigh muscle, where it is firmly secured by a bandage. The device shines light into the muscle, and light detectors measure the light intensity which is reflected back out of the muscle. This device is used to measure the oxygen content of the blood which is located within the working muscle.

### **Sessions #3-5 (total time = 1 ½ hours)**

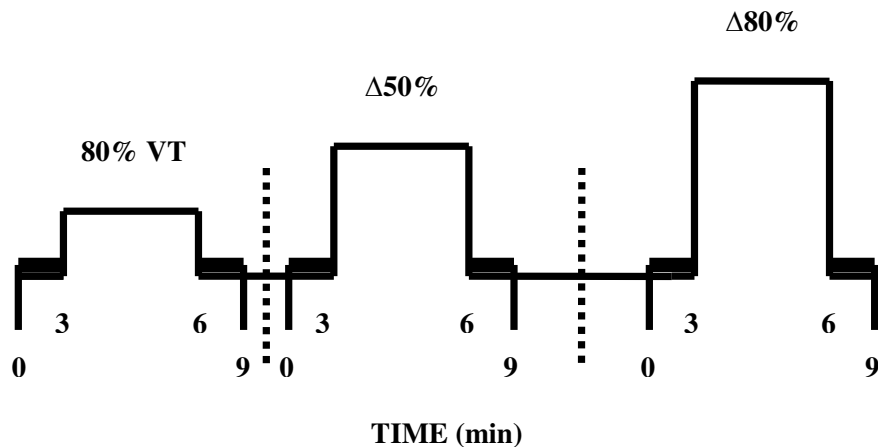
During these visits, you will perform a number of 6 minute bike rides at moderate, heavy and severe intensities as shown below in Figure 1. Each 6 min test will be preceded and followed by 3 minutes of unloaded cycling. Repeated transitions are required to ensure that the results are accurate. Throughout each exercise bout you will have the following techniques monitoring a number of physiological factors, including:

During the exercise test, you will have a number of physiological measures being performed including:

1. *Heart Rate*: similar to visit 2.
2. *Gas Analysis*: similar to visit 2.
3. *Blood analysis*: A 100µL capillary blood sample (4-5 drops) will be collected at pre-, mid and post transition by a trained sports scientist into a capillary tube for storage and analysis. Blood samples will then be inserted into iSTAT CG<sub>4+</sub> cartridges which will then be analysed using the iSTAT clinical blood analyser.
4. *Surface EMG*: similar to visit 2.
5. *Near Infrared Spectroscopy (NIRS)*: similar to visit 2.

### **Session #6**

The last visit will take place at the Rockhampton Base Hospital where you will have a resting muscle biopsy on your right thigh. An orthopaedic surgeon, Dr. Eric Hohmann will perform the biopsy procedure. A quantity of local anaesthetic will be used to numb a small area of skin, where the doctor will make a small incision and insert the biopsy needle. During the biopsy procedure you may feel somewhat of a pressure or pulling sensation as a result of the needle insertion, but no pain. Two biopsy samples will be taken for analysis from the same incision during the visit. The wound will then be cleaned and bandaged for the next 48 hours as to minimise infection. You will be given comprehensive post-operative care instructions for management of the incision. The muscle biopsy will cause minimal interference with short-term performance, and will pose no long-term loss of function.



**Figure 1:** Demonstration of exercise bouts during a square-wave transition visit.

### Analysis

A written report will be sent to you detailing and explaining your individual results and their implications. A verbal explanation of the results will also be provided after the completion of testing to inform you as to how the results relate to your training and performance. You will be asked to strictly maintain your normal diet and training load for at least the two days prior to testing. These measures will ensure that you are fresh for the test, and that diet and fatigue do not influence results.

### Risks

During testing you will be asked to perform maximal intensity exercise which may cause some discomfort. You will have been pre-screened to ensure that you do not have any existing medical conditions that may indicate that you should not undertake maximal exercise. If health risk factors are found to exist which may affect your health or contra-indicate exercise participation, then you will be referred to a medical doctor to obtain approval to participate or be excluded for the study. Some slight skin irritation may also be encountered due to the skin preparation required for the surface EMG.

Additionally, there are a number of risks associated with the use of cuff ischemia with NIRS. Whilst the likelihood of these risks is minimal it is important to be aware of these complications prior to the commencement of the study. These risks include pulmonary embolus (blockage of blood vessel in lung), skin trauma, metabolic acidosis, tourniquet pain or hypertension, arterial injury or muscle damage, or neurologic disturbance. Whilst this list may seem excessive, these complications rarely occur, and are mostly associated with cuff ischemia lasting greater than 30-60 minutes. This timeframe is

considerably longer than the expected duration of cuff ischemia proposed for the present study (~15 min). The associated risk of these complications is exceptionally low, such as only 1:13 000 people have suffered neurologic disturbance as a result of prolonged cuff ischemia of the leg. However, less serious complications such as tourniquet pain have been reported in up to 66% of patients, but only after 30-60 min of tourniquet application. Therefore, whilst arterial occlusion is associated with a number of potentially dangerous complications, the likelihood of them occurring is low, particularly with the selected use and time frame proposed to be employed in the present study.

As the muscle biopsy is a considerably invasive technique, it carries a number of risks to the subject including haematoma, skin infection, denervation of a small area of the vastus lateralis and 'delayed muscle soreness' (similar to those following unaccustomed intense exercise). The minimal risk of each complication with all required precautions taken detailed below: haematoma 2:1300, skin infection: 1:1300; minor denervation: 1:1300. Note that the instances of haematoma and skin infection were noted after four serial biopsies were taken. The delayed muscle soreness is a typical response which may last up to 48 hours which should have minimal effect on the short-term functional capacity and no long-term side effects. The risk of post-sampling infection from the instruments and post-operative care will be minimised through the taking of samples in a hygienic setting with sterilised instruments. After the biopsy is taken, the incision will be cleaned and closed by a butterfly clip, with a sterile elastic surgical stocking placed on the site for a period of 24 hours to minimise bleeding and bruising. To minimise the soreness of the biopsy, the wound will be treated with an icepack for 10-20 minutes post-sampling to aid the initial healing process. You will be given instructions on how to manage the incision to protect against infection. You will be contacted by phone after 12 and 48 hours after the procedure to check how the biopsy site is healing. After 24 hours, the incision will be inspected and a new bandage applied. The biopsy should have minimal short-term effect on your exercise capacities, and no long-term consequences.

### **Anonymity**

The confidentiality of the results of this study is assured. Under no circumstances will your name appear in publications associated with this research. Your results will be provided to you both in written and verbal form with no one else being given your results unless you request it. Hard copies of your results shall be stored in a locked filing cabinet. The information will be backed up on CD, and this will also be stored in the locked filing cabinet.

**THROUGHOUT THE COURSE OF THE RESEARCH, YOU ARE FREE TO  
WITHDRAW AT ANY TIME FOR WHATEVER REASON WITHOUT  
QUESTIONS BEING ASKED OR PENALTIES INCURRED**

## Enquiries

Any enquiries or concerns regarding the nature and/or conduct of the proposed research can be directed to the Central Queensland University's Office of Research at (07) 4923 2607. Alternatively the researchers may be contacted to discuss any concerns or queries at the contact details below:

- Ben Dascombe
  - Phone: (07) 4930 9763
  - Mobile: 0417 712 381
  - Email: [b.dascombe@cqu.edu.au](mailto:b.dascombe@cqu.edu.au)
- Dr. Peter Reaburn
  - Phone: (07) 4930 9813
  - Email: [p.reaburn@cqu.edu.au](mailto:p.reaburn@cqu.edu.au)

## Freedom to Withdraw

I have read the above information. The nature, the demands, risks and benefits of the project have been explained to me. I knowingly assume the risks involved, and understand that I may withdraw my consent and discontinue participation at any time without penalty or loss of benefit to myself. In signing this consent form I am not waiving my legal claims, rights or remedies. A copy of the consent form will be given to me.

**NAME:** \_\_\_\_\_

**SIGNATURE:** \_\_\_\_\_

**DATE:** \_\_\_\_\_

### CONTACT DETAILS:

Address: \_\_\_\_\_  
\_\_\_\_\_

Phone: (H) \_\_\_\_\_

(W) \_\_\_\_\_

(Mobile) \_\_\_\_\_

Email: \_\_\_\_\_

**INVESTIGATORS  
SIGNATURE:**

\_\_\_\_\_

**DATE:** \_\_\_\_\_

**SPECIAL CONSIDERATIONS:**

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I also agree to participate in a 30 minute time trial using the same testing techniques as those described above, which include gas analysis, blood collection, near infrared spectroscopy, electromyography and a muscle biopsy. I agree for the data gained from the 30 minute time trial to be used in collaboration with the data collected during my previous visits for publications and a doctoral thesis.

**NAME:** \_\_\_\_\_

**SIGNATURE:** \_\_\_\_\_

**DATE:** \_\_\_\_\_

**INVESTIGATORS  
SIGNATURE:** \_\_\_\_\_

**DATE:** \_\_\_\_\_

I also agree that any images of myself collected through the 30 minute time trial can be used for presentation purposes and any publications arising from the research investigation. I will receive no remuneration for such.

\_\_\_\_\_

Signature

\_\_\_\_\_

Date

## APPENDIX 2

### **Revised Physical Activity Readiness Questionnaire**

**THE EFFECTS OF TRAINING AND AGING ON PULMONARY AND  
MUSCULAR OXYGEN KINETICS**

**PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)**

Name: \_\_\_\_\_ Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

Date of birth: \_\_\_\_/\_\_\_\_/\_\_\_\_ Sex: M F (Circle one)

Address: \_\_\_\_\_

Phone (H): \_\_\_\_\_ (W): \_\_\_\_\_ (M): \_\_\_\_\_

Email: \_\_\_\_\_

1. When was the last time you had a physical examination?
2. Has any member of your family been treated for or suspected to have any of the following conditions? Please identify their relationship to you (eg. Mother, father, etc)
  - a. Diabetes YES NO \_\_\_\_\_
  - b. Heart disease/attack YES NO \_\_\_\_\_
  - c. Stroke YES NO \_\_\_\_\_
  - d. High blood pressure YES NO \_\_\_\_\_
  - e. Peripheral Artery Disease YES NO \_\_\_\_\_

3. Do you Smoke? YES NO

If no, have you quit within the last 6 months? YES NO

4. Do you know your blood fat content? YES NO

Please list:

Cholesterol:	_____	mmol/L
VLDL:	_____	mmol/L
LDL:	_____	mmol/L
HDL:	_____	mmol/L

5. Have you ever been told you have abnormal blood pressure? YES NO

Was it high or low? (please circle)

6. Are you currently taking any medication? If so what are they? (Please list)

7. If you are allergic to any foods, medications or other substances, please list them here.

8. If you have been told that you have any chronic disease or serious illness, please name them here.

9. Have you been hospitalised in the past three (3) years? Please give details.

10. During the past twelve (12) months;	YES	NO
Has a physician prescribed any medication for you?	<input type="checkbox"/>	<input type="checkbox"/>
Has your weight fluctuated more than a few kilograms?	<input type="checkbox"/>	<input type="checkbox"/>
If yes, did you attempt to control this weight change by diet and/or exercise?	<input type="checkbox"/>	<input type="checkbox"/>
Have you experienced faintness, light headedness or blackouts?	<input type="checkbox"/>	<input type="checkbox"/>
Have you experienced unusual heartbeats such as skipped beats or palpitations?	<input type="checkbox"/>	<input type="checkbox"/>
Have you experienced periods in which you heart felt as though it was racing for no apparent reason?	<input type="checkbox"/>	<input type="checkbox"/>

11. At present, do you:	YES	NO
1. Experience shortness or loss of breath when walking?	<input type="checkbox"/>	<input type="checkbox"/>
2. Experience sudden tingling, numbness or loss of feeling in your arms, hands, legs, feet or face?	<input type="checkbox"/>	<input type="checkbox"/>
3. Experience swelling in your feet or ankles?	<input type="checkbox"/>	<input type="checkbox"/>
4. Experience pains or cramps in your legs?	<input type="checkbox"/>	<input type="checkbox"/>
5. Experience any pain or discomfort in your chest?	<input type="checkbox"/>	<input type="checkbox"/>
6. Experience any pressure or heaviness in your chest?	<input type="checkbox"/>	<input type="checkbox"/>
7. Suffer from diabetes?	<input type="checkbox"/>	<input type="checkbox"/>

If yes, how is it controlled (circle)?

- Dietary means
- Insulin injection
- Oral medication
- Uncontrolled

12. Have you ever had asthma?	YES	NO
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13. How often would you characterise your stress levels as being high?

1. Occasionally
2. Frequently
3. Constantly

14. Tick the appropriate box indicating whether or not you have previous suffered from any of the following conditions?

	YES	NO
1. Myocardial Infarction	<input type="checkbox"/>	<input type="checkbox"/>
2. Arteriosclerosis	<input type="checkbox"/>	<input type="checkbox"/>
3. Heart Disease	<input type="checkbox"/>	<input type="checkbox"/>
4. Heart Block	<input type="checkbox"/>	<input type="checkbox"/>
5. Coronary Thrombosis	<input type="checkbox"/>	<input type="checkbox"/>
6. Rheumatic Heart Complications	<input type="checkbox"/>	<input type="checkbox"/>
7. Heart Attack	<input type="checkbox"/>	<input type="checkbox"/>
8. Aneurism	<input type="checkbox"/>	<input type="checkbox"/>
9. Coronary Occlusion	<input type="checkbox"/>	<input type="checkbox"/>
10. Angina	<input type="checkbox"/>	<input type="checkbox"/>
11. Heart Failure	<input type="checkbox"/>	<input type="checkbox"/>
12. Heart Murmur	<input type="checkbox"/>	<input type="checkbox"/>
13. Neuromuscular Disorder	<input type="checkbox"/>	<input type="checkbox"/>
14. Peripheral Artery Disease	<input type="checkbox"/>	<input type="checkbox"/>
15. Pulmonary Embolism	<input type="checkbox"/>	<input type="checkbox"/>
16. Deep Vein Thrombosis	<input type="checkbox"/>	<input type="checkbox"/>
17. Compartment Syndrome	<input type="checkbox"/>	<input type="checkbox"/>
18. Oedema	<input type="checkbox"/>	<input type="checkbox"/>

SCREENING CHECKLIST:

- ☐ Apparently healthy individual
- ☐ Medical examination required
- ☐ Submaximal  $\dot{V}O_2$  test required
- ☐ Diagnostic testing required
- ☐ Inadequate information given
- ☐ Exclusion from study
- ☐ Special conditions for inclusion:

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Participant: \_\_\_\_\_ Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

Exercise Physiologist: \_\_\_\_\_ Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

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Exercise recommendations/considerations

## APPENDIX 3

### **Institutional Ethics Approval**

## MEMORANDUM

*From the Office of Research*



**Secretary, Human Research Ethics Committee**

**Ph: 07 4923 2603**

**Fax: 07 4923 2600**

Email: n.turner@cqu.edu.au

19 August 2004

Mr Ben Dascombe  
Faculty of Arts, Health and Science  
Building 77, Central Queensland University  
ROCKHAMPTON QLD 4702

Dear Mr Dascombe,

**HUMAN RESEARCH ETHICS COMMITTEE APPROVAL FOR PROJECT  
H04/07-83, *THE EFFECTS OF AGING AND TRAINING ON PULMONARY  
AND MUSCULAR OXYGEN KINETICS DURING MODERATE, HEAVY AND  
SEVERE INTENSITY CYCLING EXERCISE.***

The Human Research Ethics Committee is an approved institutional ethics committee constituted in accord with guidelines formulated by the National Health and Medical Research Council (NHMRC) and governed by policies and procedures consistent with principles as contained in publications such as the joint Australian Vice-Chancellors' Committee and NHMRC *Statement and Guidelines on Research Practice*.

At its meeting on 10 August 2004, the Human Research Ethics Committee of the Central Queensland University granted ethics approval for the research activity, *The effects of aging and training on pulmonary and muscular oxygen kinetics during moderate, heavy and severe intensity cycling exercise*. (Project Number H04/07-83).

**The period of ethics approval is 16 August 2004 to 20 November 2004.**

**The approval number is H04/07-83.**

The conditions of approval for this research project are that you:

- (a) liaise with Dr Joyner or Mr Fenlon regarding the resubmission of the procedure for the control group (45-60 years); and amend the hypertension figure to Stage 1, 160/100;
- (b) amend the procedure for the biopsies to be performed by a medical practitioner in a medical facility;
- (c) amend section 4.1 to provide counselling support to participants and include the details on the Information Sheet;
- (d) conduct the research project strictly in accordance with the proposal submitted and granted ethics approval, including any amendments required to be made to the proposal by the Human Research Ethics Committee;
- (e) report immediately anything which may warrant review of ethics approval of the project, including:
  - (i) serious or unexpected adverse effects on participants;
  - (ii) proposed changes in the protocol;
  - (iii) unforeseen events that might affect continued ethical acceptability of the project;

(A written report of any adverse occurrence or unforeseen event that might affect the continued ethical acceptability of the research project must be submitted to the Chair of the Human Research Ethics Committee by no later than the next working day after recognition of an adverse occurrence/event.)

- (f) provide the Human Research Ethics Committee with a written "Final Report" by no later than 31 December 2004;
- (g) if the research project is discontinued, advise the Committee in writing within 5 working days of the discontinuation;
- (h) comply with each and all of the above conditions of approval and any additional conditions or any modification of conditions which may be made subsequently by the Human Research Ethics Committee.

Please forward relevant information (via email or memo) and relevant documentation to the Human Research Ethics Committee Secretary within 30 working days from the date of this advice. Please note that failure to comply with the conditions of approval and the *National Statement on Ethical Conduct in Research Involving Humans* may result in withdrawal of approval for the project.

A copy of the reporting pro formas may be obtained from the Human Research Ethics Committee Secretary, Nicole Turner please contact at the telephone or email given on the first page.

You are required to advise the Secretary in writing within 5 working days if this project does not proceed for any reason. In the event that you require an extension of ethics approval for this project, please make written application in advance of the end-date of this approval. The research cannot continue beyond the end date of approval unless the Committee has granted an extension of ethics approval. Extensions of approval cannot be granted retrospectively. Should you need an extension but not apply for this before the end-date of the approval then a full new application for approval must be submitted to the Secretary for the Committee to consider.

If you have any queries in relation to this approval or if you need any further information please contact the Secretary, Nicole Turner or myself.

Yours sincerely,

Associate Professor Ken Purnell  
Chair, Human Research Ethics Committee

## APPENDIX 4

### **Cuff Ischemia and Muscle Biopsy Pain Scale Results**

### MUSCLE BIOPSY PAIN AND FOLLOW-UP DATA

Subject Number	Date	Pain Rating (/10)	12 hr Follow Up	24 hr Follow Up	48 hr Follow Up	TIME BEFORE RESUMING ACTIVITY
BDMA01	10/05/2004	4	Severe cork	Major Cork	No problems	36
BDMA02	19/10/2004	1	Tender with limp - major cork	Mild soreness	No problems	48
BDMA03	10/05/2004	3	Tender with limp - major cork	Mild soreness	No problems	48
BDMA04	10/12/2004	1	Tender with limp - major cork	Tender with limp - major cork	Minor cork	3
BDMA05	10/12/2004	5	Severe cork	No problems	No problems	48
BDMA06	10/11/2004	3	Tender with limp - major cork	Minor cork	No problems	72
BDMA07	19/10/2004	1	Slight cork	No problems	No problems	36
<b>MEAN</b>		<b>2.6</b>				
BDYT01	27/09/04	3	Throbbing pain	Slight cork	No problems	32
BDYT02	28/09/04	2	Slight cork	No problems	No problems	24
BDYT03	10/04/2004	3	Major Cork	Minor cork	No problems	48
BDYT04	18/10/2004	3	Major Cork	Slight Cork	No problems	24
BDYT05	28/09/04	3	Slight cork	No problems	No problems	48
BDYT06	10/04/2004	1	Minor ache	No problems	No problems	48
BDYT07	16/11/2004	5	Minor ache	Minor cork	No problems	32
<b>MEAN</b>		<b>2.9</b>				

## CUFF ISCHEMIA PAIN DATA

Subject Number	RAMP	SWT1	SWT2	SWT3
BDMA01	5	5	5	3
BDMA02	6	7	4	3
BDMA03	5	4	4	4
BDMA04	6	2	2	2
BDMA05	5	2	3	3
BDMA06	5	5	4	4
BDMA07	3	3	2	2
<b>MA AVERAGE</b>	<b>5.0</b>	<b>4.0</b>	<b>3.4</b>	<b>3.0</b>
BDYT01	4	5	4	3
BDYT02	4	5	5	4
BDYT03	3	3	2	3
BDYT04	4	2	2	2
BDYT05	7	4	5	5
BDYT06	6	7	6	5
BDYT07	8	8	8	9
<b>YT AVERAGE</b>	<b>5.1</b>	<b>4.9</b>	<b>4.6</b>	<b>4.4</b>
<b>TOTAL AVERAGE</b>	<b>5.1</b>	<b>4.4</b>	<b>4.0</b>	<b>3.7</b>

<b>0</b>	Pain Free
<b>1</b>	Very minor annoyance - occasional minor twinges.
<b>2</b>	Minor annoyance - occasional strong twinges.
<b>3</b>	Annoying enough to be distracting.
<b>4</b>	Can be ignored if you are really involved in your work, but still distracting.
<b>5</b>	Can't be ignored for more than 30 minutes.
<b>6</b>	Can't be ignored for any length of time, but you can still go to work and participate in social activities.
<b>7</b>	Makes it difficult to concentrate, interferes with sleep You can still function with effort.
<b>8</b>	Physical activity severely limited. You can read and converse with effort. Nausea and dizziness set in as factors of pain.
<b>9</b>	Unable to speak. Crying out or moaning uncontrollably - near delirium.
<b>10</b>	Unconscious. Pain makes you pass out.