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 ± 1.7 y; $\dot{V}O_2max$= 55.2 ± 7.0 $mL \bullet kg^{-1} \bullet min^{-1}$) and seven middle-aged (MA) (age= 44.8 ± 2.7 y; $\dot{V}O_2max$= 50.2 ± 6.4 $mL \bullet kg^{-1} \bullet min^{-1}$) 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 ($\dot{V}O_2max$) 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 ± 5.1 %$\dot{V}O_2max$; MA: 70.2 ± 4.0 %$\dot{V}O_2max$; $p>0.05$) or $\dot{V}O_2max$ (Y: 3.8 ± 0.5 $L \bullet min^{-1}$, MA: 3.9 ± 0.6 $L \bullet min^{-1}$, $p>0.05$). 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, β-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 ± 0.5 W•kg BM^{-1}; MA: 3.3 ± 0.5 W•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%Δ VT-$\dot{V}O_2$max) and severe- (80%Δ VT-$\dot{V}O_2$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 (τ_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 $\dot{V}O_2$ τ_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 τ_p and $\dot{V}O_2$ τ_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 $\dot{V}O_2$ τ_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 $\dot{V}O_2$max 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 τ (τ_s) in the young cyclists, with the heavy-intensity τ_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 τ (τ_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 $\dot{V}O_2$ τ_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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Lastly, this thesis is dedicated to all those people who feel surrounded in doubt and don't think they can win – but who still bury their head and have a dig anyway.

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Eq^n 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^n 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^n 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^n 5: $A = \varepsilon \cdot [c] \cdot L \cdot B + G$

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

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

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

Eq^n 10: $MPF = LS * time + IMF$

Eq^n 11:

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

Eq^n 12:

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

Eq^n 13:

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

Eq^n 14:

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

Eq^n 15:

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

Eq^n 16:

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

Eq^n 17:

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

LIST OF ABBREVIATIONS AND NOMENCLATURE

2-OGDH	2-Oxogluterate dehydrogenase
20TT	Twenty kilometre time trial
30TT	Thirty minute time trial
ΔAbs•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_2diff	Arterio-venous oxygen difference
B	Path length of the scattering light
b•min^{-1}	Beats per minute
BLa^-	Blood lactate
[BLa^-]	Blood lactate concentration
β-HAD	Beta-hydroxyacyl-CoA dehydrogenase
BSA	Bovine serum albumin
C	Chromophore concentration
cap•mm^{-2}	Capillaries per millimetre squared
C/F	Capillary to fibre ratio
CC/F	Capillary contacts per fibre

$CC \bullet \mu m^{-2}$	Capillary contact per fibre area
CI	Confidence interval
cm	Centimetre
CO_2	Carbon dioxide
CoA	Co-enzyme A
CS	Citrate synthase
CSA	Cross-sectional area
CV%	Percentage of Co-variance
°	Degree
^{o}C	Degrees Celsius
Δ	Difference between VT and $\dot{V}O_2max$
DD_{mean}	Mean diffusion distance
DD_{max}	Maximum diffusion distance
DTNB	3,3'-dithiobis(6-nitrobenzoic acid)
ε	Chromophore extinction coefficient
EDTA	Ethylenediaminetetraacetate
$EE\dot{V}O_2$	End-exercise oxygen consumption
EEmOxy	End-exercise muscle oxygenation
EPOC	Excess post-exercise oxygen consumption
Eq^{n}	Equation
η^2	Partial eta squared
F_{sample}	Fluorescence of sample
F_{std}	Fluorescence of standard
F_IO_2	Fraction of inspired oxygen
g	Gram
G	Geometry coefficient

$g_{wt\ muscle}$	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
kΩ	Kilo-ohms
L	Litres
l	Distance between light source and detectors
$L \bullet min^{-1}$	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_2	Oxymyoglobin
$\bar{X}$	Mean
$MgCl_2$	Magnesium chloride
MHC	Myosin heavy chain
min	Minute
mL	Millilitre
$mL_{vol\ std}$	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^2	Millimetre squared
mM	Millimolar
$mmol \cdot L^{-1}$	Millimole per litre
mmol•kg w.w. $^{-1}$	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_2	Nitrogen molecule
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced)
NaOH	Sodium hydroxide
NIRS	Near infrared spectroscopy
nm	Nanometre
O_2	Oxygen molecule
p	Alpha
PAS	Periodic acid-schiff
PCr	Phosphocreatine
%	Percent
$\%\bullet W^{-1}$	Percent per watt
PFK	Phosphofructokinase
PO	Power output
pO_2	Partial pressure of oxygen
pCO_2	Partial pressure of carbon dioxide
$\dot{Q}$	Cardiac output
r	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
√	Square root
SWT	Square wave transition
SV	Stroke volume
∑	Sum
τ	Time constant
$\tau_{1/2}$	Half-life
τ_f	Off-transient time constant
τ_{fs}	Slow off-transient time constant
τ_p	Primary component time constant
τ_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
$\mu g \cdot \mu L^{-1}$	Microgram per microlitre
µL	Microlitre
$U \cdot mL^{-1}$	Units per millilitre

µm	Micrometre
μm^2	Micrometre squared
µmol	Micromole
$\mu mol \bullet g_{w.w.}^{-1} \bullet min^{-1}$	Micromole per gram wet weight per minute
$\mu mol \bullet g_{protein}^{-1} \bullet min^{-1}$	Micromole per gram of protein per minute
$\mu mol \bullet L^{-1}$	Micromole per litre
$\mu mol \bullet L^{-1} \bullet min^{-1}$	Micromole per litre per minute
vs.	Versus
$v\dot{V}O_2max$	Velocity at maximal oxygen consumption
$\dot{V}CO_2$	Volume of carbon dioxide produced
$\dot{V}E$	Volume of expired air
VL	Vastus lateralis
VM	Vastus medialis
$\dot{V}O_2$	Volume of oxygen consumed
$\dot{V}O_2$ (b)	Baseline oxygen consumption
$\dot{V}O_2max$	Maximal oxygen consumption
$\%\dot{V}O_2max$	Percentage of maximal oxygen consumption
VT	Ventilatory threshold
W	Watt
$W \bullet kg\ BM^{-1}$	Watts per kilogram body mass
wMRT	Weighted mean response time
$wMRT_f$	Off-transient weighted mean response time
y	Year