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; τ_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 τ_p in either age group. The stable $\dot{V}O_2$ and mOxy τ_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 τ_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 (± 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** [€]	**Moderate**	**Heavy**	**Severe** [€]
SWT Power (W)	178.8 ± 29.5	281.3 ± 36.1	316.7 ± 37.1	197.8 ± 22.7	316.7 ± 32.5	358.4 ± 35.6
Baseline $\dot{V}O_2$ ($mL \cdot min^{-1}$)	1118 ± 141	1180 ± 136	1423 ± 258 [§ ¥]	1293 ± 134	1386 ± 273	1560 ± 304 [§]
A_p ($mL \cdot min^{-1}$)	13202 ± 239	1953 ± 319 [#]	1733 ± 285 [§]	1336 ± 151	1829 ± 376 [#]	2054 ± 480 [§]
TD_p (s)	3.2 ± 2.1	3.9 ± 2.4	4.2 ± 1.5	5.9 ± 2.0	3.1 ± 1.4 [#]	3.1 ± 1.0 [§]
τ_p (s)	23.2 ± 6.9	23.0 ± 4.6	26.0 ± 5.8	25.5 ± 7.0	25.5 ± 5.0	30.4 ± 3.2
G_p ($mL \cdot min^{-1} \cdot W^{-1}$)	7.4 ± 0.5	6.9 ± 1.4 [#]	5.5 ± 0.8 [§ ¥]	7.5 ± 0.5	6.2 ± 1.1 [#]	6.1 ± 1.3 [§]
G_o ($mL \cdot min^{-1} \cdot W^{-1}$)	7.4 ± 0.5	8.8 ± 1.2	7.7 ± 1.3	7.5 ± 0.5	8.3 ± 1.3	6.3 ± 1.5
$EE\dot{V}O_2$ ($mL \cdot min^{-1}$)	2469 ± 363	3487 ± 510 [#]	3405 ± 418 [§]	2622 ± 239	3648 ± 345 [#]	3636 ± 363 [§]
wMRT (s)	39.2 ± 5.9	54.4 ± 11.8 [#]	38.7 ± 11.7 [¥]	36.3 ± 3.5	51.2 ± 14.5 [#]	29.2 ± 7.5 [¥]

A_p= Primary Amplitude TD_p= Primary Component Time Delay τ_p= Primary Time Constant G_p= Primary Component Gain
G_o= Overall Response Gain $EE\dot{V}O_2$ = End-Exercise $\dot{V}O_2$ wMRT= Weighted Mean Response Time

[#] significant difference between moderate and heavy-intensities ($p<0.05$); [§] significant difference between moderate and severe-intensities ($p<0.05$); [¥] significant difference between heavy and severe-intensities ($p<0.05$) [€] 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 τ_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 τ_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 $\dot{V}O_2$ τ_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 that 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 τ_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 (± 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** [€]	**Moderate**	**Heavy**	**Severe** [€]
SWT Power (W)	178.0 ± 29.5	281.0 ± 36.1	317.0 ± 37.1	198.0 ± 22.7	317.0 ± 32.5	358.0 ± 35.6
Baseline mOxy (%)	82.0 ± 8.9	78.4 ± 22.2	58.9 ± 14.1 [§ ¥]	81.8 ± 10.3	89.3 ± 3.2	78.1 ± 6.0 [¥]
A_p (%)	24.5 ± 8.5	35.2 ± 12.5	39.3 ± 14.3	32.3 ± 10.0	45.3 ± 12.4 [#]	48.9 ± 13.2
TD_p (s)	5.9 ± 2.1	5.2 ± 1.9	2.9 ± 1.3 [§ ¥]	5.2 ± 1.8	5.0 ± 1.7	2.4 ± 1.3 [§ ¥]
τ_p (s)	12.5 ± 6.1	16.0 ± 10.6	12.1 ± 6.3	15.4 ± 5.5	13.2 ± 14.8	10.3 ± 7.1
G_p (%•W^{-1})	0.15 ± 0.06	0.13 ± 0.06	0.13 ± 0.07	0.19 ± 0.65	0.16 ± 0.05	0.12 ± 0.05 [§ ¥]
G_o (%•W^{-1})	0.15 ± 0.06	0.18 ± 0.10	0.17 ± 0.07	0.26 ± 0.09	0.19 ± 0.05	0.16 ± 0.05 [§ ¥]
EEmOxy (%)	59.7 ± 14.1	31.0 ± 19.9 [#]	7.8 ± 8.4 [§ ¥]	66.5 ± 10.5	35.6± 12.2 [#]	32.3 ± 15.0 [§ ¥]
wMRT (s)	33.3 ± 9.1	47.6 ± 20.2	37.6 ± 18.2	45.7 ± 20.7	64.4 ± 37.2	34.5 ± 12.3

A_p= Primary Amplitude TD_p= Primary Time Delay τ_p = Primary Time Constant G_p= Primary Gain
G_o= Overall Gain EEmOxy= End-Exercise mOxy wMRT= Weighted Mean Response Time

[#] significant difference between moderate and heavy-intensities ($p<0.05$); [§] significant difference between moderate and severe-intensities ($p<0.05$); [¥] significant difference between heavy and severe-intensities ($p<0.05$); [€] 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 $\dot{V}O_2$ 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 τ_p. The $\dot{V}O_2$ τ_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 τ_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 $\dot{V}O_2$ 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 ($F(2,11)=7.91$, $p=0.004$, $\eta^2=613$) and severe-intensity ($F(2,11)=7.91$, $p=0.028$, $\eta^2=0.613$) SWT.

Hematological Responses

Blood pH

The mean (± SD) changes in blood pH during the three SWT intensities are shown in Table 5.3. No significant main effects of age or age x 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 (± 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**
Moderate	7.404 ± 0.022	7.389 ± 0.024	7.387 ± 0.034	7.410 ± 0.026	7.403 ± 0.020	7.412 ± 0.018
Heavy	7.408 ± 0.015	7.320 ± 0.018 #	7.218 ± 0.032 § ¥	7.418 ± 0.031	7.334 ± 0.021 #	7.237 ± 0.068 § ¥
Severe	7.383 ± 0.026	7.199 ± 0.043 #		7.376 ± 0.022	7.229 ± 0.044 #	

significant difference between 0 and 3 min; § significant difference between 3 and 6 min; ¥ 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 (± 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 x 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 (± 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**
Moderate	42.5 ± 2.5	41.8 ± 2.7	42.7 ± 7.8	41.7 ± 2.8	41.4 ± 1.6	40.9 ± 3.0
Heavy	41.2 ± 2.4	41.6 ± 2.7	36.9 ± 5.7 §	41.6 ± 2.9	40.2 ± 1.0	37.3 ± 2.4 § ¥
Severe	38.3 ± 3.5	36.4 ± 4.3		37.7 ± 3.2	36.1 ± 2.8	

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

Blood Bicarbonate

The mean (± 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 [HCO_3^-] was observed. In the young cyclists, blood [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 (± SD) blood [HCO_3^-] (mmol•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**
Moderate	26.5 ± 1.2	25.4 ± 1.2	24.4 ± 1.8 §	26.5 ± 0.9	26.2 ± 1.3	26.1 ± 1.7
Heavy	26.1 ± 1.5	20.0 ± 4.6 #	15.3 ± 2.1 § ¥	26.7 ± 1.1	21.4 ± 1.2 #	16.0 ± 1.8 § ¥
Severe	22.8 ± 1.6	12.8 ± 3.0 #		22.8 ± 3.1	15.1 ± 1.3 #	

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

The difference in the blood [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 [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, [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 (± SD) changes in [BLa^-] during the three SWT intensities are shown in Table 5.6. No significant main effect of age or age x 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 (± SD) [BLa^-] ($mmol \bullet L^{-1}$) 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**
Moderate	1.9 ± 0.7	4.2 ± 0.3 #	4.7 ± 0.9 ¥	2.0 ± 0.6	4.1 ± 2.0 #	3.1 ± 0.9 ¥
Heavy	1.9 ± 0.7	8.9 ± 1.1 #	15.3 ± 1.9 § ¥	1.8 ± 0.5	8.9 ± 1.3 #	14.4 ± 2.4 § ¥
Severe	4.7 ± 1.9	16.4 ± 0.7 §		5.3 ± 2.6	15.2 ± 1.0 §	

significant difference between 0 and 3 min; § significant difference between 3 and 6 min; ¥ 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**
$\dot{V}O_2$ τ_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	$[HCO_3^-]$ @ 0 min	0.93	0.002
$\dot{V}O_2$ wMRT	mOxy MRT	0.77	0.044	$\dot{V}O_2$ G_o	$[HCO_3^-]$ Δ0-6 min	0.91	0.004
	mOxy G_o	0.81	0.049				
	$[BLa^-]$ Δ3-6 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	$\dot{V}O_2$ τ_p	mOxy A_p	-0.84	0.019
	pH Δ0-3 min	-0.93	0.002		pH Δ0-3 min	-0.82	0.024
$\dot{V}O_2$ τ_p	mOxy τ_p	0.94	0.005		$[HCO_3^-]$ Δ0-3 min	-0.80	0.033
$\dot{V}O_2$ G_p	pO_2 @ 3 min	-0.95	0.001		$[BLa^-]$ Δ3-6 min	-0.81	0.027
	$[HCO_3^-]$ Δ0-3 min	-0.76	0.047	$\dot{V}O_2$ G_o	mOxy A_p	0.77	0.043
	$[BLa^-]$ Δ0-6 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		$[BLa^-]$ Δ0-6 min	0.89	0.007
	$[HCO_3^-]$ Δ3-6 min	-0.79	0.033		$[HCO_3^-]$ Δ0-3 min	-0.78	0.039
	pH Δ0-6 min	-0.76	0.049		$[HCO_3^-]$ Δ0-6 min	-0.81	0.027
	$[BLa^-]$ Δ0-6 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
$\dot{V}O_2$ τ_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 min	0.77	0.043
$\dot{V}O_2$ G_p	$[BLa^-]$ @ 3 min	-0.94	0.002	$\dot{V}O_2$ τ_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
	$\dot{V}O_2$ τ_p	0.83	0.040	$\dot{V}O_2$ G_p	$[HCO_3^-]$ @ 3 min	-0.81	0.028
mOxy τ_p	$[BLa^-]$ @ 3 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 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 τ	0.81	0.028	Type I %	Moderate τ_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 τ	0.87	0.026
	Heavy TD_p	-0.84	0.019	Type IIa CSA	Severe τ_p	-0.92	0.009
C:F Ratio	Moderate τ	-0.78	0.041		Severe wMRT	-0.92	0.009
	Moderate MRT	-0.85	0.015	Type IIb %	Moderate τ	0.85	0.034
CC/F	Moderate MRT	-0.81	0.028		Severe τ_p	0.77	0.044
CS activity	Moderate A_p	-0.78	0.037	CCFA	Severe τ_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_o	0.88	0.021	Type IIb CSA	Heavy A_s	0.96	0.011
Type IIb %	Moderate TD_p	0.92	0.010	Capillary Density	Heavy TD_p	-0.82	0.024
Capillary Density	Severe EEmOxy	-0.82	0.046	C:F Ratio	Severe TD_p	0.83	0.008
C:F Ratio	Moderate A_p	-0.91	0.013	CC/F	Heavy TD_p	0.78	0.039
	Heavy A_s	0.98	0.022		Heavy A_s	0.96	0.010
CC/F	Heavy A_s	0.96	0.045		Heavy wMRT	0.80	0.032
	Heavy G_s	0.99	0.006		Severe TD_p	0.83	0.020
DD_{max}	Heavy τ_p	0.89	0.016		Severe A_s	0.93	0.007
	Heavy A_s	0.99	0.010		Severe wMRT	0.80	0.032
2-OGDH Activity	Moderate τ_p	0.85	0.034	CCFA	Heavy A_s	-0.90	0.034
PFK Activity	Moderate TD_p	-0.97	0.001		Severe TD_p	0.76	0.047
LDH Activity	Heavy wMRT	0.90	0.016	DD_{max}	Heavy A_s	0.96	0.009
	Severe A_p	0.84	0.035		Heavy TD_p	0.79	0.034
				DD_{mean}	Moderate wMRT	-0.85	0.033
					Heavy A_s	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; τ_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 $\dot{V}O_2$max (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 hgiher $\dot{V}O_2$ 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 $\dot{V}O_2$max 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 $\dot{V}O_2$max, 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 ± 3 y) subjects demonstrated significantly higher maximal aerobic powers (3.8 ± 0.4 $L \bullet min^{-1}$; 49 ± 6 $mL \bullet kg^{-1} \bullet min^{-1}$) than the elderly (68 ± 3y; 2.3 ± 0.3 $L \bullet min^{-1}$; 27 ± 3 $mL \bullet kg^{-1} \bullet min^{-1}$) 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 $\dot{V}O_2$max.

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 (~6-9 $mL \bullet min^{-1} \bullet W^{-1}$) were considerably lower than those reported in previous literature (~9-11 $mL \bullet min^{-1} \bullet W^{-1}$) 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 (η^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 throuugh 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% $\dot{V}O_2$max (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 $\dot{V}O_2$max 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 $\dot{V}O_2$max 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_p was significantly faster than that observed in the $\dot{V}O_2$ TD_p 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_p. The NIRS measurement of mOxy is more instantaneous and reflects changes in intra-muscular O_2 utilisation. The changes in O_2 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_2 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 $\dot{V}O_2$ τ_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 $L \cdot min^{-1}$) demonstrated a slowed $\dot{V}O_2$ τ_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 $L \cdot min^{-1}$) cohort. More recently, concurrent training into older age has been shown to ameliorate the moderate-intensity $\dot{V}O_2$ τ_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 τ_p has previously been suggested to remain stable or to be improved with aging across exercise transitions despite a slowed $\dot{V}O_2$ τ_p response in sedentary aged subjects (DeLorey et al. 2004a; 2005). These investigators reported that the on-transient mOxy τ_p was similar between young (Y) and old (O) sedentary populations in response to a moderate-intensity (Y: 13 ± 10 s; O: 9 ± 3 s) exercise bout. The same investigators reported that the sedentary elderly cohort (8 ± 2 s) demonstrated a significantly faster mOxy τ_p than the younger subjects (14 ± 2 s) in response to heavy-intensity exercise. Other researchers have also suggested that the mOxy τ_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 τ_p demonstrated a significant effect of intensity in either age group. Thus, the present finding supports previous suggestions that the $\dot{V}O_2$ τ_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 $\dot{V}O_2$ τ_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 $\dot{V}O_2$ τ_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 τ_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 τ_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 τ_p was significantly faster than the $\dot{V}O_2$ τ_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 $\dot{V}O_2$ τ_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 $\dot{V}O_2$ τ_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 $\dot{V}O_2$ τ_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^{ω}-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 (Frandersen, 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; τ_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 $\dot{V}O_2$max and muscle histochemical and enzymatic characteristics.