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, ß-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 $\dot{V}O_2max$, 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 (± SD) physical characteristics of the young and middle-aged cyclists

Characteristic	Young	Middle-aged	Cohens D (95% CI)
Age (y)	19.6 ± 1.7	47.9 ± 2.7 *	1.91 (25.6 - 30.9)
Height (cm)	176.8 ± 3.9	178.3 ± 5.7	0.33 (-4.1 - 7.3)
Mass (kg)	69.6 ± 8.0	79.3 ± 15.7	0.85 (-2.6 - 21.9)
Σ 9 SF (mm)	93.6 ± 19.8	121.7 ± 30.0	0.98 (-1.5 - 57.6)
$\dot{V}O_2max$ ($L \cdot min^{-1}$)	3.82 ± 0.46	3.95 ± 0.60	0.25 (-0.5 - 0.8)
$\dot{V}O_2max$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	55.2 ± 7.0	50.2 ± 6.4	0.71 (-12.8 - 2.8)
VT ($L \cdot min^{-1}$)	2.68 ± 0.41	2.81 ± 0.40	0.36 (-0.3 - 0.6)
VT (%$\dot{V}O_2max$)	71.4 ± 5.1	70.2 ± 4.0	0.26 (-0.31 - 0.57)
HR_{max} ($b \cdot min^{-1}$)	194.4 ± 10.0	180.7 ± 12.1 *	1.0 (-26.6 - 0.7)
Training history (y)	9.7 ± 5.9	4.7 ± 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 (μm^2).

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

Histochemical Characteristic	Young	Middle-Aged	Cohens D (95% CI)
Type I (%)	58.3 ± 9.6	58.7 ± 16.6	0.03 (-15.7 - 16.6)
Type IIa (%)	33.7 ± 7.7	34.8 ± 12.9	0.11 (-11.5 - 13.9)
Type IIb (%)	8.0 ± 3.0	6.4 ± 4.1	0.45 (-5.9 - 2.8)
Type I CSA (μm^2)	5599 ± 1478	6759 ± 1060	0.83 (-437 - 2757)
Type IIa CSA (μm^2)	6844 ± 1993	7026 ± 1456	0.10 (-1984 - 2348)
Type IIb CSA (μm^2)	6082 ± 2534	5985 ± 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 (± 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 (cap·mm^{-2})	301.0 ± 57.4	287.0 ± 40.0	0.31 (-72.6 - 42.6)
Capillary to Fibre Ratio (C/F)	2.3 ± 0.8	2.6 ± 0.4	0.54 (-0.4 - 1.0)
Capillary Contacts per Fibre (CC/F)	4.6 ± 1.0	5.1 ± 0.3	0.66 (-0.7 - 1.4)
Capillary Contacts per Fibre Area (CC/µm^2)	7.8 ± 2.2 (10^{-4})	7.6 ± 1.2 (10^{-4})	0.16 (-2.2 - 1.7 (10^{-4}))
Maximum Diffusion Distance (µm)	14.5 ± 7.1	18.9 ± 3.8	0.75 (-2.2 - 11.1)
Average Diffusion Distance (µm)	24.6 ± 3.8	24.5 ± 1.4	0.01 (-3.4 - 3.3)

Enzyme Activities

Table 4.4 shows the maximal specific activities (µmol•$g_{protein}^{-1}$•min^{-1}) of glycolytic (PFK and LDH) and oxidative (CS, ß-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 (± SD) maximal specific glycolytic and oxidative enzyme activities (µmol•$g_{protein}^{-1}$•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 ± 5.0	41.0 ± 8.3	0.85 (-14..3 - 1.7)
LDH	242.3 ± 36.6	202.1 ± 86.1	0.60 (-117.2 - 36.8)
CS	17.0 ± 4.8	15.3 ± 5.5	0.32 (-7.6 - 4.4)
ß-HAD	1.0 ± 0.1	1.0 ± 0.3	0.37 (-0.2 - 0.4)
2-OGDH	0.10 ± 0.03	0.10 ± 0.04	0.85 (-0.007 – 0.7)

30 Minute Time Trial Results

The mean (± 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 %$\dot{V}O_2$max ($F(1,12)=6.259$, $p<0.001$, $\eta^2=0.343$). 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_{max} ($F(2,24)=52.528$, $p<0.001$, $\eta^2=0.814$) and 30TT %VO_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_{max} ($F(2,12)=13.804$, $p=0.001$, $\eta^2=0.697$) 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_{max} ($F(2,12)=94.258$, $p<0.001$, $\eta^2=0.940$) and RPO ($F(2,12)=3.943$, $p=0.048$, $\eta^2=0.397$) across the 30TT.

Table 4.5: Mean (± 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**
PO (W)	213.0 ± 37.9	206.7 ± 38.1	220.5 ± 41.1	253.7 ± 28.8 #	254.3 ± 30.0 #	263.0 ± 30.3 #
RPO ($W \cdot kg\ BM^{-1}$)	3.1 ± 0.5	3.0 ± 0.5	3.2 ± 0.6	3.2 ± 0.4	3.3 ± 0.5	3.4 ± 0.5 ¥
HR ($b \cdot min^{-1}$)	158.8 ± 18.0	169.8 ± 17.3 ¥	176.2 ± 10.2 ¥	148.2 ± 8.2	160.6 ± 9.0 ¥	166.4 ± 9.9 ¥
$\%HR_{max}$ (%)	81.7 ± 8.8	87.4 ± 8.7 ¥	90.7 ± 5.0 ¥	82.2 ± 4.9	89.0 ± 4.4 ¥	92.2 ± 3.6 ¥ §
$\dot{V}O_2$ ($mL \cdot min^{-1}$)	2712.5 ± 418.3	2840.9 ± 372.2	2844.1 ± 290.3	3233.1 ± 329.4 #	3430.0 ± 452.2 #	3422.1 ± 629.3 #
$\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	39.1 ± 4.7	40.9 ± 3.0	41.0 ± 2.5	41.4 ± 5.6	43.9 ± 7.4	43.7 ± 8.6
$\%\dot{V}O_2max$ (%)	71.3 ± 8.5	74.7 ± 8.1	75.0 ± 7.4	82.6 ± 8.6 #	87.6 ± 11.0 #	87.0 ± 13.2 #
mOxy (%)	69.9 ± 26.6	64.7 ± 23.9	50.5 ± 18.1	67.9 ± 18.3	66.5 ± 16.5	55.7 ± 18.9

PO = Power Output; RPO = Relative Power Output; mOxy = Muscle Oxygenation; HR= Heart Rate; $\dot{V}O_2$= Oxygen Consumption; $\%HR_{max}$ = % of Maximum Heart Rate; $\%\dot{V}O_2max$ = % of Maximum Oxygen Consumption

significantly different to the young cyclists ($p<0.05$); ¥ significantly different to 10 min ($p<0.05$); § significantly different to 20 min ($p<0.05$).

The mean (± 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 (± 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**
pH (AU)	7.338 ± 0.058	7.334 ± 0.056	7.287 ± 0.044 [¥] [§]	7.336 ± 0.037	7.332 ± 0.047	7.276 ± 0.040 [¥] [§]
***p*O$_2$ (mmHg)**	37.8 ± 2.6	36.3 ± 3.3	35.6 ± 3.8	35.5 ± 3.7	32.3 ± 3.9 [#]	31.5 ± 4.7 [¥]
[HCO_3^-] (mmol•L^{-1})	20.4 ± 2.6	19.4 ± 3.2	17.1 ± 2.1 [¥] [§]	19.3 ± 2.9	17.3 ± 3.5 [#]	15.0 ± 3.5 [¥] [§]
[BLa^-] (mmol•L^{-1})	8.7 ± 4.0	9.5 ± 4.3	12.6 ± 1.9 [¥] [§]	9.5 ± 3.3	10.5 ± 3.8	13.2 ± 3.5 [¥] [§]

[#] significantly different to the young cyclists ($p<0.05$); [¥] significantly different to 10 min ($p<0.05$); [§] 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, β-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•kg 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 ± 8.0 kg; MA: 79.3 ± 15.7 kg) and lower ∑9 skinfolds (Y: 93.6 ± 19.8 mm; MA: 121.7 ± 30.0 mm) than the middle-aged cyclists. Both age groups demonstrated substantially higher body mass and ∑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 $\dot{V}O_2max$ 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 ± 0.41 $L \cdot min^{-1}$; MA: 2.81 ± 0.40 $L \cdot min^{-1}$) and as a relative percentage of $\dot{V}O_2max$ (Y: 70.2 ± 4.0 $\%\dot{V}O_2max$;

MA: 71.4 ± 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 ± 7.0 mL•kg^{-1}•min^{-1}; 3.82 ± 0.46 L•min^{-1}) and middle-aged (50.2 ± 6.4 mL•kg^{-1}•min^{-1}; 3.95 ± 0.60 L•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 ± 10.6 mL•kg^{-1}•min^{-1}; MA: 37.9 ± 8.5 mL•kg^{-1}•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 ± 3 y; 3.96 ± 0.36 L•min^{-1}; Type I: 60.3 ± 9.6%; Type IIa: 33.4 ± 10.3%; Type IIb: 6.3 ± 7.1%) and masters (63 ± 6 y; 3.36 ± 0.04 L•min^{-}1; 59.6 ± 13.6%; 38.1 ± 14.7; 2.3 ± 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 ± 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 ± 3 y, n= 8) and masters (63 ± 6 y, n= 8) runners. Within the same study, a separate sub-sample of young highly-competitive runners (28 ± 3 y, n = 8) (significantly higher $\dot{V}O_2$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 O_2 delivery and rate of $\dot{V}O_2$ 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 $\dot{V}O_2$-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 $\dot{V}O_2$ 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_2 utilisation across changes in work intensity within the muscle.

Oxidative Enzymes

In the present study, the activities of CS, β-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 ± 6 y) showed significantly higher maximal activities of both SDH and β-HAD than younger (26 ± 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 ± 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 ± 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 ~ 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 $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$) or RPO ($W \cdot kg^{-1}$ BM) across the 30TT were similar between groups. However, the absolute 30TT PO (Y: 213.3 ± 41.1 W; MA: 257.0 ± 31.4 W) and $\dot{V}O_2$ (Y: 2.80 ± 0.44 $L \cdot min^{-1}$; MA: 3.36 ± 0.55 $L \cdot min^{-1}$) 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 ± 0.59 W•kg BM^{-1}; MA: 3.28 ± 0.49 W•kg BM^{-1}) and $\dot{V}O_2$ (Y: 40.23 ± 6.26 mL•kg^{-1}•min^{-1}; MA: 42.40 ± 6.95 mL•kg^{-1}•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 $\%HR_{max}$, despite the younger subjects maintaining a higher absolute HR across the 30TT. Significant effects of time in both the HR and $\%HR_{max}$ 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 $\%\dot{V}O_2max$, 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, pO_2, [HCO_3^-] and [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, [HCO_3^-] and [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 $\dot{V}O_2$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 $\dot{V}O_2max$ (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.