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Cardiovascular Adaptations Controlling Changes in V02max and VO2 Kinetics with Endurance Training in Older and Young Men and Women

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Cardiovascular Adaptations Controlling Changes in VO_{2max} and VO_2 Kinetics with Endurance Training in Older and Young Men and Women

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ABSTRACT

A reduced maximal O_2 uptake (VO_{2max}) and a slower rate of adjustment for VO_2 during the exercise on-transient have been demonstrated in older individuals compared to their younger counterparts. Endurance-exercise training elicits cardiovascular adaptations in older individuals, resulting in age-dependent limitations being reduced. The mechanisms explaining the improvements in cardiorespiratory fitness have been proposed to be sex-related. However, there is a gap of information in terms of the mechanisms and time-course of changes occurring in response to short-term endurance training. Additionally, there are few studies directly comparing physiological responses to endurance training in older and young men and women. This thesis examined the mechanisms explaining the changes in VO_{2max} and the VO_2 time constant (τVO_2) occurring in older compared with young men and women in response to a 12-week endurance training program.

The main findings were that: 1) a short-term training program with progressive increases in exercise intensity resulted in significant increases in VO_{2max} in older and young men and women; 2) the time-course of increases in VO_{2max} in response to a 12-week endurance training program was similar in older men and young men and women with changes in both maximal cardiac output and arterial-venous O_2 difference explaining the increases in VO_{2max}; however, older women showed a plateau-like response in VO_{2max} during the last 3 weeks of training and marked reliance on peripheral changes related to increased O_2 extraction throughout the training program; 3) the decrease in pulmonary τVO_2 (τVO_{2p}) in older and young men and women occurred within the first 3 weeks of training with no subsequent changes observed thereafter; 4) microvascular O_2 delivery to the active muscle sites of O_2 utilization seems to be an important constraint for the initial

slower rate of adjustment in τVO_{2p} in older and in young men and women and, although the fundamental control of VO_{2p} kinetics may take place intracellularly, by factors that were not measured in these studies, O_2 delivery appears to be a major constraint in participants with "slow" VO_2 kinetics.

In conclusion, an increase in VO_{2max} and speeding of VO_2 kinetics in older adults can be achieved with a short-term exercise training program.

Keywords: VO_2 kinetics, muscle O_2 distribution, near-infrared spectroscopy, endurance-training, aging.

CO-AUTHORSHIP

This thesis includes versions of the following manuscripts that were submitted and accepted for publication:

1. Murias JM, Kowalchuk JM & Paterson DP. Time course and mechanisms of adaptations in cardiorespiratory fitness with endurance training in older and young men (J Appl Physiol 2010 Jan; 108:621-627)

2. Murias JM, Kowalchuk JM & Paterson DP. Speeding of VO_2 kinetics with endurance training in old and young men is associated with improved matching of local O_2 delivery to muscle O_2 utilization (J Appl Physiol 2010; In Press)

3. Murias JM, Kowalchuk JM & Paterson DP. Mechanisms for increases in VO_{2max} with endurance training in older and young women (Med Sci Sports Exerc 2010; In Press)

4. Murias JM, Kowalchuk JM & Paterson DP. The time course of speeding of VO_2 kinetics in young and older women in response to endurance training (J Appl Physiol 2010; Submission # JAPPL-00270-2010)

These studies were designed by J. M. Murias and D. H. Paterson with helpful input from the advisory committee (J. M. Kowalchuk and T. J. Overend). All the data were collected and analyzed by J. M. Murias. The original manuscripts comprising this thesis were written by J. M. Murias with feedback provided by the co-authors.

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LIST OF ABBREVIATIONS

ACh	Acetylcholine
ADP	Adenosine diphosphate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
a.u.	Arbitrary units
$a\text{-}vO_{2diff}$	Arterial-venous oxygen difference
$a\text{-}vO_{2diffsub}$	Submaximal arterial-venous oxygen difference
CI	Confidence interval
CT	Continuous training
C_2H_2	Acetylene
Hb	Hemoglobin
HbO_2	Oxy-hemoglobin
Hb_{tot}	Total hemoglobin
Hct	Hematocrit
He	Helium
HHb	Deoxy-hemoglobin
HIT	High intensity training
HR	Heart rate
HR_{max}	Maximal heart rate
HR_{sub}	Submaximal heart rate
HRR	Heart rate reserve
HRT	Hormone replacement therapy

[Lac]	Lactate concentration
LV	Left ventricular
n	Number of subjects
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced form of NAD
NIRS	Near-infrared spectroscopy
NO	Nitric oxide
N_2	Nitrogen
O	Older adults (men or women)
O_2	Oxygen
O_2^-	Superoxide
P	Probability value
PCr	Phosphocreatine
PO	Power output
PO_{peak}	Peak power output
PO_{2mv}	Microvascular pressure of oxygen
Q	Cardiac output
Q_{max}	Maximal cardiac output
Q_{sub}	Submaximal cardiac output
r	Correlation value
RBC	Red blood cells
RER	Respiratory exchange ratio
ROS	Reactive oxygen species
SD	Standard deviation

SV	Stroke volume
SV_{max}	Maximal stroke volume
TD	Time delay
VCO_2	Carbon dioxide output
VO_2	Oxygen uptake
VO_{2m}	Muscle oxygen utilization
VO_{2max}	Maximal oxygen uptake
VO_{2p}	Pulmonary oxygen uptake
VO_{2peak}	Peak oxygen uptake
VO_{2sub}	Submaximal oxygen uptake
W	Watts
WR	Work rate
Y	Young adults (men or women)
Δ[HHb]	Concentration changes of deoxy-hemoglobin-myoglobin
τ	Tau (time constant)
θ_L	Estimated lactate threshold

CHAPTER I: Introduction

The capacity to perform and sustain physical exercise in adult humans declines with age (Paterson and Cunningham 1999; Paterson et al. 1999; Poole et al. 2006). Although controversy exists with regard to the mechanisms responsible for this reduced capacity and tolerance, there is no doubt that alterations in the cardiovascular system play a major role (Bassett and Howley 2000; Poole et al. 2006).

Older individuals have been shown to have a reduced maximal O_2 uptake (VO_{2max}) as well as a slower rate of adjustment for oxygen consumption (VO_2) during the on-transient of exercise (as represented by a greater VO_2 time-constant (τVO_2)) compared to their younger counterparts. Exercise training has been demonstrated to result in cardiovascular adaptations in older individuals, which have resulted in the age-dependent limitations being reduced (Paterson et al. 2004; Paterson et al. 2007). Importantly, the mechanisms responsible for the improvements in aerobic fitness have been proposed to be sex-related (Spina et al. 1996; Spina et al. 1993).

MAXIMAL O_2 UPTAKE

Measurement of VO_{2max} is used commonly to assess cardiorespiratory fitness. VO_{2max} can be defined as the highest rate at which O_2 can be taken up, transported and ultimately utilized by the tissues during severe exercise (Bassett and Howley 2000; Poole et al. 2008). Although it is believed that central components (i.e., maximal cardiac output (Q_{max})) are the main factors limiting VO_{2max}, peripheral components (i.e., peripheral diffusion gradient, mitochondrial enzyme level, capillarization) are also important (Bassett and Howley 2000; Honig et al. 1992).

An age-related decline in VO_{2max} has been established (Fitzgerald et al. 1997; Paterson et al. 1999; Paterson et al. 2004; Paterson et al. 2007; Pimentel et al. 2003; Stathokostas et al. 2004; Tanaka et al. 1997) and this loss of cardiorespiratory fitness has been linked to reductions in functional capacity and independence in the older populations (Paterson et al. 1999; Paterson et al. 2004). In older individuals, a lower VO_{2max} will result in activities of daily living being performed closer to (or even above) a functional threshold that could determine whether or not independent living is still sustainable (Paterson et al. 1999; Paterson et al. 2004; Paterson et al. 2007). Thus, a high VO_{2max} is considered to be an important component in successful aging.

Older individuals will inevitably have a reduced cardiorespiratory fitness. As noted earlier, Q_{max} is thought to be the main factor determining VO_{2max}. Considering that after the third decade of life maximal heart rate (HR_{max}) declines by approximately 0.7 beats per minute (bpm) per year (Paterson et al. 2007), a decline in VO_{2max} is to be expected even if maximal stroke volume (SV_{max}) and maximal arterial-venous O_2 difference (a-vO_{2diff}) were to be maintained. Regardless, it has been shown that reductions in both SV_{max} and maximal a-vO_{2diff} may also occur in the aged population (Lakatta 1993).

VO_2 KINETICS

Upon a step increase in power output, there is an instantaneous increase in ATP demand. Activation of oxidative phosphorylation does not occur instantaneously, but increases exponentially determined by the ATP requirement. The overall reaction sequence describing oxidative phosphorylation is as follows:

$$3ADP + 3\ Pi + NADH + H^+ + \tfrac{1}{2}\ O_2 \rightarrow 3\ ATP + NAD^+ + H_2O$$

It is believed that a slower rate of increase of oxidative phosphorylation is related to a limitation in providing any or all of the required substrates. As such, the issue of the limiting or regulating factors for VO_2 kinetics has been a matter of debate for many years, mainly between those proposing that the adjustments of VO_2 to increases in workload are related to the adequate delivery of O_2 to the muscle fibers (Hughson et al. 2001; Tschakovsky and Hughson 1999), and those suggesting a slower intracellular metabolic activation (metabolic "inertia") (Grassi 2001, 2005).

Different methodological approaches have been used to measure VO_2 kinetics in humans. Grassi et al. (Grassi et al. 1996) used the thermodilution technique to measure muscle limb blood flow directly and arterial and venous sampling for a-vO_{2diff} in order to derive muscle VO_2 in the exercising limb. This technique is invasive in nature and yet the venous O_2 content readings only provide an estimate of active muscle O_2 extraction in that the measurement includes blood returning to the venous circulation from both active and inactive fibers. Another technique used commonly to infer the rate of adjustment for muscle VO_2 requires measurements of [PCr] breakdown by 31-phosphorous magnetic resonance spectroscopy (^{31}P-MRS) (McCreary et al. 1996; Rossiter et al. 1999). However, limitations in terms of equipment requirements and exercise modalities during testing are evident. Alternatively, VO_2 kinetics can be measured at the level of the mouth (i.e. pulmonary VO_2 (VO_{2p})), where inspired and expired gases are collected for further analysis (Chin et al. 2007; DeLorey et al. 2004; Gurd et al. 2008; Jones et al. 2004). This is the most commonly used technique for measurement of VO_2 kinetics because it is non-invasive, relatively accessible for most exercise physiology laboratories, and permits measurement of VO_2 while performing different exercise modalities.

When analyzing VO_{2p} data during the transient to exercise, 3 distinct phases can be observed: Phase I (also called "cardiodynamic phase") which represents an increase in VO_{2p} caused by the elevated pulmonary circulation (due to increased Q and venous return) without reflecting the changes produced by the increase in O_2 extraction in the exercising muscles; Phase II (or the "fundamental phase") which displays a mono-exponential increase in VO_{2p} that closely reflects (within 10 %), muscle VO_2 (Grassi et al. 1996; Rossiter et al. 1999); Phase III which during exercise performed in the moderate-intensity domain occurs when steady-state is achieved.

Phase II VO_{2p} kinetics is described by its time constant during the exercise on-transient (τVO_{2p}), which represents the time taken for VO_{2p} to attain 63 % of the increase in its amplitude at steady-state (at least during exercise performed in the moderate-intensity domain). Young individuals usually display a τVO_{2p} of ~20-30 s where older subjects are often identified with longer τVO_{2p} of greater than ~40 s. However, it is important to notice that, regardless of the mechanisms controlling the rate of adjustment for VO_2 kinetics, there will be healthy young subjects showing a greater τVO_{2p} as well as healthy older individuals displaying a smaller τVO_{2p}. This means that, although some of the conditions affecting the rate at which VO_2 adjusts during the exercise on-transient may be more often present in the older population, an individual's age does not necessarily predict the length of τVO_{2p}.

RESPONSES TO ENDURANCE TRAINING EXERCISE

Changes in VO_{2max}: Although early studies failed to show significant changes in fitness in response to endurance training in older adults (Adams and DeVries 1973), subsequent research has shown consistently that, despite the age-related decline in aerobic

performance, older adults can improve their aerobic power in response to an endurance training program. In older men, training studies lasting ~6-12 months have yielded improvements in VO_{2max} ranging from 15-29 % (Coggan et al. 1993; Ehsani et al. 1991; Kohrt et al. 1991; Spina et al. 1993; Stratton et al. 1994), and even shorter term exercise training interventions of ~9-12 weeks have produced increases in VO_{2max} of ~ 6-18 % (Beere et al. 1999; Charifi et al. 2003; Gass et al. 2004; Govindasamy et al. 1992; Morris et al. 2002; Poulin et al. 1992; Takeshima et al. 2004). Spina et al. (Spina et al. 1993) demonstrated that improvements in Q_{max} and SV_{max} contributed ~2/3 of the increase in VO_{2max} in older men after 9-12 months of endurance training, with the remainder of the adaptation explained by a widened maximal a-vO_{2diff}. This demonstrated that the adaptability of the central (i.e., muscle pump) and peripheral (i.e., capillarization, oxidative enzymes) components of the cardiorespiratory system were well preserved in older men, at least in response to a long-duration training program. Similarly, Morris et al. (Morris et al. 2002) demonstrated that increases in Q_{max} in older individuals played an important role in increasing VO_{2max} in response to short-term endurance training (12 weeks), suggesting that even central adaptations can occur quickly.

In women, endurance training has been shown to increase VO_{2max} in both older (Coggan et al. 1993; Martin et al. 1990; Pedersen and Jorgensen 1978; Spina et al. 1993) and young subjects (Cunningham and Hill 1975; Cunningham et al. 1979; Ichinose et al. 2009; Pedersen and Jorgensen 1978; Spina et al. 1992b) subjects; however, the interplay between central and peripheral mechanisms underlying the increase in VO_{2max} differs between age-groups. For instance, young women have shown improvements in Q_{max} and SV_{max} (Cunningham and Hill 1975; Spina et al. 1992a) as well as maximal a-vO_{2diff} (Cunningham et al. 1979) in response to endurance training, whereas older women have

relied exclusively on increases in maximal a-vO_{2diff} in order to augment their VO_{2max} (Seals et al. 1984; Spina et al. 1993).

Interestingly, measurements of central and/or peripheral adaptations in the above mentioned studies were only taken pre- and post-training. As such, the time-course of adaptations and mechanisms explaining increments in VO_{2peak} in older and young men and women remain to be elucidated.

Another training paradigm revolves around what intensity will result in optimal adaptations in response to an endurance training program. It has been proposed that higher training intensities (75-80 % of HR reserve) are important to further increase VO_{2peak} in older adults (Ehsani et al. 1991; Seals et al. 1984). Similarly, Makrides et al. (Makrides et al. 1990) showed a 38 % increase in VO_{2peak} in older subjects in response to a 12-week interval training regime that was adjusted so that training intensity was ~ 85 % of VO_{2peak} by the third week of the program. Indeed, a plateau-like response to endurance training has been shown when the training intensity is not increased after 8 weeks of an endurance training program (O'Donovan et al. 2005). Contrary to the idea that higher training intensity may be responsible for the larger adaptations in VO_{2peak}, Gass et al. (Gass et al. 2004) proposed that, at least in older individuals, the total amount of work (and not the intensity by itself) was the key factor regulating the increase in VO_{2peak}. Based on their findings that improvements in VO_{2peak} were faster (4 versus 12 weeks) in the group training at lower intensities (50 % versus 70 % of VO_{2peak}) they suggested that higher training intensities could be above some optimal threshold for older individuals.

Changes in VO_2 kinetics: Older individuals have been shown to have a slowed Phase II VO_{2p} kinetics during the on-transient of moderate-intensity exercise (Babcock et al. 1994; Bell et al. 1999; Chilibeck et al. 1996; DeLorey et al. 2004; Gurd et al. 2008). This slower

response has been attributed to an age-related reduction in muscle blood flow as reflected by a greater ratio of change in deoxygenated hemoglobin to the change in VO_{2p} ($\Delta[HHb]/\Delta VO_{2p}$) (DeLorey et al. 2004) and also to a slower provision of substrates to the electron transport chain (Gurd et al. 2008) in older men compared to their younger counterparts.

Endurance training has been shown to result in faster VO_{2p} kinetics in both older (Babcock et al. 1994; Bell et al. 2001) and young individuals (Berger et al. 2006; Fukuoka et al. 2002; McKay et al. 2009; Phillips et al. 1995), with changes in older adults resulting in τVO_{2p} becoming similar to that observed in the untrained young (Babcock et al. 1994). Interestingly, all of the studies looking at the effects of training on phase II VO_{2p} kinetics, as well as most of the data explaining the mechanisms controlling oxidative phosphorylation have been conducted on male participants.

The mechanisms underlying the faster rate of adjustment of VO_2 in response to exercise training have not been clearly elucidated but it has been shown that they occur early in training (McKay et al. 2009). McKay et al. (McKay et al. 2009) suggested that likely an integration of both enhanced metabolic control and O_2 delivery would be responsible for the smaller τVO_{2p} observed in response to endurance training. Phillips et al. (Phillips et al. 1995), however, hypothesized that improvements in blood flow were likely responsible for a faster VO_2 kinetics observed during the first days of training. Recently, it has been proposed that endurance training results in improved endothelium-dependent and flow-mediated vasodilation in older and young humans and rats (DeSouza et al. 2000; Green et al. 2004; Haram et al. 2006; Spier et al. 2004; Spier et al. 2007) which could result in a better distribution of blood flow within the active muscles and thus, a smaller τVO_{2p} in both older and young adults. Unfortunately, not much is known

about blood flow distribution within the microcirculation of intact humans. Recently, the use of near infrared spectroscopy (NIRS) technology has permitted non-invasive inspection of muscle deoxygenation ([HHb]). By using this [HHb] signal in conjunction with VO_{2p} (reflecting muscle VO_2), inferences about muscle blood flow distribution have been possible so that its influence on the rate of adjustment of VO_2 kinetics could be discerned. Nevertheless, experiments eliciting a change in τVO_{2p} and examining its relationship with the interaction between [HHb] and VO_{2p} are required.

OVERVIEW OF STUDIES

Although several studies have been conducted to determine the cardiovascular adaptations in response to endurance training, there is a gap of information in terms of the mechanisms and time-course of changes occurring in response to short-term endurance training. Also, there is a dearth of studies directly comparing physiological responses to endurance training in older and young men and women. Thus, the present thesis was undertaken to examine the mechanisms explaining cardiovascular adaptations occurring in older and young males and females in response to a short-term (12 weeks) endurance training program.

Chapter II explores the interplay of the time-course of central versus peripheral mechanisms explaining the adaptations involving improvements in VO_{2max} during short-term endurance training directly comparing older and young male adults. It was hypothesized that: 1) Older and young men would increase VO_{2max} to a similar extent and follow a similar time-course during the duration of the exercise-training program; 2) In both older and young men, improvements in Q_{max} would explain the majority of the

increase in VO_{2max} (~2/3 of the change) whereas a widened maximal a-vO_{2diff} would be responsible for a smaller portion of the change.

Chapter III also examines the time-course and mechanisms of adaptation to improvements in VO_{2max} that occur with shorter training periods of 12 weeks or less but in older and young women. Based on previous results from long-term endurance training programs we hypothesized that: 1) the relative increase in VO_{2max} and its time-course would be similar in both older and young women; 2) increases in VO_{2max} in the older women would rely mainly on increases in a-vO_{2diff}, whereas increases in both Q_{max} and maximal a-vO_{2diff} would explain the improvements in VO_{2max} in the young women.

In chapter IV the focus was to determine the changes in the rate of adjustment of VO_2 kinetics in response to endurance training. The main goal of this study was to determine the time-course and mechanism of adaptation for a speeding of phase II VO_{2p} kinetics in older and young men, throughout a 12-week endurance training program. It was hypothesized that improvements in microvascular O_2 delivery in the exercise transient (as represented by a better matching between the rate of adjustment of muscle deoxygenation relative to phase II VO_{2p}) in response to endurance training would be associated with a faster adjustment of VO_{2p} kinetics observed early in training in both older and young men.

Finally, chapter V sought to determine the time-course of adjustment for phase II VO_{2p} kinetics in older and young women during a 12-week endurance training program. It was hypothesized that: 1) older women would have a slower phase II VO_{2p} kinetics compared to their younger counterparts at any testing time; 2) endurance training would result in speeding of phase II VO_{2p} kinetics in both older and young women with the majority of the change occurring during the first 3 weeks of training.

REFERENCES

Adams GM, DeVries HA (1973) Physiological effects of an exercise training regimen upon women aged 52 to 79. J Gerontol 28: 50-55

Babcock MA, Paterson DH, Cunningham DA (1994) Effects of aerobic endurance training on gas exchange kinetics of older men. Med Sci Sports Exerc 26: 447-452

Bassett DR, Jr., Howley ET (2000) Limiting factors for maximum oxygen uptake and determinants of endurance performance. Med Sci Sports Exerc 32: 70-84

Beere PA, Russell SD, Morey MC, Kitzman DW, Higginbotham MB (1999) Aerobic exercise training can reverse age-related peripheral circulatory changes in healthy older men. Circulation 100: 1085-1094

Bell C, Paterson DH, Kowalchuk JM, Cunningham DA (1999) Oxygen uptake kinetics of older humans are slowed with age but are unaffected by hyperoxia. Exp Physiol 84: 747-759

Bell C, Paterson DH, Kowalchuk JM, Moy AP, Thorp DB, Noble EG, Taylor AW, Cunningham DA (2001) Determinants of oxygen uptake kinetics in older humans following single-limb endurance exercise training. Exp Physiol 86: 659-665

Berger NJ, Tolfrey K, Williams AG, Jones AM (2006) Influence of continuous and interval training on oxygen uptake on-kinetics. Med Sci Sports Exerc 38: 504-512

Charifi N, Kadi F, Feasson L, Denis C (2003) Effects of endurance training on satellite cell frequency in skeletal muscle of old men. Muscle Nerve 28: 87-92

Chilibeck PD, Paterson DH, Petrella RJ, Cunningham DA (1996) The influence of age and cardiorespiratory fitness on kinetics of oxygen uptake. Can J Appl Physiol 21: 185-196

Chin LM, Leigh RJ, Heigenhauser GJ, Rossiter HB, Paterson DH, Kowalchuk JM (2007) Hyperventilation-induced hypocapnic alkalosis slows the adaptation of pulmonary O2 uptake during the transition to moderate-intensity exercise. J Physiol 583: 351-364

Coggan AR, Spina RJ, Kohrt WM, Holloszy JO (1993) Effect of prolonged exercise on muscle citrate concentration before and after endurance training in men. Am J Physiol 264: E215-220

Cunningham DA, Hill JS (1975) Effect of training on cardiovascular response to exercise in women. J Appl Physiol 39: 891-895

Cunningham DA, McCrimmon D, Vlach LF (1979) Cardiovascular response to interval and continuous training in women. Eur J Appl Physiol Occup Physiol 41: 187-197

DeLorey DS, Kowalchuk JM, Paterson DH (2004) Effect of age on O_2 uptake kinetics and the adaptation of muscle deoxygenation at the onset of moderate-intensity cycling exercise. J Appl Physiol 97: 165-172

DeSouza CA, Shapiro LF, Clevenger CM, Dinenno FA, Monahan KD, Tanaka H, Seals DR (2000) Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. Circulation 102: 1351-1357

Ehsani AA, Ogawa T, Miller TR, Spina RJ, Jilka SM (1991) Exercise training improves left ventricular systolic function in older men. Circulation 83: 96-103

Fitzgerald MD, Tanaka H, Tran ZV, Seals DR (1997) Age-related declines in maximal aerobic capacity in regularly exercising vs. sedentary women: a meta-analysis. J Appl Physiol 83: 160-165

Fukuoka Y, Grassi B, Conti M, Guiducci D, Sutti M, Marconi C, Cerretelli P (2002) Early effects of exercise training on on- and off-kinetics in 50-year-old subjects. Pflugers Arch 443: 690-697

Gass G, Gass E, Wicks J, Browning J, Bennett G, Morris N (2004) Rate and amplitude of adaptation to two intensities of exercise in men aged 65-75 yr. Med Sci Sports Exerc 36: 1811-1818

Govindasamy D, Paterson DH, Poulin MJ, Cunningham DA (1992) Cardiorespiratory adaptation with short term training in older men. Eur J Appl Physiol Occup Physiol 65: 203-208

Grassi B (2001) Regulation of oxygen consumption at exercise onset: is it really controversial? Exerc Sport Sci Rev 29: 134-138

Grassi B (2005) Delayed metabolic activation of oxidative phosphorylation in skeletal muscle at exercise onset. Med Sci Sports Exerc 37: 1567-1573

Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK, Wagner PD (1996) Muscle O2 uptake kinetics in humans: implications for metabolic control. J Appl Physiol 80: 988-998

Green DJ, Maiorana A, O'Driscoll G, Taylor R (2004) Effect of exercise training on endothelium-derived nitric oxide function in humans. J Physiol 561: 1-25

Gurd BJ, Peters SJ, Heigenhauser GJ, LeBlanc PJ, Doherty TJ, Paterson DH, Kowalchuk JM (2008) O_2 uptake kinetics, pyruvate dehydrogenase activity, and muscle deoxygenation in young and older adults during the transition to moderate-intensity exercise. Am J Physiol Regul Integr Comp Physiol 294: R577-584

Haram PM, Adams V, Kemi OJ, Brubakk AO, Hambrecht R, Ellingsen O, Wisloff U (2006) Time-course of endothelial adaptation following acute and regular exercise. Eur J Cardiovasc Prev Rehabil 13: 585-591

Honig CR, Connett RJ, Gayeski TE (1992) O2 transport and its interaction with metabolism; a systems view of aerobic capacity. Med Sci Sports Exerc 24: 47-53

Hughson RL, Tschakovsky ME, Houston ME (2001) Regulation of oxygen consumption at the onset of exercise. Exerc Sport Sci Rev 29: 129-133

Ichinose TK, Inoue Y, Hirata M, Shamsuddin AK, Kondo N (2009) Enhanced heat loss responses induced by short-term endurance training in exercising women. Exp Physiol 94: 90-102

Jones AM, Wilkerson DP, Wilmshurst S, Campbell IT (2004) Influence of L-NAME on pulmonary O2 uptake kinetics during heavy-intensity cycle exercise. J Appl Physiol 96: 1033-1038

Kohrt WM, Malley MT, Coggan AR, Spina RJ, Ogawa T, Ehsani AA, Bourey RE, Martin WH, 3rd, Holloszy JO (1991) Effects of gender, age, and fitness level on response of VO_{2max} to training in 60-71 yr olds. J Appl Physiol 71: 2004-2011

Lakatta EG (1993) Cardiovascular regulatory mechanisms in advanced age. Physiol Rev 73: 413-467

Makrides L, Heigenhauser GJ, Jones NL (1990) High-intensity endurance training in 20- to 30- and 60- to 70-yr-old healthy men. J Appl Physiol 69: 1792-1798

Martin WH, 3rd, Kohrt WM, Malley MT, Korte E, Stoltz S (1990) Exercise training enhances leg vasodilatory capacity of 65-yr-old men and women. J Appl Physiol 69: 1804-1809

McCreary CR, Chilibeck PD, Marsh GD, Paterson DH, Cunningham DA, Thompson RT (1996) Kinetics of pulmonary oxygen uptake and muscle phosphates during moderate-intensity calf exercise. J Appl Physiol 81: 1331-1338

McKay BR, Paterson DH, Kowalchuk JM (2009) Effect of short-term high-intensity interval training vs. continuous training on O_2 uptake kinetics, muscle deoxygenation, and exercise performance. J Appl Physiol 107: 128-138

Morris N, Gass G, Thompson M, Bennett G, Basic D, Morton H (2002) Rate and amplitude of adaptation to intermittent and continuous exercise in older men. Med Sci Sports Exerc 34: 471-477

O'Donovan G, Owen A, Bird SR, Kearney EM, Nevill AM, Jones DW, Woolf-May K (2005) Changes in cardiorespiratory fitness and coronary heart disease risk factors following 24 wk of moderate- or high-intensity exercise of equal energy cost. J Appl Physiol 98: 1619-1625

Paterson DH, Cunningham DA (1999) The gas transporting systems: limits and modifications with age and training. Can J Appl Physiol 24: 28-40

Paterson DH, Cunningham DA, Koval JJ, St Croix CM (1999) Aerobic fitness in a population of independently living men and women aged 55-86 years. Med Sci Sports Exerc 31: 1813-1820

Paterson DH, Govindasamy D, Vidmar M, Cunningham DA, Koval JJ (2004) Longitudinal study of determinants of dependence in an elderly population. J Am Geriatr Soc 52: 1632-1638

Paterson DH, Jones GR, Rice CL (2007) Ageing and physical activity: evidence to develop exercise recommendations for older adults. Can J Public Health 98 Suppl 2: S69-108

Pedersen PK, Jorgensen K (1978) Maximal oxygen uptake in young women with training, inactivity, and retraining. Med Sci Sports 10: 233-237

Phillips SM, Green HJ, MacDonald MJ, Hughson RL (1995) Progressive effect of endurance training on VO_2 kinetics at the onset of submaximal exercise. J Appl Physiol 79: 1914-1920

Pimentel AE, Gentile CL, Tanaka H, Seals DR, Gates PE (2003) Greater rate of decline in maximal aerobic capacity with age in endurance-trained than in sedentary men. J Appl Physiol 94: 2406-2413

Poole D, Behnke B, Musch T (2006) Capillary hemodynamics and oxygen pressures in the aging microcirculation. Microcirculation 13: 289-299

Poole DC, Wilkerson DP, Jones AM (2008) Validity of criteria for establishing maximal O2 uptake during ramp exercise tests. Eur J Appl Physiol 102: 403-410

Poulin MJ, Paterson DH, Govindasamy D, Cunningham DA (1992) Endurance training of older men: responses to submaximal exercise. J Appl Physiol 73: 452-457

Rossiter HB, Ward SA, Doyle VL, Howe FA, Griffiths JR, Whipp BJ (1999) Inferences from pulmonary O_2 uptake with respect to intramuscular [phosphocreatine] kinetics during moderate exercise in humans. J Physiol 518 (Pt 3): 921-932

Seals DR, Hagberg JM, Hurley BF, Ehsani AA, Holloszy JO (1984) Endurance training in older men and women. I. Cardiovascular responses to exercise. J Appl Physiol 57: 1024-1029

Spier SA, Delp MD, Meininger CJ, Donato AJ, Ramsey MW, Muller-Delp JM (2004) Effects of ageing and exercise training on endothelium-dependent vasodilatation and structure of rat skeletal muscle arterioles. J Physiol 556: 947-958

Spier SA, Delp MD, Stallone JN, Dominguez JM, 2nd, Muller-Delp JM (2007) Exercise training enhances flow-induced vasodilation in skeletal muscle resistance arteries of aged rats: role of PGI2 and nitric oxide. Am J Physiol Heart Circ Physiol 292: H3119-3127

Spina RJ, Miller TR, Bogenhagen WH, Schechtman KB, Ehsani AA (1996) Gender-related differences in left ventricular filling dynamics in older subjects after endurance exercise training. J Gerontol A Biol Sci Med Sci 51: B232-237

Spina RJ, Ogawa T, Coggan AR, Holloszy JO, Ehsani AA (1992a) Exercise training improves left ventricular contractile response to beta-adrenergic agonist. J Appl Physiol 72: 307-311

Spina RJ, Ogawa T, Kohrt WM, Martin WH, 3rd, Holloszy JO, Ehsani AA (1993) Differences in cardiovascular adaptations to endurance exercise training between older men and women. J Appl Physiol 75: 849-855

Spina RJ, Ogawa T, Martin WH, 3rd, Coggan AR, Holloszy JO, Ehsani AA (1992b) Exercise training prevents decline in stroke volume during exercise in young healthy subjects. J Appl Physiol 72: 2458-2462

Stathokostas L, Jacob-Johnson S, Petrella RJ, Paterson DH (2004) Longitudinal changes in aerobic power in older men and women. J Appl Physiol 97: 781-789

Stratton JR, Levy WC, Cerqueira MD, Schwartz RS, Abrass IB (1994) Cardiovascular responses to exercise. Effects of aging and exercise training in healthy men. Circulation 89: 1648-1655

Takeshima N, Rogers ME, Islam MM, Yamauchi T, Watanabe E, Okada A (2004) Effect of concurrent aerobic and resistance circuit exercise training on fitness in older adults. Eur J Appl Physiol 93: 173-182

Tanaka H, Desouza CA, Jones PP, Stevenson ET, Davy KP, Seals DR (1997) Greater rate of decline in maximal aerobic capacity with age in physically active vs. sedentary healthy women. J Appl Physiol 83: 1947-1953

Tschakovsky ME, Hughson RL (1999) Interaction of factors determining oxygen uptake at the onset of exercise. J Appl Physiol 86: 1101-1113

CHAPTER II: Time-course and mechanisms of adaptations in cardiorespiratory fitness with endurance training in older and young men

INTRODUCTION

A decline in aerobic performance with advancing age has been well documented (Fleg et al. 2005; Hollenberg et al. 2006; Paterson et al. 2007; Pimentel et al. 2003; Stathokostas et al. 2004; Tanaka et al. 1997). This decline in aerobic fitness is associated with an age-related decrease in physical functional capacity, and has been linked to reduced quality of life and loss of independence (Paterson et al. 2004) as well as cognitive function (Paterson et al. 2007). Additionally, maximal aerobic power (maximal oxygen uptake (VO_{2max})) has been shown to be an independent risk factor for all-cause and cardiovascular disease mortality (Paterson et al. 2007). Taken together, these data suggest that maintaining a high maximal aerobic power is an important component in successful healthy aging.

Training studies in older adults lasting ~6-12 months have yielded improvements in VO_{2max} ranging from 15-29% (Babcock et al. 1994b; Coggan et al. 1992; Ehsani et al. 1991; Kohrt et al. 1991; Spina et al. 1996; Spina et al. 1993; Stratton et al. 1994), and even shorter term exercise training interventions of ~9-12 weeks have produced increases in VO_{2max} of ~6-18% (Beere et al. 1999; Charles et al. 2006; Gass et al. 2004; Morris et al. 2002; Morris et al. 2003; Poulin et al. 1992). Although the percent increase in VO_{2max} in older adults has been reported to be similar to that observed young individuals (Gass et al. 2004; Govindasamy et al. 1992; Morris et al. 2002; Morris et al. 2003; Seals et al. 1984; Spina et al. 1993), direct comparisons of the effects of endurance training between older and young adults within the same training program are limited. Further, information regarding the time-course of training-induced adaptations in older compared to younger

subjects is lacking. In only a few studies has short-term endurance training (9-12 weeks) and time-course of changes in VO_{2max} been studied in older adults (Gass et al. 2004; Govindasamy et al. 1992; Morris et al. 2002) and in these studies only older adults were tested with no comparisons made to younger control training groups.

The interplay of the time-course of central versus peripheral mechanisms explaining the adaptations involving improvements in VO_{2max} during training in older compared to younger adults remain to be elucidated. Spina et al. (Spina et al. 1993) reported that improvements in Q and stroke volume (SV) contributed to the majority of the increase in VO_{2max} in older men after 9-12 months of endurance training. Others (Gass et al. 2004; Morris et al. 2002) have confirmed that improvements in maximal cardiac output (Q_{max}) in older adults occur even in response to shorter-term endurance training programs (10-12 weeks); however, only pre- and post-training measurements were taken at peak exercise. Thus, to date little is known about the time-course of central versus peripheral adaptations underlying the large changes in VO_{2max} with short-term exercise training in older adults and whether the response differs from young.

The main goal of this study was to determine the time-course and mechanisms of adaptation to a 12-week endurance training program in older (O) and young (Y) male adults. We hypothesized that: 1) Both O and Y would increase VO_{2max} to a similar extent and follow a similar time-course during the duration of the exercise-training program; 2) In both O and Y groups, improvements in Q_{max} would explain the majority of the increase in VO_{2max} (~2/3 of the change) whereas a widened a-vO_{2diff} would be responsible for a smaller portion of the change.

METHODS

Subjects: Eight O (68 ± 7 yr; mean ± SD) and 8 Y (23 ± 5 yr) men volunteered and gave written consent to participate in the study. All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All subjects were non-obese (body mass index $\leq$30 kg/m^2), non-smokers, and were physically active but none had been involved in any type of endurance training program for at least 12 months prior to the study. Additionally, no subjects were taking medications that would affect the cardiorespiratory or hemodynamic responses to exercise. Older subjects had no history of cardiovascular, respiratory or musculoskeletal diseases, were medically screened by a physician and underwent a maximal exercise stress test.

Protocol: Before training began, subjects performed a maximal cycle ergometer ramp test to exhaustion (O 15-20 W/min; Y 25 W/min) (on a Lode Corival 400 cycle-ergometer; Lode B.V., Groningen, Holland) for determination of VO_{2max} and estimation of the lactate threshold (θ_L). θ_L was defined as the VO_2 at which CO_2 output (VCO_2) began to increase out of proportion to VO_2 along with a systematic rise in minute ventilation-to-VO_2 ratio and end-tidal PO_2 whereas minute ventilation-to-VCO_2 ratio and end-tidal PCO_2 were stable. Approximately 1 min after the end of the ramp test a fingertip blood sample (~0.5 μL) was obtained to measure end-exercise blood lactate concentration using a portable device (Lactate Scout, Sports Resource Group, Hawthorne, NY). Within 5 minutes after completion of this test, subjects performed a constant-load cycling exercise to volitional fatigue at 85% of the peak power output (PO_{peak}) achieved during the ramp incremental test. This protocol (described in (Rossiter et al. 2006)) was performed to assess the attainment of VO_{2max} and to allow determination of Q_{max}. Subjects were instructed to

indicate when they thought they were ~30s from exhaustion. At that point, verbal encouragement increased and within ~15 s the measurement of Q began. VO_{2max} was defined as the highest VO_2 observed for an average of 20 consecutive seconds during either the ramp test to exhaustion or the 2-3 minute constant load at 85% of PO_{peak}. On a separate day, subjects were asked to cycle at a PO corresponding to ~90% of their pre-training θ_L and when a steady state in gas exchange was achieved Q was measured. Similar procedures were repeated after 3, 6, 9, and 12 weeks of training.

Blood tests: Prior to the start (pre-) and after 6 (mid-) and 12 weeks (post-) of training blood samples were drawn from each subject's antecubital vein for determination of hematocrit (Hct) and hemoglobin (Hb) concentration.

Training: The endurance training program consisted of 3 exercise sessions per week on a stationary cycle-ergometer (Monark Ergomedic 874E; Monark Exercise AB, Varberg, Sweeden) for a total duration of 12 weeks. Training intensity was adjusted at 3 week intervals to reflect changes in fitness level. During the first 10 weeks, each session consisted of continuous training (CT) for 45 min at a power output that elicited ~70% of the VO_{2max} observed during the most recently completed incremental ramp test. During the final 2 weeks of training (6 exercise sessions), each individual in each group (O and Y) was randomly assigned (stratified by age) to one of two sub-groups: a) CT as described above; b) high-intensity interval training (HIT), performing 10-12 exercise bouts each lasting 1-min at 90-100% of the peak power output achieved during the incremental ramp test, with 1-min rest separating bouts. Since VO_{2max} was likely to plateau after approximately 8 weeks of CT (O'Donovan et al. 2005), the HIT was used as a strategy for progressive and continued gains in the exercise program resulting in further increases in VO_{2max} favoured by peripheral adaptations (Coyle 1995).

Measurements: Gas-exchange measurements were similar to those previously described (Babcock et al. 1994a). Briefly, inspired and expired flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies VMM 110) which was calibrated before each test by using a syringe of known volume. Inspired and expired gases were sampled continuously (every 20 ms) at the mouth and analyzed for concentrations of O_2, CO_2, nitrogen (N_2), acetylene (C_2H_2), and helium (He) by mass spectrometry (Perkin Elmer MGA-1100) after calibration with precision-analyzed gas mixtures. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver et al. (Beaver et al. 1981).

Heart rate (HR) was monitored continuously by electrocardiogram using PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO) with a three-lead arrangement. Data were recorded using LabChart v4.2 (ADInstruments, Colorado Springs, CO) on a separate computer.

Q measurements: Q was measured using the acetylene (C_2H_2) open circuit inert gas washin method and analyzed using custom data acquisition software. This technique was described and validated previously (Johnson et al. 2000). Briefly, a pneumotachograph (Hans Rudolph Model 3800, Kansas City, MO; transducer, Validyne MP45-871, Northridge, CA) was attached to a non-rebreathing Y valve (Hans Rudolph 7900, Kansas City, MO), which was connected to a manual valve that allowed switching inspired gases between room air and a bag containing a mixture of C_2H_2 (0.7%), O_2 (21%), He (9%), and balance N_2. Changes in gas concentrations were aligned with gas volumes by measuring the time delay for fractional changes of the gases to occur. Throughout the sub-maximal and peak exercise measurements of Q subjects were asked to continue their normal breathing pattern when the source of inspired air was switched to the bag

containing the gas mixture and after 10 breaths the protocol was terminated. Data analysis for the calculation of Q was performed immediately after each manouver using equations reported previously (Johnson et al. 2000). The a-vO_{2diff} was calculated from the Fick equation as: a-vO_{2diff} ($mLO_2 \cdot 100mL^{-1}$ blood) = VO_2 ($L \cdot min^{-1}$) / Q ($L \cdot min^{-1}$) x 100. Stroke volume was calculated as: SV ($mL \cdot beat^{-1}$) = Q ($mL \cdot min^{-1}$) / HR (bpm).

Statistics: Data are presented as means ± SD. Independent t-tests and repeated measures analysis of variance (ANOVA) were used to determine statistical significance for the dependent variables. The ANOVA model was described as S_{16} x T_5 x A_2 such that subjects (S; number of subjects) are crossed with testing time (T; five testing times: pre-training, Week 3, Week 6, Week 9, and post-training) and age (A; older and young adults). A Tukey post-hoc analysis was used when significant differences were found for the main effects of each dependent variable. The ANOVA was analyzed by SPSS Version 12.0, (SPSS Inc., Chicago, IL). Statistical significance was declared when $p < 0.05$.

RESULTS

Subject characteristics and resting Hct and Hb values are reported in Table 2.1. Adherence to the training program was 94 ± 1% (28/30 training sessions) and 95 ± 1% (29/30 training sessions) in O and Y, respectively. Each subject completed at least 90% of the programmed training sessions (range: O = 27-29 sessions; Y = 27-30 sessions). The average training intensity (power output (PO)) per session increased significantly after each testing session in both O (i.e., week 1-3, 95 ± 31 W; week 4-6, 107 ± 31 W; week 7-9, 116 ± 32 W;) and Y (e.g., week 1-3, 183 ± 31 W; week 4-6, 198 ± 36 W; week 7-9, 207 ± 36 W); training PO was always higher in Y than in O ($p < 0.05$). In the subgroup of O and Y subjects performing the CT during the last 2 weeks of the training program (n = 7 including O and Y), a further increase in the average PO was observed compared to

week 7-9 (e.g., PO week 7-9, 152 ± 56 W (O, 92 ± 9; Y, 198 ± 48) vs. PO week 10-12, 159 ± 69 W (O, 96 ± 11; Y, 207 ± 45)). The group performing the HIT (n = 9 including O and Y) exercised at a higher average PO compared to the previous testing measurement (i.e., PO week 7-9 (continuous), 169 ± 53 W vs. week 10-12 (HIT), 285 ± 88 W); however, the estimated energy expenditure for an average of 11 ± 1 one-min bouts of exercise was ~60% lower ($p < 0.05$) for HIT than for CT. Since training type (e.g., continuous vs. HIT) did not significantly affect any of the variables of interest (i.e., PO_{peak} and maximal and sub-maximal VO_2, HR, Q, SV, and a-vO_{2diff}) the group data are combined and compared over the time-course of training.

The changes in peak exercise values in response to training are summarized in Table 2.2. PO_{peak} progressively increased from pre- to post -training in both O and Y (Table 2.2). A higher VO_{2max} was observed within 3 weeks of training in both O and Y, with further increases in VO_{2max} seen in both groups post-training. No testing time by age interactions were detected reflecting a similar rate of adaptation of VO_{2max} in both O and Y and a maintained difference between age groups across time. The percent change in VO_{2max} from pre-training to post-training was larger in O (31 ± 10%) compared to Y (18 ± 10%) adults ($p < 0.05$). The mean slope of the change in VO_{2max} was ~0.16 $L \cdot min^{-1}$ and ~0.13 $L \cdot min^{-1}$ every third week in O and Y, respectively (Figure 2.1). The VO_{2max} obtained during the ramp incremental test was similar to that observed during the 2-3 minute constant-load test to exhaustion (which was also used to determine Q_{max}) in both O and Y ($p > 0.05$). Pre- and post-training values at the end of the ramp incremental test for lactate concentration (O pre, 9.3 ± 1.1 $mmol \cdot L^{-1}$; O post, 10.9 ± 2.9 $mmol \cdot L^{-1}$; Y pre, 10.8 ± 2.0 $mmol \cdot L^{-1}$; Y post, 13.1 ± 3.0 $mmol \cdot L^{-1}$), and RER (O pre 1.20 ± 0.10; O post, 1.16 ± 0.09; Y pre,1.24 ± 0.10; Y post, 1.23 ± 0.03) were unchanged.

The HR_{max} overall response from pre- to post-intervention was unaffected by training (Table 2.2). Q_{max} was higher ($p< 0.05$) in O and Y after 3 weeks of training. A further increase in Q_{max} occurred after 9 weeks of training (Table 2.2). Maximal SV (SV_{max}) also increased significantly in both groups after 3 weeks of training. Additional improvements in SV_{max} were observed at week 9 (Table 2.2). Maximal a-vO_{2diff} was higher ($p< 0.05$) at week 3, 6 and post-training compared to pre-training in O and Y (Table 2.2). No testing time by age interactions were observed for Q_{max}, SV_{max}, and maximal a-vO_{2diff} revealing a similar rate of change in each group across time for these variables. In the O, 69% of the change in VO_{2max} from pre- to post-training was explained by the increase in Q_{max} while the remaining 31% was explained by an improved a-vO_{2diff} (calculated as the percent change in Q (or a-vO_{2diff}) divided by the total percent change in VO_{2max}). In the O, ~1/3 of the increase in VO_{2max}, Q_{max} and maximal a-vO_{2diff} occurred during the first 3 weeks of training while the remaining ~2/3 took place between week 3 and the end of the training program. The proportion of increase in VO_{2max} explained by Q_{max} (~2/3) and maximal a-vO_{2diff} (~1/3) was similar for each of these time periods (Figure 2.2). In Y, 56% of the change in VO_{2max} was attributed to a higher Q_{max} and 44% to a widened a-vO_{2diff}. In contrast to O, ~2/3 of the increase in VO_{2max} in the Y occurred within the first 3 weeks of training with the rest of the change taking place after week 3 of the program. Interestingly, the early adaptations to training in this group relied on improvements in maximal a-vO_{2diff} (~66%) while increases in Q_{max} explained the increases in VO_{2max} from week 3 to post-training (Figure 2.2).

Table 2.3 depicts the physiological responses to a constant-load submaximal exercise intensity corresponding to ~90% θ_L (O, 68 ± 15 W; Y, 128 ± 28 W). The steady-state VO_2 (VO_{2sub}) corresponding to these POs were not affected by training (O: pre-

training, 2.27 ± 0.35 $L \cdot min^{-1}$; post-training, 2.23 ± 0.35 $L \cdot min^{-1}$; Y, pre-training, 1.52 ± 0.15 $L \cdot min^{-1}$; post-training, 1.49 ± 0.17 $L \cdot min^{-1}$). Compared to pre-training, submaximal HR was lower ($p < 0.05$) after week 3 in O and Y, with no further changes observed thereafter. Submaximal Q (Q_{sub}) remained unchanged in both groups throughout the training. The Q_{sub}/VO_{2sub} was similar in O and Y and was not affected by training (O: pre-training, 7.7 ± 1.0 $L \cdot min^{-1}$; post-training, 7.9 ± 1.2 $L \cdot min^{-1}$; Y, pre-training, 7.5 ± 0.6 $L \cdot min^{-1}$; post-training, 7.4 ± 0.4 $L \cdot min^{-1}$). SV_{sub} was higher ($p < 0.05$) by week 3 compared to pre-training, with no further changes during the training program. Submaximal a-vO_{2diff} (a-$vO_{2diffsub}$) in O and Y was not affected by training.

The absolute VO_2 corresponding to θ_L ($L \cdot min^{-1}$) significantly increased after 3 weeks of training in both O and Y. A further increase in θ_L ($L \cdot min^{-1}$) was observed at week 6 and again post-training (Table 2.3) such that the pre- to post-training change was 32 ± 20% in O and 17 ± 10% in Y. There was no testing time by age interaction suggesting a similar rate of improvement in θ_L in both age groups.

Table 2.1. Subjects' characteristics and resting hematocrit and hemoglobin values.

	Age (yr)	Height (m)	Body Mass (kg)		Hct		Hb ($g \cdot dL^{-1}$)	
			Pre	Post	Pre	Post	Pre	Post
Older	68 (7)#	1.77 (0.09)	81.6 (7.6)	81.2 (7.4)	0.43 (0.03)	0.43 (0.02)	14.8 (0.8)	14.9 (0.7)
Young	23 (5)	1.78 (0.05)	79.9 (8.1)	81.1 (8.1)	0.44 (0.02)	0.44 (0.02)	15.3 (0.7)	15.4 (0.7)

Values are means ± SD. Hct, hematocrit; Hb, hemoglobin; # Significantly different from Y ($p < 0.05$).

Table 2.2. Maximal exercise responses for PO, VO_2, HR, Q, SV and a-vO_{2diff} in O and Y from pre-training through post-training.

		Pre-training	Week 3	Week 6	Week 9	Post-training
PO_{peak} (Watts)	O#	188 (44)	201 (40)*	208 (44)*†	215 (49)*†	219 (49)*†‡§
	Y	314 (41)	346 (47)*	359 (45)*†	365 (57)*†	377 (50)*†‡§
VO_{2max} ($L \cdot min^{-1}$)	O#	2.29 (0.49)	2.48 (0.42)*	2.65 (0.58)*	2.77 (0.53)*	2.95 (0.48)*†‡§
	Y	3.82 (0.47)	4.27 (0.52)*	4.22 (0.44)*	4.28 (0.49)*	4.47 (0.34)*†‡§
VO_{2max} ($mL \cdot kg \cdot min^{-1}$)	O#	28.3 (7.1)	30.7 (6.0)*	32.8 (7.6)*	34.5 (8.0)*	36.6 (6.5)*†‡§
	Y	48.0 (6.1)	53.8 (7.6)*	52.5 (6.4)*	53.1 (6.5)*	55.4 (5.5)*†‡§
HR_{max} (bpm)	O#	144 (22)	139 (23)*	141 (21)	142 (19)	145 (17)†§
	Y	189 (7)	185 (5)*	185 (5)	185 (6)	187 (7) †§
Q_{max} ($L \cdot min^{-1}$)	O#	16.8 (3.0)	18.0 (3.8)*	18.7 (4.2)*	19.8 (3.5)*†‡	20.3 (3.7)*†‡
	Y	25.9 (2.8)	26.7 (2.2)*	27.3 (2.1)*	28.6 (1.6)*†‡	28.4 (1.8)*†‡
SV_{max} ($mL \cdot beat^{-1}$)	O	122.1 (21.7)	130.4 (19.4)*	133.2 (22.0)*	140.6 (21.5)*†‡	140.2 (21.3)*†
	Y	137.3 (17.2)	144.7 (12.6)*	148.2 (15.2)*	154.6 (10.6)*†‡	152.3 (12.6)*†
Maximal a-vO_{2diff} ($mLO_2 \cdot 100mL^{-1}$ blood)	O	13.5 (2.2)	14.0 (2.2)*	14.2 (1.7)*	14.0 (1.9)	14.7 (2.1)*
	Y	14.7 (0.9)	15.8 (1.2)*	15.4 (1.3)*	14.8 (1.4)	15.7 (0.9)*

Values are means ± SD. PO_{peak}, peak power output; VO_{2max}, maximal O_2 uptake; HR_{max}, maximal heart rate; Q_{max}, maximal cardiac output; SV_{max}, maximal stroke volume; Maximal a-vO_{2diff}, maximal O_2 extraction; * Significantly different from Pre-training values ($p < 0.05$); † Significantly different from Week 3 ($p < 0.05$); ‡ Significantly different from Week 6; § Significantly different from Week 9; # Significantly different from Y ($p < 0.05$).

Table 2.3. Sub-maximal exercise responses for HR, Q, SV and a-vO_{2diff} in O and Y from pre-training through post-training.

		Pre-training	Week 3	Week 6	Week 9	Post-training
HR_{sub} (bpm)	O#	94 (14)	89 (12)*	90 (15)*	86 (9)*	88 (9)*
	Y	129 (15)	120 (15)*	116 (11)*	120 (13)*	117 (13)*
Q_{sub} ($L \cdot min^{-1}$)	O#	11.7 (2.0)	11.3 (1.5)	11.8 (2.4)	12.1 (2.0)	11.8 (2.3)
	Y	17.0 (2.5)	17.0 (2.1)	16.5 (2.4)	16.3 (2.0)	16.5 (2.6)
SV_{sub} ($mL \cdot beat^{-1}$)	O	125.3 (19.4)	128.9 (17.3)*	132.4 (23.2)*	140.7 (24.5)*	134.6 (25.4)*
	Y	132.6 (18.2)	143.6 (24.9)*	143.2 (25.3)*	136.7 (21.5)*	142.8 (25.0)*
a-vO_{2diff} ($mLO_2 \cdot 100mL^{-1}$ blood)	O	13.2 (1.8)	12.6 (1.2)	12.0 (1.6)	12.4 (1.7)	12.9 (2.0)
	Y	13.3 (1.0)	13.3 (1.0)	13.2 (1.0)	13.6 (0.9)	13.5 (0.7)
θ_L ($L \cdot min^{-1}$)	O#	1.48 (0.20)	1.70 (0.29)*	1.81 (0.29)*†	1.88 (0.34)*†	1.96 (0.40)*†‡§
	Y	2.44 (0.52)	2.58 (0.50)*	2.66 (0.44)*†	2.72 (0.49)*†	2.83 (0.49)*†‡§

Values are means ± SD. HR_{sub}, sub-maximal heart rate; Q_{sub}, sub-maximal cardiac output; SV_{sub}, sub-maximal stroke volume; a-vO_{2diff}, O_2 extraction; θ_L, estimated lactate threshold; * Significantly different from Pre-training values ($p < 0.05$); † Significantly different from Week 3 ($p < 0.05$); ‡ Significantly different from Week 6; § Significantly different from Week 9;# Significantly different from Y ($p < 0.05$).

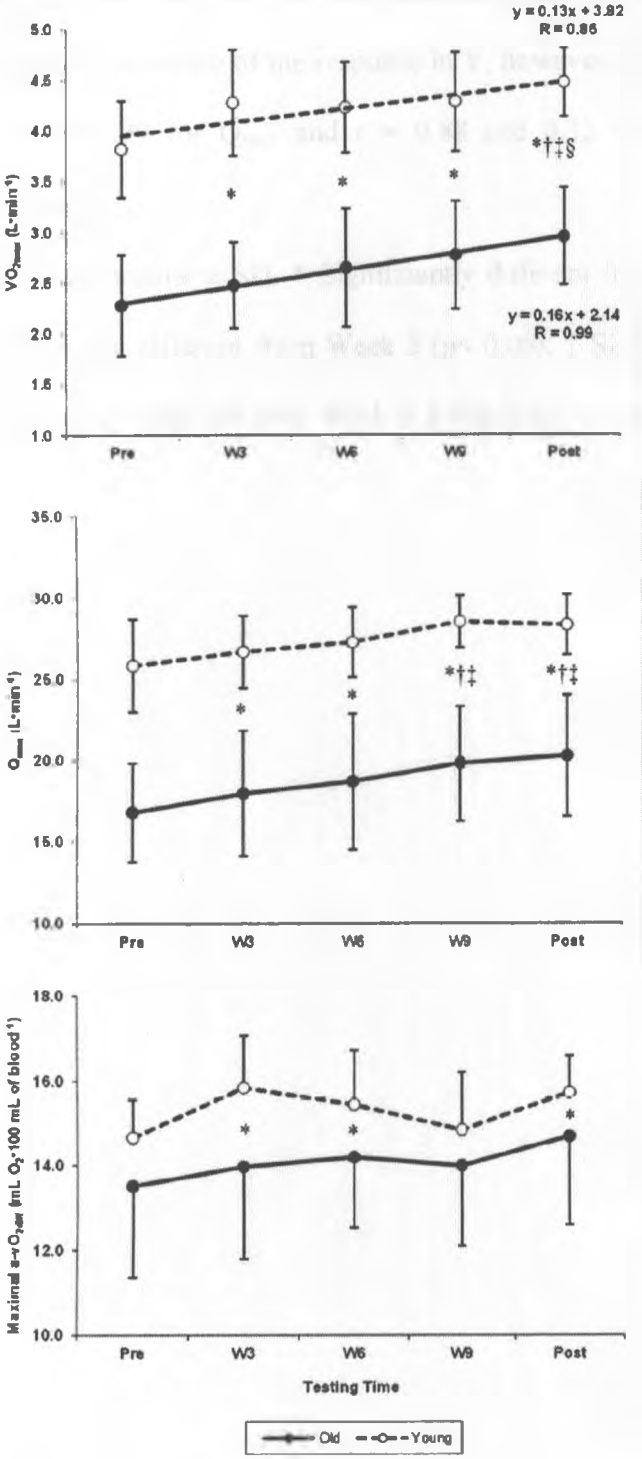

Figure 2.1. Changes in maximal VO_2, Q, and a-vO_{2diff} in response to training in O and Y.

Slopes are not calculated for the figures displaying Q_{max} and maximal a-vO_{2diff} because of the non-linear nature of the response in Y; however, the coefficient of correlation was r = 0.96 and 0.99 for Q_{max} and r = 0.88 and 0.32 for maximal a-vO_{2diff} in O and Y, respectively.

Values are means ± SD. * Significantly different from Pre-training values ($p< 0.05$); † Significantly different from Week 3 ($p< 0.05$); ‡ Significantly different from Week 6; § Significantly different from Week 9; # Significantly different from Y ($p< 0.05$).

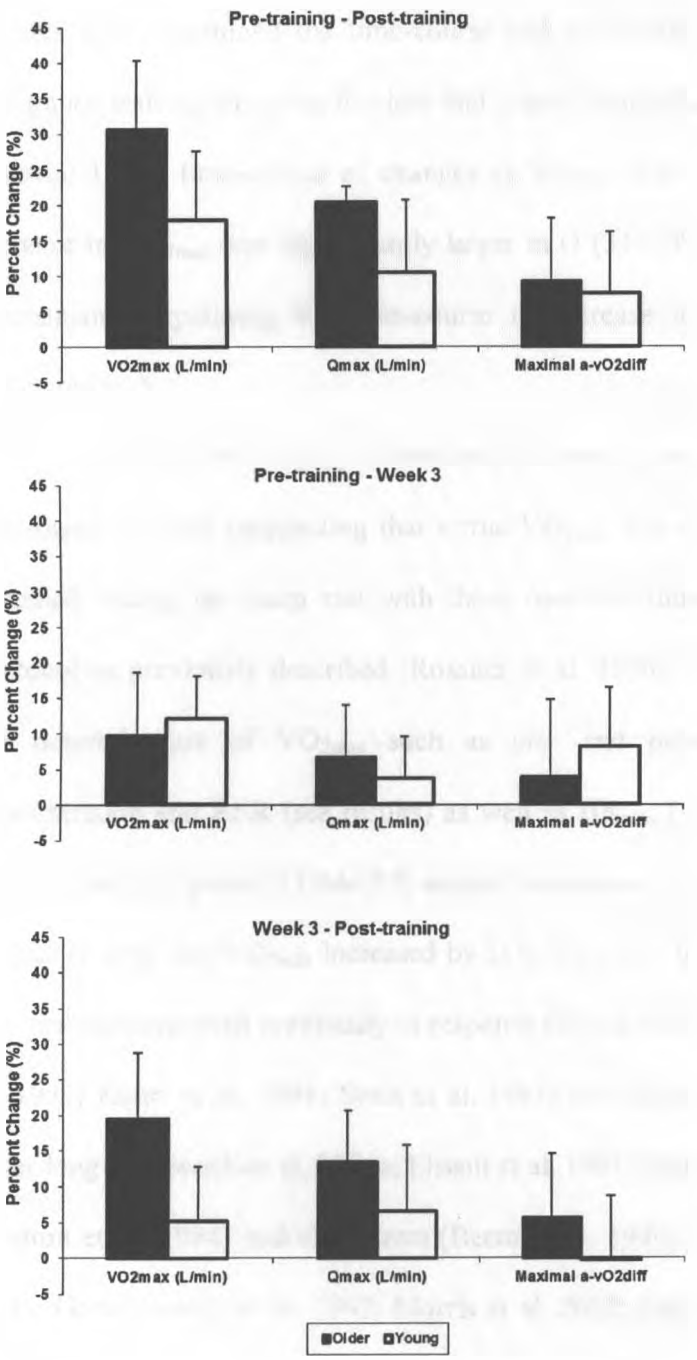

Figure 2.2. Percent changes in maximal VO_2, Q, and SV from pre-training to post-training, pre-training to week 3, and week 3 to post-training in O and Y adults. Values are means ± SD.

DISCUSSION

This study examined the time-course and mechanisms of adaptation to a 12-week endurance training program in older and young male adults. The main findings were as follows: 1) the time-course of changes in VO_{2max} was similar in O and Y; 2) the % increase in VO_{2max} was significantly larger in O (31±10%) than in Y (18±10%); 3) The mechanisms explaining the time-course of increase in VO_{2max} were different in O compared to Y.

Measurements of VO_{2max} in this study were rigorous. We confirmed no further increments in VO_2 (suggesting that a true VO_{2max} was attained) by comparing the data obtained during the ramp test with those observed during the 2-3 min constant load protocol as previously described (Rossiter et al. 2006). Additionally, secondary criteria for determination of VO_{2max} such as pre- and post-training end-exercise lactate concentration and RER (see results) as well as HR_{max} (~95% of the estimated maximal HR for each age-group) (Table 2.2) suggest a maximal effort was achieved.

Our finding that VO_{2max} increased by 31% from pre- to post-training in O is similar to the increases reported previously in response to long-term endurance training (Coggan et al. 1992; Kohrt et al. 1991; Seals et al. 1984) but higher than the increases reported in other long- (Babcock et al. 1994a; Ehsani et al. 1991; Spina et al. 1996; Spina et al. 1993; Stratton et al. 1994) and short-term (Beere et al. 1999; Charles et al. 2006; Gass et al. 2004; Govindasamy et al. 1992; Morris et al. 2002; Poulin et al. 1992) aerobic training studies in older men. The larger increase in VO_{2max} in the present study may be explained by the relatively high training intensity used (~70% of VO_{2max}) and by the frequent progression in training intensity (PO adjusted every 3 weeks). It was proposed that higher training intensities (75-80% of HR reserve (HRR)) are important to maximize increases in

VO_{2max} in older adults (Ehsani et al. 1991; Seals et al. 1984). Similarly, Makrides et al. (Makrides et al. 1990) reported a 38% increase in VO_{2max} in older subjects in response to a 12-week interval training regime where the training intensity was adjusted to ~85% of the initial VO_{2max} by the third week of the program. However, Gass et al. (Gass et al. 2004) proposed that the total amount of work, rather than training intensity, determined the increase in VO_{2max}. Considering that in the present study the total amount of work was increased by increasing the training PO, it is likely that both training intensity and total amount of work played a role in modulating the increases in VO_{2max}. In the present study, the absolute increase in VO_{2max} was similar in both O and Y, while the % increase was larger in O (O, 31%; Y, 18%) perhaps as a result of the lower absolute pre-training VO_{2max} in O. It is unlikely that the higher % increase in VO_{2max} in O reflected a relatively lower initial level of fitness compared with Y because the participants for both age groups in this study at baseline were above the mean VO_{2max} predicted for age-matched populations (ACSM 2003; Paterson et al. 1999).

Approximately 2/3 of the pre- to post-training increase in VO_{2max} in O was explained by an increase in Q_{max} with a larger post-training maximal a-vO_{2diff} accounting for the remaining ~1/3 of the change. A similar pattern of adaptation, with Q_{max} being the primary mechanism for the increase in VO_{2max}, has been previously reported in response endurance training programs in older men (Morris et al. 2002; Spina et al. 1993). What is novel about the present study is that the time-course of central and peripheral changes were tracked at 3 weeks intervals. Interestingly, the relative contribution from Q_{max} and maximal a-vO_{2diff} in explaining the larger VO_{2max} in O remained the same from pre-training to week 3 and from week 3 to post-training (the testing times at which VO_{2max} was significantly increased) suggesting that central adaptations are important in

establishing increases in VO_{2max} in O and also that these central adaptations occur rapidly (within the first 3 weeks of starting training). Considering that the overall HR_{max} response was unchanged pre- to post-training, the greater Q_{max} observed in O post-training was a consequence of a larger SV_{max} (pre- to post-training increase 16 ± 11% and 12 ± 10% in O and Y, respectively) (Table 2.2). Similarly, three weeks of training in O resulted in a reduction in HR_{sub} and an increase in SV_{sub}. Training-induced increases in SV_{sub} in O have been reported previously (Gass et al. 2004; Morris et al. 2002; Spina et al. 1993).

The larger SV_{max} could be related to an enhanced left ventricular (LV) filling, increased LV contractility, or a combination of these factors. It has been proposed that most of the increases in Q_{max} are related to an increased diastolic filling because of a more compliant left ventricle (Levine 2008), which could lead to an increased SV via the Frank-Starling mechanism (Lakatta and Levy 2003). In regard to an increased LV contractile function, it has been proposed that an enlargement of the left ventricle mass could be one of the mechanisms responsible for this adaptation (Ehsani et al. 1991; Seals et al. 1994); however it is likely this is a longer-term adaptation. Although no measures of catecholamines were obtained in this study, greater ventricular contractility following training in older adults could be related to increased sensitivity to these hormones (Spina et al. 1998), which would counteract the reported age-related decrease in catecholamine sensitivity and loss of efficiency of post-synaptic β-adrenergic signaling (Lakatta et al. 1975; Lakatta and Levy 2003).

A training-induced increase in $a\text{-}vO_{2diff}$ also provided a significant contribution to the increase in VO_{2max} in O. Even though no direct measures of peripheral adaptations are provided in the present study, previous reports have shown that a greater whole body (i.e., muscle) O_2 extraction following training in older adults could be related to improvements

in capillarization and augmented number of type IIa muscle fibers (Charles et al. 2006; Coggan et al. 1992; Hepple et al. 1997), mitochondrial enzymes activity (Bell et al. 2001; Charles et al. 2006; Coggan et al. 1992), and/or microvascular blood flow distribution (Martin et al. 1990; Sidney and Shephard 1978). Taken together, these data suggest that in older adults, both cardiac and skeletal muscle can adapt to training, and given an adequate training stimulus, this adaptation occurs relatively quickly (within 3 weeks of training) and can continue for at least 12 of weeks training.

In Y, ~60% of the increase in VO_{2max} from pre- to post-training was attributed to a greater Q_{max}, similar to that observed in O (i.e., ~66%). However, in Y the early increase in VO_{2max} (i.e., during the first 3 weeks of training) was a consequence of a greater a-vO_{2diff}. Unlike O who showed a more steady response during the 12 weeks of training, in Y, VO_{2max} remained unchanged between weeks 3 - 9, followed by an increase between weeks 9 – 12, a consequence of a greater Q_{max}. It is unclear why increases in VO_{2max} in Y men relied more on a-vO_{2diff} during the first weeks of training, but it is possible that a more effective distribution of Q in the periphery may have resulted in a better matching of O_2 delivery and utilization. Previous training studies have reported peripheral adaptations early in training in young men that would support this contention (Andersen and Henriksson 1977; Coggan et al. 1993; Denis et al. 1986; Henriksson and Reitman 1977). Since the overall HR_{max} did not change from pre- to post-training, the improvements in Q_{max} that explained the further increase in VO_{2max} with training were solely explained by a higher SV_{max}. Similarly, an improved SV was also observed at submaximal intensities as previously reported (Makrides et al. 1990; Scharhag-Rosenberger et al. 2009; Spina et al. 1992).

A training-induced increase in the VO_2 corresponding to the θ_L was observed after weeks 3, 6, and 12 in both O and Y. Similar increases in θ_L following training have been reported previously for both older (Poulin et al. 1992; Takeshima et al. 1996) and young adults (Davis et al. 1979; Ready and Quinney 1982). This improved response to submaximal exercise may be especially important in older men where certain activities of daily living may be performed above θ_L and thus qualify as "heavy" intensity and fatiguing (Paterson et al. 2007).

Based on the work of O'Donovan et al. (O'Donovan et al. 2005), we contemplated the possibility that only those men being part of the HIT group would further increase their VO_{2max}. However, both training groups (CT and HIT) showed similar improvements in response to training. This suggests that: a) when the training intensity is adjusted to reflect changes in aerobic performance, CT at an intensity of 70% of VO_{2max} remains sufficient to produce increments in VO_{2max} even after 10 weeks of performing a similar exercise training protocol; b) HIT may be a valid alternative to a chronic endurance training program even in older populations. Importantly, although a plateau response in VO_{2max} was not observed in this short-term training program, a "ceiling effect" would be expected with further endurance training.

In conclusion, we demonstrated that the time-course of adaptations in VO_{2max} was similar in O and Y men with improvements occurring as early as 3 weeks into training and continuing to the end of the program. Thus, a short-term training program yielded substantial increases in VO_{2max} in both older and young men. Increments in VO_{2max} from pre- to post-training in O were achieved through changes in Q_{max} (~2/3 of the change) as in Y. The time course of adaptation was age-dependent in that Y initially relied on increases in maximal a-vO_{2diff} (first 3 weeks) with further increases in aerobic power

being explained exclusively by a larger Q_{max} whereas O showed consistent improvements in Q_{max} (~2/3 increase) throughout the training program.

REFERENCES

ACSM (2003) ACSM's guidelines for exercise testing and prescription. Lippincot Williams & Wilkins, Philadelphia

Andersen P, Henriksson J (1977) Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. J Physiol 270: 677-690

Babcock MA, Paterson DH, Cunningham DA (1994a) Effects of aerobic endurance training on gas exchange kinetics of older men. Med Sci Sports Exerc 26: 447-452

Babcock MA, Paterson DH, Cunningham DA, Dickinson JR (1994b) Exercise on-transient gas exchange kinetics are slowed as a function of age. Med Sci Sports Exerc 26: 440-446

Beaver WL, Lamarra N, Wasserman K (1981) Breath-by-breath measurement of true alveolar gas exchange. J Appl Physiol 51: 1662-1675

Beere PA, Russell SD, Morey MC, Kitzman DW, Higginbotham MB (1999) Aerobic exercise training can reverse age-related peripheral circulatory changes in healthy older men. Circulation 100: 1085-1094

Bell C, Paterson DH, Kowalchuk JM, Moy AP, Thorp DB, Noble EG, Taylor AW, Cunningham DA (2001) Determinants of oxygen uptake kinetics in older humans following single-limb endurance exercise training. Exp Physiol 86: 659-665

Charles M, Charifi N, Verney J, Pichot V, Feasson L, Costes F, Denis C (2006) Effect of endurance training on muscle microvascular filtration capacity and vascular bed morphometry in the elderly. Acta Physiol (Oxf) 187: 399-406

Coggan AR, Spina RJ, King DS, Rogers MA, Brown M, Nemeth PM, Holloszy JO (1992) Skeletal muscle adaptations to endurance training in 60- to 70-yr-old men and women. J Appl Physiol 72: 1780-1786

Coggan AR, Spina RJ, Kohrt WM, Holloszy JO (1993) Effect of prolonged exercise on muscle citrate concentration before and after endurance training in men. Am J Physiol 264: E215-220

Coyle EF (1995) Integration of the physiological factors determining endurance performance ability. Exerc Sport Sci Rev 23: 25-63

Davis JA, Frank MH, Whipp BJ, Wasserman K (1979) Anaerobic threshold alterations caused by endurance training in middle-aged men. J Appl Physiol 46: 1039-1046

Denis C, Chatard JC, Dormois D, Linossier MT, Geyssant A, Lacour JR (1986) Effects of endurance training on capillary supply of human skeletal muscle on two age groups (20 and 60 years). J Physiol (Paris) 81: 379-383

Ehsani AA, Ogawa T, Miller TR, Spina RJ, Jilka SM (1991) Exercise training improves left ventricular systolic function in older men. Circulation 83: 96-103

Fleg JL, Morrell CH, Bos AG, Brant LJ, Talbot LA, Wright JG, Lakatta EG (2005) Accelerated longitudinal decline of aerobic capacity in healthy older adults. Circulation 112: 674-682

Gass G, Gass E, Wicks J, Browning J, Bennett G, Morris N (2004) Rate and amplitude of adaptation to two intensities of exercise in men aged 65-75 yr. Med Sci Sports Exerc 36: 1811-1818

Govindasamy D, Paterson DH, Poulin MJ, Cunningham DA (1992) Cardiorespiratory adaptation with short term training in older men. Eur J Appl Physiol Occup Physiol 65: 203-208

Henriksson J, Reitman JS (1977) Time course of changes in human skeletal muscle succinate dehydrogenase and cytochrome oxidase activities and maximal oxygen uptake with physical activity and inactivity. Acta Physiol Scand 99: 91-97

Hepple RT, Mackinnon SL, Goodman JM, Thomas SG, Plyley MJ (1997) Resistance and aerobic training in older men: effects on VO_{2peak} and the capillary supply to skeletal muscle. J Appl Physiol 82: 1305-1310

Hollenberg M, Yang J, Haight TJ, Tager IB (2006) Longitudinal changes in aerobic capacity: implications for concepts of aging. J Gerontol A Biol Sci Med Sci 61: 851-858

Johnson BD, Beck KC, Proctor DN, Miller J, Dietz NM, Joyner MJ (2000) Cardiac output during exercise by the open circuit acetylene washin method: comparison with direct Fick. J Appl Physiol 88: 1650-1658

Kohrt WM, Malley MT, Coggan AR, Spina RJ, Ogawa T, Ehsani AA, Bourey RE, Martin WH, 3rd, Holloszy JO (1991) Effects of gender, age, and fitness level on response of VO_{2max} to training in 60-71 yr olds. J Appl Physiol 71: 2004-2011

Lakatta EG, Gerstenblith G, Angell CS, Shock NW, Weisfeldt ML (1975) Diminished inotropic response of aged myocardium to catecholamines. Circ Res 36: 262-269

Lakatta EG, Levy D (2003) Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part II: the aging heart in health: links to heart disease. Circulation 107: 346-354

Levine BD (2008) VO_{2max}: what do we know, and what do we still need to know? J Physiol 586: 25-34

Makrides L, Heigenhauser GJ, Jones NL (1990) High-intensity endurance training in 20- to 30- and 60- to 70-yr-old healthy men. J Appl Physiol 69: 1792-1798

Martin WH, 3rd, Kohrt WM, Malley MT, Korte E, Stoltz S (1990) Exercise training enhances leg vasodilatory capacity of 65-yr-old men and women. J Appl Physiol 69: 1804-1809

Morris N, Gass G, Thompson M, Bennett G, Basic D, Morton H (2002) Rate and amplitude of adaptation to intermittent and continuous exercise in older men. Med Sci Sports Exerc 34: 471-477

Morris N, Gass G, Thompson M, Conforti D (2003) Physiological responses to intermittent and continuous exercise at the same relative intensity in older men. Eur J Appl Physiol 90: 620-625

O'Donovan G, Owen A, Bird SR, Kearney EM, Nevill AM, Jones DW, Woolf-May K (2005) Changes in cardiorespiratory fitness and coronary heart disease risk factors following 24 wk of moderate- or high-intensity exercise of equal energy cost. J Appl Physiol 98: 1619-1625

Paterson DH, Cunningham DA, Koval JJ, St Croix CM (1999) Aerobic fitness in a population of independently living men and women aged 55-86 years. Med Sci Sports Exerc 31: 1813-1820

Paterson DH, Govindasamy D, Vidmar M, Cunningham DA, Koval JJ (2004) Longitudinal study of determinants of dependence in an elderly population. J Am Geriatr Soc 52: 1632-1638

Paterson DH, Jones GR, Rice CL (2007) Ageing and physical activity: evidence to develop exercise recommendations for older adults. Can J Public Health 98 Suppl 2: S69-108

Pimentel AE, Gentile CL, Tanaka H, Seals DR, Gates PE (2003) Greater rate of decline in maximal aerobic capacity with age in endurance-trained than in sedentary men. J Appl Physiol 94: 2406-2413

Poulin MJ, Paterson DH, Govindasamy D, Cunningham DA (1992) Endurance training of older men: responses to submaximal exercise. J Appl Physiol 73: 452-457

Ready AE, Quinney HA (1982) Alterations in anaerobic threshold as the result of endurance training and detraining. Med Sci Sports Exerc 14: 292-296

Rossiter HB, Kowalchuk JM, Whipp BJ (2006) A test to establish maximum O_2 uptake despite no plateau in the O_2 uptake response to ramp incremental exercise. J Appl Physiol 100: 764-770

Scharhag-Rosenberger F, Meyer T, Walitzek S, Kindermann W (2009) Time course of changes in endurance capacity: A 1-yr training study. Med Sci Sports Exerc

Seals DR, Hagberg JM, Hurley BF, Ehsani AA, Holloszy JO (1984) Endurance training in older men and women. I. Cardiovascular responses to exercise. J Appl Physiol 57: 1024-1029

Seals DR, Hagberg JM, Spina RJ, Rogers MA, Schechtman KB, Ehsani AA (1994) Enhanced left ventricular performance in endurance trained older men. Circulation 89: 198-205

Sidney KH, Shephard RJ (1978) Frequency and intensity of exercise training for elderly subjects. Med Sci Sports 10: 125-131

Spina RJ, Miller TR, Bogenhagen WH, Schechtman KB, Ehsani AA (1996) Gender-related differences in left ventricular filling dynamics in older subjects after endurance exercise training. J Gerontol A Biol Sci Med Sci 51: B232-237

Spina RJ, Ogawa T, Kohrt WM, Martin WH, 3rd, Holloszy JO, Ehsani AA (1993) Differences in cardiovascular adaptations to endurance exercise training between older men and women. J Appl Physiol 75: 849-855

Spina RJ, Ogawa T, Martin WH, 3rd, Coggan AR, Holloszy JO, Ehsani AA (1992) Exercise training prevents decline in stroke volume during exercise in young healthy subjects. J Appl Physiol 72: 2458-2462

Spina RJ, Turner MJ, Ehsani AA (1998) Beta-adrenergic-mediated improvement in left ventricular function by exercise training in older men. Am J Physiol 274: H397-404

Stathokostas L, Jacob-Johnson S, Petrella RJ, Paterson DH (2004) Longitudinal changes in aerobic power in older men and women. J Appl Physiol 97: 781-789

Stratton JR, Levy WC, Cerqueira MD, Schwartz RS, Abrass IB (1994) Cardiovascular responses to exercise. Effects of aging and exercise training in healthy men. Circulation 89: 1648-1655

Takeshima N, Kobayashi F, Watanabe T, Tanaka K, Tomita M, Pollock ML (1996) Cardiorespiratory responses to cycling exercise in trained and untrained healthy elderly: with special reference to the lactate threshold. Appl Human Sci 15: 267-273

Tanaka H, Desouza CA, Jones PP, Stevenson ET, Davy KP, Seals DR (1997) Greater rate of decline in maximal aerobic capacity with age in physically active vs. sedentary healthy women. J Appl Physiol 83: 1947-1953

CHAPTER III: Mechanisms for increases in VO_{2max} with endurance training in older and young women

INTRODUCTION

Aerobic training has been shown to increase maximal oxygen uptake (VO_{2max}) in both older (Coggan et al. 1992; Martin et al. 1990; Seals et al. 1984; Spina et al. 1996; Spina et al. 1993a) and younger (Cunningham et al. 1979; Pedersen and Jorgensen 1978; Spina et al. 1992a; Spina et al. 1992b) women. However, the relative contribution of central (cardiac output (Q)) and peripheral (arterial-venous O_2 difference ($a\text{-}vO_{2diff}$)) mechanisms underlying the increases in VO_{2max} are not fully understood. For instance, in young women, increases in VO_{2max} in response to endurance training have been attributed to increases in maximal Q (Q_{max}) and maximal stroke volume (SV_{max}) (Cunningham and Hill 1975; Spina et al. 1992a; Spina et al. 1992b) as well as maximal $a\text{-}vO_{2diff}$ (Cunningham et al. 1979), whereas in older women only increases in maximal $a\text{-}vO_{2diff}$ have been reported (Seals et al. 1984; Spina et al. 1996; Spina et al. 1993a). Importantly, these studies were all long-term exercise training programs (6 to 12 months) with no short-term endurance training studies (8-12 weeks) elucidating the mechanisms underlying the significant improvements in VO_{2max} in older women. Thus, the time-course and mechanisms of adaptation to improvements in VO_{2max} that occur with shorter training periods of 12 weeks or less in older (and younger) women remain unknown.

An age-related decline in VO_{2max} (Fitzgerald et al. 1997; Hollenberg et al. 2006; Paterson et al. 2007; Stathokostas et al. 2004; Tanaka et al. 1997) may contribute to a reduction in functional capacity, and thus, lead to a loss of independence in older individuals (Paterson et al. 2004). It has been proposed that there is a functional fitness

musculoskeletal diseases, were medically screened by a physician and underwent a maximal exercise stress test.

Protocol: Before training began, subjects performed a maximal cycle ergometer ramp test to exhaustion (O 12-15 W/min; Y 20 W/min) on a Lode Corival 400 cycle-ergometer (Lode B.V., Groningen, Holland) for determination of VO_{2max} and estimation of the lactate threshold (θ_L). θ_L was defined as the VO_2 at which CO_2 output (VCO_2) began to increase out of proportion to VO_2 along with a systematic rise in minute ventilation-to-VO_2 ratio and end-tidal PO_2 whereas minute ventilation-to-VCO_2 ratio and end-tidal PCO_2 were stable. Approximately 1 min after the end of the ramp test, a fingertip blood sample (~0.5 μL) was obtained to measure end-exercise blood lactate concentration using a portable device (Lactate Scout, Sports Resource Group, Hawthorne, NY). Within five minutes after completion of this test, subjects performed a constant-load cycling exercise to volitional fatigue at 85% of the peak power output (PO_{peak}) achieved during the ramp incremental test. This protocol (described in (Rossiter et al. 2006)) was performed to assess the attainment of VO_{2max} and to allow determination of Q_{max}. Subjects were instructed to indicate when they thought they were ~30 s from exhaustion. At that point, verbal encouragement increased and within ~15 s the measurement of Q began. VO_{2max} was defined as the highest VO_2 observed for an average of 20 consecutive seconds during either the ramp test to exhaustion or the 2-3 minute constant load at 85% of PO_{peak}. On a separate day, subjects were asked to cycle at a PO corresponding to ~90% of their pre-training θ_L and when a steady state in gas exchange was achieved Q was measured. Similar procedures were repeated after 3, 6, 9, and 12 weeks of training.

Blood tests: Prior to the start (pre-) and after 6 (mid-) and 12 weeks (post-) of training blood samples were drawn from each subject's antecubital vein for determination of hematocrit (Hct) and hemoglobin (Hb) concentrations.

Training: The endurance training program consisted of 3 exercise sessions per week on a stationary cycle-ergometer (Monark Ergomedic 874E; Monark Exercise AB, Varberg, Sweeden) for a total duration of 12 weeks. During the first 10 weeks, each session consisted of CT for 45 min at a power output that elicited ~70% of the VO_{2max} observed during the incremental ramp test. During the final 2 weeks of training (6 exercise sessions), each individual in each group (O and Y) was randomly assigned (stratified by age) to one of two sub-groups: a) CT as described above; b) high-intensity interval training (HIT), performing 10-12 exercise bouts each lasting 1-min at 90-100% of the peak power output achieved during the incremental ramp test, with 1-min rest separating bouts. Training intensity was adjusted at 3 week intervals to reflect changes in fitness level. Since VO_{2max} was likely to plateau after approximately 8 weeks of CT (O'Donovan et al. 2005), the HIT was used as a strategy for progressive and continued gains in the exercise program resulting in further increases in VO_{2max} favoured by peripheral adaptations (Coyle 1995).

Measurements: Gas-exchange measurements were similar to those previously described (Babcock et al. 1994). Briefly, inspired and expired flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies VMM 110) which was calibrated before each test by using a syringe of known volume. Inspired and expired gases were sampled continuously (every 20 ms) at the mouth and analyzed for concentrations of O_2, CO_2, nitrogen (N_2), acetylene (C_2H_2), and helium (He) by mass spectrometry (Perkin Elmer MGA-1100) after calibration with precision-analyzed gas

mixtures. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver et al. (Beaver et al. 1981).

Heart rate (HR) was monitored continuously by electrocardiogram using PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO) with a three-lead arrangement. Data were recorded using LabChart v4.2 (ADInstruments, Colorado Springs, CO) on a separate computer.

Q measurements: Q was measured using the acetylene (C_2H_2) open circuit inert gas washin method and analyzed using custom data acquisition software. This technique was described and validated previously (Johnson et al. 2000). Briefly, a pneumotachograph (Hans Rudolph Model 3800, Kansas City, MO; transducer, Validyne MP45-871, Northridge, CA) was attached to a non-rebreathing Y valve (Hans Rudolph 7900, Kansas City, MO), which was connected to a manual valve that allowed switching inspired gases between room air and a bag containing a mixture of C_2H_2 (0.7%), O_2 (21%), He (9%), and balance N_2. Changes in gas concentrations were aligned with gas volumes by measuring the time delay of the gases as described above. Throughout the sub-maximal and peak exercise measurements of Q subjects were asked to continue their normal breathing pattern when the source of inspired air was switched to the bag containing the gas mixture and after 10 breaths the protocol was terminated. Data analysis for the calculation of Q was performed immediately after each manouver using equations reported previously (Johnson et al. 2000). The $a\text{-}vO_{2diff}$ was calculated from the Fick equation as: $a\text{-}vO_{2diff}$ ($mLO_2 \cdot 100mL^{-1}$ blood) = VO_2 ($L \cdot min^{-1}$) / Q ($L \cdot min^{-1}$) x 100. Stroke volume was calculated as: SV ($mL \cdot beat^{-1}$) = Q ($mL \cdot min^{-1}$) / HR (bpm).

Statistics: Data are presented as means ± SD. Independent t-tests and repeated measures analysis of variance (ANOVA) were used to determine statistical significance for the

dependent variables. A Tukey post-hoc analysis was used when significant differences were found for the main effects of each dependent variable. The ANOVA was conducted by SPSS Version 15.0, (SPSS Inc., Chicago, IL). Statistical significance was declared when $p < 0.05$.

RESULTS

Pre- and post-training subjects' characteristics and resting Hct and Hb are depicted in Table 3.1. Compliance with the training program was 94 ± 4% (28/30 training sessions) in O and 98 ± 3% (29/30 training sessions) in Y, and each subject completed at least 27 of the programmed training sessions (i.e., 90%). The average training power output (PO) per session significantly increased after each testing session in both O (e.g., week 1-3, 64 ± 5 W; week 4-6, 71 ± 9 W; week 7-9, 76 ± 9 W) and Y (e.g., week 1-3, 121 ± 13 W; week 4-6, 134 ± 14 W; week 7-9, 142 ± 15 W) and PO was always higher in Y than in O ($p < 0.05$). In the subgroup of O and Y subjects performing the continuous training during the final weeks of the program (n = 7 including O and Y), the average PO per session significantly increased compared to week 7-9 (e.g., PO week 7-9, 111 ± 33 W vs. PO week 10-12, 116 ± 34 W. In the group performing the HIT (n = 7 including O and Y) exercise intensity was higher compared to the previous 3 weeks (e.g., PO week 7-9 (continuous) = 116 ± 40 W vs. week 10-12 (HIT) = 199 ± 76 W); however, the estimated energy expenditure for an average of 11 ± 1 one-minute bouts of exercise was significantly decreased during HIT, by ~60% compared to the energy expenditure of the 45-min continuous bout.

Table 3.2 summarizes the changes in peak and maximal exercise values in response to training. PO_{peak} significantly increased at each testing session from pre-training to post-training in both O and Y women. A higher VO_{2max} ($L \cdot min^{-1}$) was observed within 3

weeks of training in both O and Y, the magnitude of which was larger in Y. Further significant changes in VO_{2max} in both groups occurred from week 3 to week 6 and from week 6 to week 9. When changes in VO_{2max} data were expressed relative to body mass ($mL \cdot kg^{-1} \cdot min^{-1}$), a similar response was observed; however, there was also a significant main effect from week 9 to post-training accompanied by a testing-time by age interaction that demonstrated a continued increase in VO_{2max} in Y women with no further changes observed in the O group (Figure 3.1). The percent change in VO_{2max} from pre-training to post-training was similar in O (17 ± 14%) compared to Y (22 ± 6%) adults ($p > 0.05$). The VO_{2max} obtained during the ramp test was similar to that observed during the 2-3 minute constant-load test to exhaustion (which was also used to determine Q_{max}) in both O and Y ($p > 0.05$).

Maximal HR (HR_{max}) remained unchanged with training in both O and Y women. As observed in Figure 3.1, Q_{max} was higher ($p< 0.05$) post-training compared to pre-training, week 3, and week 6, as well as at week 9 compared to week 6. An age by testing time interaction between week 6 and week 9 indicates a significant improvement in Q_{max} in Y but not in O women that was maintained at the post-training measurement. SV_{max} displayed a similar response as that observed for Q_{max}. Maximal a-vO_{2diff} was higher ($p< 0.05$) at week 6, 9 and post-training compared to pre-training and also at week 6 compared to week 3. There was an age by testing time interaction between pre-training and week 3 reflecting the reliance on a-vO_{2diff} in Y women early in training. Further interactions between week 6 and week 9 and week 9 and post-training represent the higher reliance on a-vO_{2diff} in O later in training.

Figure 3.2 describes the relative contribution of Q_{max} and maximal a-vO_{2diff} to the increase in VO_{2max}. In the O, 65% of the change in VO_{2max} from pre- to post-training was

explained by the increase in a-vO_{2diff} while the remaining 35% was explained by an improved Q_{max} (calculated as the percent change in Q (or a-vO_{2diff}) divided by the total percent change in VO_{2max}). In Y, 55% of the improvement in VO_{2max} was attributed to a higher Q_{max} and 45% to a widened a-vO_{2diff}. Interestingly, the small (3%) but significant change in VO_{2max} during the first 3 weeks of training in the older women relied exclusively on a non-significant improvement in Q_{max}. In Y women, an 8% increase in VO_{2max} during the first 3 weeks of training was attributed to a widened a-vO_{2diff} (Figure 3.2). The remainder of the change (i.e., from week 3 to post-training) in VO_{2max} in O (14%) and Y (13%) women was mostly explained by improvements in maximal a-vO_{2diff} in O in contrast to Q_{max} in Y (Figure 3.2). Training type (e.g., continuous vs. HIT) over the latter period of training did not significantly affect any of the variables of interest at maximal exercise (e.g., PO_{peak}, VO_{2max}, HR_{max}, Q_{max}, SV_{max}, and maximal a-vO_{2diff}).

Table 3.3 depicts changes in response to training during sub-maximal intensity exercise (at a given absolute PO; O, 47 ± 6 W and Y, 73 ± 9 W). VO_2 in O (e.g., pre-training, 1.18 ± 0.16 $L \cdot min^{-1}$; post-training, 1.25 ± 0.18 $L \cdot min^{-1}$) and in Y (e.g., pre-training, 1.50 ± 0.19 $L \cdot min^{-1}$; post-training, 1.46 ± 0.20 $L \cdot min^{-1}$) was unchanged over the course of training. Sub-maximal HR (HR_{sub}) significantly decreased after 3 weeks of training in both O and Y with no further changes observed thereafter. Sub-maximal Q (Q_{sub}) remained unchanged in both groups throughout testing. A significant increase in sub-maximal SV (SV_{sub}) was evident at week 9 and post-training compared to pre-training in O and Y ($p < 0.05$). Sub-maximal a-vO_{2diff} (a-$vO_{2diffsub}$) was not affected by training. θ_L ($L \cdot min^{-1}$) was higher in Y compared to O ($p < 0.05$). The absolute θ_L ($L \cdot min^{-1}$) significantly increased after 3 weeks of training in both O and Y. A further increase in θ_L was observed at week 9 and post-training (Table 3.3). There was a training-type

interaction effect with θ_L being significantly increased in the HIT group (θ_L continuous: week 9, 1.64 ± 0.30 $L \cdot min^{-1}$, post-training, 1.65 ± 0.33 $L \cdot min^{-1}$; θ_L HIT: week 9, 1.73 ± 0.47 $L \cdot min^{-1}$, post-training, 1.80 ± 0.47 $L \cdot min^{-1}$).

Table 3.1. Subjects' characteristics and resting hematocrit and hemoglobin values in older and young women.

	Age (yr)	Height (m)	Body Weight (kg)		Hct		Hb ($g \cdot dL^{-1}$)	
			Pre	Post	Pre	Post	Pre	Post
Older (n = 6)	69 (7)#	1.63 (0.03)	71.8 (5.5)	71.3 (5.8)	0.37 (0.03)	0.38 (0.02)	13.1 (0.9)	13.0 (0.8)
Young (n = 8)	25 (5)	1.66 (0.05)	65.6 (15.2)	65.4 (15.5)	0.40 (0.02)	0.40 (0.01)	13.6 (0.4)	13.8 (0.3)

Values are means ± SD. Hct, hematocrit; Hb, hemoglobin; # Significantly different from Y ($p < 0.05$).

Table 3.2. Maximal exercise responses for PO, VO_2, HR, Q, SV, a-vO_{2diff} and [Lac] in O and Y from pre-training through post-training.

		Pre-training	Week 3	Week 6	Week 9	Post-training
PO_{peak} (Watts)	O#	119 (15)	125 (18) *	129 (17) *†	135 (16) *†‡	140 (23) *†‡§
	Y	211 (26)	238 (33)[a]	248 (30)	256 (31)	265 (35)
VO_{2max} ($L \cdot min^{-1}$)	O#	1.73 (0.25)	1.77 (0.25) *	1.86 (0.20) *†	2.02 (0.28) *†‡	2.01 (0.29) *†‡
	Y	2.65 (0.34)	2.87 (0.34)[a]	3.03 (0.42)	3.11 (0.38)	3.22 (0.37)
VO_{2max} ($mL \cdot kg \cdot min^{-1}$)	O#	23.9 (2.1)	24.6 (2.3) *	26.1 (2.3) *†	28.2 (3.7) *†‡	28.3 (4.3) *†‡§
	Y	41.2 (4.7)	44.7 (5.8)[a]	46.9 (5.4)	48.3 (6.0)	50.6 (6.8)[d]
HR_{max} (bpm)	O#	150 (18)	150 (18)	154 (18)	153 (15)	155 (15)
	Y	184 (5)	183 (4)	181 (5)[b]	181 (8)	182 (6)
Q_{max} ($L \cdot min^{-1}$)	O#	15.8 (1.6)	16.8 (2.2)	15.6 (2.7)	15.6 (1.5) ‡	16.7 (1.6) *†‡
	Y	21.0 (3.0)	20.4 (2.6)	20.6 (2.6)	23.4 (2.9)[c]	23.2 (1.5)
SV_{max} ($mL \cdot beat^{-1}$)	O	106.2 (14.6)	112.6 (18.3)	102.3 (18.8)	102.9 (13.6) ‡	109.5 (16.6) *‡
	Y	114.3 (16.6)	112.0 (15.1)	114.0 (14.5)	130.1 (20.5)[c]	127.8 (9.5)
Maximal a-vO_{2diff} ($mLO_2 \cdot 100mL^{-1}$ blood)	O#	11.0 (1.8)	10.7 (1.4)	12.1 (1.5) *†	12.8 (0.9) *	12.0 (1.7) *
	Y	12.7 (1.1)	14.0 (0.9)[a]	14.7 (1.1)	13.3 (1.3)[c]	13.9 (1.6)[d]
[Lac] ($mmol \cdot L^{-1}$)	O#	7.9 (1.9)	9.1 (1.3)	7.9 (1.9)	7.0 (1.1)	7.7 (1.4)
	Y	10.8 (2.0)	12.1 (2.4)	13.8 (3.8)	12.0 (1.5)	13.1 (3.0)

Values are means ± SD. PO_{peak}, peak power output; VO_{2max}, maximal O_2 uptake; HR_{max}, maximal heart rate; Q_{max}, maximal cardiac output; SV_{max}, maximal stroke volume; Maximal a-vO_{2diff}, maximal a-vO_{2diff}; [Lac], Lactate concentration; # (in column 2) denotes a significant main effect for age ($p < 0.05$); Symbols (*†‡§) in the rows between O and Y for each testing time denote a significant testing-time main effect: * Different from Pre-training ($p < 0.05$); † Different from Week 3 ($p < 0.05$); ‡ Different from Week 6; § Different from Week 9 ($p < 0.05$); [a-d] symbols denote a significant testing-time by age interaction ($p < 0.05$): [a] Pre-training-week 3 interaction ($p < 0.05$); [b] Week 3-week 6 interaction ($p < 0.05$); [c] Week 6-week 9 interaction ($p < 0.05$); [d] Week 9-Post-training interaction ($p < 0.05$).

Table 3.3. Sub-maximal exercise responses for VO_2, HR, Q, SV and a-vO_{2diff} in O and Y from pre-training through post-training.

		Pre-training	Week 3	Week 6	Week 9	Post-training
VO_{2sub}	O#	1.18 (0.16)	1.14 (0.12)	1.14 (0.07)	1.20 (0.19)	1.25 (0.18)
	Y	1.50 (0.19)	1.47 (0.21)	1.46 (0.18)	1.42 (0.18)	1.46 (0.20)
HR_{sub} (bpm)	O	111 (25)	107 (19) *	103 (17) *	104 (16) *	104 (14) *
	Y	124 (8)	114 (10)	113 (9)	111 (8)	111 (8)
Q_{sub} ($L \cdot min^{-1}$)	O	10.7 (1.7)	10.5 (1.9)	10.5 (0.9)	10.6 (1.4)	10.9 (1.8)
	Y	12.2 (1.6)	12.0 (1.8)	11.8 (1.8)	11.8 (1.8)	11.9 (1.4)
SV_{sub} ($mL \cdot beat^{-1}$)	O	98.4 (17.4)	98.7 (14.4)	104.1 (12.2)	103.6 (13.9) *	106.7 (22.7) *
	Y	99.0 (13.3)	106.4 (21.4)	104.6 (18.0)	106.8 (17.2)	108.1 (15.4)
a-$vO_{2diffsub}$ ($mLO_2 \cdot 100mL^{-1}$ blood)	O#	11.1 (1.4)	11.5 (1.2)	10.9 (0.8)	11.3 (0.5)	11.5 (1.3)
	Y	12.3 (0.9)	12.3 (1.2)	12.5 (0.9)	12.1 (1.1)	12.3 (1.1)
θ_L ($L \cdot min^{-1}$)	O#	1.12 (0.15)	1.26 (0.15) *	1.28 (0.14) *	1.36 (0.17) *†‡	1.38 (0.17) *†‡§
	Y	1.57 (0.20)	1.71 (0.24)	1.83 (0.30)	1.93 (0.30)	1.99 (0.30)

Values are means ± SD. VO_{2sub}, submaximal VO_2; HR_{sub}, submaximal heart rate; Q_{sub}, submaximal cardiac output; SV_{sub}, submaximal stroke volume; a-$vO_{2diffsub}$, submaximal a-vO_{2diff}; θ_L, estimated lactate threshold; # (in column 2) denotes a significant main effect for age ($p < 0.05$); Symbols (*†‡§) in the rows between O and Y for each testing time denote a significant testing-time main effect: *

Significantly different from Pre-training values ($p < 0.05$); † Significantly different from Week 3 ($p < 0.05$); ‡ Significantly different from Week 6; § Significantly different from Week 9; # Significantly different from Y ($p < 0.05$).

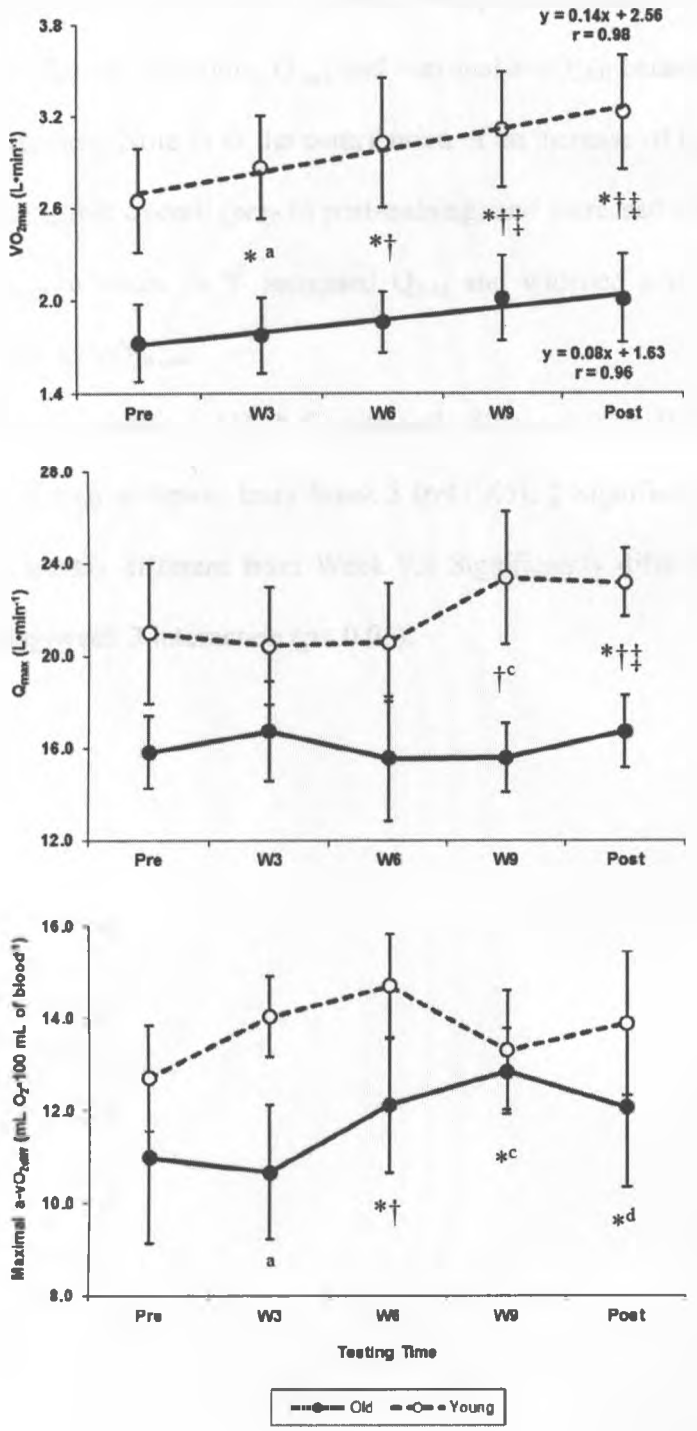

Figure 3.1. Changes in maximal VO_2, Q, and a-vO_{2diff} in response to training in O and Y.

Linear regression on the VO_{2max} versus time data depict a 140 $mL \cdot min^{-1}$ increase every 3 weeks in Y and 80 $mL \cdot min^{-1}$ increase every three weeks in O. Slopes were not calculated for the figures displaying Q_{max} and maximal a-vO_{2diff} because of the non-linear nature of the response. Note in O the contribution of an increase of Q_{max} at week 3 to the increase in VO_{2max},but overall (pre- to post-training) and increased a-vO_{2diff} in O with little change in Q_{max}, whereas in Y increased Q_{max} and widened a-vO_{2diff} both contributed to the increase in VO_{2max}.

Values are means ± SD. * Significantly different from Pre-training values ($p < 0.05$); † Significantly different from Week 3 ($p < 0.05$); ‡ Significantly different from Week 6; § Significantly different from Week 9;# Significantly different from Y ($p < 0.05$); [a] Pre-training-week 3 interaction ($p < 0.05$).

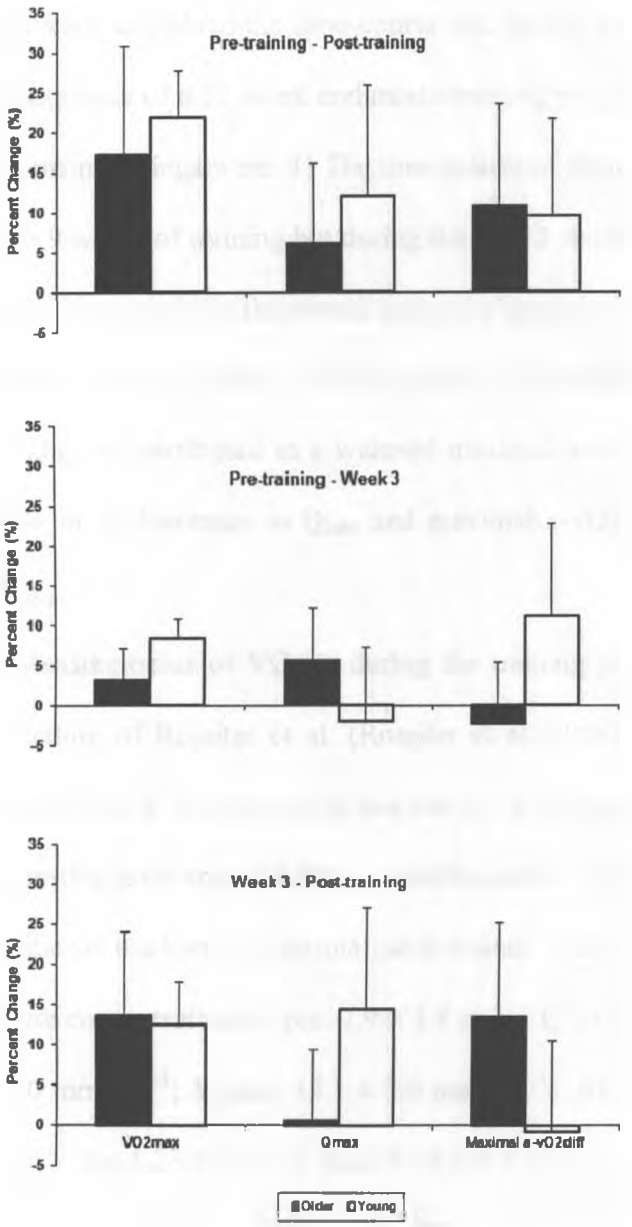

Figure 3.2. Percent changes in maximal VO_2, Q, and a-vO_{2diff} from pre-training to post-training, pre-training to week 3, and week 3 to post-training in O and Y adults.

Values are means ± SD.

DISCUSSION

This study examined the time-course and factors contributing to increases in VO_{2max} as a consequence of a 12-week endurance training program in older and young female adults. The main findings were: 1) The time-course of changes in VO_{2max} was similar in O and Y up to 9 weeks of training but during the final 3 weeks of training VO_{2max} increased only in the Y. Nevertheless, the overall percent change in VO_{2max} from pre- to post-training was similar in both groups; 2) The majority of the increase in VO_{2max} from pre- to post-training was attributed to a widened maximal a-vO_{2diff} in O (~2/3 of the improvement), while in Y, increases in Q_{max} and maximal a-vO_{2diff} contributed equally to the greater VO_{2max}.

Measurements of VO_{2max} during the training program were rigorous. Following the procedure of Rossiter et al. (Rossiter et al. 2006), we demonstrated no differences in VO_{2max} during the ramp test and the 2-3 min constant-load test to exhaustion, thereby suggesting attainment of VO_{2max}. Additionally, achievement of VO_{2max} was supported by traditional markers of maximal performance such as pre- and post-training end-exercise lactate concentration (O pre, 7.9 ± 1.9 mmol·L^{-1}; O post, 7.7 ± 1.4 mmol·L^{-1}; Y pre, 10.8 ± 2.0 mmol·L^{-1}; Y post, 13.1 ± 3.0 mmol·L^{-1}), RER (O pre 1.17 ± 0.07; O post, 1.18 ± 0.09; Y pre,1.23 ± 0.05; Y post, 1.19 ± 0.03) (data not reported in the results section) as well as a HR_{max} (~100% and ~95% of the estimated maximal HR for O and Y, respectively; Table 3.2).

The observation that VO_{2max} increased by ~17% from pre- to post-training in older women agrees with increases reported previously in response to short-term (8-12 weeks, 15%-17%) (Haykowsky et al. 2005; Perini et al. 2002; Takeshima et al. 2004) and long-term (6-12 months, 15%-23%) (Kohrt et al. 1991; Martin et al. 1990; Spina et al. 1996;

Spina et al. 1993a; Spina et al. 1993b) endurance training programs in older women. The present study emphasizes that older women can undertake and adapt to a relatively vigorous exercise program and that when training intensity is adjusted to accommodate increases in fitness, significant gains in VO_{2max} can be achieved in as early as 3 weeks and continue for up to 9 weeks training. Paterson and coworkers (Paterson et al. 2004; Paterson et al. 2007) demonstrated that a higher VO_{2max} in healthy independent older adults decreased the risk of becoming dependent by 14% per each $mL \cdot kg^{-1} \cdot min^{-1}$. Therefore, the ~4.5 $mL \cdot kg^{-1} \cdot min^{-1}$ training-induced increase in VO_{2max} in the present study may be expected to reduce the risk of becoming dependent by ~60%.

In the older women of the present study, no further changes in VO_{2max} were observed between 9 and 12 weeks of training despite the progressive increase in training intensity. Perhaps a longer duration training program was required in this older female group; however studies reporting on longer duration training programs (6-12 months) have not observed greater increases in VO_{2max} than those reported in this study.

Q_{max} was not significantly changed by 12 weeks of training in the older women. Thus, the majority of the increase in VO_{2max} observed in this group was not explained by central adaptations. Previous reports also failed to show improvements in left ventricular dimensions or diastolic filling dynamics (Haykowsky et al. 2005; Spina et al. 1996) in older women. More specifically, Spina et al. (Spina et al. 1993a) showed that older women increased their VO_{2max} with long-term training with no central adaptations; in the present study a similar magnitude of increase in VO_{2max} with shorter-term training was also attributable to a widened maximal a-vO_{2diff} with minimal change in Q_{max} and maximal SV, whereas in young women on the same training program the increased VO_{2max} was equally explained by a larger Q_{max} and widened maximal a-vO_{2diff}.

Additionally, in older men (undertaking the same exercise training program and using similar measurement techniques) (Murias et al. 2010) a large increase in VO_{2max} was explained mainly by an increased Q_{max} but also by a widened maximal a-vO_{2diff}. Thus, the present data confirm in a short-term training study the limited central adaptation previously reported with long-term endurance training in older women in spite of a substantial increase in VO_{2max}. Thus, the majority of the training-induced increase in VO_{2max} in the older women was explained by a widened a-vO_{2diff}. This is consistent with other studies showing a dependence on peripheral adaptations (Spina et al. 1993a; Spina et al. 1993b) explained by training-related increases in muscle capillarization and oxidative enzymes in older women (Coggan et al. 1992).

Although increases in VO_{2max} in older women relied on a larger maximal a-vO_{2diff}, the absolute level of a-vO_{2diff} extraction in the older women was significantly lower than that observed in young women, and, also, significantly lower than that reported previously for older and young men (Murias et al. 2010). One reason for this could be a lower delivery of O_2 in older women. Assuming an O_2 carrying capacity of 1.34 mL of O_2 per gram of Hb, the total arterial O_2 content of the older women would be 17.4 $mLO_2 \cdot 100mL^{-1}$ blood whereas the young women would have an O_2 content of 18.5 $mLO_2 \cdot 100mL^{-1}$ blood. The second explanation is a lesser O_2 extraction; older women had not only the lowest absolute a-vO_{2diff} but their relative O_2 extraction at maximal exercise was also smaller (69%) compared to young women (75%) (or compared to older men (74%) and young men (76%) from our previous study) (Murias et al. 2010). One reason for this could be a diminished oxidative capacity. In this regard, Conley et al. (Conley et al. 2000) have shown that losses of both mitochondrial content and function occur in older adults (both women and men). Another reason for older women having a reduced maximal a-vO_{2diff}

could be related to limitations to oxygen transport to and within the active muscles. It has been shown that older women have a reduced leg vascular conductance that is partly responsible for a lower leg blood flow during sub-maximal and maximal exercise (Parker et al. 2008; Proctor et al. 2004). Also, endurance training was shown to produce large increases in peak leg blood flow and vascular conductance in older men, whereas in older women blood flow increases were much more variable and not always affected by training (Martin et al. 1990). Thus, although increases in VO_{2max} in the older women relied upon a widened a-vO_{2diff}, this adaptation with training may be limited as both the arterial O_2 delivery and the O_2 extraction are lower than in young women (or in older or young men).

Taken together, these data suggest that the adaptation of VO_{2max} in older women may be limited not only by lack of central adaptation but also by peripheral factors such that a ceiling effect occurs. Thus, a plateau-like response in the increase of VO_{2max} after 9 weeks of training should not be surprising, as the maximal potential for widening of a-vO_{2diff} may have been obtained by 9 weeks. Our data showed that during the first three weeks of the training program a small but significant increase in VO_{2max} in the older group was supported by a non-statistically significant increase in Q_{max} that may represent an early adaptation related to a decrease in afterload. Additionally, we showed a central adaptation in older women with HR_{sub} being reduced after 3 weeks of training which resulted in a larger SV_{sub}. This supports the idea that central adaptations may play a role in the increase in VO_{2max} early in training in older women. In young women, the total increase in VO_{2max} during the first 3 weeks of training was attributed to an increase in maximal a-vO_{2diff} as previously reported in young men (Murias et al. 2010). Although it is unclear why this occurred, it is plausible that an improved distribution of cardiac output

due to adaptations in the periphery may have resulted in better distribution and utilization of the available O_2. In this regard, other endurance training studies in young men have found increases in muscle capillarization (Andersen and Henriksson 1977; Denis et al. 1986) and in oxidative enzymes (Coggan et al. 1993; Gibala et al. 2006; Henriksson and Reitman 1977; Svedenhag et al. 1983) occurring within the first 2-10 weeks of training. Further increases in VO_{2max} in young women were the result of a larger Q_{max} without changes in maximal a-vO_{2diff}. Considering that HR_{max} remained unchanged in response to the training program, the improvements in Q_{max} were explained solely by a greater SV_{max}. These data are in agreement with previous reports showing increments in Q_{max} and SV_{max} in young women in response to endurance training (Cunningham and Hill 1975; Spina et al. 1992a; Spina et al. 1992b).

Another finding from this study is that in both older and young women the absolute θ_L increased after 3 weeks of training with further increases seen at week 6, and post-training. Increases in VO_2 at the θ_L have been shown previously for both older (Poulin et al. 1992; Takeshima et al. 1996) and young individuals (Davis et al. 1979; Ready and Quinney 1982). This training adaptation is particularly meaningful in older women because for this population certain activities of daily living may be performed above θ_L (particularly when VO_{2max} is low) which could result in disturbance of cellular homeostasis and premature fatigue. A VO_2 increase of ~260 $mL \cdot min^{-1}$ at θ_L in older women after only 12 weeks of training is of functional relevance, since a greater θ_L will help older individuals remain living independently.

In the present study, we speculated that a HIT intervention during the final weeks of the training program would allow VO_{2max} to further increase. However, we found that the type of training (HIT or CT) did not affect the response to maximal exercise. Although

the HIT intervention was not powered to observe any differential effects in older versus young women, these results suggest that: a) after 10 weeks of training, continuous training at an intensity of 70% of VO_{2max} is still a sufficient stimulus to produce increments in VO_{2max} in young women, at least when training intensities are adjusted (every 3 weeks) to reflect changes in aerobic performance; b) older women may have limitations to further increase their VO_{2max} after 9 weeks of training regardless of the training type, at least in response to a short-term training intervention. This does not preclude that longer training durations may be required for older women to further increase their VO_{2max} as seen in young women. Finally, an interaction reflecting a higher θ_L in HIT compared to CT was observed during sub-maximal intensity measurements, which could be interpreted as a positive effect of the HIT intervention.

Limitations of the study: A limitation of this study is the low number of subjects. Nevertheless, it is important to note that the statistical power of our two-way repeated measures ANOVA was always larger than 0.8 for the "main effects" and "interaction effects" of each variable.

Another limitation could be related to the fact that that our measures of a-vO_{2diff} are derived from measures of Q and VO_2. Although the acetylene open circuit inert gas washin technique has been shown to be a valid method for measuring Q (Johnson et al. 2000), the reader should be aware that this is an indirect methodology for estimating Q.

In conclusion, this study demonstrated the effectiveness of a short-term (12 weeks) exercise training programs with vigorous intensity (~70% VO_{2max}) and regular progression. Although older and young women had a similar time-course of adaptations in VO_{2max} during the first 9 weeks of the endurance training program, only young women showed a continued increase in VO_{2max} after that time point, with older women displaying

a plateau response. The majority of the improvement in VO_{2max} in older women and almost half of the change in VO_{2max} in young women were attributed to a widened a-vO_{2diff}. Given the reliance of peripheral adaptations in older women in improving VO_{2max}, training programs in this population should be specific to those muscle groups needed in daily aerobic activities (i.e., legs: walking, stairs climbing) thereby maintaining function and independence in older women. Despite their reliance on a widened a-vO_{2diff}, older women displayed a restricted O_2 extraction percentage that could be responsible for the lack of further increase in VO_{2max} during the last two weeks of training.

REFERENCES

Andersen P, Henriksson J (1977) Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. J Physiol 270: 677-690

Babcock MA, Paterson DH, Cunningham DA (1994) Effects of aerobic endurance training on gas exchange kinetics of older men. Med Sci Sports Exerc 26: 447-452

Beaver WL, Lamarra N, Wasserman K (1981) Breath-by-breath measurement of true alveolar gas exchange. J Appl Physiol 51: 1662-1675

Coggan AR, Spina RJ, King DS, Rogers MA, Brown M, Nemeth PM, Holloszy JO (1992) Skeletal muscle adaptations to endurance training in 60- to 70-yr-old men and women. J Appl Physiol 72: 1780-1786

Coggan AR, Spina RJ, Kohrt WM, Holloszy JO (1993) Effect of prolonged exercise on muscle citrate concentration before and after endurance training in men. Am J Physiol 264: E215-220

Conley KE, Esselman PC, Jubrias SA, Cress ME, Inglin B, Mogadam C, Schoene RB (2000) Ageing, muscle properties and maximal O_2 uptake rate in humans. J Physiol 526 Pt 1: 211-217

Coyle EF (1995) Integration of the physiological factors determining endurance performance ability. Exerc Sport Sci Rev 23: 25-63

Cunningham DA, Hill JS (1975) Effect of training on cardiovascular response to exercise in women. J Appl Physiol 39: 891-895

Cunningham DA, McCrimmon D, Vlach LF (1979) Cardiovascular response to interval and continuous training in women. Eur J Appl Physiol Occup Physiol 41: 187-197

Davis JA, Frank MH, Whipp BJ, Wasserman K (1979) Anaerobic threshold alterations caused by endurance training in middle-aged men. J Appl Physiol 46: 1039-1046

Denis C, Chatard JC, Dormois D, Linossier MT, Geyssant A, Lacour JR (1986) Effects of endurance training on capillary supply of human skeletal muscle on two age groups (20 and 60 years). J Physiol (Paris) 81: 379-383

Fitzgerald MD, Tanaka H, Tran ZV, Seals DR (1997) Age-related declines in maximal aerobic capacity in regularly exercising vs. sedentary women: a meta-analysis. J Appl Physiol 83: 160-165

Gibala MJ, Little JP, van Essen M, Wilkin GP, Burgomaster KA, Safdar A, Raha S, Tarnopolsky MA (2006) Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. J Physiol 575: 901-911

Haykowsky M, McGavock J, Vonder Muhll I, Koller M, Mandic S, Welsh R, Taylor D (2005) Effect of exercise training on peak aerobic power, left ventricular morphology, and muscle strength in healthy older women. J Gerontol A Biol Sci Med Sci 60: 307-311

Henriksson J, Reitman JS (1977) Time course of changes in human skeletal muscle succinate dehydrogenase and cytochrome oxidase activities and maximal oxygen uptake with physical activity and inactivity. Acta Physiol Scand 99: 91-97

Hollenberg M, Yang J, Haight TJ, Tager IB (2006) Longitudinal changes in aerobic capacity: implications for concepts of aging. J Gerontol A Biol Sci Med Sci 61: 851-858

Johnson BD, Beck KC, Proctor DN, Miller J, Dietz NM, Joyner MJ (2000) Cardiac output during exercise by the open circuit acetylene washin method: comparison with direct Fick. J Appl Physiol 88: 1650-1658

Kohrt WM, Malley MT, Coggan AR, Spina RJ, Ogawa T, Ehsani AA, Bourey RE, Martin WH, 3rd, Holloszy JO (1991) Effects of gender, age, and fitness level on response of VO_{2max} to training in 60-71 yr olds. J Appl Physiol 71: 2004-2011

Martin WH, 3rd, Kohrt WM, Malley MT, Korte E, Stoltz S (1990) Exercise training enhances leg vasodilatory capacity of 65-yr-old men and women. J Appl Physiol 69: 1804-1809

Murias JM, Kowalchuk JM, Paterson DH (2010) Time-course and mechanisms of adaptations in cardiorespiratory fitness with endurance training in older and young men. J Appl Physiol (In Press)

O'Donovan G, Owen A, Bird SR, Kearney EM, Nevill AM, Jones DW, Woolf-May K (2005) Changes in cardiorespiratory fitness and coronary heart disease risk factors following 24 wk of moderate- or high-intensity exercise of equal energy cost. J Appl Physiol 98: 1619-1625

Parker BA, Smithmyer SL, Pelberg JA, Mishkin AD, Proctor DN (2008) Sex-specific influence of aging on exercising leg blood flow. J Appl Physiol 104: 655-664

Paterson DH, Govindasamy D, Vidmar M, Cunningham DA, Koval JJ (2004) Longitudinal study of determinants of dependence in an elderly population. J Am Geriatr Soc 52: 1632-1638

Paterson DH, Jones GR, Rice CL (2007) Ageing and physical activity: evidence to develop exercise recommendations for older adults. Can J Public Health 98 Suppl 2: S69-108

Pedersen PK, Jorgensen K (1978) Maximal oxygen uptake in young women with training, inactivity, and retraining. Med Sci Sports 10: 233-237

Perini R, Fisher N, Veicsteinas A, Pendergast DR (2002) Aerobic training and cardiovascular responses at rest and during exercise in older men and women. Med Sci Sports Exerc 34: 700-708

Poulin MJ, Paterson DH, Govindasamy D, Cunningham DA (1992) Endurance training of older men: responses to submaximal exercise. J Appl Physiol 73: 452-457

Proctor DN, Koch DW, Newcomer SC, Le KU, Smithmyer SL, Leuenberger UA (2004) Leg blood flow and VO_2 during peak cycle exercise in younger and older women. Med Sci Sports Exerc 36: 623-631

Ready AE, Quinney HA (1982) Alterations in anaerobic threshold as the result of endurance training and detraining. Med Sci Sports Exerc 14: 292-296

Rossiter HB, Kowalchuk JM, Whipp BJ (2006) A test to establish maximum O_2 uptake despite no plateau in the O_2 uptake response to ramp incremental exercise. J Appl Physiol 100: 764-770

Seals DR, Hagberg JM, Hurley BF, Ehsani AA, Holloszy JO (1984) Endurance training in older men and women. I. Cardiovascular responses to exercise. J Appl Physiol 57: 1024-1029

Spina RJ, Miller TR, Bogenhagen WH, Schechtman KB, Ehsani AA (1996) Gender-related differences in left ventricular filling dynamics in older subjects after endurance exercise training. J Gerontol A Biol Sci Med Sci 51: B232-237

Spina RJ, Ogawa T, Coggan AR, Holloszy JO, Ehsani AA (1992a) Exercise training improves left ventricular contractile response to beta-adrenergic agonist. J Appl Physiol 72: 307-311

Spina RJ, Ogawa T, Kohrt WM, Martin WH, 3rd, Holloszy JO, Ehsani AA (1993a) Differences in cardiovascular adaptations to endurance exercise training between older men and women. J Appl Physiol 75: 849-855

Spina RJ, Ogawa T, Martin WH, 3rd, Coggan AR, Holloszy JO, Ehsani AA (1992b) Exercise training prevents decline in stroke volume during exercise in young healthy subjects. J Appl Physiol 72: 2458-2462

Spina RJ, Ogawa T, Miller TR, Kohrt WM, Ehsani AA (1993b) Effect of exercise training on left ventricular performance in older women free of cardiopulmonary disease. Am J Cardiol 71: 99-104

Stathokostas L, Jacob-Johnson S, Petrella RJ, Paterson DH (2004) Longitudinal changes in aerobic power in older men and women. J Appl Physiol 97: 781-789

Svedenhag J, Henriksson J, Sylven C (1983) Dissociation of training effects on skeletal muscle mitochondrial enzymes and myoglobin in man. Acta Physiol Scand 117: 213-218

Takeshima N, Kobayashi F, Watanabe T, Tanaka K, Tomita M, Pollock ML (1996) Cardiorespiratory responses to cycling exercise in trained and untrained healthy elderly: with special reference to the lactate threshold. Appl Human Sci 15: 267-273

Takeshima N, Rogers ME, Islam MM, Yamauchi T, Watanabe E, Okada A (2004) Effect of concurrent aerobic and resistance circuit exercise training on fitness in older adults. Eur J Appl Physiol 93: 173-182

Tanaka H, Desouza CA, Jones PP, Stevenson ET, Davy KP, Seals DR (1997) Greater rate of decline in maximal aerobic capacity with age in physically active vs. sedentary healthy women. J Appl Physiol 83: 1947-1953

CHAPTER IV: Speeding of VO_2 kinetics with endurance training in old and young men is associated with improved matching of local O_2 delivery to muscle O_2 utilization

INTRODUCTION

The rate of adjustment of the primary component (phase II) of pulmonary VO_2 (VO_{2p}) closely reflects the adjustments of oxidative metabolism at the skeletal muscle level (Grassi et al. 1996; McCreary et al. 1996; Rossiter et al. 1999). The phase II VO_{2p} kinetics during the on-transient of moderate-intensity exercise is slower in older compared to younger adults (Babcock et al. 1994b; Bell et al. 1999; Chilibeck et al. 1996; DeLorey et al. 2005). A slower adjustment of oxidative metabolism results in a larger O_2 deficit and greater reliance on substrate-level phosphorylation to provide ATP in sufficient amounts to sustain any given activity. As such, older adults may experience premature fatigue and reduced tolerance to exercise (DeLorey et al. 2007).

In some conditions (e.g., hypoxia, β-adrenergic receptor blockade, reduced arterial perfusion) and subject groups (e.g., those with chronic heart failure, peripheral vascular disease, diabetes) it has been suggested that O_2 delivery may constrain VO_2 kinetics (Hughson et al. 2001; Poole et al. 2008; Tschakovsky and Hughson 1999). Although bulk delivery of O_2 to the exercising limb does not seem to limit the rate of adjustment of VO_2 kinetics (Bell et al. 2001), the local distribution of blood flow within the active muscles appears to be critical in the matching of O_2 delivery to O_2 utilization during the kinetic phase of adjustment to the increased energy demand with exercise (duManoir et al. 2010). Near-infrared spectroscopy (NIRS) data in young adults have shown that the rate of deoxygenation is very rapid (DeLorey et al. 2003) and suggest that the rate of increase in microvascular blood flow in the active tissues is slow compared to the increase in muscle

O_2 utilization (VO_{2m}) (DeLorey et al. 2003; Harper et al. 2006). An age-related reduction in local (microvascular) blood flow is reflected by a greater ratio of change in deoxygenated hemoglobin to the change in VO_{2p} ($\Delta[HHb]/\Delta VO_{2p}$) in older compared to younger men (DeLorey et al. 2004) as well as a transiently greater fall in microvascular PO_2 (PO_{2mv}) in older compared to young rats (Behnke et al. 2005). Thus older adults rely more on O_2 extraction during the on-transient of exercise probably because of a lower local (microvascular) blood flow-to-VO_{2m} ratio.

Endurance training results in faster VO_{2p} kinetics in both older (Babcock et al. 1994a; Bell et al. 2001) and younger (Berger et al. 2006; Fukuoka et al. 2002; McKay et al. 2009; Phillips et al. 1995) individuals. However, the mechanisms underlying that faster response have not been clearly elucidated nor has the time-course of adaptation of VO_{2p} been established in older adults. Information on the time-course of the training-induced speeding of VO_2 kinetics may shed light on factors limiting the adjustment of VO_{2m} and how and whether their contributions are influenced by aging and training status. As such, the main goal of this study was to determine the time-course and mechanism of adaptation for phase II VO_{2p} in older and younger men, throughout a 12-week endurance training program. We hypothesized that there would be an improved microvascular O_2 delivery in the exercise transient in response to the endurance training that would be associated with a faster adjustment of VO_{2p} kinetics observed early in training in both older and young adults. Improvements in microvascular O_2 delivery would be indicated by a better matching between the rate of adjustment of muscle deoxygenation relative to phase II VO_{2p} (i.e. the $\Delta[HHb]/\Delta VO_{2p}$ ratio), which represents a decreased reliance on O_2 extraction for a given VO_{2p}.

METHODS

Subjects: Eight older (O) (68 ± 7 yr; mean ± SD) and 8 young (23 ± 5 yr) adult men volunteered and gave written consent to participate in the study. Descriptive and baseline data from these subjects were given in a previous report looking at central and peripheral adaptations to endurance training in the same men (Murias et al. (2010); the reader is referred to this paper for further information on increases in maximal VO_{2p}, cardiac output and arterial-venous O_2 difference). All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All subjects were non-obese (body mass index $\leq$ 30 kg/m^2), non-smokers, and were physically active but none had been involved in any type of endurance training program for at least 12 months prior to the study. Additionally, no subjects were taking medications that would affect the cardiorespiratory or hemodynamic responses to exercise. Subjects had no history of cardiovascular, respiratory or musculoskeletal diseases, and older subjects were medically screened by a physician and underwent a maximal exercise stress test prior to participation.

Protocol: Before training began, subjects reported to the laboratory on two separate occasions. On day one, a maximal cycle ergometer ramp test (O 15-20 W/min; Y 25 W/min) was performed (Lode Corival 400; Lode B.V., Groningen, Holland) for determination of peak VO_2 (VO_{2peak}) and the estimated lactate threshold (θ_L). θ_L was defined as the VO_2 at which CO_2 production (VCO_2) began to increase out of proportion in relation to VO_2 with a systematic rise in minute ventilation-to-VO_2 ratio and end-tidal PO_2 whereas minute ventilation-to-VCO_2 ratio and end-tidal PCO_2 were stable. After this test, subjects returned to the laboratory on a different day to perform step transitions in work rate (WR) from 20 W to a moderate-intensity WR that elicited a VO_2 corresponding

to 90% θ_L. Each subject performed two sets, each including two step transitions consisting of 6 min of pedalling at 20 W, 6 min at 90% θ_L, 8 min at 20 W, and another 6 min at 90% θ_L. Each set was separated by a 30 min period of rest sitting on a chair. Identical procedures were repeated after weeks 3, 6, 9, and 12 of training. However, since the step-transitions were performed at the same absolute intensity as the initial tests, the order for the ramp test and the step-transitions was assigned randomly. At least 24 h were allowed between the ramp-test and the step transitions.

Training: The endurance training program consisted of 3 exercise sessions per week on a stationary cycle-ergometer (Monark Ergomedic 874E; Monark Exercise AB, Varberg, Sweeden) for a total duration of 12 weeks. Training intensity was adjusted at 3 week intervals to reflect changes in fitness level. During the first 10 weeks, each session consisted of continuous training (CT) for 45 min at a power output that elicited ~70% of the VO_{2peak} observed during the incremental ramp test. During the final 2 weeks of training (6 exercise sessions), each individual in each group (O and Y) was randomly assigned (stratified by age) to one of two sub-groups: a) CT as described above; b) high-intensity interval training (HIT), performing 10-12 exercise bouts each lasting 1-min at 90-100% of the peak power output achieved during the incremental ramp test, with 1-min rest separating bouts. Since VO_{2peak} was likely to plateau after approximately 8 weeks of CT (O'Donovan et al. 2005), and this study was also designed to look at changes in VO_{2peak} with training (see (Murias et al. 2010); data not further considered in the present study), the HIT was used as a strategy for progressive and continued gains in the exercise program resulting in further increases in VO_{2peak} favoured by peripheral adaptations (Coyle 1995).

Measurements: Gas-exchange measurements were similar to those previously described (Babcock et al. 1994b). Briefly, inspired and expired flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies VMM 110) which was calibrated before each test by using a syringe of known volume. Inspired and expired gases were sampled continuously (every 20 ms) at the mouth and analyzed for concentrations of O_2, CO_2, and N_2 by mass spectrometry (Perkin Elmer MGA-1100) after calibration with precision-analyzed gas mixtures. Changes in gas concentrations were aligned with gas volumes by measuring the time delay for a square-wave bolus of gas passing the turbine to the resulting changes in fractional gas concentrations as measured by the mass spectrometer. Data collected every 20 ms were transferred to a computer, which aligned concentrations with volume information to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver et al. (Beaver et al. 1981).

Heart rate (HR) was continuously monitored by electrocardiogram using PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO) with a three-lead arrangement. Data were recorded using LabChart v4.2 (ADInstruments, Colorado Springs, CO) on a separate computer.

Local muscle oxygenation profiles of the quadriceps vastus lateralis muscle were made with NIRS (Hamamatsu NIRO 300, Hamamatsu Photonics, Hamamatsu, Japan). Optodes were placed on the belly of the muscle midway between the lateral epicondyle and greater trochanter of the femur. The optodes were housed in an optically dense plastic holder and secured on the skin surface with tape and then covered with an optically dense, black vinyl sheet, thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to minimize movement of the optodes.

The physical principles of tissue spectroscopy are described in detail by Elwell (Elwell 1995) and the manner in which these are applied have been explained by DeLorey et al. (DeLorey et al. 2003). Briefly, one fiber optic bundle carried the NIR-light produced by the laser diodes to the tissue of interest while a second fiber optic bundle returned the transmitted light from the tissue to a photon detector (photomultiplier tube) in the spectrometer. Four different wavelength laser diodes (775, 810, 850, and 910 nm) provided the light source. The diodes were pulsed in a rapid succession and the light was detected by the photomultiplier tube for online estimation and display of the concentration changes from the resting baseline oxy-hemoglobin (HbO_2), deoxy-hemoglobin (HHb), and total hemoglobin (Hb_{tot}). In this study, we used an interoptode spacing of 5 cm. Given the uncertainty of the optical path length in the vastus lateralis at rest and during exercise, NIRS data are presented as delta (Δ) arbitrary units (a.u.). NIRS-derived signal was zero set prior to the onset of exercise while subjects were quietly seated on the cycle ergometer. The raw attenuation signals (in optical density units) were transferred to a computer for later analysis. Changes in light intensities were recorded continuously at 2 Hz.

Data analysis: VO_2 data were filtered by removing aberrant data points that lay outside 4 SD of the local mean. The data for each transition then were linearly interpolated to 1 s intervals and time-aligned such that time zero represented the onset of exercise. Data from each transition were ensemble-averaged to yield a single, averaged response for each subject. This transition was further time-averaged into 10 s bins to provide a single time-averaged response for each subject. The on-transient response for VO_2 was fitted using a mono-exponential model of the form:

$$Y_{(t)} = Y_{Bsln} + Amp\ (1 - e^{-(t-TD)/\tau}), \text{ equation (1)}$$

where $Y_{(t)}$ represents VO_2 at any time (t); Y_{Bsln} is the baseline VO_2 during 20 W cycling; Amp is the steady-state increase in VO_2 above the baseline value; τ is the time-constant defined as the duration of time for VO_2 to increase to 63% of the steady-state increase; and TD is the time delay (such that the model is not constrained to pass through the origin). The phase I-phase II transition was constrained to a constant of 25 s. Data were modeled from the beginning of phase II to 4 min (240 s) of the step-transition. The model parameters were estimated by least-squares nonlinear regression (Origin, OriginLab Corp., Northampton, MA, USA) in which the best fit was defined by minimization of the residual sum of squares and minimal variation of residuals around the Y-axis (Y = 0). The 95% confidence interval (CI) for the estimated time constant was determined after preliminary fit of the data with Bsln, Amp, and TD constrained to the best-fit values and the τ allowed to vary.

Heart rate data were determined from the R-R interval on a second-by-second basis and edited and modeled in the same manner as the VO_2 data described above. The on-transient HR response was modeled from the onset of exercise to 240 s using the exponential model described in equation (1).

The NIRS-derived Δ[HHb] data were time aligned and ensemble averaged to 5-s bins to yield a single response for each subject. The Δ[HHb] profile has been described to consist of a time delay at the onset of exercise, followed by an increase in the signal with an "exponential-like" time-course (DeLorey et al. 2003). The time delay for the Δ[HHb] response (TD-Δ[HHb]) was determined using second-by-second data and corresponded to the time between the onset of exercise and the first point at which the Δ[HHb] signal started to systematically increase. Determination of the TD-Δ[HHb] was made on individual trials and averaged to yield a single value for each individual. The Δ[HHb]

data were modeled from the end of the TD-Δ[HHb] to 90 s of the transition using an exponential model as described in equation 1. The τ[HHb] described the time course for the increase in Δ[HHb], while the overall time course of Δ[HHb] from the onset of exercise was described by the effective Δ[HHb] (τ'Δ[HHb] = TD-Δ[HHb] + τΔ[HHb]).

The second-by-second Δ[HHb] and VO_{2p} data were normalized for each subject (0-100% of the response). The normalized VO_{2p} was left shifted by 20s to account for the phase I-phase II transition so that the onset of exercise coincided with the beginning of phase II VO_{2p}, which has been previously described to coincide with muscle VO_2 within 10% (Rossiter et al. 1999). Data were further averaged into 5s bins for statistical comparison of the rate of adjustment for Δ[HHb] and ΔVO_{2p}. Additionally, an overall $\Delta[HHb]/\Delta VO_{2p}$ ratio for the adjustment during the exercise on-transient was derived for each individual as the average value from 20-150 s into the transition. The start point was selected to be 20 s to begin beyond the physiological TD-Δ[HHb] derived from NIRS. An end point of 150 s was selected to ensure that both the Δ[HHb] and ΔVO_{2p} signals had already reached 100% of their amplitudes.

Statistics: Data are presented as means ± SD. Paired and unpaired t-tests and repeated measures analysis of variance (ANOVA) were used to determine statistical significance for the dependent variables. The ANOVA model was described as $S_{16} \times T_5 \times A_2$ such that subjects (S; number of subjects) are crossed with testing time (T; five testing times: pre-training, Week 3, Week 6, Week 9, and post-training) and age (A; older and young adults). A Tukey post-hoc analysis was used when significant differences were found for the main effects of each dependent variable. Pearson product moment correlation coefficients were used to determine the degree of association between key variables. The

ANOVA and correlation coefficients were analyzed by SPSS Version 15.0, (SPSS Inc., Chicago, IL). Statistical significance was declared when $p < 0.05$.

RESULTS

Subject characteristics and pre-training peak exercise values are listed in Table 4.1. Compliance with the training program was 94 ± 1 % (28/30 training sessions) and 95 ± 1 % (29/30 training sessions) in O and Y, respectively. VO_{2peak} was significantly increased by 3 weeks in both O (by 10 ± 9 %) and Y (by 12 ± 6 %). Further training resulted in a total improvement from pre- to post-training that represented 31 ± 10 % in O and 18 ± 10 % in Y. As noted in "Methods", groups were split after the 10th week of training; however, since training type (i.e., continuous vs. interval) did not affect any of the kinetic parameters, data will not be presented separately.

VO_2 kinetics: O had a greater phase II VO_2 time constant (τVO_{2p}) compared with Y. Pre-training τVO_{2p} was 43 ± 11 s in O and 34 ± 8 s in Y. The τVO_{2p} decreased significantly by 3 weeks training in both O (35 ± 9 s) and Y (22 ± 8 s) with no further changes seen with continued training (Figure 4.1, Table 4.2). After 3 weeks of training, τVO_{2p} in O was similar to that observed in Y pre-training. No testing time by age interactions were detected reflecting a similar rate of adaptation of VO_{2p} kinetics in both O and Y and a maintained difference between age groups across time.

The amplitude of the increase in VO_{2p} across all testing times was lower in O (0.55 ± 0.29 $L \cdot min^{-1}$) compared with Y (1.23 ± 0.20 $L \cdot min^{-1}$) reflecting the lower WR in the O (i.e., O: 68 ± 15 W; Y: 128 ± 28 W). No changes in the functional VO_{2p} gain were observed with training (with a mean overall $\Delta VO_{2p}/\Delta WR$ in O, 11.4 ± 1.3 $mL \cdot min^{-1} \cdot W^{-1}$, and in Y, 11.5 ± 0.9 $mL \cdot min^{-1} \cdot W^{-1}$).

HR kinetics: The τHR was greater in O compared with Y. After 3 weeks of training τHR decreased in O and in Y and it remained decreased compared with pre-training for the remainder of the study (Table 4.2). In O, τHR was not different from τVO_{2p} at any testing time. In Y, the only significant difference was a greater τHR compared to τVO_{2p} at week 6.

Δ[HHb] kinetics: The amplitude of the increase in Δ[HHb] (O: 10 ± 5 a.u.; Y: 13 ± 6 a.u.) as well as τΔ[HHb] (O: 13 ± 6 s; Y: 11 ± 2 s) were similar in both groups. The overall time course of Δ[HHb], as reflected by the τ'Δ[HHb] was longer ($p< 0.05$) in O (21 ± 7 s) compared with Y (17 ± 3 s). No changes in response to training were observed for TD-Δ[HHb], τΔ[HHb], or τ'Δ[HHb] in either O or Y (Table 4.2).

Pre-training, τ'Δ[HHb] adjustment was shorter than τVO_{2p} ($p< 0.05$) in both O and Y (Table 4.2, Figure 4.2 Panel 1 A and Figure 4.3 Panel 1 A), which resulted in the calculated $\Delta[HHb]/\Delta VO_{2p}$ ratio displaying a transient "overshoot" during the exercise on-transient relative to the subsequent steady state level (Figure 4.2 Panel 2 A and Figure 4.3 Panel 2 A). After 3 weeks training in Y, the τ'Δ[HHb] and τVO_{2p} were similar (Table 4.2), and the $\Delta[HHb]/\Delta VO_{2p}$ "overshoot" was attenuated (Figure 4.3 Panels 1 and 2 B). With further training, the "overshoot" was eliminated in Y (Figure 4.3 Panels 1 and 2 C, D, E). In O, after 3 weeks of training the $\Delta[HHb]/\Delta VO_{2p}$ "overshoot" (Figure 4.2 Panel 2 B) also was attenuated, however in this group, the τ'Δ[HHb] remained shorter ($p< 0.05$) than the τVO_{2p} (Table 4.2). No further attenuations in the $\Delta[HHb]/\Delta VO_{2p}$ "overshoot" were observed with continued training in O (Figure 4.2 Panel 2 C, D, E). The reductions in τVO_{2p} in both O and Y with training were closely associated with a lowered $\Delta[HHb]/\Delta VO_{2p}$ ratio during the exercise on-transient (r: O = 0.93, $p< 0.05$; Y = 0.98, $p< 0.05$; Figure 4.4).

Table 4.1. Subject characteristics and peak exercise responses.

	n	Age (yr)	Height (cm)	Body mass (kg)	Peak WR (W)	Peak HR (bpm)	VO_{2peak} ($L \cdot min^{-1}$)	VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$)
Old	8	68±7	177±9	82±8	188±44	144±22	2.3±0.5	28±7
Young	8	23±5*	178±5	80±8	314±41*	189±7*	3.8±0.5*	48±6*

Values are means ± SD; n, no. of subjects; WR, work rate; HR, heart rate; VO_{2peak}, peak oxygen uptake. * $p < 0.05$ compared to old.

Table 4.2. Kinetics parameters for VO_2, HR, and Δ[HHb] in O and Y from pre-training through post-training.

		Pre-training	Week 3	Week 6	Week 9	Post-training
Phase II τVO_{2p} (s)	O#	43 ± 10	35 ± 9*	34 ± 8*	33 ± 8*	32 ± 7*
	Y	34 ± 8	22 ± 8*	19 ± 6*	20 ± 7*	19 ± 7*
τHR (s)	O#	49 ± 15	35 ± 10*	38 ± 10*	31 ± 10*	31 ± 11*†
	Y	45 ± 14	28 ± 10*	27 ± 7*‡	29 ± 13*	26 ± 7*†
τΔ[HHb] (s)	O	13 ± 11	15 ± 7	11 ± 4	13 ± 4	12 ± 4
	Y	11 ± 4	12 ± 2	10 ± 2	11 ± 2	10 ± 1
TD-Δ[HHb] (s)	O#	7 ± 3	9 ± 2	8 ± 2	8 ± 1	9 ± 1
	Y	6 ± 1	6 ± 1	6 ± 1	6 ± 2	7 ± 1
τ'Δ[HHb] (s)	O#	20 ± 12‡	24 ± 8‡	19 ± 4‡	21 ± 4‡	19 ± 4‡
	Y	17 ± 4‡	18 ± 2	16 ± 2	17 ± 3	16 ± 1

Values are means ± SD. HR, heart rate; HHb, deoxygenated hemoglobin; τ, time constant of response; TD, time delay; τ'Δ[HHb], sum of effective τΔ[HHb] and TD-Δ[HHb]; * Significantly different from Pre-training values ($p < 0.05$); † Significantly different from Week 6 ($p < 0.05$); ‡ Significantly different from Phase II τVO_{2p} at the same testing time ($p < 0.05$); # Significantly different from Y ($p < 0.05$).

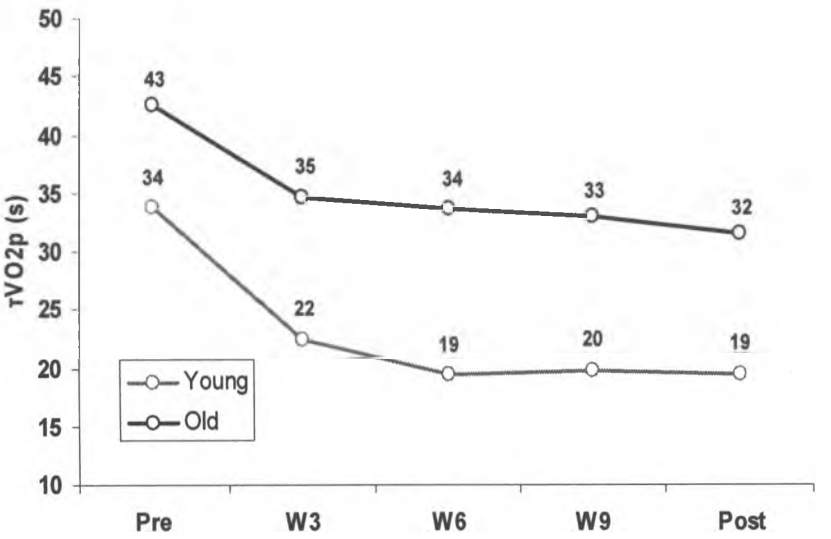

Figure 4.1. Changes in the phase II VO_2 time constant (τVO_{2p}) over the course of the endurance training program in older and young adults.

* $p < 0.05$ compared to pre-training.

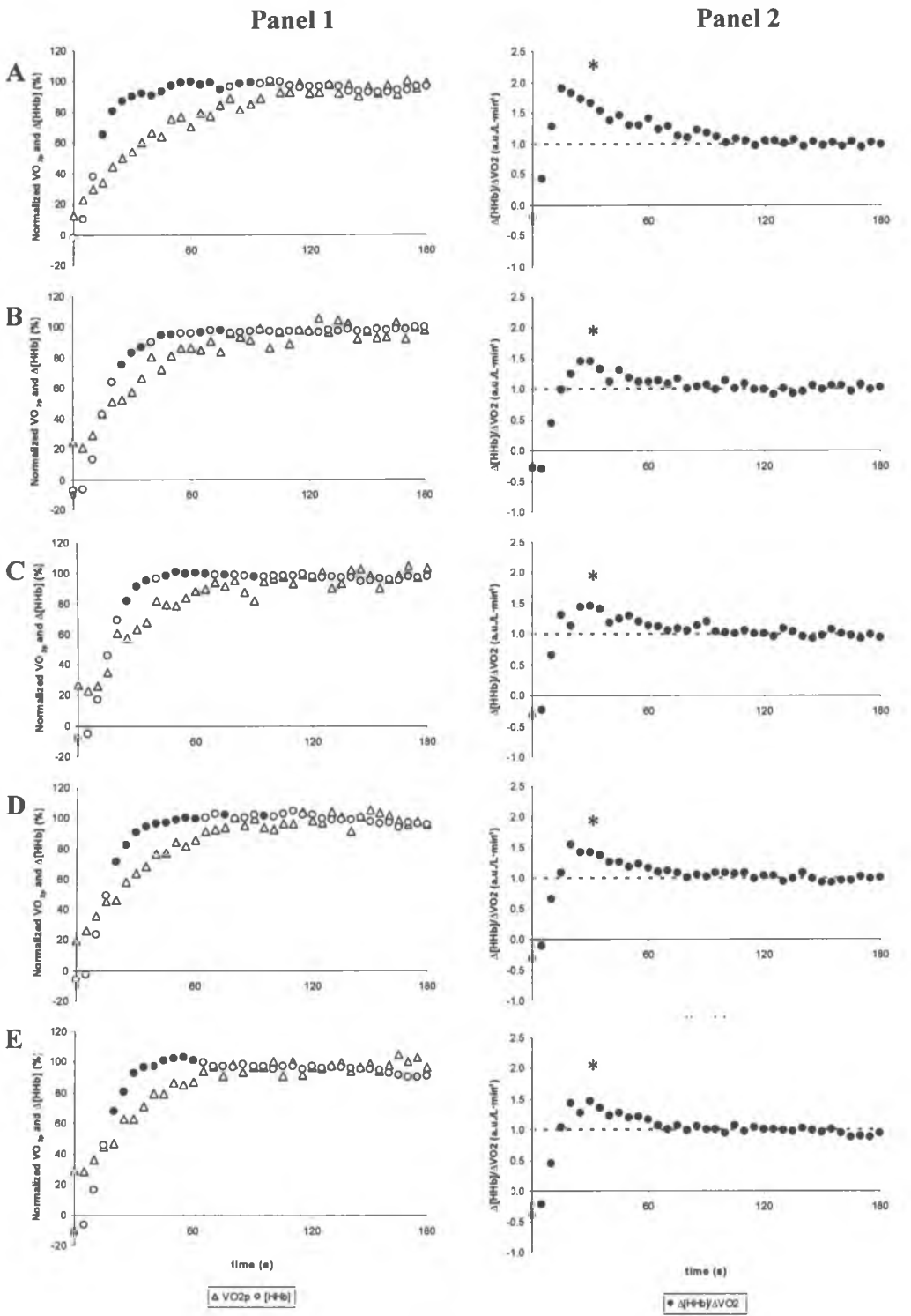

Figure 4.2. Panel 1, group mean profiles for the adjustment of Δ[HHb] (circles) and VO_{2p} (triangles; left shifted such that data from phase I VO_{2p} was not included) during the

initial 180 s of a step-transition in work rate in older adults pre-training (A), at week 3 (B), week 6 (C), week 9 (D), and post-training (E).

Filled circles denote time points at which the relative increase of Δ[HHb] is greater than the relative increase of VO_{2p} ($p < 0.05$). Panel 2, group mean profiles for the adjustment of $\Delta[HHb]/\Delta VO_{2p}$ during the initial 180 s of a step-transition in work rate in older adults pre-training (A), at week 3 (B), week 6 (C), week 9 (D), and post-training (E).

* $\Delta[HHb]/\Delta VO_{2p}$ significantly different from 1.0 ($p < 0.05$).

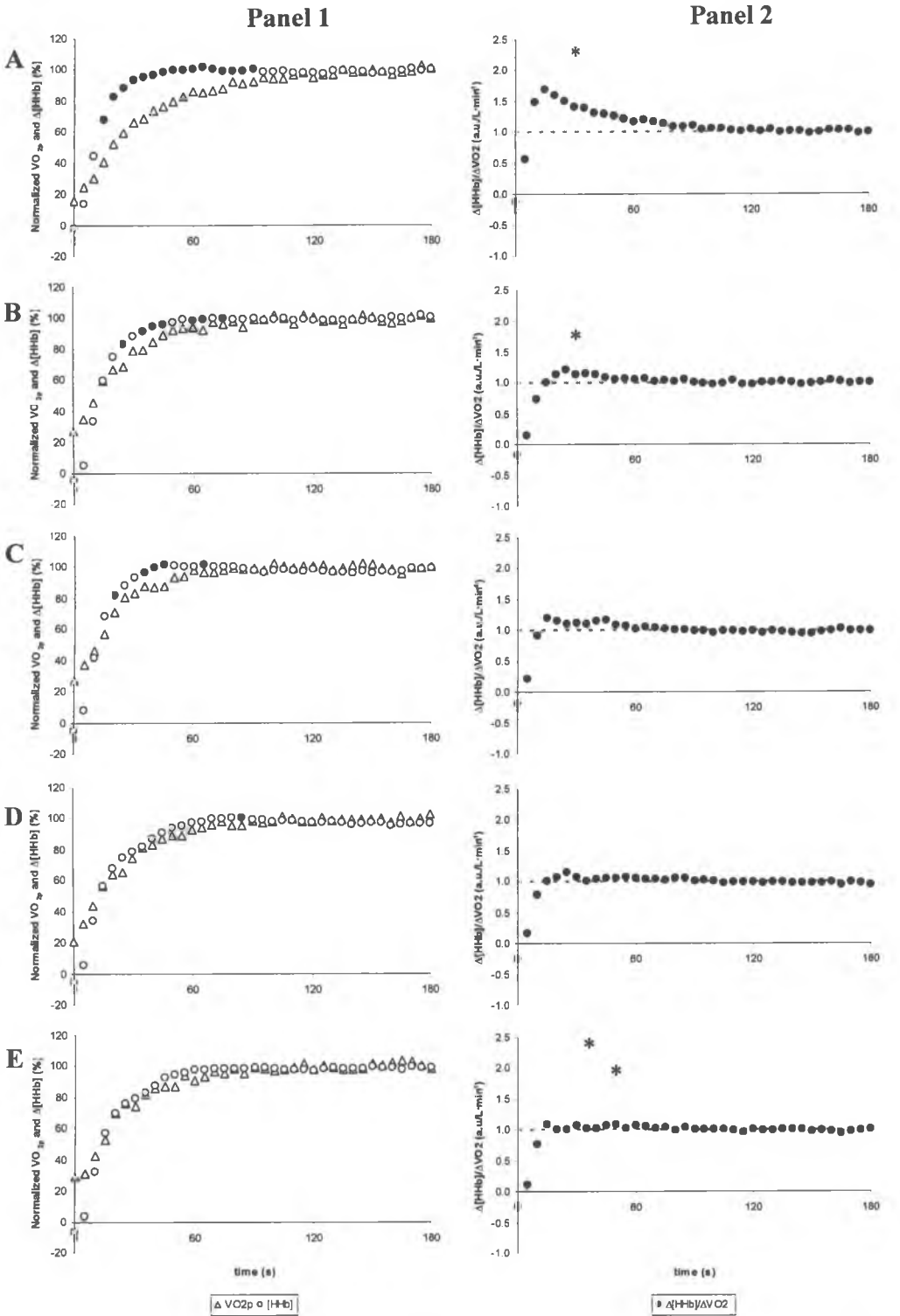

Figure 4.3. Panel 1, group mean profiles for the adjustment of Δ[HHb] (circles) and VO_{2p} (triangles; left shifted such that data from phase I VO_{2p} was not included) during the

initial 180 s of a step-transition in work rate in young adults pre-training (A), at week 3 (B), week 6 (C), week 9 (D), and post-training (E).

Filled circles denote time points at which the relative increase of Δ[HHb] is greater than the relative increase of VO_{2p} ($p < 0.05$). Panel 2, group mean profiles for the adjustment of $\Delta[HHb]/\Delta VO_{2p}$ during the initial 180 s of a step-transition in work rate in young adults pre-training (A), at week 3 (B), week 6 (C), week 9 (D), and post-training (E).

* $\Delta[HHb]/\Delta VO_{2p}$ significantly different from 1.0 ($p < 0.05$).

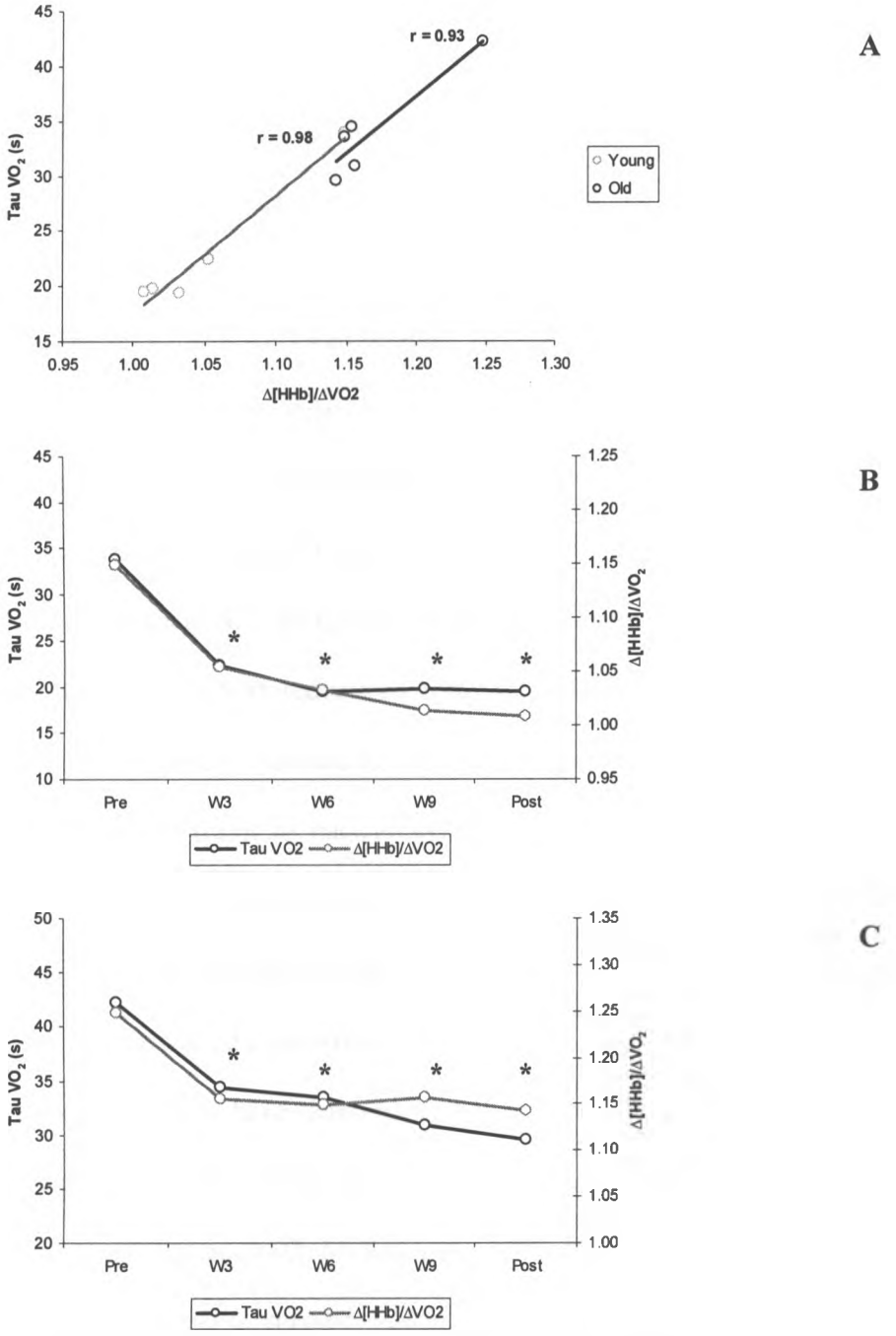

Figure 4.4. A, correlation between changes in $\Delta[HHb]/\Delta VO_{2p}$ and τVO_{2p} in response to training for O and Y; B, time course of changes in $\Delta[HHb]/\Delta VO_{2p}$ and τVO_{2p} in Y; C, time course of changes in $\Delta[HHb]/\Delta VO_{2p}$ and τVO_{2p} in O.

* $p < 0.05$ compared to pre-training.

DISCUSSION

This study examined the effects of a 12-week endurance training program on the time-course of adaptation of VO_{2p} during transitions to moderate-intensity exercise in O and Y healthy adults. The main findings were as follows: 1) The decrease in τVO_{2p} in both O and Y occurred within the first 3 weeks of training with no significant changes seen thereafter; 2) After 3 weeks of training, an attenuation in the $\Delta[HHb]/\Delta VO_{2p}$ "overshoot" during the on-transient, relative to the subsequent steady-state level, suggests a better matching of microvascular blood flow and O_2 distribution and muscle O_2 utilization in the exercise transient in both O and Y. 3) Continued training beyond 3 weeks in both O and Y was not associated with further apparent improvement in the matching of blood flow-to-O_2 utilization or with reductions in τVO_{2p}. 4) In Y, by week 6 O_2 utilization and phase II VO_{2p} were closely matched ($\Delta[HHb]/\Delta VO_{2p}$ ~1.0) suggesting that the possibility of a O_2 delivery constraint to muscle VO_2 kinetics was ameliorated, whereas in O further improvements in the matching of $\Delta[HHb]/\Delta VO_{2p}$ were not achieved in response to the training program and thus no additional changes in τVO_{2p} were observed, indicating that O_2 delivery remained a constraint.

Previous studies have demonstrated that endurance training results in a faster VO_{2p} kinetics in both older (Babcock et al. 1994a; Bell et al. 2001) and young (Berger et al. 2006; Fukuoka et al. 2002; McKay et al. 2009; Phillips et al. 1995) adults. However, the time-course of this adaptation has only been explored in young (McKay et al. 2009; Phillips et al. 1995) and middle-aged (Fukuoka et al. 2002), but not in older adults. In younger groups a faster VO_{2p} occurred in as little as 2 to 4 days of endurance training (McKay et al. 2009; Phillips et al. 1995) with improvements continuing up to 30 days after the start of the training program (Phillips et al. 1995) with no further changes

observed after 30, 60, and 90 days of training (Fukuoka et al. 2002). The present data demonstrated faster VO_{2p} kinetics with endurance training in older adults (as well as young) within the first 3 weeks with no further significant changes thereafter (6, 9, 12 weeks). Taken together, these data show that the rate of adaptation of oxidative phosphorylation at exercise onset after the initiation of an endurance training program occurs within 3 weeks of training regardless of age and may continue up to 30 days (4 weeks) with no further change observed with up to 12 weeks. Further studies are warranted to clarify the short-term (e.g., < 3 weeks of training) time course of adaptation of τVO_{2p} in older adults.

What mechanisms or regulatory factors might constrain the VO_2 kinetics in both older and younger subjects and explain the faster VO_2 kinetics with exercise training? A limitation in O_2 delivery to the active tissues has been proposed as one of the likely mechanisms regulating the rate of adaptation of oxidative phosphorylation (Hughson et al. 2001; Poole et al. 2008; Tschakovsky and Hughson 1999). Phillips et al. (Phillips et al. 1995) hypothesized that a faster femoral artery blood velocity in the absence of increases in muscle oxidative enzyme activity (Green et al. 1991) was the mechanism responsible for the reduction in τVO_{2p} observed early in training in young subjects. From the present study, using the HR as an estimate of "central" blood flow it was notable that τHR was similar to τVO_{2p} in O and Y at any testing time (with the only exception being Y at week 6) suggesting that the time course of increase of central blood flow and thus central O_2 delivery was matched to muscle O_2 utilization. Training resulted in a decreased τHR in both O and Y. Importantly, τHR is only an indirect estimation of O_2 delivery. Measures of muscle conduit artery blood flow kinetics during the transition to exercise show that the rate of adjustment is similar to or faster than that of VO_{2p} (duManoir et al. 2010;

Harper et al. 2006; MacPhee et al. 2005). Indeed, in a training study of older adults that resulted in a faster VO_2 kinetics, the kinetics of femoral artery mean blood velocity was unchanged (Bell et al. 2001). Thus, bulk delivery of O_2 to the exercising limb does not seem to be limiting τVO_{2p} or the adaptation to training that speeds VO_2 kinetics.

Despite evidence showing that the kinetics of bulk delivery of O_2 in the limbs is appropriate to meet the metabolic requirements of the active tissues, blood flow responses to exercise are not only mediated by changes in cardiac output or conduit artery flow but also by the effects of the muscle pump and various vasoactive metabolites and hormones regulating the level of constriction and dilation within the microvascular resistance vessels (Dinenno et al. 1999; Dinenno et al. 2001; Muller-Delp 2006; Proctor and Parker 2006). Recent advances with NIRS have allowed continuous assessment of muscle deoxygenation as an index of the matching of microvascular O_2 delivery-to-muscle O_2 utilization. DeLorey et al. (DeLorey et al. 2003, 2004), applying this measure in conjunction with measurements of VO_2 kinetics, showed that the rate of adjustment of the NIRS-derived Δ[HHb] signal was faster than the adjustment of phase II VO_{2p} and that this response was exacerbated in older adults, indicating a greater fractional O_2 extraction and thus poorer blood flow distribution. Similarly, Harper et al. (Harper et al. 2006) reported that in young adults performing moderate-intensity knee-extension exercise, femoral artery blood flow adjusted faster than the estimated capillary blood flow. Thus, although the rate of adjustment of blood flow in the conduit artery matches that observed for VO_2, the kinetics of microvascular blood flow may have a slower time-course and therefore, limit the rate of adjustment of VO_2 kinetics. With training of young subjects, based on a faster adjustment of phase II VO_{2p} with unchanged adjustment in Δ[HHb], McKay et al. (McKay et al. 2009) speculated that training-induced decreases in the τVO_{2p} were

explained, at least in part, by better matching of muscle O_2 delivery to O_2 utilization. The present data showed that pre-training, $\tau'\Delta[HHb]$ was shorter than τVO_{2p} ($p< 0.05$) in both O and Y (Table 4.2, Figure 4.2 Panel 1 A and Figure 4.3 Panel 1 A), which resulted in the $\Delta[HHb]/\Delta VO_{2p}$ ratio displaying a transient "overshoot" relative to the subsequent steady-state level (Figure 4.2 Panel 2 A and Figure 4.3 Panel 2 A). This transient "overshoot" in the $\Delta[HHb]/\Delta VO_{2p}$ ratio (values > 1.0) is consistent with a greater microvascular fractional O_2 extraction per unit VO_{2p} compared to the exercise steady-state (values = 1.0), and reflects a lower O_2 delivery relative to muscle O_2 utilization in the area of the NIRS probe (slower adjustment of microvascular blood flow).

The young subjects in this study displayed "slower" VO_{2p} kinetics than normally observed in relatively fit, young adults (τVO_{2p} ~20 s). The adjustment of VO_2 in this group may be constrained by a mismatch between local muscle perfusion and metabolism that requires O_2 extraction to increase rapidly during the exercise on-transient due to a slow increase in local muscle O_2 delivery. In this regard, untrained, sedentary young (as well as older) adults, as used in the present study, may exhibit a reduced endothelium-dependent vasodilation compared to those who regularly perform aerobic exercise (DeSouza et al. 2000), which could contribute to a poorer microvascular blood flow and the transient "overshoot" in the $\Delta[HHb]/\Delta VO_{2p}$ ratio reported in this study. Animal studies have shown that endothelium-dependent vasodilation (using acetylcholine (ACh)) and flow- (shear stress) induced vasodilation were reduced in feed arteries and in 1A arterioles of soleus muscles (oxidative) of old but not of young rats (Muller-Delp et al. 2002), which could contribute to an impaired blood flow distribution in the older adults in this study. Interestingly, at rest and during steady-state submaximal exercise total hindlimb blood flow was similar in old and young rats, but blood flow distribution to

oxidative muscles was reduced and distribution to glycolytic muscles was increased in older animals (Musch et al. 2004), suggesting that the matching of blood flow and O_2 delivery to O_2 utilization in active fibers of the old rats may be compromised. $\Delta[HHb]/\Delta VO_{2p}$ data presented in the present study support the idea of older individuals having a maldistribution of blood flow within the active muscles at the start of exercise.

After 3 weeks of training, the $\tau'\Delta[HHb]$ in Y was unchanged compared to pre-training but now was similar to τVO_{2p} (Table 4.2). Also, during the transition to exercise, the "overshoot" in the $\Delta[HHb]/\Delta VO_{2p}$ profile was attenuated in this group (Figure 4.3 Panels 1 and 2 B) and was not evident after further training (Figure 4.3 Panels 1 and 2 C, D, E) suggesting that local blood flow and O_2 delivery were better matched to the O_2 requirement of the active muscle. Older adults, also showed an attenuated $\Delta[HHb]/\Delta VO_{2p}$ "overshoot" after 3 weeks of training (Figure 4.2 Panel 2 B); however, $\tau'\Delta[HHb]$ adaptation remained faster than τVO_{2p} (Table 4.2) and, unlike the response in Y, the "overshoot" in the $\Delta[HHb]/\Delta VO_{2p}$ profile was not eliminated with continued training in O (Figure 4.2 Panel 2 C, D, E). What mechanism might explain the improvements in the matching of local O_2 delivery to muscle O_2 utilization within 3 weeks in both older and younger individuals? Changes to ACh-mediated and flow-induced vasodilation represent an important factor that may have influenced the time-course of adaptation of VO_2 kinetics to training in older and younger groups. Older and middle-aged men who regularly perform endurance exercise (DeSouza et al. 2000; Taddei et al. 2000) or who completed 3 months of aerobic exercise (DeSouza et al. 2000) demonstrate a greater ACh-mediated vasodilatory response as compared to their sedentary counterparts. Interestingly, exercise training was shown to restore both endothelium- (Spier et al. 2004) and flow-dependent (Spier et al. 2007) vasodilation in soleus muscle arterioles from old

and young rats. However, training in young rats resulted in improvements beyond those observed in the old trained animals (Spier et al. 2004; Spier et al. 2007), supporting the finding of the present study that training adaptations did not continue beyond 3 weeks in O, and with 12 weeks of training, or even longer-term training (Babcock et al. 1994a) the τVO_2 does not achieve values reached in the trained young. Rapid improvements in both endothelium and flow-mediated vasodilation were reported 12-24 h after a single bout of exercise and were sustained for 1-2 days, however, unlike the acute response, chronic exercise training induces adaptations that are two-fold higher and more long-lasting, remaining for up to 1 week post-exercise (Green et al. 2004; Haram et al. 2006). Therefore, enhancement of endothelium and flow-mediated vasodilation in both O and Y adults may be mainly responsible for the faster blood flow adjustment at exercise onset, and thereby lead to an attenuation or elimination of the "overshoot" in the $\Delta[HHb]/\Delta VO_{2p}$ profile that is seen in the present study with exercise training. The high correlation and similar time course of changes in $\Delta[HHb]/\Delta VO_{2p}$ and τVO_{2p} (Figure 4.4) in both O and Y further supports the notion that an improved O_2 distribution within the microvasculature plays a major role in the changes observed in τVO_{2p}.

Another mechanism that may affect the vasodilatory responses to exercise is increased accumulation of reactive oxygen species (ROS). Increased ROS have been proposed to affect the NO-mediated signalling and bioavailability in older subjects due to their binding affinity to NO (Taddei et al. 2000). Taddei et al. (2000) showed that treating older sedentary individuals with antioxidants restored the vasodilatory capacity of NO inhibited by L-NMMA; this suggests that the age-related endothelial dysfunction is at least in part caused by oxidative stress-induced reduction in NO bioavailability. Indeed, administration of antioxidants eliminated the PO_{2mv} undershoot observed in older rats'

spinotrapezius muscle during a transition from rest to moderate intensity exercise (Herspring et al. 2008).

Age-related changes in capillary structure have been proposed to affect gas exchange between the capillary and the muscle fiber (Coggan et al. 1992). However, recent data showed that the capillary structure is not compromised with age (Mathieu-Costello and Hepple 2002). In fact, the ratio of capillary-to-fiber surface contact to oxidative capacity has been shown to be substantially higher in the older rats (Hepple 2000). As such, an O_2 diffusion limitation in the old would not be explained by a reduced structural capacity for O_2 transfer per se but rather depend on the flux and distribution of red blood cells (RBC) within the capillary bed. In this regard, older rats have a decreased lineal density of RBC-perfused capillaries lying adjacent to a fiber (which determines the potential for blood-myocyte O_2 flux) and compensate for this, at least at rest, by increasing individual capillary RBC velocity and flux such that O_2 delivery (as quantified by $RBC \cdot mm^{-1} \cdot s^{-1}$) is similar in both old and young (Russell et al. 2003). However, during electrically-induced contractions older rats do not show the increased capillary RBC velocity and flux observed in young rats (Copp et al. 2009). These alterations in capillary hemodynamics in the older rats are likely to reduce the convective and diffusive transport of O_2 to the myocyte.

Several studies have suggested that the locus of control for oxidative phosphorylation resides intracellularly (Grassi et al. 1998a; Grassi et al. 1998b). At the onset of exercise, the rate of increase in VO_2 is determined by the PCr shuttle attenuating the increase in ADP accumulation in the mitochondria (Walsh et al. 2005). Additionally, NO production competing with O_2 for the binding site of cytochrome c oxidase has been proposed to play a role in regulating the rate at which VO_2 adapts (Jones et al. 2003; Kindig et al. 2002).

Substrate supply, in particular related to PDH activity, has also been thought to be one of the mechanisms related to the rate of VO_2 increase (Grassi et al. 2002; Gurd et al. 2006, 2008; Rossiter et al. 2003). Although these intracellular factors (as cited above) may be the main ones controlling oxidative phosphorylation and eliciting a VO_2 kinetics of less than ~20 s, for those with slower VO_2 kinetics the present data suggest the matching of microvascular O_2 delivery to the metabolic demand is a key factor determining or constraining the rate at which VO_{2p} adjusts (i.e., τVO_2). Nevertheless, we cannot eliminate the possibility that changes in mitochondrial oxidative capacity (with training) may alter phosphorylation and/or redox potential and thereby influence the driving of oxidative phosphorylation.

In conclusion, this study demonstrated that 3 weeks of endurance training resulted in a significant decrease in τVO_{2p} in both older and young adults with no significant reductions in τVO_{2p} seen during the subsequent 9 weeks of training. Additionally, τVO_{2p} measured after 3 weeks of training in O was similar to that observed in Y before the start of training. Finally, an improved $\Delta[HHb]/\Delta VO_{2p}$ ratio (reflecting a better matching of O_2 distribution within the tissues) was associated with the reduction in τVO_{2p} observed in both O and Y. Although these data do not preclude that the basic control to VO_{2p} kinetics resides within intracellular factors that were not measured in this study, it suggests that with "slower" VO_2 kinetics the rate of adaptation of VO_2 may be constrained by O_2 availability related to the matching of microvascular O_2 delivery to muscle VO_2.

REFERENCES

Babcock MA, Paterson DH, Cunningham DA (1994a) Effects of aerobic endurance training on gas exchange kinetics of older men. Med Sci Sports Exerc 26: 447-452

Babcock MA, Paterson DH, Cunningham DA, Dickinson JR (1994b) Exercise on-transient gas exchange kinetics are slowed as a function of age. Med Sci Sports Exerc 26: 440-446

Beaver WL, Lamarra N, Wasserman K (1981) Breath-by-breath measurement of true alveolar gas exchange. J Appl Physiol 51: 1662-1675

Behnke BJ, Delp MD, Dougherty PJ, Musch TI, Poole DC (2005) Effects of aging on microvascular oxygen pressures in rat skeletal muscle. Respir Physiol Neurobiol 146: 259-268

Bell C, Paterson DH, Kowalchuk JM, Cunningham DA (1999) Oxygen uptake kinetics of older humans are slowed with age but are unaffected by hyperoxia. Exp Physiol 84: 747-759

Bell C, Paterson DH, Kowalchuk JM, Moy AP, Thorp DB, Noble EG, Taylor AW, Cunningham DA (2001) Determinants of oxygen uptake kinetics in older humans following single-limb endurance exercise training. Exp Physiol 86: 659-665

Berger NJ, Tolfrey K, Williams AG, Jones AM (2006) Influence of continuous and interval training on oxygen uptake on-kinetics. Med Sci Sports Exerc 38: 504-512

Chilibeck PD, Paterson DH, Petrella RJ, Cunningham DA (1996) The influence of age and cardiorespiratory fitness on kinetics of oxygen uptake. Can J Appl Physiol 21: 185-196

Coggan AR, Spina RJ, King DS, Rogers MA, Brown M, Nemeth PM, Holloszy JO (1992) Histochemical and enzymatic comparison of the gastrocnemius muscle of young and elderly men and women. J Gerontol 47: B71-76

Copp SW, Ferreira LF, Herspring KF, Musch TI, Poole DC (2009) The effects of aging on capillary hemodynamics in contracting rat spinotrapezius muscle. Microvasc Res 77: 113-119

Coyle EF (1995) Integration of the physiological factors determining endurance performance ability. Exerc Sport Sci Rev 23: 25-63

DeLorey DS, Kowalchuk JM, Paterson DH (2003) Relationship between pulmonary O_2 uptake kinetics and muscle deoxygenation during moderate-intensity exercise. J Appl Physiol 95: 113-120

DeLorey DS, Kowalchuk JM, Paterson DH (2004) Effect of age on O_2 uptake kinetics and the adaptation of muscle deoxygenation at the onset of moderate-intensity cycling exercise. J Appl Physiol 97: 165-172

DeLorey DS, Kowalchuk JM, Paterson DH (2005) Adaptation of pulmonary O_2 uptake kinetics and muscle deoxygenation at the onset of heavy-intensity exercise in young and older adults. J Appl Physiol 98: 1697-1704

DeLorey DS, Paterson DH, Kowalchuk JM (2007) Effects of ageing on muscle O_2 utilization and muscle oxygenation during the transition to moderate-intensity exercise. Appl Physiol Nutr Metab 32: 1251-1262

DeSouza CA, Shapiro LF, Clevenger CM, Dinenno FA, Monahan KD, Tanaka H, Seals DR (2000) Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. Circulation 102: 1351-1357

Dinenno FA, Jones PP, Seals DR, Tanaka H (1999) Limb blood flow and vascular conductance are reduced with age in healthy humans: relation to elevations in sympathetic nerve activity and declines in oxygen demand. Circulation 100: 164-170

Dinenno FA, Tanaka H, Stauffer BL, Seals DR (2001) Reductions in basal limb blood flow and vascular conductance with human ageing: role for augmented alpha-adrenergic vasoconstriction. J Physiol 536: 977-983

duManoir GR, DeLorey DS, Kowalchuk JM, Paterson DH (2010) Kinetics of VO_2 limb blood flow and regional muscle deoxygenation in young adults during moderate intensity, knee-extension exercise. Eur J Appl Physiol 108: 607-617

Elwell C (1995) A Practical Users Guide To Near Infrared Spectroscopy., London: Hamamatsu Photonics KK

Fukuoka Y, Grassi B, Conti M, Guiducci D, Sutti M, Marconi C, Cerretelli P (2002) Early effects of exercise training on on- and off-kinetics in 50-year-old subjects. Pflugers Arch 443: 690-697

Grassi B, Gladden LB, Samaja M, Stary CM, Hogan MC (1998a) Faster adjustment of O_2 delivery does not affect VO_2 on-kinetics in isolated in situ canine muscle. J Appl Physiol 85: 1394-1403

Grassi B, Gladden LB, Stary CM, Wagner PD, Hogan MC (1998b) Peripheral O_2 diffusion does not affect VO_2on-kinetics in isolated insitu canine muscle. J Appl Physiol 85: 1404-1412

Grassi B, Hogan MC, Greenhaff PL, Hamann JJ, Kelley KM, Aschenbach WG, Constantin-Teodosiu D, Gladden LB (2002) Oxygen uptake on-kinetics in dog gastrocnemius in situ following activation of pyruvate dehydrogenase by dichloroacetate. J Physiol 538: 195-207

Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK, Wagner PD (1996) Muscle O2 uptake kinetics in humans: implications for metabolic control. J Appl Physiol 80: 988-998

Green DJ, Maiorana A, O'Driscoll G, Taylor R (2004) Effect of exercise training on endothelium-derived nitric oxide function in humans. J Physiol 561: 1-25

Green HJ, Jones S, Ball-Burnett ME, Smith D, Livesey J, Farrance BW (1991) Early muscular and metabolic adaptations to prolonged exercise training in humans. J Appl Physiol 70: 2032-2038

Gurd BJ, Peters SJ, Heigenhauser GJ, LeBlanc PJ, Doherty TJ, Paterson DH, Kowalchuk JM (2006) Prior heavy exercise elevates pyruvate dehydrogenase activity and speeds O_2 uptake kinetics during subsequent moderate-intensity exercise in healthy young adults. J Physiol 577: 985-996

Gurd BJ, Peters SJ, Heigenhauser GJ, LeBlanc PJ, Doherty TJ, Paterson DH, Kowalchuk JM (2008) O_2 uptake kinetics, pyruvate dehydrogenase activity, and muscle deoxygenation in young and older adults during the transition to moderate-intensity exercise. Am J Physiol Regul Integr Comp Physiol 294: R577-584

Haram PM, Adams V, Kemi OJ, Brubakk AO, Hambrecht R, Ellingsen O, Wisloff U (2006) Time-course of endothelial adaptation following acute and regular exercise. Eur J Cardiovasc Prev Rehabil 13: 585-591

Harper AJ, Ferreira LF, Lutjemeier BJ, Townsend DK, Barstow TJ (2006) Human femoral artery and estimated muscle capillary blood flow kinetics following the onset of exercise. Exp Physiol 91: 661-671

Hepple RT (2000) Skeletal muscle: microcirculatory adaptation to metabolic demand. Med Sci Sports Exerc 32: 117-123

Herspring KF, Ferreira LF, Copp SW, Snyder BS, Poole DC, Musch TI (2008) Effects of antioxidants on contracting spinotrapezius muscle microvascular oxygenation and blood flow in aged rats. J Appl Physiol 105: 1889-1896

Hughson RL, Tschakovsky ME, Houston ME (2001) Regulation of oxygen consumption at the onset of exercise. Exerc Sport Sci Rev 29: 129-133

Jones AM, Wilkerson DP, Koppo K, Wilmshurst S, Campbell IT (2003) Inhibition of nitric oxide synthase by L-NAME speeds phase II pulmonary VO_2 kinetics in the transition to moderate-intensity exercise in man. J Physiol 552: 265-272

Kindig CA, McDonough P, Erickson HH, Poole DC (2002) Nitric oxide synthase inhibition speeds oxygen uptake kinetics in horses during moderate domain running. Respir Physiol Neurobiol 132: 169-178

MacPhee SL, Shoemaker JK, Paterson DH, Kowalchuk JM (2005) Kinetics of O_2 uptake, leg blood flow, and muscle deoxygenation are slowed in the upper compared with lower region of the moderate-intensity exercise domain. J Appl Physiol 99: 1822-1834

Mathieu-Costello O, Hepple RT (2002) Muscle structural capacity for oxygen flux from capillary to fiber mitochondria. Exerc Sport Sci Rev 30: 80-84

McCreary CR, Chilibeck PD, Marsh GD, Paterson DH, Cunningham DA, Thompson RT (1996) Kinetics of pulmonary oxygen uptake and muscle phosphates during moderate-intensity calf exercise. J Appl Physiol 81: 1331-1338

Proctor DN, Parker BA (2006) Vasodilation and vascular control in contracting muscle of the aging human. Microcirculation 13: 315-327

Rossiter HB, Ward SA, Doyle VL, Howe FA, Griffiths JR, Whipp BJ (1999) Inferences from pulmonary O_2 uptake with respect to intramuscular [phosphocreatine] kinetics during moderate exercise in humans. J Physiol 518 (Pt 3): 921-932

Rossiter HB, Ward SA, Howe FA, Wood DM, Kowalchuk JM, Griffiths JR, Whipp BJ (2003) Effects of dichloroacetate on VO_2 and intramuscular ^{31}P metabolite kinetics during high-intensity exercise in humans. J Appl Physiol 95: 1105-1115

Russell JA, Kindig CA, Behnke BJ, Poole DC, Musch TI (2003) Effects of aging on capillary geometry and hemodynamics in rat spinotrapezius muscle. Am J Physiol Heart Circ Physiol 285: H251-258

Spier SA, Delp MD, Meininger CJ, Donato AJ, Ramsey MW, Muller-Delp JM (2004) Effects of ageing and exercise training on endothelium-dependent vasodilatation and structure of rat skeletal muscle arterioles. J Physiol 556: 947-958

Spier SA, Delp MD, Stallone JN, Dominguez JM, 2nd, Muller-Delp JM (2007) Exercise training enhances flow-induced vasodilation in skeletal muscle resistance arteries of aged rats: role of PGI2 and nitric oxide. Am J Physiol Heart Circ Physiol 292: H3119-3127

Taddei S, Galetta F, Virdis A, Ghiadoni L, Salvetti G, Franzoni F, Giusti C, Salvetti A (2000) Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes. Circulation 101: 2896-2901

Tschakovsky ME, Hughson RL (1999) Interaction of factors determining oxygen uptake at the onset of exercise. J Appl Physiol 86: 1101-1113

Walsh B, Howlett RA, Stary CM, Kindig CA, Hogan MC (2005) Determinants of oxidative phosphorylation onset kinetics in isolated myocytes. Med Sci Sports Exerc 37: 1551-1558

CHAPTER V: The time course of speeding of VO_2 kinetics in young and older women in response to endurance training

INTRODUCTION

The study of pulmonary O_2 uptake (VO_{2p}) kinetics provides insight into the potential factors regulating mitochondrial oxidative phosphorylation. In healthy individuals, the rate at which VO_2 adjusts to a new energetic demand is controlled intracellularly (Behnke et al. 2001; Grassi 2001; Poole et al. 2007) but may be constrained by the rate of delivery of O_2 to the active muscle fibers (Gurd et al. 2008; Hughson et al. 2001; Tschakovsky and Hughson 1999).

Older individuals have a longer Phase II VO_{2p} time constant (τVO_{2p}) during the on-transient of moderate-intensity exercise compared to younger adults (Babcock et al. 1994b; Bell et al. 1999; Chilibeck et al. 1996; DeLorey et al. 2005). Endurance training has been demonstrated to result in faster VO_{2p} kinetics in both older (Babcock et al. 1994a; Bell et al. 2001) and young (Fukuoka et al. 2002; Phillips et al. 1995) healthy men. In a recent study we reported that after only 3 weeks of endurance training the adjustment of VO_2 at the onset of exercise became faster in both the older and young males and that this short training time was enough for older adults to achieve a VO_2 kinetics response similar to that observed in the young individuals before the start of training (Murias et al. 2010b). In that study we also reported that during transitions to moderate-intensity exercise, a better matching of microvascular blood flow-to-O_2 utilization in the active muscles seemed to be responsible for the speeding of VO_{2p} kinetics in response to training (Murias et al. 2010b).

Surprisingly, there is little information on VO_2 kinetics in women. Gurd et al. (Gurd et al. 2007) examined the effects of menstrual cycle on τVO_{2p} in young women, and

Stathokostas et al. (2008) studied the effects of hormone replacement therapy on τVO_{2p} in older women. However, to our knowledge, there is no information on changes in the rate of adjustment of VO_{2p} kinetics in response to endurance training in older and young women. Interestingly, recent studies suggest that blood flow distribution may differ depending on gender and age (Parker et al. 2008a). Unlike older men (Proctor et al. 2003b), normally active older women have a blunted vascular conductance and hyperemic response (Parker et al. 2008a; Proctor et al. 2003a), whereas young female adults show a femoral blood flow and vascular conductance that is better than that observed even in young male adults (Parker et al. 2007). Additionally, cardiovascular adaptations to endurance training in older (Spina et al. 1993) but not in young (Murias et al. 2010a) women have been shown to rely mainly on a wider arterial-venous O_2 difference ($a\text{-}vO_{2diff}$) with no increases in cardiac output. This markedly different cardiovascular response in older compared to young women could affect the matching of O_2 delivery to O_2 utilization and thus, the rate of adjustment of VO_{2p} in response to a step-transition in the moderate-intensity domain.

Based on the contention that older and young women represent the lower (blunted blood flow) and upper (high blood flow) ends of the spectrum in terms of vascular responsiveness to exercise and considering that the distribution of blood flow within the active muscles has been shown to play an important role in rate of adjustment of oxidative phosphorylation (DeLorey et al. 2007; Murias et al. 2010b), and that endurance training has been demonstrated to speed VO_{2p} kinetics in young and old men (Murias et al. 2010b), we sought to determine the time-course of adjustment for phase II VO_{2p} kinetics in older and younger women during a 12-week endurance training program. We hypothesized that: 1) older women would have a slower phase II VO_{2p} kinetics compared

to their younger counterparts at any testing time; 2) endurance training would result in speeding of phase II VO_{2p} kinetics in both older and young women with the majority of the change occurring during the first 3 weeks of training; 3) the speeding of VO_{2p} kinetics in response to endurance training in older women would not be enough to reduce τVO_{2p} to the values observed in the young women before training started.

METHODS

Subjects: Six older (O) (69 ± 7 yr; mean ± SD) and 8 young (Y) (25 ± 5 yr) adult women volunteered and gave written consent to participate in the study. Descriptive and baseline data from these subjects were given in a previous report examining central and peripheral adaptations to endurance training in the same women (Murias et al. 2010a); the reader is referred to this paper for further information on increases in maximal VO_{2p}, cardiac output and arterial-venous O_2 difference). All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All subjects were non-obese (body mass index ≤ 30 kg·m^{-2}), non-smokers, and were physically active but none of them had been involved in any type of endurance training program for at least 12 months prior to the study. Additionally, no subjects were taking medications that would affect the cardiorespiratory or hemodynamic responses to exercise. Subjects had no history of cardiovascular, respiratory or musculoskeletal diseases, and older women were medically screened by a physician and underwent a maximal exercise stress test.

Protocol: Before training began, subjects reported to the laboratory on two separate occasions. On day one, a maximal cycle ergometer ramp test (O 15-20 W/min; Y 25 W/min) was performed (Lode Corival 400; Lode B.V., Groningen, Holland) for determination of peak VO_2 (VO_{2peak}) and the estimated lactate threshold (θ_L). θ_L was

defined as the VO_2 at which CO_2 output (VCO_2) began to increase out of proportion in relation to VO_2 with a systematic rise in minute ventilation-to-VO_2 ratio and end-tidal PO_2 whereas minute ventilation-to-VCO_2 ratio and end-tidal PCO_2 were stable. After this test, subjects returned to the laboratory on a different day to perform step transitions in work rate (WR) from 20 W to a moderate-intensity WR that elicited a VO_2 corresponding to 90% θ_L. Each subject performed two repetitions of the following protocol: 6 min cycling at 20 W, 6 min at 90% θ_L, 8 min at 20 W, and 6 min at 90% θ_L; transitions were performed continuously and each repetition was separated by 30 min rest with the subject sitting on a chair. Identical procedures were repeated after 3, 6, 9, and 12 weeks of training. However, since the step-transitions were performed at the same absolute power output as the initial tests, the order for the ramp test and the step-transitions was assigned randomly. At least 24 h were allowed between the ramp-test and the step transitions.

Training: The endurance training program consisted of 3 exercise sessions per week on a stationary cycle-ergometer (Monark Ergomedic 874E; Monark Exercise AB, Varberg, Sweeden) for a total duration of 12 weeks. Training intensity was adjusted at 3 week intervals to reflect changes in fitness. During the first 10 weeks, each session consisted of continuous training (CT) for 45 min at a power output that elicited ~70% of the VO_{2peak} observed during the incremental ramp test. During the final 2 weeks of training (6 exercise sessions), to maintain an emphasis increasing intensity some subjects performed high-intensity interval training (HIT), which consisted of 10-12 exercise bouts each lasting 1-min at 90-100% of the peak power output achieved during the previous incremental ramp test, each separated by 1-min resting recovery.

Measurements: Gas-exchange measurements were similar to those previously described (Babcock et al. 1994b). Briefly, inspired and expired flow rates were measured using a

low dead space (90 mL) bidirectional turbine (Alpha Technologies VMM 110) which was calibrated before each test by using a syringe of known volume. Inspired and expired gases were sampled continuously (every 20 ms) at the mouth and analyzed for concentrations of O_2, CO_2, and N_2 by mass spectrometry (Perkin Elmer MGA-1100) after calibration with precision-analyzed gas mixtures. Changes in gas concentrations were aligned with gas volumes by measuring the time delay for a square-wave bolus of gas passing the turbine to the resulting changes in fractional gas concentrations as measured by the mass spectrometer. Data collected every 20 ms were transferred to a computer, which aligned concentrations with volume information to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver et al. (1981).

Heart rate (HR) was monitored continuously by electrocardiogram using PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO) with a three-lead arrangement. Data were recorded using LabChart v4.2 (ADInstruments, Colorado Springs, CO) on a separate computer.

Local muscle oxygenation profiles of the quadriceps vastus lateralis muscle were made with NIRS (Hamamatsu NIRO 300, Hamamatsu Photonics, Hamamatsu, Japan). Optodes were placed on the belly of the muscle midway between the lateral epicondyle and greater trochanter of the femur. The optodes were housed in an optically dense plastic holder and secured on the skin surface with tape and then covered with an optically dense, black vinyl sheet, thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to minimize movement of the optodes.

The physical principles of tissue spectroscopy are described in detail by Elwell (Elwell 1995) and the manner in which these are applied has been explained by DeLorey et al.

(DeLorey et al. 2003). Briefly, one fiber optic bundle carried the NIR-light produced by the laser diodes to the tissue of interest while a second fiber optic bundle returned the transmitted light from the tissue to a photon detector (photomultiplier tube) in the spectrometer. Four different wave lengths laser diodes (775, 810, 850, and 910 nm) provided the light source. The diodes were pulsed in a rapid succession and the light was detected by the photomultiplier tube for online estimation and display of the concentration changes from the resting baseline oxy-hemoglobin (HbO_2), deoxy-hemoglobin (HHb), and total hemoglobin (Hb_{tot}). In this study, we used an interoptode spacing of 5 cm. Given the uncertainty of the optical path length in the vastus lateralis at rest and during exercise, NIRS data are presented as delta (Δ) arbitrary units (a.u.). NIRS-derived signal was zero set prior to the onset of exercise while subjects were quietly seated on the cycle ergometer. The raw attenuation signals (in optical density units) were transferred to a computer for later analysis. Changes in light intensities were recorded continuously at 2 Hz.

Data analysis: VO_2 data were filtered by removing aberrant data points that lay outside 4 SD of the local mean. The data for each transition then were linearly interpolated to 1 s intervals and time-aligned such that time zero represented the onset of exercise. Data from each transition were ensemble-averaged to yield a single, averaged response for each subject. This transition was further time-averaged into 10 s bins to provide a single time-averaged response for each subject. The on-transient response for VO_2 was modelled using a mono-exponential of the form:

$$Y_{(t)} = Y_{Bsln} + Amp\ (1 - e^{-(t-TD)/\tau}), \text{ equation (1)}$$

where $Y_{(t)}$ represents VO_2 at any time (t); Y_{Bsln} is the baseline VO_2 during 20 W cycling; Amp is the steady-state increase in VO_2 above the baseline value; τ is the time-constant

defined as the duration of time for VO_2 to increase to 63% of the steady-state increase; and TD is the time delay (such that the model is not constrained to pass through the origin). The phase I-phase II transition was constrained to 20 s. Data were modeled from the beginning of phase II to 4 min (240 s) of the step-transition. The model parameters were estimated by least-squares nonlinear regression (Origin, OriginLab Corp., Northampton, MA, USA) in which the best fit was defined by minimization of the residual sum of squares and minimal variation of residuals around the Y-axis (Y = 0). The 95% confidence interval (CI) for the estimated time constant was determined after preliminary fit of the data with Bsln, Amp, and TD constrained to the best-fit values and the τ allowed to vary.

Heart rate data were determined from the R-R interval on a second-by-second basis and edited and modeled in the same manner as described above for VO_2 data. The on-transient HR response was modeled from the onset of exercise to 240 s using the mono-exponential model described in equation (1).

The NIRS-derived Δ[HHb] data were time-aligned and ensemble-averaged to 5-s bins to yield a single response for each subject. The Δ[HHb] profile is described as consisting of a time delay at the onset of exercise, followed by an increase in the signal with an "exponential-like" time-course (DeLorey et al. 2003). The time delay for the Δ[HHb] response (TD-Δ[HHb]) was determined using second-by-second data as the duration between the onset of exercise and the first point at which the Δ[HHb] signal started to systematically increase. The Δ[HHb] data were modeled from the end of the TD-Δ[HHb] to 90 s of the transition using an exponential model as described in equation 1. The $\tau\Delta$[HHb] described the time course for the increase in Δ[HHb], while the overall time

course of Δ[HHb] from the onset of exercise was described by the effective Δ[HHb] (τ'Δ[HHb] = TD-Δ[HHb] + τΔ[HHb]).

The second-by-second Δ[HHb] and VO_{2p} data were normalized for each subject (0-100% of the response). The normalized VO_{2p} was left-shifted by 20s to account for the phase I-phase II transition so that the beginning of phase II VO_{2p}, which has been previously described to coincide with muscle VO_2 within 10% (Grassi et al. 1996) coincided with the onset of exercise. Data were further averaged into 5s bins for statistical comparison of the rate of adjustment for Δ[HHb] and ΔVO_{2p}. Additionally, an overall $\Delta[HHb]/\Delta VO_{2p}$ ratio for the adjustment during the exercise on-transient was derived for each individual as the average value from 20-150 s into the transition. The start point was selected to be 20 s to begin beyond the physiological TD-Δ[HHb] derived from NIRS. An end point of 150 s was selected to ensure that both the Δ[HHb] and ΔVO_{2p} signals had reached steady-state (~98 to 100% of their response amplitudes).

Statistics: Data are presented as means ± SD. Paired and unpaired t-tests and a two-way repeated measures analysis of variance (ANOVA) were used to determine statistical significance for the dependent variables. A Tukey post-hoc analysis was used when significant differences were found for the main effects of each dependent variable. Pearson product moment correlation coefficients were used to determine the degree of association between key variables. The ANOVA and correlation coefficients were analyzed by SPSS Version 15.0, (SPSS Inc., Chicago, IL). Statistical significance was declared when $p < 0.05$.

RESULTS

Subject characteristics and pre-training peak exercise values are listed in Table 5.1. Compliance with the training program was 94 ± 4 % (28/30 training sessions) and 98 ± 3

% (29/30 training sessions) in O and Y, respectively, with all subjects completing at least 90 % of the programmed training sessions. As noted in 'Methods' groups were split after the 9th week of training; however, since training type (i.e., continuous vs. interval) did not affect any kinetic parameter, data were combined. The training program resulted in a significant increase in VO_{2peak} in O and Y (Murias et al. 2010a).

VO_2 kinetics: The phase II VO_2 time constant (τVO_{2p}) was greater ($p < 0.05$) in O (55 ± 16 s) than in Y (31 ± 8 s). The τVO_{2p} decreased by approximately 30-35% ($p < 0.05$) after 3 weeks training in both O (35 ± 12 s) and Y (22 ± 4 s) with a further decrease observed at week 9 of training compared to week 3 (O, 32 ± 12 s; Y, 18 ± 5 s) (Figure 5.1, Table 5.2); the τVO_{2p} observed after 3 weeks of training in O was similar to that observed in Y pre-training. There was no testing time by age interactions reflecting a similar time-course for the decrease in τVO_{2p} in O and Y over the course of training.

The VO_{2p} amplitude was lower in O (0.29 ± 0.08 $L \cdot min^{-1}$) compared with Y (0.57 ± 0.12 $L \cdot min^{-1}$) reflecting the lower work rate (WR) in O (O, 47 ± 6 W; Y, 73 ± 9 W). The VO_{2p} amplitudes were unchanged over the course of training reflecting the unchanged WR at each testing time, and resulted in a similar VO_{2p} gain ($\Delta VO_{2p}/\Delta WR$) in O (10.0 ± 1.4 $mL \cdot min^{-1} \cdot W^{-1}$) and Y (10.6 ± 1.0 $mL \cdot min^{-1} \cdot W^{-1}$) over the course of training.

HR kinetics: The τHR was greater ($p < 0.05$) in O compared with Y. After 3 weeks training τHR decreased from 62 ± 21 s to 53 ± 13 s in O and from 45 ± 13 s to 31 ± 11 s in Y, with a further decrease in τHR ($p < 0.05$) observed after 6 weeks training (O, 47 ± 12 s; Y, 22 ± 7 s) (Table 5.2).

There was no relationship between the decrease in τHR and the decrease in τVO_2 from pre- to 3 weeks training for either O ($r = 0.19$; $p > 0.05$) or Y ($r = 0.46$; $p > 0.05$). In O, τHR was larger than τVO_{2p} at week 3 of training ($p < 0.05$). In Y τHR was larger than

τVO_{2p} pre-training, at week 9 and post-training ($p < 0.05$; p values for week 3 and week 6 = 0.06 and 0.09, respectively).

Δ[HHb] kinetics: Due to technical issues related to NIRS data acquisition in the older women, NIRS data were only available for the young women. In Y, after the step increase in work rate, a TD-Δ[HHb] of 8 ± 2 s was observed pre-training. No change in TD-Δ[HHb] occurred as a result of training (Table 5.2). The amplitude of the increase in Δ[HHb] was also unaffected by training (e.g., Pre-training: 4 ± 3 a.u.; Post-training: 3 ± 2 a.u.). The τΔ[HHb] was similar across testing times; however, there was a trend ($p = 0.08$) towards a faster adjustment with training (Table 5.2). Despite this trend, the overall change of the effective Δ[HHb] (τ'Δ[HHb] = TD-Δ[HHb] + τΔ[HHb]) was not affected by training (Table 5.2). In fact, the τ'Δ[HHb] adjustment progressed from being faster (albeit not significant) to being significantly slower than the adjustment of τVO_{2p} (Table 5.2; Figure 5.2 A to E).This resulted in the $\Delta[HHb]/\Delta VO_{2p}$ displaying a small pre-training transient "overshoot" relative to the subsequent steady state level (Figure 5.2 Panels 1 and 2 A) that was abolished during the subsequent testing times (Figure 5.2 Panels 1 and 2 B to E).

Table 5.1. Subject characteristics and peak exercise responses

	n	Age (yr)	Height (cm)	Body mass (kg)	Peak WR (W)	Peak HR (bpm)	VO_{2peak} ($L \cdot min^{-1}$)	VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$)
Older	6	69±7	163±3	72±6	119±15	150±18	1.73±0.25	22.9±2.3
Young	8	25±5*	166±5	66±15	211±26*	187±3*	2.65±0.34*	41.2±4.7*

Values are means ± SD; n, no. of subjects; WR, work rate; HR, heart rate; VO_{2peak}, peak oxygen uptake. * $p < 0.05$ compared to old.

Table 5.2. Kinetics parameters for VO_2, HR, in O and Y women and Δ[HHb] in Y women from pre-training through post-training.

		Pre-training	Week 3	Week 6	Week 9	Post-training
Phase II τVO_{2p} (s)	O#	55 ± 16	35 ± 12*	35 ± 10*	32 ± 12*†	33 ± 8*
	Y	31 ± 8	22 ± 4*	17 ± 5*	17 ± 5*†	17 ± 4*
τHR (s)	O#	62 ± 21	53 ± 13*‡	47 ± 12*†	41 ± 11*†	41 ± 14*†
	Y	45 ± 13‡	31 ± 11*	22 ± 7*†	26 ± 9*†‡	22 ± 4*†‡
τΔ[HHb] (s)	Y	17 ± 3	15 ± 7	12 ± 3	13 ± 7	11 ± 4
TD-Δ[HHb] (s)	Y	8 ± 4	7 ± 1	8 ± 2	8 ± 2	9 ± 1
τ'Δ[HHb] (s)	Y	25 ± 5	22 ± 7	20 ± 3	21 ± 7	21 ± 3‡

Values are means ± SD. HR, heart rate; HHb, deoxygenated hemoglobin; τ, time constant of response; TD, time delay; τ'Δ[HHb], sum of τΔ[HHb] and TD Δ[HHb]; * Significantly different from Pre-training values ($p < 0.05$); † Significantly different from Week 6 ($p < 0.05$); ‡ Significantly different from Phase II τVO_{2p} at the same testing time ($p < 0.05$); # Significantly different from Y ($p < 0.05$).

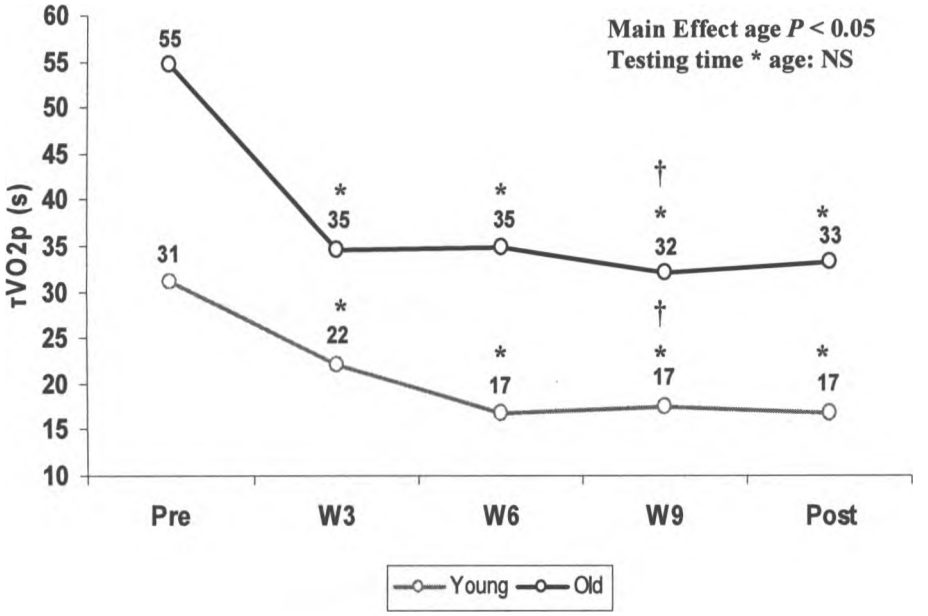

Figure 5.1. Changes in the phase II VO_2 time constant (τVO_{2p}) over the course of the endurance training program in older and young women.

* $p < 0.05$ to pre-training; † $p < 0.05$ to Week 3.

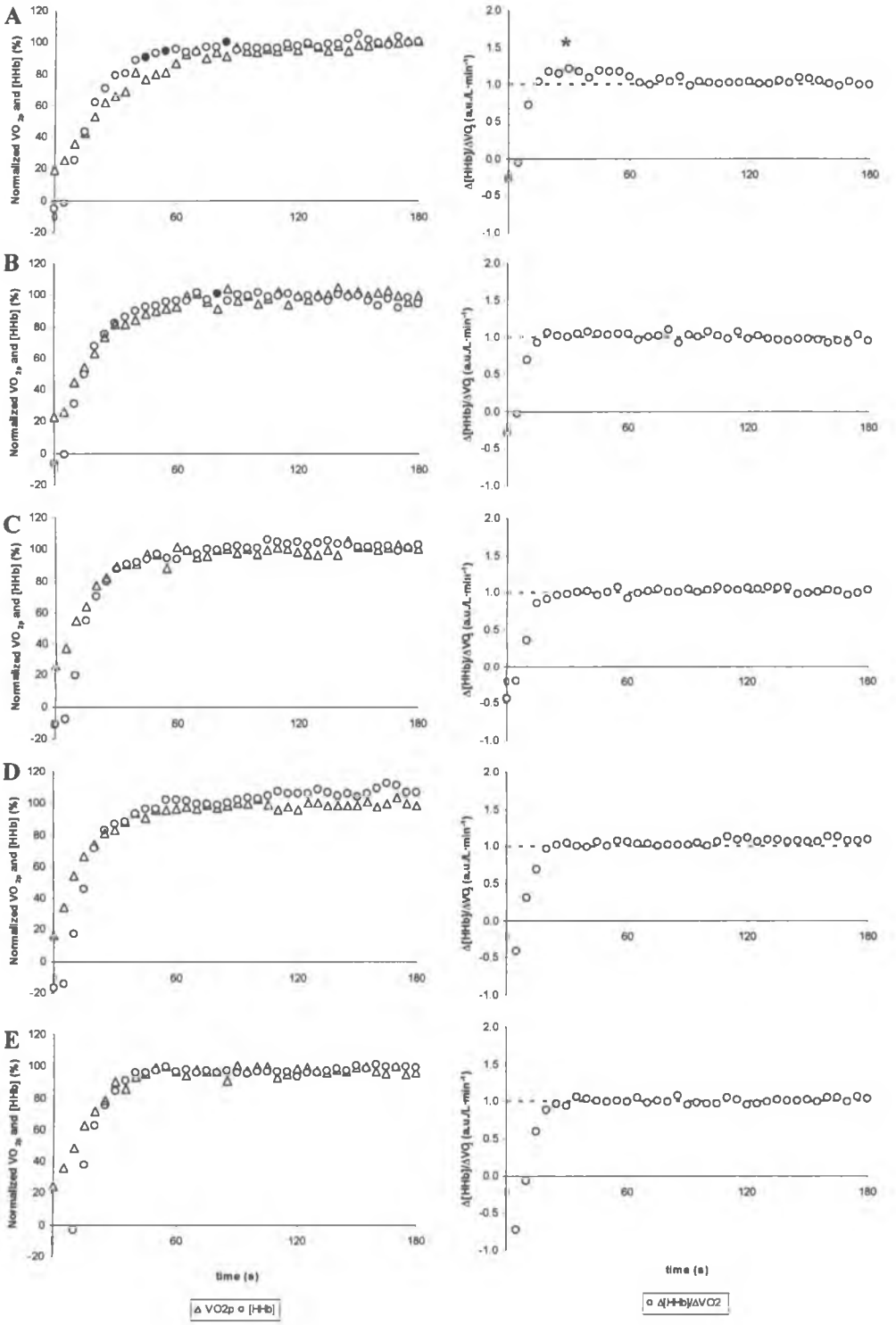

Figure 5.2. Panel 1, group mean profiles for the adjustment of Δ[HHb] (circles) and VO_{2p} (triangles; left shifted such that data from phase I VO_{2p} was not included) during the

initial 150 s of a step-transition in work rate in young women pre-training (A), at week 3 (B), week 6 (C), week 9 (D), and post-training (E). Filled circles denote time points at which the relative increase of Δ[HHb] is greater than the relative increase of VO_{2p} ($p < 0.05$). Panel 2, group mean profiles for the adjustment of $\Delta[HHb]/\Delta VO_{2p}$ during the initial 150 s of a step-transition in work rate in older adults pre-training (A), at week 3 (B), week 6 (C), week 9 (D), and post-training (E).

* $\Delta[HHb]/\Delta VO_{2p}$ significantly different from 1.0 ($p < 0.05$).

DISCUSSION

The main goal of this study was to investigate the time-course of adaptation of τVO_{2p} induced by endurance training in O and Y women. The main findings were as follows: 1) Older women had slower VO_{2p} kinetics than young women in response to step-transitions in work rate within the moderate intensity domain; 2) the τVO_{2p} was reduced as a consequence of endurance training in both age groups, with the greatest reduction in τVO_{2p} seen within the first 3 weeks of training; 3) contrary to our hypothesis after 3 weeks training in older women, the τVO_{2p} was reduced to the extent that it was similar to that observed in the younger women prior to the start of training; 4) in young women, the reduction in τVO_{2p} with an unchanged $\tau'\Delta[HHb]$ resulted in a reduction in the $\Delta[HHb]/\Delta VO_{2p}$ "overshoot" during the on-transient relative to the subsequent steady-state level, suggesting that the matching of microvascular blood flow-to-O_2 utilization was improved, thus requiring less reliance on O_2 extraction to support the muscle O_2 requirement during the exercise transient after 3 weeks of training. Additionally, based on our previously reported data which showed a greater submaximal arterial-venous O_2 differences ($a\text{-}vO_{2diff}$) for a given VO_{2p} in O compared to Y women (Murias et al. 2010a); see later discussion) it is suggested that an inadequate blood flow adjustment may limit VO_{2p} kinetics in O women as well.

This is the first study to explore the rate of adaptation with training of VO_{2p} kinetics in O and Y women. Previous studies have shown that endurance training results in a faster VO_{2p} kinetics in both O (Babcock et al. 1994a; Bell et al. 2001; Murias et al. 2010b) and Y (Fukuoka et al. 2002; McKay et al. 2009; Murias et al. 2010b; Phillips et al. 1995) men. These changes were shown to occur as quickly as 2 days (McKay et al. 2009) or 4 days (Phillips et al. 1995) after the start of a endurance training program, with improvements

continuing for 2 to 4 weeks of training but with no further changes observed thereafter (up to 12 weeks) (Fukuoka et al. 2002; Murias et al. 2010b; Phillips et al. 1995). The findings of the present study demonstrate that the training-induced adaptations reported in older and young men are also seen in O and Y women, with reductions in τVO_{2p} occurring rapidly and continuing for ~30 days after the start of an endurance training program.

It was hypothesized previously that a greater τVO_{2p} in older individuals could be related to a mismatch between O_2 delivery and O_2 utilization within the active muscles (DeLorey et al. 2004). Recently, we proposed that an improved microvascular O_2 distribution (as represented by a reduction in the transient $\Delta[HHb]/\Delta VO_{2p}$ "overshoot" relative to the subsequent steady-state level) was responsible for the reduction in τVO_{2p} in O and Y men (Murias et al. 2010b). This transient "overshoot" in the $\Delta[HHb]/\Delta VO_{2p}$ ratio (values > 1.0) is consistent with a greater microvascular fractional O_2 extraction per unit VO_{2p} compared to the exercise steady-state (values = 1.0), and reflects a lower microvascular blood flow and O_2 delivery relative to muscle O_2 utilization in the area of the NIRS probe (slower adjustment of microvascular blood flow). In the present study we speculated that older and young women would represent, respectively, an age group having an impaired or delayed blood flow adjustment at the start of exercise, and a group with a more rapid and adequate blood flow adjustment in response to exercise. This contrast between exercise blood flow responses in older compared to younger women thus would provide a model to study the adequacy of blood flow distribution in limiting the adjustment of muscle O_2 uptake (as reflected in VO_{2p} kinetics response). Pre-training data in Y women showed that with a tendency for a smaller $\tau'\Delta[HHb]$ compared to τVO_{2p} there was a small transient "overshoot" in $\Delta[HHb]/\Delta VO_{2p}$ (relative to steady-state values)

during the transition to moderate-intensity exercise (Table 5.2; Figure 5.2 Panels 1 and 2 A), but from 3 weeks to the end of training, the "overshoot" was abolished in young women (Table 5.2; Figure 5.2 Panels 1 and 2 B to E). The progressive reduction in τVO_{2p} over the course of training without a concomitant reduction in $\tau'\Delta$[HHb] suggests that a training-induced speeding of microvascular blood flow kinetics likely contributed to the faster adjustment of muscle O_2 utilization with training in young women. Indeed, post-training $\tau'\Delta$[HHb] was actually greater than τVO_{2p} suggesting that microvascular blood flow during the on-transient was actually in excess of that observed during the steady-state (Table 5.2). The training-induced changes in VO_{2p} and Δ[HHb] seen in young women in the present study are similar to those training-induced changes reported for young men in our previous study (Murias et al. 2010b). Parker et al. reported that leg blood flow and vascular conductance were greater in young women compared to young men (Parker et al. 2007) and to older women (Parker et al. 2008a). Taken together, these data support the idea that younger women have a greater blood flow and blood flow matching to O_2 utilization, such that at a given VO_2, O_2 extraction and a-vO_{2diff} are reduced.

In the present study, we were unable to detect any NIRS-derived signals in the older women. A methodological consideration related to the use of NIRS is that propagation of light through the tissue is influenced not only by the muscle but also by the subcutaneous fat. A thick adipose tissue layer observed in the O group (caliper-derived measurement of adipose thickness in the thigh in O in this study was ~37 ± 7 mm, whereas in young women thigh skinfold thickness is reported as being ~20 mm (Clasey et al. 1999)) results in greater "scattering" of light within the adipose tissue layer and less light returning to or detected by the receiving optode, thus lack of signal and erroneous measurements (van

Beekvelt et al. 2001). Nevertheless, as part of the overall study of endurance training in older and young women we measured cardiac output (Q), by open circuit acetylene method (Johnson et al. 2000) during the steady-state of moderate-intensity exercise (these data were detailed in another paper (Murias et al. 2010a)). In relation to the present analysis the calculated a-vO_{2diff} per VO_{2p} (a-vO_{2diff}/VO_{2p}), reflecting whole body O_2 extraction for a given VO_{2p}, can be used as a proxy for muscle O_2 extraction, and thus reflect muscle perfusion. Interestingly, in the moderate-intensity steady-state a-vO_{diff}/VO_{2p} was significantly higher in O compared to Y at any testing time throughout the training program (e.g., Pre-training: O, 41 ± 12 $mL \cdot 100mL^{-1}$ $blood \cdot (L \cdot min\ O_2)^{-1}$; Y, 22 ± 4 $mL \cdot 100mL^{-1}$ $blood \cdot (L \cdot min\ O_2)^{-1}$; Post-training: O, 40 ± 12 $mL \cdot 100mL^{-1}$ $blood \cdot (L \cdot min\ O_2)^{-1}$; Y, 22 ± 4 $mL \cdot 100mL^{-1}$ $blood \cdot (L \cdot min\ O_2)^{-1}$). Although this measure is based on systemic cardiac output and does not provide information during the transition to exercise, these measures demonstrate a greater reliance on O_2 extraction (i.e., a wider a-vO_{2diff}) for a given steady-state VO_2 during moderate-intensity exercise in older women, suggesting a lower blood flow in the active muscles. Others also have demonstrated a reduced leg blood flow and vascular responsiveness in older compared to young women and speculated on a greater reliance on O_2 extraction (Parker et al. 2008a; Proctor et al. 2003a). Parker et al. (Parker et al. 2008b) reported that the overall adjustment for O_2 extraction (derived from NIRS Δ[HHb] data) was not different in older and young women performing knee-extension exercise at different sub-maximal intensities; this similar overall Δ[HHb] adjustment in older and young women, in presence of a slower VO_{2p} adjustment in the O (as seen in this study) would result in a transient "overshoot" in $\Delta[HHb]/\Delta VO_{2p}$ relative to the steady-state situation, representing a poorer blood flow in the microvasculature during the exercise on-transient. Collectively,

these data of a greater reliance on O_2 extraction for a given VO_2 in older women suggest a blood flow and O_2 delivery constraint to VO_2 kinetics in the older women that seemed to be partially but not fully resolved with training.

Several studies have shown that endothelium-dependent and flow-mediated vasodilation are diminished in post-menopausal women because of the loss of circulating estrogens, and that hormone replacement therapy (HRT) results in a reduction or abolishment of these negative effects in the conduit vessels (Austin 2000; Moreau et al. 2003) as well as in the microvasculature (Peterson et al. 2000). As such, a poorer blood flow distribution within the microvasculature and likely a slower rate of adjustment for oxidative phosphorylation would be expected in older women not using HRT. However, recent data have shown no differences in τVO_{2p} between a control group of older women compared to a group receiving HRT (Stathokostas et al. 2009). Thus, a slower rate of VO_{2p} adjustment observed in the old women may not be related to estrogen deficiency. Other factors such as age-related increases in reactive oxygen species (ROS) (i.e., superoxide (O_2^-)) that could affect the NO-mediated signalling and bioavailability could be responsible for the sluggish vascular responsiveness (Taddei et al. 2000). Nevertheless, more research is needed investigating the age-related effects of loss of estrogens on control of exercise blood flow and its relationship with VO_2 kinetics.

An enhanced endothelium and flow-mediated vasodilatory response in both older and young adults may be one factor improving blood flow distribution and thus, speeding VO_{2p} kinetics. Older and middle-aged men involved in regular endurance exercise exhibit a greater ACh-mediated vasodilatory response as compared to their sedentary counterparts (DeSouza et al. 2000; Taddei et al. 2000). Similarly, endurance training restored the loss of endothelium-mediated vasodilation in old and middle-age men

(DeSouza et al. 2000). In animals, endothelium-dependent and flow-mediated vasodilation was reduced in feed arteries and 1A arterioles of oxidative muscles in old but not in young rats (Muller-Delp et al. 2002). Exercise training in older animals restored endothelium-dependent and flow-mediated vasodilation, and in young rats vascular responsiveness was improved beyond pre-training values (Spier et al. 2004; Spier et al. 2007). Also, endothelial responsiveness to ACh improves rapidly in response to acute and chronic exercise, with chronically trained animals having a two-fold larger and longer lasting (~1 week vs. 1-2 day) increase in vascular responsiveness compared to those animals evaluated after acute exercise (Haram et al. 2006). Taken together, these data suggest that an improved endothelium-dependent and flow-mediated vasodilation in response to training could result in improvements in blood flow distribution within the muscles. However, although these mechanisms are likely to occur in young women, they may not be that evident in older women (Parker and Proctor 2008; Parker et al. 2008a).

The rate of adjustment of τHR is often used as a proxy of central delivery of O_2 to the tissues. In this study, τHR was smaller after 3 weeks of training and then further decreased after 6 weeks of training in both O and Y women. Nevertheless, there was no relationship between changes in τHR and τVO_{2p} in either O or Y women. Also, young women had a significantly slower τHR pre-training, at week 9, and post-training compared to the τVO_{2p} adjustment; however, the estimated microvascular blood flow was improved with training to the point that an excess in blood flow during the exercise on-transient (relative to steady-state) was suggested post-training. In this regard, studies have shown that the adjustment of blood flow even in the conduit arteries (closer to the active muscles) differs from that seen in the microcirculation (Ferreira et al. 2005; MacPhee et al. 2005). These results show that muscle blood flow responses are not only mediated by

changes in cardiac output or conduit artery flow but also by the effects of the muscle pump and various vasoactive metabolites and hormones regulating the level of constriction and dilation in the vasculature (Dinenno et al. 1999; Dinenno et al. 2001; Muller-Delp 2006; Proctor and Parker 2006).

The mitochondrial content of ADP likely provides an important signal for activation of oxidative phosphorylation at the onset of exercise with the PCr shuttle (Whipp and Mahler 1980) serving as a spatial and temporal buffer attenuating the increase in intracellular ADP and thus slowing the adjustment of oxidative phosphorylation. Activation of intracellular enzymes to provide Acetyl CoA and electrons (in the form of reducing equivalents) to the tricarboxylic acid cycle and electron transport chain may also influence the rate of adjustment of muscle VO_2 kinetics (Grassi et al. 1998a; Grassi et al. 1998b; Grassi et al. 2002; Rossiter et al. 2003). Nevertheless, data from the present (and also from our previous (Murias et al. 2010b)) study suggest that VO_2 kinetics become faster through an improved microvascular O_2 delivery, as indicated by the measure of a better matched microvascular deoxygenation relative to the rate of adjustment of VO_2. In young women it appears that short-term training can remove any constraint imposed by O_2 delivery resulting in a τVO_2 in the ~20 s range, whereas in older women the τVO_2 was reduced from 55 s to 33 s, but the constraint to the rate of adjustment of VO_2 kinetics was not fully resolved.

In conclusion, this study demonstrated that pulmonary O_2 uptake (and muscle O_2 utilization) was faster in both O and Y women after only three weeks of endurance training with no significant changes observed thereafter. Additionally, 3 weeks of training resulted in the τVO_{2p} in O women being similar to that observed in Y women pre-training. Inequalities in O_2 distribution may contribute to the initial slower rate of

adjustment in τVO_{2p} in both O and Y women. Although these results are similar to those observed in Y and O men, our data indicated a better microvascular blood flow in Y women compared to Y men and also suggested a poorer O_2 distribution in O women. Although these data do not preclude that the fundamental control to VO_{2p} kinetics may be attributed to intracellular factors that were not measured in this study, they suggest that O_2 delivery appears to be a constraint in those with "slow" kinetics or indeed VO_2 kinetics of > ~20 s.

REFERENCES

Austin CE (2000) Chronic and acute effects of oestrogens on vascular contractility. J Hypertens 18: 1365-1378

Babcock MA, Paterson DH, Cunningham DA (1994a) Effects of aerobic endurance training on gas exchange kinetics of older men. Med Sci Sports Exerc 26: 447-452

Babcock MA, Paterson DH, Cunningham DA, Dickinson JR (1994b) Exercise on-transient gas exchange kinetics are slowed as a function of age. Med Sci Sports Exerc 26: 440-446

Beaver WL, Lamarra N, Wasserman K (1981) Breath-by-breath measurement of true alveolar gas exchange. J Appl Physiol 51: 1662-1675

Behnke BJ, Kindig CA, Musch TI, Koga S, Poole DC (2001) Dynamics of microvascular oxygen pressure across the rest-exercise transition in rat skeletal muscle. Respir Physiol 126: 53-63

Bell C, Paterson DH, Kowalchuk JM, Cunningham DA (1999) Oxygen uptake kinetics of older humans are slowed with age but are unaffected by hyperoxia. Exp Physiol 84: 747-759

Bell C, Paterson DH, Kowalchuk JM, Moy AP, Thorp DB, Noble EG, Taylor AW, Cunningham DA (2001) Determinants of oxygen uptake kinetics in older humans following single-limb endurance exercise training. Exp Physiol 86: 659-665

Chilibeck PD, Paterson DH, Petrella RJ, Cunningham DA (1996) The influence of age and cardiorespiratory fitness on kinetics of oxygen uptake. Can J Appl Physiol 21: 185-196

Clasey JL, Kanaley JA, Wideman L, Heymsfield SB, Teates CD, Gutgesell ME, Thorner MO, Hartman ML, Weltman A (1999) Validity of methods of body composition assessment in young and older men and women. J Appl Physiol 86: 1728-1738

DeLorey DS, Kowalchuk JM, Paterson DH (2003) Relationship between pulmonary O_2 uptake kinetics and muscle deoxygenation during moderate-intensity exercise. J Appl Physiol 95: 113-120

DeLorey DS, Kowalchuk JM, Paterson DH (2004) Effect of age on O_2 uptake kinetics and the adaptation of muscle deoxygenation at the onset of moderate-intensity cycling exercise. J Appl Physiol 97: 165-172

DeLorey DS, Kowalchuk JM, Paterson DH (2005) Adaptation of pulmonary O_2 uptake kinetics and muscle deoxygenation at the onset of heavy-intensity exercise in young and older adults. J Appl Physiol 98: 1697-1704

DeLorey DS, Paterson DH, Kowalchuk JM (2007) Effects of ageing on muscle O_2 utilization and muscle oxygenation during the transition to moderate-intensity exercise. Appl Physiol Nutr Metab 32: 1251-1262

DeSouza CA, Shapiro LF, Clevenger CM, Dinenno FA, Monahan KD, Tanaka H, Seals DR (2000) Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. Circulation 102: 1351-1357

Dinenno FA, Jones PP, Seals DR, Tanaka H (1999) Limb blood flow and vascular conductance are reduced with age in healthy humans: relation to elevations in sympathetic nerve activity and declines in oxygen demand. Circulation 100: 164-170

Dinenno FA, Tanaka H, Stauffer BL, Seals DR (2001) Reductions in basal limb blood flow and vascular conductance with human ageing: role for augmented alpha-adrenergic vasoconstriction. J Physiol 536: 977-983

Elwell C (1995) A Practical Users Guide To Near Infrared Spectroscopy., London: Hamamatsu Photonics KK

Ferreira LF, Townsend DK, Lutjemeier BJ, Barstow TJ (2005) Muscle capillary blood flow kinetics estimated from pulmonary O2 uptake and near-infrared spectroscopy. J Appl Physiol 98: 1820-1828

Fukuoka Y, Grassi B, Conti M, Guiducci D, Sutti M, Marconi C, Cerretelli P (2002) Early effects of exercise training on on- and off-kinetics in 50-year-old subjects. Pflugers Arch 443: 690-697

Grassi B (2001) Regulation of oxygen consumption at exercise onset: is it really controversial? Exerc Sport Sci Rev 29: 134-138

Grassi B, Gladden LB, Samaja M, Stary CM, Hogan MC (1998a) Faster adjustment of O_2 delivery does not affect VO_2 on-kinetics in isolated in situ canine muscle. J Appl Physiol 85: 1394-1403

Grassi B, Gladden LB, Stary CM, Wagner PD, Hogan MC (1998b) Peripheral O_2 diffusion does not affect VO_2on-kinetics in isolated insitu canine muscle. J Appl Physiol 85: 1404-1412

Grassi B, Hogan MC, Greenhaff PL, Hamann JJ, Kelley KM, Aschenbach WG, Constantin-Teodosiu D, Gladden LB (2002) Oxygen uptake on-kinetics in dog gastrocnemius in situ following activation of pyruvate dehydrogenase by dichloroacetate. J Physiol 538: 195-207

Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK, Wagner PD (1996) Muscle O2 uptake kinetics in humans: implications for metabolic control. J Appl Physiol 80: 988-998

Gurd BJ, Peters SJ, Heigenhauser GJ, LeBlanc PJ, Doherty TJ, Paterson DH, Kowalchuk JM (2008) O_2 uptake kinetics, pyruvate dehydrogenase activity, and muscle deoxygenation in young and older adults during the transition to moderate-intensity exercise. Am J Physiol Regul Integr Comp Physiol 294: R577-584

Gurd BJ, Scheid J, Paterson DH, Kowalchuk JM (2007) O_2 uptake and muscle deoxygenation kinetics during the transition to moderate-intensity exercise in different phases of the menstrual cycle in young adult females. Eur J Appl Physiol 101: 321-330

Haram PM, Adams V, Kemi OJ, Brubakk AO, Hambrecht R, Ellingsen O, Wisloff U (2006) Time-course of endothelial adaptation following acute and regular exercise. Eur J Cardiovasc Prev Rehabil 13: 585-591

Hughson RL, Tschakovsky ME, Houston ME (2001) Regulation of oxygen consumption at the onset of exercise. Exerc Sport Sci Rev 29: 129-133

Johnson BD, Beck KC, Proctor DN, Miller J, Dietz NM, Joyner MJ (2000) Cardiac output during exercise by the open circuit acetylene washin method: comparison with direct Fick. J Appl Physiol 88: 1650-1658

MacPhee SL, Shoemaker JK, Paterson DH, Kowalchuk JM (2005) Kinetics of O_2 uptake, leg blood flow, and muscle deoxygenation are slowed in the upper compared with lower region of the moderate-intensity exercise domain. J Appl Physiol 99: 1822-1834

McKay BR, Paterson DH, Kowalchuk JM (2009) Effect of short-term high-intensity interval training vs. continuous training on O_2 uptake kinetics, muscle deoxygenation, and exercise performance. J Appl Physiol 107: 128-138

Moreau KL, Donato AJ, Tanaka H, Jones PP, Gates PE, Seals DR (2003) Basal leg blood flow in healthy women is related to age and hormone replacement therapy status. J Physiol 547: 309-316

Muller-Delp JM (2006) Aging-induced adaptations of microvascular reactivity. Microcirculation 13: 301-314

Muller-Delp JM, Spier SA, Ramsey MW, Delp MD (2002) Aging impairs endothelium-dependent vasodilation in rat skeletal muscle arterioles. Am J Physiol Heart Circ Physiol 283: H1662-1672

Murias JM, Kowalchuk JM, Paterson DH (2010a) Mechanisms for increases in VO_{2max} with endurance training in older and young women. Med Sci Sports Exerc (In Press)

Murias JM, Kowalchuk JM, Paterson DH (2010b) Speeding of VO_2 kinetics with endurance training in old and young men is associated with improved matching of local O_2 delivery to muscle O_2 utilization. J Appl Physiol (In Press)

Parker B, Proctor D (2008) Commentary on Viewpoint: Exercise and cardiovascular risk reduction: time to update the rationale for exercise? Considering the role of sex in modulating direct effects of exercise on the vasculature. J Appl Physiol 105: 778

Parker BA, Smithmyer SL, Pelberg JA, Mishkin AD, Herr MD, Proctor DN (2007) Sex differences in leg vasodilation during graded knee extensor exercise in young adults. J Appl Physiol 103: 1583-1591

Parker BA, Smithmyer SL, Pelberg JA, Mishkin AD, Proctor DN (2008a) Sex-specific influence of aging on exercising leg blood flow. J Appl Physiol 104: 655-664

Parker BA, Smithmyer SL, Ridout SJ, Ray CA, Proctor DN (2008b) Age and microvascular responses to knee extensor exercise in women. Eur J Appl Physiol 103: 343-351

Peterson LR, Courtois M, Peterson LF, Peterson MR, Davila-Roman VG, Spina RJ, Barzilai B (2000) Estrogen increases hyperemic microvascular blood flow velocity in postmenopausal women. J Gerontol A Biol Sci Med Sci 55: M174-179

Phillips SM, Green HJ, MacDonald MJ, Hughson RL (1995) Progressive effect of endurance training on VO_2 kinetics at the onset of submaximal exercise. J Appl Physiol 79: 1914-1920

Poole DC, Ferreira LF, Behnke BJ, Barstow TJ, Jones AM (2007) The final frontier: oxygen flux into muscle at exercise onset. Exerc Sport Sci Rev 35: 166-173

Proctor DN, Koch DW, Newcomer SC, Le KU, Leuenberger UA (2003a) Impaired leg vasodilation during dynamic exercise in healthy older women. J Appl Physiol 95: 1963-1970

Proctor DN, Newcomer SC, Koch DW, Le KU, MacLean DA, Leuenberger UA (2003b) Leg blood flow during submaximal cycle ergometry is not reduced in healthy older normally active men. J Appl Physiol 94: 1859-1869

Proctor DN, Parker BA (2006) Vasodilation and vascular control in contracting muscle of the aging human. Microcirculation 13: 315-327

Rossiter HB, Ward SA, Howe FA, Wood DM, Kowalchuk JM, Griffiths JR, Whipp BJ (2003) Effects of dichloroacetate on VO_2 and intramuscular ^{31}P metabolite kinetics during high-intensity exercise in humans. J Appl Physiol 95: 1105-1115

Spier SA, Delp MD, Meininger CJ, Donato AJ, Ramsey MW, Muller-Delp JM (2004) Effects of ageing and exercise training on endothelium-dependent vasodilatation and structure of rat skeletal muscle arterioles. J Physiol 556: 947-958

Spier SA, Delp MD, Stallone JN, Dominguez JM, 2nd, Muller-Delp JM (2007) Exercise training enhances flow-induced vasodilation in skeletal muscle resistance arteries of aged rats: role of PGI2 and nitric oxide. Am J Physiol Heart Circ Physiol 292: H3119-3127

Spina RJ, Ogawa T, Kohrt WM, Martin WH, 3rd, Holloszy JO, Ehsani AA (1993) Differences in cardiovascular adaptations to endurance exercise training between older men and women. J Appl Physiol 75: 849-855

Stathokostas L, Kowalchuk JM, Petrella RJ, Paterson DH (2008) Maximal and submaximal aerobic fitness in postmenopausal women: influence of hormone-replacement therapy. Appl Physiol Nutr Metab 33: 922-928

Stathokostas L, Kowalchuk JM, Petrella RJ, Paterson DH (2009) Moderate and heavy oxygen uptake kinetics in postmenopausal women. Appl Physiol Nutr Metab 34: 1065-1072

Taddei S, Galetta F, Virdis A, Ghiadoni L, Salvetti G, Franzoni F, Giusti C, Salvetti A (2000) Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes. Circulation 101: 2896-2901

Tschakovsky ME, Hughson RL (1999) Interaction of factors determining oxygen uptake at the onset of exercise. J Appl Physiol 86: 1101-1113

van Beekvelt MC, Borghuis MS, van Engelen BG, Wevers RA, Colier WN (2001) Adipose tissue thickness affects in vivo quantitative near-IR spectroscopy in human skeletal muscle. Clin Sci (Lond) 101: 21-28

Whipp BJ, Mahler M (1980) Dynamics of pulmonary gas exchange during exercise. In: West JB (ed) Pulmonary Gas Exchange, Vol II, Academic, New York, pp. 33-95

CHAPTER VI: Summary, limitations and future directions

SUMMARY

The overall goal of this thesis was to examine which cardiovascular adaptations were responsible for the expected increases in VO_{2max} and the faster rate of adjustment for VO_2 kinetics in response to a short-term endurance training program in older and young men and women.

Chapters II and III determined the time-course and mechanisms of adaptations in cardiorespiratory fitness in response to endurance training in older and young men, and older and young women, respectively. Previous studies had reported that cardiovascular responses to exercise could be not only age- but also sex-dependent (Parker et al. 2008) and that different strategies in terms of the mechanisms explaining increases in VO_{2max} were used in older men compared to older women (Spina et al. 1993), at least in response to long-duration endurance-training programs (6-12 months). We were interested in determining if the rate of increase in VO_{2max} as well as the mechanisms responsible for that increase were similar in response to a short-term endurance training program compared to those reported previously for long-term endurance training (Ehsani et al. 1991; Kohrt et al. 1991; Spina et al. 1996; Spina et al. 1993).

The main findings from chapter II were that: 1) although the percentage increase in VO_{2max} was significantly larger in older men (31±10%) than in young men (18±10%), a short-term training program with progressive increases in exercise intensity yielded significant increases in VO_{2max} in both older and young men that were similar to or even higher than those reported for long-term endurance training programs (Ehsani et al. 1991; Kohrt et al. 1991; Makrides et al. 1990; Seals et al. 1984; Spina et al. 1993); 2) the time-

course of increases in VO_{2max} was similar in older and young men; however, the mechanisms explaining the time-course of this increase were different such that older men showed consistent improvements in Q_{max} (explaining ~2/3 of the increase in VO_{2max}) throughout the training program, whereas the young men initially relied on increases in maximal a-vO_{2diff} (first 3 weeks) with further increases in aerobic power being explained exclusively by a larger Q_{max}.

The conclusions from chapter III were as follows: 1) even though the overall percent change in VO_{2max} from pre- to post-training was similar in older (17±14%) and young (22±6%) women, the time-course of increases in VO_{2max} was similar only up to 9 weeks of training with VO_{2max} only increasing in the young women during the final 3 weeks of training; 2) as previously suggested in response to long-term endurance training (Spina et al. 1993) the majority of the increase in VO_{2max} from pre- to post-training was explained by a widened maximal a-vO_{2diff} in older women (~2/3 of the improvement), whereas young women relied equally in increases in Q_{max} and maximal a-vO_{2diff} in order to increase their VO_{2max}.

What is novel about these two studies is that they showed that a short-term endurance training program can be as effective as a long-duration training intervention in older and young men and women. Another important addition to the literature is that although these studies confirm central and peripheral contributions to the increase in VO_{2max} previously reported for older and young men and women, they also describe the time-course of those changes within a 12-week period of training. This is relevant for adequate design of endurance-training programs targeting specific groups.

In chapters IV and V the focus of this thesis was the study of the responses of short-term endurance training on VO_2 kinetics in older and young men and women. Within the

past years, several studies have suggested that O_2 distribution within the active tissues could play an important role in controlling the rate of adjustment of VO_2 (DeLorey et al. 2004; Hughson et al. 2001; Tschakovsky and Hughson 1999). Also, some studies have demonstrated that endurance training results in ameliorated vascular responsiveness (DeSouza et al. 2000; Green et al. 2004; Spier et al. 2004; Spier et al. 2007) which could result in improved O_2 distribution within the muscles and thus a smaller τVO_2. As such, the studies described in these two chapters aimed to gain further understanding about the role of the matching of O_2 delivery to muscle VO_2 (as interpreted from the changes in the normalized $\Delta[HHb]/\Delta VO_2$ ratio) as a mechanism controlling the rate of adjustment of VO_2 kinetics.

In chapter IV it was concluded that: 1) the decrease in τVO_{2p} in both older and young men occurred within the first 3 weeks of training with no subsequent changes observed thereafter; 2) an improved $\Delta[HHb]/\Delta VO_{2p}$ ratio (reflecting a better matching of O_2 delivery to O_2 utilization within the tissues) was associated with the reduction in τVO_{2p} observed in both older and young men.

Chapter V similarly concluded that: 1) pulmonary O_2 uptake (reflecting muscle O_2 utilization) adjusted more rapidly in both older and young women after only 3 weeks of endurance training with no significant changes observed thereafter; 2) inequalities in O_2 distribution may contribute to the initial slower rate of adjustment in τVO_{2p} in both older and young women. Taken together, these two manuscripts suggested that, although the fundamental control of VO_{2p} kinetics may take place intracellularly by factors that were not measured in these studies, O_2 delivery appears to be a constraint in those with "slow" kinetics, or in fact VO_2 kinetics of $> \sim 20$ s.

The most relevant additions to the field of VO_2 kinetics from these studies are confirming that changes in response to endurance training occur rapidly (within the first 3 weeks) with no further changes observed thereafter, and that improved matching of O_2 delivery to muscle VO_2 is an important mechanism controlling the rate of adjustment of VO_2 kinetics. Despite the limitations imposed by age, these observations seem to apply to both older and young individuals.

In summary, these series of studies demonstrated that the magnitude of increases in VO_{2max} and speeding of VO_2 kinetics are similar in older and young men and women. Interestingly, although older men and young men and women displayed a rather similar time-course of adaptations in response to a 12-week endurance training program, older women showed a plateau-like response in VO_{2max} after 9 weeks of training and marked reliance on peripheral changes related to increased O_2 extraction as previously proposed (Spina et al. 1993).

LIMITATIONS

One limitation in the present study is that the changes observed in systemic a-vO_{2diff} are derived from measurements of systemic Q using an indirect methodology that is subject to variability. However, the C_2H_2 open circuit technique has been validated (Johnson et al. 2000) and we have been able to repeat measurements with a variability of ~1 $L \cdot min^{-1}$.

Another limitation in this set of studies is related to the use of NIRS in that the area of muscle "interrogation" represents only a small region over the surface of the active muscle (quadriceps) to examine the rate of adjustment of Δ[HHb]. Although some studies have shown that the magnitude and time-course of the Δ[HHb] signal remain unaltered within different portions of the vastus lateralis muscle (duManoir et al. 2010), heterogeneities have been shown to exist from one site of inspection to another (Koga et

al. 2007). Additionally, with the NIRS system used in these studies we were restricted to assumptions of the optical path length of the near-infrared light (which may be affected by local blood flow and cellular volume and ionic changes occurring during muscle contractions (Hamaoka et al. 2007). As such, data were expressed in arbitrary units. Nevertheless, this limitation becomes minimized by the fact that the Δ[HHb] data were normalized for each individual as a proportion of the full-scale amplitude of the signal from the loadless cycling to steady-state for comparison of its rate of adjustment compared with that of VO_{2p}.

In the studies in chapter IV and V, observations were made in terms of an improved matching of muscle O_2 delivery to muscle VO_2. These observations rely on the interpretation of the $\Delta[HHb]/\Delta VO_{2p}$ data. We used Δ[HHb] as a proxy variable for muscle O_2 extraction, thus reflecting changes in the a-vO_{2diff}. Changes in the rate of adjustment of the normalized VO_{2p} (representing muscle VO_2) in relation to the responses of the rate of adjustment of the normalized Δ[HHb] are likely determined by changes in the rate of adjustment of muscle blood flow. However, no direct measurements of muscle O_2 delivery were made.

It was proposed that O_2 distribution within the active muscles plays an important role in the rate of adjustment of VO_2, especially in those subjects displaying a τVO_{2p} larger than ~20 s. Also, it was suggested that the fundamental control of VO_2 kinetics may reside intracellularly. However, no measurements were taken during the on-transient of exercise that could provide with a better understanding of what those intracellular components could be.

FUTURE DIRECTIONS

The studies described in chapters II and III suggested that moderately vigorous to vigorous intensities are important for maintaining the increases in VO_{2max} in response to training in both older and young subjects. Although a HIT intervention was included during the final weeks of the endurance training program, these studies were not powered to actually determine the sex by testing time interaction. Additionally, only 2 weeks of HIT training were performed. Based on the positive results observed in young individuals (Gibala et al. 2006; Laursen and Jenkins 2002; McKay et al. 2009) and the initial observations from this thesis, a study designed to compare HIT versus CT in older and young subjects would seem appropriate.

Our data from chapters IV and V suggest that muscle O_2 distribution is a key factor determining the rate of adjustment of VO_2 kinetics (at least when τVO_{2p} is larger than ~20 s). A logical progression would be to examine the rate of adjustment of VO_{2p} in subjects with a "slow" VO_2 kinetics before and after an intervention aimed to increase blood flow in the periphery, perhaps by drug-induced vasodilation (e.g., tadalafil, sildenafil, or vardenafil).

REFERENCES

DeLorey DS, Kowalchuk JM, Paterson DH (2004) Effect of age on O_2 uptake kinetics and the adaptation of muscle deoxygenation at the onset of moderate-intensity cycling exercise. J Appl Physiol 97: 165-172

DeSouza CA, Shapiro LF, Clevenger CM, Dinenno FA, Monahan KD, Tanaka H, Seals DR (2000) Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. Circulation 102: 1351-1357

duManoir GR, DeLorey DS, Kowalchuk JM, Paterson DH (2010) Kinetics of VO_2 limb blood flow and regional muscle deoxygenation in young adults during moderate intensity, knee-extension exercise. Eur J Appl Physiol 108: 607-617

Ehsani AA, Ogawa T, Miller TR, Spina RJ, Jilka SM (1991) Exercise training improves left ventricular systolic function in older men. Circulation 83: 96-103

Gibala MJ, Little JP, van Essen M, Wilkin GP, Burgomaster KA, Safdar A, Raha S, Tarnopolsky MA (2006) Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. J Physiol 575: 901-911

Green DJ, Maiorana A, O'Driscoll G, Taylor R (2004) Effect of exercise training on endothelium-derived nitric oxide function in humans. J Physiol 561: 1-25

Hamaoka T, McCully KK, Quaresima V, Yamamoto K, Chance B (2007) Near-infrared spectroscopy/imaging for monitoring muscle oxygenation and oxidative metabolism in healthy and diseased humans. J Biomed Opt 12: 062105

Hughson RL, Tschakovsky ME, Houston ME (2001) Regulation of oxygen consumption at the onset of exercise. Exerc Sport Sci Rev 29: 129-133

Johnson BD, Beck KC, Proctor DN, Miller J, Dietz NM, Joyner MJ (2000) Cardiac output during exercise by the open circuit acetylene washin method: comparison with direct Fick. J Appl Physiol 88: 1650-1658

Koga S, Poole DC, Ferreira LF, Whipp BJ, Kondo N, Saitoh T, Ohmae E, Barstow TJ (2007) Spatial heterogeneity of quadriceps muscle deoxygenation kinetics during cycle exercise. J Appl Physiol 103: 2049-2056

Kohrt WM, Malley MT, Coggan AR, Spina RJ, Ogawa T, Ehsani AA, Bourey RE, Martin WH, 3rd, Holloszy JO (1991) Effects of gender, age, and fitness level on response of VO_{2max} to training in 60-71 yr olds. J Appl Physiol 71: 2004-2011

Laursen PB, Jenkins DG (2002) The scientific basis for high-intensity interval training: optimising training programmes and maximising performance in highly trained endurance athletes. Sports Med 32: 53-73

Makrides L, Heigenhauser GJ, Jones NL (1990) High-intensity endurance training in 20- to 30- and 60- to 70-yr-old healthy men. J Appl Physiol 69: 1792-1798

McKay BR, Paterson DH, Kowalchuk JM (2009) Effect of short-term high-intensity interval training vs. continuous training on O_2 uptake kinetics, muscle deoxygenation, and exercise performance. J Appl Physiol 107: 128-138

Parker BA, Smithmyer SL, Pelberg JA, Mishkin AD, Proctor DN (2008) Sex-specific influence of aging on exercising leg blood flow. J Appl Physiol 104: 655-664

Seals DR, Hagberg JM, Hurley BF, Ehsani AA, Holloszy JO (1984) Endurance training in older men and women. I. Cardiovascular responses to exercise. J Appl Physiol 57: 1024-1029

Spier SA, Delp MD, Meininger CJ, Donato AJ, Ramsey MW, Muller-Delp JM (2004) Effects of ageing and exercise training on endothelium-dependent vasodilatation and structure of rat skeletal muscle arterioles. J Physiol 556: 947-958

Spier SA, Delp MD, Stallone JN, Dominguez JM, 2nd, Muller-Delp JM (2007) Exercise training enhances flow-induced vasodilation in skeletal muscle resistance arteries of aged rats: role of PGI2 and nitric oxide. Am J Physiol Heart Circ Physiol 292: H3119-3127

Spina RJ, Miller TR, Bogenhagen WH, Schechtman KB, Ehsani AA (1996) Gender-related differences in left ventricular filling dynamics in older subjects after endurance exercise training. J Gerontol A Biol Sci Med Sci 51: B232-237

Spina RJ, Ogawa T, Kohrt WM, Martin WH, 3rd, Holloszy JO, Ehsani AA (1993) Differences in cardiovascular adaptations to endurance exercise training between older men and women. J Appl Physiol 75: 849-855

Tschakovsky ME, Hughson RL (1999) Interaction of factors determining oxygen uptake at the onset of exercise. J Appl Physiol 86: 1101-1113

APPENDIX I: Copy of ethics approval

Western

Office of Research Ethics
The University of Western Ontario
Room 00045 Dental Sciences Building, London, ON, Canada N6A 5C1
Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. D.H. Paterson
Review Number: 13244 **Revision Number:** 1
Review Date: July 26, 2007 **Review Level:** Expedited
Protocol Title: Exercise training adaptations in VO2 kinetics, cardiac output, muscle deoxygenation and capillarization in older adults
Department and Institution: Kinesiology, University of Western Ontario
Sponsor:
Ethics Approval Date: August 2, 2007 **Expiry Date:** April 30, 2009
Documents Reviewed and Approved: revised study methodology, revised inclusion/exclusion criteria, revised sample size, revised Letter of Information and Consent, and revised study advertisements
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

[redacted]

Chair of HSREB: Dr. John W. McDonald
Deputy Chair: Susan Hoddinott

Ethics Officer to Contact for Further Information

☑ Jennifer McEwen	☐ Denise Grafton (	☐ Ethics Officer

cc: ORE File

This is an official document. Please retain the original in your files.

APPENDIX II: Copy of letter of information and consent form

LETTER OF INFORMATION

Exercise training adaptations in VO_2 kinetics, cardiac output, muscle deoxygenation and capillarization in older adults.

Principal Investigator: Donald H. Paterson, PhD

Purpose of the Study:

You are being invited to participate in a study that examines how much and how quickly the aerobic (or oxygen) system in your body adapts to exercise training. This study will compare responses in young adults (18-40 yr old) and older adults (60-85 yr old). Endurance exercise training has been shown to improve aerobic fitness as well as to increase the speed at which the aerobic system is activated at the onset of exercise. Central (i.e. blood delivery from the heart to the muscles) and/or peripheral (i.e. O_2 utilized by the muscle) factors may both be involved in these improvements. However, the time at which changes occur during the exercise training program and differences within each age group need further study.

Participation in this study requires you to visit the research laboratory at the Canadian Centre for Activity and Aging (Arthur and Sonia Labatt Health Science Centre, Room 313) on a maximum of 65 different occasions for a length of approximately 25 weeks (45 training sessions plus approximately 15-20 testing sessions, depending on your time availability during each one of these). Taking part in this study represents an extensive time commitment. However, each training session will not last longer than 60 minutes and the testing sessions will last a maximum of 3 hours.

A total of 24-36 adults (12-18 young and 12-18 older) will be invited to participate in this study. In order to participate you must be between 18-40 (young) or 60-85 (older) years of age and healthy. You will not be able to participate in the study if you have been diagnosed previously with any respiratory (i.e. chronic obstructive pulmonary disease), cardiovascular (i.e. coronary heart disease), metabolic (i.e. diabetes) or neurological (i.e. Parkinson's disease) disease; or you are currently taking prescribed medication that may affect your cardiovascular responses to exercise; or you are a smoker; or you respond to the exercise protocol in an irregular manner (i.e. chest pains, nausea, dizziness, shortness of breath, excessive awareness of breathing, or inability to maintain required pedal cadence – represented by the revolutions per minute at which the cycle pedals spin while you exercise); or cannot tolerate the exercise protocol.

Research Testing Protocol:

During the first visit to the laboratory you will complete an incremental test on a stationary bicycle, which is a test to measure your maximal aerobic fitness level. In an incremental test the intensity of exercise increases gradually throughout the test until you

are physically unable to continue exercising because the intensity is either too high or too uncomfortable. The test will begin with the exercise intensity being very light and easy (very little resistance) and then, the exercise intensity will gradually and continuously increase until you are unable to continue because of fatigue, or until you wish to stop. This visit should last approximately 1 hour.

On a subsequent visit, a series of 4 moderate-intensity tests will be completed (repeat testing is required in order to ensure the accuracy and reliability of the data). You will begin by riding the stationary bicycle at a very light intensity (baseline exercise at 20 watts, which would be equivalent to riding your own bicycle at a very slow speed). After this accommodation period, the intensity will be increased to a level considered to be moderate-intensity exercise (exercise in the moderate domain could theoretically be performed indefinitely and should not produce signs of fatigue). You will ride at this intensity for 6 minutes, followed by a 6 minute cool-down at a light intensity and then another moderate intensity ride. You then will be given a 20 min rest, after which you will be asked to repeat the same two moderate-intensity exercises.

At the end of this part of the protocol you will move to a chair and, after a brief period of rest (approximately 10 minutes) you will be asked to perform a maximal voluntary contraction (MVC). The MVC will involve a maximal contraction of your leg where you will try to "push" your foot into the floor as hard as possible until the investigator tells you to relax. This maneuver will allow us to measure the highest and lowest levels of oxygen within your thigh muscle.

On another day (this could be either before or after the moderate-step tests) two resting muscle biopsies will be obtained from your thigh muscle (quadriceps). Analysis of these samples will allow us to know the activity of enzymes regulating aerobic metabolism and the amount of blood vessels (capillaries) supplying your muscles with oxygen. Additionally, some measures of your heart will be performed by using a non-invasive technique (Echocardiography or Ultrasound). This technique is similar to that used by obstetricians to see a fetus inside the womb.

During the incremental and the moderate-intensity tests you will be asked to perform some breathing maneuvers where you will breathe a known concentration of carbon dioxide, oxygen, and/or acetylene for approximately 10-20 seconds for each maneuver. This will allow us to estimate your cardiac output (i.e. the amount of blood pumped out by your heart over a given period of time) at different exercise intensities.

Before starting the exercise training program, 25 mL or approximately 5 teaspoons of venous blood will be withdrawn from your forearm.
The maximal incremental test, the 4 moderate-intensity tests, venous blood sampling, and heart measurements will be repeated after 3, 7, 11, and 15 weeks of training. Muscles biopsies will be taken again from your thigh muscle after 7 and 15 weeks (mid- and post-training). Five and 10 weeks after the exercise training program is completed, you will be asked to repeat all testing procedures.

Exercise Training:

All exercise training will be performed on a stationary bicycle. You will be training for 15 consecutive weeks. During the first 11 weeks, the training intensity will represent 70% of your performance during the maximal incremental test. Although more intense than the exercise you performed during the moderate-intensity tests, this exercise intensity is expected to produce fatigue only after approximately 1-2 hours of exercise. For the remaining 4 weeks of training you will either continue with the same training as you have been performing or you will be assigned to a higher intensity training group. This higher intensity training will consist of bouts of exercise (i.e.: 1 minute pedaling against resistance and 1 minute of unloaded pedaling) at approximately 90% of your maximal capacity as evaluated during the incremental test. Although more demanding than the continuous training previously performed, the pauses in between bouts of exercise and the shorter total duration of the workout will allow you to satisfactorily complete each session. Training sessions will be performed 3 times a week and you will exercise for approximately 25-35 min during the first 2 weeks, with duration progressively increasing until you are able to train continuously for 45 min. If you are part of the group exercising at a higher intensity, the training session will have a total duration of approximately 25-30 minutes for the last 4 weeks of training.

Research Procedures:

During each of the exercise tests you will be required to wear a nose-clip (to prevent you from breathing through your nose) and a rubber mouthpiece (similar to breathing through a snorkel or diving mask). This will enable us to measure the air that you breathe in and out. You may experience some initial discomfort from wearing the nose-clip and mouthpiece.

During each of the exercise tests the oxygenation of your leg muscle will be measured using near-infrared spectroscopy which projects light into a specific location of your leg muscles and measures the amount of light coming out at another location. A small piece of equipment will be placed on your leg approximately midway between your hip and your knee. It will be secured with tape, covered to prevent light from entering or leaving the area, and bound with elastic bandage to minimize movement. You might experience a bit of discomfort by having this equipment secured to your leg during the exercise period. However, this is a non-invasive procedure. Additionally, oxygenation of your blood will be measured using infrared oximetry (a non-invasive measure similar to that performed by nurses when you go to visit a doctor at the hospital) with the probe clipped onto your earlobe or finger.

During 5 visits (pre-training, midway through training, immediately after training, 5 and 10 weeks after training was completed), muscle will be removed from your thigh (quadriceps) by means of a needle biopsy. Muscle biopsies will be taken by a physician trained in this technique. While you are resting quietly on a bed, an anesthetic will be applied locally to freeze the skin over your thigh muscle at the sites where the biopsies will be taken. During each of these visits, two small incisions (approximately 1 cm) will be made through your skin and into your muscle at a point approximately midway

between your hip and knee. There may be some discomfort associated with the biopsy procedure (like someone pressing hard into your muscle) but you should experience no pain. There is some blood loss associated with the biopsy procedure (less than 1 mL per biopsy; 0.2 teaspoon; 0.033 fluid oz.). There may be light bruising of the leg muscle but this will generally fade within a couple of days. **Please refer to the Muscle Biopsy Information Sheet for more information regarding this procedure**.

During the study, cardiac output (i.e. the amount of blood pumped out by your heart over a given period of time) will be measured non-invasively at rest and during exercise using the acetylene (C_2H_2) open-circuit and/or CO_2 rebreathing techniques. With the open circuit technique you will complete approximately 10 breathing cycles inhaling from a bag containing a known concentration of gases and exhaling to the room. With the rebreathing techniques, you will be breathing into and out of a bag (rebreathing) also containing a known concentration of gases. Each rebreathing maneuver will last approximately 10-20 s, after which you will start breathing room air again.

Heart rate and rhythm will be continuously monitored by electrocardiogram. One electrode will be placed on each of the following areas: left chest, right chest, and left side under your ribs and connected to an electrocardiograph. The electrodes use adhesive tape to secure to the skin. There are no known risks or discomforts associated with this procedure.

During the incremental and the moderate-intensity tests, blood lactate concentration will be measured by means of a portable lactate analyzer. A drop of blood from one of your fingertips (approximately 25 μL) will be taken for each analysis. The tip of the finger will be pricked with a lancet at the end of each minute during exercise.

As described before, venous blood samples will be withdrawn from your forearm during this study. A total amount of 25 mL of blood (approximately 5 teaspoons) will be withdrawn to run a complete blood count and to examine your liver function, lipid profile, and HbA1C (glycosylated hemoglobin).

Possible Risks and Discomforts:

Any exercise carries a slight risk of heart attack or may be uncomfortable if you are unfit or not used to exercise. The risk of a cardiac event (heart attack, dysrhythmias, etc.) in a mixed subject population (healthy low risk and unhealthy high risk patients together) is approximately 6:10,000; however, the risk decreases in a previously healthy (i.e. young moderately active) population (adapted from ACSM's Guidelines for Exercise Testing and Prescription). There might be some minor discomfort during the exercise testing. You may experience increased awareness of breathing, muscle pain and/or fatigue, increased sweating, or a general feeling of fatigue or nausea, all of which are not unexpected consequences of exercise.

The local anesthetic or "freezing" used in the biopsy procedure is the same anesthetic used for most surgical or dental procedures performed under "local" anesthesia. The risk of allergic reaction or other untoward effect is estimated at less than 1:10,000.

If the site of muscle biopsy becomes more tender and redness and/or swelling develops in that area over the 5-7 days after the biopsy you should seek medical attention immediately. You should also report this change to the research person supervising your study as soon as possible.

During the CO_2 rebreathe you may experience some light headedness or dizziness due to the higher level of inhaled CO_2. You also may begin to feel a bit warmer during this time. This is a normal response and will disappear within 6-10 seconds once breathing from room air is restored.

There may be some pain or discomfort related to the venous blood sampling and/or fingertip prick.

Participation in this study requires an extensive time commitment which may be inconvenient for you at some point during the study.

Benefits of Participation:

This is a basic physiology/biochemistry study and, as such, there will be no direct benefits received as a consequence of participating in the study. However, due to the nature of the exercise training there may be some beneficial cardiovascular adaptations (increased fitness); however, these may only be temporary and disappear within a few weeks of the completion of the study. If you are interested, the rational for conducting the research and theory and significance of each of the tests will be explained, as will your individual results from each of the tests. You will also have the opportunity to learn about and better understand your physiological responses to an exercise situation.

Other Pertinent Information:

You are encouraged to ask questions regarding the purpose of the study, specific measures or outcomes of your exercise test, or overall findings and conclusions from this research study.

Confidentiality:

Records from the study are confidential and will be stored securely at the testing facility. They will be available for analysis within the research group. Although biopsies will be analyzed in a laboratory at University of Calgary, the results will be sent and stored in our laboratory in the Canadian Centre for Activity and Aging. No other agencies or individuals will have access to the collected data. Your records are listed according to an identification number rather than by your name. Published reports resulting from this study will not identify you by name.

Voluntary Participation:

Participation in this study is voluntary. You may refuse to participate or withdraw from the study at any time with no effect on your future care and/or academic or employment status.

You will be given a copy of this letter of information and signed consent forms. You do not waive any legal rights by signing the consent form. If you have any questions regarding this study please contact Dr. Donald Paterson [redacted] at the Canadian Centre for Activity and Aging, Sonia and Arthur Labatt Health Sciences Building, The University of Western Ontario, London. If you have any question about the conduct of this study or your rights as a research subject you may contact the Director of the Office of Research Ethics, The University of Western Ontario, 519[redacted].

LETTER OF INFORMED CONSENT

Exercise training adaptations in VO_2 kinetics, cardiac output, muscle deoxygenation and capillarization in older adults.

Principal Investigator: Donald H. Paterson, PhD

I have read the Letter of Information and Muscle Information Sheet and Screening Form, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

Participant:

_____ _____

Name (please print) Signature

_____

Date

Investigator (Person Responsible for Obtaining Informed Consent):

_____ _____

Name (please print) Signature

_____

Date

Muscle Biopsy Information Sheet

Canadian Centre for Activity and Aging
School of Kinesiology, Faculty of Health Sciences
Department of Physical Medicine and Rehabilitation,
Faculty of Medicine and Dentistry
The University of Western Ontario

You have volunteered to take part in a research study that requires you to undergo a muscle biopsy. This is a commonly performed procedure in research studies and for the medical diagnosis of muscle disease. The procedure will be performed by a medical doctor trained to perform muscle biopsies or a specially trained researcher directly supervised by a medical doctor.

The muscle biopsy involves the removal of a small piece of muscle tissue from one of the muscles in your leg using a sterile hollow needle. The area over the outside of your lower thigh muscle (vastus lateralis muscle) will be carefully cleaned. A small amount of local freezing (anesthetic) will be injected into and under the skin. You will likely experience a burning sensation while the freezing is injected. Then a small, 4 – 5 mm incision will be made in your skin in order to create an opening for the biopsy needle. There is often a small amount of bleeding from the incision, but this is usually minimal.

The biopsy needle will then be inserted through the incision into the thigh muscle and a small piece of muscle (100 – 200 mg), about the size of a pencil eraser, will be quickly removed and the needle taken out. During the time that the sample is being taken (about 5 seconds), you may feel the sensation of deep pressure in your thigh and on some occasions this is moderately painful. However, the discomfort very quickly passes and you are able of performing exercise and daily activities. There may be some minimal bleeding when the needle is removed which may require application of pressure for a few minutes.

Following the biopsy, the incision will be closed with sterile tape (steri-strips), and wrapped with a tensor bandage. You should refrain from excessive muscle use for the remainder of the day. Once the freezing wears off, your leg may feel tight and often there is the sensation of a deep bruise or "Charlie Horse". Pain killers such as Acetaminophen (e.g. Tylenol) or Ibuprofen (e.g. Advil) are acceptable if you experience pain associated with the biopsy. It is also beneficial to periodically apply an ice pack to the biopsy site the following day, as this will help to reduce any swelling and any residual soreness. The following day your leg may feel uncomfortable when going down stairs. The tightness in the muscle usually disappears within 2 days and subjects routinely begin exercising at normal capacity within 2 days. In order to allow the incisions to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 4 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided for at least 4 days following the biopsy procedure.

Seven to ten days after the biopsy you will be asked to visit Dr. Doherty either at his office at RM 066, St. Mary's Building, St. Joseph's Health Centre, or at the Canadian Centre for Activity and Ageing so that he can assess the biopsy site and see how it is healing.

Potential Risks

- The local freezing will likely result in a burning feeling in the thigh at the time of the injection. This will last only 5 – 10 seconds. There is an extremely low risk of allergic reaction to the local injection (1 in 1 million).
- The chance of a local skin infection in less than 1 in 1000. Carefully cleaning the skin and keeping the area clean and dry until the skin heals will minimize this.
- Most subjects experience local soreness and stiffness in the leg for two or three days after the biopsy similar to a deep bruise or Charlie Horse. There is a very low risk of internal bleeding at the biopsy site which can result in more prolonged pain and stiffness in the leg.
- On occasion, a small lump of scar tissue may form under the site of the incision, but this normally disappears within 2-3 months, or within a few weeks if massaged. A small visible scar often remains from the biopsy incision.
- There is the possibility of a small area of numbness (about the size of a one dollar coin) around the biopsy site. This usually resolves over 5 – 6 months. There is a very low risk (estimated at less than 1/5000) of damage to a small nerve branch to the muscle. This would result in partial weakness of the vastus lateralis muscle (one of four muscles that straightens the knee) and would likely have no impact on day-to-day activities. Nerve injuries like this usually resolve in 8 – 12 months, but there is a theoretical risk of mild leg weakness.

Concerns or Problems

Infection can be serious, if you experience excessive redness, swelling or infection around the biopsy site or pain or stiffness in your leg you must contact Dr. Doherty right away. Dr. Doherty will be available 24 hours a day to answer any of your concerns or questions about the biopsy.

Dr. Tim Doherty: [redacted]

[redacted]

However, if for some reason, you are not able to contact Dr. Doherty then you should contact your family doctor or go to the Emergency Department.

Please keep this Information Sheet until such time as your biopsy site has fully healed.

MUSCLE BIOPSY SUBJECT SCREENING FORM

To help us ensure your safety and wellbeing please answer the following questions.

1. Have you ever had a negative or allergic reaction to local freezing (e.g. during dental procedures)?

   No ☐ Yes ☐

2. Do you have any tendency toward easy bleeding or bruising (e.g with minor cuts or shaving)?

   No ☐ Yes ☐

3. Are you currently taking any medications that may increase the chance of bleeding or bruising (e.g. Aspirin, Coumadin, Anti-inflammatories, Plavix)?

   No ☐ Yes ☐

4. Have you ever fainted or do you have a tendency to faint when undergoing or watching medical procedures?

   No ☐ Yes ☐

5. Will you contact Dr. Tim Doherty directly if you have any concerns about the biopsy site including: excessive redness, swelling, infection, pain or stiffness of the leg?

   No ☐ Yes ☐

6. Are you willing to visit Dr. Tim Doherty 7 – 10 days following the biopsy at either his office or the Canadian Centre for Activity and Aging for an assessment of the biopsy site?
   No ☐ Yes ☐

Subject Name (print) :_____

Subject Signature :_____

Date :_____

Signature of Person
Conducting Assessment: _____

APPENDIX III: Permission to reproduce previously published material

Request for Permission to Reproduce Previously Published Material

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Affiliation: The University of Western Ontario

University Address (for PhD students): Arthur and Sonia Labatt Health Sciences Building, London, Ontario, Canada, N6A 5B9

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Article or Chapter Title: Speeding of VO2 kinetics with endurance training in old and young men is associated with improved matching of local O2 delivery to muscle O2 utilization

Journal or Book Title: Journal of Applied Physiology

Volume: 108 Page No(s): 913-22 Figure No(s): Table No(s):

Year: 2010 DOI: 10.1152/japplphysiol.01355.2009

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Affiliation: The University of Western Ontario

University Address (for PhD students): Arthur and Sonia Labatt Health Sciences Building, London, Ontario, Canada, N6A 5B9

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[redacted]

E-mail permission from Medicine and Sciences in Sports and Exercise.

Original request

Dear Kenneth Wilson,

Thanks for you quick reply. I tried to get permission through the website you refer me to (I actually was on the phone with a representative from "rightslink") but this will not be possible until the manuscript is published in the journal. Since I have to submit my thesis within the next few weeks, I was wondering if you could tell me how I can get permission to use the "In Press" manuscript as a part of my dissertation.

Sincerely,

Juan Murias

Response

Mr. Murias,

You may refer to your article as “in press.” Use the parts of the article that you need in your thesis.

Kenneth O. Wilson
Director of Editorial Services
American College of Sports Medicine