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The Effects of Age and Long-Term Endurance Training on VO2 Kinetics

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Graduate Program in Kinesiology

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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THE EFFECTS OF AGE AND LONG-TERM ENDURANCE TRAINING ON VO₂ KINETICS

(Thesis format: Integrated Article)

by

Tyler M. Grey

Graduate Program in Kinesiology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

The School of Graduate and Postdoctoral Studies
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ABSTRACT

The kinetics of the adjustment of pulmonary oxygen uptake ($VO_2$) was examined during step transitions from 20 W to moderate-intensity cycling in young (Y), middle-aged (M), and older (O) endurance trained and untrained men. $VO_2p$ was measured breath-by-breath and changes in deoxygenated hemoglobin ([HHb]) were measured by near-infrared spectroscopy. $VO_2p$ and [HHb] were modeled with a monoexponential model. The kinetic time constant for $VO_2$ ($τ_{VO_2p}$) was not different across age-groups ($P > 0.05$) in the trained group (17 ± 8, 18 ± 5, and 20 ± 5 s, in Y, M, and O, respectively). For untrained, $τ_{VO_2p}$ was greater ($P < 0.05$) only in the O (26 ± 7, 24 ± 7, and 42 ± 11 s for Y, M, and O, respectively). The overall adjustment of [HHb] was faster than $τ_{VO_2p}$ in O untrained, resulting in an [HHb]/$VO_2p$ “overshoot” during the exercise transient; this may reflect a microvascular blood flow limitation. The present study suggests that long-term endurance training can abolish the age-related slowing of $τ_{VO_2p}$ via improved matching of local $O_2$ delivery to muscle $VO_2$.

Keywords: $O_2$ uptake kinetics, aging, trained, near-infrared spectroscopy
CO-AUTHORSHIP STATEMENT

This study was designed by M.D. Spencer, T.M. Grey and D.H. Paterson with input from the advisory committee (J.M. Kowalchuk and G.R. Belfry). The majority of the data were collected and analyzed by T.M. Grey with the assistance of M.D. Spencer and J.M. Murias. T.M. Grey wrote the original manuscript for the study. D.H. Paterson provided financial support and editorial feedback.
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LIST OF TERMS AND ABBREVIATIONS

[ADP] – adenosine diphosphate concentration

AMP – amplitude of the response

[ATP] – adenosine triphosphate concentration

a-vO$_2$ difference – difference between arterial and venous oxygen content

BSLN – baseline

CO$_2$ – carbon dioxide

DCA – dichloroacetate

ETC – Electron transport chain

F$_i$O$_2$ – fraction of inspired O$_2$

[HbO$_2$] – oxyhemoglobin, measure of muscle oxygenation concentration

[HHb] – deoxyhemoglobin, measure of muscle deoxygenation concentration

HR – heart rate

$\theta_L$ – lactate threshold

M – middle-aged

MOD – moderate intensity exercise domain

MT – middle-aged trained group

MuT – middle-aged untrained group

NIRS – near infra-red spectroscopy

NOS – nitric oxide synthase

O – older

O$_2$ – oxygen

OT – older trained group

OuT – older untrained group

PaO$_2$ – arterial partial pressure of O$_2$
PCO₂ – partial pressure of carbon dioxide

[PCr] – phosphocreatine concentration

PDH – pyruvate dehydrogenase

PO₂ – partial pressure of oxygen

Q – cardiac output

RER – respiratory exchange ratio

SD – standard deviations

τ – time constant; time required to attain 63% of the steady-state response

τ’ – effective time constant (τ + TD)

TCA – tricarboxylic acid cycle

TD – time delay

VCO₂ – volume of carbon dioxide

VO₂ – volume of oxygen uptake

VO₂max – maximal oxygen uptake; measure of maximal aerobic power

VO₂m – muscle oxygen uptake

VO₂p – pulmonary oxygen uptake

W – watts

WR – work rate

Y – young

YT – young trained group

YuT – young untrained group
CHAPTER 1

1 REVIEW OF THE LITERATURE

1.1 INTRODUCTION

The study of VO\(_2\) (volume of oxygen (O\(_2\)) uptake) and its regulation is important as oxidative metabolism is the principle means by which the human organism generates energy to do work in all but the most short-lived activities. VO\(_2\) is measured as the difference between the volume of O\(_2\) inspired and O\(_2\) expired at the mouth. This pulmonary measure allows us to determine the relative level of exertion at the exercising muscle. The present thesis focuses on comparisons of aerobic function with aging (young, middle-aged, and older), in both endurance trained and untrained men. Two important aerobic functions of the cardiovascular system are: 1) maximal aerobic power, and 2) rate of adjustment of O\(_2\) uptake and utilization (VO\(_2\) kinetics) in response to a change in work rate (energy demand) from baseline to sub-maximal exercise. Maximal aerobic power (VO\(_2\)max) represents the maximum capacity of the whole body to transport and use oxygen during incremental exercise to fatigue; thus, the cardiorespiratory fitness of an individual is heavily determined by an individual’s maximal VO\(_2\) uptake. The second measure of aerobic function, VO\(_2\) kinetics, is the measure of the rate of adjustment of O\(_2\) uptake and utilization at the muscle during sub-maximal exercise. Whereas VO\(_2\)max relies heavily on bulk blood flow (cardiac output) and O\(_2\) delivery to the exercising muscle, the VO\(_2\) kinetic profile is determined mostly by: a) microvascular O\(_2\) delivery and b) metabolic substrate utilization and enzymatic activation at the muscle. Thus, the VO\(_2\) kinetic profile alludes to the physiological mechanisms active at the exercising muscle that regulate O\(_2\) uptake during the on-transient to sub-maximal exercise. A faster VO\(_2\) kinetic profile represents the body’s ability to obtain more energy from aerobic metabolism (an essentially endless supply of energy) earlier, with less reliance on anaerobic metabolism (which produces fatigue-causing metabolites). Therefore, for many performance outcomes, ranging from those of endurance athletes to that of older adults accomplishing daily tasks, it is advantageous to have a faster VO\(_2\) kinetic profile. In the end, VO\(_2\)max and VO\(_2\) kinetics are two different measures of aerobic function and both have differing underlying mechanisms that govern the response to exercise.
1.2 AEROBIC FUNCTION AND AGING

1.2.1 Maximal Aerobic Power

Maximal aerobic function (VO$_{2\text{max}}$) can be defined by the Fick Equation (equation 1):

$$\text{VO}_{2\text{max}} = Q \times (a-v\text{O}_2 \text{ difference})$$

Equation 1

where $Q$ is cardiac output (product of heart rate (HR) and stroke volume) or tissue blood flow, and $a-v\text{O}_2$ difference is the difference between arterial and venous $\text{O}_2$ content and characterizes the tissue’s ability to extract oxygen from the circulating blood. Thus, VO$_{2\text{max}}$ is determined by both the capacity for oxygen delivery and for oxygen utilization. As we age the functional capacity of the cardiovascular system decreases, resulting in a decline in VO$_{2\text{max}}$ (Betik & Hepple, 2008). The rate of decline per decade in healthy sedentary men appears to range between ~10 to 15% (Paterson & Cunningham, 1999; Paterson et al., 1999; Rogers et al., 1990; Stathokostas et al., 2004; Trappe et al., 1996), whereas females range from ~7 to 12% (Fitzgerald et al., 1997; Paterson et al., 1999; Stathokostas et al., 2004; Tanaka et al., 1997). The decline in VO$_{2\text{max}}$ with age is most likely due to a combination of a compromised capacity for both oxygen delivery and oxygen utilization (Murias et al., 2011a). Thus, the decline can partially be attributed to a decrease in $Q$, which some researchers have attributed to the natural decline in maximal heart rate (HR) (Fuchi et al., 1989; Pimental et al., 2003). However, Hagberg et al. (1985) and others (Beere et al., 1999; Rogers et al., 1990; Trappe et al., 1996) have found that in healthy older individuals the decline in VO$_{2\text{max}}$ may be attributed to both a decline in HR (and consequently $Q$) and $a-v\text{O}_2$ difference. Nevertheless, the decline in VO$_{2\text{max}}$ with age is generally determined by the decline in $Q$ (Rowell, 1974).

1.2.2 VO$_2$ Kinetics

Physiology and Measurement of Oxygen Uptake Kinetics

Immediately following the onset of exercise, a higher adenosine triphosphate (ATP) requirement exists within the active muscles (Rossiter et al., 1999). Whereas ATP demand increases instantaneously, indicators of oxidative phosphorylation such as muscle oxygen uptake (VO$_{2\text{m}}$) and pulmonary oxygen uptake (VO$_{2\text{p}}$) have been observed to be relatively slow (Grassi et al., 1996); thus, the remaining energy demand is met by phosphocreatine (PCr) hydrolysis and, to a lesser extent, anaerobic glycolysis/glycogenolysis (Whipp & Wasserman, 1972). Furthermore, if the exercising work rate (WR) is to be maintained for extended periods of time, the prolonged ATP demand must be met through oxidative phosphorylation. Given that the
exercise transition remains below the lactate threshold ($\theta_L$) and within the moderate-intensity domain (MOD), $VO_2$ will illustrate a multi-phase exponential response to meet the ATP demand. Thus, a delay is created between the demand for ATP (instantaneous) and the matched production of ATP through oxidative phosphorylation; when the production of ATP matches the demand for ATP, a steady-state will be attained (Whipp, 1971). The exponential increase in $VO_2$ before steady-state is reached (usually within 120 to 240 seconds in MOD (Whipp, 1971)), is referred to as phase II $VO_2$, which can be described quantitatively with a time constant ($\tau$).

The $\tau$ of the phase II $VO_2$ ($\tau VO_2$) response from the onset of exercise represents the time it takes to achieve 63% of $\Delta VO_2$ (the change in $VO_2$ to steady state at new work rate). The response may also be characterized by its overall amplitude, which is the change in $VO_2$ from baseline to the steady-state achieved following the exercise transition. Knowledge of both the amplitude and $\tau VO_2$ allows for the estimation of the $O_2$ deficit, which reflects the muscle’s reliance on non-oxidative pathways (PCr hydrolysis and glycolysis/glycogenolysis) for energy production during exercise transitions to MOD (DeLorey et al., 2007; Paterson & Whipp, 1991). Therefore, an advantage exists with lesser $\tau VO_2$ values as the transition to steady-state is shortened and there is a reduced reliance on non-oxidative pathways.

The implication that measures of $VO_2$ directly reflect the measures of oxygen uptake at the muscle ($VO_{2m}$) has been confirmed. Researchers have used a few different techniques to assess the approximation of human $VO_{2m}$ (Grassi et al., 1996; Koga et al., 2005), the most common measure being $VO_{2p}$. Grassi et al. (1996) observed the $VO_{2p}$ response to be within ~10% of $VO_{2m}$ in vivo via invasive measures of conduit artery blood flow and a-vo$O_2$ difference across the exercised muscle. Magnetic resonance spectroscopy has also been used to show: a) a tight coupling between [PCr] breakdown and $VO_{2m}$ and b) similar kinetic responses between [PCr] breakdown and the adjustment of $VO_{2p}$ (Chilibeck et al., 1998; McCreary et al., 1996; Rossiter et al., 1999). Therefore, the non-invasive measures of $VO_{2p}$ are validated to be useful for investigating the regulation of $O_2$ consumption at the level of the muscle.

Pulmonary measures of $VO_2$ are collected breath-by-breath during the step transitions in work rate. Data from three continuous transitions (Spencer et al., 2011) of baseline to MOD are interpolated to 1 s intervals, time-aligned, and ensemble averaged to yield a single response. The $VO_2$ kinetic response is fitted with a mono-exponential model of the form (equation 2):

$$VO_{2p}(t) = VO_{2BLN} + \text{Amp}[1 - e^{-(t-TD)/\tau}]$$

*Equation 2*
where $VO_{2p}$ is $VO_2$ at any time (t); $VO_{2BSLN}$ is baseline $VO_2$; Amp is the steady-state increase in $VO_2$ above baseline; TD is the time delay; and $\tau$ is the phase II $VO_2$ time constant.

**Factors Limiting Oxygen Uptake Kinetics**

In order to prevent a fall in intra-cellular [ATP], the rate at which ATP is utilized must be met by the rate of ATP production. Since sustained exercise is greatly dependent on $O_2$ uptake, oxidative phosphorylation is relied upon heavily to produce the necessarily rate of ATP production. The overall reaction describing oxidative phosphorylation can be summarized by equation 3:

$$NADH + H^+ + \frac{1}{2} O_2 + 3 ADP + 3 P_i \rightarrow 3 ATP + NAD^+ + H_2O \quad \text{Equation 3}$$

During exercise, if any of the substrates required for oxidative phosphorylation (NADH, $O_2$, and ADP) are not readily available, then the rate of activation of oxidative phosphorylation may be limited and as a result $VO_2$ kinetics could be slowed. Grassi et al. (2011) have shown that $VO_2$ kinetics are tightly regulated/controlled by mechanisms linked to increased [ADP] and the PCr shuttle system; briefly, PCr breakdown appears to delay or attenuate the increase in [ADP], thereby reducing a more rapid activation of oxidative phosphorylation. To show this they used creatine kinase inhibitors (in canines) to reduce PCr breakdown and increase [ADP] more rapidly, which resulted in faster $VO_2$ kinetics (Grassi et al., 2011). Nevertheless, beyond the basic mechanisms that regulate the rate of increase in oxidative phosphorylation at the exercising muscle, the other determinants of the $VO_2$ kinetic profile are based on a combination of physiological factors; phase II $VO_2$ kinetics are mainly limited by (1) $O_2$ delivery to and within the exercising muscle, and (2) an intracellular control on “metabolic inertia”/‘sluggish’ activation of enzymes and provision of substrates for oxidative phosphorylation.

**(1) Oxygen Delivery**

Studies investigating the effect of $O_2$ delivery on $VO_2$ kinetics have designed experiments that impair $O_2$ transport by varying methods. Beta-adrenergic receptor blockade slowed $VO_2$ kinetics in MOD by reducing heart rate and subsequently $O_2$ transport (Hughson, 1984). Several research groups have also slowed $VO_2$ kinetics with hypoxia (lower fraction of inspired $O_2$ ([FiO$_2$]) and thus reducing arterial partial pressure of oxygen (PaO$_2$)) across varying work rates (DeLorey et al., 2004c; Hughson & Kowalchuk, 1995; Spencer et al., 2012; Springer et al., 1991). A change in body position also produces a change in $O_2$ delivery (Hughson et al., 1993;
MacDonald et al., 1998); slower kinetics are a result of exercising in the supine position (most likely due to reduced perfusion pressure), whereas in the upright position gravity increases driving pressure for arterial blood to perfuse into the working leg muscles (MacDonald et al., 1998). Furthermore, combining interventions that augment both convective O₂ delivery and metabolic substrate provision via heavy priming exercise (i.e. MOD1-HVY-MOD2 protocol) but with the addition of hypoxia (through reduced FiO₂ which presumably maintains O₂ delivery (as an increase in blood flow compensation) but impairs PaO₂ and the vascular muscle O₂ flux gradient) have resulted in lengthened τVO₂ despite the priming effects of increase metabolic substrate provision (Spencer et al., 2012). These results suggest that O₂ delivery is a major factor in the limitation of the rate of oxidative phosphorylation.

However, when attempting to speed VO₂ kinetics via increased O₂ availability, especially in the MOD, there seems to be no impact on τVO₂. In the pump-perfused dog hindlimb model, Grassi and colleagues showed no speeding of VO₂ kinetics despite improving bulk convective blood flow and peripheral diffusive O₂ delivery (Grassi et al., 1998); although, it needs to be considered that dog gastrocnemius muscle is highly oxidative (more than in humans) and differs in capillarization and blood flow distribution (Grassi et al., 1998). Studies examining the effects of hyperoxia (FiO₂ >50%) on VO₂ kinetics in MOD found slightly but not significantly faster kinetics in one study (MacDonald et al., 1997), and no effect in others (Bell et al., 1999; Hughson & Kowalchuk, 1995). However, these results should be considered with caution as hyperoxia also causes systemic vasoconstriction, which will result in reducing blood flow to maintain total O₂ delivery (MacDonald et al., 1997).

Recent studies conducted in our laboratory (DeLorey et al., 2004a,b,c, 2007; Murias et al., 2010, 2011a,b, 2012; Spencer et al., 2011, 2012) have used near-infrared spectroscopy (NIRS) to measure tissue oxygenation at the exercising limb (i.e. microvascular O₂ delivery); the general conclusion is that (for individuals with τVO₂p > ~ 20 s) the rate of adjustment of VO₂ is mainly constrained by the matching of local O₂ distribution to the muscle (Murias et al., 2011b). Refer to next section for full description of NIRS.

**Near-Infared Spectroscopy**

The use of NIRS has generated a method to effectively measure tissue oxygenation via non-invasive observation of microvascular hemoglobin/myoglobin. Infra-red light is used to measure the presence of oxygenated hemoglobin [HbO₂] and deoxygenated hemoglobin [HHb]
within the tissue. Thus, the quantification of specific changes in [HbO₂] and [HHb] can be utilized to provide an index of O₂ extraction during transitions from baseline to MOD. Therefore, the NIRS method provides an insight into the local microvascular O₂ delivery at the working muscle and the rate of O₂ utilization.

(2) Substrate Utilization

Following a step-increase in work rate, there must be an increase in the provision of electrons and reducing equivalents (i.e. NADH, FADH₂) to the mitochondrial electron transport chain (ETC), in order for oxidative phosphorylation to increase. For the concentration of NADH to rise there must be an increase in either the breakdown of fat (via β-oxidation) or the production of pyruvate (from glycolytic pathways). Increases in pyruvate consequently requires increases in the tightly regulated pyruvate dehydrogenase (PDH) production of acetyl-CoA and flux through the tricarboxylic acid cycle (TCA cycle). The hypothesis that a sluggish activation of substrate utilization causes a slowing of VO₂ kinetics would suggest that if augmenting O₂ delivery causes no perceptible changes in τVO₂, then the limitation must lie within the metabolic pathways. Pyruvate dehydrogenase has been studied as a potential site of regulation for oxidative phosphorylation (Bangsbo et al., 2002; Grassi et al., 2002; Howlett et al., 1999; Jones et al., 2004; Rossiter et al., 2003); the mitochondrial PDH complex is responsible for regulating the entry of carbohydrate-derived substrate into the TCA cycle and the provision of reducing equivalents to the ETC. Conflicting evidence exists in the literature in both human and canine models. By increasing the activation of PDH via a pharmacological intervention (dichloroacetate: DCA) or heavy-priming exercise there was a significant decrease of the contribution of substrate-level phosphorylation during MOD (Gurd et al., 2006; Howlett et al., 1999). These findings would suggest that the reduction of substrate-level phosphorylation would stem from a more rapid activation of both oxidative phosphorylation and muscle O₂ utilization. However, experiments in humans (Bangsbo et al., 2002; Jones et al., 2004; Rossiter et al., 2003) failed to demonstrate faster VO₂ kinetics following prior PDH activation by DCA supplementation. Furthermore, Grassi et al. (2002) used an isolated dog gastrocnemius muscle to show that despite an improved metabolic efficiency (i.e. PCr sparing), there were no effects on τVO₂. This lack of consensus suggests that the hypothesis of sluggish activation of substrate could result in slow VO₂ kinetics, however O₂ delivery appears to be a primary factor related to a slow VO₂ kinetic response.
The literature shows an age-related slowing of VO$_2$ kinetics (~7 s/decade (Babcock et al., 1992)) and consistently slowed VO$_2$ kinetics in older compared to young groups (Babcock et al., 1994b; Bell et al., 1999; Chilibeck et al., 1996; Cunningham et al., 1993; Murias et al., 2010). Unlike other studies on younger subjects that indicate a control and limit of VO$_2$ kinetics in the rate of muscle oxidative metabolism (via oxygen delivery and substrate utilization) (Grassi et al., 1996), the slower VO$_2$ kinetics in older adults most likely reflects a limitation in O$_2$ delivery to the exercising muscle (Chilibeck et al., 1996; Murias et al., 2010). This reduced ability to deliver O$_2$ to the muscle with aging is most likely partially due to the paralleled slower heart rate kinetics in older adults (Cunningham et al., 1993). Additionally, researchers in our laboratory (DeLorey et al., 2004a; Murias et al., 2010) have shown an age-related reduction in microvascular blood flow (reflected by a greater ratio of change in deoxygenated hemoglobin to change in VO$_2$) in older men; thus, older adults rely more on O$_2$ extraction during transition to MOD, probably due to lower microvascular blood flow. Furthermore, Musch et al. (2004) studying aged rats found a redistribution of muscle blood flow during submaximal exercise in older compared to young, which could contribute to reduced O$_2$ delivery. Therefore, evidence suggests that a potential deterioration of microvascular O$_2$ delivery exists in the aging human population. Recently, Murias and colleagues revealed that mechanisms exist, other than bulk blood flow (Q), which could limit aerobic function (Murias et al., 2010); thus, it is important to examine both VO$_2$max and VO$_2$ kinetics in order to characterize the change in aerobic function in aging populations.

1.3 AEROBIC FUNCTION AND AGING IN ENDURANCE TRAINED

1.3.1 Maximal Aerobic Power

The above section outlines the effects of aging on regular healthy individuals. It is of interest to examine what effects endurance training has on the aging population. Numerous studies have showed that endurance-trained men have higher VO$_2$max values than age-matched untrained men (Fuchi et al., 1989; Hagberg et al., 1985; Pimental et al., 2003; Rogers et al., 1990; Trappe et al., 1996; Wilson & Tanaka, 2000). As outlined above (refer to section 1.2.1), regular healthy men experience a natural decline in VO$_2$max at a rate of ~10 to 15% per decade (Paterson & Cunningham, 1999; Paterson et al., 1999; Rogers et al., 1990; Stathokostas et al., 2004; Trappe et al., 1996); whereas, endurance-trained men appear to attenuate (~6 to 10% per
decade) the expected age-related decline in relative VO$_2$max (Fuchi et al., 1989; Hagberg et al., 1985; Pimental et al., 2003; Rogers et al., 1990; Trappe et al., 1996; Wilson & Tanaka, 2000). The reduction in VO$_2$max in healthy men can be attributed to a reduction in Q (Fuchi et al., 1989; Pimental et al., 2003), which is partially attributed to the natural decline in maximal HR. Other researchers have found that the decline in both maximal HR and a-vO$_2$ difference contribute to the reduced VO$_2$max with age (Beere et al., 1999; Hagberg et al., 1985; Rogers et al., 1990; Trappe et al., 1996). Older endurance trained men lessened the reduction in the VO$_2$max by attenuation of the decline in Q (via elevated stroke volume to compensate for the decline in maximal HR) and a-vO$_2$ difference (Hagberg et al., 1985), compared to their healthy counterparts. The endurance-trained do show a reduction in maximal HR with age (Fuchi et al., 1989; Hagberg et al., 1985; Trappe et al., 1996), but also the degree of loss in VO$_2$max has been attributed to the reduction in training intensity or volume (Pimental et al., 2003; Rogers et al., 1990).

Interestingly, the above-mentioned studies tested chronically endurance-trained men, however other researchers (Beere et al., 1999; Murias et al., 2011a) have seen similar adaptations from only 3 to 6 months of endurance training in regular healthy older men. Murias et al. (2011a) found increased capillarization (reflecting improved O$_2$ delivery) and citrate synthase activity following 12 weeks of endurance training; these results suggest there is a potential for an increased capacity of O$_2$ to be utilized and distributed within the active muscle. These studies have shown that regular healthy older men could increase VO$_2$max from endurance training by increasing a-vO$_2$ difference (Beere et al., 1999) or both a-vO$_2$ difference and Q (with improvements in Q representing approximately 2/3 of the difference) (Murias et al., 2011a).

Therefore, endurance training can attenuate (or slightly reverse) the effects of aging by retaining a greater capacity for O$_2$ delivery and O$_2$ utilization with chronic endurance training (or following a 3 to 6 month bout of endurance training).

1.3.2 VO$_2$ Kinetics

Endurance exercise training has been shown to result in a speeding of VO$_2$ kinetics following the onset of moderate intensity exercise both in young (Fukuoka et al., 2002; Koppo et al., 2004; Murias et al., 2010) and older men (Babcock et al., 1994a; Bell et al., 2001a; Berger et al., 2006a; Fukuoka et al., 2002; Murias et al., 2010), and older women (Dogra et al., 2013).

Endurance training in the elderly has shown substantial (38 – 48%) reductions in τVO$_2$ such that
values approach those found in younger subjects (Babcock et al., 1994a; Bell et al., 2001a; Fukuoka et al., 2002; Murias et al., 2010). This speeding (especially in older adults) is likely a result of physiological adaptations that improve muscle O\textsubscript{2} availability (i.e., enhanced muscle perfusion or blood flow) (Murias et al., 2010). These data also suggest that the slowing of VO\textsubscript{2} kinetics in older individuals is related, to a large extent, to a reduction in physical activity and/or aerobic fitness with age (Berger et al., 2006a). DeSouza et al. (2000) found that middle-aged and older endurance-trained men were able to reverse the age-related loss in endothelium-dependent vasodilation to that of younger endurance-trained men. This maintained endothelium vasodilatory response could possibly provide a mechanism by which improved O\textsubscript{2} delivery (via O\textsubscript{2} diffusion at the capillaries) in endurance-trained men could speed VO\textsubscript{2} kinetics.

Interestingly, the amount by which the slowing of VO\textsubscript{2} kinetics can be attenuated is dependent upon the type of training; Berger et al. (2006a) examined both endurance- and sprint-trained athletes from ages 46 to 85 and found that endurance trained athletes maintained similar \(\tau\text{VO}_2\) values to that of healthy younger individuals, whereas sprint trained athletes had a slowing of VO\textsubscript{2} kinetics with age (however all \(\tau\text{VO}_2\) values in the sprint group were still below the age-matched sedentary counterparts). Additionally, just one bout of heavy-priming exercise will speed VO\textsubscript{2} kinetics in both young (Spencer et al., 2012) and older men (DeLorey et al., 2004b; Scheuermann et al., 2002), presumably as a result of improved O\textsubscript{2} delivery (Scheuermann et al., 2002).

Although it is well known that VO\textsubscript{2max} is lower and \(\tau\text{VO}_2\text{p}\) is greater in older compared to young individuals, little data exists on middle-aged men. It has also been shown that endurance training will increase VO\textsubscript{2max} (Beere et al., 1999; Hagberg et al., 1985; Pimental et al., 2003; Rogers et al., 1990; Trappe et al., 1996) and lower \(\tau\text{VO}_2\text{p}\) (Berger et al., 2006a; DeSouza et al., 2000; McKay et al., 2009; Murias et al., 2010) in young and older individuals. Therefore, the purpose of the study herein was to examine the VO\textsubscript{2} responses in young, middle-aged, and older, endurance trained and untrained men. It was hypothesized that there would be a continuous increase in \(\tau\text{VO}_2\text{p}\) from young to older men, in both the trained and untrained groups, with \(\tau\text{VO}_2\text{p}\) values in the trained groups lower than the untrained counterparts. Additionally, it was hypothesized that the [HHb] in groups with slow VO\textsubscript{2} kinetics would reveal a greater muscle deoxygenation for a given VO\textsubscript{2} during the exercise transient, representing a sluggish microvascular O\textsubscript{2} delivery.
CHAPTER 2

2  THE EFFECTS OF AGE AND LONG-TERM ENDURANCE TRAINING ON VO₂ KINETICS

2.1  INTRODUCTION

Studies of the physiological response to exercise in different age groups, particularly young and older, have been conducted to infer that age-related changes exist (Jackson et al., 1995; Murias et al., 2010; Wilson & Tanaka, 2000). Additionally, comparisons of highly trained versus more sedentary groups of different ages have allowed the assessment as to whether age-related changes may be in part due to lack of physical activity or whether long-term physical activity prevents or reduces these losses. In particular, many studies have examined the age-related changes in VO₂max in highly trained and untrained individuals (Jackson et al., 1995; Pimental et al., 2003; Stathokostas et al., 2004; Wilson & Tanaka, 2000). However, for the aerobic parameter of VO₂ kinetics, few studies have examined the differences across age groups and particularly in chronically trained and untrained men in different age groups.

The VO₂ kinetic profile during the transition to moderate-intensity exercise is slower in older individuals compared to young healthy men (Babcock et al., 1992, 1994b; Murias et al., 2010); thus, older individuals display a larger O₂ deficit and may experience premature fatigue (DeLorey et al., 2007). The slower VO₂ kinetics associated with aging may be associated with a limitation in O₂ delivery to the exercising muscle (Murias et al., 2010). Endurance training exercise has been shown to speed VO₂ kinetics in young (Koppo et al., 2004; Murias et al., 2010), middle-aged (Berger et al., 2006a; Fukuoka et al., 2002), and older (Babcock et al., 1994a; Bell et al., 2001a; Berger et al., 2006a; Murias et al., 2010) men. However, it remains unclear whether the age-related slowing of VO₂ kinetics is due to aging per se or a lack of physical activity. Since VO₂ kinetics is a measure of differing physiological regulations/control of aerobic metabolism, analysis of VO₂ kinetics will shed further light on changes with age and the influence of differing levels of physical activity.

A number of studies have examined the time course adjustment of VO₂p during the transitions to moderate-intensity exercise in young and older men (Chilibeck et al., 1997; DeLorey et al., 2004a, 2007; Gurd et al., 2008; Murias et al., 2010); however, there has been
limited study of the VO$_{2p}$ kinetics of middle-aged and older men (Berger et al., 2006a; Fukuoka et al., 2002). Thus, the main goal of this study was to examine the age-related differences in VO$_2$ kinetics of untrained young, middle-aged and older groups of men, and to compare to age-matched groups of endurance trained men. Additionally, the goal was to determine if the same mechanism responsible for slower VO$_2$ kinetics in older men (i.e. an O$_2$ delivery limitation) (Murias et al., 2010) exists in other groups with slow VO$_2$ kinetics. We hypothesized that there would be a continuous increase in the phase II VO$_{2p}$ time constant (τVO$_{2p}$) from young to middle-aged to older men, in both the trained and untrained groups, with τVO$_{2p}$ values in the trained groups always lower than the untrained counterparts; thus, the slowed VO$_2$ kinetics with age would not be largely attenuated in endurance trained. It was further hypothesized that in groups with slower VO$_2$ kinetics there would be an associated rapid rate of muscle deoxygenation suggesting an O$_2$ delivery limitation at the microvascular/active muscle level.

2.2 METHODS

Participants: 36 healthy men volunteered and gave written consent to participate in this study. Additionally the data of 15 healthy men from studies completed previously (≤ 3 years prior) in the lab using similar equipment and protocol were retrieved. Subjects were separated into three groups: young (18 – 35 yr), middle-aged (40 – 59 yr), and older (60 – 85 yr). Each group was further separated into two categories, trained and untrained, yielding six groups: young trained (YT) and untrained (YuT), middle-aged trained (MT) and untrained (MuT), and older trained (OT) and untrained (OuT). All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All participants were non-smokers and were not taking medications that would affect the cardiorespiratory or hemodynamic responses to exercise.

Subject Training Status: The untrained (recreationally active) men were not actively training or participating in an exercise training program, and were recruited by publically-posted flyers. The endurance trained men were competitive and/or actively training cyclists and were recruited by flyers posted at their cycling clubs. All trained cyclists had been training for ≥ 5 years in YT and ≥ 10 years in MT and OT, and typically cycled at least 5 times/wk for > 300 km•wk$^{-1}$. 
**Protocol:** On day one, participants reported to the laboratory to perform a ramp incremental test (30 W·min⁻¹ for YT and MT, 25 W·min⁻¹ for YuT, MuT, and OT, and 20 W·min⁻¹ for OuT) to the limit of tolerance on a cycle ergometer (model: H-300-R Lode; Lode B.V., Groningen, Holland) for determination of maximal VO₂ (VO₂max) and the estimated lactate threshold (θₐ); the ramp portion of the protocol was initiated following 4 minutes of cycling at 20 W (watts). Peak VO₂ (VO₂max) was determined as the maximal 20 s averaged VO₂ₚ value during the last 60 s of the ramp incremental test. The maximal HR and RER (respiratory exchange ration) values during the ramp incremental test were obtained by averaging the final 30 seconds of the trial. θₐ was determined by visual inspection as the VO₂ at which CO₂ (carbon dioxide) output (VCO₂) began to increase out of proportion in relation to VO₂, with a systematic rise in minute ventilation-to-VO₂ ratio and end-tidal PO₂ (partial pressure of O₂) whereas minute ventilation-to-VCO₂ ratio and end-tidal PCO₂ (partial pressure of CO₂) were stable (Beaver et al., 1986).

From the results of this ramp test, a moderate-intensity work rate (WR) was selected to elicit a VO₂ equivalent to ~80% of the VO₂ at θₐ (MOD). On a second laboratory session, subjects completed three continuous transitions from cycling at baseline (20 W) to cycling at MOD, each for 6 minutes. The cycling transitions between baseline and MOD were initiated as a “step” change. Subjects were instructed to maintain a pedal rate between 60 – 70 RPM throughout the trial.

**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 using a syringe of known volume. Inspired and expired gases were continuously sampled (50 Hz) at the mouth and analyzed for concentrations of O₂, CO₂, and N₂ by mass spectrometry (Innovision, AMIS 2000, Lindvedvej, Denmark) 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 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).

HR was monitored continuously by electrocardiogram (three-lead arrangement) using
PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO). Data were recorded using LabChart v6.1 (ADInstruments, Colorado Springs, CO) on a separate computer.

Local muscle deoxygenation ([HHb]) of the quadriceps vastus lateralis muscle was monitored continuously with a frequency-domain multi-distance NIRS system (Oxiplex TS, Model 95205, ISS, Champaign, IL, USA) as previously described by Murias et al. (2012). Briefly, the arrangement for the present study included a single channel consisting of eight laser diodes operating at two wavelengths (\(\lambda = 690\) and 828 nm, four at each wavelength) which were pulsed in a rapid succession, and a photomultiplier tube. The lightweight plastic NIRS probe (connected to laser diodes and photomultiplier tube by optical fibers) consisted of two parallel rows of light emitter fibers and one detector fiber bundle; the source-detector separations for this probe were 2.0, 2.5, 3.0, and 3.5 cm for both wavelengths. The probe was placed on the belly of the muscle midway between the lateral epicondyle and greater trochanter of the femur; it was covered with an optically-dense, black vinyl sheet, thus minimizing the intrusion of extraneous light and secured in place with an elastic strap tightened to prevent movement of the probe. NIRS measurements were collected continuously for the entire duration of each trial. This allowed for continuous measurement of absolute concentration changes of oxyhemoglobin ([HbO\(_2\)]) and [HHb].

The near-infrared spectrometer was calibrated at the beginning of each testing session following a warm-up period of at least 20 min. The calibration was done with the probe placed on a calibration block (phantom) with absorption (\(\mu_A\)) and reduced scattering coefficients (\(\mu_s'\)) previously measured; thus, correction factors were determined and were automatically implemented by the manufacturer’s software for the calculation of the \(\mu_A\) and \(\mu_s'\) for each wavelength during the data collection. Calculation of [HHb] reflected continuous measurements of \(\mu_s'\) made throughout each testing session (i.e., constant scattering value not assumed). Data were stored online at an output frequency of 25 Hz, but were reduced to 1 s bins for all subsequent analyses within the present study.

Data analysis: VO\(_{2p}\) data were filtered by removing aberrant data points that lay outside 4 standard deviations (SD) of the local mean. Data for each repetition were then linearly interpolated to 1 s intervals, time-aligned such that time zero represented each transition and ensemble-averaged to yield a single averaged response for each subject. These averaged
responses were further time-averaged into 5 s bins. The on-transient responses for VO$_{2p}$ were modelled using the following equation:

$$Y(t) = Y_{BSLN} + A \left(1 - e^{-(t-TD)/\tau}\right); \text{ [Equation 1]}$$

where $Y(t)$ represents the VO$_{2p}$ at any given time ($t$); $Y_{BSLN}$ is the steady state baseline value of $Y$ before an increase in WR; $A$ is the amplitude of the increase in $Y$ above $Y_{BSLN}$; $\tau$ represents the time required to attain 63% of the steady-state amplitude; and TD represents the mathematically generated time delay through which the exponential model is predicted to intersect $Y_{BSLN}$. After excluding the initial 20 s of data from the model, while still allowing TD to vary freely (in order to optimize accuracy of parameter estimates), VO$_{2p}$ data were modeled to 4 min (240 s) of the step-transition; this ensured that each subject had attained a VO$_{2p}$ steady-state, yet did not bias the model fit during the on-transient (Bell et al., 2001b). 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 for the estimated time constant was determined after preliminary fit of the data with $Y_{BSLN}$, $A$, and TD constrained to the best-fit values and the $\tau$ allowed to vary.

The [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. The time delay for the [HHb] response (TD[Hb]) was determined using second-by-second data and corresponded to the time, after the onset of exercise, at which the [HHb] signal began a systematic increase from its nadir value. Determination of the TD[Hb] was made on individual trials and averaged to yield specific values for each individual. The [HHb] data were modeled using Equation 1; the fitting window for the “exponential” response spanned from the end of the TD[Hb] to 90 s into each transition. As described previously (duManoir et al., 2010), different fitting strategies ranging from 90-180 s into a transition resulted in minimal differences in estimates of $\tau$[HHb]. Baseline [HHb] ([HHb]$_{BSLN}$) values were computed as the mean value in the 60 s prior to a transition. Whereas the $\tau$[HHb] described the time course for the increase in [HHb], the overall change of the effective [HHb] ($\tau'[HHb] = TD[Hb] + \tau[Hb]$) described the overall time course of the [HHb] from the onset of the step transition.

Calculations of the [HHb]/VO$_{2p}$ ratio were similar to those previously described (Murias
et al., 2011b, 2012). Briefly, the second-by-second [HHb] and VO2p data were normalized for each subject (0% representing the 20 W baseline value, and 100% representing the post-transition steady-state). This normalization procedure was undertaken so that the specific time course of adjustment in the respective signals could be considered without concern for signal amplitude. The normalized VO2p was left-shifted 20 s to account for the phase I-Phase II transition, so that the onset of exercise coincided with the beginning of phase II VO2p (Murias et al., 2011b), which has been previously described to correspond with muscle VO2 (VO2m) within 10% (Grassi et al., 1996). Data were further averaged into 5-s bins for statistical comparison of the rate of adjustment for [HHb] and VO2p. Additionally, an overall average [HHb]/VO2p ratio for the adjustment period during the exercise on-transient was derived for each individual as the average of the twenty-one 5 s ratio values from 20 to 120 s (approximating the start of the [HHb]/VO2p “overshoot” to the time point at which the ratio reached the steady-state value of 1.0 in all groups). The limitations of this analysis are detailed in Murias et al. (2011b).

Statistics: Data are presented as means ± SD. Two-way analysis of variance (ANOVA) was used to determine statistical significance for the dependent variables. Tukey post-hoc tests were used when significant differences were found for the main effects and to quantify the strength of relationships between variables. All statistical analyses were performed using SPSS Version 18.0, (SPSS Inc., Chicago, IL). Statistical significance was declared when P < 0.05.

2.3 RESULTS

Subject characteristics and peak exercise values are listed in Table 1. Subjects reached volitional fatigue during the ramp incremental test with the mean HR data at, or no less than 5 beats•min⁻¹ below, the age-predicted maximum and mean RER data greater than 1.2 for all groups. The training program data of each of the endurance-trained groups are listed in Table 2. The endurance-trained men reported cycling for 435, 309, and 304 km•wk⁻¹ in the YT, MT, and OT groups, respectively. The trained cyclists were also long-term endurance athletes and had been training for 6, 15, and 23 years prior to testing in the YT, MT, and OT groups, respectively.

Individual VO2max data, as well as group means and SD, are presented in Fig. 1. VO2max significantly (P < 0.05) decreased with age in both trained (66.0 ± 7.6, 55.3 ± 7.3, and 45.5 ± 9.0 mL•kg⁻¹•min⁻¹, for YT, MT, and OT, respectively) and untrained (49.9 ± 3.7, 45.3 ±
7.9, and 29.0 ± 5.3 mL•kg⁻¹•min⁻¹, for YuT, MuT, and OuT, respectively) groups. VO₂max was significantly (P < 0.05) greater in each trained group compared to the corresponding age-matched untrained group. The estimated lactate thresholds were: 2.8 ± 0.5, 2.4 ± 0.3, and 2.1 ± 0.2 L•min⁻¹ for the trained groups (YT, MT, and OT, respectively) and 2.2 ± 0.3, 2.2 ± 0.2, and 1.6 ± 0.3 L•min⁻¹ for the untrained groups (YuT, MuT, and OuT, respectively).

**VO₂ Kinetics.** Individual τVO₂p data, as well as group means and SD, are presented in Fig. 2. Phase II τVO₂p was similar between the trained and untrained groups for both Y and M groups (Table 3). τVO₂p did not significantly increase with age in the trained group; whereas, τVO₂p was not different between YuT and MuT but it was greater (P < 0.05) in the OuT compared to YuT, MuT, and OT (Table 3). Based on the assigned MOD work rates, VO₂p AMP significantly (P < 0.05) decreased with age in both trained and untrained groups, and was greater (P < 0.05) in each trained group compared to the corresponding age-matched untrained group (Table 3). VO₂p TD was significantly (P < 0.05) lower in the untrained compared to trained groups (for Y and O).

**[HHb] Kinetics.** τ [HHb] as well as the overall time course of [HHb], reflected as τ' [HHb], were longer (P < 0.05) in the untrained compared to the trained groups (for M and O; Table 4). The TD[HHb] was longer (P < 0.05) in the OT compared to the YT.

The normalized (%) responses of [HHb] and VO₂p adjustments to the step-transition in work rate are presented in Fig. 3. Greater adjustment in [HHb] compared to VO₂p resulted in a small transient “overshoot” in [HHb]/VO₂p for YuT, MT, OT and a relatively large value in OuT (Fig. 4); however, only the OuT overshoot was significantly greater when compared across age and against the trained counterpart. Furthermore, the overshoot in the YuT, MT and OT groups was relatively short (from 25 to ≤ 35 s), whereas the OuT overshoot extended from 20 to 75 s.
Table 1: Subject characteristics and peak exercise responses

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age (yr)</th>
<th>Body Mass (kg)</th>
<th>Height (cm)</th>
<th>VO(_2)max (L•min(^{-1}))</th>
<th>HR max (beats•min(^{-1}))</th>
<th>RER max</th>
</tr>
</thead>
<tbody>
<tr>
<td>YT</td>
<td>8</td>
<td>24 ± 6</td>
<td>68 ± 9</td>
<td>177 ± 5</td>
<td>4.5 ± 0.5</td>
<td>195 ± 7</td>
<td>1.26 ± 0.06</td>
</tr>
<tr>
<td>MT</td>
<td>9</td>
<td>52 ± 5</td>
<td>80 ± 7</td>
<td>179 ± 7</td>
<td>4.4 ± 0.4</td>
<td>176 ± 5†</td>
<td>1.21 ± 0.08</td>
</tr>
<tr>
<td>OT</td>
<td>9</td>
<td>64 ± 3</td>
<td>77 ± 12</td>
<td>181 ± 8</td>
<td>3.4 ± 0.4†‡</td>
<td>165 ± 6‡</td>
<td>1.21 ± 0.05</td>
</tr>
<tr>
<td>YuT</td>
<td>8</td>
<td>23 ± 4</td>
<td>80 ± 9</td>
<td>181 ± 6</td>
<td>4.0 ± 0.4</td>
<td>194 ± 3</td>
<td>1.32 ± 0.08</td>
</tr>
<tr>
<td>MuT</td>
<td>9</td>
<td>52 ± 2</td>
<td>80 ± 9</td>
<td>175 ± 6</td>
<td>3.6 ± 0.4*</td>
<td>173 ± 8†</td>
<td>1.24 ± 0.06</td>
</tr>
<tr>
<td>OuT</td>
<td>8</td>
<td>68 ± 5</td>
<td>85 ± 11</td>
<td>174 ± 7</td>
<td>2.5 ± 0.6*†‡</td>
<td>154 ± 9‡</td>
<td>1.33 ± 0.10</td>
</tr>
</tbody>
</table>

Values are means ± SD. YT, young trained; MT, middle-aged trained; OT, older trained; YuT, young untrained; MuT, middle-aged untrained; OuT, older untrained; HR, heart rate; RER, respiratory exchange ratio. *, significantly different from age-matched trained group (P < 0.05) †, significantly different from training-matched young group (P < 0.05); ‡, significantly different from training-matched middle group (P < 0.05).
Table 2: Group average training program data for the endurance-trained men

<table>
<thead>
<tr>
<th>Trained</th>
<th># of rides (wk(^{-1}))</th>
<th>Weekly Distance (km(\times)wk(^{-1}))</th>
<th>Years Training (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YT</td>
<td>6.1 ± 0.7</td>
<td>435 ± 180</td>
<td>6.0 ± 2.9</td>
</tr>
<tr>
<td>MT</td>
<td>5.0 ± 0.9</td>
<td>309 ± 84</td>
<td>15.4 ± 6.5</td>
</tr>
<tr>
<td>OT</td>
<td>4.8 ± 0.4</td>
<td>304 ± 71</td>
<td>23.0 ± 6.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. YT, young trained; MT, middle-aged trained; OT, older trained.
Table 3: VO$_{2p}$ kinetic parameters for the transition to moderate-intensity exercise

<table>
<thead>
<tr>
<th></th>
<th>VO$_{2p}$BSLN (L·min$^{-1}$)</th>
<th>VO$_{2p}$AMP (L·min$^{-1}$)</th>
<th>TD VO$_{2p}$ (s)</th>
<th>τVO$_{2p}$ (s)</th>
<th>CI$^{95}$ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YT</td>
<td>0.98 ± 0.22</td>
<td>1.32 ± 0.33</td>
<td>16.1 ± 1.1</td>
<td>17.0 ± 7.5</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>MT</td>
<td>1.03 ± 0.20</td>
<td>1.07 ± 0.28†</td>
<td>16.1 ± 3.1</td>
<td>18.1 ± 5.3</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>OT</td>
<td>0.93 ± 0.15</td>
<td>0.82 ± 0.2†‡</td>
<td>15.7 ± 1.8</td>
<td>19.8 ± 5.4</td>
<td>3.3 ± 1.7</td>
</tr>
<tr>
<td>YuT</td>
<td>1.06 ± 0.18</td>
<td>1.00 ± 0.21*</td>
<td>11.2 ± 5.8*</td>
<td>25.7 ± 6.6</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>MuT</td>
<td>0.97 ± 0.13</td>
<td>0.92 ± 0.22*†</td>
<td>14.5 ± 5.3</td>
<td>24.4 ± 7.4</td>
<td>2.6 ± 1.2</td>
</tr>
<tr>
<td>OuT</td>
<td>0.91 ± 0.12</td>
<td>0.55 ±0.21*†‡</td>
<td>10.2 ± 8.0*</td>
<td>42.0 ± 11.3*†‡</td>
<td>4.8 ± 3.0</td>
</tr>
</tbody>
</table>

Values are means ± SD. YT, young trained; MT, middle-aged trained; OT, older trained; YuT, young untrained; MuT, middle-aged untrained; OuT, older untrained; VO$_{2p}$, pulmonary VO$_2$; BSLN, baseline; AMP, amplitude; TD, time delay; τ, time constant of response; CI$^{95}$, 95% confidence interval of τVO$_{2p}$. *, significantly different from age-matched trained group (P < 0.05); †, significantly different from training-matched young group (P < 0.05); ‡, significantly different from training-matched middle group (P < 0.05).
Table 4: [HHb] kinetic parameters for the transition to moderate-intensity exercise

<table>
<thead>
<tr>
<th>Group</th>
<th>TD[HHb] (s)</th>
<th>τ[HHb] (s)</th>
<th>τ'[HHb] (s)</th>
<th>[HHb]/VO$_{2p}$</th>
</tr>
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<td>YT</td>
<td>9.0 ± 2.1</td>
<td>10.8 ± 2.9</td>
<td>19.8 ± 2.5</td>
<td>1.01 ± 0.07</td>
</tr>
<tr>
<td>MT</td>
<td>10.1 ± 1.8</td>
<td>7.7 ± 2.0</td>
<td>17.9 ± 2.6</td>
<td>1.04 ± 0.05</td>
</tr>
<tr>
<td>OT</td>
<td>12.3 ± 3.1†</td>
<td>7.7 ± 3.0</td>
<td>20.0 ± 3.5</td>
<td>1.04 ± 0.04</td>
</tr>
<tr>
<td>YuT</td>
<td>9.4 ± 1.8</td>
<td>10.9 ± 2.9</td>
<td>20.3 ± 3.5</td>
<td>1.05 ± 0.03</td>
</tr>
<tr>
<td>MuT</td>
<td>10.2 ± 2.1</td>
<td>12.9 ± 8.5*</td>
<td>23.1 ± 8.8*</td>
<td>1.02 ± 0.09</td>
</tr>
<tr>
<td>OuT</td>
<td>10.3 ± 3.3</td>
<td>12.7 ± 5.9*</td>
<td>23.0 ± 6.6*</td>
<td>1.30 ± 0.13§</td>
</tr>
</tbody>
</table>

Values are means ± SD. YT, young trained; MT, middle-aged trained; OT, older trained; YuT, young untrained; MuT, middle-aged untrained; OuT, older untrained; [HHb], deoxygenated hemoglobin concentration; TD, time delay; τ, time constant of response; τ'[HHb], sum of τ[HHb] and TD[HHb].*, significantly different from age-matched trained group (P < 0.05); †, significantly different from training-matched young group (P < 0.05); §, [HHb]/VO$_{2p}$ significantly different from 1.0 (P < 0.05).
Fig. 1. Individual and average group VO$_2$max (mL·kg$^{-1}$·min$^{-1}$) values. Group values are mean ± SD.
Fig. 2. Individual and average group $\tau \text{VO}_2p$ (s) values. Group values are mean ± SD.
Fig. 3. Group mean profiles for the adjustment of [HHb] and VO$_{2p}$ (left shifted such that data from phase I VO$_{2p}$ were not included) during the step transition to MOD. ⋄, time points at which the relative increase of [HHb] is greater than the relative increase of VO$_{2p}$ (P < 0.05).
Fig. 4. Group mean profiles for the adjustment of \([\text{HHb}]/\text{VO}_2p\) during the step transition to MOD. •, time points at which the relative increase of [HHb] is greater than the relative increase of \(\text{VO}_2p\) (P < 0.05).

*\([\text{HHb}]/\text{VO}_2p\) significantly different from 1.0 (P < 0.05).
2.4 DISCUSSION

Numerous studies have examined the time course adjustment of VO$_{2p}$ during the transitions to moderate-intensity exercise in young and older men (Chilibeck et al., 1997; DeLorey et al., 2004a, 2007; Gurd et al., 2008; Murias et al., 2010). Additionally, a couple of studies have reported the VO$_2$ kinetics of middle-aged and older men (Berger et al., 2006a; Fukuoka et al., 2002). The present study examined the VO$_2$ kinetic profiles of young, middle-aged, and older endurance trained and untrained men. The main findings were as follows: 1) in the untrained groups, $\tau$VO$_{2p}$ did not differ significantly between young to middle-aged, but was substantially greater in the older group; 2) in the chronically endurance-trained groups, $\tau$VO$_{2p}$ did not change appreciably across age, and was always less than the untrained groups (only significantly ($P < 0.05$) less than older untrained group); 3) there was a significant [HHb]/VO$_{2p}$ “overshoot” during the exercise transition in the older untrained group indicating an O$_2$ delivery limitation accompanying the slow VO$_2$ kinetics.

Many studies have reported the age-related decline in VO$_{2\text{max}}$ (Beere et al., 1999; Betik & Hepple, 2008; Fleg et al., 2005; Jackson et al., 1995; Pimental et al., 2003; Stathokostas et al., 2004; Trappe et al., 1996; Wilson & Tanaka, 2000). The VO$_{2\text{max}}$ values in this study decreased as a function of age for both training groups ($\sim$5mL·kg$^{-1}$·min$^{-1}$ per decade), with each group having a significantly lower VO$_{2\text{max}}$ than the preceding training-matched younger group, and with endurance trained group values consistently greater than untrained, which is in accordance with the literature (Jackson et al., 1995; Pimental et al., 2003; Stathokostas et al., 2004; Wilson & Tanaka, 2000). Both trained and untrained groups showed similar rates of decline (8 – 9% per decade) in VO$_{2\text{max}}$, which is also similar to the literature ($\sim$6 – 10%) (Fleg et al., 2005; Pimental et al., 2003; Stathokostas et al., 2004; Trappe et al., 1996; Wilson & Tanaka, 2000). Previous cross-sectional (Pimental et al., 2003) and longitudinal (Fleg et al., 2005) studies have revealed an accelerated decline in VO$_{2\text{max}}$ with age, which also occurs in the present study. Fleg et al. (2005) found a 3 – 6% decline per decade in the 20s and 30s, and >20% per decade in the 70s and later; in the present study, the declines between young and middle-aged were 6 and 3% (for trained and untrained, respectively), and between middle-aged and older were 15 and 23% (for trained and untrained, respectively). Jackson et al. (1995) have partially attributed the accelerated decline in VO$_{2\text{max}}$ to an increase in body fat with age; thus, the increase in body mass (although
not significant) with age in the present study partially contributes to the greater decline in VO$_2$max at older age. The untrained subjects of the present study were recreationally active men, and had VO$_2$max values for young, middle-aged, and older, respectively, that were 14, 23 and 7% greater than the averages reported for an age-matched population (ACSM, 2013). Endurance trained groups were heavily trained and had VO$_2$max values for young, middle-aged, and older, respectively, that were 35, 40, and 36% greater than the averages reported for an age-matched population (ACSM, 2013). It should be considered that although training distances decreased with age, the level of activity in the middle-aged and older group (>10 hours•week$^{-1}$) was still considerably beyond that of recreationally active individuals at any age.

A number of studies have measured τVO$_2$p values in young untrained men (Berger et al., 2006b; Gurd et al., 2006, 2008; Koppo et al., 2004; McKay et al., 2009; Murias et al., 2010, 2012; Spencer et al., 2011, 2012); our group mean τVO$_2$p values in young (26 s) are in agreement with these studies which reported a range between 21 and 34 s. Additionally, a number of studies have examined τVO$_2$p values in older untrained men (Berger et al., 2006a; Chilibeck et al., 1996; Gurd et al., 2008; Murias et al., 2010); our group mean τVO$_2$p values in the old untrained (42 s) are also similar to that of the literature which reported a range between 40 and 55 s. Furthermore, a number of training studies have measured the change in τVO$_2$p following endurance-training programs lasting ≤12 weeks (Berger et al., 2006b; Fukuoka et al., 2002; Murias et al., 2010). These three training studies have shown a speeding of τVO$_2$p to an average of ~23 s in young (Berger et al., 2006b; Murias et al., 2010) and ~33 s in older (Fukuoka et al., 2002; Murias et al., 2010) men, whereas our values in the chronically endurance trained are lower in both young (17 s) and particularly in the older (20 s) group. The trained men of the present study have been training for more than a few years (averaging 6 in young and 23 years in older) and are considered long-term, chronically endurance trained, which is most likely the reason for the faster VO$_2$ kinetics. Evidence to support this is found in one cross-sectional study in young (Koppo et al., 2004) and one in old (Berger et al., 2006a) looking at long-term, chronically endurance trained men. In these studies τVO$_2$p values of 12 s for young (Koppo et al., 2004) and 29 s for older (Berger et al., 2006a) are fast relative to untrained, which is in accordance with our results (17 and 20 s for young and older, respectively). Therefore, studies of transient endurance training (i.e. ≤12 week endurance training programs) in men did not show τVO$_2$p values reduced to as low as values seen in our long-term endurance trained young and older men. Thus, long-
term endurance training appears to prevent the age-related slowing of VO₂ kinetics, despite an age-related decline in VO₂max.

Few studies exist that have measured VO₂ kinetics in the middle-aged population. Berger et al. (2006a) and Fukuoka et al. (2002) measured τVO₂p in untrained middle-aged men and found values averaging ~48 s, which are greater than the present study (24 s). This discrepancy is possibly due to the difference in relative physical activity of the groups as the subjects in the present study were recreationally active and the subjects in their studies were sedentary. In our middle-aged group we found that in the chronic endurance training group the τVO₂p (18 s) demonstrated fast VO₂ kinetics with a τ similar to that of younger endurance trained (17 s); whereas, other studies in middle-aged endurance-trained men did not reach such low values (Berger et al., 2006a; Fukuoka et al., 2002). Research groups examining middle-aged endurance-trained men via cross-sectional study (Berger et al., 2006a) or following a 90-day endurance-training program (Fukuoka et al., 2002) found a speeding of τVO₂p to only 25 (Berger et al., 2006a) and 29 s (Fukuoka et al., 2002). In the end, the chronically endurance trained men of the present study were able to prevent the age-related slowing of VO₂ kinetics, whereas transient endurance training programs are unable to speed VO₂ kinetics to that of faster young endurance-trained.

Is the age-related slowing of VO₂ kinetics attributable to lack of physical activity with age or rather aging itself? VO₂max declined at similar rates in both trained and untrained groups, despite the endurance trained individuals being heavily active at all ages; thus, the decline in VO₂max does not necessarily indicate a decline in physical activity, but rather the natural loss in maximal cardiac output (Fuchi et al., 1989; Hagberg et al., 1985, Rowell, 1974; Wilson & Tanaka, 2000). However, in individuals with chronically high levels of physical activity, the age-related increase in τVO₂p was abolished, thus maintaining a VO₂ kinetic profile similar to that of the young endurance trained. Therefore, individuals who are long-term heavily active are able to abolish the change in τVO₂p with age, and since τVO₂p is a sub-maximal measure, it is possible that a different mechanism exists (other than the mechanisms determining VO₂max) that governs the VO₂ kinetic response with age.
What mechanism or regulatory factor might constrain VO$_2$ kinetics with age, which is abolished in long-term endurance training? A limitation in O$_2$ delivery to the exercising muscle has been proposed as a likely mechanism regulating the rate of adaptation of oxidative phosphorylation (Murias et al., 2010, 2011b, 2012, 2014; Poole et al., 2008; Spencer et al., 2012). It has been hypothesized (Phillips et al., 1995) that faster femoral artery blood velocity (from endurance training) was responsible for reductions in τVO$_{2p}$; however, measures of muscle conduit artery blood flow kinetics in young healthy adults have shown that the rate of adjustment is similar to or faster than that of VO$_{2p}$ (duManoir et al., 2010; MacPhee et al., 2005). Furthermore, in a training study of older adults that resulted in faster VO$_2$ kinetics, the kinetics of femoral artery mean blood velocity remained unchanged following training (Bell et al., 2001a). Therefore, bulk O$_2$ delivery does not seem to be limiting VO$_2$ kinetics or the adaptation to training that results in faster VO$_2$ kinetics. Recent advancements in NIRS have allowed a continuous assessment of tissue deoxygenation at the exercising muscle, providing an index of O$_2$ extraction and an insight into local microvascular O$_2$ delivery. Our laboratory has applied this measure in conjunction with VO$_2$ kinetics, to show a faster adjustment of the [HHb] signal than the adjustment of phase II VO$_{2p}$ in individuals with relatively slow kinetics (τVO$_{2p}$ > 20 s) (DeLorey et al., 2004a; Murias et al., 2011b, 2012; Spencer et al., 2012); this is represented by a transient [HHb]/VO$_{2p}$ “overshoot.” The overshoot relative to the [HHb]/VO$_{2p}$ ratio established at the steady-state response (ratio = 1.0) indicates a greater fractional O$_2$ extraction and thus poorer blood flow distribution to the active muscle. In the present study, only the older untrained group demonstrated a significant [HHb]/VO$_{2p}$ overshoot throughout the transition to moderate-intensity exercise; thus, older untrained men appear to have an O$_2$ delivery limitation that is prevented by chronic endurance training.

It was noted in the present study that a “true” [HHb]/VO$_{2p}$ overshoot was shown only in the older untrained group. The young untrained and both middle-aged and older trained groups showed a brief overshoot that occurred relatively early in the exercise transition (from 25 to ≤ 35 s), whereas in the older untrained group the overshoot extended from 20 to 75 s. It is noteworthy to mention that two subjects in each of the middle-aged and older endurance trained groups had a “true” overshoot of the [HHb] signal relative to its steady-state at ~90 s into exercise (i.e. considerable overshoot in the [HHb] to that of ~140 – 160% of steady-state), potentially leading to an overall group overshoot. Therefore, the overshoot in these groups (excluding older
untrained) are likely not representative of an O\textsubscript{2} distribution limitation to the working muscle groups occurring throughout the adjustment toward the steady-state relationship of the [HHb]/VO\textsubscript{2p}.

The present study suggests that long-term or chronic training is required to maintain a VO\textsubscript{2} kinetic profile similar to young endurance trained; whereas a previous study by Murias et al. (2010) examined the change in VO\textsubscript{2} kinetics following a 12-week endurance training intervention in older adults. Murias et al. (2010) showed that in older individuals there is an O\textsubscript{2} limitation that was reduced (following 3 weeks of endurance training) to that of regular healthy young, but with no change thereafter. The present study showed that chronically endurance trained older men displayed kinetics faster than young untrained and similar to young endurance-trained men, and the transient training study by Murias et al. (2010) could not speed VO\textsubscript{2} kinetics beyond young untrained. Consequently, it appears that chronic endurance training can prevent and abolish the O\textsubscript{2} delivery limitation, whereas transient multi-week training regimes only partially improve the VO\textsubscript{2} kinetic limitation.

The O\textsubscript{2} delivery limitation (poorer microvascular blood flow) in the older untrained group, represented as the transient overshoot in the [HHb]/VO\textsubscript{2p} ratio, could be explained by a reduced endothelium-dependent vasodilation compared to younger or more active individuals (DeSouza et al., 2000). In regard to aging, animal studies have shown that endothelium-dependent vasodilation was reduced in feed arteries and 1A-arterioles of oxidative soleus muscles in older but not young rats (Muller-Delp et al., 2002), which could contribute to an impaired blood flow distribution. Interestingly, exercise training was shown to restore both flow- (Spier et al., 2007) and endothelium- (Spier et al., 2004) dependent vasodilation in the soleus muscle arterioles of older rats. In humans, DeSouza et al. (2000) tested both chronically endurance-trained individuals and sedentary individuals after 3 months of aerobic endurance training, and found that endurance training could restore (in sedentary older men following endurance training) or prevent (in the chronically endurance trained) the age-related decline in endothelium-dependent vasodilation. This amelioration potentially occurs via a nitric oxide synthase (NOS) dependent mechanism, in which the active tissue increases endothelium-NOS protein expression (Seals et al., 2008; Spier et al., 2004). Therefore, enhancement of endothelium-dependent vasodilation may be responsible for improved blood flow delivery at
exercise onset, and thereby abolish the [HHb]/VO$_2$p overshoot that is seen in older untrained men and absent in older trained men of the present study.

Complimentary to functional changes at the active muscle, structural improvements (i.e. increased capillarization) have also been measured following endurance training (Coggan et al., 1990, 1992; Murias et al., 2011a). Murias et al. (2011a) found increases in capillarization of 20-30% in young and 30-40% in older males within a 12-week endurance training program. Additionally, Coggan et al. (1992) have shown similar improvements in capillary density (increases of 21%) following 9-12 months of endurance training in older men. In chronically trained masters athletes, Coggan et al. (1990) found capillary densities that were similar to that of training-matched young athletes. The results of these studies suggest that short- and long-term endurance training is associated with substantial gains in capillarization, which reflects better O$_2$ delivery and the potential for improved O$_2$ distribution. Increases in capillarization indicate that a larger surface area is available for O$_2$ exchange, suggesting an elevated O$_2$ flux capacity exists between the capillaries and the muscle fibers (Hepple et al., 1997). These structural changes, accompanied by the improvements in endothelium-NOS protein expression, indicate that endurance training profoundly improves blood flow delivery and thus O$_2$ flux at the onset of exercise.

_Limitations:_

Although the present study was interested in the three age groups, in order to distinguish age-related changes in VO$_2$ kinetics across all ages a greater representation of (trained and untrained) men aged 30 to 45 in particular, would be required; larger and more homogenous groups (in terms of activity level), which better represent the average population would be more ideal. A limitation also lies in the subject recruitment. The untrained individuals were recreationally active, and although not participating in systemic training program, were involved in an active lifestyle; it is difficult to know whether the “degree” of activity was similar across age groups and this could affect whether the VO$_2$max and the $\tau$VO$_2$p were different between age-groups. Nevertheless, based on their relative cardiorespiratory fitness (i.e. VO$_2$max values that were 7 – 23% above average) the untrained men seem to be similar representations of the population at each age group. It would be of interest to study sedentary individuals, however a difficulty lies within recruiting a truly sedentary population. Additionally, the endurance trained
cyclists are not uniformly trained; each individual cyclist abides by his own fitness regime, which varies with age in distance and number of rides per week. Nevertheless, all trained cyclists were heavily active with cardiorespiratory fitness levels (VO2max) 35 – 40% greater than the average population.

Conclusion:

In summary, the age-related slowing of VO2 kinetics can be attenuated with short-term endurance training (Murias et al., 2010) in young and older; however, the present study demonstrated that long-term endurance training is required to abolish the age-related slowing of VO2 kinetics and maintain τVO2p values comparable to young endurance trained. The slower VO2 kinetics in the older untrained group was associated with an [HHb]/VO2p overshoot, indicating an O2 delivery limitation. The older endurance trained group (with τVO2p values similar to young endurance trained) did not present a “true” [HHb]/ VO2p overshoot, suggesting long-term endurance training provides functional (enhanced endothelium-dependent vasodilation) as well as structural (increased capillarization) improvements in order to abolish the O2 delivery limitation associated with normal aging.
REFERENCE LIST


APPENDIX A: ETHICS APPROVAL NOTICE

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Donald Paterson
Review Number: 18148
Review Level: Full Board
Approved Local Adult Participants: 48
Approved Local Minor Participants: 0
Protocol Title: Elite cyclists vs. untrained controls: comparing the VO2 kinetics response in young, middle aged and older men.
Department & Institution: Kinesiology, University of Western Ontario
Sponsor: Natural Sciences and Engineering Research Council

Ethics Approval Date: September 12, 2011
Expiry Date: August 31, 2012

Documents Reviewed & Approved & Documents Received for Information:

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This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (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 study on the approval date noted above. The membership of this HSREB 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.

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