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

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

THE EFFECTS OF AGE AND LONG-TERM ENDURANCE TRAINING ON VO_2 KINETICS

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by

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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 (τVO_{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, τVO_{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 τVO_{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 τVO_{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_iO_2 – fraction of inspired O_2

[HbO_2] – oxyhemoglobin, measure of muscle oxygenation concentration

[HHb] – deoxyhemoglobin, measure of muscle deoxygenation concentration

HR – heart rate

θ_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

PCO_2 – partial pressure of carbon dioxide

[PCr] – phosphocreatine concentration

PDH – pyruvate dehydrogenase

PO_2 – 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

VCO_2 – volume of carbon dioxide

VO_2 – volume of oxygen uptake

VO_2max – maximal oxygen uptake; measure of maximal aerobic power

VO_{2m} – muscle oxygen uptake

VO_{2p} – 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_2max) 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_2max 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_2max 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_2max) can be defined by the Fick Equation (equation 1):

$VO_2max = Q \times (a\text{-}vO_2 \text{ difference})$ *Equation 1*

where Q is cardiac output (product of heart rate (HR) and stroke volume) or tissue blood flow, and a-vO_2 difference is the difference between arterial and venous O_2 content and characterizes the tissue's ability to extract oxygen from the circulating blood. Thus, VO_2max 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_2max (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_2max 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_2max may be attributed to both a decline in HR (and consequently Q) and a-vO_2 difference. Nevertheless, the decline in VO_2max 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_{2m}) and pulmonary oxygen uptake (VO_{2p}) 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 (θ_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 (τ).

The τ of the phase II VO_2 (τVO_2) response from the onset of exercise represents the time it takes to achieve 63% of ΔVO_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 τVO_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 τVO_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_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):

$$VO_{2p}(t) = VO_{2BSLN} + Amp[1 - e^{-(t-TD)/\tau}] \qquad \textit{Equation 2}$$

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 τ 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:

$$NADH + H^+ + ½\ O_2 + 3\ ADP + 3\ P_i \rightarrow 3\ ATP + NAD^+ + H_2O \qquad \textit{Equation 3}$$

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 O_2 delivery and metabolic substrate provision via heavy priming exercise (i.e. MOD1-HVY-MOD2 protocol) but with the addition of hypoxia (through reduced FiO_2 which presumably maintains O_2 delivery (as an increase in blood flow compensation) but impairs PaO_2 and the vascular muscle O_2 flux gradient) have resulted in lengthened τVO_2 despite the priming effects of increase metabolic substrate provision (Spencer et al., 2012). These results suggest that O_2 delivery is a major factor in the limitation of the rate of oxidative phosphorylation.

However, when attempting to speed VO_2 kinetics via increased O_2 availability, especially in the MOD, there seems to be no impact on τVO_2. In the pump-perfused dog hindlimb model, Grassi and colleagues showed no speeding of VO_2 kinetics despite improving bulk convective blood flow and peripheral diffusive O_2 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 (FiO_2 >50%) on VO_2 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 O_2 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 O_2 delivery); the general conclusion is that (for individuals with τVO_{2p} > ~ 20 s) the rate of adjustment of VO_2 is mainly constrained by the matching of local O_2 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 [HbO_2] and deoxygenated hemoglobin [HHb]

within the tissue. Thus, the quantification of specific changes in [HbO_2] and [HHb] can be utilized to provide an index of O_2 extraction during transitions from baseline to MOD. Therefore, the NIRS method provides an insight into the local microvascular O_2 delivery at the working muscle and the rate of O_2 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, $FADH_2$) 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 VO_2 kinetics would suggest that if augmenting O_2 delivery causes no perceptible changes in τVO_2, 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 O_2 utilization. However, experiments in humans (Bangsbo et al., 2002; Jones et al., 2004; Rossiter et al., 2003) failed to demonstrate faster VO_2 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 τVO_2. This lack of consensus suggests that the hypothesis of sluggish activation of substrate could result in slow VO_2 kinetics, however O_2 delivery appears to be a primary factor related to a slow VO_2 kinetic response.

VO_2 Kinetics and Aging

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_{2p}) 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_2max 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_2max 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_2max 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_2max (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_2max 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_2max 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_2max 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_2max 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_2max 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_2 availability (i.e., enhanced muscle perfusion or blood flow) (Murias et al., 2010). These data also suggest that the slowing of VO_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_2 delivery (via O_2 diffusion at the capillaries) in endurance-trained men could speed VO_2 kinetics.

Interestingly, the amount by which the slowing of VO_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 τVO_2 values to that of healthy younger individuals, whereas sprint trained athletes had a slowing of VO_2 kinetics with age (however all τVO_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_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_2 delivery (Scheuermann et al., 2002).

Although it is well known that VO_2max is lower and τVO_{2p} 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_2max (Beere et al., 1999; Hagberg et al., 1985; Pimental et al., 2003; Rogers et al., 1990; Trappe et al., 1996) and lower τVO_{2p} (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_2 responses in young, middle-aged, and older, endurance trained and untrained men. It was hypothesized that there would be a continuous increase in τVO_{2p} from young to older men, in both the trained and untrained groups, with τVO_{2p} values in the trained groups lower than the untrained counterparts. Additionally, it was hypothesized that the [HHb] in groups with slow VO_2 kinetics would reveal a greater muscle deoxygenation for a given VO_2 during the exercise transient, representing a sluggish microvascular O_2 delivery.

CHAPTER 2

2 THE EFFECTS OF AGE AND LONG-TERM ENDURANCE TRAINING ON VO_2 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 VO_2max 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 VO_2 kinetics, few studies have examined the differences across age groups and particularly in chronically trained and untrained men in different age groups.

The VO_2 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 O_2 deficit and may experience premature fatigue (DeLorey et al., 2007). The slower VO_2 kinetics associated with aging may be associated with a limitation in O_2 delivery to the exercising muscle (Murias et al., 2010). Endurance training exercise has been shown to speed VO_2 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 VO_2 kinetics is due to aging per se or a lack of physical activity. Since VO_2 kinetics is a measure of differing physiological regulations/control of aerobic metabolism, analysis of VO_2 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 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); 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 $km \bullet wk^{-1}$.

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 VO_2 (VO_2max) and the estimated lactate threshold (θ_L); the ramp portion of the protocol was initiated following 4 minutes of cycling at 20 W (watts). Peak VO_2 (VO_2max) was determined as the maximal 20 s averaged VO_{2p} 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. θ_L was determined by visual inspection as the VO_2 at which CO_2 (carbon dioxide) 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 (partial pressure of O_2) whereas minute ventilation-to-VCO_2 ratio and end-tidal PCO_2 (partial pressure of CO_2) were stable (Beaver et al., 1986).

From the results of this ramp test, a moderate-intensity work rate (WR) was selected to elicit a VO_2 equivalent to ~80% of the VO_2 at θ_L (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 O_2, CO_2, and N_2 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 (λ = 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 (μ_A) and reduced scattering coefficients (μ_s') previously measured; thus, correction factors were determined and were automatically implemented by the manufacturer's software for the calculation of the μ_A and μ_s' for each wavelength during the data collection. Calculation of [HHb] reflected continuous measurements of μ_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:

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

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}; τ 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 τ 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[HHb]) 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[HHb] 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[HHb] 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 τ[HHb]. Baseline [HHb] ($[HHb]_{BSLN}$) values were computed as the mean value in the 60 s prior to a transition. Whereas the τ[HHb] described the time course for the increase in [HHb], the overall change of the effective [HHb] (τ'[HHb] = TD[HHb] + τ[HHb]) 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 VO_{2p} 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 VO_{2p} 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 VO_{2p} (Murias et al., 2011b), which has been previously described to correspond with muscle VO_2 (VO_{2m}) 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 VO_{2p}. Additionally, an overall average [HHb]/VO_{2p} 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]/VO_{2p} "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 VO_2max data, as well as group means and SD, are presented in Fig. 1. VO_2max 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. VO_2max 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).

VO_2 Kinetics. Individual τVO_{2p} data, as well as group means and SD, are presented in Fig. 2. Phase II τVO_{2p} was similar between the trained and untrained groups for both Y and M groups (Table 3). τVO_{2p} did not significantly increase with age in the trained group; whereas, τVO_{2p} 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, $VO_{2p\ AMP}$ 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). $VO_{2p\ TD}$ 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 VO_{2p} adjustments to the step-transition in work rate are presented in Fig. 3. Greater adjustment in [HHb] compared to VO_{2p} resulted in a small transient "overshoot" in [HHb]/VO_{2p} 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

	n	Age (yr)	Body Mass (kg)	Height (cm)	VO_2max (L•min^{-1})	HR max (beats•min^{-1})	RER max
YT	8	24 ± 6	68 ± 9	177 ± 5	4.5 ± 0.5	195 ± 7	1.26 ± 0.06
MT	9	52 ± 5	80 ± 7	179 ± 7	4.4 ± 0.4	176 ± 5†	1.21 ± 0.08
OT	9	64 ± 3	77 ± 12	181 ± 8	3.4 ± 0.4†‡	165 ± 6†‡	1.21 ± 0.05
YuT	8	23 ± 4	80 ± 9	181 ± 6	4.0 ± 0.4	194 ± 3	1.32 ± 0.08
MuT	9	52 ± 2	80 ± 9	175 ± 6	3.6 ± 0.4*	173 ± 8†	1.24 ± 0.06
OuT	8	68 ± 5	85 ± 11	174 ± 7	2.5 ± 0.6*†‡	154 ± 9†‡	1.33 ± 0.10

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

Trained	# of rides (wk^{-1})	Weekly Distance ($km \bullet wk^{-1}$)	Years Training (yr)
YT	6.1 ± 0.7	435 ± 180	6.0 ± 2.9
MT	5.0 ± 0.9	309 ± 84	15.4 ± 6.5
OT	4.8 ± 0.4	304 ± 71	23.0 ± 6.8

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

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

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

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

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 ($P < 0.05$).

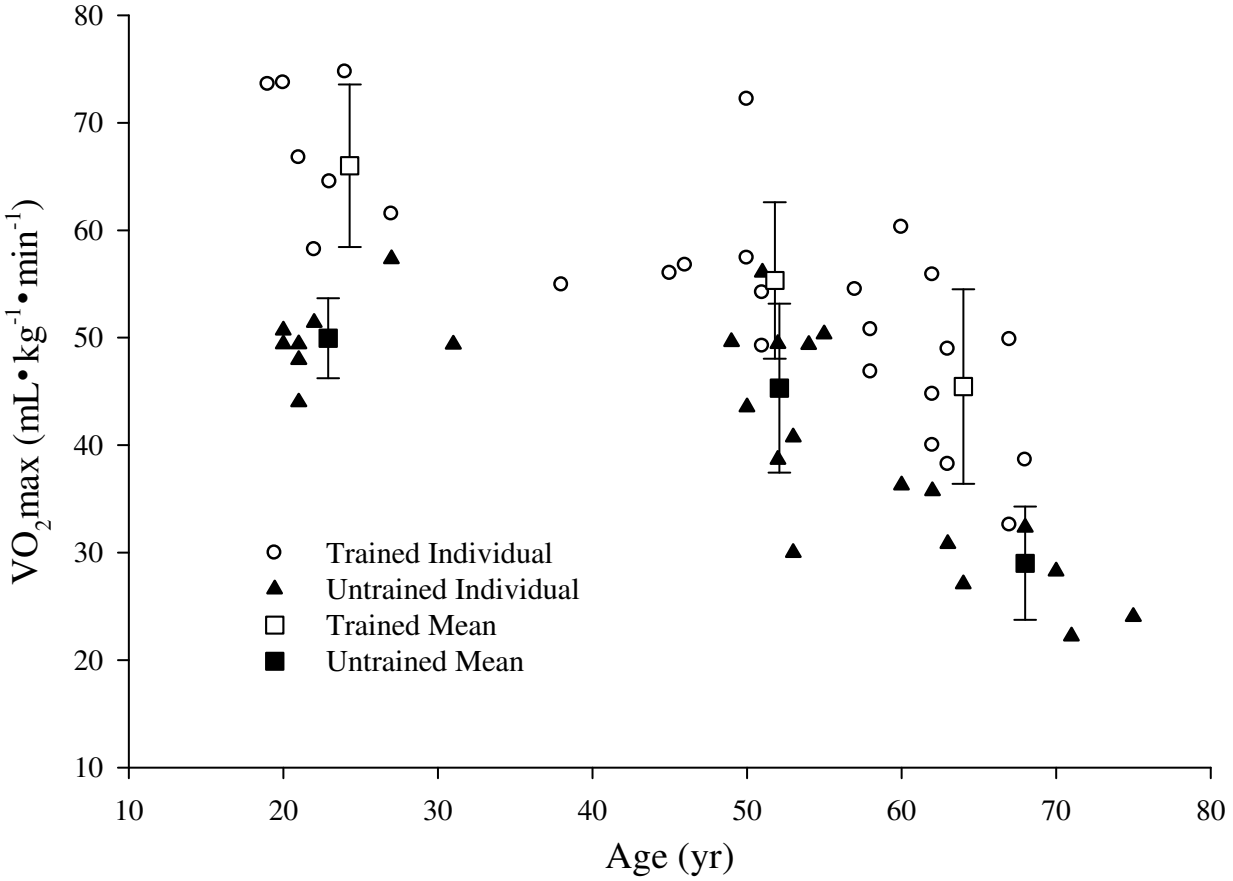

Fig. 1. Individual and average group VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$) values. Group values are mean ± SD.

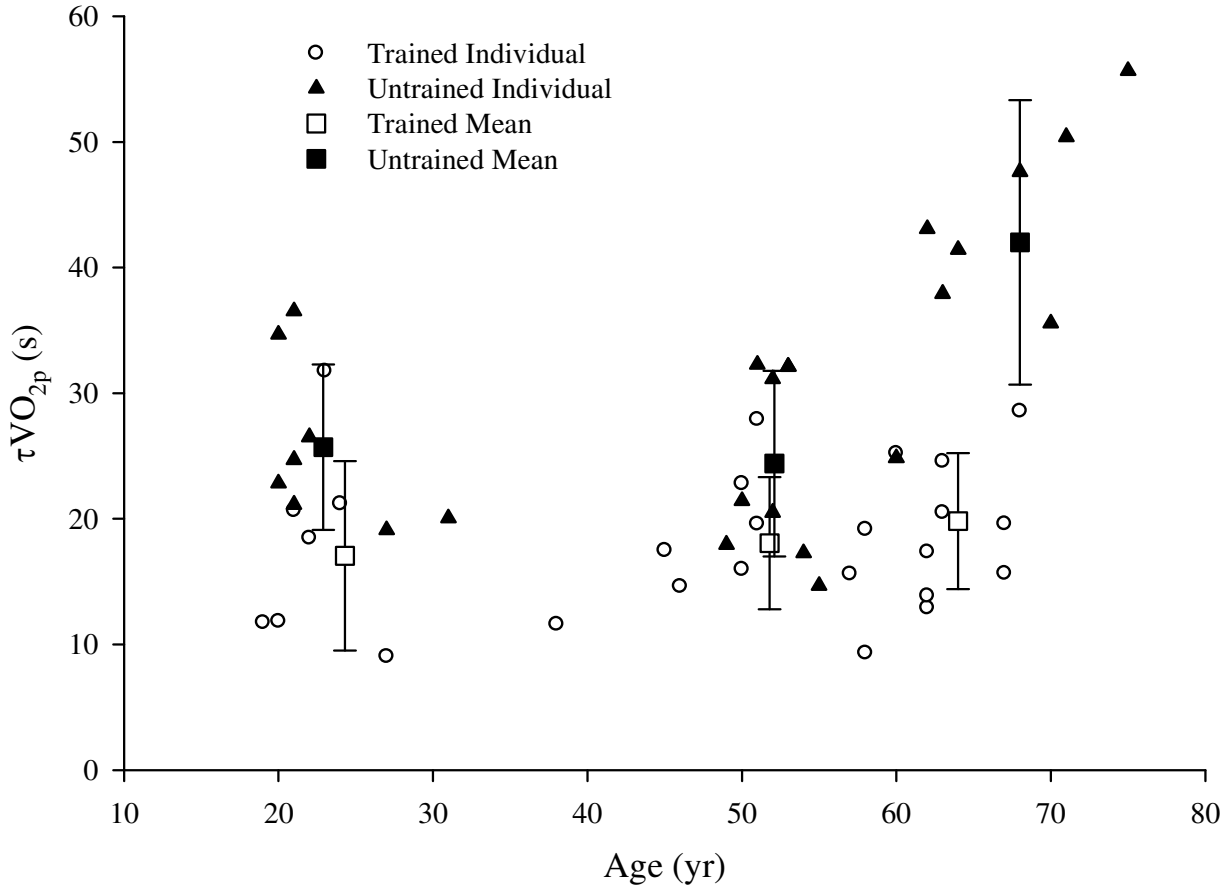

Fig. 2. Individual and average group τVO_{2p} (s) values. Group values are mean ± SD.

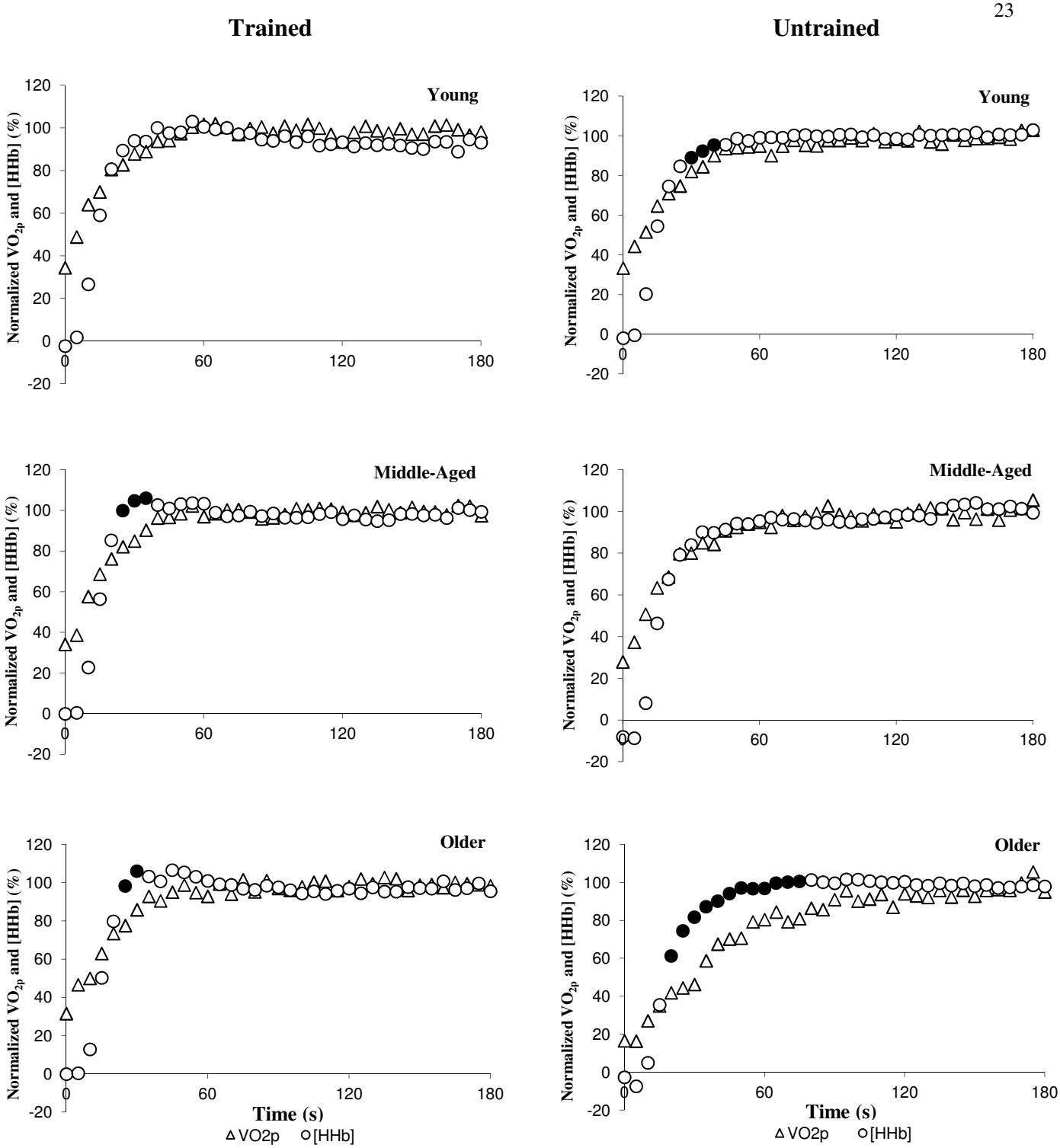

Fig. 3. Group mean profiles for the adjustment of [HHb] and VO_{2p} (left shifted such that data from phase I VO_{2p} were not included) during the step transition to MOD. •, time points at which the relative increase of [HHb] is greater than the relative increase of VO_{2p} ($P < 0.05$).

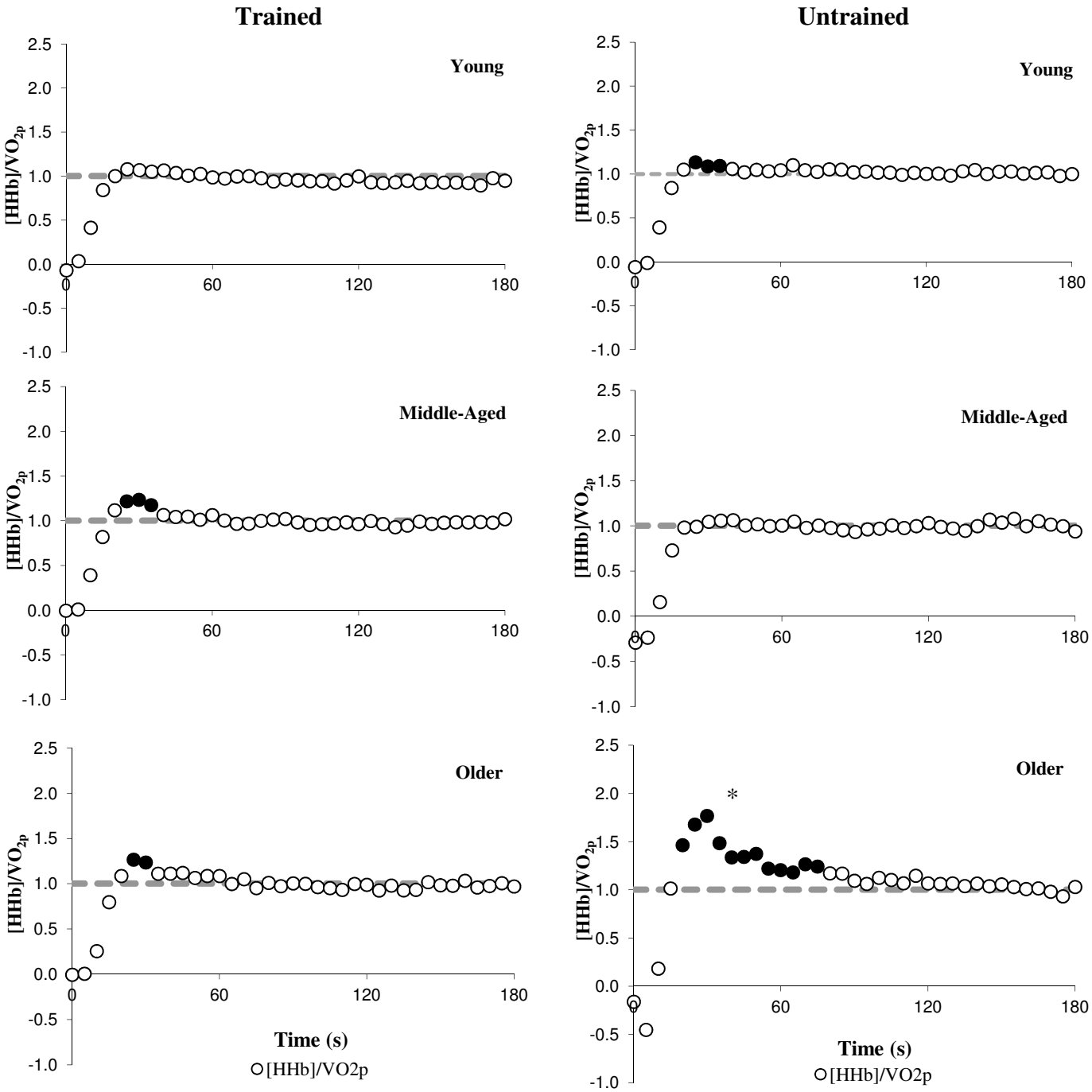

Fig. 4. Group mean profiles for the adjustment of $[HHb]/VO_{2p}$ during the step transition to MOD. ●, time points at which the relative increase of [HHb] is greater than the relative increase of VO_{2p} ($P < 0.05$). *$[HHb]/VO_{2p}$ significantly different from 1.0 ($P < 0.05$).

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, τVO_{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, τVO_{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_2max (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_2max values in this study decreased as a function of age for both training groups (~5mL•kg^{-1}•min^{-1} per decade), with each group having a significantly lower VO_2max 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_2max, which is also similar to the literature (~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_2max 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_2max 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_2max at older age. The untrained subjects of the present study were recreationally active men, and had VO_2max 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_2max 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_{2p} 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_{2p} 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_{2p} 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_{2p} 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_{2p} 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_{2p} 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 el al., 2006a) looking at long-term, chronically endurance trained men. In these studies τVO_{2p} 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_{2p} 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 VO_2 kinetics, despite an age-related decline in VO_2max.

Few studies exist that have measured VO_2 kinetics in the middle-aged population. Berger et al. (2006a) and Fukuoka et al. (2002) measured τVO_{2p} 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 τVO_{2p} (18 s) demonstrated fast VO_2 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 τVO_{2p} 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 VO_2 kinetics, whereas transient endurance training programs are unable to speed VO_2 kinetics to that of faster young endurance-trained.

Is the age-related slowing of VO_2 kinetics attributable to lack of physical activity with age or rather aging itself? VO_2max 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 VO_2max 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 τVO_{2p} was abolished, thus maintaining a VO_2 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 τVO_{2p} with age, and since τVO_{2p} is a sub-maximal measure, it is possible that a different mechanism exists (other than the mechanisms determining VO_2max) that governs the VO_2 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 ($\tau VO_{2p} > 20$ 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 $\leq$ 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_2 distribution limitation to the working muscle groups occurring throughout the adjustment toward the steady-state relationship of the $[HHb]/VO_{2p}$.

The present study suggests that long-term or chronic training is required to maintain a VO_2 kinetic profile similar to young endurance trained; whereas a previous study by Murias et al. (2010) examined the change in VO_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_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_2 kinetics beyond young untrained. Consequently, it appears that chronic endurance training can prevent and abolish the O_2 delivery limitation, whereas transient multi-week training regimes only partially improve the VO_2 kinetic limitation.

The O_2 delivery limitation (poorer microvascular blood flow) in the older untrained group, represented as the transient overshoot in the $[HHb]/VO_{2p}$ 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_{2p} 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_2max and the τVO_{2p} were different between age-groups. Nevertheless, based on their relative cardiorespiratory fitness (i.e. VO_2max 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 (VO_2max) 35 – 40% greater than the average population.

Conclusion:

In summary, the age-related slowing of VO_2 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 VO_2 kinetics and maintain τVO_{2p} values comparable to young endurance trained. The slower VO_2 kinetics in the older untrained group was associated with an [HHb]/VO_{2p} overshoot, indicating an O_2 delivery limitation. The older endurance trained group (with τVO_{2p} values similar to young endurance trained) did not present a "true" [HHb]/ VO_{2p} overshoot, suggesting long-term endurance training provides functional (enhanced endothelium-dependent vasodilation) as well as structural (increased capillarization) improvements in order to abolish the O_2 delivery limitation associated with normal aging.

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APPENDIX A: ETHICS APPROVAL NOTICE

Research Western

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:

Document Name	Comments	Version Date
UWO Protocol		
Letter of Information & Consent		2011/08/23
Other	Script- Non student and student volunteers	

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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Name:	Tyler M. Grey, B.Sc., M.Sc. Candidate
Post-secondary Education and Degrees:	The University of Western Ontario London, Ontario, Canada 2012-2014 M.Sc. Kinesiology The University of Western Ontario London, Ontario, Canada 2007-2011 B.Sc. Kinesiology
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Grey TM, Redguard DA, Wengle RS, Wegscheider PK. Effect of plantar flexor muscle fatigue on postural control (2013). *Western Undergraduate Research Journal: Health and Natural Science*. 3: 1-7.

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