MEAN BLOOD VELOCITY AND OXYGEN UPTAKE KINETICS IN OLDER AND YOUNGER MEN

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ABSTRACT

Oxygen uptake ($\dot{V}O_2$) kinetics have been shown to be slower in older compared to younger adults during the on-transient of exercise involving a large muscle mass (cycling) but similar during small muscle mass exercise (plantar flexion). This may be explained by a limited capacity to transport oxygen during large muscle mass activity in these older adults. The purpose of the present study was to examine the kinetics of $\dot{V}O_2$ and mean blood velocity (MBV) in older and younger men during the on-transient of single leg knee extension (KE) exercise. Six healthy older (77±6 yr) and six healthy younger (27±3 yr) men performed constant load single leg knee extension (KE) exercise (30 extensions/min) transitions from loadless work to a work rate eliciting a $\dot{V}O_2$ of approximately 60% of their peak KE $\dot{V}O_2$ ($\dot{V}O_{2pkKE}$). Breath-by-breath alveolar $\dot{V}O_2$ data, collected using a mass spectrometer, were time aligned and ensemble averaged. The $\dot{V}O_2$ on-transient was modelled with a single exponential from *phase 2* onset at 20 s to end-exercise. Femoral artery MBV was determined using a 4 MHz pulsed wave Doppler ultrasound probe placed directly over the femoral artery, distal to the inguinal ligament. MBV data were averaged over 2 second intervals (1 contraction cycle) and fit with a single exponential model. The time constants (τ) of the least squares regression models were used to describe the changes in $\dot{V}O_2$ and MBV dynamics. $\dot{V}O_{2pkKE}$ was significantly lower in old (1.00 ± 0.19 l•min^{-1}) than in young adults (1.51 ± 0.32 l•min^{-1}). $\tau\dot{V}O_2$ was significantly slower in old (91.2 ± 13.4 s) compared to young adults (36.6 ± 6.0 s) ($P<0.05$). In contrast, τMBV was not different between the two groups (old, 25.3 ± 2.9 s; young, 20.2 ± 2.5 s, $P>0.05$). Correlations of $\tau\dot{V}O_2$ and τMBV were not significant (for all

subjects combined, $r = 0.23$; $P = 0.47$). Furthermore, no differences were observed between unloaded and steady state exercise MBV responses in old or young adults. With the observed similarity in MBV response coupled with the considerably faster rate of increase in MBV than $\dot{V}O_2$, it was concluded that the transport of blood to the exercising muscle was not limiting the kinetics of $\dot{V}O_2$ in the old or in the young. Discounting a blood flow limitation, the slowing of $\dot{V}O_2$ kinetics evidenced in older men may be rate controlled by the inertia of muscle oxidative metabolism.

Keywords: older adults, knee extension exercise, oxygen uptake kinetics, mean blood velocity, Doppler ultrasound, femoral artery.

This work is dedicated to my father the architect,
who with pencil, ruler and precision,
shaped many a building;
who with love, compassion and guidance,
shaped a boy into a man.

Thank you for always having room for me
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CHAPTER I

INTRODUCTION

Subsequent to the onset of constant load exercise, alveolar oxygen uptake ($\dot{V}O_2$) increases to reflect the increase in muscle oxidative metabolism (Whipp *et al.*, 1982; Paterson & Whipp, 1991; Rossiter *et al.*, 1999). The fundamental, or primary *phase 2* component of the increase of $\dot{V}O_2$ is characterized by linear, first-order dynamics (Whipp *et al.*, 1986) such that the time constant (τ) of the *phase 2* response is invariant in the moderate to heavy work intensity domains (Barstow & Mole, 1991; Barstow *et al.*, 1993). Efforts to elucidate the regulatory mechanism(s) responsible for controlling the rate of adaptation of *phase 2* $\dot{V}O_2$ have resolved to a dichotomy of support with two opposing sides: one purporting that the non-steady state increase in $\dot{V}O_2$ is limited by the transport of oxygen (O_2) to the muscle (Hughson, 1990; Tschakovsky & Hughson, 1999); the other suggesting the rate of adaptation is determined by muscle O_2 utilization, such that the kinetics of $\dot{V}O_2$ are limited by the inertia of muscle oxidative metabolism (Whipp & Mahler, 1980; Grassi *et al.*, 1996; Grassi *et al.*, 1998b).

The kinetics of $\dot{V}O_2$ have been shown to be slower in older compared to younger adults (Cunningham *et al.*, 1993; Babcock *et al.*, 1994b; Chilibeck *et al.*, 1996). Whereas slowed kinetics in old compared to young have been demonstrated during both cycling and treadmill running, both groups evidence a similar rate of adaptation during plantar flexion

exercise relative to younger subjects (Chilibeck *et al.*, 1996). The similarity observed in the adaptation time course in both older and younger individuals during plantar flexion exercise may be explained by the frequent recruitment of this muscle group, as this may serve to preserve a well-trained state and thus offset the age-associated slowing evidenced during quadriceps exercise in older adults (Chilibeck *et al.*, 1998). Accordingly, previous work has demonstrated that aerobic training can speed $\dot{V}O_2$ kinetics, such that the response time of an older subject will begin to approach that of a younger fit individual (Babcock *et al.*, 1994a).

The slower $\dot{V}O_2$ kinetics in older adults may reflect a limited ability to transport O_2 in these individuals. Slower heart rate (HR) kinetics and significant correlations between τHR and $\tau\dot{V}O_2$ are thought to indicate that a slowly adjusting cardiac output at the onset of exercise may be responsible for the slowed $\tau\dot{V}O_2$ observed in older subjects (Cunningham *et al.*, 1993; Chilibeck *et al.*, 1996). Further, Petrella and colleagues (Petrella *et al.*, 1999) have shown that sedentary older adults may experience slowed $\dot{V}O_2$ kinetics consequent to poor diastolic function and cardiac filling. Following improvements in cardiac function through ingestion of a calcium channel blocker, Petrella et al. (1999) showed a significantly faster $\tau\dot{V}O_2$ in their older subjects, suggested to result from improved O_2 transport. In contrast to these findings, Bell et al. (1999) attempted to increase O_2 delivery by having their older subjects breathe hyperoxic gas while exercising, but observed no improvement in $\tau\dot{V}O_2$, suggesting O_2 delivery to the muscle was not the limitation.

The prolonged time course of adaptation in $\dot{V}O_2$ exhibited by older adults may be due to age-related structural or biochemical changes at the level of the muscle. Coggan et al. (1992a) observed significant decrements in both muscle capillarization as well as in oxidative

enzyme activity in sedentary older versus younger individuals. Thus, the slowed $\dot{V}O_2$ response in older adults may be explained by compromised functioning of the oxidative machinery.

The use of single leg knee extension (KE) exercise affords a convenient approach to study the on-transient kinetics of gas exchange as $\dot{V}O_2$ measured at the mouth can be obtained in concert with estimates of blood flow to the working quadriceps muscle. This method has been documented in the literature (Shoemaker *et al.*, 1994; Shoemaker *et al.*, 1996b; MacDonald *et al.*, 1998) in studies examining the cardiorespiratory and hemodynamic responses during exercise. The model allows investigation to be directed at an isolated muscle which is capable of eliciting an adequate elevation in whole body energy turnover, thus facilitating the collection of breath-by-breath changes in gas exchange. Furthermore, single leg KE exercise would not be expected to overly tax the central O_2 transport system, yet would recruit a muscle group that would not be expected to remain trained with age. Therefore, the single leg model presents an attractive means to undertake kinetic investigation in older adults.

Recently, the use of pulsed Doppler ultrasound has gained increased support as a means whereby non-invasive measures of changes in blood delivery to the working muscle during both on- and off-transients can be approximated (Shoemaker *et al.*, 1994; Shoemaker *et al.*, 1996b; Hughson *et al.*, 1997; MacDonald *et al.*, 1998). This technology has permitted the simultaneous determination of beat-by-beat changes in blood velocity and alveolar gas exchange kinetics during dynamic exercise. Furthermore, recent work examining femoral artery blood flow in rhythmically contracting knee extensor muscles has shown that femoral

artery diameter is not altered during exercise (Radegran, 1997; MacDonald *et al.*, 1998). It follows then that changes in blood flow during single leg KE exercise can be reasonably approximated by changes in Doppler ultrasound measures of muscle blood velocity. To date, studies have shown that, in young adults, the rate of response in femoral artery blood velocity (or flow) is faster than $\tau\dot{V}O_2$ during leg extension exercise (Shoemaker *et al.*, 1994; MacDonald *et al.*, 1998). In combination with reports of rapid increases in the adaptation in leg blood flow at the start of rhythmic KE exercise in young adults (Walloe & Wesche, 1988; Eriksen *et al.*, 1990), these data suggest that the kinetics of $\dot{V}O_2$ during leg extension exercise may not be dependent on the rate of O_2 supply.

The purpose of the present study was to compare the kinetics of $\dot{V}O_2$ and mean blood velocity (MBV) during the on-transient of single leg KE exercise in both older and younger men. Consistent with the findings of previous studies, it was hypothesized that MBV would exhibit faster kinetics than $\dot{V}O_2$ in the young adults. If older men demonstrated a similar rapid response in MBV, then the expected slowing of $\dot{V}O_2$ kinetics in these individuals would not be due to a blood flow (or O_2 delivery) limitation. Rather, the slowed $\dot{V}O_2$ response may originate as a consequence of a maldistribution of blood flow or an impediment in the diffusive transfer of O_2 within the tissue, or may be limited by the rate of O_2 utilization at the level of the muscle (metabolic inertia). Should slowed $\dot{V}O_2$ kinetics be evidenced along with a concomitant slowing in MBV kinetics, it would suggest the influence of an O_2 transport limitation in older men.

The prolonged period of adaptation in $\dot{V}O_2$ in older subjects suggests that during the transition from rest to exercise, energetic requirements in these individuals are sustained

through a greater reliance upon anaerobic metabolism. Increasing the proportional contribution of anaerobic resynthesis of ATP introduces the possibility of early fatigue in older adults. By considering the performance of daily activities to be analogous to a series of exercise transitions, a better understanding of mechanisms that control the rate of adaptation of $\dot{V}O_2$ may promote the development of more effective strategies to enhance the quality of life in later years.

CHAPTER 2

REVIEW OF LITERATURE

2.1 The Kinetics of Oxygen Uptake

Following the onset of a step increase in work rate, oxygen uptake ($\dot{V}O_2$) kinetics describe the rate of increase in $\dot{V}O_2$ as it adjusts to meet the energy demands of the exercise. The profile of the $\dot{V}O_2$ response is described by three distinct phases, each of which is manifest by a different mechanism in the adjustment process (Whipp *et al.*, 1982; Casaburi *et al.*, 1989; Paterson & Whipp, 1991; Barstow *et al.*, 1994b; Chilibeck *et al.*, 1997). *Phase I* is described by a rapid and abrupt increase in $\dot{V}O_2$ following the onset of exercise while mixed venous and end-tidal gas tensions remain relatively stable (Weissman *et al.*, 1982). The 'cardiodynamic' *phase I* increase in $\dot{V}O_2$ is the result of (and directly proportional in magnitude to) an increase in pulmonary blood flow owing to an increase in cardiac output ($\dot{Q}$) (Whipp *et al.*, 1982). The duration of *phase I* is ~20 seconds and is thought to reflect the transit delay before gas tension changes in venous blood from the contracting muscles are expressed at the lungs.

Following *phase I*, blood-borne gas signals [decreased venous oxygen (O_2) content and changes in gas partial pressures ($P_{ET}O_2$ and $P_{ET}CO_2$) from muscle metabolism] from the working muscles are reflected by changes in gas exchange at the lungs, indicating the onset of *phase 2* of the response.

For perturbations within the domain of moderate intensity the required $\dot{V}O_2$ is equal to, or less than, the subject's anaerobic threshold (Θ_{an}) and consequently such tasks can be performed without induction of a sustained lactic acidosis (Whipp *et al.*, 1986; Gaesser & Poole, 1996). Under such conditions, the rate of oxidative energy production (i.e., mitochondrial resynthesis of ATP) can readjust to adequately supply the energetic demands of the working musculature. Within the domain of moderate intensity, the increase in $\dot{V}O_2$ is approximated to follow a monoexponential time course:

$$\dot{V}O_2(t) = \dot{V}O_2(ss) \{1 - e^{-[(t-\delta)/\tau]}\}$$

where $\dot{V}O_2(t)$ is the increase of $\dot{V}O_2$ above the prior steady state (or rest) value at time t; $\dot{V}O_2(ss)$ is the increase in $\dot{V}O_2$ from the prior level, or the amplitude of $\dot{V}O_2$; τ represents the time constant of the response (i.e., the time required to reach 63% of the overall response in $\dot{V}O_2$); and δ represents an early delay that proceeds the exponential rise to steady state (Whipp *et al.*, 1982; Whipp *et al.*, 1986; Gaesser & Poole, 1996).

Thus, for moderate intensity exercise, the *phase 2* increase in $\dot{V}O_2$ is characterized by a single exponential equation (Whipp *et al.*, 1982) with a τ of approximately 30 to 45 seconds, which does not vary appreciably with work rate (Davies *et al.*, 1972; Whipp *et al.*, 1986; Casaburi *et al.*, 1989; Gaesser & Poole, 1996). The τ of this phase has been referred to as the 'primary' or 'early' component for $\dot{V}O_2$ (Paterson & Whipp, 1991; Barstow *et al.*, 1993; Barstow, 1994a).

Moderate intensity tasks show a continual increase in $\dot{V}O_2$ throughout *phase 2* until an eventual steady state is reached. This plateau in $\dot{V}O_2$ is termed *phase 3*, and represents the point where oxidative mechanisms of ATP rephosphorylation are matched to the energy

demands of the exercise perturbation. For work rates greater than those of moderate intensity (i.e., > Θ_{an}), *phase 3* describes an additional gradual increase in $\dot{V}O_2$ termed the slow component (Whipp *et al.*, 1986; Paterson & Whipp, 1991; Barstow *et al.*, 1993; Womack *et al.*, 1995; Gaesser & Poole, 1996; Barstow *et al.*, 1996). Most investigators have suggested a delayed emergence of the slow component, suggesting it begins ~80-120 seconds into exercise (Paterson & Whipp, 1991; Barstow & Mole, 1991; Barstow *et al.*, 1993). Some have purported this slow component to originate simultaneously with the onset of the *phase 2* response (Macdonald *et al.*, 1997).

During heavy intensity exercise, investigators have attempted to mathematically describe the more complex $\dot{V}O_2$ response using various models (including one, two, or three components) and selective fitting windows. Paterson and Whipp (1991) demonstrated that the early kinetic phase of the response was best approximated by fitting the data to 3 minutes, and indicated that a monoexponential fit of this primary component was equally good for work rates above and below Θ_{an}. The results showed a slightly slower time constant when comparing supra- to sub-Θ_{an} work rates. Casaburi et al. (1989) also found a biexponential model (where the primary component was fit separately from the slow component) to produce a significantly better fit of the $\dot{V}O_2$ during heavy exercise. In the studies of Barstow et al. (1991; 1993), the τ value for the early component was largely invariant and did not differ significantly from that observed for moderate exercise, compared to the somewhat slower τ observed by Paterson and Whipp (1991) for heavy exercise.

The kinetics of the primary component (*phase 2*) of $\dot{V}O_2$ measured at the lung are of particular interest as they are thought to reflect metabolic control processes exerted at the

level of the working muscles. Similarity in pulmonary measures of $\dot{V}O_2$ obtained in concert with serial measures of arteriovenous O_2 differences (thus allowing computation of $\dot{V}O_2$) across the exercising muscle have demonstrated that changes in gas exchange at the mouth (pulmonary or alveolar $\dot{V}O_2$) closely reflect muscle O_2 uptake (Poole *et al.*, 1992; Grassi *et al.*, 1996). The use of ^{31}P-nuclear magnetic resonance spectroscopy (^{31}P-MRS) has illustrated a close temporal similarity between the kinetics of $\dot{V}O_2$ and the rate of change in phosphocreatine (PCr) during the on- and off-transitions to moderate intensity exercise (Barstow *et al.*, 1994b; McCreary *et al.*, 1996; Rossiter *et al.*, 1999). The observed symmetry in PCr and $\dot{V}O_2$ kinetics reflects the first-order nature of respiratory control and provides further confirmation that pulmonary gas exchange data measured during the transient *phase 2* response adequately reflect the dynamics of muscle respiration ($\dot{Q}O_2$) during exercise (Barstow *et al.*, 1994b; Chilibeck *et al.*, 1998; Tschakovsky & Hughson, 1999; Whipp *et al.*, 1999).

2.2 Factors Limiting the On-Transient Kinetics of $\dot{V}O_2$

Whereas mathematical modeling has provided a means to quantify the time course of adaptation of $\dot{V}O_2$ to a work stimulus, to date the control mechanisms that govern the rate of increase in $\dot{V}O_2$ remain unclear. Debate over the point of limitation has been resolved into two distinct and directly contrasting views suggesting that *1*) $\dot{V}O_2$ is limited by the rate of convective delivery of O_2 to the exercising muscles (Davies *et al.*, 1972; Hughson, 1990; Hughson *et al.*, 1993; Shoemaker *et al.*, 1994; Hughson *et al.*, 1996; MacDonald *et al.*, 1998; Tschakovsky & Hughson, 1999); or, *2*) the kinetics of $\dot{V}O_2$ are governed by the metabolic

inertia of oxidative flux dictated at the level of the muscle (Whipp & Mahler, 1980; Grassi *et al.*, 1996; Grassi *et al.*, 1997; Grassi *et al.*, 1998a; Grassi *et al.*, 1998b).

Hughson et al. (1993) compared the response in $\dot{V}O_2$ between upright and supine cycling to supine combined with the application of lower body negative pressure (LBNP). In their study, it was postulated that their model of applying LBNP would increase perfusion and thus O_2 delivery during the on-transient to exercise. Their results demonstrated that slowed $\dot{V}O_2$ kinetics during supine cycling (τ~40s) were accelerated following the application of LBNP (τ~27s) to rates similar to those observed during upright pedaling (τ~25s). Further work (Hughson *et al.*, 1996) comparing rhythmic forearm exercise performed both above and below the heart indicated that increases in muscle $\dot{V}O_2$ (determined from venous blood estimates of O_2 extraction) were faster when the working arm was exercised at a level below the heart. In that study, the authors observed a close correlation between the rate of adjustment in $\dot{V}O_2$ and increases in limb blood flow, the latter of which also responded more rapidly with the arm exercising below heart level. In the same regard, similar observations illustrating a temporal dependence of $\dot{V}O_2$ on the rate of limb blood flow have been made during dynamic leg exercise (MacDonald *et al.*, 1998). MacDonald et al. (1998) manipulated perfusion pressure to the working legs by having the subject sit upright or supine while exercising. Estimates of limb blood flow, computed from Doppler ultrasound measures, showed a more rapid increase in blood flow in the upright position, and coincided with a faster mean response time (a weighted mean of the time constant and time delay for each exponential term from the model fit of the data) for the adjustment of $\dot{V}O_2$ when compared to supine exercise. Taken together, the results of the aforementioned studies amalgamate to

expose a system where the adjustment in $\dot{V}O_2$ following the onset of an external workload is potentially limited by circulatory O_2-transport mechanisms.

That $\dot{V}O_2$ on-kinetics are determined by the rate of adjustment of O_2 delivery is not without opposition. Conflicting evidence stemming from studies directed at elucidating the point of control in the time course of adaptation of $\dot{V}O_2$ have purported that the rate of adjustment is set by inertia or "sluggishness" of skeletal muscle oxidative metabolism (Grassi *et al.*, 1996; Grassi *et al.*, 1997; Timmons *et al.*, 1998; Grassi *et al.*, 1998a; Grassi *et al.*, 1998b; Grassi, 2000). In these studies, manipulations of the rate of increase in O_2 supply (either through arterial blood flow or central $\dot{Q}$) did not affect $\dot{V}O_2$ kinetics, thus demonstrating that the profile of the $\dot{V}O_2$ on-transient responds independently of convective and diffusive transfer of O_2.

In an effort to obtain direct, muscle-level measures describing the relationship between $\dot{V}O_2$ kinetics and the rate of delivery of O_2 to the muscle, Grassi and colleagues undertook a series of experiments employing in situ canine muscle (1998a; 1998b) and trained human subjects (1996). In the first of these studies, a constant-infusion thermodilution technique allowed for determination of rapid measures of leg blood flow along with serial measurements of arteriovenous O_2 differences across the working muscle during the non-steady state transition to sub-threshold cycling in young men (Grassi *et al.*, 1996). With these direct measures of $\dot{V}O_2$ on-kinetics, the authors demonstrated that a significant increase in blood flow (and O_2 transport) failed to alter the rate of O_2 extraction in the early phase of the transient.

The observation that $\dot{V}O_2$ kinetics were not constrained by the bulk delivery of O_2 in the first 10-15 seconds of exercise were further corroborated by subsequent work conducted in the isolated canine gastrocnemius (Grassi *et al.*, 1998a). Similar to their previous study employing human models, the authors implemented an experimental design which afforded a direct means for determination of O_2 delivery and muscle $\dot{V}O_2$ on-kinetics. Moreover, the model permitted the investigators to eliminate any delay in convective O_2 delivery during the transient phase of the response by pump perfusion (manual roller pump control into conduit artery). The response to repeated rectangular bouts of electrically stimulated isometric contractions illustrated that accelerated kinetics of O_2 delivery did not speed the kinetics of $\dot{V}O_2$ (Grassi *et al.*, 1998a). The results of the aforementioned studies showed that the rate of bulk O_2 delivery did not appear to impart limitation on the kinetics of muscle $\dot{V}O_2$.

'Delivery' of O_2 to the muscles involves both blood-borne transport in the vasculature as well as diffusive transit from capillaries to mitochondria. That this final stage in the pathway of O_2 delivery does not limit muscle $\dot{V}O_2$ kinetics was demonstrated in a recent study (Grassi *et al.*, 1998b) which also employed electrically stimulated canine gastrocnemius exercise. To enhance the diffusive flux of O_2, the capillary-to-mitochondria O_2-gradient was augmented via the inspiration of a hyperoxic (100%) gas administered concomitantly with pharmaceutically mediated allosteric inhibition of hemoglobin (Hb). While the above conditions did improve diffusive movement of O_2 and increased O_2 off-loading from Hb, Grassi et al. (1998b) did not observe any significant changes in $\dot{V}O_2$ kinetics in response to square wave muscle contraction perturbations. Taken together, the combined results of the aforementioned studies (Grassi *et al.*, 1996; Grassi *et al.*, 1998a; Grassi *et al.*, 1998b) argue

strongly against the notion of $\dot{V}O_2$ kinetics being limited by convective or diffusive O_2 transport.

If O_2 delivery does not appear to constrain the rate of adjustment of $\dot{V}O_2$, then the alternative hypothesis suggesting that $\dot{V}O_2$ on-kinetics are determined by the metabolic inertia of muscle oxidative metabolism must be considered. In their examination of O_2 consumption following isometric tetanic contractions in frog sartorius muscle, Kushmerick and Paul (1976) observed a time course of PCr resynthesis that was not detectably different than that for changes in O_2 recovery consumption. They concluded that mitochondrial oxidation may be controlled in part by the level of ADP available during the transition from the unstimulated to the contractile state. This viewpoint has been extended through the work of Whipp and Mahler (1980), who applied a systems-analysis approach to examine the mechanisms that couple ATP production by oxidative metabolism to the reactions that describe its subsequent breakdown in muscle. Based on the initial hypothesis that O_2 consumption and ATP hydrolysis operate as a first-order system, their analysis derived a control scheme whereby muscle $\dot{V}O_2$ may be determined by the operating of the PCr system. In their model of respiratory control, muscle O_2 consumption changed in parallel with changes in muscle PCr content via the creatine phosphokinase (CPK) pathway. Thus, $\dot{V}O_2$ kinetics may be rate controlled by limiting intramuscular factors.

Recent work by Timmons et al. (1998) examined the potential influence of cellular oxidative enzymes on the rate of increase in $\dot{V}O_2$ at the onset of exercise by considering the activity of the inner mitochondrial membrane-bound pyruvate dehydrogenase complex (PDC). Increased activity of PDC via the administration of dichloroacetate in humans appeared to

elicit significant reductions in PCr degradation following the onset of submaximal exercise. This implies that the activation of mitochondrial respiration may be limited by the availability of acetyl groups, and may strongly determine the extent of the O_2 deficit (and hence potentially $\dot{V}O_2$ kinetics) at the onset of exercise (Timmons *et al.*, 1998).

2.3 Arterial Blood Flow and $\dot{V}O_2$ Kinetics

Efforts to elucidate the limiting factor in the adaptation of $\dot{V}O_2$ following a change in power output may benefit greatly from the inclusion of simultaneous estimates of changes in blood flow during the transition phase. Such information could provide an index of the rate of change of blood flow (and thus O_2 delivery) to the working musculature, which may provide further insight into the point of control. Recently, with advances both in the electronics field and microcomputer processing speeds, there has been a proliferation of research conducted describing the use of Doppler ultrasound to understand the transient responses and adjustments of the circulation at rest and during steady state exercise, as well as during the non-steady state adaptation. This technology provides a non-invasive means by which estimates of blood flow, including its direction and character, can be obtained. Although some systems employ Doppler ultrasound for imaging applications, the following review will be restricted to the role of the technology only as it pertains to the evaluation of blood flow.

2.3.1 Doppler Ultrasound - Principle and Instrumentation

The use of ultrasound to assess blood flow is founded upon the Doppler Effect. Stated simply, the Effect involves observing the change in frequency or wavelength due to motion. In the fields of diagnostics and research, it is the change in frequency between transmitted and received ultrasound signals (i.e., signals above the range of human hearing, about 20 kHz), that gives rise to a description of movement and speed within the area of interrogation. Keeping in focus with its application to hemodynamic assessment, Doppler ultrasound involves the conversion of an electrical signal into sound waves by a piezoelectric element present in the transducer. The sound waves are directed into tissue where they encounter and are subsequently reflected by moving erythrocytes, a process referred to as Rayleigh Tyndall scattering (Atkinson & Berry, 1974; Burns & Jaffe, 1985). When the backscattered sound reaches the receiving transducer, a difference in frequency between the emitted and returned signal is detected, and describes the Doppler shift (Δf):

$$\Delta f = \frac{2 f_o v \cos\theta}{c} \qquad (1)$$

where Δf is the change in ultrasonic frequency; f_o is the transmitted frequency of the incident ultrasound beam; v is the velocity of the moving erythrocytes; θ is the angle between the direction of movement of the target and the incident ultrasound beam; and c is a constant that represents the speed of propagation of ultrasound in human tissue; and the factor of *2* is included to account for the fact that a change in frequency occurs twice, once between the source and reflector and again between reflector and receiving transducer (Gill, 1985; Burns & Jaffe, 1985; Nelson & Pretorius, 1988). In tissues other than lung and bone, the average

value for c has been listed at 1540 m/s (Chauveau *et al.*, 1985; Kremakau, 1990). Some Doppler systems provide information in terms of velocity rather than frequency, which is arrived at through knowledge of the Doppler angle and the rearrangement of Equation (1):

$$v = \frac{\Delta f c}{2 f_o \cos\theta} \tag{2}$$

Strictly speaking, Doppler ultrasound provides either frequency or velocity measures. Blood flow measures are obtained by calculating the product of ultrasound measures of blood velocity and vessel cross sectional area (Gill, 1985), as described by the equation:

$$\text{blood flow} = v \bullet \pi r^2 \tag{3}$$

Doppler information can be obtained through either a *Continuous Wave* (CW) or *Pulsed Wave* (PW) technique. The former method describes a system where the transmitted signal is generated continuously. Reflected echos are collected using a second, separate transducer. The transmitting and receiving transducers are usually mounted within the same housing and are angled inwardly relative to one another, such that the sampled volume is defined as the region where their respective beams (incident and reflected) overlap. Continuous wave systems relay information regarding both the direction and speed of blood movement without limitation (see Section 2.3.2). However, as CW systems provide information on the entire volume defined by the overlapping region of transmitted and received beams, the depth from which blood velocity data are gathered cannot be controlled. Consequently, all moving objects in the area of overlap will be interrogated, and thus the

exact source of the Doppler signal cannot be determined (Nelson & Pretorius, 1988; Gill, 1990).

Pulsed wave systems incorporate short bursts of ultrasound as opposed to the continuous emissions of the CW technique. In a PW setup, both transmission and reception of signals is achieved using a single transducer element. A generator gate is included within the PW instrument which allows for short pulses of ultrasound to be emitted. By controlling the time interval from which echos are received, the operator can restrict the depth from which velocity data is sampled. The sensitive zone of insonification during PW sampling is termed the 'range gate'(Burns & Jaffe, 1985; Meire & Farrant, 1995).

2.3.2 Limitations and Sources of Error in the Use of Doppler Ultrasound

Measurements obtained through Doppler ultrasound velocimetry can be limited by a number of factors connected to both instrumentation and technique. Firstly, as has been noted above, CW Doppler systems sample everything present in the area of overlap of the two transducer beams. However, this eliminates any means for spatial resolution and strongly impedes the ability to resolve sampling depth. The time interval 'gating' present in PW systems overcomes this problem, but can introduce error into measurement through aliasing of high Doppler frequencies (Nelson & Pretorius, 1988; Kremakau, 1990). The Nyquist Theorem (Gill, 1985; Burns & Jaffe, 1985) states that a Doppler signal can only be correctly reconstructed when the frequency of the reflected signal is no greater than half the sampling rate or *pulse repetition frequency* (PRF). Aliasing results when the frequency of the Doppler shift exceeds the Nyquist limit. Frequencies in excess of the PRF are aliased and will appear

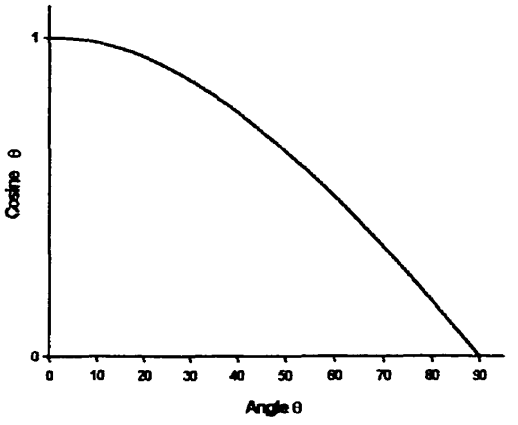

Figure 2.1. Angle θ is the angle between the direction of flow and the direction of ultrasound propagation.

entirely different (Gill, 1985), resulting in an underestimation of the true frequency (and thus velocity) of the interrogated blood sample. As aliasing is a consequence of sampling rate, it can often by corrected by increasing the beam/vessel angle, increasing the PRF, reducing the ultrasonic frequency (by changing to a lower frequency transducer) and thus lower Δf (Burns & Jaffe, 1985), or by using a CW system, which can measure a wide range of velocities without limit (Nelson & Pretorius, 1988).

A second form of uncertainty in Doppler-calculated blood velocity originates from error in estimating the angle between insonating beam and vessel. In their development of a calibration procedure of beat-by-beat estimates of blood flow from Doppler velocimetry with strain-gauge measurements, Tschakovsky et al. (1995) proposed that differences in insonation angle were the likely cause of significant day-to-day variability in ultrasound flow measurements. As is indicated in Equations (1) and (2), determination of Δf or v requires a measure of the Doppler angle, defined in the form of a cosine (Figure 2.1). When the angle

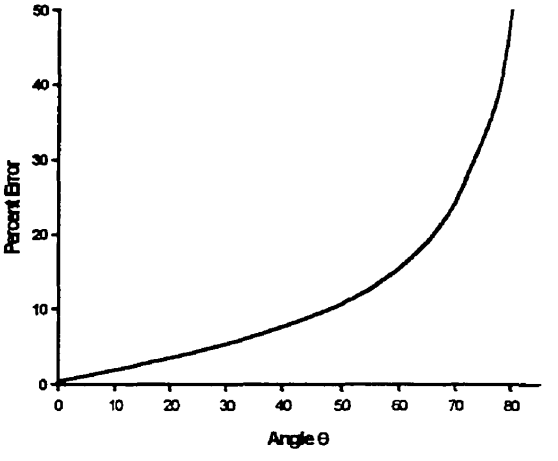

Figure 2.2. The effect of a 5-degree error in the measurement of the angle between ultrasound beam and direction of flow on the estimate of flow velocity. Adapted from Burns & Jaffe (1985).

formed between the ultrasound beam and blood vessel is 0°, the cosine is 1 and the observed Doppler shift is maximal. Conversely, as the angle approaches 90° (i.e., transducer perpendicular to vessel), the detected shift becomes increasingly small, to the point where the Δf signal disappears. Of consequence, Doppler measurements are not reliably achieved at Doppler angles greater than about 60 to 70 degrees (Kremakau, 1990). For smaller angles, the cosine function does not vary considerably, such that Doppler angles between 0° and 20° will only underestimate velocity by a maximum of ±3% (Gill, 1985). However, the effect of error in the determination of θ for larger angles will have a more profound influence (Figure 2.2), where an uncertainty of 5% in θ at 70 ° can induce an error of 19% in the calculated blood velocity (Burns & Jaffe, 1985). In light of the strong potential influence of variations in θ on velocity, it is recommended that ultrasonic flowmetry be conducted using Doppler angles less than 60° (Burns & Jaffe, 1985; Nelson & Pretorius, 1988; Kremakau, 1990).

A final concern with regard to the error in Doppler ultrasound estimates surrounds variation present in the reflected signal. Each moving erythrocyte will give rise to a range of frequencies as it crosses the ultrasound beam. When this individual effect is summed for all moving red blood cells under interrogation, a phenomenon known as *transit-time broadening* results (Burns & Jaffe, 1985) which can increase the noise in the resultant Doppler signal. Similarly, random error in measurement from improper beam-vessel alignment as well as internal interference from adjacent vessels and tissues can further reduce the quality of ultrasound flowmetry (Gill, 1985). Day-to-day variability from such sources can be reduced by having several repeated measurements conducted by the same person (Shoemaker *et al.*, 1996b) and then averaging the response (Chauveau *et al.*, 1985; Tschakovsky *et al.*, 1995).

Despite the limitations inherent in the use of Doppler ultrasound, evidence has been presented to support the accuracy of the technique. Comparisons of ultrasound-determined velocity with those obtained using perivascular electromagnetic flowmetry (Chauveau *et al.*, 1985) and magnetic resonance imaging (Zananiri *et al.*, 1993) have revealed no significant differences in the values obtained. Further work by Radegran (1997) has shown close agreement between rest and exercise measures of femoral flow obtained through Doppler and thermodilution techniques across several work rates. Thus, as long as the potential sources of error are observed and several repeated measurements are conducted (and averaged), Doppler ultrasound can represent an effective and non-invasive method to assess arterial flow.

2.3.3. Simultaneous Doppler Ultrasound Velocimetry and $\dot{V}O_2$ Kinetics

Recently, many investigators have employed Doppler ultrasound to evaluate the rate of adaptation in blood velocity during the on-transient to exercise during both rhythmic leg (Walloe & Wesche, 1988; Eriksen *et al.*, 1990; Shoemaker *et al.*, 1996b) and arm (Tschakovsky *et al.*, 1995; Shoemaker *et al.*, 1996a; Shoemaker *et al.*, 1997) exercise. There have also been several reports (Shoemaker *et al.*, 1994; Hughson *et al.*, 1996; MacDonald *et al.*, 1998) where Doppler ultrasound estimates of the rate of hemodynamic adjustment have been coupled with simultaneous measurements of $\dot{V}O_2$ to better understand the relationship between O_2 delivery and O_2 uptake kinetics.

Many of the aforementioned Doppler ultrasound studies have been reported in terms of mean blood velocity (MBV). When measures of flow have been desired, investigators have conducted echo Doppler imaging to measure arterial diameter in order to subsequently

calculate blood flow (Hughson *et al.*, 1996; Shoemaker *et al.*, 1996a; Shoemaker *et al.*, 1997; MacDonald *et al.*, 1998). Interestingly, two recent reports have demonstrated that in the femoral artery, vessel diameter remains unchanged between rest and exercise (MacDonald *et al.*, 1998) for several different workloads (Radegran, 1997).

Studies that have examined the time course of blood velocity and/or flow to a step increase in work rate have evidenced very rapid rates of adaptation (Walloe & Wesche, 1988) compared to the kinetics of $\dot{V}O_2$. Following the beginning of two-legged rhythmic exercise (3-5 cm heel lifts while lying supine), Eriksen et al. (1990) have shown $\dot{Q}$ to reach 80% or more of its steady state value in about 10 s, and femoral flow to attain 90% of its maximal plateau value in about 15 s. Somewhat slower rates of adjustment in femoral artery blood velocity have been reported in other studies, describing τ values of 14 to 35 s (Shoemaker *et al.*, 1994; Shoemaker *et al.*, 1996b). Variability in response times has been further demonstrated during forearm exercise, where values for mean response time have ranged from ~30s (Hughson *et al.*, 1996) to 75 s (Shoemaker *et al.*, 1996a). The discrepancy between rates of increase in MBV observed may likely reflect differences in work intensity, contraction rate, or study design.

Rate of contraction and workload may be strong determinants of differences in the adaptation of blood velocity. This has been demonstrated by Shoemaker et al. (1996; 1997), where positioning the forearm above the heart and increasing power output were both shown to slow the rate of adjustment in forearm blood velocity/flow. Similarly, MacDonald et al. (1998) demonstrated that the mean response time of adaptation in femoral artery blood flow was slower in the supine (27.6 ± 3.9s) compared to upright position (17.3 ± 4.0s) during knee

extension exercise in young men. In the studies of both MacDonald et al. (MacDonald *et al.*, 1998) and Hughson et al. (Hughson *et al.*, 1996), $\dot{V}O_2$ kinetics were found to be slower in conditions that promoted slower rates of adjustment in blood flow. Thus, these data extrapolate to support the notion that O_2 availability may limit the adaptation of muscle $\dot{V}O_2$.

2.4 Ageing and $\dot{V}O_2$ Kinetics

The kinetics of $\dot{V}O_2$ for moderate intensity exercise have been shown to be slowed as a function of age (Babcock *et al.*, 1994b). The protracted response observed in elderly subjects has been demonstrated during both square wave and sinusoidal forcing tests (Cunningham *et al.*, 1993), and has been reproduced repeatedly during large muscle mass activities such as cycling and treadmill running (Chilibeck *et al.*, 1996; Bell *et al.*, 1999). However, Chilibeck et al. (1996; 1997) found that the trend of a slowed rate of adaptation of $\dot{V}O_2$ in older individuals did not hold constant for smaller muscle mass activities (e.g., rhythmic plantar flexion exercise), although the older adults in their studies performed regular walking exercise. The observed similarity in time course of $\dot{V}O_2$ in older and younger subjects suggests that the plantar flexors may remain well trained in the (ambulatory) elderly, as the muscle group would experience daily recruitment during stepping, walking, and stair climbing. Such an effect has been demonstrated by Babcock et al. (1994a), who presented data from older men showing that 24 weeks of endurance training accelerated the kinetics of ventilation and gas exchange to values which approached those reported for fit young subjects. Therefore, the prolonged response in the kinetics of $\dot{V}O_2$ observed in older adults may arise

from an increasingly sedentary lifestyle associated with increasing age, rather than as a consequence of simply growing older (Paterson, 1992).

The decline in $\dot{V}O_2$ kinetics observed in older adults may be related to changes present at the level of the muscle in these subjects, affecting either transport through the microcirculation or intramuscular oxidative capacity, or some combination of the two. In moderately active subject groups, Chilibeck et al. (1997) examined the relationship between muscle capillarization and $\dot{V}O_2$ kinetics in the lateral gastrocnemius of older and younger men and women. Similar $\dot{V}O_2$ on-kinetics were observed in young (44.8 ± 9.7s) and old (47.7 ± 19.0s) during moderate intensity plantar flexion exercise, along with no evidence of any differences in muscle capillarization, fiber area, or diffusion distances between subject groups. However, these findings have not been consistent within the literature, as Coggan et al. (1992a) found significant reductions in both capillarization and mitochondrial enzyme activity in older adults. However, following 9-12 months of endurance training, it appears that these losses can be reversed (Coggan *et al.*, 1992b). Sustained training in the elderly may even increase the activity of mitochondrial enzymes to levels in excess of the very fit young (Coggan *et al.*, 1990).

The slower $\dot{V}O_2$ kinetics in older adults may alternatively reflect a limitation in central O_2 delivery to the exercising muscles. Chilibeck et al. (1996) suggested that impaired delivery of O_2 may be linked to age-related reductions in central cardiac output, as was inferred through their observation of slowed $\dot{V}O_2$ and HR kinetics in older adults. A putative relationship between τHR and $\tau\dot{V}O_2$ has been further suggested with the work of Cunningham et al. (1993) but was not supported in the findings of Babcock et al. (1994b), who found no

significant slowing of HR kinetics with age. The conflicting results of these two studies may reflect differences in exercise protocol (square wave vs. sinusoidal forcing) or in relative fitness of the subjects.

In a related investigation, Petrella et al. (1999) observed a faster $\tau\dot{V}O_2$ after the administration of the calcium channel blocker verapamil to older sedentary and hypertensive men and women. Although the pharmacological intervention did not induce accelerated HR kinetics, increases in cardiac filling and stroke volume were evidenced in these subjects. These results would purport that in older groups with below average fitness, cardiac function (and thus the ability to deliver O_2) may limit the $\dot{V}O_2$ response during the non-steady state adaptation to an exercise stimulus. In opposition to these findings are the recent results of Bell et al. (1999), who observed no improvements in $\dot{V}O_2$ kinetics following hyperoxic breathing in older men performing cycling exercise. The apparent ineffectiveness of hyperoxia to speed $\dot{V}O_2$ kinetics has also been demonstrated in younger subjects (Macdonald *et al.*, 1997), indicating that O_2 transport, which would presumably be enhanced in hyperoxia (as a result of increased arterial PO_2), may not be limiting in the non-steady state adaptation of $\dot{V}O_2$.

2.5 Summary

Oxygen uptake kinetics during the on-transient of exercise to a moderate intensity step change in power output are described by a 3-phase response. *Phase 2*, also referred to as the primary component of $\dot{V}O_2$, can be approximated to follow a monoexponential time course for exercise intensities within the moderate domain. During heavy intensity exercise, *phase 3* describes the delayed onset of a slow component after the primary response. Several

studies have shown the time constant for the primary component of the $\dot{V}O_2$ response to be invariant of exercise intensity, and thus its value will remain unchanged in heavy domain work tasks. It still remains unclear what controls the rate of adaptation in $\dot{V}O_2$ following the onset of exercise. Current theories have suggested that the rate of uptake of O_2 may be determined by transport limitations or by the intramuscular oxidative machinery. In older adults, the kinetics of $\dot{V}O_2$ have been shown to be slowed as a function of age, but the exact causes for the prolonged response remain to be elucidated. Doppler ultrasound velocimetry may provide insight into mechanisms controlling the rate of adaptation of $\dot{V}O_2$, and may help to expand our current knowledge regarding the age-related slowing of $\dot{V}O_2$ on-kinetics.

CHAPTER 3

METHODS

3.1 Subjects

Six older men (77±6 years) and six younger men (27±3 years) volunteered for participation in the study. Subjects comprised individuals from within the community and graduate students at the university. All were healthy and were not taking any medication known to influence cardiorespiratory function. The older men were recruited as part of an endurance training study being conducted at the same facility. The data used in the current study represent baseline (pre-training) measurements for these older men, and were obtained during the initial testing of that investigation. Lifestyles of subjects in both groups ranged from sedentary to moderately active. All subjects gave informed consent to participate in this study, which was approved by the Health Science Review Board for Research Involving Human Subjects.

3.2 Peak $\dot{V}o_2$ Test: Two Leg Cycle Ergometer Exercise ($\dot{V}o_{2pkCE}$)

Subjects performed a ramp exercise test to volitional fatigue on an electrically braked cycle ergometer (Lode, model H300R) for determination of two leg cycle ergometer peak $\dot{V}O_2$ ($\dot{V}O_{2pkCE}$). Older subjects performed the test under the supervision of a physician and were

monitored with a 12-lead ECG. Following 4 minutes of loadless cycling (power output of ~15 W), work rate increased as a ramp function at a rate of 10-12 W/min for old or 25 W/min for young subjects. Fatigue was defined as the point at which the subjects were no longer able to maintain a cycling cadence of 60 rpm. $\dot{V}O_{2pkCE}$ was established from gas exchange data as the highest $\dot{V}O_2$ achieved averaged over the 10 second interval of breath-by-breath measurements at the end of the test.

3.3 Ergometer Design

Exercise testing was conducted using a custom-built single-leg knee extension ergometer adapted after the model described by Andersen et al. (1985). A Monark cycle ergometer (Model 814 E) was substituted in place of a Krogh ergometer. Subjects were seated on a bench with a fully adjustable backrest allowing adjustment of both tilt and fore-aft positioning. This ensured that subjects of different heights and leg lengths could be accommodated equally well. The ergometer was adjusted such that all subjects performed knee extensions with the hips flexed at an angle of approximately 120°.

Single leg knee extension exercise consisted of pushing against a padded bar attached to a lever arm. An aluminum rod connected the lever arm to a pedal of the cycle ergometer. The cycle ergometer freewheel was converted to a fixed-wheel hub such that flywheel and pedals rotated in unison. Thus, with knee extension, the cycle pedal would rotate one-half turn after which flywheel momentum would return the lever arm to the starting position. Thus, the quadriceps group were the only muscles recruited for the exercise. Work rate was manipulated by adding weights to the basket suspended above the Monark flywheel (as with

normal cycle ergometry). Unloaded exercise was achieved via manual rotation of the cycle ergometer pedals by the investigator. There was no attachment between the extending lower leg and padded bar of the ergometer; subjects were instructed to keep the two in contact by following the movement of the bar during the unloaded segment of the protocol.

Subjects were instructed to maintain a cadence of 30 knee extensions per minute throughout the exercise tests. The two-second contraction cycle (1-second extension, 1-second relaxation) was guided by a metronome that provided both audible and visual signaling to the subject, and was further ensured by having the investigators monitor the revolutions-per-minute output on the ergometer computer screen. Each knee extension required movement of the lower leg from approximately 95° to 160°, corresponding to one full revolution of the ergometer pedals. Subjects were instructed to keep their hands relaxed and flat on the bench alongside their hips, and were not permitted to grasp the sides of the bench or prop themselves up while performing the extensions.

3.4 Peak $\dot{V}O_2$ Test: Single leg Knee Extension Exercise ($\dot{V}O_{2pkKE}$)

Each subject performed an incremental exercise test for determination of single leg knee extension peak $\dot{V}O_2$ ($\dot{V}O_{2pkKE}$). The test consisted of 3 minutes of loadless exercise followed by an increase in work rate to 15 W. This was maintained for 2 minutes, after which the work rate was increased by a further 3 W for older or 6 W for younger subjects every 2 minutes. Increases to work rate were achieved by the addition of weights (with 3 W corresponding to the addition of 0.1 kg) to the basket suspended above the ergometer

freewheel. The test ended when subjects were no longer able to maintain the required rate of 30 extensions per minute.

3.5 Constant Load Knee Extension Exercise

Subjects were required to perform a number of repeats of constant load knee extension exercise. Each repeat consisted of 6 minutes of unloaded effort followed by a step increase in work rate to an intensity equal to approximately 60% of $\dot{V}O_{2pkKE}$. This was maintained for a period of 6 minutes and was followed by a final 6 minutes of unloaded recovery. Two square wave transitions were performed in sequence followed by a rest period of approximately 30-40 minutes, followed by a repeat of the two square waves. All 6 old and 2 young subjects completed a total of 6 exercise transitions across 2 separate visits to the laboratory, performing two sets of successive square wave transitions the first day and one set the following day. The remaining 4 young completed a total of 4 exercise transitions during one visit to the laboratory, performed as two sets of successive square waves.

3.6 Calibration, Equipment, and Data Acquisition

Calibration of equipment was completed prior to each testing session. Inspired and expired flow rates were measured using a low dead space (90 ml) bidirectional turbine (Alpha Technologies VMM 110), which was calibrated daily using a syringe of known volume (0.990 liter). Respired gases were sampled continuously (every 20 ms) at the mouth and analyzed for concentrations of O_2, CO_2, and N_2 by a mass spectrometer (Perkin Elmer MGA-1100) calibrated daily against precision-analyzed gas mixtures. Changes in gas concentration

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. Collected data were converted from analog to digital format and stored on a computer for later processing and analysis. Breath-by-breath alveolar gas exchange data were calculated using the algorithms of Beaver et al. (1981).

Femoral artery MBV data were determined by using pulsed Doppler ultrasound velocimetry (Multigon Industries, Model 500M). A flat probe with an operating frequency of 4 MHz and a fixed angle of insonation of 45° was placed on the skin over the common femoral artery, approximately 2 - 3 cm distal to the inguinal ligament. The ultrasound gate was maintained at full width to ensure complete insonation of the entire vessel cross section with approximately constant intensity (Gill, 1985). Beat-by-beat MBV was calculated by integrating the total area under the MBV profile with the marked QRS complex of the ECG tracing to indicate the end of one heartbeat. MBV and ECG data were all recorded at a frequency of 200 Hz and stored on a computer for later analysis.

Blood samples were collected at exercise onset as well as at minute 3, 4.5, and 6 during one of the six square wave repeats. A final sample was drawn at the end of 6 minutes of unloaded recovery (i.e., 12 minutes after work onset). Blood was sampled using a percutaneous Teflon catheter (Angiocath, 21 gauge) inserted into a dorsal vein of the hand. The hand and forearm were wrapped in a warm heating pad which was worn for the duration of the exercise protocol. Arterialized venous samples (McLoughlin *et al.*, 1992) were collected using heparinized syringes (3 cc) and immediately stored in an ice bath. Concentrations of plasma lactate were determined using a blood gas-electrolyte analyzer

(Nova Stat Profile 9 Plus gas-electrolyte analyzer, Nova Biomedical Canada) or a glucose-lactate analyzer (YSI 2300, Yellow Springs Instruments Company, Inc.), both of which were calibrated at regular intervals during sample analysis.

3.7 Data Analysis

Breath-by-breath data were interpolated to 1-s intervals. MBV data were interpolated to 2-s intervals (1 contraction cycle). Square wave repeats were time aligned and ensemble averaged to yield a single data set for each subject. Averaged response data were fit using a monoexponential model in the general form:

$$Y(t) = A_0 + A\left\{1 - e^{-[(t-TD)/\tau]}\right\}$$

where Y represents $\dot{V}O_2$ or MBV at any time (t), A_0 is the model estimate of baseline, A is the amplitude of the increase in Y above the baseline value, and TD and τ represent the time delay and time constant of the response, respectively. The τ value reflects the time to reach 63% of the amplitude of the steady state response as derived from the exponential model. The total lag time (TLT) was calculated as the sum of τ and TD. The $\dot{V}O_2$ response was quantified with a monoexponential curve fit from 20 seconds after exercise onset, to exclude the *phase I* response, until the end of exercise. MBV data were fit using a monoexponential model encompassing the last two minutes of unloaded work through to end-exercise. All mathematical curve fitting was performed using a least squares non-linear regression where model fit was determined by the regression line that yielded the lowest residual sum of squares (RSS). In certain cases where noise or variation in the data created difficulty in model

fitting and achieving a minimized RSS, the model fit of the data was attempted for fitting windows of 3, 4, or 5 minutes into exercise as opposed to attempting to fit the full 6 minute exercise period. The optimal fit for each case determined by the observed goodness of fit through the on-transient phase of interest (*phase 2*). In cases where the $\dot{V}O_2$ response evidenced a slow component, the fitting window was reduced to avoid the inclusion of this delayed onset component into the regression, ensuring that the model fit was constrained to include only the primary (*phase 2*) component.

3.8 Statistical Analyses

Comparisons of physiological responses were evaluated using a one-way analysis of variance (ANOVA) with between-group measures for age. Differences in on-transient kinetic responses of $\dot{V}O_2$ and MBV (τ and TLT) across the two groups were assessed using a two-way ANOVA. Pairwise multiple comparisons among the mean values of the factor levels within age (old vs. young) and response parameter (τ and TLT for $\dot{V}O_2$ and MBV) were performed using a Student-Newman-Keuls post hoc analysis. The level of significance was set at $P<0.05$. The relationship between $\tau\dot{V}O_2$ and τMBV was examined with a Pearson product-moment coefficient of correlation.

CHAPTER 4

RESULTS

4.1 Subject Characteristics and Tests of Peak $\dot{V}O_2$

Six older men and six younger men participated in this study. The mean (±SD) ages of the subjects were 77(±6) and 27(±3) years for the older and younger groups, respectively (Table 1). The two groups were similar in terms of height and body mass. Peak $\dot{V}O_2$ determined from the two leg ramp cycle ergometer test ($\dot{V}O_{2pkCE}$) in the older group (2.02 ± 0.81 l•min^{-1}) was significantly lower than in the young (3.97 ± 0.60 l•min^{-1})(Table 1). The older subjects also demonstrated a significantly lower peak $\dot{V}O_2$ ($\dot{V}O_{2pkKE}$) during the incremental knee extension exercise test (1.00 ± 0.19 l•min^{-1}) compared to the younger men (1.51 ●0.32 l•min^{-1})(Table 1). Peak work rates for the incremental one leg test were 21.5 and 46 W for old and young, respectively. The peak O_2 uptakes during KE represented 38% in young and 50% in old of the mean peak $\dot{V}O_2$ elicited during two leg cycling ($\dot{V}O_{2pkCE}$). The highest HR observed during the KE test averaged 111 beats•min^{-1} in old and 117 beats•min^{-1} in young, corresponding to 64% in young and 74% in old of the of the peak HR achieved in the cycling test.

4.2 $\dot{V}O_2$ and HR Responses

For the constant load one leg exercise test, the mean $\dot{V}O_2$ response measured during unloaded knee extension exercise was significantly lower in the old compared to the young

group (403.6 ± 19.5 vs. 523.3 ± 38.6 ml•min^{-1}). During the constant square wave exercise, the $\dot{V}O_2$ was significantly lower in old compared to young at the midpoint (minute 3) of the constant load (668.7 ± 22.1 vs 870.6 ± 58.8 ml•min^{-1}), and at the end of exercise (725.1 ± 13.9 vs 880.1 ± 52.9 ml•min^{-1}), reflecting the lower WR in the old (15 W) versus young (21 W). Evaluating the difference in O_2 uptake determined at minute 3 and end exercise illustrated a small increase in $\dot{V}O_2$ (slow component) present in the older men which tended to be greater than that observed in the younger subjects ($\Delta\dot{V}O_{2(6-3)}$ = 56.4 ± 18.3 vs. 9.6 ± 12.3 ml•min^{-1}, P = 0.059). This magnitude of the $\Delta\dot{V}O_{2(6-3)}$ in the older subject group, determined through linear regression of the 3 to 6 minute interval, averaged 22 ml•min^{-1} each minute.

Mean heart rate responses during both loadless (old 81.4 ± 3.7, young 70.8 ● 7.1 beats•min^{-1}) and throughout the steady state exercise transition (6 min, old 98.5 ± 5.1, young 84.2 ± 7.9 beats•min^{-1}) were not significantly different between the two age groups. The unloaded to end-exercise HR increase averaged 17 beats•min^{-1} in old and 13 beats•min^{-1} in young. End exercise HR averaged 71 (young) and 88% (old) of HR_{pkKE}.

4.3 $\dot{V}O_2$ Kinetics

Three of the old and all six of the young men were observed to reach a steady state in $\dot{V}O_2$ at the end of the 6 minute square wave bout. The slower on-transient $\dot{V}O_2$ response in one leg exercise of an older subject compared to a younger subject is shown in Figure 4.1. Baseline $\dot{V}O_2$ (A_0 from the model fit, at approximately 20 s) in young (669.7 ± 45.5 ml•min^{-1}) was significantly greater than in older men (485.7 ± 16.7 ml•min^{-1}). However, the total amplitude in $\dot{V}O_2$ (A) was similar between the two groups (234.4 ± 24.9 and 205.2 ± 22.8

$ml \cdot min^{-1}$ for old and young, respectively)(Table 4.2). To account for these differences, the $\dot{V}O_2$ response of an older and younger subject are depicted as normalized for the total amplitude representing 100% and the old and young response compared in Figure 4.2.

The non-linear least squares regression model fit a significantly slower mean $\tau\dot{V}O_2$ of 91.2 (± 13.4) s in the older group when compared to the 36.6 (± 6.0) s time constant in the young (Table 4.2). The length of the TD component of fit was not significantly different between the two groups. With the relatively similar residual sum of squares of the line of best fit to the data, the monoexponential model (constrained to begin 20 s after work onset) provided an appropriate and equally good description of the response in both subject groups (Fig 4.3 and 4.4).

4.4 MBV Kinetics

Baseline MBV for loadless exercise (Table 4.3) was similar in old (21.2 ± 1.8 $cm \cdot s^{-1}$) and young groups (18.1 ± 2.7 $cm \cdot s^{-1}$). Comparable values were also apparent in the steady state of exercise, where MBV averaged 39.8 (± 3.5) $cm \cdot s^{-1}$ in the old and 33.3 (± 3.0) $cm \cdot s^{-1}$ in the young men. The rate of adaptation in MBV following the onset of work was not different between the two age groups (Table 4.3, Figures 4.5 and 4.6), with group mean τMBV values averaging 25.3 (± 2.9) s in old and 20.2 (± 2.5) s in young. There were no significant differences in parameter estimates (τ, TD, TLT) of the MBV response in either of the two groups. On average, in the young subjects the MBV response produced a signal with more noise and variability, as evidenced by a greater scattering of residuals about the non-linear regression line (Table 4.3, Figure 4.7).

Comparison of the time constants of the two response parameters showed that τMBV responded much more quickly than $\tau\dot{V}O_2$ in both groups. Pearson product-moment coefficient of correlation revealed no significant relationship between τMBV and $\tau\dot{V}O_2$ in either the old ($r = 0.10$; $P = 0.85$) or the young ($r = -0.66$; $P = 0.15$), or for all subjects combined ($r = 0.23$; $P = 0.47$).

4.5 Plasma Lactate

Blood samples were obtained from four of the six subjects in both the older and younger group. The mean concentration of plasma lactate ([La^-]) taken at exercise onset (minute 6) was significantly higher in the old (2.2 ± 0.3 mmol•L^{-1}) compared to the young men (1.0 ± 0.1 mmol•L^{-1}). However, at all other sampling times throughout exercise and following recovery, plasma lactate concentration was found to not differ significantly between the two groups. During exercise, [La^-] rose to 3.3 (± 0.3) and 2.3 (± 0.8) mmol•L^{-1} in old and young, respectively, and reached concentrations of 3.6 (± 0.4) mmol•L^{-1} (old) and 2.4 ± 1.2 mmol•L^{-1} in (young) by end exercise. Following 6 minutes of recovery, mean [La^-] increased slightly in the old (4.0 ± 0.4 mmol•L^{-1}) and decreased in the young (2.0 ± 1.0 mmol•L^{-1}), but overall there were no differences in final concentration between the two groups.

Table 4.1. Subject physical characteristics and measures of gas exchange for peak cycling and knee extension exercise tests.

Subject	Age (yr)	Height (cm)	Body Mass (kg)	$\dot{V}O_{2pkCE}$ ($l \cdot min^{-1}$)	$\dot{V}O_{2pkKE}$ ($l \cdot min^{-1}$)
Young (n=6)					
2541	29	180	84.0	4.26	1.51
3436	28	186	95.0	3.23	1.52
3168	30	178	89.0	4.76	1.63
3720	23	171	69.0	3.41	1.26
3722	29	172	74.0	3.76	1.56
3697	23	187	107.5	4.42	1.59
Mean	27	179	86.4	3.97	1.51
(SD)	(3)	(7)	(14.1)	(0.60)	(0.32)
Old (n=6)					
1239	74	181	82.0	2.29	1.24
3704	83	175	69.0	1.60	0.99
1721	78	181	89.7	2.08	1.05
3892	66	166	88.0	2.06	0.81
1933	79	176	90.3	2.03	0.76
0520	83	176	74.5	2.06	1.15
Mean	77	176	82.3	2.02*	1.00*
(SD)	(6)	(5)	(8.8)	(0.81)	(0.19)

$\dot{V}O_{2pkCE}$, peak $\dot{V}O_2$ for cycle ergometer ramp exercise test; $\dot{V}O_{2pkKE}$, peak $\dot{V}O_2$ for incremental single leg knee extension exercise test.

* Significant difference between age groups ($P<0.05$)

Table 4.2. Summary of parameter estimates of the model fit for oxygen uptake ($\dot{V}O_2$) response during moderate intensity knee extension exercise in young and old men.

Parameter	Young	Old
A_0 ($ml \cdot min^{-1}$)	669.7 (45.5)	485.7* (16.7)
TD (s)	-1.4 (3.9)	8.2 (5.7)
$\tau\dot{V}O_2$ (s)	36.6 (6.0)	91.2* (13.4)
TLT (s)	35.2 (4.2)	99.4* (13.5)
A ($ml \cdot min^{-1}$)	205.2 (22.8)	234.4 (24.9)
RSS ($\times 10^5$)	2.9 (0.6)	2.9 (0.8)

Data are means (SE). A_0, $\dot{V}O_2$ at 20 s post-exercise onset; $\tau\dot{V}O_2$, time constant; TLT, total lag time; A, amplitude; RSS, residual sum of squares of model fit.

* Significantly different than young ($P<0.05$).

Table 4.3. Summary of parameter estimates of the model fit for mean blood velocity (MBV) response during moderate intensity knee extension exercise in young and old men.

Parameter	Young	Old
A_0 ($cm \cdot s^{-1}$)	18.1	21.2
	(2.7)	(1.8)
TD (s)	-0.3	-1.8
	(9.6)	(6.1)
τMBV (s)	20.2	25.3
	(2.5)	(2.9)
TLT (s)	19.8	23.5
	(9.2)	(5.3)
A ($cm \cdot s^{-1}$)	15.2	18.6
	(1.6)	(3.9)
RSS (x 10^3)	4.9	2.8
	(1.0)	(0.6)

Data are means (SE). A_0, MBV during unloaded knee extension exercise; τMBV, time constant; TLT, total lag time; A, amplitude; RSS, residual sum of squares of model fit.

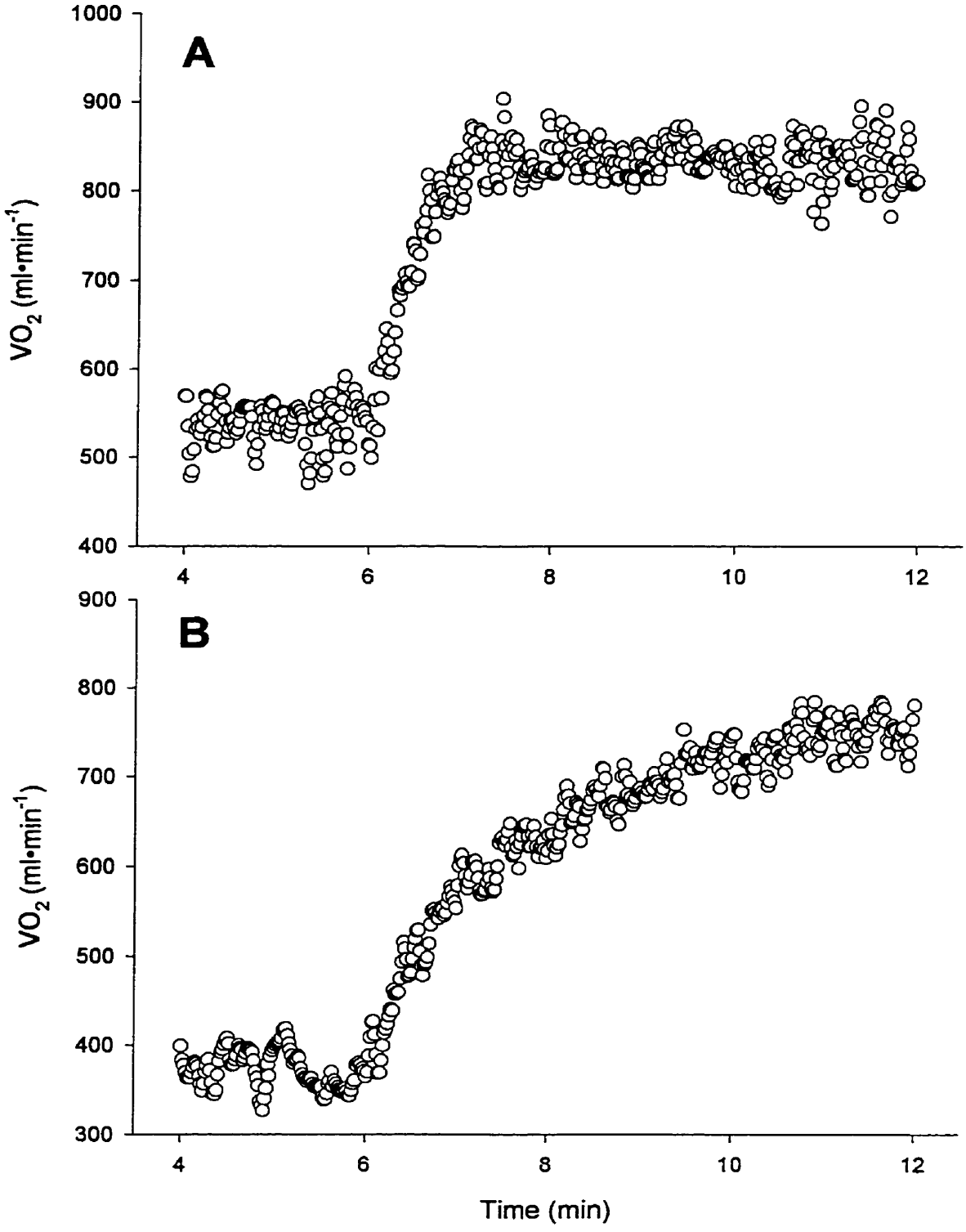

Figure 4.1. Individual mean oxygen uptake response to square wave knee extension exercise in a single young (**A**) and old (**B**) subject. Data are 1 s averages and represent the ensemble average of six repeats. Exercise onset is at minute 6.

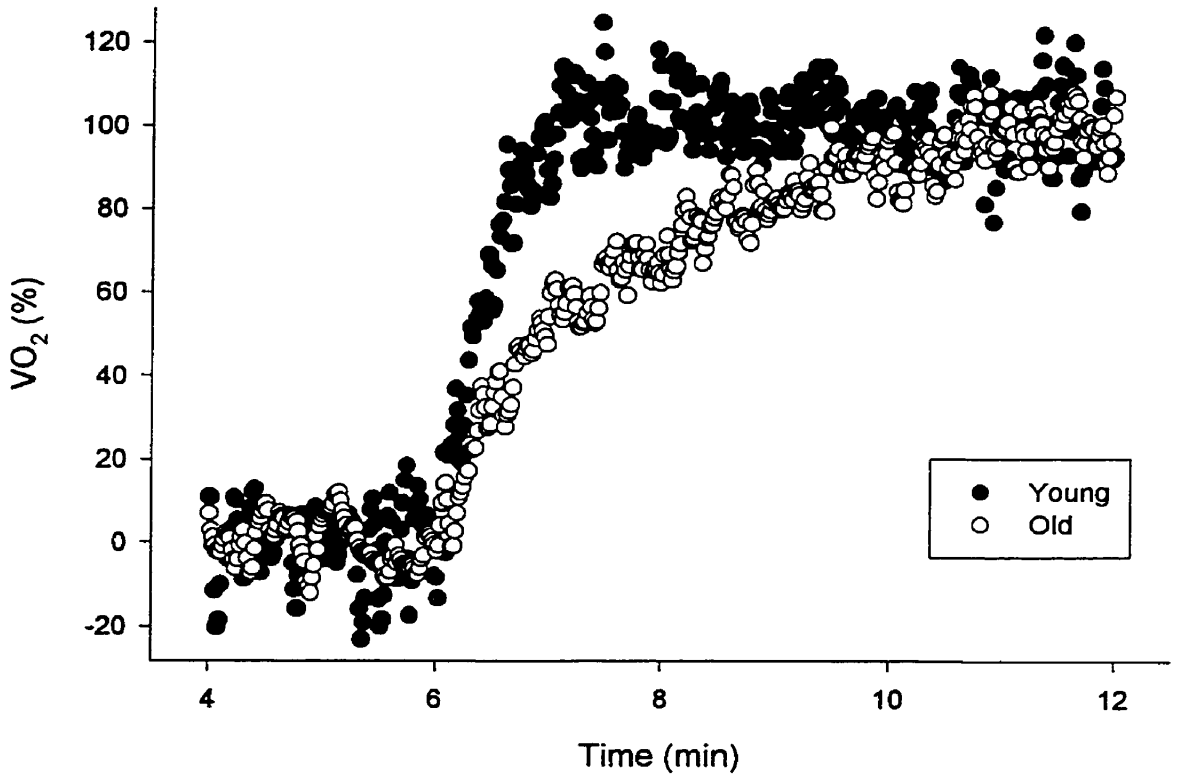

Figure 4.2. Normalized plots of oxygen uptake response to square wave knee extension exercise in a single young (solid circles) and old (open circles) subject. Data represent the ensemble average of six repeats. Exercise onset is at minute 6.

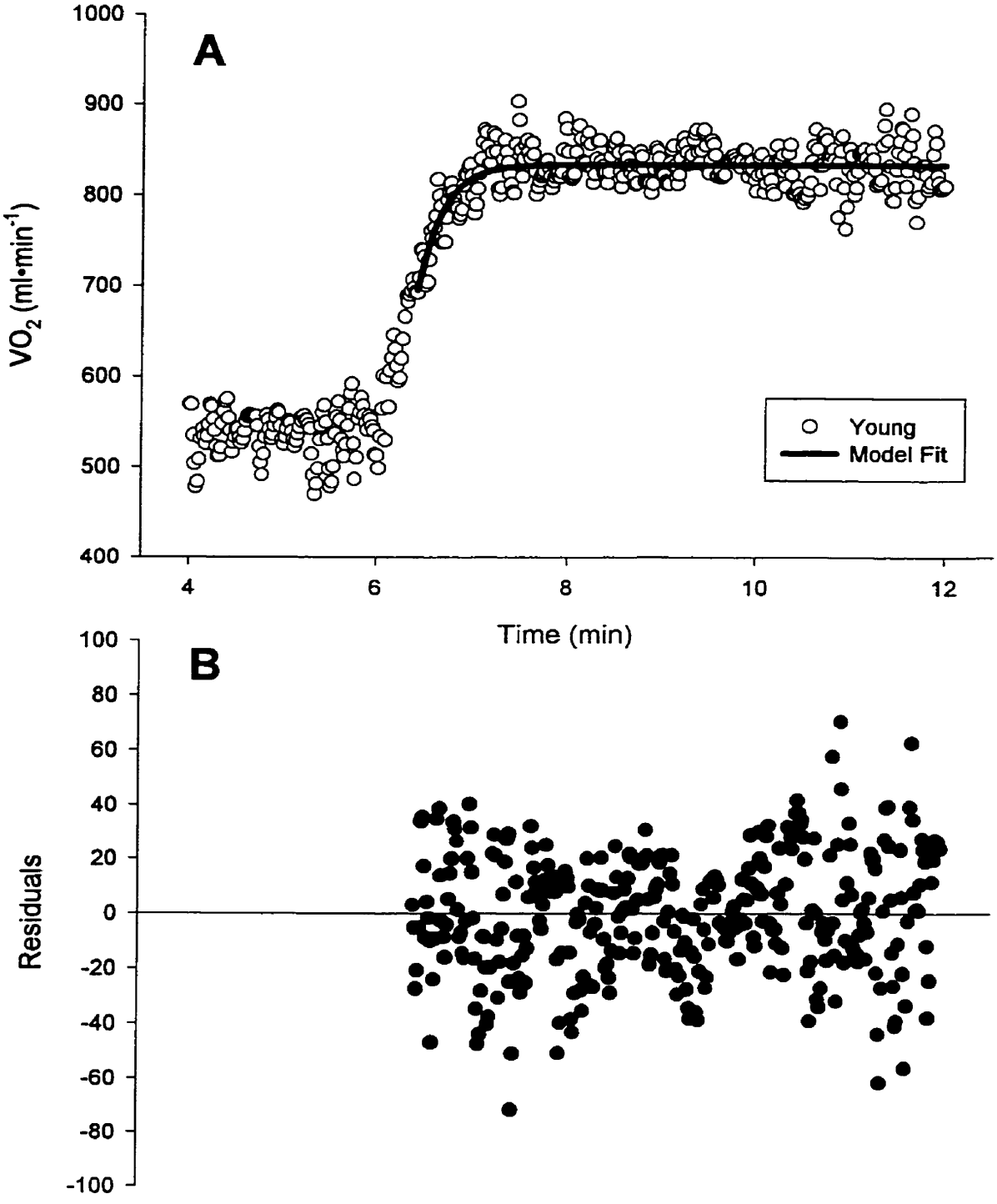

Figure 4.3. Oxygen uptake response to square wave knee extension exercise in a single young subject. Panel **A** shows breath-by-breath data (open circles) and least squares regression (solid line). Panel **B** shows plot of residuals.

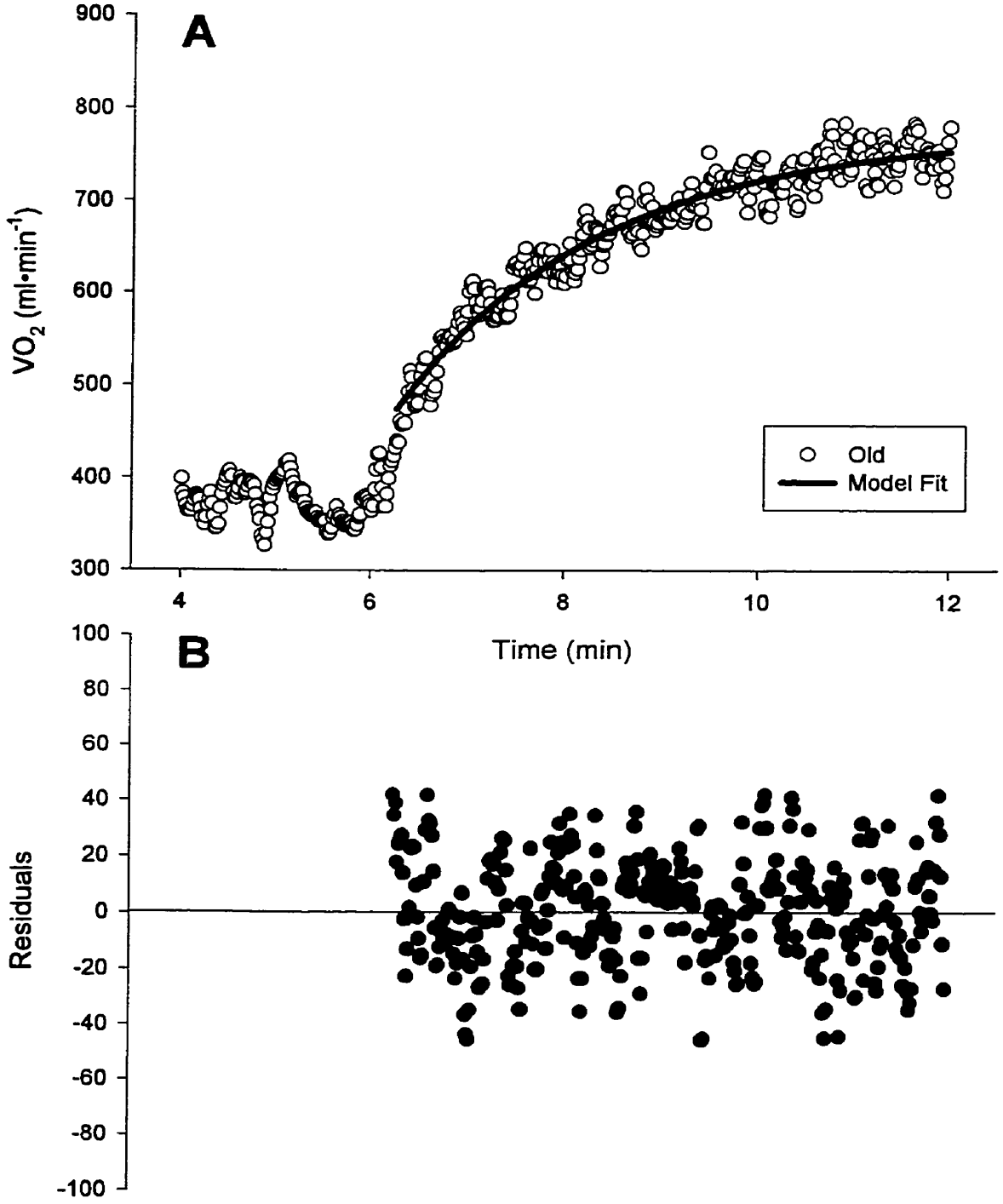

Figure 4.4. Oxygen uptake response to square wave knee extension exercise in a single old subject. Panel **A** shows breath-by-breath data (open circles) and least squares regression (solid line). Panel **B** shows plot of residuals.

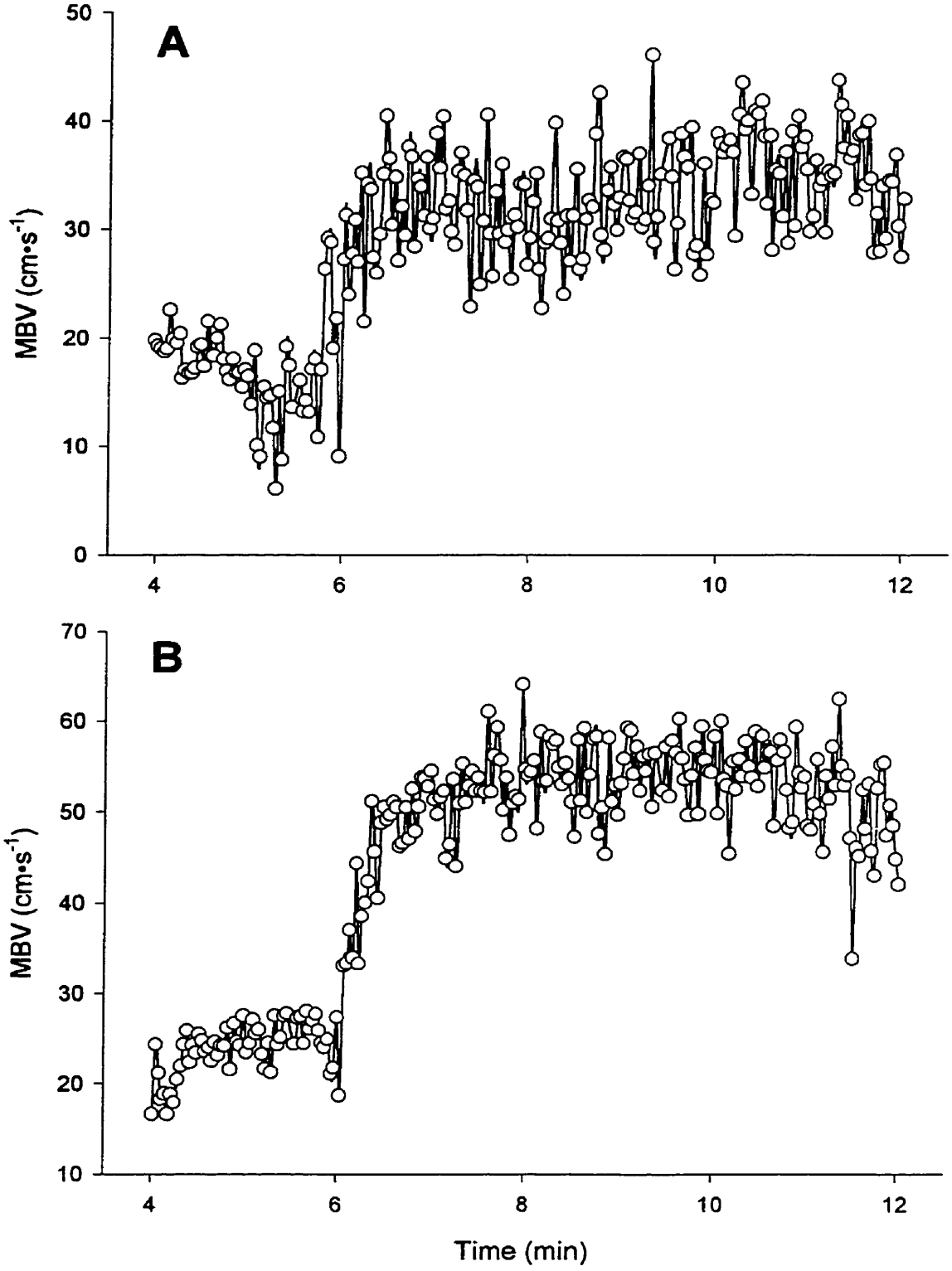

Figure 4.5. Individual mean blood velocity response to square wave knee extension exercise in a single young (**A**) and old (**B**) subject. Data are 2 s means (1 contraction cycle) and represent the ensemble average of six repeats. Exercise onset is at minute 6.

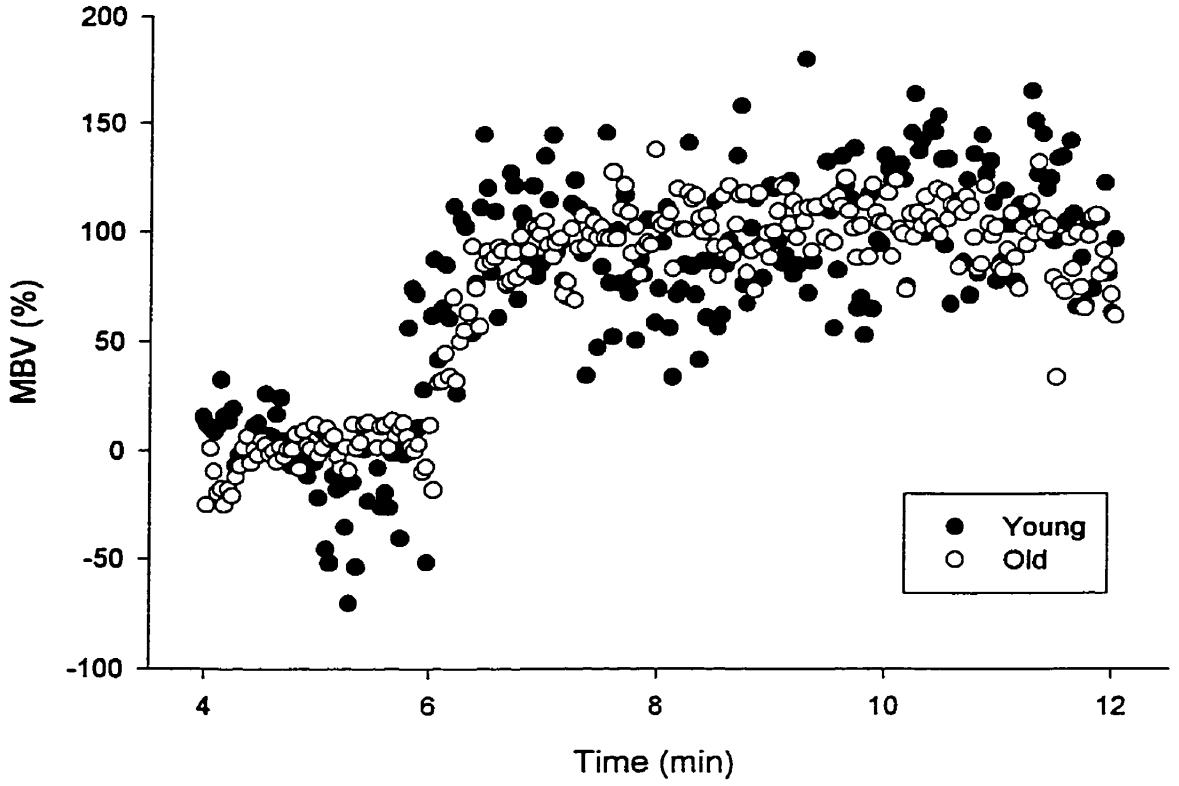

Figure 4.6. Normalized plots of mean blood velocity response to square wave knee extension exercise in a single young (solid circles) and old (open circles) subject. Data are 2 s means and represent the ensemble average of six repeats. Exercise onset is at minute 6.

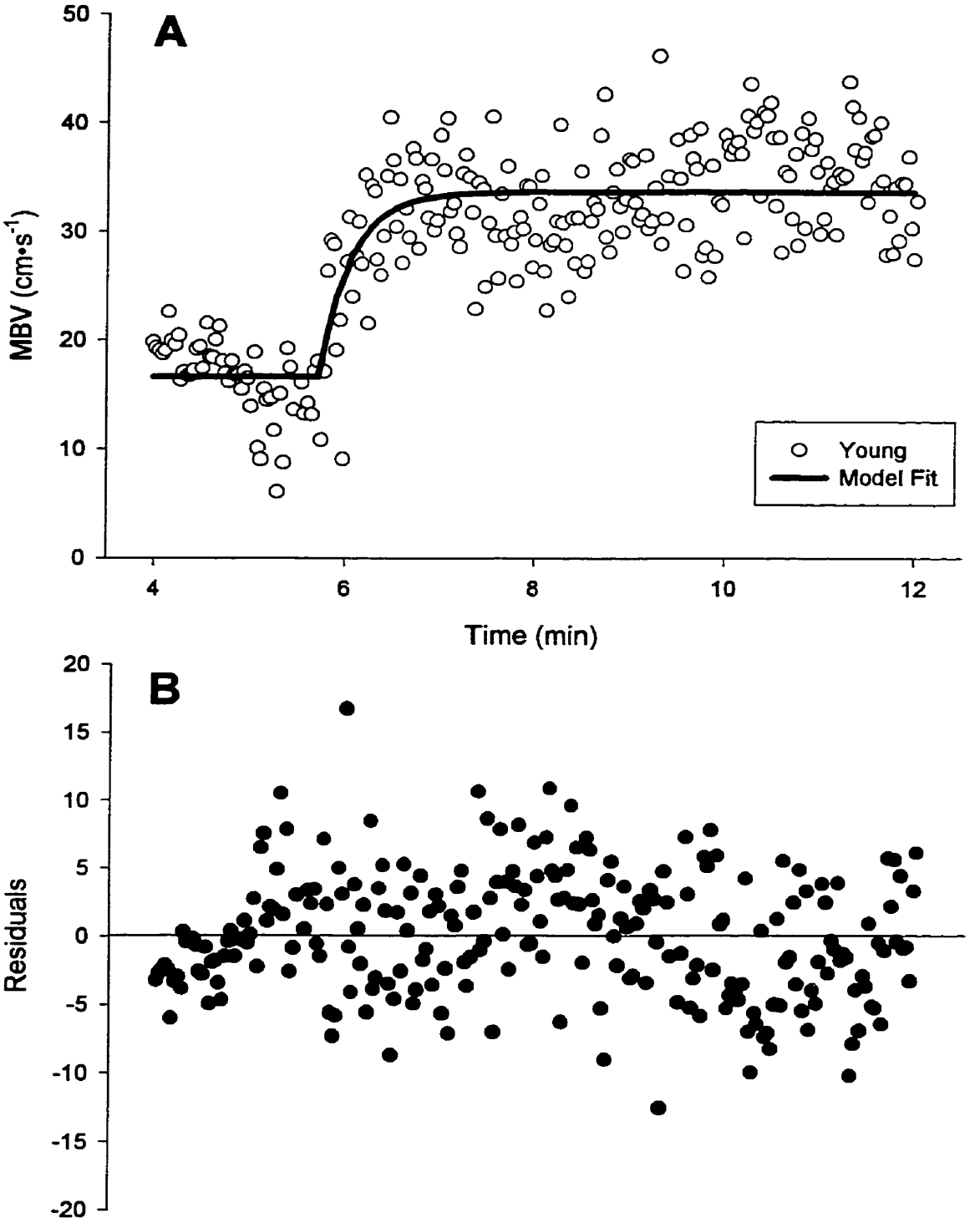

Figure 4.7. Mean blood velocity response to square wave knee extension exercise in a single young subject. Panel **A** shows beat-by-beat data (open circles) and least squares regression (solid line). Panel **B** shows plot of residuals.

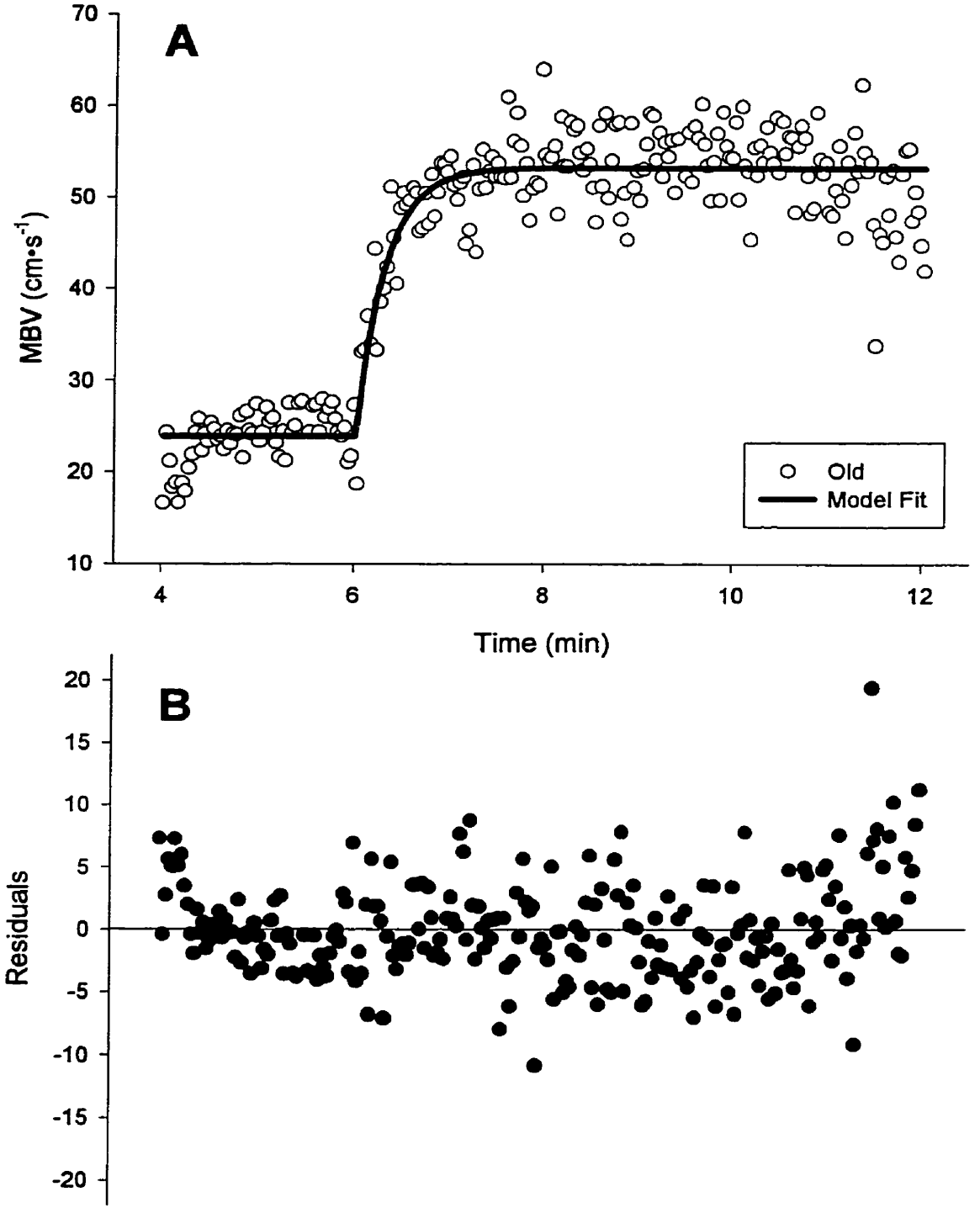

Figure 4.8. Mean blood velocity response to square wave knee extension exercise in a single old subject. Panel **A** shows beat-by-beat data (open circles) and least squares regression (solid line). Panel **B** shows plot of residuals.

CHAPTER 5

DISCUSSION

The present study was undertaken to examine the kinetics of $\dot{V}O_2$ and of mean blood velocity (MBV) during single leg knee extension exercise. Older men were compared with young to discern whether slow $\dot{V}O_2$ kinetics at the exercise on-transient in the old reflected a slowed kinetics of MBV. For the constant load knee extension exercise, significantly slower $\dot{V}O_2$ kinetics were observed in older compared to younger men. However, old and young exhibited similar rates of adjustment of MBV at the exercise on-transient and a similar magnitude of increase for the steady state MBV. The τMBV was significantly faster than $\tau\dot{V}O_2$ in both old and young. These data suggest that transport of blood to the exercising muscle was not the limiting factor slowing the time course of adaptation in $\dot{V}O_2$ in older subjects during KE exercise.

The use of a knee extension exercise model was chosen to allow for study of a relatively isolated muscle group (quadriceps) during exercise, along with measures of MBV in the femoral artery. Furthermore, use of the relatively large muscle mass of the upper leg (compared to arm or lower leg exercise) provided a greater amplitude of response, permitting curve fit analysis of the gas exchange response during exercise. The observed $\dot{V}O_2$ response in the steady state of the constant load, one-legged knee extension exercise approximated 725 $ml \cdot min^{-1}$ in old and 880 $ml \cdot min^{-1}$ in young, representing 73% (old) and 58% (young) of the

peak one-legged $\dot{V}O_2$. Similar proportions of the peak cycling responses were witnessed to occur in both groups and [La^-] was well below that described for critical power in young and old (Overend *et al.*, 1992), suggesting that the exercise was well within sustainable limits. At the onset of square wave exercise (i.e., after 6 minutes of loadless work), the [La^-] was significantly higher in the old compared to young. This may be related to a greater early lactate accumulation in the older men during the transition from rest to exercise at the onset of loadless KE work. Alternatively, a reduced muscle mass in the old may have rendered the task of loadless extensions (lifting of the lower leg) to be of greater relative intensity for these individuals compared to the young group.

The design was for a similar relative work rate in old and young and a $\dot{V}O_2$ response in the moderate intensity domain, to facilitate model fitting of the $\dot{V}O_2$ data. In fact, the relative work rates were higher in the old due to a limitation of the minimum work rate which could be set on the ergometer. Thus, in the old, the $\dot{V}O_2$ response showed a slight "slow component" and the post-exercise blood lactate showed a Δ[La^-] of 1.8 mmol•L^{-1} in the old compared with 1.4 mmol•L^{-1} in young. The larger Δ[La^-] in old may be the consequence of a greater contribution from early La^- accumulation related to the slower rise in $\dot{V}O_2$ at the start of exercise. Thus, the old would be categorized as exercising at slightly above anaerobic threshold, on the verge of the heavy intensity domain, but certainly well below severe exercise or critical power, wherein blood lactate would increase throughout exercise and there would be a large "slow component" of $\dot{V}O_2$. Despite this difference in relative exercise intensity of old and young, it is unlikely to affect the analysis of kinetics. In the current study, responses that displayed a delayed onset slow component were analyzed with a reduced fitting window

(to exclude the slow component), such that the regression was fit to describe only the primary (*phase 2*) component of the response. In fitting the primary component of the $\dot{V}O_2$ response (*phase 2*), Barstow and colleagues (1991; 1993) observed τ to be constant across both moderate and heavy intensity exercise. Paterson and Whipp (1991) found slower *phase 2* kinetics with heavy work, however the difference in τ from moderate exercise was only 8.9 seconds, and their exercise was at $\Delta 50$ (a $\dot{V}O_2$ midway between Θ_{an} and $\dot{V}O_{2max}$), close to the "severe" exercise domain. Thus, in the present study, the determination of $\tau\dot{V}O_2$ and the kinetics of MBV related to the exercise demand should not be appreciably affected by the slightly different relative work rates of old and young.

The slowed $\dot{V}O_2$ kinetics observed in older subjects is consistent with most previous reports for older adults for studies of cycling (Cunningham *et al.*, 1993; Babcock *et al.*, 1994b) or treadmill (Chilibeck *et al.*, 1996) exercise. Chilibeck et al. (1996; 1997) found $\dot{V}O_2$ kinetics not to be slowed in older adults during the small muscle mass exercise of plantar flexion. They suggested this could be consequent to the minimal cardiovascular requirement of a small muscle mass. Alternatively, it was suggested that plantar flexor muscles experiencing daily activity may have preserved muscle metabolic responses. In the present study, the quadriceps, as used in cycling, are unlikely to be as "trained" as the plantar flexors. Thus the slowed kinetics may reflect responses in muscle metabolism.

The limitations or mechanisms of the slowed $\dot{V}O_2$ kinetics with age remain unclear. Some investigators have hypothesized that the slowing of $\dot{V}O_2$ kinetics in older adults may be related to an impaired central delivery of O_2 (or kinetics of $\dot{Q}$ increase at the exercise on-transient), inferred through slowed HR kinetics (Cunningham *et al.*, 1993; Chilibeck *et al.*,

1996). In addition, these investigators reported a significant correlation between $\tau\dot{V}O_2$ and τHR. Further support for a centrally limited O_2 delivery has been presented by Petrella et al. (1999). Their study demonstrated that sedentary older adults with impaired diastolic function showed slow $\dot{V}O_2$ kinetics. However, pharmacological intervention using a calcium channel blocker was shown to improve diastolic function and cardiac filling at rest, and to speed the $\dot{V}O_2$ kinetics of the exercise response. Although HR kinetics were not changed, it was suggested improved diastolic function would yield an improved stroke volume and central blood flow at exercise onset, overcoming a limitation of blood flow delivery to the exercising muscles. Whereas these studies may suggest a central circulatory limitation, there were no direct measures of the $\dot{Q}$ kinetics, and no consideration of the factor of distribution of the available blood flow to exercising muscle.

In the present study, the uncertainty attributed to indirect estimates of circulatory adequacy at the onset of exercise was circumvented by using Doppler ultrasound to gain an index of peripheral blood flow in the artery supplying the exercising muscle group. The Doppler MBV measures provided an indication of local circulatory adjustment. If cardiac output or blood flow distribution at exercise onset was related to the slowed $\dot{V}O_2$ on-kinetics in the old men, it would be expected that MBV would adapt more slowly in this group compared to the young. MBV showed a similar time constant response in both old (25.3 ± 2.9 $cm \cdot s^{-1}$) and young (20.2 ± 2.5 $cm \cdot s^{-1}$). Furthermore, the two groups were not different in terms of steady state MBV at baseline or during exercise. The time course of adaptation in MBV response observed in the current study was similar to, or slightly slower than that of

other reports employing a knee extension exercise model, with values of MBV within 16 to 18 $cm \cdot s^{-1}$ (Shoemaker *et al.*, 1996b; MacDonald *et al.*, 1998).

The Doppler instrumentation used in the current study did not provide a means to determine arterial diameter. The only assessment of hemodynamic adjustment obtained was of the change in femoral artery blood velocity. Nevertheless, several recent reports have indicated that femoral artery diameter remains the same throughout the rest-to-exercise transition (Shoemaker *et al.*, 1996b; Radegran, 1997; MacDonald *et al.*, 1998), averaging about 10.2 mm. If this diameter estimate holds true for older individuals, estimates of the magnitude of leg blood flow (LBF) can be made in the current investigation ($LBF = MBV \cdot \pi r^2$). With an observed steady state exercise blood velocity of 40 and 33 $cm \cdot s^{-1}$ for old and young, blood flow would have approximated 1960 $ml \cdot min^{-1}$ in old and 1632 $ml \cdot min^{-1}$ in young for an exercise $\dot{V}O_2$ of 725 and 880 $ml \cdot min^{-1}$, respectively. In studying young individuals, MacDonald et al. (1998) reported a comparable KE exercise LBF of 2043 $ml \cdot min^{-1}$ for a $\dot{V}O_2$ of 907 $ml \cdot min^{-1}$. The blood flow calculations from the present study yield a $LBF/\dot{V}O_2$ of 1.85 to 2.70, which is in close agreement to the ratio of 2.25 calculated from the data of MacDonald et al. (1998). However, the extent to which the presently calculated flow can be compared is limited, particularly given that exercise in the current study involved only the quadriceps, whereas MacDonald et al. (1998) employed a protocol requiring both quadriceps extension and hamstrings flexion.

Is the LBF adequate to support the increased O_2 demand of the exercising muscles? The relationship between blood flow and oxygen uptake has been established for cycling and treadmill exercise as the $\dot{Q}$-$\dot{V}O_2$ regression. For older adults, Thomas et al. (1993) found $\dot{Q}$

$= 4.64\ \dot{V}O_2 + 4.21$. According to this relationship, for the amplitude of $\dot{V}O_2$ response of 234 $ml \cdot min^{-1}$ in $\dot{V}O_2$ observed in the older men between unloaded and steady state exercise, the required increase in $\dot{Q}$ would be 1.09 $L \cdot min^{-1}$. Doppler measures showed MBV in the old to increase by about 19 $cm \cdot s^{-1}$, corresponding to a 0.93 $L \cdot min^{-1}$ increase in leg blood flow (assuming constant arterial diameter $\cong$ 10.2 mm). This estimate of LBF suggests that of the total increase in $\dot{Q}$ required to support the exercise $\dot{V}O_2$, approximately 85% was distributed to the femoral artery of the exercising leg. This figure agrees with the estimates discussed by Rowell (1974), whereby following the redistribution of $\dot{Q}$ away from splanchnic and non-exercising regions, an 85% distribution of $\dot{Q}$ to working muscle would be expected as adequate to support exercise demands (Rowell, 1974). Additionally, Eriksen et al. (1990) have shown that, consequent to its redistribution, blood flow to the exercising limbs increases to a greater extent than predicted from the $\dot{Q}$ alone. That is, a greater peripheral than central flow increase upon exercise, even in moderate work, was evident. Thus, it appears in the present study that the calculated LBF is appropriate for the exercise task, indicating that central blood flow can adequately supply the quadriceps during moderate one leg KE exercise.

The current data indicate that the rate of increase in MBV was considerably faster than that of $\dot{V}O_2$ and the kinetics of MBV appeared not to be limiting for the $\dot{V}O_2$ kinetics in the old, or for that matter in the young. Somewhat contrasting evidence has been provided by Hughson et al. (1996), who demonstrated slower $\dot{V}O_2$ kinetics concomitant with slower adjustment of limb blood flow during supine forearm exercise above versus below the horizontal body position. In a similar regard, $\dot{V}O_2$ and leg blood flow were shown to increase at a greater rate during upright compared to supine knee extension exercise (MacDonald *et*

al., 1998). In both of these studies, a dependence of $\dot{V}O_2$ on blood flow was inferred from the results of a manipulation of muscle perfusion pressure. Differences in exercise protocol may be important in explaining the unsupportive findings of the current investigation. The one-legged exercise of MacDonald et al. (1998) required contractions on knee extension and on flexion, compared to the present protocol of muscle contraction on extension and relaxation on flexion. The more continuous contractions of their protocol may have impeded blood flow, whereas the current design may be more analogous to the rhythmic dynamic contractions of aerobic exercise such as cycling or walking and running. In the current investigation, where such hindrances to blood flow were not expected to occur, MBV kinetics were consistently faster than $\tau\dot{V}O_2$. Nevertheless, slowed $\dot{V}O_2$ kinetics were observed in the older subjects. As has been suggested by Segal (1992), the conduit arteries (e.g. femoral artery) do not represent active sites of flow control to the exercising muscle.

During constant load cycle ergometry at an intensity engendering a slow component of $\dot{V}O_2$, Poole et al. (1991) observed that leg blood flow increased gradually during exercise. In the present study, where the older adults evidenced a $\dot{V}O_2$ slow component, a gradual increase in blood flow might be expected, similar to that seen in young adults by Pool et al. (1991). However, blood velocity in the older subjects demonstrated a rapid increase and attainment of steady state which persisted until the end of exercise. The inconsistency between the current findings and those of Poole et al. (1991) may related to differences in blood flow distribution within the active muscle. It has been demonstrated that both vascular conductance (Dinenno *et al.*, 1999) and the ability to redistribute blood flow away from splanchnic and renal circulations (Ho *et al.*, 1997) are reduced in older men. Taken together,

these data would imply that although similar blood flow responses were observed at the level of the femoral artery in older and younger men, distribution to and within the muscle may be compromised in older adults, and may not be apparent in Doppler measures of "bulk" flow.

A different point of control (other than blood flow supply gleaned from measures of femoral artery MBV) may be exerting a regulatory influence on the rate of increase in $\dot{V}O_2$. Whereas gross delivery of blood flow (and presumably O_2) to the working muscles does not appear related to the slowed $\dot{V}O_2$ response observed in older subjects, we cannot exclude the possibility that increases in $\dot{V}O_2$ at the start of exercise may be limited through the transit of O_2 from artery to mitochondria. Coggan et al. (1992a) have shown lower levels of capillarization in sedentary older compared to younger adults, which may impose a limitation on the rate of O_2 diffusion. Recent work (Chilibeck *et al.*, 1997) has demonstrated that moderately active older men and women, who exhibit equally fast $\dot{V}O_2$ on-kinetics during plantar flexion exercise compared to younger subjects, also showed similar levels of muscle capillarization and O_2 diffusion distances from capillary to muscle fiber. These data would imply that slower $\dot{V}O_2$ kinetics in the unfit old may have been limited by the rate of O_2 diffusion from capillary to muscle fiber. However, in the isolated dog gastrocnemius preparation of Grassi et al. (1998b), the enhancement of peripheral O_2 diffusion was not shown to improve $\dot{V}O_2$ kinetics. This has recently been corroborated in work with older adults (Bell *et al.*, 1999), where increases to the "driving pressure" of O_2 from hyperoxic gas breathing has also shown no effect on the time constant of the $\dot{V}O_2$ response. These findings, taken together with the results of the present study, would imply that neither peripheral O_2 diffusion nor bulk

blood flow delivery to exercising muscle appear to cause the slowing of kinetics observed in the old.

In summary, single leg KE exercise was used to examine the kinetics of $\dot{V}O_2$ in both older and younger men while simultaneously measuring the rate of adaptation in femoral artery MBV during moderate intensity square wave exercise. Consistent with the literature, the results demonstrated a significantly slower $\tau\dot{V}O_2$ in the older compared to younger subjects. Analysis of the MBV response showed similar rates of adaptation and a faster τMBV versus $\tau\dot{V}O_2$ in both old and young. These findings extrapolate to suggest that during exercise, blood flow delivery (and O_2 transport) does not appear to limit $\dot{V}O_2$ kinetics in either old or young populations. In consideration of the reported age-related reductions in both mitochondrial content and oxidative capacity (Beyer *et al.*, 1984; Coggan *et al.*, 1992a), the slowed rate of $\dot{V}O_2$ adaptation present in older adults may be controlled by muscle O_2 utilization (Whipp & Mahler, 1980; Grassi *et al.*, 1996). Under this paradigm, slowed $\dot{V}O_2$ kinetics in the old would be related to factors governing the inertia of muscle oxidative metabolism.

CHAPTER 6

CONCLUSIONS AND LIMITATIONS

6.1 Conclusions

The present study was undertaken to investigate the relationship between the kinetics of $\dot{V}O_2$ and femoral artery mean blood velocity (MBV) during the on-transient of square wave knee extension (KE) exercise in both older and younger men. It was hypothesized that older subjects would demonstrate a slower rate of adaptation in $\dot{V}O_2$ relative to the young. If both old and young demonstrated similar rates of adjustment in MBV (and thus presumably femoral blood flow), then the slower kinetics of $\dot{V}O_2$ in the older group would not be due to a blood (and O_2) delivery limitation. Should older men evidence a slower time course of adaptation in MBV compared to young, it would suggest that the slower $\dot{V}O_2$ kinetics observed in the old may be limited by O_2 transport.

Based on the findings of the present study, it can be concluded that:

1. During submaximal single leg KE exercise, the kinetics of $\dot{V}O_2$ were observed to be significantly slower in older relative to younger men.
2. The response profile of MBV was similar in both old and young, with no significant differences apparent in either the time constant (τMBV) or in the amplitude of the steady state response.

3. In both older and younger adults, the time constant for MBV (τMBV) was significantly faster than that for $\dot{V}O_2$ ($\tau\dot{V}O_2$). Furthermore, τMBV and $\tau\dot{V}O_2$ were not significantly correlated in either the old or the young, or across all subjects pooled together.

Thus, circulatory adaptations in blood flow to the exercising muscle did not impose the limit to the rate of oxygen uptake kinetics of knee extension exercise, and do not explain the slowed $\dot{V}O_2$ kinetics in old.

6.2 Limitations

Due to limitations in the ability to adjust the minimum resistance on the KE ergometer, the relative work rates were higher in the old compared to young, resulting in the emergence of a small "slow component" in the older subjects. It has been demonstrated in young individuals that a prior bout of supra-Θ_{an} exercise induces faster $\dot{V}O_2$ kinetics during subsequent supra-Θ_{an} exercise (Gerbino *et al.*, 1996). With the older men working slightly above the anaerobic threshold (Θ_{an}), the sequential design of the exercise protocol (requiring the performance of two consecutive square wave bouts, each followed by 6 minutes of loadless exercise) may have brought about metabolic changes causing a speeding of the $\dot{V}O_2$ kinetics during the second square wave on-transient. As the young subjects were working at a moderate intensity, it is not expected that their results were affected in this manner.

In the current study, metabolically driven increases in vasodilation and muscle blood flow at the start of the second square wave, coupled with a lower muscle pH and thus potential rightward shift of the oxygen dissociation curve may have accelerated the observed kinetics of both MBV and $\dot{V}O_2$ in the older group (Gerbino *et al.*, 1996). As all square wave

repeats were incorporated into a single averaged response, inclusion of the second of the two consecutive bouts into the average may have accelerated the overall response kinetics. If the mechanism proposed by Gerbino et al. (1996) was present in the older men during exercise, a different interpretation of the observed results may be necessary. In the assumption that kinetics were speeded in the second exercise transition, $\dot{V}O_2$ kinetics in the old may have actually been slower than that suggested by modelling the averaged response. This would provide further support for the conclusion that the rate of adaptation of $\dot{V}O_2$ is slowed with increasing age. However, if the first square wave induced a residual vasodilation which persisted through to the onset of the second on-transient, then the measured rate of adaptation in blood flow (and presumably MBV), may have also been accelerated relative to the first exercise bout in the old. With the potential for a faster MBV response in the old, the conclusion of similarity in MBV responses may be questioned. Nevertheless, the warm-up effect of hastening the MBV response in the old is unlikely to be of a magnitude sufficient to account for the degree of slowing in $\dot{V}O_2$ kinetics in the old, or to suggest any limitation in blood flow delivery.

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APPENDICES

APPENDIX 1
PARAMETERS OF MODEL FIT FOR $\dot{V}O_2$ RESPONSE

Table A.1.1 Response parameters in older men (n=6).

Subject	A_0	A_1	TD	τ	TLT	TA	RSS	MSSE
1721	461.25	311.90	2.40	122.29	124.70	773.15	231844.77	690.01
3892	499.60	149.34	35.99	82.15	117.55	648.94	74828.65	479.67
1933	474.79	299.30	-3.77	126.89	123.13	744.09	119919.88	356.90
1239	526.32	212.60	2.16	40.89	43.05	738.92	410010.75	738.92
3704	529.30	206.30	10.52	65.20	75.72	735.60	631701.27	1880.06
0520	423.20	231.82	2.57	109.60	112.17	655.02	273977.49	922.67
Mean	485.74	235.21	8.21	91.17	99.39	720.95	290380.47	936.60
(SE)	(16.70)	(24.98)	(5.74)	(13.99)	(13.46)	(22.82)	(83671.22)	(229.45)

Table A.1.2 Response parameters in younger men (n=6).

Subject	A_0	A_1	TD	τ	TLT	TA	RSS	MSSE
3722	545.95	195.47	-18.16	49.42	31.25	741.42	205032.90	1340.80
3720	550.51	199.56	-0.82	34.33	33.51	750.07	103587.30	664.00
3168	661.90	186.40	7.51	24.34	31.85	848.30	449993.90	1047.30
3436	723.58	204.05	3.74	45.06	48.80	927.63	365450.10	1087.60
3697	839.80	308.04	-6.28	51.61	45.33	1147.84	501240.80	1491.80
2541	696.38	137.65	5.69	14.81	20.50	834.03	165002.50	491.10
Mean	669.69	205.20	-1.39	36.60	35.21	874.88	298384.58	1020.43
(SE)	(45.51)	(22.82)	(3.92)	(6.03)	(4.22)	(61.39)	(66604.58)	(156.77)

APPENDIX 2
PARAMETERS OF MODEL FIT FOR MBV RESPONSE

Table A.2.1. Response parameters in older men (n=6).

Subject	A_0	A_1	TD	τ	TLT	TA	RSS	MSSE
1721	15.08	30.40	-2.41	23.30	20.89	45.47	1080.26	7.50
3892	16.65	18.83	11.63	32.66	44.28	35.48	3590.34	25.11
1933	23.96	29.32	0.82	18.17	18.99	53.28	3568.93	15.32
1239	26.04	13.78	7.78	19.25	27.12	39.82	4987.50	26.96
3704	21.20	7.80	-30.60	34.86	4.26	29.00	856.54	6.25
0520	24.24	11.35	1.89	23.57	25.46	35.63	2496.83	10.95
Mean	21.20	18.58	-1.80	25.30	26.50	39.78	2763.40	15.35
(SE)	(1.81)	(3.86)	(6.12)	(2.83)	(5.31)	(3.50)	(653.69)	(3.62)

Table A.2.2. Response parameters in younger men (n=6).

Subject	A_0	A_1	TD	τ	TLT	TA	RSS	MSSE
3722	10.93	21.09	-14.13	31.50	17.37	32.01	2710.93	15.67
3720	28.44	17.60	47.19	17.47	64.66	46.04	5116.84	35.53
3168	23.58	11.82	-12.94	20.01	7.06	35.40	4820.40	20.69
3436	16.60	17.06	-15.75	20.43	4.68	33.66	4810.93	20.65
3697	13.21	10.61	-8.35	18.34	10.00	23.82	2792.10	27.37
2541	15.90	13.25	2.11	13.20	15.32	29.15	9681.53	42.28
Mean	18.11	15.24	-0.31	20.16	19.85	33.35	4988.79	27.03
(SE)	(2.70)	(1.63)	(9.86)	(2.50)	(9.17)	(3.03)	(1034.61)	(4.14)