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Central Cardiorespiratory And Peripheral Metabolic Influences On Kinetics Of Oxygen Uptake In Older Humans

Philip David Chilibeck

CENTRAL CARDIORESPIRATORY AND PERIPHERAL METABOLIC INFLUENCES ON KINETICS OF OXYGEN UPTAKE IN OLDER HUMANS

By Philip D. Chilibeck

Faculty of Kinesiology

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collecting and analyzing the NMRS data, for assisting me with the writing of those portions of our papers and for her continued friendship; and 2) Brad Hansen, who stands alone as *the* perfect lab technician. He is the glue that holds everything together in our labs at the Centre for Activity and Ageing.

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Abstract

The purpose of this thesis was to determine which factors influence oxygen uptake ($\dot{V}O_2$) kinetics during exercise in old individuals. These factors may include central O_2 delivery (i.e. cardiac output kinetics), the rate of O_2 delivery at the exercising muscle, and the rate at which mitochondria can use O_2. Fitness level may also influence $\dot{V}O_2$ kinetics, by affecting the above factors. These factors were estimated by measuring heart rate (HR) kinetics, muscle capillarization, and citrate synthase (CS) activity, respectively, in groups of old ($\sim$65y) and young ($\sim$26y) individuals. Capillarization and CS activity of the lateral gastrocnemius were compared to $\dot{V}O_2$ and PCr kinetics (which reflect kinetics of muscle O_2 consumption), measured during ankle plantar flexion, while HR kinetics were compared to $\dot{V}O_2$ kinetics during plantar flexion, cycling, and treadmill exercise. The influence of fitness levels on $\dot{V}O_2$ kinetics during cycling was also assessed in old and young groups of individuals. Old and young individuals had similar measures for capillarization, CS activity, and HR, PCr, and $\dot{V}O_2$ kinetics during plantar flexion exercise. Old adults had slower HR and $\dot{V}O_2$ kinetics during cycling and treadmill exercise, compared to young adults. Old individuals, who were relatively fit for their age, had $\dot{V}O_2$ kinetics which were similar to fit young subjects.

These studies show that peripheral muscle oxidative capacity (as determined by enzyme activities, capillarization and PCr dynamics), and $\dot{V}O_2$

kinetics during exercise of a small muscle mass (plantar flexors), accustomed to daily activity, are maintained with age. Cardiorespiratory kinetics of the old individuals are slower during exercise involving large muscle mass (i.e. treadmill walking or cycling), which places a greater demand on central O_2 delivery; however these kinetics can be greatly improved with moderate improvements in fitness level.

It is speculated that peripheral oxidative capacity (i.e. mitochondrial capacity or capillarization) is dominant in controlling $\dot{V}O_2$ kinetics during exercise of a small muscle mass in old individuals, since these measures were similar, along with $\dot{V}O_2$ kinetics, in the old compared to young subjects. Central O_2 transport (cardiac output kinetics, as estimated by HR kinetics) appears to limit $\dot{V}O_2$ kinetics during larger muscle mass exercise (cycling and treadmill walking) in older individuals.

Acknowledgements

Many individuals are deserving of my gratitude for their assistance in the completion of my studies, and right off I would like to apologize to anyone I have missed in the following acknowledgements.

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collecting and analyzing the NMRS data, for assisting me with the writing of those portions of our papers and for her continued friendship; and 2) Brad Hansen, who stands alone as *the* perfect lab technician. He is the glue that holds everything together in our labs at the Centre for Activity and Ageing.

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Chapter 1

Introduction

The adjustment of oxygen uptake ($\dot{V}O_2$) during moderate intensity cycling exercise is slower in old compared to young individuals (Cunningham et al., 1993; Babcock et al., 1994a). This implies that physiological processes controlling the $\dot{V}O_2$ response to workrate changes are affected by age. One purpose of this thesis was to identify which processes are affected and whether they alter $\dot{V}O_2$ kinetics. Slow $\dot{V}O_2$ kinetics of old individuals may be related to slower rates of O_2 transport to exercising muscle or slower rates of utilization of O_2 by mitochondria. Cunningham et al. (1993) have shown that heart rate (HR) kinetics are slower in old individuals, implying that kinetics of cardiac output (Q) and therefore central O_2 delivery are slower in old individuals. The rate of peripheral O_2 transport and utilization may also be slower with age, as capillarization (Coggan et al., 1992b) and activity level of oxidative enzymes (Coggan et al., 1992b; Keh-Evans et al., 1992) are reduced. These measurements (HR kinetics, oxidative enzyme activity, and muscle capillarization) were made in old and young individuals, for comparison to their $\dot{V}O_2$ kinetics.

All of these factors can be improved with increased fitness level: $\dot{V}O_2$ kinetics (Babcock et al., 1994b) and measurements of muscle oxidative capacity (Meredith et al., 1989; Coggan et al., 1992a) of trained old individuals are

similar to young counterparts. A second purpose of this thesis was to determine the degree to which $\dot{V}O_2$ kinetics differ with different levels of fitness in old compared to young individuals, given that fit old have greatly improved muscle oxidative capacity. This was accomplished by cross-sectional comparisons of old and young individuals of varying levels of fitness.

1.1 Thesis Outline

This thesis examines the factors which may influence $\dot{V}O_2$ kinetics in old individuals: oxidative enzyme activity, muscle capillarization, HR kinetics, and fitness levels.

Chapter 2 provides a review of literature which is comprised of sections describing: 1) measurement of $\dot{V}O_2$ kinetics, 2) factors limiting central O_2 transport in old individuals, 3) factors limiting peripheral O_2 transport in old individuals, and 4) reductions in capacity of mitochondria of old adults to utilize O_2.

The first two studies (Chapters 3 and 4) compare in old and young individuals, measurements of oxidative enzyme activity and capillarization of the lateral gastrocnemius, and $\dot{V}O_2$ and PCr kinetics during ankle plantar flexion exercise. By studying a muscle group accustomed to daily activity (i.e. walking), it can be determined whether chronic activity can offset negative effects that age may have on these variables.

The third study (Chapter 5) examines the influence of exercise involving

differing muscle mass on the relationship between age and $\dot{V}O_2$ kinetics. Kinetics of $\dot{V}O_2$ and HR are compared during exercise involving a large muscle mass (cycling and treadmill walking), and an exercise involving a small muscle mass (plantar flexion). If HR (an estimate of cardiac output) kinetics are limiting to $\dot{V}O_2$ kinetics in old individuals, HR and $\dot{V}O_2$ kinetics may be slower (than young individuals) during cycling and treadmill walking, which place larger demands on central O_2 delivery, but not during plantar flexion exercise, which requires a smaller amplitude of response from the central cardiovascular system. If chronic activity offsets the negative effect of age on $\dot{V}O_2$ kinetics, old individuals should have faster $\dot{V}O_2$ kinetics during walking and plantar flexion, than during cycling, since the former two exercises involve muscle groups used on a daily basis.

The final study (Chapter 6) investigates the influences of age and relative fitness level ($\dot{V}O_2$max adjusted for age and sex) on the kinetics of $\dot{V}O_2$ during cycling. It is hypothesized that $\dot{V}O_2$ kinetics will be slower with age, but that relative fitness level will override the ageing effect, so that fit older individuals will have similar kinetics when compared to their young counterparts.

The first two studies (Chapters 3 and 4) have been submitted for publication to the *Journal of Applied Physiology*. Preliminary results, upon which the study of Chapter 5 is based, have been published in *Advances in Experimental Medicine and Biology* (volume 393, pp. 195-200, 1995), the entire study of Chapter 5 is in press in the *Journal of Applied Physiology* and the

study of Chapter 6 is in press in the *Canadian Journal of Applied Physiology*.

The final chapter (Chapter 7) provides a general summary of the four studies and their implications.

Chapter 2

Review of Literature

2.1 Measurement of $\dot{V}O_2$ kinetics

Measurement of the dynamics of non-steady state physiological responses at the onset or termination of exercise offer the advantage of understanding of control mechanisms of responses (i.e. oxygen uptake) to the disturbances of homeostasis. This allows the "assembling [of] a physiological model of the system's control" (Whipp and Ward, 1990). The increase in oxygen uptake ($\dot{V}O_2$) during exercise reflects the integrated action of respiratory, circulatory, and metabolic changes. Linnarsson (1974) was one of the first investigators to apply the concept of "control engineering" to better understand which physiological processes influence the dynamics of $\dot{V}O_2$ in the non-steady state. Linnarsson (1974) refers to these physiological processes as a "black box". Changes applied to the system (i.e. changes in exercise workrate) are referred to as input functions, while changes in the time course of $\dot{V}O_2$ are referred to as the output function. By comparing patterns of input and output, properties of the interposed system may be studied. Relationships between input and output functions can be used to develop mathematical models to describe the dynamic behaviour of $\dot{V}O_2$ (Linnarsson, 1974).

The adjustment rate of aerobic metabolism can be approximated by

measuring the adjustment rate of $\dot{V}O_2$ measured at the lung, during the transition from one workrate to another (Figure 1). The rate of adjustment is estimated by calculating the rate constant (k) [Linnarsson, 1974], time constant (τ) [Whipp et al., 1982], or half-time (Cerretelli et al., 1977) of the $\dot{V}O_2$ response, using a single component exponential model, or by determining the total lag time (TLT) from a multiple-component exponential model (Hughson et al., 1988) [the use of one model over another is discussed below]. Total lag time is determined as the weighted sum of the time delays and τ's from each exponential component in this type of fit (Hughson et al., 1988). The τ of a monoexponential fit model or TLT from a double exponential model, is equal to the time required for $\dot{V}O_2$ to change from baseline to approximately 63% of the final steady state, whereas the half-time ($t_{1/2}$) is the time required for 50% of the change. The τ or $t_{1/2}$ are used as estimates of the rate of $\dot{V}O_2$ adjustment, rather than measuring the total time for $\dot{V}O_2$ to reach steady state, because the exact time at which steady state is attained is often difficult to determine, as $\dot{V}O_2$ changes are very slight at the end of the monoexponential plateau. Rate constant, τ and $t_{1/2}$ are related to each other as:

$$\tau = 1/k = t_{1/2} / 0.693$$

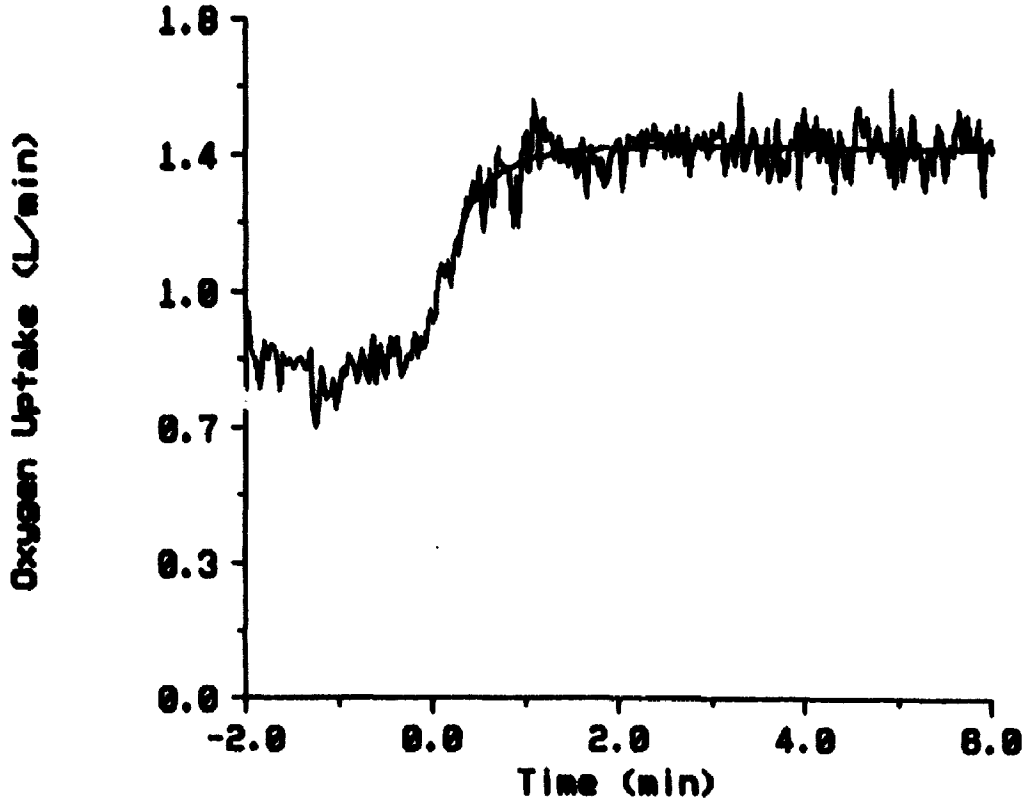
These kinetic parameters are used to describe the kinetics of the $\dot{V}O_2$ response.

Figure 1. $\dot{V}O_2$ adjustment during the transition from one workrate to another.

The monoexponential increase in $\dot{V}O_2$ to a steady state is represented by the equation:

$$\dot{V}O_2 (t) = a_0 + a_1(1-e^{-|t-TD/\tau|}),$$

where a_0 = $\dot{V}O_2$ at baseline, a_1 = $\dot{V}O_2$ amplitude, t = time, TD = time delay, and τ = time constant


Oxygen Uptake (L/min)
1.8
1.4
1.0
0.7
0.3
0.0
-2.0
0.0
2.0
4.0
6.0
Time (min)

2.1.1 Breath-by-Breath Measurement

The method used to collect gas exchange for estimation of kinetics throughout this thesis involves breath-by-breath measurements. Linnarsson (1974), as well as several other early investigators (Auchincloss et al., 1966; Beaver et al., 1973) stressed the importance of breath-by-breath systems for the analyses of $\dot{V}O_2$ kinetics. This involves the use of rapid-response transducers for the generation of signals for flow, and fractional concentrations of gases (Linnarsson 1974). Fast-response gas analyses allow a detailed look at the time course of $\dot{V}O_2$ change, and a more precise characterization of kinetics than systems that do not use breath-by-breath measures (Beaver et al., 1973; Linnarsson, 1974).

2.1.2 Algorithms for estimating alveolar gas exchange

When estimating breath-by-breath alveolar $\dot{V}O_2$, from measurements at the mouth, corrections must be made for changes in lung gas stores. This is important during kinetic analyses if one wishes to estimate changes in muscle O_2 consumption from $\dot{V}O_2$ measured at the mouth, since changes in lung gas stores will dissociate muscle from lung measurements during the transition to exercise. Over a long period of measurement, this is not a problem, as gas stores in the lung fluctuate and total lung gas exchange averages to the same value as alveolar gas exchange. When analyzing gas exchange on a breath-to-breath basis, however, discrepancies between $\dot{V}O_2$ measured at the mouth and alveolar

$\dot{V}O_2$ (which is representative of muscle O_2 consumption) become apparent (Beaver et al., 1981). Algorithms, which account for changes in lung gas stores, have been developed to correct for these discrepancies (Beaver et al., 1981; Swanson and Sherrill, 1983). The algorithm of Beaver et al. (1981) makes corrections for both changes in lung volumes and alveolar gas concentrations. Whereas this model uses resting functional residual capacity for the lung volume term, Swanson's algorithm yields estimates of "effective lung volume" and "effective pulmonary blood flow" (Swanson & Sherrill, 1983; Swanson, 1990). Effective lung volume and blood flow refer to "the value of end-expiratory volume and blood flow that effectively participates in breath-by-breath gas exchange" (Swanson & Sherrill, 1983; Swanson, 1990). Swanson (1990) has demonstrated that this algorithm yields a measurement of gas exchange with a higher signal to noise ratio than the method of Beaver et al. (1981).

2.1.3 Improving signal to noise ratio of $\dot{V}O_2$ transients

One difficulty in modelling $\dot{V}O_2$ kinetics arises when the signal to noise ratio of the response to a workrate is small, for example, in older subjects (Babcock et al., 1994a) or cardiac transplant recipients (Paterson et al., 1994). One method of reducing noise in the response is to overlay (time align and average) several responses to repeats of the same exercise perturbation. Linnarsson (1974), one of the first investigators to introduce this concept for this application, determined that the random noise of a response is reduced by a

factor of $\sqrt{n}$, where n is the number of repeats of the workrate perturbation. Lamarra et al. (1987) presented an equation to determine the number of repeats of a protocol that should be overlaid, based on the confidence interval necessary for an estimate of $\tau\dot{V}O_2$. Here, the confidence interval around the estimate (i.e. of τ) refers to a statistical expression for error in estimating τ (Lamarra, 1990). Specifically, the confidence interval for an estimate is the "range of values for an estimate, which have a fixed probability of containing the true value of the parameter" (Lamarra, 1990). Using τ as a parameter, for example, a 95% confidence interval of $\pm$10s implies that 95 times out of 100, the estimate is within $\pm$10s of the true value of τ. For a desired confidence interval, the number of repeats needed depends on the noise of a subject's breath-by-breath response and the steady state gain of the subject's response (i.e. steady state minus baseline $\dot{V}O_2$) to a given workrate. This can be expressed with the equation:

$$n = (L \cdot S/k \cdot Yss)^2,$$

where n refers to the number of repeats, L is a constant (which is equal to 47.5, for a 95% confidence interval), S is the standard deviation of the breath-by-breath response (which is determined at steady state), K is the confidence interval desired (i.e. $\pm$10s) and Yss is the steady-state ga.n of the response to the workrate (i.e. $\dot{V}O_2$ at steady-state minus $\dot{V}O_2$ at baseline) [Lamarra et al., 1987]. Others have rearranged the equation, to determine the 95% confidence interval for estimates of τ, based on a fixed number of repeats of exercise tests

(Paterson et al., 1994).

2.1.4 Modelling of $\dot{V}O_2$ kinetics

Physiologists often choose a specific model to describe $\dot{V}O_2$ kinetics based on what they believe is the controlling process for the rate of $\dot{V}O_2$ adjustment to exercise (i.e. a structural model; Swanson, 1990). Conversely, several models may be compared to determine which best fits the $\dot{V}O_2$ response (i.e. empirical model; Swanson, 1990), and then inferences as to which physiological processes are controlling, can be made based on the best model.

Some prefer a monoexponential model (i.e. Cerretelli et al., 1980), based on the belief that the controlling process for $\dot{V}O_2$ kinetics is through the stimulation of mitochondrial respiration by monoexponential changes in a specific muscle metabolite (discussed below) [Mahler, 1985]. Others prefer models of higher order, based on the belief that the rate of O_2 adjustment is dependent on a complex integration of factors (i.e. respiratory, circulatory and metabolic changes) [Linnarsson, 1974; Hughson et al., 1988].

One popular modification is to fit only the second phase of the $\dot{V}O_2$ response, ignoring the first 15 to 20 seconds (Whipp et al., 1982). The first 15 to 20 seconds are thought to be influenced primarily by an increase in pulmonary perfusion, at exercise onset. This has been termed the "phase 1" response (Whipp et al., 1982; Whipp & Ward, 1990). Phase 2 of the $\dot{V}O_2$ response starts after 15-20 seconds and corresponds to the monoexponential

increase of $\dot{V}O_2$ to a steady-state. This portion of the response is thought to be influenced by the additional component of changes in mixed venous gas tensions resulting from increased tissue metabolism (Whipp et al., 1982). It is hypothesized that the phase 2 response reflects muscle O_2 consumption (Whipp et al., 1982). The final phase of the response, the steady state phase of $\dot{V}O_2$, has been termed "phase 3".

Linnarsson (1974) described a number of factors to consider when deciding which model best fits a physiological response: 1) the better model will result in an appreciable reduction in the sum of squared differences between the model fit and the response, 2) the better model will result in a less negative negative time delay, 3) the two τ's of a second order model should be distinctly different, 4) with group averaging, the sum of squared differences between model fit and response should be reduced in proportion to the number of subjects. If it is only slightly reduced, the order of the model is too low.

Increasing the number of parameters in a model almost always leads to a better fit to the data points (reduction in sum of squared residuals) because this allows more flexibility to the curve fitting procedure. Motulsky and Ransnas (1987) give a description of a simple statistical test to determine whether a higher order model provides a significantly better fit to the data. This test involves the determination of an F ratio:

$$F = [(SS_1 - SS_2) / (df_1 - df_2)] / [SS_2 / df_2],$$

where SS is the sum of squares differences between the model fit and the

A number of other changes with ageing, which alter O_2 carrying capacity of arterial blood, may be linked to slower rates of central O_2 transport. Red blood cell volume, and thus haemoglobin quantity may be reduced with ageing (Sjostrand, 1949; Wennesland et al., 1959). Alterations in haemoglobin O_2 saturation of young humans have resulted in changes in $\dot{V}O_2$ kinetics: when carbon monoxide loading was used to increase carboxyhaemoglobin levels (thus reducing the amount of haemoglobin saturated with O_2), subjects had a slower rate of $\dot{V}O_2$ adjustment to exercise (Koike et al., 1990). Alterations in haemoglobin resulting in a reduced O_2 transport to working muscle, may therefore be one mechanism by which kinetics are slowed with ageing, but this has yet to be tested.

Changes in the respiratory system with ageing may also reduce arterial O_2 content (Levitzky, 1984). There may be a loss of alveolar surface area (Thurlbeck, 1967) and a less efficient matching of ventilation and perfusion in the lung (Holland et al., 1968), resulting in a reduced arterial PO_2 (Sorbini et al., 1968; Muiesan et al., 1971) with ageing. Whether or not these changes affect $\dot{V}O_2$ kinetics, and the possibility of reversing these changes with training in old humans, requires further study.

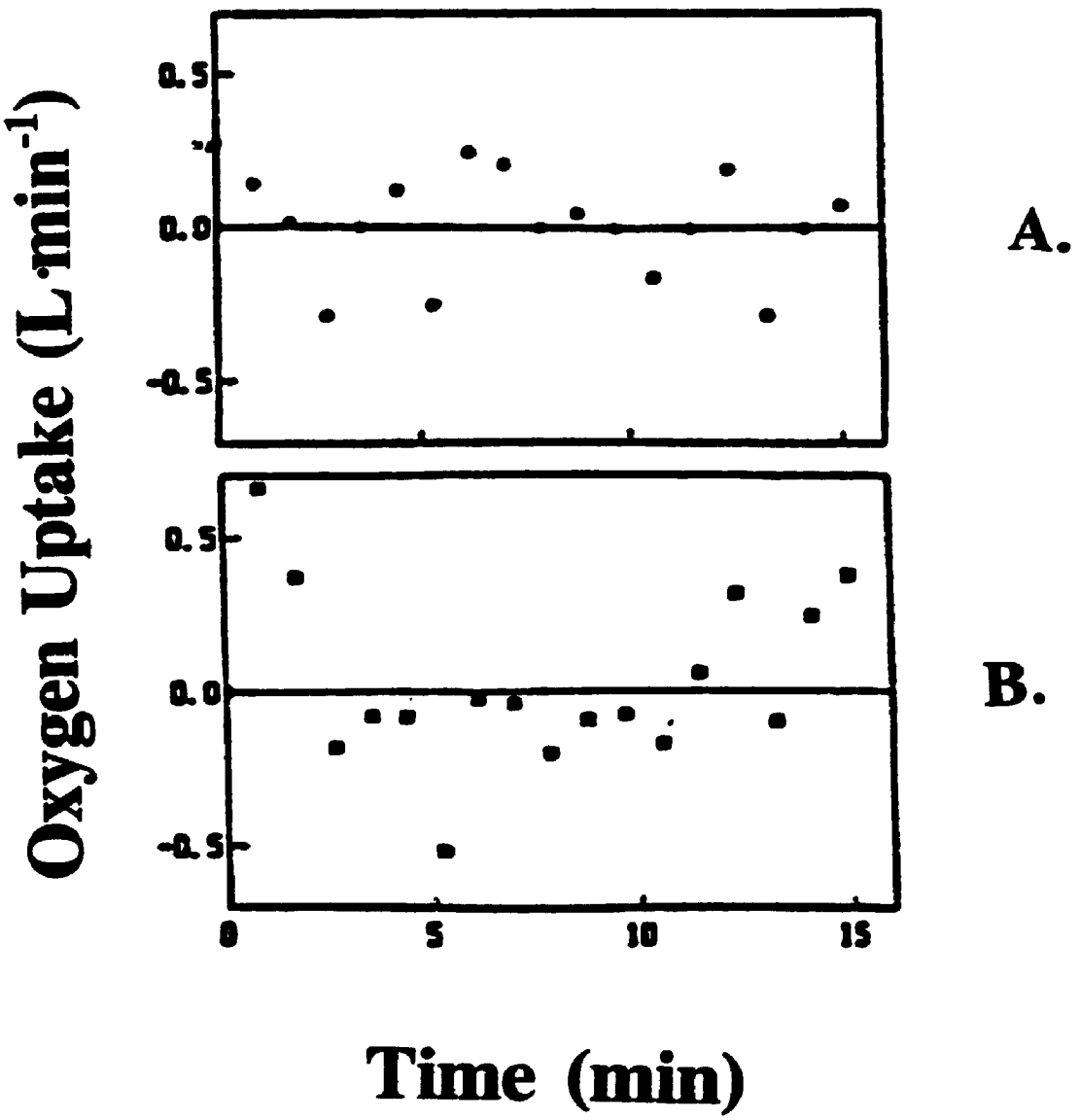
Although not related to central O_2 transport, one other change at the lung that may affect kinetics with ageing is worth mentioning. A reduced efficiency of lung mechanics, resulting in an increased cost of breathing in old individuals (Johnson et al., 1991) may result in "extra O_2" consumption, and a delay in the

Figure 2.

a. Residual plot from data fit with an appropriate model

b. Residual plot from data fit with an inappropriate model

(Figure modified from Motulsky and Ransnas, 1987)


Oxygen Uptake (L·min-1)
0.5
0.0
-0.5
A.
0.5
0.0
-0.5
B.
0
5
10
15
Time (min)

this does not imply that one factor is limiting to $\dot{V}O_2$ kinetics in old subjects, but rather that one factor predominates. They suggested that either the rate of O_2 utilization at the mitochondria, or the rate of O_2 transport could be the dominant limiting factor (Cunningham et al., 1993). The model used in this thesis was fit from the 20s portion of the $\dot{V}O_2$ response, omitting the portion of the response which corresponds to the muscle to lung transport delay (Whipp et al., 1982). With this fit, $\dot{V}O_2$ measured at the lungs should reflect oxygen consumption at the muscle.

2.2 $\dot{V}O_2$ Kinetics of Aged Humans

The kinetics of $\dot{V}O_2$ with moderate intensity cycling are slower in older than younger individuals during the adjustment to exercise (Cunningham et al., 1993; Babcock et al., 1994a). A slower $\dot{V}O_2$ adjustment increases the reliance on anaerobic glycolysis to meet energy requirements. This increases the formation of lactic acid (Linnarsson et al., 1974) and may result in increased sensations of fatigue. A slower $\dot{V}O_2$ recovery may be interpreted as a delay in the replenishment of phosphate energy stores by aerobic metabolism (McCully et al., 1993) or a delay in the oxidative removal of metabolic waste products, such as lactic acid (Donovan and Brooks, 1983), following exercise.

Slower $\dot{V}O_2$ dynamics may be due to a slower rate of O_2 transport to the working muscle (Linnarsson, 1974; Hughson, 1990), or a slower rate of O_2 utilization as a result of slower rates of activation of mitochondrial enzymes

within the muscle (Whipp and Mahler, 1980; Pendergast et al., 1980). $\dot{V}O_2$ measured at the lungs following a transport delay from active muscle, and given only minor changes in oxygen uptake of non-exercising tissues, reflects oxygen consumption of the working muscle during exercise (Whipp et al., 1982). Oxygen is consumed at the mitochondria during the process of oxidative phosphorylation which regenerates ATP used during muscle contraction. The process of oxidative phosphorylation can be summarized by the following equation (Chance et al., 1985; McCully et al., 1988):

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

The rate of this reaction is limited by the activity of the mitochondrial enzymes involved in oxidative metabolism and the concentration of the substrates (ADP, Pi, NADH, O_2) used in the reaction. Thus, the slower $\dot{V}O_2$ dynamics of older individuals may be due to slower utilization of metabolic substrates (ADP, Pi or NADH), in conjunction with lower enzyme activities, or a slower rate of O_2 delivery to the mitochondria for use in oxidative phosphorylation.

A moderate duration of aerobic training of old individuals resulted in significantly faster $\dot{V}O_2$ kinetics, so that rates of $\dot{V}O_2$ adjustment were similar to those seen in young individuals (Babcock et al., 1994b) . Whether this is due to an improvement in systems involved with O_2 transport or O_2 utilization remains unclear.

The following sections provide a review of the influences of O_2 transport and O_2 utilization on $\dot{V}O_2$ kinetics, the influences of ageing on physiological systems affecting O_2 transport and utilization, and the effects of training on these systems in aged individuals. The goal of this review is to provide an understanding of the mechanisms by which $\dot{V}O_2$ kinetics may be slowed with ageing and the mechanisms by which these dynamics may be improved with training of old individuals.

2.3 Limitations by Central O_2 Transport

Cardiac output (Q) during the steady state of submaximal exercise is not compromised in older individuals (Paterson, 1992; Thomas et al., 1993). There is only a slightly reduced Q to $\dot{V}O_2$ relation, during exercise, in old (Thomas et al., 1993) compared to young individuals (Faulkner et al., 1977). Thomas et al. (1993) found a Q-$\dot{V}O_2$ slope of 4.6 to 4.8 in old individuals, while Faulkner et al. (1977) found a Q-$\dot{V}O_2$ slope of 5.2 to 5.9 in young individuals. The influence of ageing on the rate of Q adjustment to exercise, however, has not been examined. A slowing in the rate of Q adjustment to exercise in older adults could be one of the factors contributing to slower $\dot{V}O_2$ kinetics in this group. Experiments with young individuals, where perturbations have been used to alter Q dynamics, have resulted in proportional changes in $\dot{V}O_2$ kinetics. Hughson and Inman (1986) demonstrated that $\dot{V}O_2$ kinetics during arm exercise could be made faster by circulatory occlusion of the legs. These authors hypothesized that

there was an augmentation in the effective central blood volume, permitting an increase in Q during the exercise transient. Beta-blockade, which slows Q kinetics, also has been shown to slow $\dot{V}O_2$ kinetics (Hughson and Kowalchuk, 1991). When heart rate (HR) kinetics were altered by transitions from different levels of exercise (Hughson and Morrissey, 1983), or by pacing of programmable pacemakers in cardiac patients (Casaburi et al., 1989), $\dot{V}O_2$ kinetics were similarly altered. Furthermore, the slow $\dot{V}O_2$ kinetics of cardiac transplant recipients are presumably secondary to their minimal HR response at exercise onset (Paterson et al., 1994). Thus, slowing of kinetics of Q (central O_2 delivery) in old individuals may play a role in the slowing of their $\dot{V}O_2$ kinetics.

In the present thesis, HR kinetics were used as an estimate of Q kinetics. While this may not be the most precise estimate of Q kinetics, it has been shown that the increase in Q at the start of exercise is accomplished mainly by an increase in HR when exercise transitions are from loadless or moderate exercise (as done in the present thesis), as opposed to rest, whereas changes in stroke volume (SV) are minimal (DeCort et al., 1991; Yoshida et al., 1993). Furthermore, it has been suggested that SV may actually transiently decrease at the onset of exercise performed in the supine position (Hughson et al., 1991), which is the position used with the main exercise model (supine plantar flexion) in the first few studies of this thesis.

Cunningham et al. (1993) demonstrated that HR kinetics during the adjustment to cycling were slower in old compared to young individuals, in

conjunction with a slower $\tau\dot{V}O_2$. Results of Babcock et al. (1994a) did not replicate the finding of a slower τHR in old individuals, during transition to moderate intensity cycling, but did show that improvements in τHR with training of their old group were significantly correlated with improvements in $\tau\dot{V}O_2$ ($r=0.78$) [Babcock et al., 1994b], suggesting a link between improvements in central Q adjustment and $\dot{V}O_2$ dynamics.

A lower total blood volume in old compared to young individuals (Davy and Seals, 1994) may also be a factor affecting the rate of Q adjustment and $\dot{V}O_2$ kinetics. A lower blood volume may impair left ventricular filling, which would lower stroke volume and cardiac output (via the Frank-Starling mechanism) during exercise, resulting in impaired O_2 delivery and a slowing of $\dot{V}O_2$ kinetics. Total blood volume has been found to be higher in trained compared to untrained old individuals (Stevenson et al., 1994; Carroll et al., 1995) and may contribute to the faster VO_2 kinetics found with training (Babcock et al., 1994b). A link between the two would have to be confirmed with measurement during the same training study. In young individuals, short-term training (four days), which produced increases in blood volume (Green et al., 1987), resulted in faster $\dot{V}O_2$ kinetics (Phillips et al., 1995) and a more efficient coupling of PCr breakdown to $\dot{V}O_2$ (a smaller breakdown of PCr without any change in $\dot{V}O_2$ early in exercise [Green et al., 1995]). Here, breakdown of high energy phosphates is thought to activate mitochondrial respiration during the transition to exercise (Marsh et al., 1993a).

A number of other changes with ageing, which alter O_2 carrying capacity of arterial blood, may be linked to slower rates of central O_2 transport. Red blood cell volume, and thus haemoglobin quantity may be reduced with ageing (Sjostrand, 1949; Wennesland et al., 1959). Alterations in haemoglobin O_2 saturation of young humans have resulted in changes in $\dot{V}O_2$ kinetics: when carbon monoxide loading was used to increase carboxyhaemoglobin levels (thus reducing the amount of haemoglobin saturated with O_2), subjects had a slower rate of $\dot{V}O_2$ adjustment to exercise (Koike et al., 1990). Alterations in haemoglobin resulting in a reduced O_2 transport to working muscle, may therefore be one mechanism by which kinetics are slowed with ageing, but this has yet to be tested.

Changes in the respiratory system with ageing may also reduce arterial O_2 content (Levitzky, 1984). There may be a loss of alveolar surface area (Thurlbeck, 1967) and a less efficient matching of ventilation and perfusion in the lung (Holland et al., 1968), resulting in a reduced arterial PO_2 (Sorbini et al., 1968; Muiesan et al., 1971) with ageing. Whether or not these changes affect $\dot{V}O_2$ kinetics, and the possibility of reversing these changes with training in old humans, requires further study.

Although not related to central O_2 transport, one other change at the lung that may affect kinetics with ageing is worth mentioning. A reduced efficiency of lung mechanics, resulting in an increased cost of breathing in old individuals (Johnson et al., 1991) may result in "extra O_2" consumption, and a delay in the

attainment of steady state during constant load work. This has been suggested as a mechanism responsible for the O_2 drift and longer $\dot{V}O_2$ kinetics during exercise above the ventilatory threshold ($V_{1\,1}$) in young individuals (Roston et al., 1987). Cross-sectional data indicates that this situation may be improved with an increase in the fitness of older individuals (Johnson et al., 1991).

In summary, central O_2 transport may be slower in old individuals due to a slower Q adjustment to exercise, lower blood volume, reduced haemoglobin, and changes in the respiratory system. Whether these affect $\dot{V}O_2$ kinetics and whether training can improve these variables in old individuals, remains to be tested.

2.4 Limitations by Peripheral O_2 Transport

$\dot{V}O_2$ kinetics are affected by perturbations designed to alter peripheral blood flow. In young individuals, $\dot{V}O_2$ kinetics were found to be slower with exercise performed in a supine versus upright position, where the supine position is thought to result in a reduction of perfusion in the exercising legs (Convertino et al., 1984; Hughson et al., 1993). Kinetics of $\dot{V}O_2$ were slower, despite an elevation of Q in this position, suggesting that perfusion of the exercising legs had a greater influence on $\dot{V}O_2$ adjustment to exercise, than did central O_2 transport. When lower body negative pressure was applied to the legs in this supine position, presumably causing an increase in perfusion, $\dot{V}O_2$ kinetics were faster (Hughson et al., 1993).

This evidence that $\dot{V}O_2$ kinetics appear to be affected by changes in peripheral muscle perfusion may provide another explanation for the slowing of $\dot{V}O_2$ kinetics with age. Several investigators have shown that systemic vascular conductance is reduced during exercise in old, compared to young individuals. Vascular conductance, estimated from changes in Q and mean arterial pressure, was found to be reduced during cycling (Makrides et al., 1990) and treadmill (Hagberg et al., 1985) exercise in old individuals. Consistent with a reduced vascular conductance, Wahren et al. (1974) found that blood flow, measured by indicator dilution, increased less during cycling in old compared to young individuals. This was compensated for by an increased arterial-femoral venous O_2 difference in the old individuals. McCully et al. (1994a) found similar results during treadmill exercise, where haemoglobin oxygen saturation, measured by near infrared spectroscopy at the gastrocnemius, was progressively reduced in old, but not young individuals. Although $\dot{V}O_2$ kinetics were not measured, one could assume that with this increased O_2 extraction throughout exercise, the time required for muscle O_2 consumption to reach steady state levels would be increased in the old individuals. Whether the kinetics of blood flow are actually affected in old individuals has yet to be tested. A reduced perfusion in old individuals may be due to a variety of factors:

1) Some (Parizkova et al., 1971; Coggan et al., 1992b), but not all (Grimby et al., 1982), studies have shown that muscle capillarization is reduced in old, compared to young adults.

2) Skeletal muscle of older individuals has been shown to consist of greater amounts of "nonmuscle" (fat and connective) tissue, than young individuals (Rice et al., 1989; Overend et al., 1992); and

3) There is a decreased beta-adrenoreceptor sensitivity with age (Van Brummelen et al., 1981), which would reduce arterial relaxation during exercise.

A reduced capillarization and increased amount of "non-muscle" tissue would not only decrease the amount of blood flow that could be directed to active muscle during exercise, but it would also increase the diffusion distances for O_2 transport from capillary to mitochondria (Snyder, 1987), and reduce the effective surface area for O_2 exchange (Leinonen, 1980). Taken together, these changes would cause a slowing of $\dot{V}O_2$ kinetics in old individuals.

While simple counts of capillary numbers can be obtained from human muscle biopsies, other factors difficult to measure in the human may play a significant role in the rate of O_2 delivery to working muscle. Kinetics of O_2 delivery may be affected by branching patterns in the capillary network, length of capillary paths and capillary interconnections (Plyley et al., 1976). Another factor may be the rate of capillary recruitment at the onset of exercise, which has been shown to be substantial in dog gracilis muscle (Honig et al., 1980). Further, diffusion distance, which depends on capillary numbers (Snyder, 1987), may be inaccurate in assessing O_2 diffusibility. Honig et al. (1984) hypothesized that the principle gradient for O_2 diffusion is across the capillary to the

sarcolemma (muscle fibre membrane), rather than across the muscle cell interior, as was hypothesized from early classical studies (Krogh, 1918, 1919). Tissue gradients for O_2 are small, as myoglobin acts as a buffer to keep PO_2 relatively uniform throughout the muscle cell (Honig et al., 1984). There may also be diffusion interaction between muscle cells, which would complicate estimates of diffusion from capillaries alone. The PO_2 gradient from inactive to working fibres is substantial and the surface area for exchange is great compared to that of capillaries (Honig et al., 1984). With their difficulty of measurement, it is difficult to assess the effects of ageing on these factors in humans.

The potential for improvements in peripheral O_2 transport with training in old adults may be one mechanism by which $\dot{V}O_2$ kinetics are improved. Marked improvements in muscle capillarization (Coggan et al., 1992a), and vascular conductance during exercise (Makrides et al., 1990; Martin et al., 1990) have been demonstrated with endurance training of old individuals. Again, an association between these variable and improved kinetics needs to be verified through measurement in the same training study. Physical training did not produce an improvement in beta-adrenoceptor density, which may contribute to control of resistance vessel opening during exercise, in aged human skeletal muscle (Martin et al., 1989). Improvement in this variable may therefore be ruled out as a factor involved with the improvement of $\dot{V}O_2$ kinetics after training old individuals. This does not rule out, however, the possibility that

beta-receptor sensitivity is modified with training.

In summary, a reduced perfusion of exercising muscle in old individuals caused by reduced capillarization, greater amounts of fat and connective tissue, and an impaired arterial relaxation may prolong $\dot{V}O_2$ kinetics. Whether improvement in these variables with training can improve $\dot{V}O_2$ kinetics remains to be tested.

2.5 Limitations by Utilization of O_2

A number of investigators dispute the theory that O_2 delivery is limiting during exercise transients and favour a limitation by rate of mitochondrial O_2 utilization. Some have observed that there is an absence of hypoxic loci, as assessed by spectroscopic measurement of myoglobin saturation, during rest to moderate workrate transitions in dog muscle preparations (Connett et al., 1984; Gayeski et al., 1985), suggesting that O_2 transport to the mitochondria is not limiting to the process of oxidative phosphorylation in these instances. Others have observed that blood flow adjustment to exercise (stimulation of dog gastrocnemius) is faster than $\dot{V}O_2$ adjustment and have interpreted this O_2 delivery as being too fast to limit the rate of O_2 uptake (Piiper et al., 1968). Similarly in humans, Cerretelli et al. (1980) have estimated muscle blood flow kinetics an order of magnitude faster than $\dot{V}O_2$ kinetics (although the xenon-133 clearance method for estimating blood flow kinetics in these studies have been criticized [Marcus et al., 1981] and blood flow kinetics measured by Doppler [Shoemaker et al., 1994] and thermodilution techniques [Grassi et al., 1996]

have been found to be longer). These investigators (Cerretelli et al., 1980; Piiper et al., 1968) favour theories where oxidative phosphorylation is limited by the delivery of a metabolic substrate (i.e. ADP, Pi, creatine [CR]), from contraction at the myofibrils (Bessman and Carpenter, 1985; Mahler, 1985; Meyer, 1988), to the mitochondria, rather than by the delivery of O_2 . In this situation, the rate of O_2 utilization by the mitochondria is limited by the activity rate of the enzymes controlling reactions that use these substrates for aerobic production of ATP.

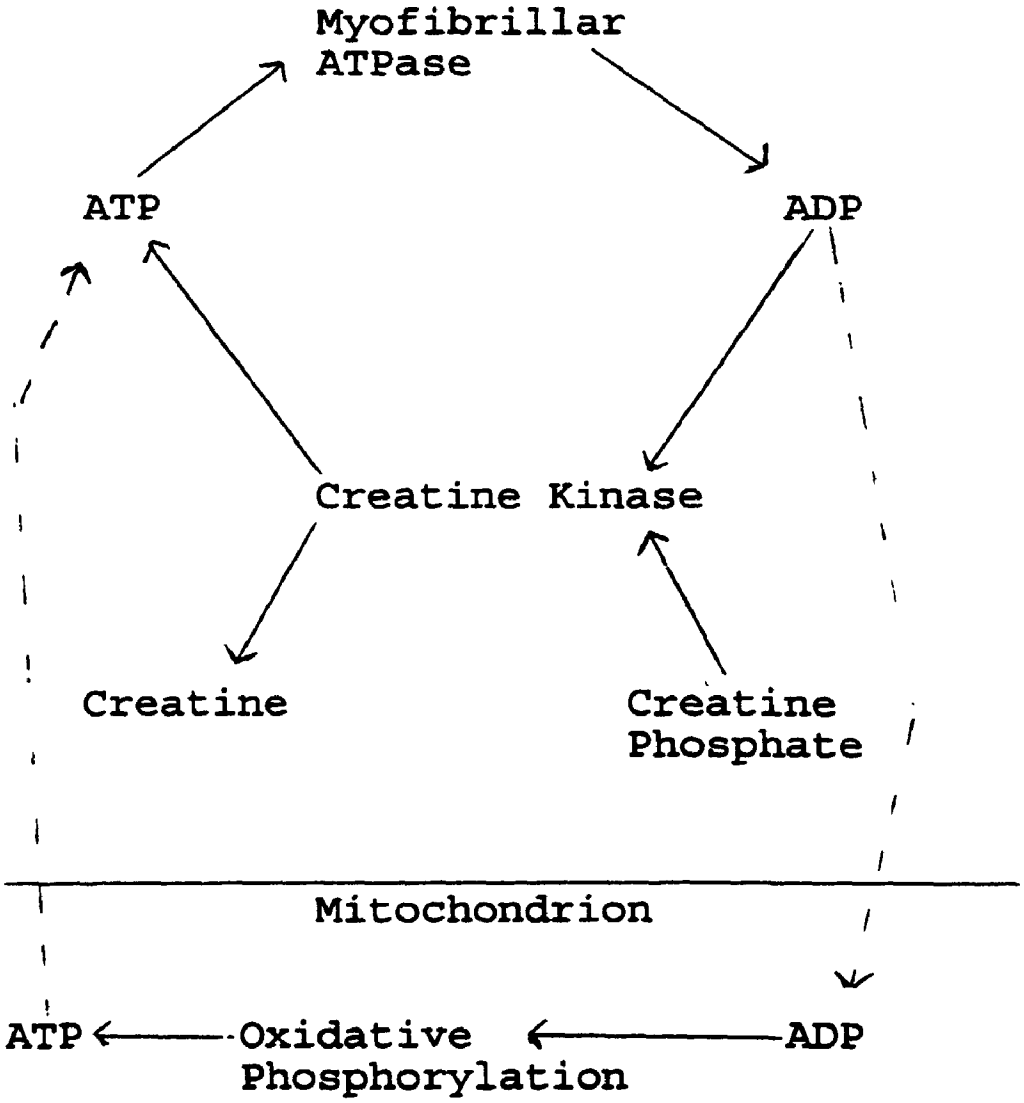
A number of theories have been put forward as to which metabolites or combinations of metabolites control mitochondrial respiration. These control themes are summarized, in simplified form, in the following section.

2.5.1 Theories of control of mitochondrial respiration by metabolites produced during contractions

In early studies of mitochondrial respiratory control, it was found that ADP added to a medium containing isolated mitochondria stimulated respiration (Chance & Williams, 1955). It was postulated that ADP, produced by the breakdown of ATP at the myofilaments, diffuses to the mitochondria, and enters the mitochondria through an adenine nucleotide translocase in the inner membrane, to stimulate oxidative phosphorylation (Figure 3).

A modification of the above theory is that the adenine nucleotide translocase in the inner mitochondrial membrane, which exchanges ADP for

Figure 3. Control of mitochondrial respiration by [ADP]


Myofibrillar
ATPase
ATP
ADP
Creatine Kinase
Creatine
Creatine
Phosphate
Mitochondrion
ATP
Oxidative
Phosphorylation
ADP

ATP produced by oxidative phosphorylation, is limiting to aerobic respiration (Klingenberg, 1980). In this "adenine translocase" hypothesis, the extramitochondrial [ADP]/[ATP] ratio determines the rate of the translocase reaction, and ultimately, oxidative phosphorylation.

A third hypothesis, termed the "near equilibrium hypothesis", suggests that the rate of mitochondrial respiration is dependent on the extramitochondrial [ADP][Pi]/[ATP] ratio, as well as the intramitochondrial [NADH]/[NAD^+] ratio (Wilson, 1994). During oxidative phosphorylation, ATP is produced at three different sites by the transfer of reducing equivalents down the respiratory chain, driving the subsequent phosphorylation of ADP. This hypothesis implies that the reactions of the first two sites (oxidation of NADH with conversion of ADP to ATP and transfer of reducing equivalents) are near equilibrium and that the reactions of the third site (conversion of O_2 and ADP to H_2O and ATP) are irreversible; this is the rate determining step in the entire reaction. Although the last step (phosphorylation by the cytochrome oxidase reaction) is rate limiting, the level of substrate (reducing equivalents) for this reaction is determined by the reactions at the previous two sites.

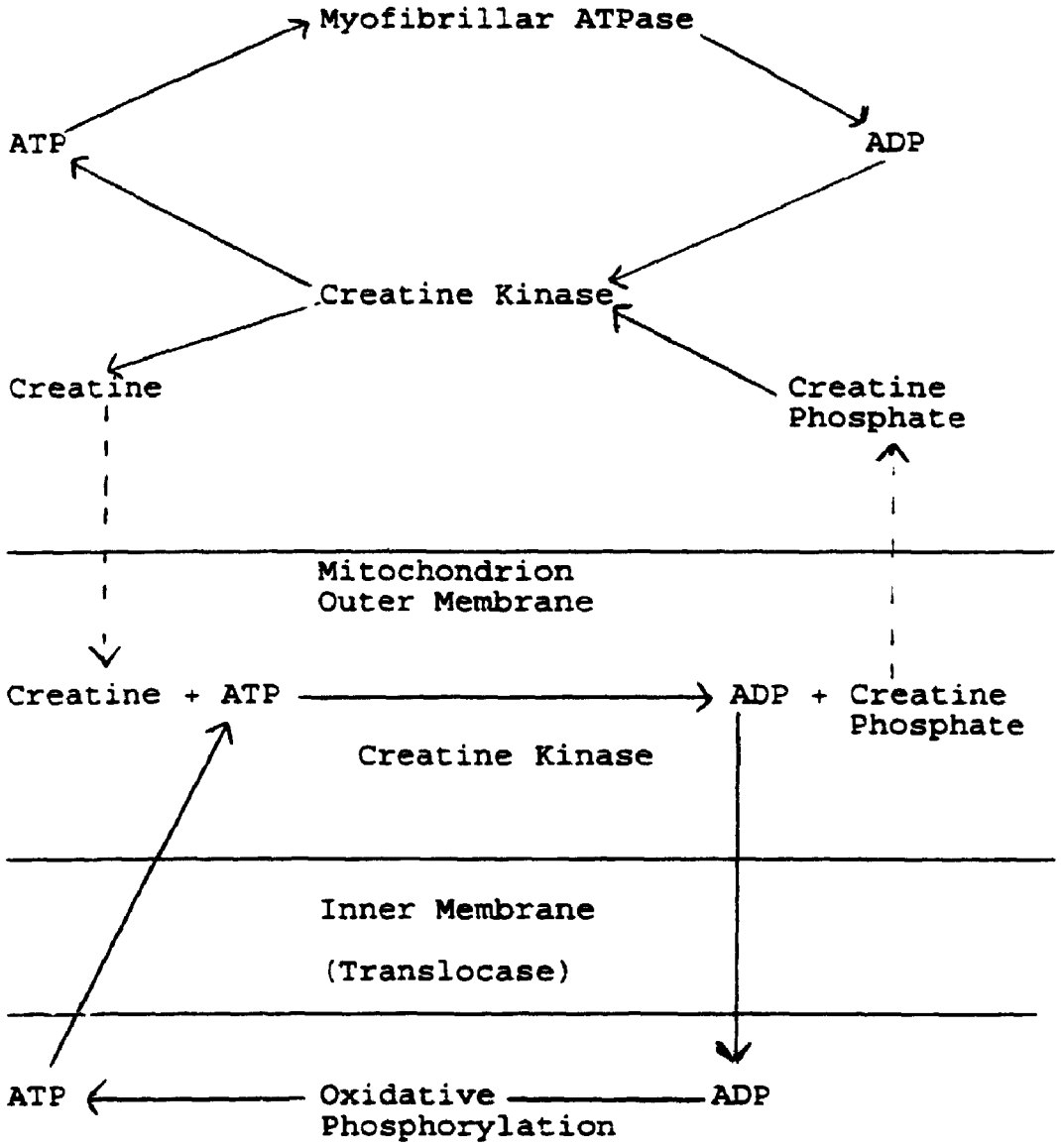
The finding that most of the ADP within a cell is bound to actin (Seraydarian et al., 1962), so that free ADP within the cytosol is very low, cast doubt on the ability of ADP to diffuse from the myofibril to the mitochondria. With the discovery of a mitochondrial creatine kinase (CK) isozyme (Jacobs et al., 1964) and the observation that respiration could be stimulated by theaddition

of Cr and ATP to a medium containing mitochondria, Bessman and Fonyo (1966) proposed what is termed the "creatine shuttle hypothesis". Here, Cr acts as a shuttle between myofibril and mitochondria to control respiration (Figure 4). With this shuttle, Cr, produced at sites of energy utilization within a cell (i.e. myofibrillar ATPase), by the creatine kinase (CK) reaction (creatine phosphate + ADP → Cr + ATP) diffuses to the mitochondria. The Cr is phosphorylated via the mitochondrial CK isozyme, by the reverse of the CK reaction (Cr + ATP → creatine phosphate + ADP), where ADP and ATP are compartmentalized and transported to and from the mitochondria by the adenine nucleotide translocase. The creatine phosphate formed is shuttled outside the mitochondria to sites of energy utilization, to re-phosphorylate ADP produced by the breakdown of ATP, thus completing the cycle.

In a modification of the above theory, Meyer et al. (1984) proposed that the creatine kinase reaction plays a role in the facilitated diffusion of ADP to mitochondria, and of ATP back to the myofibril. In this theory, the CK reaction acts as a "high-energy phosphate buffer".

Features of the above theories have been integrated through *in vivo* experiments by Connett and Honig (1989). They stimulated dog gracilis muscle at various frequencies to elicit different rates of muscle oxygen consumption and measured metabolites following quick freezing at different time intervals. They found that changes in muscle oxygen consumption could be predicted by changes in PCr, Cr and Pi or by changes in [ADP][Pi]/[ATP], but they

Figure 4. Control of mitochondrial respiration by the creatine shuttle


Myofibrillar ATPase
ATP
ADP
Creatine Kinase
Creatine
Creatine Phosphate
Mitochondrion Outer Membrane
Creatine + ATP
ADP + Creatine Phosphate
Creatine Kinase
Inner Membrane
(Translocase)
ATP
Oxidative Phosphorylation
ADP

eliminated the possibility of independent control by [ADP]. One important implication from these experiments is that Pi/PCr changes, which can be non-invasively followed by ^{31}P-nuclear magnetic resonance (^{31}P-NMR), may be assessed to gain an understanding into the control of muscle O_2 consumption during exercise transients in humans.

2.5.2 PCr kinetics and the effects of age

Two ^{31}P-NMR studies have measured the kinetics of PCr, which are thought to represent the kinetics of muscle O_2 consumption, during the recovery from exercise in old compared to young individuals (Taylor et al., 1984; McCully et al., 1993). Taylor et al. (1984) measured kinetics in muscle of the forearm, following exercise of the finger flexors, and found no difference in recovery rates for PCr between young and old individuals. In contrast, McCully et al. (1993) found significantly slower kinetics during recovery from ankle plantar flexion exercise, with τPCr averaging 57s and 31s for old and young groups, respectively. The slow τPCr of old humans is supported by two additional studies, where kinetics were measured in old groups only. Keller et al. (1985) and Hands et al. (1986) measured time constants for PCr of 53s and 48s, respectively, during recovery from ankle plantar flexion. The differences between these and the study of Taylor et al. (1984) may be due to the different muscle groups studied. In addition, Taylor et al. (1984) used a simple bulb squeezing exercise that was not graded to any individual criteria. The time

constants for PCr during recovery for old and young individuals found by McCully et al. (1993) and those for old humans found by others (Keller et al., 1985; Hands et al., 1986), are very similar to the $\tau\dot{V}O_2$ during on-transients to cycle exercise in old and young individuals (Babcock et al., 1994a), suggesting that slower regulation of oxidative phosphorylation by metabolic substrate is limiting to muscle $\dot{V}O_2$ kinetics in old humans. Whether there is an association between PCr and $\dot{V}O_2$ kinetics would require measurement during the same study, with the same exercise for both measurements.

Slower PCr kinetics along with slower $\dot{V}O_2$ kinetics in old individuals, does not however, rule out the possibility that there is limitation due to slower O_2 transport during exercise transients. Chance et al. (1986b) have shown that Pi/PCr reaches higher levels during hypoxic conditions. This implies that phosphate metabolism can be affected by O_2 delivery; therefore slower kinetics of phosphate metabolism may be due to slower O_2 delivery. Furthermore, measurement of PCr kinetics in patients suffering from peripheral vascular disease, where blood flow is restricted, reveals longer kinetics in recovery than controls (Keller et al., 1985; Hands et al., 1986). Oxygen transport limitation, therefore, may have effects on both PCr and $\dot{V}O_2$ kinetics. Evidence presented in the next section, however, suggests that in most cases, PCr kinetics are related to the rate of oxidative enzyme activity in the mitochondria.

A limited number of studies have explored the effects of training on PCr kinetics in old humans. McCully et al. (1991) had elderly subjects perform a

seven week training program, which involved the performance of toe raises, twice per day. This had no effect on improving PCr kinetics measured following plantar flexion exercise. The authors described this training program as "mild" which suggests that the training may have been of insufficient intensity to be effective. Marsh et al. (1993), however, found that mild exercise training of the forearm delayed the intracellular threshold (the breakpoint in Pi/PCr during a ramp exercise test). This suggests that changes in PCr during exercise can be effected by mild endurance training. In a cross-sectional study, McCully et al. (1994a) found that the kinetics of haemoglobin oxygen resaturation following exercise, which is related to PCr kinetics (McCully et al., 1994b), were similar in a trained group of old individuals ($\tau \sim 27$s) compared to a young group ($\tau \sim 22$s). This suggests that, like $\dot{V}O_2$ kinetics ($\tau\dot{V}O_2 = 32$s in trained old individuals) [Babcock et al., 1994b], PCr kinetics of old humans can be improved to a rate which is similar to fit young individuals.

2.5.3 Mitochondrial capacity: its relationship to PCr kinetics

That PCr kinetics are related to mitochondrial capacity is supported by three general findings:

1) The activity of the mitochondrial isozyme of creatine kinase (CK), thought by some to control the rate of adjustment of mitochondrial respiration as part of the creatine shuttle (Whipp and Mahler, 1980), is higher in aerobically trained versus sedentary individuals (Sylven et al., 1984), and is increased

following endurance training (Apple and Rogers, 1986). Endurance training results in higher levels of mitochondrial CK, as mitochondrial number and size are increased. Assuming that the creatine shuttle is in effect (Whipp and Mahler, 1980), this may result in faster PCr kinetics, as there is an increase in the surface area available for Cr interaction with CPK and shorter diffusion distances for Cr from myofibril to mitochondria (Gollnick, 1986).

2) PCr kinetics are faster in specifically trained muscle groups. Runners have faster ^{31}P-NMR measured PCr kinetics during exercise of the plantar flexors (McCully et al., 1992), femoral flexors (Yoshida and Watari, 1993), and knee extensors (Takahashi et al., 1995), while rowers have faster kinetics during wrist flexion (McCully et al., 1989). This implies that factors within the muscle, rather the central O_2 delivery, control the rate of PCr kinetics. This does not rule out the possibility, however, that peripheral O_2 delivery is enhanced in these individuals (i.e. through increased capillarization).

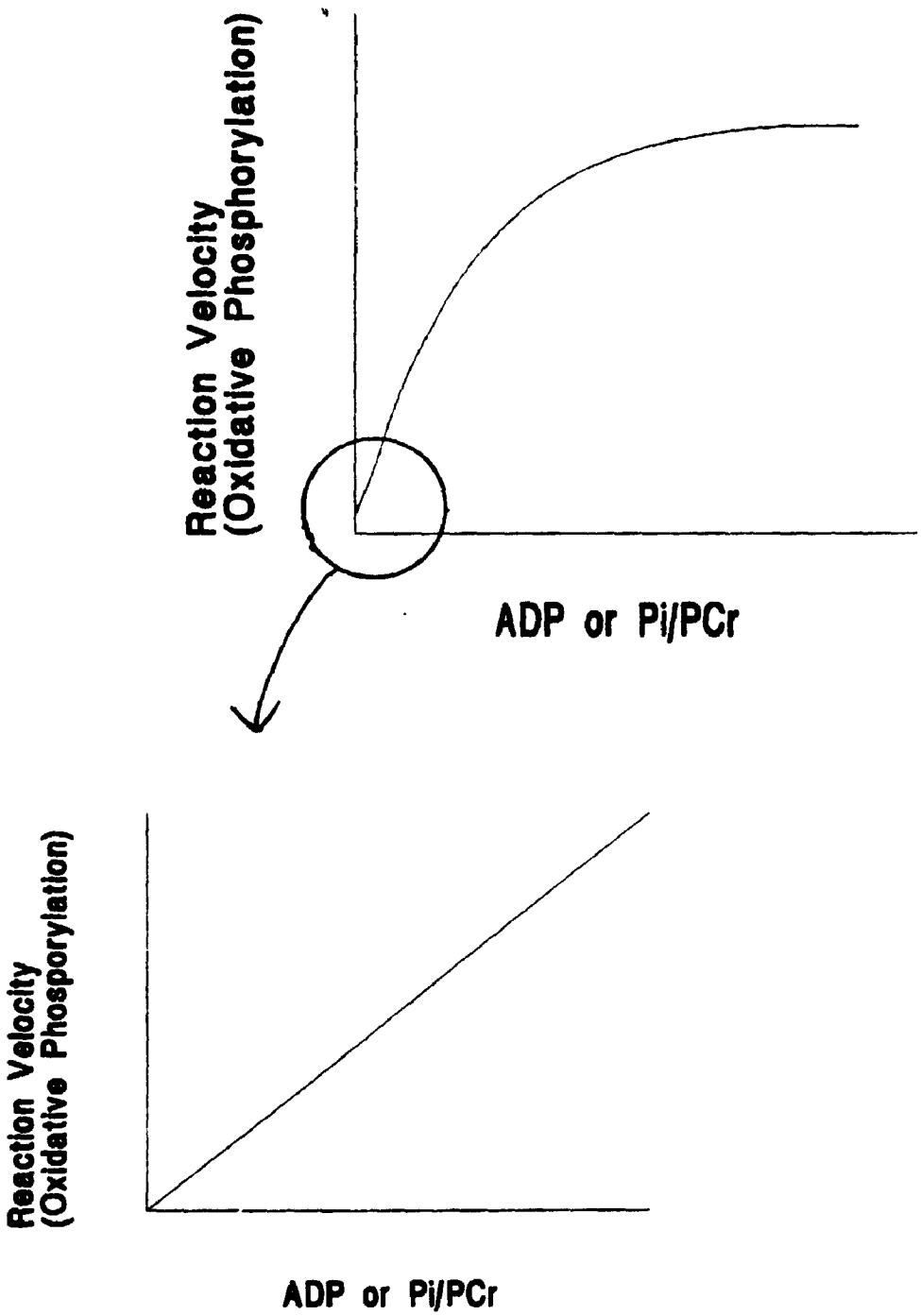
3) During chronic electrical stimulation of rabbit fast twitch muscle, the increase in mitochondrial CK has a time course and extent of change which is similar to that of other mitochondrial enzymes (Schmitt and Pette, 1985) and the rate of PCr kinetics during recovery from exercise is proportional to citrate synthase activity in both young (Jansson et al., 1990) and old (McCully et al., 1993) individuals. This implies that PCr kinetics are related to mitochondrial capacity (i.e. with greater mitochondrial size and number, there is an increased chance of Cr interacting with mitochondrial CK and there is a shorter diffusion

distance from myofibril to mitochondria for Cr).

2.5.4. First order proportionality between muscle metabolites and O_2 consumption: a model for the control of $\dot{V}O_2$ kinetics

The idea that $\dot{V}O_2$ kinetics depend on the activation of mitochondrial oxidative phosphorylation by a metabolic substrate originates with the experiments by Chance and Williams (1955), where serial additions of increasing concentrations of ADP resulted in a "Michaelis-Menten" response of respiratory activity. Michaelis-Menten kinetics are characterized by an increase in reaction velocity (i.e. of the enzymes involved in oxidative phosphorylation) when substrate (ADP) is added to the reaction, in increasing concentrations (Chance et al., 1985) [Figure 5]. At low concentrations of substrate, reaction velocity increases with increasing concentration of substrate. This portion of the substrate-reaction velocity curve is essentially linear (circled portion of Figure 5), that is, the velocity is directly proportional to the substrate concentration. This region of the curve is termed the region of "first-order" kinetics. Once substrate concentration reaches high levels, reaction velocity no longer increases (it "plateaus"), and substrate concentration is no longer limiting. Under normal situations, such as moderate intensity exercise, it is thought that cytosolic metabolic substrate (ADP or Pi/PCr) is kept at low levels, while delivery of O_2 is sufficient to keep its concentration at high levels. Thus, metabolic substrate lies on the low portion of its curve with reaction velocity, in the first-order

Figure 5. Michaelis-Menten control of oxidative phosphorylation by ADP (Pi/PCr). The circled, enlarged portion represents the region of "first order" kinetics.


Reaction Velocity
(Oxidative Phosphorylation)
ADP or Pi/PCr
Reaction Velocity
(Oxidative Phosporylation)
ADP or Pi/PCr

region (Chance et al., 1986b).

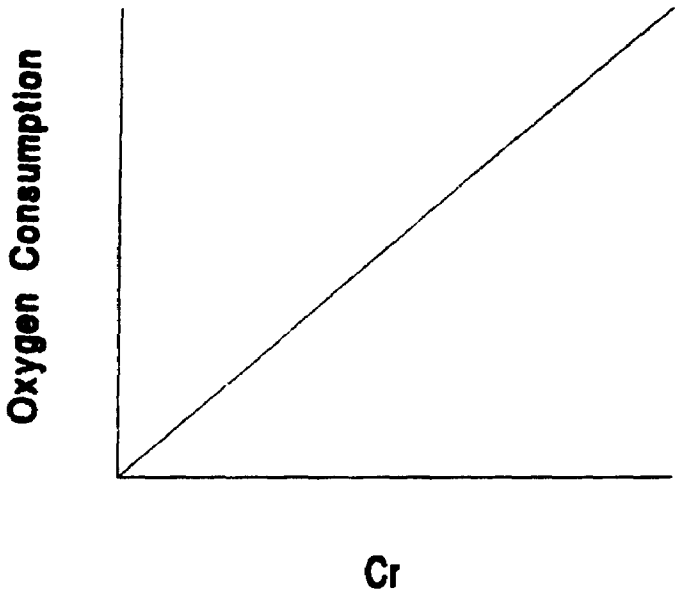
Evidence for control of mitochondrial respiration by first-order kinetics, with an equivalent proportionality between muscle oxygen consumption and PCr level, is given by Mahler (1985), who studied changes following tetanus of frog sartorius muscle. After tetanus, during which an impulse-like increase occurs in the rate of ATP hydrolysis, the rate of muscle O_2 consumption reaches a peak and then declines monoexponentially. This monoexponential decrease implies that the reactions that couple O_2 consumption to cytosolic ATP hydrolysis behave as a first order system. This concept is outlined in Figure 6. A first order system is shown in Figure 6a, where the velocity of the reactions of oxidative phosphorylation (represented by rate of O_2 consumption on the y-axis) increases linearly with substrate concentration (which is represented by Cr, from the breakdown of PCr, on the x-axis). Following an impulse increase in workrate, a certain amount of substrate (Cr) is rapidly introduced, and reaction velocity (oxygen consumption) reaches a certain level in response to the substrate. In a first order system, O_2 consumption will decrease monoexponentially with time, as substrate (Cr) is removed by oxidative rephosphorylation to PCr (Figure 6b). Looking at Figure 6b, over the first small interval of time, following the impulse, the reaction velocity (O_2 consumption) decreases rapidly, because much substrate is consumed (due to the initial rapid reaction velocity initiated by introduction of substrate). As substrate concentration (Cr) is reduced, reaction velocity is lower and substrate is used up

Figure 6

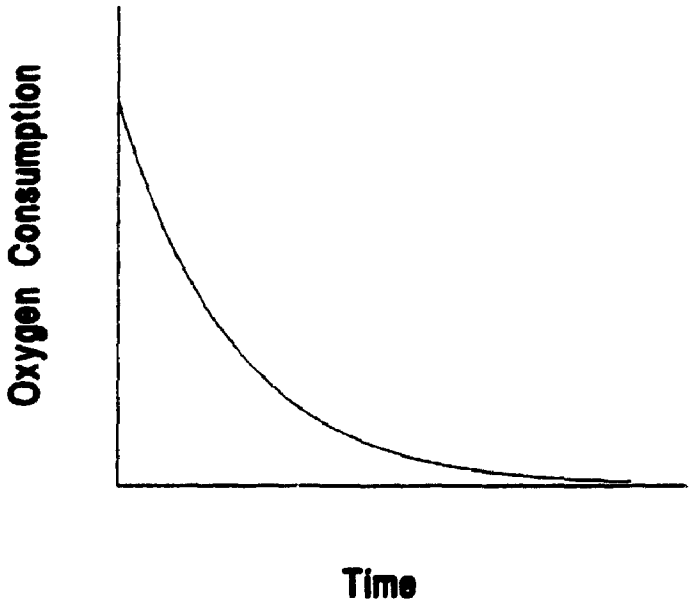
a. 1st-order kinetics of Cr vs. O_2 consumption

b. Monoexponential decline in O_2 consumption during recovery from an impulse increase in workrate

a



b.

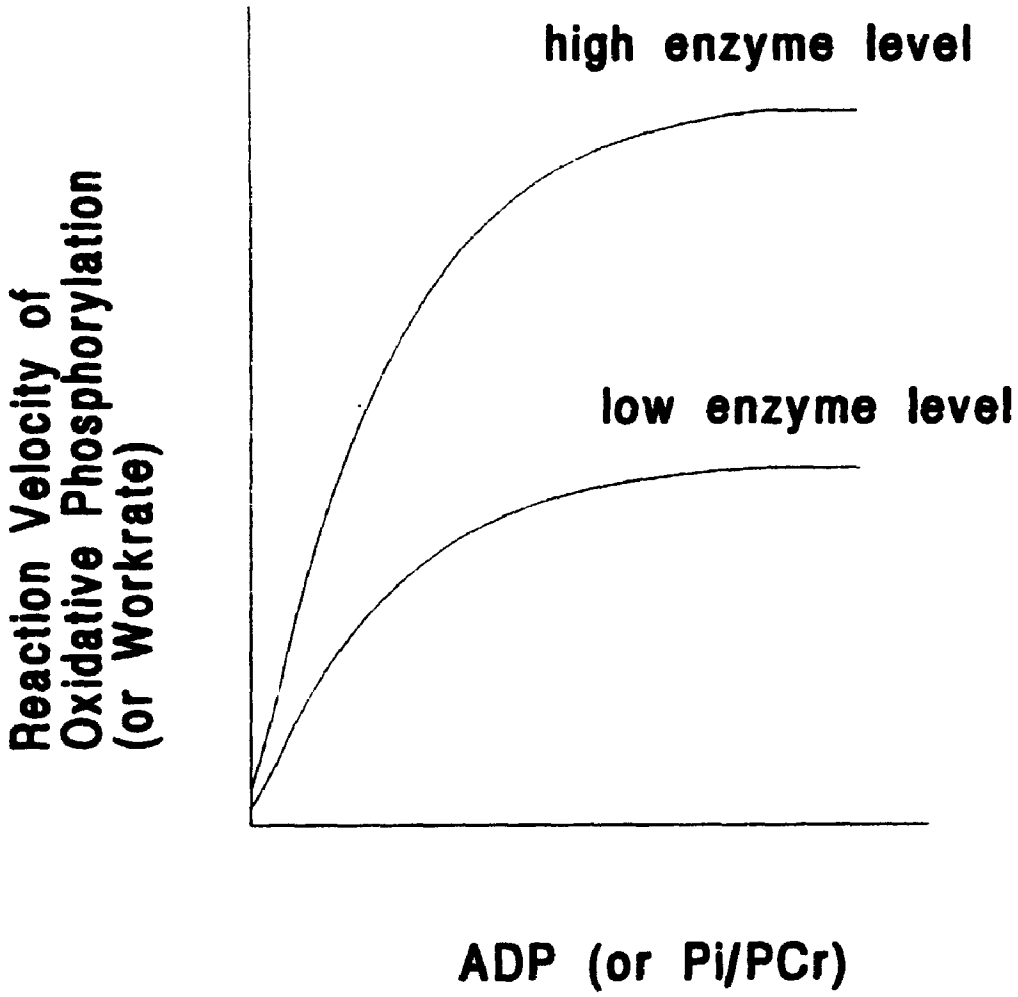


at a reduced rate, so that the decrease in reaction velocity (due to substrate reduction) is slower over the next unit of time, until a point is reached where reaction velocity (O_2 consumption) has "plateaued" or reached baseline. Using this same reasoning, a number of investigators have cited the monoexponential changes of $\dot{V}O_2$ (measured at the lung) at the onset and offset of constant intensity exercise, as evidence of control of muscle O_2 consumption by first order kinetics with a metabolic substrate (Cerretelli et al., 1980; Henry, 1951; Whipp and Mahler, 1980).

In a system with first-order behaviour, the rate of increase in muscle oxygen consumption to a steady-state, in response to a constant workrate, is determined by the level of enzyme within the mitochondria. This concept, is illustrated in Figure 7. With a lower level of enzyme, the reaction velocity (i.e. of oxidative phosphorylation) is lower at any given level of substrate concentration (Gollnick and Saltin, 1982). At the start of exercise, ATP is broken down at the myofibril, and metabolic substrate (represented by ADP for simplicity), which stimulates oxidative phosphorylation, builds up in the cytoplasm. With lower enzyme levels, ADP is used by oxidative phosphorylation at a slower rate (lower reaction velocity), resulting in a slower production of ATP. The time for aerobic production of ATP to match utilization at the myofibril will take longer, resulting in a slower attainment of steady-state O_2 consumption. This would be reflected as a slower $\dot{V}O_2$ kinetics.

The graph of Figure 7 has been modified according to work by Chance

Figure 7. Effect of enzyme level on reaction velocity


high enzyme level
low enzyme level
Reaction Velocity of Oxidative Phosphorylation (or Workrate)
ADP (or Pi/PCr)

et al. (1986a), with Pi/PCr, measured by ^{31}P-NMR, on the x-axis (instead of ADP), and workrate (which changes in proportion to reaction velocity of oxidative phosphorylation) on the y-axis. In their study, sedentary young individuals lie on the lower curve and move to the upper curve, following a training program. This implies that kinetics have been improved with training. Similar Pi/PCr versus workrate plots show that old individuals lie on a lower curve in comparison to younger subjects (Coggan et al., 1993), implying slower kinetics. Cross-sectional comparisons of old trained versus untrained individuals (Coggan et al., 1993) and longitudinal training of older individuals (Marsh et al., 1993b), indicate that with training, old individuals can move from the lower to the higher curve, again implying an improvement in kinetics. Sedentary older individuals have been shown to have lower levels of oxidative enzymes, in conjunction with their position on the Pi/PCr-workrate plot, when compared to young subjects (Coggan et al., 1993), providing *in vitro* support for *in vivo* findings. Most studies, but not all (Grimby et al., 1982), support the notion that old individuals have lower oxidative enzyme levels, compared to young individuals (Essen-Gustavsson and Borges, 1986; Trounce et al., 1989; Coggan et al., 1992b; Keh-Evans et al., 1992); this may be reflected in slower $\dot{V}O_2$ kinetics. Study of the relationship between oxidative enzyme activities and $\dot{V}O_2$ kinetics in old individuals is needed. Phillips et al. (1995) did not find a relationship between oxidative enzyme activity and $\dot{V}O_2$ kinetics in young individuals, suggesting that linear first-order control of $\dot{V}O_2$ kinetics by the

activity of mitochondrial enzyme reactions, may be an oversimplification. In a first order system with dynamic linearity, the time course of the system output must scale everywhere with input (Lamarra, 1990). It has been shown that $\dot{V}O_2$ kinetics are best described by a non-linear dynamic system, in that $\dot{V}O_2$ kinetics differ with different forcing functions (Hughson et al., 1988) or differ when preceded by prior exercise states (Hughson and Morrisey, 1982). This also suggests that control of kinetics is more complicated than models of first-order proportionality between metabolic substrate and O_2 consumption (Hughson, 1990).

With endurance training, older individuals show significant increases in level of oxidative enzymes (Suominen et al., 1977; Coggan et al., 1992a), which approach the levels seen in young individuals (Coggan et al., 1992b). When old and young subjects were trained for the same duration at similar relative workrates, old individuals showed significant improvement in muscle oxidative capacity, in comparison to young individuals, despite similar gains between old and young individuals in levels of VO_{2max} (Meredith et al., 1989). Similar results are seen when comparing masters athletes to performance-matched young individuals: the masters athletes have higher levels of oxidative enzymes, despite lower levels of VO_{2max} (Houston and Green, 1981; Coggan et al., 1990). This suggests that the major adaptation in older individuals in response to aerobic training is an elevation in level of oxidative enzymes, as opposed to improvements in central O_2 transport (which should be reflected in

higher levels of VO_{2max}). This suggests that a large part of the improvement in $\dot{V}O_2$ kinetics of old individuals with training may be due to improvements in oxidative enzyme activity (or peripheral O_2 transport, as capillarization is also elevated in masters athletes, compared to young; Houston and Green, 1981; Coggan et al., 1990). This would have to be confirmed with measures of oxidative enzymes and $\dot{V}O_2$ kinetics over a longitudinal training study.

2.6 Application of Measurement of $\dot{V}O_2$ Kinetics

The ability of an individual to perform aerobic exercise is often assessed by measuring parameters such as maximal oxygen uptake (VO_{2max}) and ventilatory or anaerobic threshold ($V_{E}T$) [Whipp et al., 1981], which are considered measures of the maximal capacity of the cardiorespiratory system ($\dot{V}O_{2max}$), and the maximal workrate that can be performed before significant activation of anaerobic glycolysis ($V_{E}T$). During everyday activities these conditions are rarely encountered, except during intense exercise or long duration continuous exercise. One is more likely to experience moderate changes in metabolic states during continual adjustments to, and recovery from, tasks of daily living (Whipp and Ward, 1990). The rate of metabolic adjustment to and recovery from moderately intense exercise may therefore be a more appropriate measure of an individual's ability to cope with daily tasks.

In the present thesis, an attempt is made to determine the influences of central O_2 transport (estimated by HR kinetics), capacity for peripheral O_2 transport (estimated by measures of capillarization), and rate of mitochondrial O_2 utilization (estimated from citrate synthase activity level) on $\dot{V}O_2$ kinetics in old humans. The influence of training on offsetting any negative effects of ageing on these variables is studied by using muscle groups accustomed to daily activity (plantar flexors) and comparing old and young individuals of varying fitness levels.

Chapter 3

The Effects of Age on $\dot{V}O_2$ and PCr Kinetics During Exercise

3.1 Abstract

The purpose of this study was to compare kinetics of oxygen uptake ($\dot{V}O_2$) and phosphocreatine (PCr) during the adjustment to and recovery from moderate intensity plantar flexion exercise in groups of moderately active old (n=10, 66.9y) and young (n=10, 27.5y) individuals. Phase 2 $\dot{V}O_2$ kinetics, measured breath-by-breath, were similar in the two groups, with time constants (τ) averaging 46.3, 38.1, and 46.3, 40.7s for on- and off- transients to exercise in young and old groups, respectively. These were similar to corresponding PCr kinetics, measured by ^{31}P-nuclear magnetic resonance spectroscopy, which averaged 50.6, 42.0, 39.8, and 37.6s. $\tau\dot{V}O_2$-on correlated with τPC n, for combined groups (r=0.53; P=0.015). Citrate synthase activity, measured from biopsies of the lateral gastrocnemius, was similar in both groups, but not correlated to any of the kinetic measurements. It is concluded that: 1) $\dot{V}O_2$ and PCr kinetics during exercise of a muscle group accustomed to daily activity are not compromised in old humans 2) Citrate synthase activity, as a marker for mitochondrial oxidative capacity, is not critical in the control of oxidative kinetics 3) PCr kinetics reflect kinetics of muscle O_2 consumption and are expressed at the lung ($\dot{V}O_2$ kinetics) after a transit delay.

3.2 Introduction

Kinetics of phosphocreatine (PCr) breakdown at the start of exercise may play a role in the control of mitochondrial respiration, either directly (Bessman & Fonyo, 1966) or through proportional changes with other cytosolic modulators (Connett & Honig, 1989). PCr kinetics are thought to reflect the kinetics of muscle oxygen consumption, and following a transport delay are expressed at the lung as $\dot{V}O_2$ kinetics (Whipp & Mahler, 1980). Few investigators have measured PCr kinetics during the transition to exercise in humans (Binzoni et al., 1992; Marsh et al., 1993; Yoshida & Watari, 1993), and none have studied the effects of ageing on these kinetics. McCully et al. (1993) have shown that PCr kinetics during the recovery from plantar flexion exercise are longer in old compared to young individuals. These values are similar to the longer kinetics for $\dot{V}O_2$ adjustment to cycling exercise observed in old individuals (Cunningham et al., 1993; Babcock et al., 1994a). Slower PCr kinetics may be due to a reduced mitochondrial capacity in old individuals, as demonstrated by the significant correlation between lower levels of citrate synthase (CS) activity and longer PCr recovery (McCully et al., 1993). Lower levels of CS reflect a loss of mitochondrial content and a loss of sensitivity to cytosolic modulators of mitochondrial respiration (Dudley et al., 1987). Others have failed to find slower PCr recovery kinetics with ageing (Taylor et al., 1984). Also, $\dot{V}O_2$ kinetics (Babcock et al., 1994b; Chilibeck et al., 1996) and mitochondrial enzyme activities (Meredith et al., 1989; Coggan et al., 1992a) in

moderately active old individuals are not different from young individuals. This suggests that slower kinetics and a reduced mitochondrial capacity are the result of sedentary lifestyles of old humans, rather than the aging process per se. The primary purpose was to measure the kinetics of PCr and $\dot{V}O_2$ during the transition to exercise, as well as oxidative enzyme activity, for comparison of moderately active old to young individuals.

The second purpose was to observe if $\dot{V}O_2$ and PCr kinetics, measured during the same exercise protocol, were correlated, as suggested from animal models (Piiper et al., 1968; Marconi et al., 1982) and computer simulations (Barstow et al., 1990; Cochrane & Hughson, 1992). It has previously been shown that the time constants (τ) for PCr and $\dot{V}O_2$ kinetics during plantar flexion exercise are similar, but a correlation between the two variables was not found, with a small number of young subjects (McCreary et al., 1996).

3.3 Methods

Ten young (4 females, and 6 males, aged 27.5±2.0y, 77.0±16.2 kg) and 10 old (8 females and 2 males, aged 66.9±3.7y, 67.6±11.5 kg) subjects participated in this study, which was approved by the University of Western Ontario Review Board for Research Involving Human Subjects. Lifestyles of subjects ranged from sedentary to moderately active. Most older subjects reported walking as their main form of exercise. These individuals were recruited from activity classes, where group walking was performed two to

three times per week for approximately 30 minutes per day. Subjects had been participating in this class for an average of 18 months (with a range of 9 to 35 months).

Subjects performed a ramp test on a cycle ergometer, for determination of peak $\dot{V}O_2$. The methods are described in detail elsewhere (Chilibeck et al., 1996).

Subjects performed a series of plantar flexion exercise tests, which included: 1) a progressive ramp test, for determination of peak work rate and work rate at the intracellular threshold (IT). This threshold corresponds to the point at which there is a transition from moderate change in Pi/PCr and pH to a rapid change in both variables, and is thought to correspond to the work rate at which there is an increased contribution from anaerobic metabolism (Marsh et al., 1991). 2) Constant load square wave exercise tests, with work rates corresponding to 80% of IT, during which changes in PCr were monitored by ^{31}P-Nuclear Magnetic Resonance Spectroscopy (^{31}P-MRS), or changes in $\dot{V}O_2$ were monitored, breath-by-breath. Tests with ^{31}P-MRS and $\dot{V}O_2$ measures were performed on separate days. All tests were completed on a custom built ergometer, and consisted of unilateral ankle plantar flexion, during which the subject, in a supine position, pushed against a foot pedal to lift a load via a pulley system at a frequency of 0.5 Hz . This involved one plantar flexion contraction, and then passive movement of the foot through dorsi flexion to return the load to the starting position, over 2 seconds). Mechanical stops were

placed on the pulley to define the distance through which the load was lifted from 5° of dorsi flexion to 35° of plantar flexion. A metronome was used to assist the subject in maintaining the proper cadence. The subject's leg was stabilized by velcro straps with the knee slightly flexed (less than 10° of flexion).

3.3.1 ^{31}P-MRS

^{31}P spectra collected during both the progressive resistance (ramp) and constant load (square wave) protocols were acquired at 1.5 T with a Siemans Helicon imaging and spectroscopy system and a dual tuned (^{31}P and ^{1}H) surface coil. Placement of the surface coil over the belly of the lateral gastrocnemius was guided by transverse relaxation (T_2) weighted images obtained previously (McCreary et al., 1996). These images, along with surface electromyographic measurements indicated that the lateral gastrocnemius was the predominant muscle used during the plantar flexion protocol (McCreary et al., 1996). The magnetic field homogeneity was adjusted until the proton signal from water produced a peak with a full width at half maximum of 20-30 Hz (0.3-0.4 ppm) and was approximately Lorentzian in shape.

Phosphorous spectra were acquired at rest, throughout exercise and during recovery. Time averaged free induction decays (FIDs) with 2048 complex data points were collected with a dwell time of 125 μs, resulting in a spectral width of $\pm$4000 Hz. A nominal 46° pulse was used with repetition time of 1000 ms. Time averaged FIDs were left shifted 3 points, to remove broad

components from the spectra, and a 5 Hz exponential filter applied. Peak areas and positions were calculated from the 1024 points in the FID using an iterative, non-linear least-squares routine based on the VARPRO algorithm (Van Der Veen et al., 1988), which fit Lorentzian line shapes to each peak. No correction for partial saturation was made since this was a serial study determining the kinetic time constants from relative changes in PCr and Pi and absolute quantities were not necessary. It was assumed that T_1 relaxation of PCr and Pi remains constant throughout exercise. Intracellular pH was calculated to within $\pm$0.05 pH units from the chemical shift between Pi and PCr peaks (Taylor et al., 1983).

The peak areas and positions determined for each spectrum were assumed to represent metabolite and pH data at the midpoint of the acquisition time for the spectrum. This assumption is true during steady state conditions, however, during exercise transients, the metabolite levels change in an exponential manner. Thus, the linear assumption is not correct under non-steady state conditions. Marsh and co-workers (1993) have addressed this problem previously. They found that, in the worst case, a linear approximation over 32s introduced a 10% error in the time position. This small error had negligible effects on the determination of response time constants. Since the linear approximation in this experiment is over a shorter period (20s or 6s), the error in the time position was likely less than 10%.

3.3.2 Progressive Ramp Exercise Test

During the progressive (ramp) supine plantar flexion exercise test, ^{31}P spectra were acquired every 10s (10 FIDs per spectrum) during a 2 minute warm-up period with 0 load, and throughout the progressive test. The load was continuously increased at a rate of 1.08 $kg \cdot min^{-1}$ by pumping water into a container with a peristaltic pump until the subject reached volitional fatigue or could no longer maintain cadence or a full range of motion.

To identify the onset of intracellular acidosis during the ramp exercise, pH was plotted as a function of work rate. Piecewise linear regression was used to fit the data and determine the power output at which the slope of the best fit line changed significantly. This point was referred to as the intracellular threshold (Marsh et al., 1991).

3.3.3 Determination of PCr Kinetics

PCr kinetics were determined from constant load (square wave), moderate intensity supine plantar flexion tests, which were five minutes in duration. Work rates during these tests corresponded to the work rate at ~80% of subjects' IT (determined from the ramp test) and averaged 3.7 ± 1.5 and 1.7 ± 0.5 watts for young and old groups, respectively. Since the young group had a higher IT, relative to peak work rate (~70% of peak work rate), compared to the old group (~60% of peak work rate), square wave work rates, relative to peak power, were ~55% of peak power for the young group, and ~46% of peak power for the old group. Square wave tests were repeated three

times, separated by seven minutes of loadless plantar flexion. Phosphorous spectra were acquired throughout these tests. Five FIDs were time averaged for the spectra collected during the square wave protocol for an acquisition time of 6s per spectrum. The data buffer was written to the hard disk after each off-transient and took approximately 2 minutes.

PCr data were normalized to the total ^{31}P signal (the sum of PCr, Pi, ATP, phosphomonoester and phosphodiester peak areas) and plotted as a function of time for each phase. PCr responses to the three repeated square waves were time aligned for improvement in the signal to noise ratio. Lamarra et al. (1987) have demonstrated that averaging of physiological data from repeated tests allows for a more precise characterization of non-steady state kinetics. Averaged PCr responses were fit with a simple, three parameter exponential growth or decay model:

$$PCr(t) = PCr_0 e^{(-t/\tau)} + PCr_{ss} \qquad (a)$$

$$PCr(t) = PCr_{ss}\{1-e^{(-t/\tau)}\} + PCr_0 \qquad (b)$$

where PCr_0 is the value of PCr at t=0, PCr_{ss} is the steady state value of PCr, and τ is the ensemble response time constant. Individual on- and off-transients were fit with the same functions to determine the PCr_0 values. The relative PCr levels at time zero were assigned the steady state value of the previous phase of the test. Linear regression analysis was applied to the data collected during the first loadless exercise to determine the PCr level at time t=0, for the first on-transient.

3.3.4 $\dot{V}O_2$ Kinetics

The supine plantar flexion square wave tests were repeated, on separate days, for measurement of $\dot{V}O_2$ transients, with work rates identical to those used in the ^{31}P-MRS test. On-transients were five minutes in duration, separated by six minutes of loadless plantar flexion. Transients were from loadless exercise, as opposed to rest, to minimize the phase 1 portion of the $\dot{V}O_2$ response (Whipp et al., 1982). Subjects were blinded to changes in load to minimize anticipatory responses.

Measurement of $\dot{V}O_2$ was performed using a modification of the methods of Babcock et al. (1994a). Inspired and expired gas flows were measured using a low dead space (90 mL) bi-directional turbine (Alpha Technologies, VMM 110) calibrated by a 3.01 L syringe and gas concentrations were measured by a mass spectrometer (Airspec 2000 MGR 9N) calibrated 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. Data collected every 20 ms were converted from analog to digital format and stored for later processing by a microcomputer. Breath-by-breath alveolar gas exchange data were calculated using the algorithms of Beaver et al. (1981).

Subjects performed 12 square wave repeats over four to five sessions. Breath-by-breath data from the repeats were interpolated to 1 second, time

aligned and averaged to improve the signal to noise ratio. The average $\dot{V}O_2$ responses for each subject were fit with a 3 parameter, first order (monoexponential) model, starting at the phase 2 portion of the response (20s), for both transition to and recovery from exercise. The calculation was performed as an iterative process by micro-computer to find the best fit using the following equation:

$$\dot{V}O_2(t) = \dot{V}O_{2_{ss}}\{1-e^{-[(t-\delta)/\tau]}\} + \dot{V}O_2^0,$$

where t represents any time; $\dot{V}O_{2_{ss}}$ is the steady state gain in $\dot{V}O_2$, $\dot{V}O_2^0$ is the value of $\dot{V}O_2$ at t=0, and τ and δ are the time constant and time delay of the response, respectively. In this model, the first 20s of data are excluded, as this is thought to correspond to the transit delay for gases from muscle to lung following the start of exercise (Whipp et al., 1982). Although phase I is variable for individual subjects, data were fit from 20s for all subjects, as the small amplitude of the $\dot{V}O_2$ response and the use of loadless exercise prior to increase in workrate make it difficult to determine the exact time at which phase 2 begins (Whipp et al., 1982; McCreary et al., 1996).

3.3.5 Citrate Synthase Activity

Needle biopsies were obtained from the lateral head of the gastrocnemius of seven young and seven old subjects, who volunteered for this portion of the study. Muscle samples were frozen in liquid N_2 and stored at -80°C. Maximal activity of Citrate Synthase (CS) was determined according to a modification of the method of Srere (1969). Although CS is not the most appropriate enzyme

for estimation of capacity for oxidative phosphorylation, it changes in proportion to mitochondrial content and those enzymes directly involved in control of oxidation phosphorylation (i.e. cytochrome oxidase) [Dudley et al., 1987]. Briefly, muscle samples were weighed and homogenized in 20 vol of 600 mM NaCl, 15 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl; pH 7.5) and analyzed at 30°C by spectrophotometry (Philips Pye Unicam, PU 8800). Protein concentration was determined by the method of Lowry et al. (1951). All enzyme assays were performed at the same time to minimize interassay variability.

3.3.6 Statistics

Comparisons between PCr and $\dot{V}O_2$ kinetics, on- and off- transients, and these values for old and young groups, were made with a 3 factor analysis of variance (ANOVA), with age as a between group factor, and τPCr versus $\tau\dot{V}O_2$ and transient (on- versus off-) as within subjects factors. Using the methods of Lamarra et al. (1987), the 95% confidence intervals for estimates of τPCr and $\tau\dot{V}O_2$ were calculated, based on the number of square wave repeats, the response amplitudes and standard deviations of steady state response fluctuations. Comparisons between young and old groups for CS activity were made using a 1-factor ANOVA. Pearson-product correlations were performed to test for relationships between CS activity, PCr and $\dot{V}O_2$ kinetics. The reduced major axis model of Anderson et al. (1986) was used to adjust the slope of regression lines, for significant correlations, since both x and y variables are

estimated with error. Significance for each test was set at $P < 0.05$. All results are expressed as means$\pm$SD.

3.4 Results

Peak $\dot{V}O_2$ from cycle ramp tests was more than twice as high in the young as compared to the old group (43.6$\pm$8.1 versus 20.5$\pm$2.6 mL·kg^{-1}·min^{-1}; $P < 0.01$).

Resting intramuscular pH of the lateral gastrocnemius muscle group was similar in young (7.06$\pm$0.04) and old (7.05$\pm$0.02) groups, as was resting Pi/PCr (0.20$\pm$0.05 vs. 0.21$\pm$0.06, for young and old groups, respectively).

Transients for PCr breakdown and $\dot{V}O_2$ adjustment at the start of exercise and for PCr resynthesis and $\dot{V}O_2$ recovery following exercise are shown in Figure 8, for a young subject and Figure 9 for an older subject. The Pi/PCr ratio during steady state was not different between young (0.82$\pm$0.35) and old (0.57$\pm$0.27) groups. The slightly higher mean for the young group is expected from their higher absolute work rates (McCully et al., 1993). Relative depletion of PCr by the end of exercise was not different between young (29.8$\pm$11.7%) and old (22.6$\pm$10.3%) groups, nor was end exercise pH (6.90$\pm$0.08 versus 6.96$\pm$0.09 for young and old groups, respectively). This indicates that relative work rates were equally stressful in both groups. There were no significant differences for τPCr and $\tau\dot{V}O_2$ during adjustment to and recovery from exercise, between young and old groups (Table 1). Using the equations developed by Lamarra et al. (1987), the 95% confidence intervals for

$\tau\dot{V}O_2$ and τPCr were $\pm$8.9s and $\pm$3.4s for the young group and $\pm$10.1s and $\pm$5.0s for the old group. This is based on $\dot{V}O_2$ steady state gains of 0.108 and 0.083 L·min^{-1} for young and old groups, respectively, with a standard deviation for breath-by-breath fluctuation of 0.070 and 0.061 L·min^{-1} for young and old groups. The steady state gain for PCr breakdown during exercise was 11.7 and 9.3 (arbitrary units) for young and old groups, respectively, with a standard deviation for steady state PCr measures of 1.47 and 1.69, for young and old groups.

Young and old groups had similar maximal activities for CS (33.7$\pm$13.7 versus 33.9$\pm$16.1 mmol·g protein^{-1}·min^{-1}, for young and old groups respectively), indicating that the old group had maintained their mitochondrial oxidative capacity. Correlations between CS activity and PCr or $\dot{V}O_2$ kinetics, for combined groups (n=14), did not reach significance (r = -0.33 to 0.22). The highest correlation for either group was CS versus $\tau\dot{V}O_2$ for the adjustment to exercise in the young group (r = -0.65; P=0.11).

Time constants for PCr versus $\dot{V}O_2$ were not different for individual nor combined groups, nor for on- versus off- transients (Table 1). For combined groups, τPCr and $\tau\dot{V}O_2$ during the same exercise transient were almost identical (Table 1), and were correlated for the on- transient (Figure 10a), but not the off- transient (Figure

Figure 8. Averaged $\dot{V}O_2$ breath-by-breath response for 12 transitions a) to and b) recovery from moderate intensity plantar flexion for a young individual and averaged PCr changes for 3 transitions c) to and d) recovery from the same exercise task in the same individual. Best fit monoexponential curves are shown; $\tau\dot{V}O_2$-on and -off and τPCr-on and -off were 37.1, 38.2, 41.7, and 26.8s, respectively, for this individual.

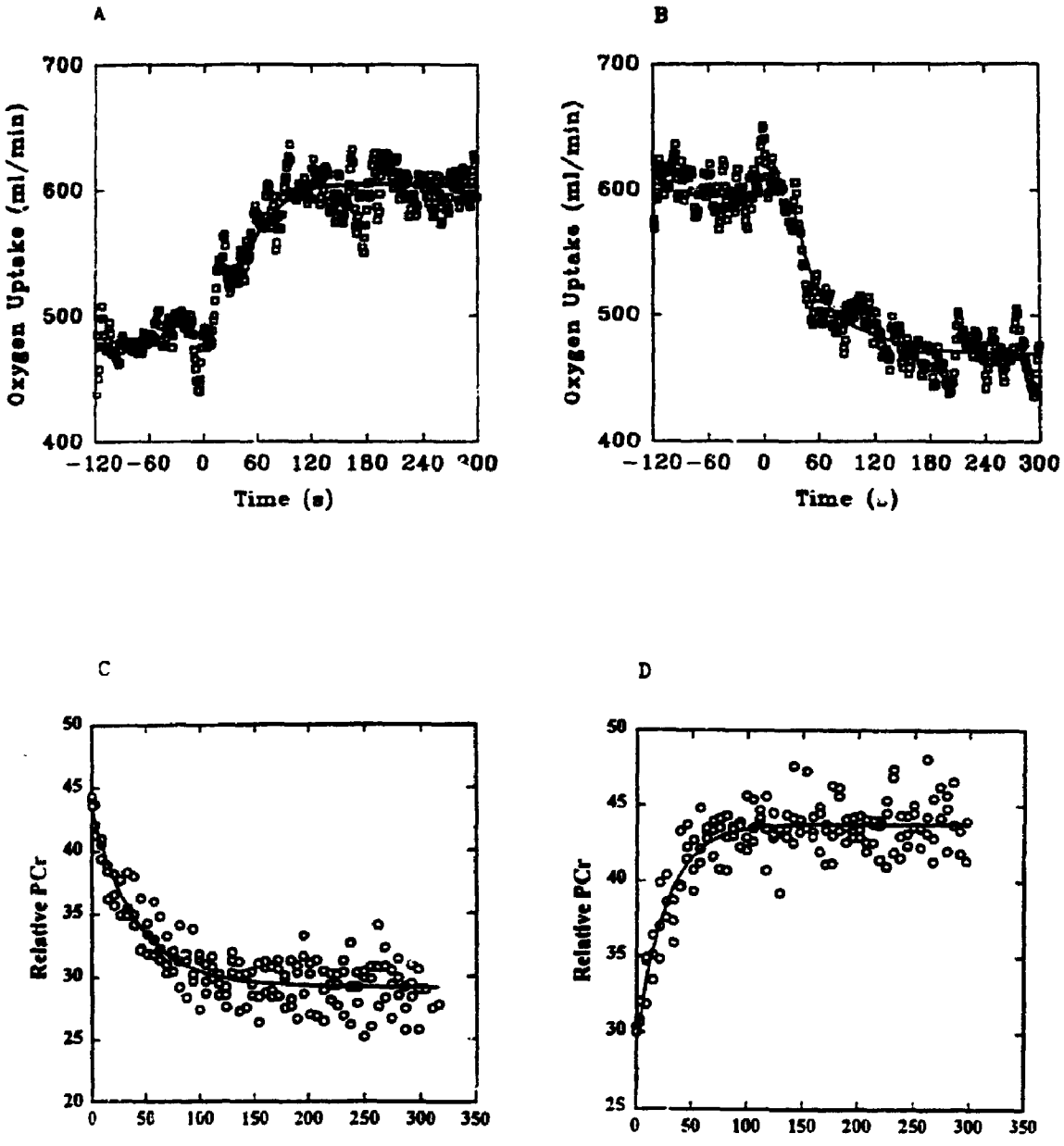

A
B
C
D
Oxygen Uptake (ml/min)
Time (s)
Relative PCr
700
600
500
400
-120
-60
0
60
120
180
240
300
50
45
40
35
30
25
20
100
150
200
250
350

Figure 9. Averaged $\dot{V}O_2$ and PCr responses, as in Figure 8, for an older individual. a) $\tau\dot{V}O_2$-on = 42.7s b) $\tau\dot{V}O_2$-off = 29.3s c) τPCr-on = 34.6s d) τPCr-off = 26.5s

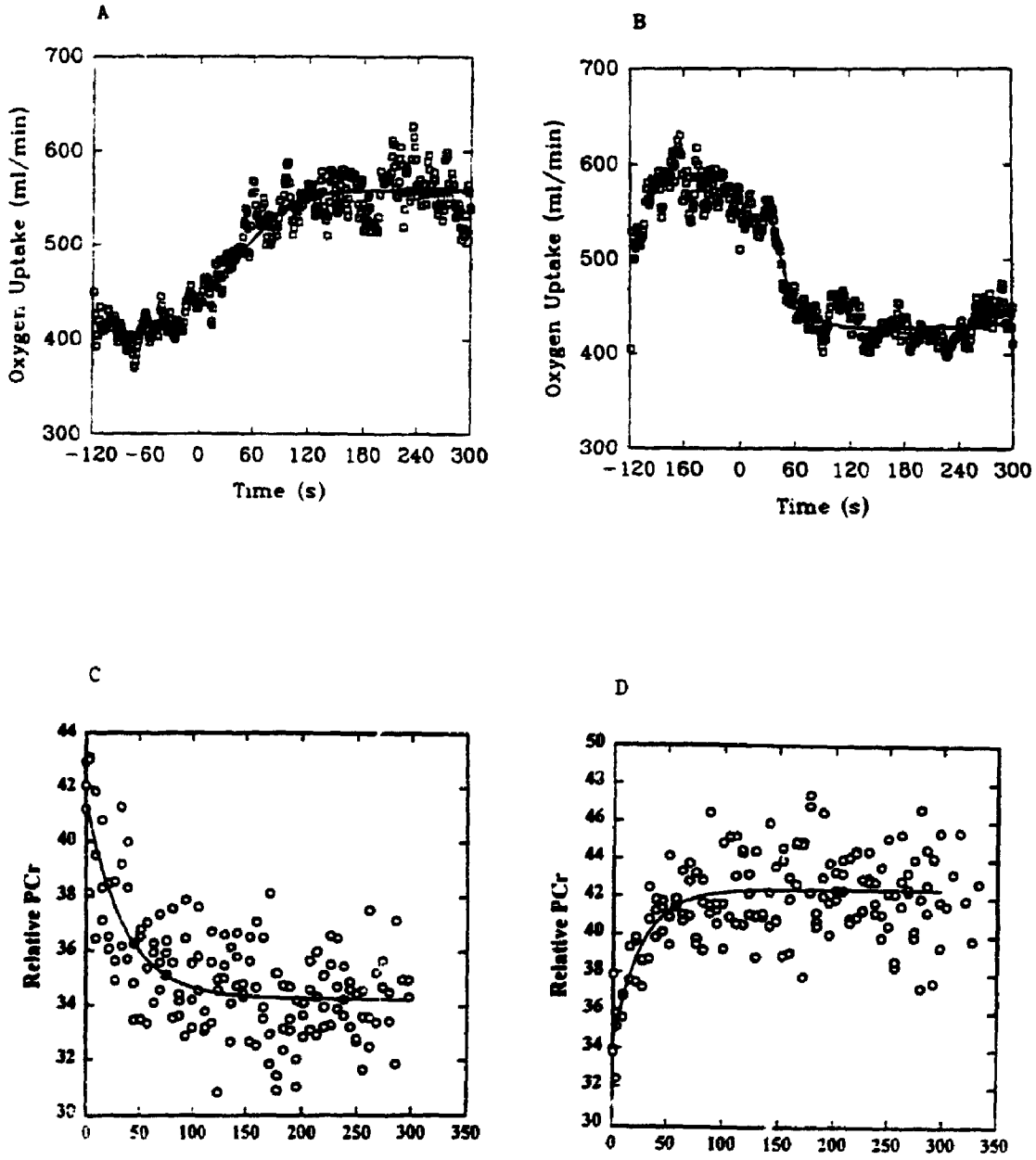

A
Oxygen Uptake (ml/min)
700
600
500
400
300
−120 −60 0 60 120 180 240 300
Time (s)
B
Oxygen Uptake (ml/min)
700
600
500
400
300
−120 160 0 60 120 180 240 300
Time (s)
C
Relative PCr
44
42
40
38
36
34
32
30
0 50 100 150 200 250 300 350
D
Relative PCr
50
48
46
44
42
40
38
36
34
32
30
0 50 100 150 200 250 300 350

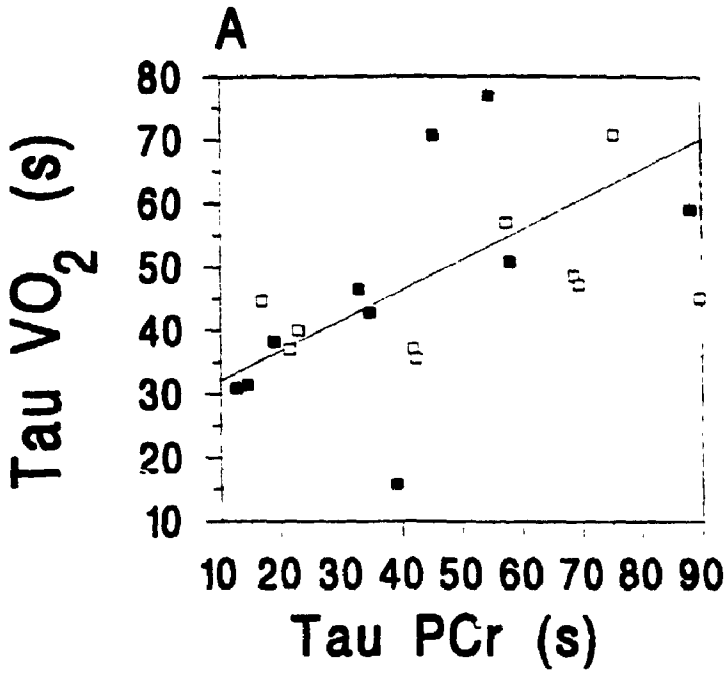
Table 1. Kinetics of $\dot{V}O_2$ and PCr in old and young subjects

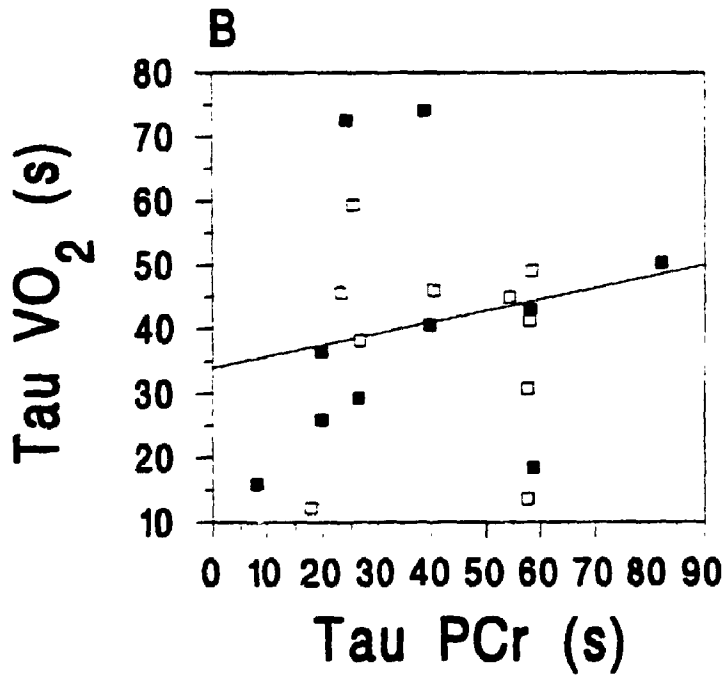
	On-Transition		Off-Transition	
	$\tau\dot{V}O_2$ (s)	τPCr (s)	$\tau\dot{V}O_2$ (s)	τPCr (s)
Young (n=10)	46.3±10.2	50.6±24.0	38.1±14.4	42.0±16.1
Old (n=10)	46.3±17.8	39.8±22.0	40.7±19.2	37.6±21.6
Groups Combined (n=20)	46.3±14.5	45.2±23.6	39.4±17.1	39.8±19.2

All values are means±SD

Figure 10. a) Correlation between τPCr and $\tau\dot{V}O_2$ during the adjustment to exercise: $\tau\dot{V}O_2$ = 0.33 (τPCr) + 31.5; r = 0.53; (P = 0.015). The slope of this regression, corrected according to the reduced major axis model of Anderson et al. (1986) = 0.62. Data points are individual subjects, where open squares = young subjects and closed squares = old subjects.

b) Correlation between τPCr and $\tau\dot{V}O_2$ during recovery from exercise (r = 0.11; NS).


A
Tau VO_2 (s)
80
70
60
50
40
30
20
10
10 20 30 40 50 60 70 80 90
Tau PCr (s)


B
Tau VO_2 (s)
80
70
60
50
40
30
20
10
0 10 20 30 40 50 60 70 80 90
Tau PCr (s)

3.5 Discussion

Peak $\dot{V}O_2$ for old and young subjects indicated that both groups were of "average" fitness levels for their age (Paterson, 1992). However, both groups were considered to be moderately active, as the old group participated in walking exercise on a regular basis, and the young group was comprised of kinesiology students.

The results indicate that $\dot{V}O_2$ and PCr kinetics, measured during exercise of a muscle group (plantar flexors) used during everyday activity, are not slower in old compared to young individuals (Table 1). The results for PCr kinetics are in contrast to those of McCully et al. (1993), who found slower rates of PCr recovery following plantar flexion exercise in a group of old versus young individuals. The slow PCr kinetics in older adults is supported by studies of Keller et al. (1985: τPCr = 53s) and Hands et al. (1986; τPCr = 48s). The differing results between the present study and that of McCully's may be due to the fitness levels of old and young groups. Despite McCully's old subjects having a slightly higher absolute $\dot{V}O_2$peak, they were of lower relative fitness level compared to the old subjects of the present study ($\dot{V}O_2$ peak= 17.9 vs. 20.5 $ml \cdot kg^{-1} \cdot min^{-1}$) and their young group was of slightly higher relative fitness level, compared to the young group of the present study ($\dot{V}O_2$ peak = 49.5 vs. 43.6 $ml \cdot kg^{-1} \cdot min^{-1}$). In a later study, McCully et al. (1994a) found that the rate of haemoglobin resaturation following plantar flexion exercise, as measured by near infrared spectroscopy, was similar in a group of moderately active older

subjects (enrollees in an exercise program for older adults) compared to a young group. Haemoglobin resaturation kinetics have been shown to reflect PCr recovery kinetics (McCully et al., 1994b), suggesting that this moderately active older population had fast PCr kinetics, similar to the group in the present study.

Participation in walking exercise by the old individuals may have been enough to offset any declines in peripheral muscular function; this is evident in their similar levels of CS activity when compared to the young group. Others have shown that peripheral muscular function of old individuals can be improved with moderate training to levels similar to those found in young individuals. Coggan et al. (1992a) have shown that oxidative enzyme activities and muscle capillarization can be increased to levels comparable to young individuals (Coggan et al., 1992b) with a program of moderate walking and jogging exercise. Oxidative capacity of skeletal muscle, determined in vitro, has been shown to be elevated in old to levels similar to those found in young individuals, after each has undergone similar aerobic training programs (Meredith et al., 1989). In both of the above training studies (Meredith et al., 1989; Coggan et al., 1992a), older individuals still had levels for $\dot{V}O_2$ peak which were lower than young individuals. This implies that old humans retain the capacity for peripheral muscle adaptation despite a decline in the capacity for improving central cardiovascular function with training. This is supported by the finding that the older individuals had similar kinetics for $\dot{V}O_2$ and PCr during plantar flexion exercise (Table 1), but slower $\dot{V}O_2$ kinetics during cycling

compared to young individuals as found in a previous study (Chilibeck et al., 1995). Cycling exercise places a greater stress on the cardiovascular system and may be limited by central factors (i.e. kinetics of cardiac output), rather than peripheral factors, as is exercise of a smaller muscle mass such as the plantar flexors (Hughson, 1990).

It was hypothesized that the rate of PCr kinetics would be related to mitochondrial capacity (estimated by activity of CS) as was found by McCully et al. (1993) for their older group and by others in young groups (Jansson et al., 1990). Higher mitochondrial number and size, as produced by endurance training, results in higher levels of the mitochondrial isoform of creatine phosphokinase (CPK) (Apple & Rogers 1986; Schmitt & Pette, 1985). Mitochondrial CPK is thought to play an important role in the control of respiration, as Cr acts as a high energy phosphate shuttle from myofibril to CPK at the mitochondria (Bessman & Fonyo, 1966; Whipp & Mahler, 1980). An increase in mitochondrial size and number may result in faster PCr kinetics, as there is an increase in the surface area available for Cr interaction with CPK and shorter diffusion distances for Cr from myofibril to mitochondria (Gollnick, 1986). However, a correlation between our estimate of mitochondrial capacity (citrate synthase activity) and PCr or $\dot{V}O_2$ kinetics was not found during either the transition to or recovery from exercise. This is similar to the findings of Phillips et al. (1995) and Green et al. (1995), who found faster $\dot{V}O_2$ kinetics and smaller depletion of PCr early in exercise, following short-term endurance

training, where there was an absence of change in mitochondrial capacity, as measured by CS activity. Other factors, besides mitochondrial capacity, may therefore control PCr and $\dot{V}O_2$ kinetics during exercise. These may include muscle fibre recruitment patterns or blood flow kinetics, which would affect the rate of oxygen delivery to exercising tissue. The latter theory is supported by the finding that individuals suffering from peripheral vascular disease have prolonged PCr kinetics during recovery (Keller et al., 1985), and that the rate of PCr recovery following muscle stimulation of perfused rat hindlimb is dependent on oxygen delivery through the perfusate (Idstrom et al., 1985). Thus, oxygen delivery to the mitochondria during exercise transients may limit the rate of oxidative phosphorylation. Whether PCr kinetics during the on-transient is effected by O_2 delivery remains to be tested. Measurement of blood flow kinetics (Shoemaker et al., 1994) in combination with PCr kinetics could determine whether O_2 delivery is limiting during exercise transients in humans.

McCreary et al. (1996) previously found similar time constants for $\tau\dot{V}O_2$ and τPCr adjustment to and recovery from plantar flexion exercise and predicted that these would correlate if measured in a large number of subjects heterogeneous for fitness level or age. The results of the present study (Figure 10a) support this hypothesis, for the on-transient to exercise. It has been assumed that PCr kinetics reflect the kinetics of muscle oxygen consumption (Whipp & Mahler, 1980; Marsh et al., 1993) and that this is expressed at the lungs, after a transit delay, as the phase 2 $\dot{V}O_2$ response (Whipp et al., 1982).

Computer simulations of transients to and from exercise have predicted a similarity between kinetics of muscle O_2 consumption and the phase 2 $\dot{V}O_2$ response (Barstow et al., 1990; Cochrane & Hughson, 1992). These investigators altered variables which are thought to cause a dissociation of the kinetics of muscle O_2 consumption from those of lung $\dot{V}O_2$ (i.e. blood flow kinetics, venous blood volume, blood flow to non-working tissues) and found that as long as these variables were within physiological ranges, only a minor dissociation between kinetics of muscle O_2 consumption and lung $\dot{V}O_2$ was apparent. Barstow et al. (1994) provided in vivo, although indirect, support for the model predictions by showing that PCr kinetics, measured during plantar flexion exercise, were similar to phase 2 $\dot{V}O_2$ kinetics measured during cycling. The findings of the present study and others (McCreary et al., 1996; Table 1, and Figure 10) provide further support for a similarity between muscle and lung $\dot{V}O_2$ kinetics, with measures of PCr and $\dot{V}O_2$ kinetics during the same exercise task. A significant correlation between the two, however, cannot be interpreted as cause and effect. There is substantial evidence to show that $\dot{V}O_2$ kinetics during the on-transient to exercise can be altered by various manipulations (Hughson and Kowalchuk, 1991, 1995), but very little (if any) to show that these manipulations affect PCr kinetics.

Although τPCr and $\tau\dot{V}O_2$ during recovery were similar (Table 1), a significant correlation between these two measures was not found (Figure 10b). Oxidative metabolism following exercise is required for PCr repletion and

clearance of lactic acid (Donovan & Brooks, 1983). Any build up of lactic acid during exercise may therefore cause a dissociation between kinetics of O_2 consumption and PCr repletion. Although exercise intensity was set below the IT, both young and old groups had a slight reduction in pH by the end of exercise (pH = 6.90 in the young group and 6.96 in the old group), indicating that there may have been some lactic acid accumulation during exercise. A decrease in pH may also cause a distortion in the kinetics of PCr repletion (Bendahan et al., 1990), since H^+ ions cause a shift in the creatine kinase equilibrium that favours PCr breakdown.

The following conclusions can be drawn from this study:

1) Moderately active old individuals have similar PCr and $\dot{V}O_2$ kinetics as young counterparts, during exercise of a muscle group (plantar flexors) accustomed to daily activity.

2) These older individuals retain mitochondrial capacity, measured as citrate synthase activity, suggesting that the capacity for peripheral muscular adaptation is maintained in older adults.

3) PCr and $\dot{V}O_2$ kinetics are probably influenced by factors other than mitochondrial capacity, such as O_2 transport to exercising muscle. PCr kinetics are slower during recovery in subjects with peripheral vascular disease (Keller et al., 1985) and breathing hypoxic gas mixtures slows $\dot{V}O_2$ kinetics during the adjustment to exercise (Hughson and Kowalchuk, 1995). However, the effects of alterations of O_2 delivery on kinetics of PCr during on-transients remains to

be tested.

4) PCr kinetics, measured at the exercising muscle, are similar to phase 2 $\dot{V}O_2$ kinetics, measured at the lung.

Chapter 4

Relationship Between Muscle Capillarization, O_2 Diffusion Distance, and $\dot{V}O_2$ Kinetics in Old and Young Individuals

4.1 Abstract

The relationship between muscle capillarization, estimated O_2 diffusion distance from capillary to mitochondria, and O_2 uptake ($\dot{V}O_2$) kinetics was studied in 11 young (25.9y) and 9 old (66.0y) adults. $\dot{V}O_2$ kinetics were determined by calculating the time constants (τ) for the phase 2 $\dot{V}O_2$ adjustment to and recovery from the average of 12 repeats of a 6 minute, moderate intensity plantar flexion exercise test. Muscle capillarization was determined from cross-sections of biopsy material taken from lateral gastrocnemius. Young and old groups had similar $\dot{V}O_2$ kinetics ($\tau\dot{V}O_2$-on=44 vs 48s; $\tau\dot{V}O_2$-off=33 vs 44s, for young and old groups, respectively), muscle capillarization, and estimated O_2 diffusion distances. Muscle capillarization expressed as capillary density or average number of capillary contacts per fibre / average fibre area and the estimates of diffusion distance were significantly correlated to $\dot{V}O_2$-off kinetics in the young individuals ($r=-0.68$ to -0.83; $p<0.05$). It is concluded that: 1) capillarization and $\dot{V}O_2$ kinetics during exercise of a muscle group accustomed to everyday activity (i.e. walking), are well maintained in old individuals, 2) in the young individuals, recovery of $\dot{V}O_2$ following exercise is

faster with a greater capillary supply over a given muscle fibre area or shorter O_2 diffusion distances.

4.2 Introduction

The kinetics of oxygen uptake ($\dot{V}O_2$) adjustment to moderate intensity exercise have been reported for differing exercise perturbations (eg. supine vs. upright, arms vs. legs) [Hughson et al., 1993; Cerretelli et al., 1977], and in subject groups of differing fitness levels (Babcock et al., 1994b; Chilibeck et al., 1996). Nevertheless debate continues regarding the control mechanisms of $\dot{V}O_2$ kinetics. Various studies have suggested that the limiting factor in the control of $\dot{V}O_2$ kinetics may be the rate of response of the central circulation (Hughson & Kowalchuk, 1991), blood flow distribution to the active muscle (Hughson et al., 1993), or the sites of metabolic control of muscle oxidative phosphorylation (Mahler, 1985). Hughson et al. have used perturbations of circulatory occlusion to non-exercising limbs (Hughson & Imman, 1986), or lower body negative pressure (Hughson et al., 1993) to show that $\dot{V}O_2$ kinetics can be highly influenced by changes in peripheral circulation. Recent studies (Shoemaker et al., 1994) have used Doppler measures of arterial blood velocity to estimate the rate of change in muscle blood flow in relation to $\dot{V}O_2$ kinetics during the adjustment to or recovery from exercise. The present study was designed to use another approach to examine the relationship of peripheral circulation to $\dot{V}O_2$ kinetics by investigating the relationship between muscle

capillarization and $\dot{V}O_2$ kinetics.

Recent studies in this laboratory (Cunningham et al., 1993; Babcock et al., 1994a) have shown that the kinetics of $\dot{V}O_2$ adjustment to moderate intensity exercise are slower in sedentary old compared to young individuals. The reasons for the slowed transition kinetics in old individuals are not clear. The rate of peripheral oxygen delivery to working muscle may be one factor, as muscle capillarization has been found to be reduced in old compared to young subjects (Coggan et al., 1992b). Slower kinetics and reduced capillarization may be due to sedentary living, as opposed to the ageing process, as moderate aerobic training improves both measures to levels seen in young individuals (Coggan et al., 1992a; Babcock et al., 1994b). The purpose to this study was to measure $\dot{V}O_2$ kinetics during exercise and capillarization for comparison of moderately active old and young groups.

4.3 Methods

4.3.1 Subjects

$\dot{V}O_2$ kinetics during plantar flexion exercise and muscle capillarization of the lateral gastrocnemius were measured in nine old and 11 young individuals. Subject characteristics are listed in Table 2. Subjects were moderately active, but not well trained. Older subjects reported walking as the primary form of exercise. Values for maximal oxygen uptake indicated that subjects were of average fitness for their respective age groups (Paterson, 1992). All gave

Table 2. Subject characteristics

	Age (y)	Mass (kg)	Height (cm)	VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)
Young (n=5 males, 6 females)	25.9±2.1	77.7±16.4	174.5±10.0	43.6±9.3
Old (n=2 males, 7 females)	66.0±6.3	70.7±13.3	165.7±8.8	20.6±2.5

All values are means±SD

informed consent to participate in this study which was approved by the University Review Board for Research Involving Human Subjects.

4.3.2 Exercise Tests

For determination of peak work rate, subjects initially performed exercise tests to fatigue on a custom built plantar flexion ergometer. This involved pushing on a foot pedal, to lift a weight attached by a pulley system, at a frequency of 0.5 Hz. Work rate was increased as a ramp function with increments averaging 0.3 $W \cdot min^{-1}$ for old and 0.6 $W \cdot min^{-1}$ for young subjects.

For determination of $\dot{V}O_2$ kinetics, subjects performed 12 six-minute square-wave transitions to and from ankle plantar flexion exercise, over three to four separate laboratory visits . The intensity was set at 45% of peak work rate, which averaged 1.6 and 3.4 W for old and young subjects, respectively. This work rate could be considered moderate, as further ramp testing determined that the work rates were below the subjects' intracellular threshold, as determined by ^{31}P-NMRS (Marsh et al., 1991). This threshold is considered the point at which anaerobic glycolysis is increased, as evidenced by an increased rate of change in pH (Marsh et al., 1991). A large number of transitions were performed in order to improve the signal to noise ratio (Lamarra et al., 1987), in light of the small amplitude of the $\dot{V}O_2$ response with the small muscle group exercise. Transitions were separated by six minutes of loadless plantar flexion and were initiated manually by the experimenter. Subjects were blinded to the initiation

and termination of square waves.

$\dot{V}O_2$ was measured using a modification of the methods of Babcock et al. (1992a). Inspired and expired gas flows were measured using a low deadspace (90mL) bi-directional turbine (Alpha Technologies, VMM 110) calibrated by a 3.01 L syringe and gas concentrations were measured by a mass spectrometer (Airspec 2000 MGR 9N) calibrated 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. Data collected every 20 ms were converted from analog to digital format and stored for later processing by a microcomputer.

Breath-by-breath alveolar gas exchange data were calculated using the algorithms of Beaver et al. (1981). Breath-by-breath data were interpolated to 1-s, with square wave repeats time aligned and averaged. Averaged responses for each subject were fit using a first order (monoexponential) model of the form:

$$Y(t) = a\{1-e^{-[(t-\delta)/\tau]}\},$$

where Y represents $\dot{V}O_2$ at time (t); and a, δ and τ are the amplitude, time delay and time constant of the response, respectively. The monoexponential curves were fit starting at 20s into the transition (i.e. approximate start of phase 2) of the $\dot{V}O_2$ response (Whipp et al., 1982). Solutions for a, τ, and δ were derived from an iterative optimization computer routine.

4.3.3 Muscle Capillarization

Needle biopsy samples were obtained from the lateral head of the right gastrocnemius of each subject. These were oriented longitudinally in embedding media, frozen in liquid N_2-cooled isopentane, and stored in liquid N_2. Frozen sections were cut, at a width of 10 μm, on a microtome cryostat (Leitz Lauda 1720) mounted on glass coverslips, and analyzed for capillarization by staining with periodic acid-Schiff's reagent, according to the method of Anderson (1975). Sections were magnified, and projected on an image analyzer (Quantimet 520), for counting of capillaries. The number of muscle fibres in sections averaged 134 ± 63 (range 44-255). Muscle capillarization was expressed as capillary density (total number of capillaries in a section divided by the total area), capillary to fibre ratio (C/F) (total number of capillaries in a section divided by the number of fibres), the average number of capillaries in contact with each fibre (CC), and the average number of capillaries in contact with each fibre divided by the average fibre area (CC/FA). Average and maximal diffusion distances for oxygen from capillary to muscle fibre were estimated using the equations developed by Snyder (1990), for capillaries distributed in random arrays:

maximal diffusion distance = [0.415 + 0.477 / (capillary to fibre ratio)] x √average fibre cross-sectional area

average diffusion distance = [0.207 + 0.232 / (capillary to fibre ratio)] x √average fibre cross-sectional area

These diffusion distances are based on the cumulative frequency of the area of each fibre within a measured distance from a capillary. Maximal diffusion distance is the distance where 95% of the fibre area is served by a capillary, whereas average diffusion distance is the distance where 50% of the fibre area is served by a capillary (Snyder, 1987). Equations for random, rather than square or hexagonal arrays, were used, based on the results (see below) for capillary to fibre ratio and average number of capillary contacts per fibre, according to descriptions of arrays by Plyley and Groom (1975).

4.3.4 Statistics

All results are expressed as means ± SD. Confidence intervals for parameter estimation of $\tau\dot{V}O_2$ were calculated, based on the $\dot{V}O_2$ response amplitude and the SD of breath-by-breath $\dot{V}O_2$ fluctuation, as described by Lamarra et al. (1987). Comparisons of $\tau\dot{V}O_2$ were made using a three-factor ANOVA with age (old vs. young groups) and sex (males vs. females) as between subjects factors and repeated measures on square-wave transient (on vs. off). Comparisons of capillarization and diffusion distances were made using a 2-factor (age x sex) ANOVA. Pearson product correlations were used to compare $\tau\dot{V}O_2$ with measures of muscle capillarization and diffusion distances. Slopes for significant regressions were corrected using the method of Anderson et al. (1986) where x and y variables are assumed to be estimated with error. $P < 0.05$ was accepted as significant.

4.4 Results

No effects due to sex were found for any measures. The inclusion of only two male subjects, however, does result in a high probability of a type 2 error in our ANOVA. Coggan et al. (1992b), using larger sample sizes, found differences between males and females; therefore, our finding of a lack of effect due to sex must be interpreted with caution. For simplicity, all results are presented with subjects grouped by age (old vs. young groups).

$\dot{V}O_2$-on and -off responses to plantar flexion exercise for an old and young subject, along with monoexponential fits, are depicted in Figures 11 and 12, respectively. Results for $\tau\dot{V}O_2$, summarized in Table 3, showed no significant difference in $\tau\dot{V}O_2$ between young and old individuals, and within age groups no difference between on- and off- kinetics. Using the equations developed by Lamarra et al. (1987), the 95% confidence intervals for estimation of this parameter (for both on- and off- transients) were $\pm$11.5s and $\pm$8.4s for old and young groups, respectively. This is based on the $\dot{V}O_2$ steady state amplitudes which averaged 0.08 and 0.11 L·min^{-1} for old and young groups, respectively, and the SD of breath-by-breath fluctuations, which averaged 0.067 L·min^{-1} for both old and young groups.

Muscle capillarization, fibre areas, and diffusion distances are summarized in Table 4. No differences were found between old and young groups for any of the variables.

For combined groups, $\tau\dot{V}O_2$ was generally faster with increased muscle

capillarization, with correlation coefficients (r) between various measures of muscle capillarization and $\dot{V}O_2$ kinetics ranging from -0.23 to -0.59 (Table 5). Correlations for capillary density versus $\tau\dot{V}O_2$-off (r = -0.48) and CC/FA versus $\tau\dot{V}O_2$-off (r = -0.59) were significant ($P < 0.05$). The correlation between maximal or average diffusion distance versus $\tau\dot{V}O_2$-off approached significance ($P = 0.052$). For individual groups, capillary density, CC/FA, and diffusion distances were significantly correlated with $\tau\dot{V}O_2$-off in the young group only (Table 5). There were no significant relationships between $\tau\dot{V}O_2$ and capillarization for the older group (Table 5). Figure 13 shows the individual points for the relationships of $\tau\dot{V}O_2$-off kinetics with capillary density, CC/FA, and maximal diffusion distance. The slopes for the regressions from the Pearson-product correlations were -0.11, -73.5, and 1.26 for Figure 13 a, b, and c, respectively. Based on the fact that both x and y variables for each graph were estimated with error, the corrected slopes, based on the method of Anderson et al. (1986) were -0.16, -88.6, and 1.85, for Figure 13 a, b, and c, respectively.

Figure 11. A. $\dot{V}O_2$ during on-transition to ankle plantar flexion in an older individual, along with monoexponential fit ($\tau\dot{V}O_2$ = 47s) B. $\dot{V}O_2$ during off-transition ($\tau\dot{V}O_2$ = 37s)

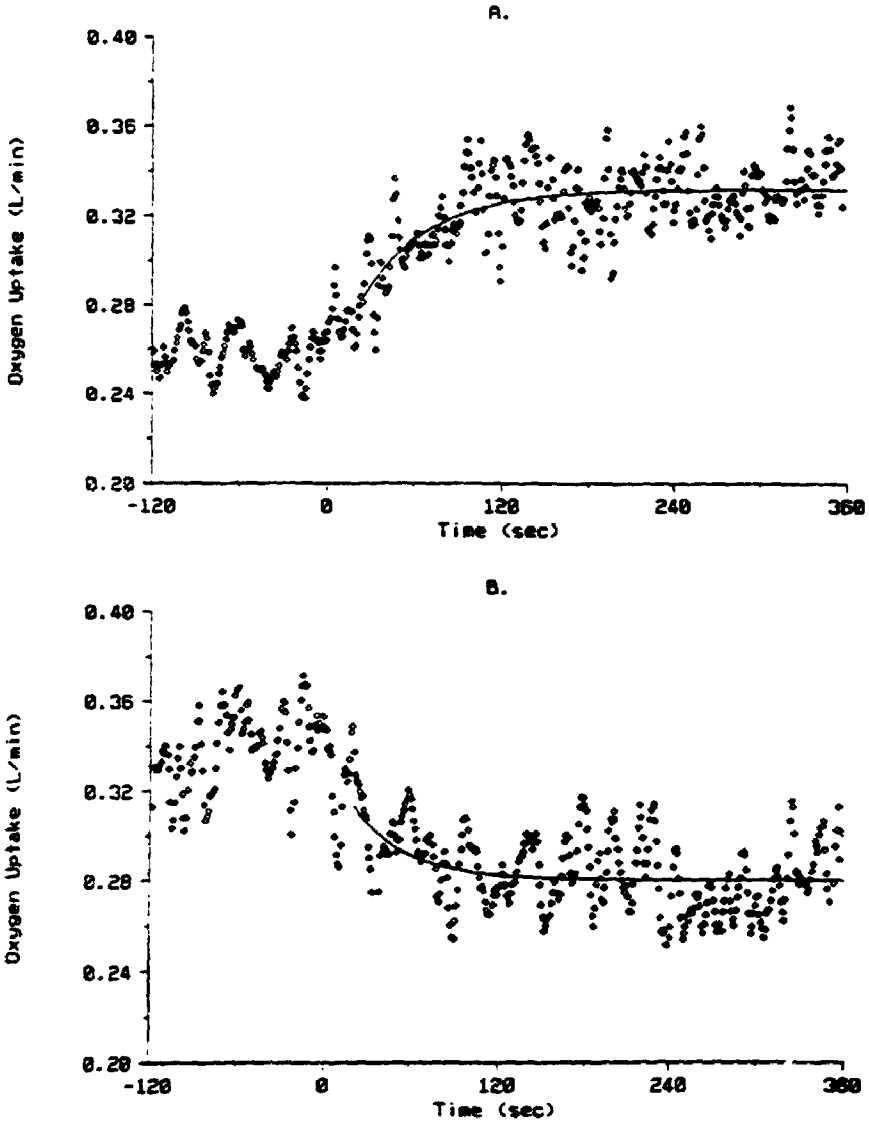

A.
0.40
0.36
0.32
0.28
0.24
0.20
Oxygen Uptake (L/min)
-120
0
120
240
360
Time (sec)
B.
0.40
0.36
0.32
0.28
0.24
0.20
Oxygen Uptake (L/min)
-120
0
120
240
360
Time (sec)

Figure 12. A. $\dot{V}O_2$ during on-transition to ankle plantar flexion in a young individual, along with monoexponential fit ($\tau\dot{V}O_2$ = 39s) B. $\dot{V}O_2$ during off-transition ($\tau\dot{V}O_2$ = 43s)

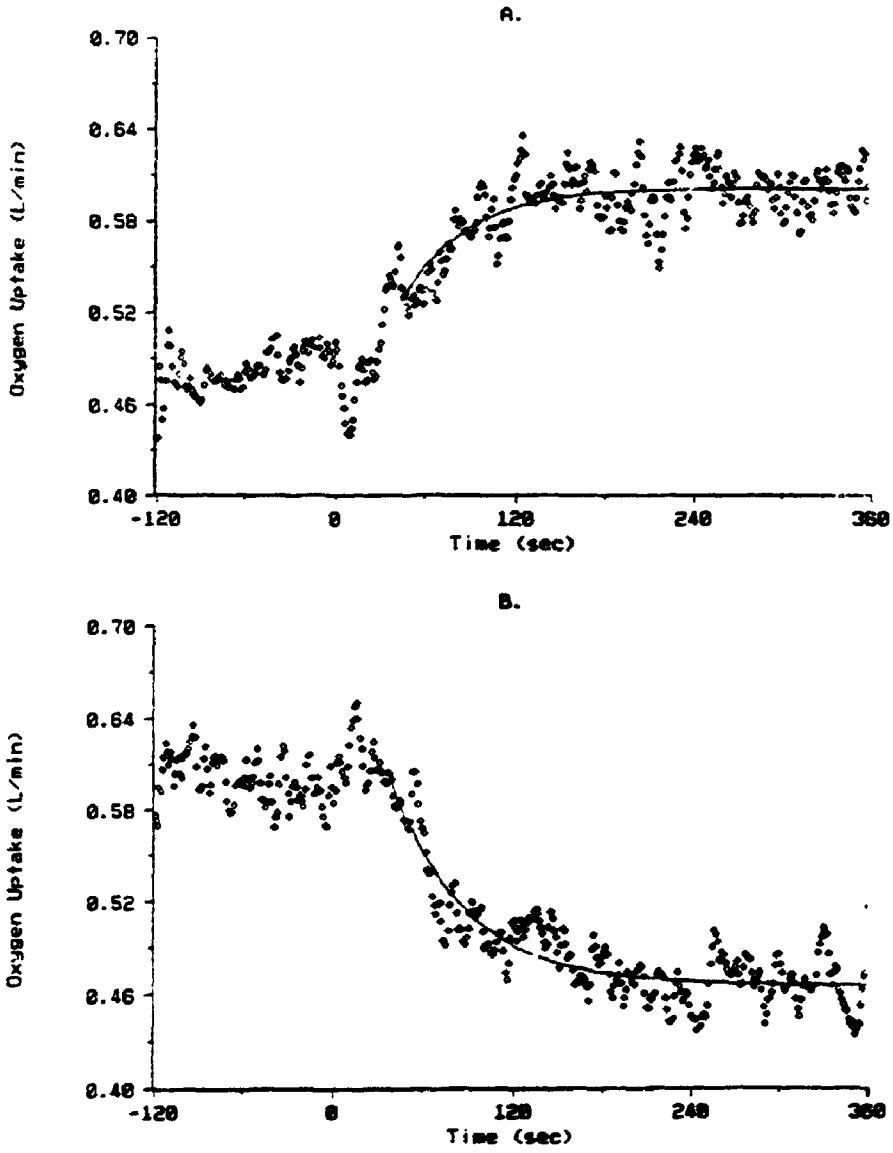

A.
0.70
0.64
0.58
0.52
0.46
0.40
Oxygen Uptake (L/min)
-120
0
120
240
360
Time (sec)
B.
0.70
0.64
0.58
0.52
0.46
0.40
Oxygen Uptake (L/min)
-120
0
120
240
360
Time (sec)

Table 3. $\dot{V}O_2$ kinetics for plantar flexion exercise in young and old individuals

	$\tau\dot{V}O_2$-on (s)	$\tau\dot{V}O_2$-off (s)
Young	44.8±9.7	33.1±16.6
Old	47.7±19.0	44.1±18.8

All values are means±SD

Table 4. Capillarization, fibre areas and estimates of O_2 diffusion distances in lateral gastrocnemius of young and old groups

	Caps·mm^{-2}	C·F^{-1}	CC	CC·FA^{-1} (μm^{-2}·10^{-3})	FA (μm^2)	maxDD (μm)	avgDD (μm)
Young	295 ± 105	1.93 ± 0.30	3.58 ± 0.64	0.54 ± 0.19	7175 ± 2389	55.7 ± 8.9	27.5 ± 4.4
	(181-513)	(1.5-2.6)	(2.3-4.4)	(0.29-0.86)	(4310-10970)	(42.3-68.6)	(20.9-33.9)
Old	242 ± 50	1.72 ± 0.52	3.35 ± 0.89	0.46 ± 0.11	7047 ± 1431	59.1 ± 4.3	29.2 ± 2.1
	(180-336)	(1.2-3.0)	(2.1-5.5)	(0.31-0.61)	(5263-9022)	(53.1-66.1)	(26.2-32.6)

All values are means ± SD; ranges are shown in brackets

Caps·mm^{-2} = capillary density (capillaries·mm^{-2})

C·F^{-1} = capillary to fibre ratio

CC = average number of capillary contacts per fibre

CC·FA^{-1} = average number of capillary contacts per fibre · average fibre area^{-1}

FA = fibre area

maxDD = estimated maximal O_2 diffusion distance

avgDD = estimated average O_2 diffusion distance

Table 5. Correlations between measures of capillarization and $\dot{V}O_2$ kinetics

	Caps·mm^{-2}	C·F^{-1}	CC	CC·FA^{-1}	DD
Young					
$\tau\dot{V}O_2$-on	-0.29	-0.37	-0.51	-0.43	0.15
$\tau\dot{V}O_2$-off	-0.68*	-0.20	-0.36	-0.83*	0.68*
Old					
$\tau\dot{V}O_2$-on	-0.22	-0.25	-0.35	-0.39	0.29
$\tau\dot{V}O_2$-off	0.07	-0.24	-0.31	-0.10	-0.17
Combined					
$\tau\dot{V}O_2$-on	-0.23	-0.29	-0.40	-0.36	0.19
$\tau\dot{V}O_2$-off	-0.48*	-0.28	-0.37	-0.59*	0.44

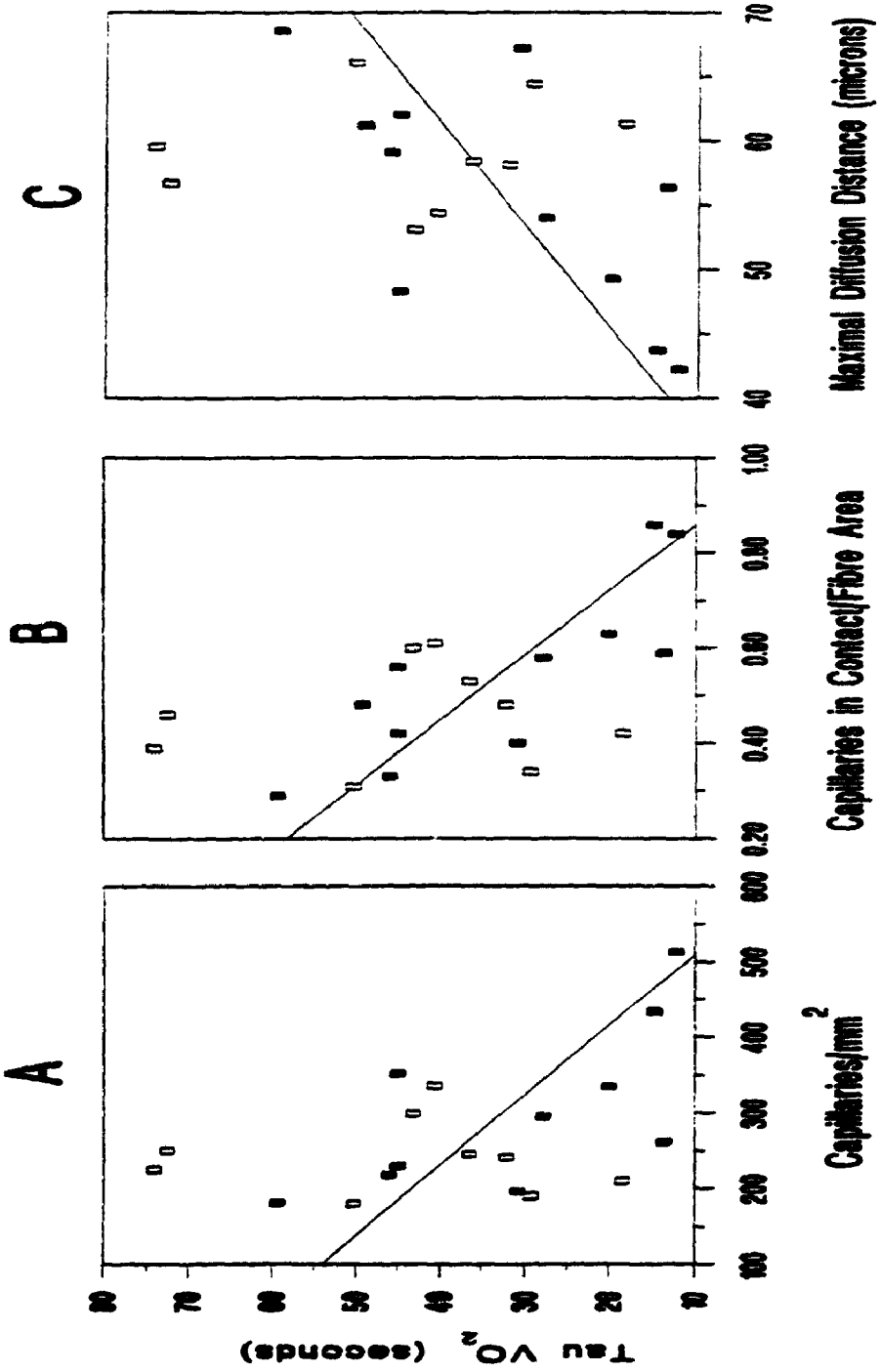
* $P < 0.05$

DD = maximal or average diffusion distance; see Table 4 for all other abbreviations

Figure 13. A. Correlation between capillary density and $\tau\dot{V}O_2$-off. Young group (closed squares) r = -0.68 (P = 0.02); Old group (open squares) r = 0.07 (NS); Combined groups r = -0.48 (P = 0.03).

B. Correlation between CC/FA and $\tau\dot{V}O_2$-off. Young group (closed squares) r = -0.83 (P = 0.002); Old group (open squares) r = -0.10 (NS); Combined groups r = -0.59 (P = 0.007).

C. Correlation between estimated O_2 diffusion distance and $\tau\dot{V}O_2$-off. Young group (closed squares) r = 0.68 (P = 0.02); Old group (open squares) r = -0.17 (NS); Combined groups r = 0.44 (NS; P = 0.052).


A
B
C
Tau VO_2 (seconds)
80
70
60
50
40
30
20
10
100
200
300
400
500
600
0.20
0.40
0.60
0.80
1.00
40
50
60
70
Capillaries/mm^2
Capillaries in Contact/Fibre Area
Maximal Diffusion Distance (microns)

4.5 Discussion

As mentioned in Chapter 3, $\dot{V}O_2$ kinetics during plantar flexion exercise were not different between old and young groups. In an earlier study, with the same group of subjects, $\dot{V}O_2$ kinetics during cycle ergometry were slower in old individuals (Chilibeck et al., 1995), in agreement with previous results from this laboratory (Cunningham et al., 1993; Babcock et al., 1994a). The differences between results may be due to the training of the muscle group tested. The plantar flexors are used to a great extent in everyday activity (i.e. walking) and may be relatively well trained in the old group. Babcock et al. (1994b) have shown that specific training of older subjects can substantially improve the rate of $\dot{V}O_2$ kinetics to levels similar to those of young fit individuals. Differences between cycling and plantar flexion tests may also indicate that the supine plantar flexion technique fails to identify differences in kinetics, even though they may exist. Hughson et al. (1993) have shown that $\dot{V}O_2$ kinetics are slower during supine exercise in young individuals. The response to supine exercise in old individuals is untested, therefore, their exists the possibility that $\dot{V}O_2$ kinetics are slower in young, but not old during supine exercise, cancelling any differences between the groups which may exist.

Usually measurements of $\dot{V}O_2$ kinetics have been performed during cycle exercise, as opposed to the plantar flexion exercise used in the present study. Plantar flexion exercise was used in order to compare $\dot{V}O_2$ kinetics with capillarization of a specific muscle (the lateral gastrocnemius).

Electromyographic (EMG) analyses have shown that cycle exercise involves the recruitment of many muscle groups of the leg (Jorge & Hull, 1986; Green & Patla, 1992); therefore, comparison of kinetics with biopsy data of one muscle group (i.e. the vastus lateralis) may be invalid. Results from a previous study, where muscle recruitment was assessed with magnetic resonance imaging and EMG, demonstrated that the gastrocnemius is the dominant muscle used during submaximal ankle plantar flexion, with minimal contribution from other muscle groups, such as the soleus or quadriceps (McCreary et al., 1994).

The results for capillarization of the lateral gastrocnemius were similar to those of both the old and young groups of Coggan et al. (1992b), with the exception of a higher capillary to fibre ratio for the old individuals of this study. The averages for each measure of capillarization tended to be lower in old compared to young groups (Table 4). Whereas differences between the groups of the present study were not significant, Coggan et al. (1992b), with a larger sample size, detected significant differences with lower levels of capillarization in lateral gastrocnemius of old individuals. Based on measurements of VO_{2max}, the old subjects of Coggan et al. (1992b) were "more fit" than those in the present thesis, which may seem confounding, since they found differences between their old and young groups, whereas we did not. VO_{2max} was measured by cycle ergometry in the present thesis, whereas Coggan et al (1992b) used a treadmill protocol. As the subjects of the present thesis were accustomed to walking exercise, their VO_{2max} may be higher, if measured by treadmill, as

opposed to cycle ergometry. When assessing capillary data across studies, it has been suggested that the best measure to use for comparison is capillary to fibre ratio, since a measure such as capillary density is highly influenced by muscle fibre size, which in turn may be affected by shrinkage during histochemical preparation techniques which may vary between laboratories (Plyley & Groom, 1975). Capillary to fibre ratio of the young (1.93) and old (1.72) groups of the present study (Table 4) were in the middle of the range from the literature for capillary to fibre ratios of lateral gastrocnemius for young individuals (range = 1.11-2.51) [Makitie, 1977; Anderson & Kroese, 1978; Cabric et al., 1987; Coggan et al., 1992b] and old individuals (range = 1.48-1.96) [Hammersten et al., 1980; Henriksson et al., 1980]. It is therefore concluded that the capillary results of this study were representative of typical old and young subjects. Studies comparing muscle capillarization (of the quadriceps) in old versus young humans have provided mixed results. An early study by Parizkova et al. (1971) showed a reduced capillary to fibre ratio in an old compared to a young group, but other studies have shown no significant differences with age (Grimby et al., 1982; Denis et al., 1986, Jakobsson et al., 1990). Differences between studies may be due to the training status of groups compared. Muscle capillarization appears to be very sensitive to training in old individuals, as demonstrated by Coggan et al. (1992a). Moderate training of older subjects resulted in substantial increases in muscle capillarization, to levels similar to those of their young group (Coggan et al., 1992b), despite the old individuals still having

substantially lower levels of VO_{2max}. The fact that most of the old subjects performed moderate levels of walking on a daily basis could have been a sufficient stimulus to offset any loss of capillarization with ageing.

While capillary counts between young and old subjects of the present study were not different, differences in capillary structure cannot be assessed with the techniques used. It has been suggested that there may be an alteration in capillary basement lamina with ageing (Ballard et al., 1979); this may affect diffusibility of O_2 through the capillary membrane. Studies comparing ultrastructural differences in capillaries or diffusibility of substances through capillary membranes (Leinonen, 1980) are lacking in older populations.

The present data show that measures of capillarization were significantly correlated with $\dot{V}O_2$ kinetics only when expressed in relation to fibre area (Table 5 and Figure 13). Capillarization, expressed as CC/FA had the strongest correlation with $\dot{V}O_2$ kinetics (Table 5, and Figure 13). This measurement gives an index of capillary supply to fibre area, accounting for the effects of diffusion (Plyley, 1989). Estimates of diffusion distances, based on the equations of Snyder (1990), differ in that C/F, rather than CC is used in relation to fibre area. As suggested by Plyley (1989), C/F, and capillary density, are global indices of capillarization and yield little information on the capillary supply of individual fibres. Therefore, CC/FA appears to offer a better assessment of O_2 delivery. Nevertheless, measurement of capillary numbers alone may be an oversimplification. Kinetics of O_2 delivery may also be affected by branching

patterns in the capillary network, length of capillary path., and capillary interconnections (Plyley et al., 1976), all of which would have to be measured in muscle sections cut longitudinally as opposed to transversely (Plyley et al., 1976). Another factor may be the rate of capillary recruitment, which has been shown to affect O_2 transport to dog gracilis muscle at the onset of exercise (Honig et al., 1980). Estimates of diffusion distance from capillary to cell interior, as done in this study, may be inaccurate in assessing O_2 diffusibility. Honig et al. (1984) hypothesized that the principal gradient for O_2 diffusion is across the capillary to the sarcolemma, rather than across the muscle cell interior. Tissue gradients for O_2 are small, as myoglobin acts as a buffer to keep PO_2 relatively uniform throughout the muscle cell (Honig et al., 1984). There may also be diffusion interaction between muscle cells, which would complicate estimates of diffusion from capillaries alone. The PO_2 gradient from inactive to working fibres is substantial and the surface area for exchange is great compared to that of capillaries (Honig et al., 1984).

It was hypothesized that $\dot{V}O_2$ kinetics may be related to the degree of muscle capillarization, since it has been shown that kinetics can be highly influenced by changes in peripheral circulation (Hughson & Inman, 1986; Hughson et al., 1993). With increased capillarization, diffusion distances from capillary to muscle fibre interior are shorter (Snyder, 1987) and there is an increased surface area for O_2 exchange (Leinonen, 1980), which together should decrease the transport time of O_2 to mitochondria. In the present study,

correlations between $\dot{V}O_2$ kinetics and capillarization per fibre area, were in a direction indicating faster kinetics with increased capillarization and shorter diffusion distances (Table 5). The modest correlations between simple measures of capillarization or diffusion distances and $\dot{V}O_2$ kinetics may be accounted for by the complicating factors described above.

However, the data do reveal two important aspects to consider. First, the findings show a significant relationship between $\dot{V}O_2$ kinetics and capillarization only in relation to the off-kinetics, not the on-kinetics, and second, this relationship is significant in the young, but not the old group.

The finding that capillarization was significantly correlated with $\dot{V}O_2$-off kinetics, but not $\dot{V}O_2$-on kinetics suggests that O_2 delivery may have a greater influence on $\dot{V}O_2$ recovery than $\dot{V}O_2$ adjustment to exercise. This is supported by Idstrom et al. (1985) who found that the rate of recovery of phosphocreatine (PCr) after contractions of perfused rat hindlimb, was related to O_2 supply through the perfusate, while the rate of PCr breakdown at the start of exercise was not (although PCr data were collected over 1.5 minute intervals at the onset of exercise, making estimates of breakdown rates inaccurate). Here, PCr kinetics are thought to reflect kinetics of muscle O_2 consumption (Barstow et al., 1994). $\dot{V}O_2$ was not, however, examined in this study, and it is unknown whether alterations which affect $\dot{V}O_2$ kinetics (Hughson and Kowalchuk, 1991, 1995) will also affect PCr kinetics. An O_2 dependence of $\dot{V}O_2$ kinetics during recovery is supported by models based on computer simulations of unsteady

states of exercise, which predict that O_2 delivery is limiting to $\dot{V}O_2$-off kinetics in situations where rate of cardiac output recovery is fast (Barstow et al., 1990). That $\dot{V}O_2$ kinetics are dependent on blood flow during recovery, but not during the onset of exercise is also implied by Shoemaker et al. (1994), who have demonstrated that the kinetics of blood flow recovery from knee extension exercise have a similar time course compared to $\dot{V}O_2$ recovery, while the rate of blood flow adjustment to exercise differs from that of $\dot{V}O_2$ adjustment. While tissue gradients for O_2 may be small during exercise on-transients due to myoglobin buffering (Honig et al., 1984), this might not be the case during recovery from exercise. With a relatively rapid decrease in blood flow during recovery, O_2 transport may limit oxidative metabolism (Barstow et al., 1990), resulting in a desaturation of myoglobin, and larger tissue O_2 gradients. In this instance, O_2 diffusion distance from capillary to fibre may affect kinetics of $\dot{V}O_2$.

If capillary recruitment is a factor affecting O_2 transport at the onset of exercise (Honig et al., 1980), this could explain the lack of correlation between capillarization measurements and $\dot{V}O_2$ kinetics during this transient as opposed to the recovery from exercise. During recovery, previously recruited capillaries may remain open, and capillarization may therefore have a greater effect on $\dot{V}O_2$ kinetics. Differences between the number of capillaries open at the end versus the beginning of exercise could explain the differences in the relationships between capillarization and $\dot{V}O_2$ kinetics during on- and off-

transients in the present study. The staining technique used for capillaries in the present study stains for all capillaries at rest, but does not give information on the number of capillaries which may be open during exercise transients. Furthermore, factors besides capillarization may have greater effects on blood flow adjustment at the start of exercise. These could include vascular responsiveness to adrenergic stimuli and vasodilating substances produced during metabolism.

It was hypothesized that capillarization would be lower in the old group, and that this would result in slow $\dot{V}O_2$ kinetics. However, measures of capillarization in the old group of this study were not reduced and capillarization was unrelated to $\dot{V}O_2$ kinetics. $\dot{V}O_2$ kinetics of old individuals might be more related to the rate of central O_2 transport (cardiac output kinetics) or the rate at which muscle can utilize the O_2 delivered to it. Babcock et al. (1994b) found a correlation between improvements in $\tau\dot{V}O_2$ and τHR ($r=0.78$) with training of old subjects, supporting the former hypothesis.

The findings of this study indicate that:

1) $\dot{V}O_2$ kinetics and muscle capillarization are well maintained in old individuals for a muscle group (the plantar flexors) used extensively during everyday activity.

2) Correlations between $\dot{V}O_2$ kinetics during recovery and muscle capillarization are strongest when capillarization is related to fibre area, thus accounting for diffusion distances.

3) Rate of O_2 delivery is probably affected by the interaction of many factors (i.e. capillary path lengths, capillary branching, capillary recruitment patterns, and O_2 diffusion from adjacent muscle cells), and estimating capacity for O_2 delivery from individual measures of capillarization may be an oversimplification.

4) Capillarization has a stronger relationship with $\dot{V}O_2$-off than $\dot{V}O_2$-on kinetics. This may be related to increased reliance on O_2 transport in recovery due to a reduced myoglobin saturation, or removal of metabolic waste products following exercise.

5) Capillarization has a stronger relationship with $\dot{V}O_2$ kinetics in young than old individuals. Other factors, such as rate of oxidative enzyme reactions or cardiac output kinetics, may limit $\dot{V}O_2$ kinetics in the elderly.

Chapter 5

Cardiorespiratory Kinetics During Exercises of Different Muscle Groups and Mass in Old and Young Individuals

5.1 Abstract

The purpose was to compare cardiorespiratory kinetics during moderate intensity (below ventilatory threshold) exercises of different muscle groups (double leg cycling versus treadmill walking and single leg ankle plantar flexion) in old and young subjects. Oxygen uptake ($\dot{V}O_2$) during exercise transitions was measured breath-by-breath and the phase 2 portion of the response was fit by a monoexponential for determination of the time constant (τ) of $\dot{V}O_2$. Two separate studies were performed: In study 1, 12 old (aged 66.7y) and 16 young (aged 26.3y) subjects were compared during cycling and ankle plantar flexion exercise, and in study 2, five old (aged 69.6y) and five young (aged 24.4y) subjects were compared during cycling and treadmill walking. $\dot{V}O_2$ transients during square-wave cycling exercise were significantly slower in the old compared with the young groups. In contrast, $\dot{V}O_2$ kinetics did not differ between old and young individuals during plantar flexion exercise. Heart rate kinetics followed the same pattern, with τHR being significantly slower in the old versus young groups during transitions to cycling, but not plantar flexion. In study 2, $\tau\dot{V}O_2$ and τHR during on-transients to treadmill square-wave exercise

were significantly slower in the old compared to the young group, but $\tau\dot{V}O_2$ was significantly faster during treadmill than cycling in the old individuals. The differences with ageing between the modes of exercise may be related to the muscle mass involved, and the circulatory demands. On the other hand, slowed $\dot{V}O_2$ kinetics with age appear to occur in a mode (cycling) in which the muscles are not accustomed to the activity, while in a mode of normal activity (walking) and with the muscle groups (plantar flexors) accustomed to the activity, $\dot{V}O_2$ kinetics are not slowed to the same degree with age.

5.2 Introduction

The kinetics of oxygen uptake ($\dot{V}O_2$) adjustment to moderate intensity cycling exercise are slowed as a function of age (Cunningham et al., 1993; Babcock et al., 1994a). This implies that older individuals must rely on anaerobic systems, to a greater extent, to meet energy requirements during transitions to exercise, increasing the possibility of early fatigue. The reasons for the slowed transition kinetics, in the old compared with young individuals, are not clear. Certainly, exercise training will improve the kinetics, such that trained old persons can demonstrate response rates to moderate exercise similar to values in untrained young individuals (Babcock et al., 1994b). Whether this improvement is due to faster rates of adjustment of central blood flow at onset of exercise or to improved muscle function with respect to O_2 delivery and utilization, is unknown. The purpose of this study was to examine

cardiorespiratory kinetics in groups of old and young subjects, during exercises of muscle groups, which differed in their habitual use and muscle groups of differing mass and central blood flow requirements. Two separate studies were performed: Comparisons of $\dot{V}O_2$ kinetics between old and young groups during cycling versus 1) unilateral ankle plantar flexion, and 2) treadmill exercise. Slower kinetics during cycling, but not plantar flexion exercise, in old individuals would suggest that kinetics are maintained in a muscle group used in daily activity and that muscle function is preserved with respect to peripheral O_2 delivery and utilization. If kinetics during walking were unaffected by age, it would suggest a maintenance by daily activity, whereas if slowed, it would suggest that with larger muscle mass exercise, slowed kinetics may be related to slower adjustment of central blood flow.

5.3 Methods

5.3.1 Subjects

In the first study, $\dot{V}O_2$ kinetics were measured during cycle and unilateral ankle plantar flexion exercise in 12 older subjects (10 females and two males, aged 66.7±6.7 y) compared to 16 younger subjects (seven females and nine males, aged 26.3±2.5 y). Lifestyles of subjects ranged from sedentary to moderately active. Most older subjects reported walking as their main form of exercise.

In the second study, $\dot{V}O_2$ kinetics were measured during cycle and

treadmill exercise in five older males (aged 69.6±4.3 y) compared to five younger subjects (three females and two males, aged 24.4±3.1 y). The young subjects were moderately active, and the older subjects were participating in a thrice weekly walk-jog exercise program. All gave informed consent to participate in this study which was approved by the University Review Board for Research Involving Human Subjects.

5.3.2 Exercise Tests

For all exercise tests, $\dot{V}O_2$ was measured using a modification of the methods of Babcock et al. (1994a). Inspired and expired gas flows were measured using a low deadspace (90mL) bi-directional turbine (Alpha Technologies, VMM 110) calibrated by a 3.01 L syringe and gas concentrations were measured by a mass spectrometer (Airspec 2000 MGR 9N for study 1, and Perkin Elmer, MGA-1100 for study 2) calibrated 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. Heart rate was recorded by a modified V_5 lead and converted by cardio-tachometer to an analogue signal alternating with each heart beat. Data collected every 20 ms were converted from analog to digital format and stored for later processing by a microcomputer. Breath-by-breath alveolar gas exchange data were calculated using the algorithms of Beaver et al. (1981).

Subjects initially performed a ramp exercise test on an

electromagnetically braked cycle ergometer (Lode), for determination of ventilatory threshold (V_ET) and maximal oxygen uptake ($\dot{V}O_2max$). The ramp protocol was designed to produce fatigue within 12 minutes, with workrate increases averaging 12 $W \cdot min^{-1}$ for old, and 25 $W \cdot min^{-1}$ for young subjects. Ventilatory threshold was determined as the point at which there was a systematic increase in the ventilatory equivalent of O_2 and also in the $P_{ET}O_2$, with no concomitant rise in the ventilatory equivalent for CO_2 or a decrease in the $P_{ET}CO_2$. Subjects in study 1 also performed ramp exercise tests on a custom built plantar flexion ergometer, in a semi-supine position, for determination of peak workrate. This involved pushing on a foot pedal, to lift a weight attached by a pulley system, at a frequency of 0.5 Hz. Workrate increments averaged 0.3 $W \cdot min^{-1}$ for old and 0.6 $W \cdot min^{-1}$ for young subjects. Subjects in study 2 also had $\dot{V}O_2max$ and V_ET measured during a ramp test on a treadmill. This test was initiated from a 0% grade at 0.47 $m \cdot s^{-1}$ (28.3 $m \cdot min^{-1}$) and designed to elicit similar $\dot{V}O_2$ increments as in the cycle ramp tests. The treadmill grade and speed were both adjusted with early increments dominated by changes in grade and later increments by changes in speed. As with the cycle ramp test, the protocol was designed to produce fatigue within 12 minutes.

Approximately one week following the ramp tests, subjects performed three repeats of a square-wave (each lasting six minutes for study 1, and five minutes for study 2) on the cycle ergometer, with workrate set at an intensity corresponding to 90% of V_ET. This intensity corresponded to approximately 100

W for young and 40 W for old subjects. The square wave tests were separated by six minutes of loadless cycling (0 W, pedalling at 60 rpm), and initiated under computer control.

Over three to four separate laboratory visits, subjects in study 1 performed 12 six-minute square-wave transitions to and from ankle plantar flexion exercise, at an intensity set at 45% of peak workrate. This averaged 1.6 and 3.4 W for old and young subjects, respectively. This workrate could be considered moderate, as further ramp testing determined that the workrates were below the subjects' intracellular threshold, as determined by ^{31}P-NMRS (Marsh et al., 1991). Transitions were separated by six minutes of loadless plantar flexion and were initiated manually by the experimenter. Subjects were blinded to the initiation and termination of square waves. In the plantar flexion exercise, a greater number of transitions were performed (than during the cycling tests), in order to improve the signal to noise ratio (Lamarra et al., 1987), in light of the small amplitude of the $\dot{V}O_2$ response with the small muscle group exercise. The number of repetitions required was based on the $\dot{V}O_2$ response amplitude and the SD of breath-by-breath $\dot{V}O_2$ fluctuation, as described by Lamarra et al. (1987), to achieve a confidence interval for parameter estimation of $\tau\dot{V}O_2$ similar to the confidence interval for the cycle test.

Each subject in study 2 performed three repeats of a five minute square wave on a treadmill, at a workrate corresponding to 90% of $V_{E}T$. The speed of the treadmill was set at 3.3 mph (1.5 $m \cdot s^{-1}$) for all subjects, with the percent

grade adjusted to elicit a workrate requiring a $\dot{V}O_2$ similar to that of each individual's cycling square wave. Square wave repeats were separated by a 5 minute period of walking on the spot beside the running treadmill, at a rate to elicit a $\dot{V}O_2$ similar to the loadless cycling protocol. Subjects were notified when the work period was approaching, and this period was recorded as having started when the subject's lead foot hit the treadmill. Although knowledge of an approaching work period may result in anticipatory responses, which could affect cardiorespiratory responses at the start of exercise (Linnarsson, 1974), we believe these were minimal, as HR and $\dot{V}O_2$ responses appeared normal (see results). For the treadmill results, only on-transient kinetics were measured. No attempt was made to do off-kinetics.

Breath-by-breath data were interpolated to 1-s, with square wave repeats time aligned and averaged. Averaged responses for each subject were fit using a first order (monoexponential) model of the form:

$$Y(t) = a\{1-e^{-[(t-\delta)/\tau]}\},$$

where Y represents either $\dot{V}O_2$ or HR at time (t); and a, δ and τ are the amplitude, time delay and time constant of the response, respectively. The monoexponential curves were fit from the start of the phase 2 (20s) portion of the $\dot{V}O_2$ response (Whipp et al., 1982) and from time 0 (initiation of the square wave) for heart rate data. Solutions for a, τ, and δ were derived from an iterative optimization computer routine.

5.3.3 Statistics

All results are expressed as means ± SD. For study 1, comparisons of $\tau\dot{V}O_2$ of old and young groups were made using a three-factor ANOVA with repeated measures on square-wave transient (on vs. off) and type of exercise (cycle vs. plantar flexion ergometry). For study 2, comparisons of $\tau\dot{V}O_2$ of old and young groups were made using a two-factor ANOVA with repeated measures for type of exercise (cycle vs. treadmill). For both studies, comparisons of τHR on-transients of old and young groups were made using a two-factor ANOVA with repeated measures on type of exercise. Tukey post-hoc testing was used to determine differences between individual means. Simple correlations were used to compare $\tau\dot{V}O_2$ and τHR of old and young groups for cycle and plantar flexion exercise, in study 1. $P < 0.05$ was accepted as significant.

5.4 Results

Values for $\dot{V}O_2$max from all tests are reported in Table 6. Young groups had significantly higher values than old groups for cycle and treadmill exercise.

On- and off- transients of averaged repeats and the mono-exponential fits are shown in Figure 14 for cycle ergometry , Figure 15 for ankle plantar flexion, and Figure 16 for treadmill walking, for an old and young subject. For the group average results of study 1, on the different modes, the calculated 95% confidence intervals for $\tau\dot{V}O_2$ were: cycle, old individuals 9.5s, young

individuals 3.5s; plantar flexion, old individuals 11.7s, young individuals 9.1s. In study 2, the estimate of $\tau\dot{V}O_2$ had a similar confidence interval for that of cycling and treadmill walking in old (10s) and young (4s) individuals.

Average $\tau\dot{V}O_2$ results for cycle and ankle plantar flexion exercise (study 1) are shown in Table 7. The $\tau\dot{V}O_2$ was significantly slower in old compared to young individuals during cycle on- and off- transients. There were no significant differences between young and old individuals for the $\tau\dot{V}O_2$ of the plantar flexion exercise.

Average $\tau\dot{V}O_2$ results for cycle and treadmill exercise (study 2) are shown in Table 8. Again, $\tau\dot{V}O_2$ transients were significantly slower in old compared with young individuals during the cycling and treadmill exercise (Table 8). Within the old group, the $\tau\dot{V}O_2$ on-transients during plantar flexion and during treadmill walking were significantly faster than during cycle ergometry (Tables 7 and 8); these differences were not observed in young individuals.

Average τHR results for on- transients are shown in Table 9 for cycling and ankle plantar flexion (study 1) and in Table 10 for cycling and treadmill walking (study 2). For three of the older subjects, HR data were too noisy or of insufficient amplitude for analysis during either plantar flexion or cycle ergometry; thus they were excluded (Table 9). HR transients were significantly slower during cycling (Table 9 and 10) and treadmill (Table 10) in old compared to young individuals, but significantly faster in old individuals during

the plantar flexion exercise, and not different from young individuals (Table 9).

Significant correlations were found between $\tau\dot{V}O_2$ and τHR, for the young group, during both cycle ($r=0.71$; $P<0.0006$) and plantar flexion ($r=0.63$; $P<0.008$) exercise, and for the old group during cycling ($r=0.62$; $P<0.013$), but not plantar flexion ($r=0.13$; $P=0.7$) exercise. The $\tau\dot{V}O_2$-τHR relationship for treadmill exercise was not assessed due to smaller subject numbers.

Figure 14: Averaged response of $\dot{V}O_2$ to 3 square wave repeats of cycling at 90% of $V_{E}T$, along with monoexponential fit, for **A.** on-transient of a young subject ($\tau\dot{V}O_2$ = 26 s), **B.** off-transient of a young subject ($\tau\dot{V}O_2$ = 28 s), **C.** on-transient of an old subject ($\tau\dot{V}O_2$ = 65 s) and **D.** off-transient of an old subject ($\tau\dot{V}O_2$ = 45 s). $\dot{V}O_2$ amplitudes, from baseline to steady state, averaged 1.13 and 0.32 $L \cdot min^{-1}$ for young and old groups, respectively.

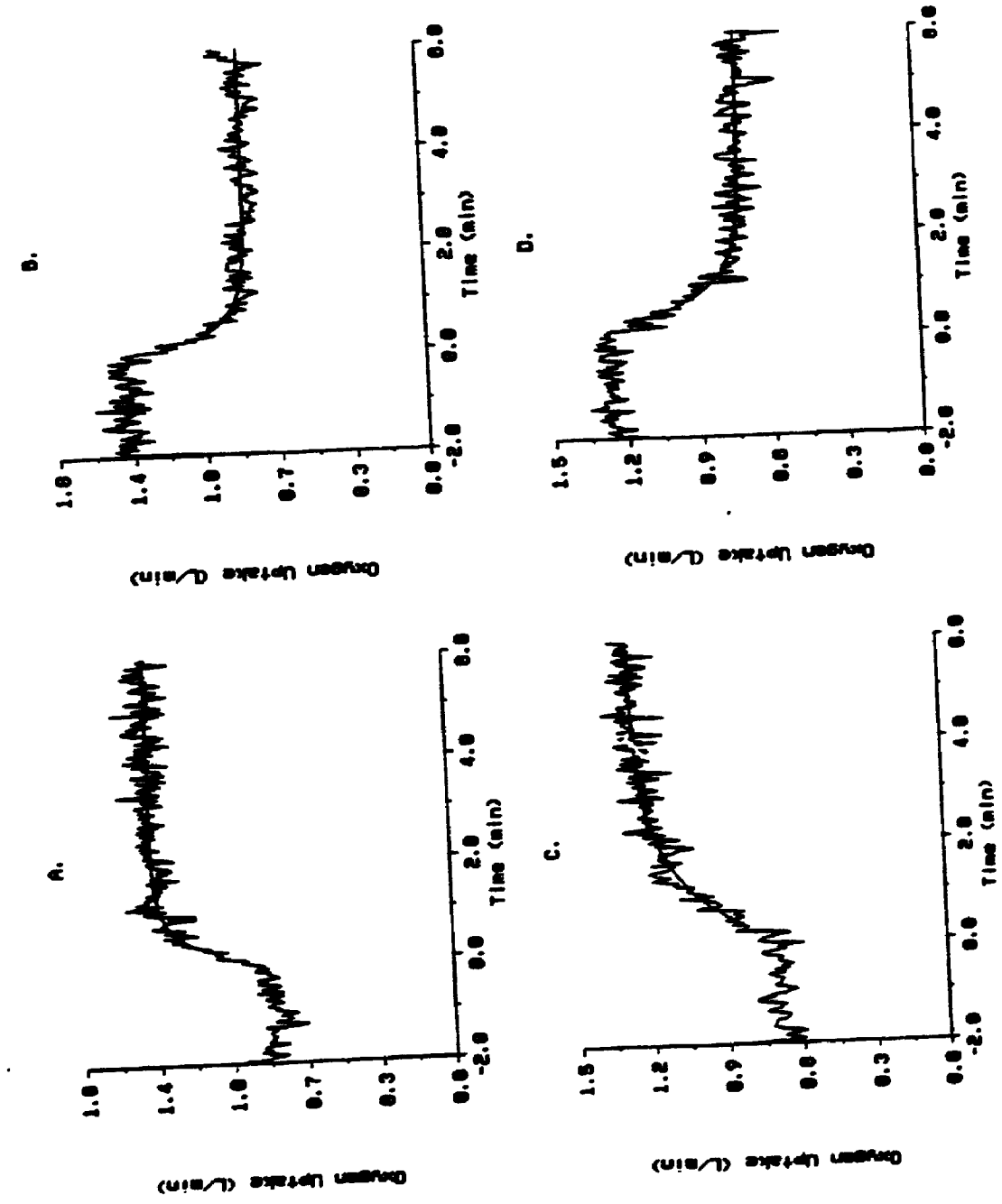

A.
B.
C.
D.
Oxygen Uptake (L/min)
Time (min)
1.6
1.4
1.0
0.7
0.3
0.0
-2.0
0.0
2.0
4.0
6.0
1.5
1.2
0.9
0.6

Figure 15: Averaged responses of $\dot{V}O_2$ to 12 square wave repeats of ankle plantar flexion at 45% peak workrate, along with monoexponential fit, for **A.** on-transient of a young subject ($\tau\dot{V}O_2$ = 32 s), **B.** off-transient of a young subject ($\tau\dot{V}O_2$ = 15 s), **C.** on-transient of an old subject ($\tau\dot{V}O_2$ = 31 s), **D.** off-transient of an old subject ($\tau\dot{V}O_2$ = 16 s). $\dot{V}O_2$ amplitudes from baseline to steady state, averaged 0.11 and 0.08 $L \cdot min^{-1}$ for young and old groups, respectively.

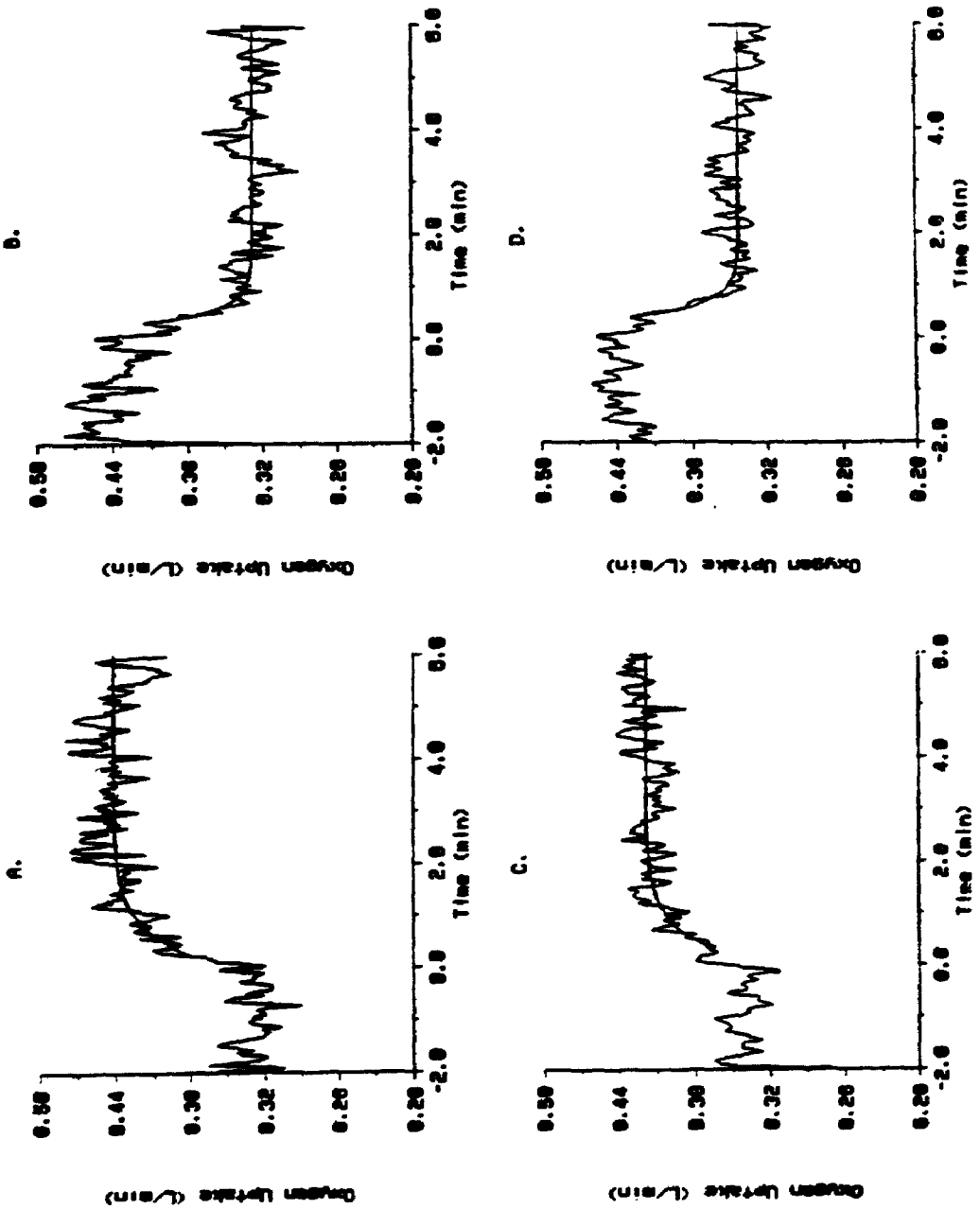
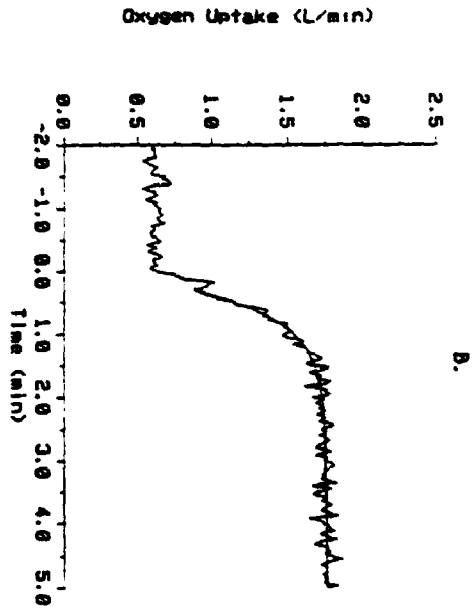
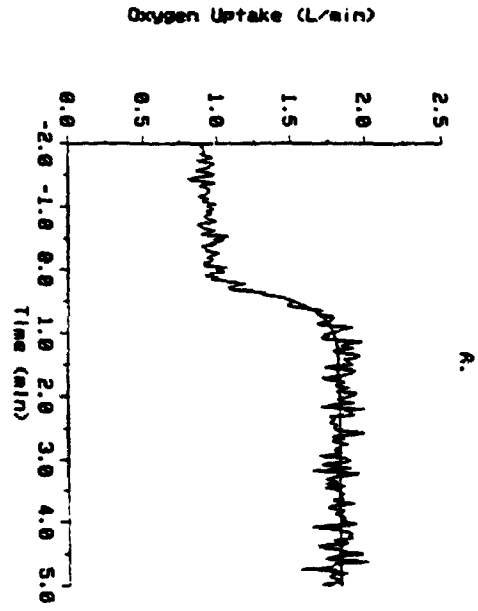

A.
B.
C.
D.
Oxygen Uptake (L/min)
Time (min)
0.50
0.44
0.38
0.32
0.26
-2.0
0.0
2.0
4.0
6.0

Figure 16: Averaged responses of $\dot{V}O_2$ to three square wave repeats of treadmill walking at 90% of V_{LT}, along with monoexponential fit, for **A.** on-transient of a young subject ($\tau\dot{V}O_2$ = 19 s), **B.** on-transient of an old subject ($\tau\dot{V}O_2$ = 29 s).


Oxygen Uptake (L/min)
0.0
0.5
1.0
1.5
2.0
2.5
-2.0 -1.0 0.0 1.0 2.0 3.0 4.0 5.0
Time (min)

B.


Oxygen Uptake (L/min)
0.0
0.5
1.0
1.5
2.0
2.5
-2.0 -1.0 0.0 1.0 2.0 3.0 4.0 5.0
Time (min)

A.

Table 6. $\dot{V}O_2max$ of old versus young groups for cycle and treadmill exercise

	Cycle $\dot{V}O_2max$		Treadmill $\dot{V}O_2max$	
	$L \cdot min^{-1}$	$mL \cdot kg^{-1} \cdot min^{-1}$	$L \cdot min^{-1}$	$mL \cdot kg^{-1} \cdot min^{-1}$
Study 1				
Young (n=16)	3.25±0.91*	43.0±8.3*	-	-
Old (n=12)	1.45±0.43	20.4±2.7	-	-
Study 2				
Young (n=5)	2.67±0.68*	40.1±3.9*	3.29±0.98*	49.4±4.8*
Old (n=5)	2.25±0.25	27.9±3.3	2.57±0.12	32.2±3.2

Values are means±SD

*Significantly different from old individuals ($P<0.025$)

Table 7. $\tau\dot{V}O_2$ (s) for plantar flexion and cycle exercise in young and old subjects (study 1)

	Plantar Flexion		Cycle Ergometry	
	On-Transient	Off-Transient	On-Transient	Off-Transient
Young (n=16)	44.2±11.7	35.0±13.8	40.3±13.2	35.0±6.7
Old (n=12)	44.7±16.4	41.8±18.9	63.6±16.6*¶§	49.3±18.1*

Values are means±SD

*Significantly different between age groups ($P<0.025$)

¶Significantly different between exercise modes ($P<0.01$)

§Significantly different between on- and off- transients ($P<0.05$)

Table 8. $\tau\dot{V}O_2$ (s) for on-transients to treadmill and cycle exercise in young and old subjects (study 2)

	Treadmill (Walking)	Cycle Ergometry
Young (n=5)	23.0±8.0	25.5±4.9
Old (n=5)	34.3±7.1*	44.8±6.6*¶

All values are means±SD

*Significantly different between age groups ($P<0.05$)

¶Significantly different between exercise modes ($P<0.05$)

Table 9. τHR (s) for on-transients to ankle plantar flexion and cycle exercise in young and old subjects (study 1)

	Plantar Flexion	Cycle Ergometry
Young (n=16)	35.3±28.0	46.4±22.3
Old (n=9)	32.6±29.3	73.1±36.4*¶

All values are means±SD

*Significantly different between age groups ($P<0.05$)

¶Significantly different between exercise modes ($P<0.01$)

Table 10. τHR (s) for on-transients to treadmill and cycle exercise in young and old subjects (study 2)

	Treadmill (Walking)	Cycle Ergometry
Young (n=5)	19.2±6.3	23.2±8.1
Old (n=5)	36.4±6.7*	31.4±12.8*

All values are means±SD

* Significantly different between age groups ($P<0.05$)

5.5 Discussion

The results for the ramp tests (Table 6) indicated that young subjects in both studies and old subjects in study 1 were of "average" fitness for their age, while old subjects in study 2 were of "above average" fitness for their age (Paterson et al., 1992), as expected. That peak $\dot{V}O_2$ during treadmill exercise was greater than cycle exercise for both young and old groups of study 2 was not surprising, as these groups were more accustomed to walking and jogging types of exercise than cycling.

The finding of slower $\dot{V}O_2$ kinetics during cycling (Tables 7 and 8) in the old compared to the young groups is in agreement with previous results (Cunningham et al., 1993; Babcock et al., 1994a). Slower $\dot{V}O_2$ kinetics were also observed in the older subjects during treadmill walking on-transients. Old subjects of study 2 (Table 8) appear to have faster $\dot{V}O_2$ kinetics than old subjects of study 1 (Table 7). This may be attributed to their higher fitness levels (Table 6). It has previously been demonstrated that moderate training of old individuals results in marked improvements in kinetics (Babcock et al., 1994b).

Slowing of $\dot{V}O_2$ kinetics may be due to slower cardiac output kinetics, and thus O_2 delivery to exercising muscle at the beginning of exercise. Heart rate kinetics, used to approximate cardiac output dynamics (DeCort et al., 1991), were found to be significantly slower in old versus young individuals during cycling and treadmill exercise (Tables 9 and 10). As well, significant

correlations were found between $\tau\dot{V}O_2$ and τHR in old and young groups during cycling. This relationship of $\tau\dot{V}O_2$ with τHR is in agreement with the findings of Cunningham et al. (1993), but differs from those of Babcock et al. (1994a), who found no significant slowing of heart rate kinetics with age.

With no change in HR kinetics in the older subjects, Babcock et al. (1994a) attributed slowing of $\dot{V}O_2$ dynamics during cycling to factors at the level of the muscle. These could include muscle capillarization, which could affect the rate of delivery of O_2 to the active muscle, or activity level of oxidative enzymes which could affect the dynamics of O_2 utilization at the mitochondria. With regard to the quadriceps, the major muscle group used during cycling exercise, in old compared to young individuals, some investigators have found lower levels of capillarization (Parizkova et al., 1971) and higher amounts of "non-muscle" tissue (fat and connective tissue) [Overend et al., 1992] (which may limit oxygen diffusion), and lower levels of oxidative enzymes (Essen-Gustavsson et al., 1986; Merideth et al., 1989; Trounce et al., 1989). Many studies, however, have shown no differences in these factors, at the quadriceps, with ageing (Larsson and Karlson, 1978; Orlander et al., 1978; Aniasson and Gustavson, 1981; Grimby et al., 1982; Denis et al., 1986; Borges and Essen-Gustavson, 1989). Thus, whether peripheral factors are responsible for the slower O_2 adjustment to cycling exercise in old individuals remains open to question.

It was found that $\dot{V}O_2$ kinetics during exercise (plantar flexion) of a

muscle group used on a daily basis were not slower in the old compared to young individuals (Table 7). Most investigators have shown that activity levels of oxidative enzymes (Coggan et al., 1992b; Keh-Evans et al., 1992; Coggan et al., 1993; McCully et al., 1993) and capillarization (Coggan et al., 1992b) of the major muscle used during plantar flexion, the gastrocnemius, are decreased, and the amount of "non-muscle" tissue of the plantar flexor compartment is increased (Rice et al., 1989) in old compared to young individuals. One would therefore expect oxygen transport to the gastrocnemius muscle, or oxygen utilization within the muscle, to be slower with age, resulting in slowed $\dot{V}O_2$ kinetics. As well, studies with ^{31}P-NMRS have shown that the Pi/PCr ratio is increased during ramp exercise of the plantar flexors (Coggan et al., 1993), and PCr resynthesis following exercise is prolonged (Keller et al., 1985; McCully et al., 1993) in old humans. An increased Pi/PCr ratio during exercise is thought to reflect decreased muscle oxidative capacity (Marsh et al., 1991), and PCr kinetics are thought to correspond to muscle respiratory kinetics (Whipp and Mahler, 1980; Marsh et al., 1993). Not all studies have found reductions in oxidative capability in old humans: Henriksson et al. (1980) reported activity levels of succinate dehydrogenase, and muscle capillarization, that were similar to measures for young gastrocnemius (Green et al., 1981; Coggan et al., 1992), and Jakobsson et al. (1990) found no reduction in capillarization of another muscle of the lower leg, the tibialis anterior, in old versus young individuals. Differences between studies may be due to level of training of subjects. It has

been suggested that Scandinavian studies may include older subjects of better training status, due to their more active lifestyles than North Americans (Rogers and Evans, 1993); however, studies comparing the lifestyles of old individuals in these two populations are lacking. The subjects of study 1 were not well trained, based on their $\dot{V}O_2$max, but many of them reported that they were moderately active and used walking as their main form of exercise. For a muscle group used in walking and accustomed to daily activity (ankle plantar flexors), $\dot{V}O_2$ kinetics do not appear to be slowed with age (Table 7). This is further supported by the results of study 2: $\dot{V}O_2$ kinetics during walking were significantly faster than during cycling in an older group of subjects, who were accustomed to regular walking exercise (Table 8). Thus, slowed $\dot{V}O_2$ kinetics with age seem to occur mainly in an exercise mode (cycling) in which muscle groups are not accustomed to the activity.

With regard to the effects of regular physical activity, Babcock et al. (1994b) have demonstrated that cycling $\dot{V}O_2$ kinetics in old subjects were faster following specific training (6 months) and approached values reported in young subjects. Coggan et al. (1992) have demonstrated that training for 9-12 months resulted in increases in activity of oxidative enzymes and capillarization of the lateral gastrocnemius to levels equal to or surpassing those in young individuals (1992b). Marsh et al. (1993b) have shown that the Pi/PCr ratio during exercise can be substantially reduced (increased oxidative capacity) in old muscle following moderate training over three months. Finally, in agreement with the

finding of better performance during plantar flexor or treadmill walking than cycling exercise in old individuals, Nakao et al. (1989) have shown that muscular endurance of the plantar flexors is maintained to a better degree with age than is muscular endurance of the quadriceps.

One other explanation for the finding that $\dot{V}O_2$ kinetics are slowed during cycling, and to some extent during treadmill walking, but not plantar flexion, in the old compared to young individuals, may be the different circulatory demands of a large versus small muscle mass. During exercise of a small muscle mass, it is unlikely that cardiovascular function is challenged, as perfusion is in excess of metabolic demand (Anderson and Saltin, 1985), while, in whole body exercise, demand for O_2 can outstrip its supply (Hughson, 1990). Thus, O_2 supply may be limiting to $\dot{V}O_2$ kinetics even in submaximal moderate intensity leg cycling, but certainly not during ankle plantar flexion. One could argue, however, that kinetics of $\dot{V}O_2$ during cycling at moderate exercise intensities may not be O_2 limited, as Hughson and Kowalchuk (1995) recently demonstrated that $\dot{V}O_2$ kinetics during sub-ventilatory threshold cycling exercise could not be made faster by breathing hyperoxic gas mixtures. If O_2 supply were limiting $\dot{V}O_2$ kinetics during the ankle plantar flexion exercise, one would expect that $\dot{V}O_2$ kinetics during exercise in this semi-supine position would be slower compared to upright cycling: Hughson et al. (1993) have demonstrated that $\dot{V}O_2$ kinetics are slower during supine compared to upright cycling and that this may be due to a reduced perfusion of exercising legs. In the present study,

$\dot{V}O_2$ kinetics were not slower during supine plantar flexion compared to upright cycling (Table 7); therefore, O_2 supply does not appear to be limiting during ankle plantar flexion. One could, however, argue that $\dot{V}O_2$ kinetics should be faster with exercise of the plantar flexors, since they are used to a greater extent on a daily basis, than muscles used during cycling. This may have offset any slowing of kinetics due to the supine nature of the plantar flexion exercise. If O_2 supply limits kinetics during large but not small muscle mass exercise, a slower kinetics of cardiac output in old compared to young individuals, would cause $\dot{V}O_2$ kinetics to be affected during exercise of a large muscle mass (cycling or walking), but not during exercise of a small muscle mass (plantar flexion). The finding that HR kinetics are slowed with age during cycling and treadmill walking, and correlated to $\dot{V}O_2$ kinetics during cycling, but not plantar flexion, in the old individuals, supports this concept. This does not rule out the possibility, however, that the rate of O_2 supply in the periphery may affect $\dot{V}O_2$ kinetics during plantar flexion, independent of cardiac output kinetics.

Differences in absolute exercise intensity between plantar flexion and cycling and the characteristics of the heart rate response during these exercises may also account for differences in kinetics, for the old group. During exercise that results in small changes in HR (such as plantar flexion), the increase in HR is dominated by withdrawal of vagal tone, which tends to be a fast process (Maciel et al., 1986). The larger change in HR during cycling is dominated by sympathetic activation, which may be a slower process in old individuals, as

demonstrated by an impaired beta-adrenergic receptor responsiveness (Van Brummelen et al., 1981). If this is the case, and $\dot{V}O_2$ kinetics are dependent on HR kinetics, old subjects should have slower kinetics during cycling, but not plantar flexion, as we have found (Tables 7 to 10). Vagal tone may be affected by ageing, but studies comparing old to young individuals have been equivocal, whereas a larger number of studies have shown that sympathetic activation is blunted in old subjects (Seals et al., 1994).

A final explanation for the finding of significantly faster $\dot{V}O_2$ kinetics during plantar flexion and treadmill walking than during cycling in the old individuals could be related to the recruitment of different muscle fibre types during a frequently used movement pattern (walking) compared with recruitment patterns during a seldom used movement pattern (cycling) [Sale, 1987]. For oxygen utilization, recruitment of type I muscle fibres, compared to type II fibres, is more efficient. Type II fibres have been shown to elicit longer kinetics for oxygen consumption recovery from exercise during animal experiments (Crow and Kushmerick, 1982). Although, in theory, recruitment of type II fibres during cycling would violate the size principle, it has been shown that type IIa, as well as type I fibres are recruited early during moderate intensity cycling, based on the glycogen depletion technique (Green et al., 1990).

The finding of nearly identical $\dot{V}O_2$ kinetics during plantar flexor and cycling exercise in the young subjects of this study is supported by Barstow et al. (1994) who found that $\dot{V}O_2$ kinetics during cycling were similar to PCr

kinetics during plantar flexion exercise. Comparisons between $\dot{V}O_2$ and PCr kinetics must, however, be interpreted with caution, as there is evidence to show that $\dot{V}O_2$ kinetics can be altered by various manipulations (Hughson et al., 1993; Hughson and Kowalchuk, 1995) but very little evidence to show that these manipulations affect PCr kinetics. Comparisons of the gastrocnemius and vastus lateralis within young subjects have shown that these muscles have similar levels of oxidative enzyme activity (Gollnick et al., 1974; Green et al., 1981) and capillarization (Green et al., 1981). If these factors affect $\dot{V}O_2$ kinetics, they may account for the similar $\dot{V}O_2$ kinetics during plantar flexion and cycling exercise in the young group.

In summary, it was found that $\dot{V}O_2$ kinetics are slowed with age during cycling, but not ankle plantar flexion exercise. Although $\dot{V}O_2$ kinetics of the old during treadmill walking were also significantly slower compared to young individuals, they were faster than during cycling exercise in the old individuals. The differences with ageing between types of exercise may be related to the muscle mass involved, and the circulatory demands, or slowed $\dot{V}O_2$ kinetics with age may only occur in a mode (cycling) in which the muscle groups are not accustomed to the activity. For a muscle group used in walking (ankle plantar flexors), $\dot{V}O_2$ kinetics are not slowed.

Chapter 6

The Influence of Age and Cardiorespiratory Fitness on Kinetics of Oxygen Uptake

6.1 Abstract

The purpose of this study was to determine the influences of ageing and cardiorespiratory fitness on kinetics of oxygen uptake ($\dot{V}O_2$) during the transition to exercise of moderate intensity. Twenty-nine old (68.9 y) and 16 young (26.3 6y) individuals initially performed a ramp exercise test on a cycle ergometer for determination of ventilatory threshold (V_{LT}) and maximal $\dot{V}O_2$ ($\dot{V}O_2max$). On a separate day, subjects performed three transitions to and from a six minute constant load, at an intensity corresponding to 90% of V_{LT}, for determination of $\dot{V}O_2$ kinetics. Breath-by-breath $\dot{V}O_2$ transients were time aligned and averaged and fit with a monoexponential equation, starting at 20 seconds (phase 2) of the response, for determination of the time constant of $\dot{V}O_2$ ($\tau\dot{V}O_2$). The young group had a significantly faster $\tau\dot{V}O_2$ (40.3 s versus 54.8 s for the old group) [$p<0.01$]. $\tau\dot{V}O_2$ was significantly correlated with $\dot{V}O_2max$ for both young ($r= -0.85$) and old ($r= -0.59$) groups; however the slope of the $\tau\dot{V}O_2$-$\dot{V}O_2max$ regression equation was steeper for the old group (-1.74 vs. -1.39 for the young group), indicating that fit older subjects had $\dot{V}O_2$ kinetics that approached those of fit young individuals, despite the old individuals having

lower levels of $\dot{V}O_2max$. A multiple linear regression indicated that relative fitness ($\dot{V}O_2max$ adjusted for age and sex) was the strongest significant predictor of $\tau\dot{V}O_2$, followed by sex and age. Although $\dot{V}O_2$ kinetics are definitely slowed with age, relative levels of cardiorespiratory fitness also have a great influence on the dynamic response of $\dot{V}O_2$. Older individuals can achieve $\dot{V}O_2$ kinetics similar to young fit subjects, with moderate improvements in fitness.

6.2 Introduction

The adjustment of oxygen uptake ($\dot{V}O_2$) at the onset of constant load, moderate-intensity exercise is slower in older individuals (Babcock et al., 1994a; Cunningham et al., 1993), faster in individuals of higher levels of cardiorespiratory fitness (Powers et al., 1985; Zhang et al., 1991), and can be accelerated with training of sedentary young (Cerretelli et al., 1979; Hagberg et al., 1980; Hickson et al., 1978; Yoshida et al., 1992) and old (Babcock et al., 1994b) subjects. Studies of aerobic training of sedentary young individuals which have increased maximal oxygen uptake ($\dot{V}O_2max$) by 20-38%, have resulted in decreases of 21-28% for the time constant (τ) of $\dot{V}O_2$ adjustment at the start of exercise (Cerretelli et al., 1979; Hagberg et al., 1980; Hickson et al., 1978; Yoshida et al., 1992). Training of older men results in similar relative increases in $\dot{V}O_2max$, but a much larger reduction, of 49%, for $\tau\dot{V}O_2$ (Babcock et al., 1994b). Although $\dot{V}O_2max$ of these trained older men was lower than that seen in average younger individuals, $\tau\dot{V}O_2$ averaged 32 s, which

is similar to values for fit young subjects. Thus it seems that with a moderate improvement in cardiorespiratory fitness, the age-related slowing of $\tau\dot{V}O_2$ can be substantially reduced. We hypothesize that older individuals respond differently to training with respect to improvements in $\dot{V}O_2$ kinetics, and that an individual's relative level of cardiorespiratory fitness, rather than age, has a greater influence on $\tau\dot{V}O_2$. The purpose of this study was to test this hypothesis by comparing $\dot{V}O_2$ kinetics for large groups of old and young individuals, of varying degrees of cardiorespiratory fitness.

6.3 Methods

Twenty-nine old (16 females and 13 males; aged 68.9±5.8y; body mass, 73.4±12.1 kg; height, 169.7±9.6 cm) and 16 young (seven females and nine males; aged 26.3±2.5y; body mass, 75.6±16.0 kg; height, 173.9±10.1 cm) subjects gave informed consent to participate in this study which was approved by the University Review Board for Research Involving Human Subjects.

For all exercise tests, $\dot{V}O_2$ was measured using a modification of the methods of Babcock et al. (1994a). Inspired and expired gas flows were measured using a low deadspace (90mL) bi-directional turbine (Alpha Technologies, VMM 110) calibrated by a 3.01 L syringe, and gas concentrations were measured by a mass spectrometer (Airspec 2000 MGR 9N or Perkin Elmer, MGA-1100) calibrated 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. Heart rate was recorded by a modified V_5 lead and converted by cardio-tachometer to an analogue signal alternating with each heart beat. Data collected every 20 ms were converted from analog to digital format and stored for later processing by a microcomputer. Breath-by-breath alveolar gas exchange data were calculated using the algorithms of Beaver et al. (1981).

Subjects initially performed a ramp exercise test on an electromagnetically braked cycle ergometer (Lode), for determination of ventilatory threshold (V_ET) and $\dot{V}O_2max$. The ramp protocol was designed to produce fatigue within 12 minutes, with increasing workrates averaging 12 $W \cdot min^{-1}$ for old, and 25 $W \cdot min^{-1}$ for young subjects. Ventilatory threshold was determined as the point at which there was a systematic increase in the ventilatory equivalent of O_2 and also in the $P_{ET}O_2$, with no concomitant rise in the ventilatory equivalent for CO_2 or decrease in the $P_{ET}CO_2$.

Approximately one week following the ramp test, subjects performed three repeats of a six minute square wave, with constant workrate set at an intensity corresponding to 90% of V_ET. This intensity corresponded to approximately 100 W for young and 40 W for old subjects. The square wave tests were separated by six minutes of loadless cycling (0 W, pedalling at 60 rpm), and initiated under computer control.

Breath-by-breath data were interpolated to 1-s, with square wave repeats

time aligned and averaged for improvement in the signal to noise ratio. Averaged responses for each subject were fit using a first order (monoexponential) model of the form:

$Y(t) = a\{1-e^{-1((t-\delta)/\tau 1}\}$,

where Y represents either $\dot{V}O_2$ or HR at time (t); and a, δ and τ are the amplitude, time delay and time constant of the response, respectively. The monoexponential curves were fit from 20 seconds (the start of the phase 2 portion) of the $\dot{V}O_2$ response (Whipp et al., 1982) and from time 0 for heart rate data. Residuals from the fits of the data of Babcock et al. (1994a) suggest that phase 1 may be absent or not detectable (with only three repetitions of the square wave forcing function) in older individuals. As a test of whether the phase 1 portion of the response would alter kinetics, the monoexponential curve was fit from time 0 of the $\dot{V}O_2$ response and compared to the phase 2 fit. Solutions for a, τ, and δ were derived from an iterative optimization computer routine.

$\dot{V}O_2$max, $V_{I\!I}T$, $\tau\dot{V}O_2$, and τHR of old versus young groups were compared using analysis of variance (ANOVA). The $\tau\dot{V}O_2$'s derived from fits from time 0 and from 20 seconds were compared within age groups by ANOVA. Simple correlations were performed for the relationship of $\tau\dot{V}O_2$ with $\dot{V}O_2$max, $V_{I\!I}T$ and τHR for the old and young groups. Differences with age for the relationship between cardiorespiratory fitness and $\dot{V}O_2$ kinetics were tested by comparing the slopes of the linear regression of $\tau\dot{V}O_2$ versus $\dot{V}O_2$max for old

versus young groups, using a Student's t-test (Zar, 1984). The slopes were adjusted, based on the fact that both x and y variables of the regression are estimated with error, by using the method of Anderson et al. (1986). These slopes were also compared with a Student's t-test. Factors which contributed to the variability in $\dot{V}O_2$ kinetics were determined by performing a multiple linear regression for $\tau\dot{V}O_2$ with age, sex, relative fitness, and τHR as independent variables. Age and τHR were entered as ratio variables, and sex was entered as a nominal variable (females were assigned the number 0; males the number 1). Relative fitness was determined by dividing individuals into lower, middle, and upper tertiles, according to percentile rankings for $\dot{V}O_2$max for different age groups and sex from the Canada Fitness Survey (1983). This was entered into the regression as an ordinal variable, where individuals in the 67th to 100th percentile for $\dot{V}O_2$max, for their respective age group and sex, were assigned the number 1, individuals in the 34th to 66th percentile were assigned the number 2, and individuals in the 0 to 33rd percentile were assigned the number 3. The level of significance for all statistical tests was set at $p < 0.05$.

6.4 Results

Responses of $\dot{V}O_2$ to the onset of the square wave workload, along with the phase 2 monoexponential fit, are shown for a young unfit and a young fit subject in Figure 17, and for an old unfit and fit subject in Figure 18. The gain in $\dot{V}O_2$ was larger for young subjects (as expected from the greater workrate),

with $\dot{V}O_2$ increasing to a steady state more rapidly in the young compared to unfit old individuals. In general, $\tau\dot{V}O_2$ of fitter old subjects approached values seen in fit young individuals.

The young group had a significantly higher $\dot{V}O_2$max and V_LT, and faster $\tau\dot{V}O_2$ (40.3 vs. 54.8s) than the old group, as a whole (Table 11). There were no significant differences in τHR between the young and old individuals (Table 11). Although reproducibility of data obtained from single ramp work tests has been questioned (Hughson & Inman, 1986), the determination of V_LT, for the purpose of setting square wave workloads at a moderate intensity, seemed to be adequate, as during the square wave tests, a slow component of increasing $\dot{V}O_2$, characteristic of workloads above V_LT (Paterson & Whipp, 1991), was minimal. Within groups. $\tau\dot{V}O_2$ did not differ whether the monoexponential model was fit from time 0 or from the 20 seconds (young $\tau\dot{V}O_2$= 38.5$\pm$8.0 vs. 40.3$\pm$13.6s; old $\tau\dot{V}O_2$= 55.4$\pm$17.5 vs. 54.8$\pm$18.5s), indicating that exclusion of a possible phase 1 did not affect the analyses of the relationship of age with $\tau\dot{V}O_2$. The "goodness of fit" of the monoexponential model, as described by the square root of the sum of squares of the residuals divided by the square root of the number of data points (i.e. 340 points for the 340 seconds of phase 2 $\dot{V}O_2$, and 360 points for the 360 seconds of HR data), was not significantly different between old and young groups (by ANOVA). The average residuals (for $\dot{V}O_2$; young 0.07$\pm$0.02, old 0.06$\pm$0.02, and for HR; young 2.39$\pm$1.37, old 2.60$\pm$1.57) were similar to those reported by Babcock et al. (1994a) for young and old

groups.

Significant correlations were found for the relationship of $\tau\dot{V}O_2$ with $\dot{V}O_2max$, $V_E T$ and τHR, for both young and old groups (Table 12). The relationship of $\tau\dot{V}O_2$ with $\dot{V}O_2max$ was much stronger than the $\tau\dot{V}O_2$-$V_E T$ correlation.

Individual data for $\tau\dot{V}O_2$ versus $\dot{V}O_2max$, for young and old groups, along with linear regression equations are shown in Figure 19. The slopes of the regression lines for old and young groups were significantly different ($p<0.001$); the greater slope for the old group (-1.74 versus -1.39 for the young group) indicated that for a given difference in $\dot{V}O_2max$, the old group showed a greater difference in $\dot{V}O_2$ kinetics. When influential outliers were eliminated from this analysis (one young individual with $\dot{V}O_2max=25$ and two old individuals with $\dot{V}O_2max > 36$), correlations between $\tau\dot{V}O_2$ and $\dot{V}O_2max$ for young and old groups remained significant ($R=-0.77$, $p<0.001$, and $R=-0.50$, $p=0.007$) and the difference between the slopes of the regression lines for old and young groups was even greater (-1.9 vs. -1.1). Although it appeared that the relationship between $\tau\dot{V}O_2$ and $\dot{V}O_2max$ for the old group may have been non-linear, a hyperbolic or logistic fit of the data did not provide a significantly better fit than the linear regression, as determined by the F-ratio of the residual sum of squares of each fit (Motulsky & Ransnas, 1987).

Multiple linear regression for $\tau\dot{V}O_2$ indicated that relative cardiorespiratory fitness, sex, and age, but not τHR, were significant

explanatory variables of the variance in $\tau\dot{V}O_2$ (multiple r = 0.75; $p<0.0001$), independently accounting for 13.3%, 9.7% and 6.4% of the variance, respectively. The incremental proportion of variance explained as each independent variable was added to the model is shown in Table 13 and graphically in Figure 20. Simple correlation coefficients among the explanatory variables are shown in Table 14.

Figure 17: Averaged response of $\dot{V}O_2$ to 3 square wave transitions at an intensity corresponding to 90% of $V_E T$, along with monoexponential fit, for **A.** an unfit young subject ($\tau\dot{V}O_2$= 48 s) and **B.** a fit young subject ($\tau\dot{V}O_2$= 25 s).

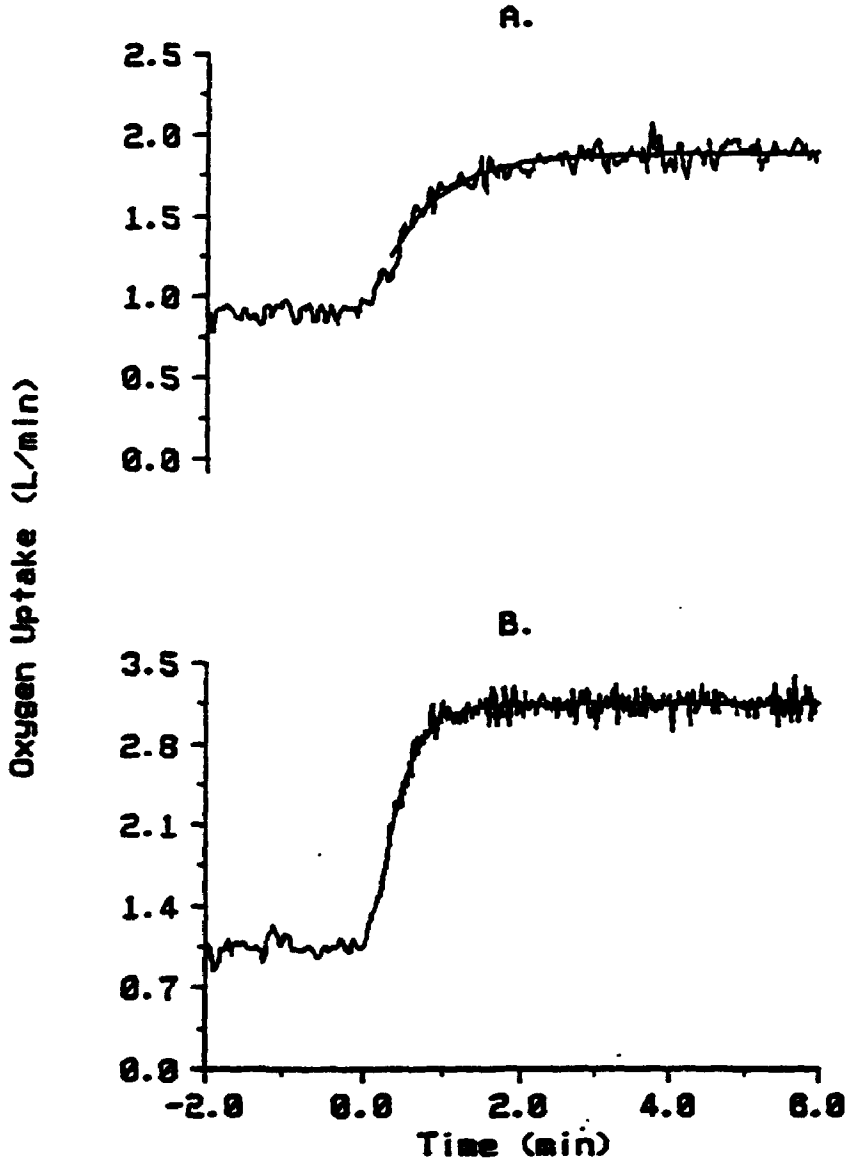

A.
2.5
2.0
1.5
1.0
0.5
0.0
B.
3.5
2.8
2.1
1.4
0.7
0.0
Oxygen Uptake (L/min)
-2.0
0.0
2.0
4.0
6.0
Time (min)

Figure 18: Averaged response of $\dot{V}O_2$ to 3 square wave transitions at an intensity corresponding to 90% of $V_{L}T$, along with monoexponential fit, for **A.** an unfit old subject ($\tau\dot{V}O_2$ = 66 s) and **B.** a fit old subject ($\tau\dot{V}O_2$ = 32 s).

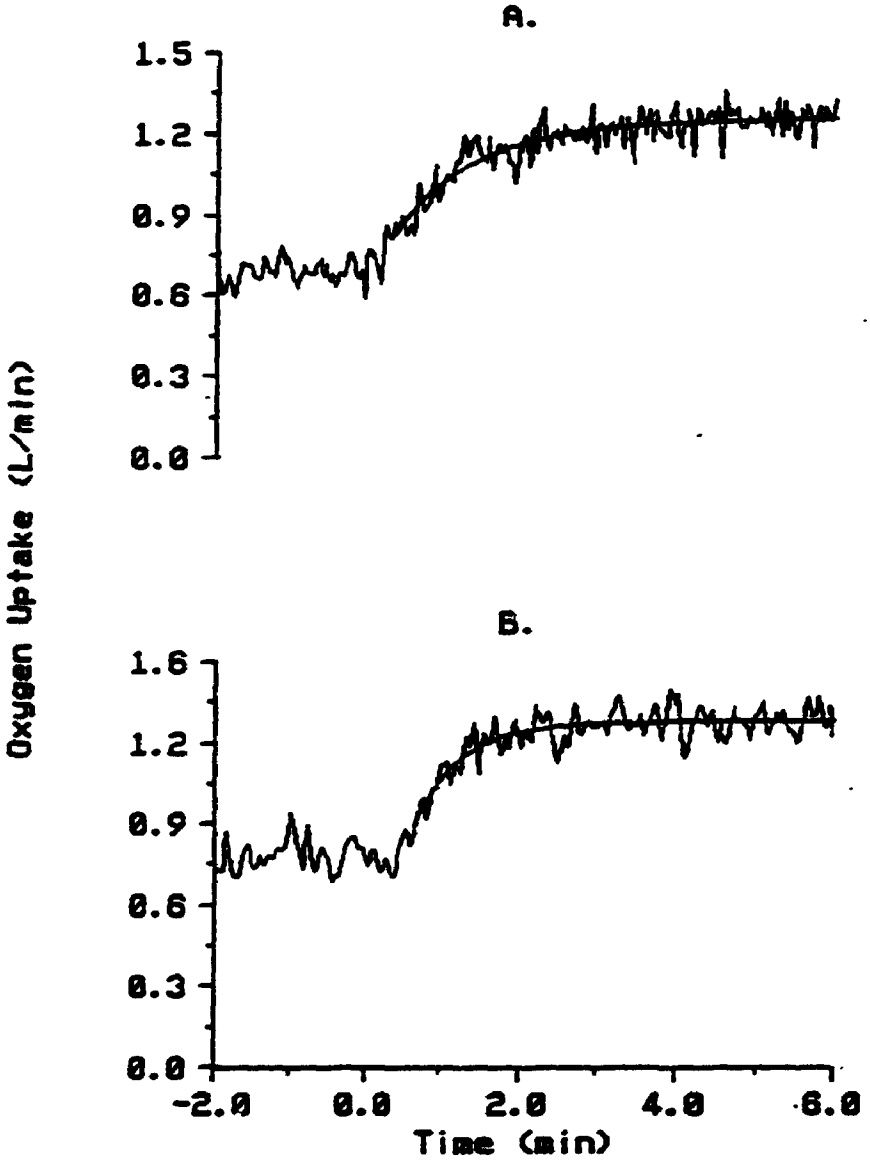

A.
1.5
1.2
0.9
0.6
0.3
0.0
B.
1.6
1.2
0.9
0.6
0.3
0.0
-2.0
0.0
2.0
4.0
6.0
Time (min)
Oxygen Uptake (L/min)

Table 11. Maximal oxygen uptake, V_ET, and $\dot{V}O_2$ and HR kinetics of young vs. old groups

	$\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	V_ET ($mL \cdot kg^{-1} \cdot min^{-1}$)	$\tau\dot{V}O_2$ (s)	τHR (s)
Young (n=16)	43.0±8.3[¶]	25.8±3.4[¶]	40.3±13.6[¶]	46.4±22.3
Old (n=29*)	24.7±6.2	16.5±3.[illegible]	54.8±18.5	54.4±29.7

Values are mean±SD

*For τHR, n=26 (HR data for 3 old subjects were too noisy for analysis)

[¶]Significantly different from old individuals ($p<0.01$)

Table 12. Correlations of $\tau\dot{V}O_2$ with $\dot{V}O_2max$, V_ET, and τHR for old and young groups

	$\dot{V}O_2max$	V_ET	τHR
Young (n=16)			
$\tau\dot{V}O_2$	-0.85 ($p<0.0001$)	-0.62 ($p<0.02$)	0.64 ($p<0.008$)
Old (n=29*)			
$\tau\dot{V}O_2$	-0.59 ($p<0.001$)	-0.39 ($P<0.03$)	0.47 ($p<0.014$)

*For τHR, n=26

Figure 19: Relationship between oxygen uptake kinetics and $\dot{V}O_2max$ for young and old groups:

Young $\tau\dot{V}O_2 = 99.9 - 1.39\ (\dot{V}O_2max)$; $R = -0.85$ ($p < 0.0001$); Corrected slope = 1.63; Standard error of the estimate = 7.4

Old $\tau\dot{V}O_2 = 97.8 - 1.74\ (\dot{V}O_2max)$; corrected slope = 2.9; $R = -0.59$ ($p < 0.001$); Standard error of the estimate = 15.3

The slope of the regression line (from the Pearson-product correlation and the reduced major axis model of Anderson et al. [1986]) for the old group is significantly steeper than that of the young group ($p < 0.001$).

Tau $\dot{V}O_2$ = time constant for $\dot{V}O_2$ kinetics

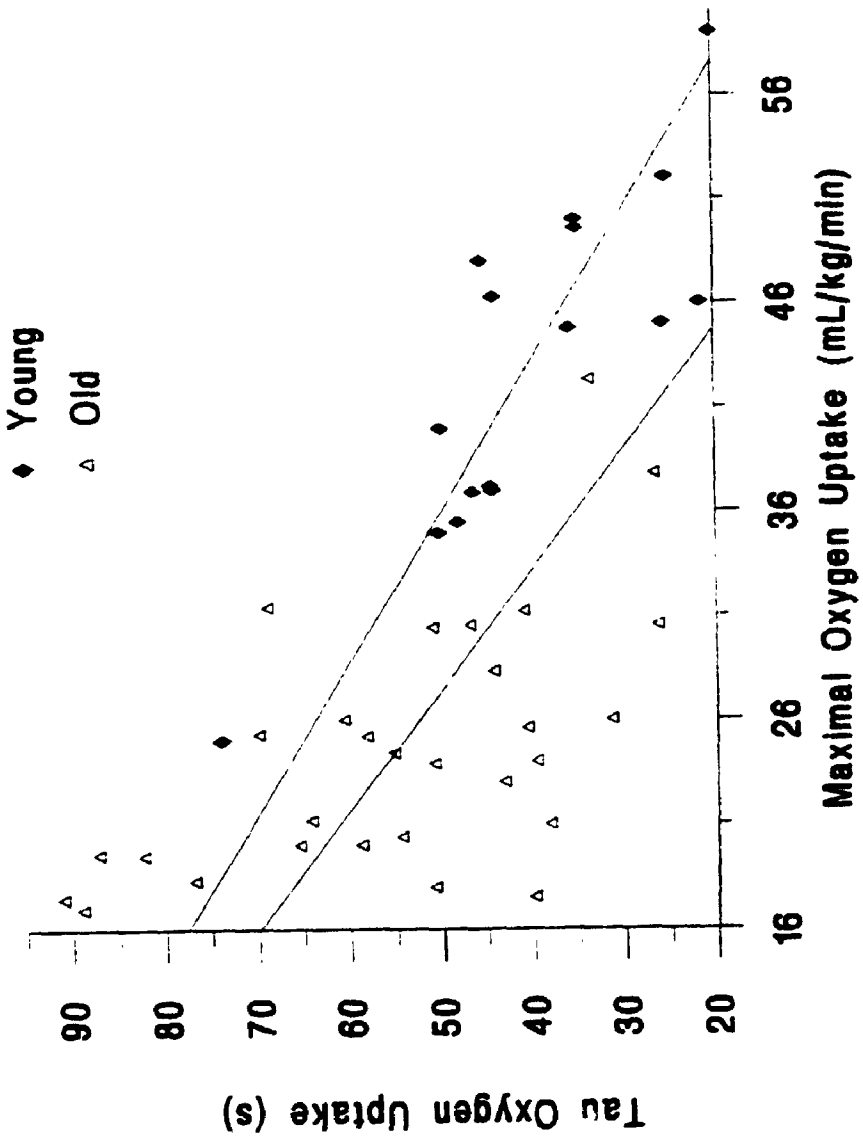

Young
Old
Tau Oxygen Uptake (s)
90
80
70
60
50
40
30
20
16
26
36
46
56
Maximal Oxygen Uptake (mL/kg/min)

Table 13. Multiple regression analysis of explanatory variables for $\tau\dot{V}O_2$

Dependent Variable	Explanatory Variable	p value	Incremental % Variation Explained	Overall % Variation Explained
$\tau\dot{V}O_2$	Relative Fitness	0.002	31.4%	31.4%
	Sex	0.007	16.3%	47.7%
	Age	0.025	7.2%	54.9%
	τHR	0.21	1.9%	56.8%

Table 14. Correlation coefficients for explanatory variables used in multiple regression analysis for determinants of $\tau\dot{V}O_2$

	Relative Fitness	Sex	Age	τHR
$\tau\dot{V}O_2$	0.56*	0.41*	0.40*	0.52*
Relative Fitness		0.01	0.14	0.53*
Sex			0.15	0.24
Age				0.19

*$p < 0.05$

Where

relative fitness: high = 1

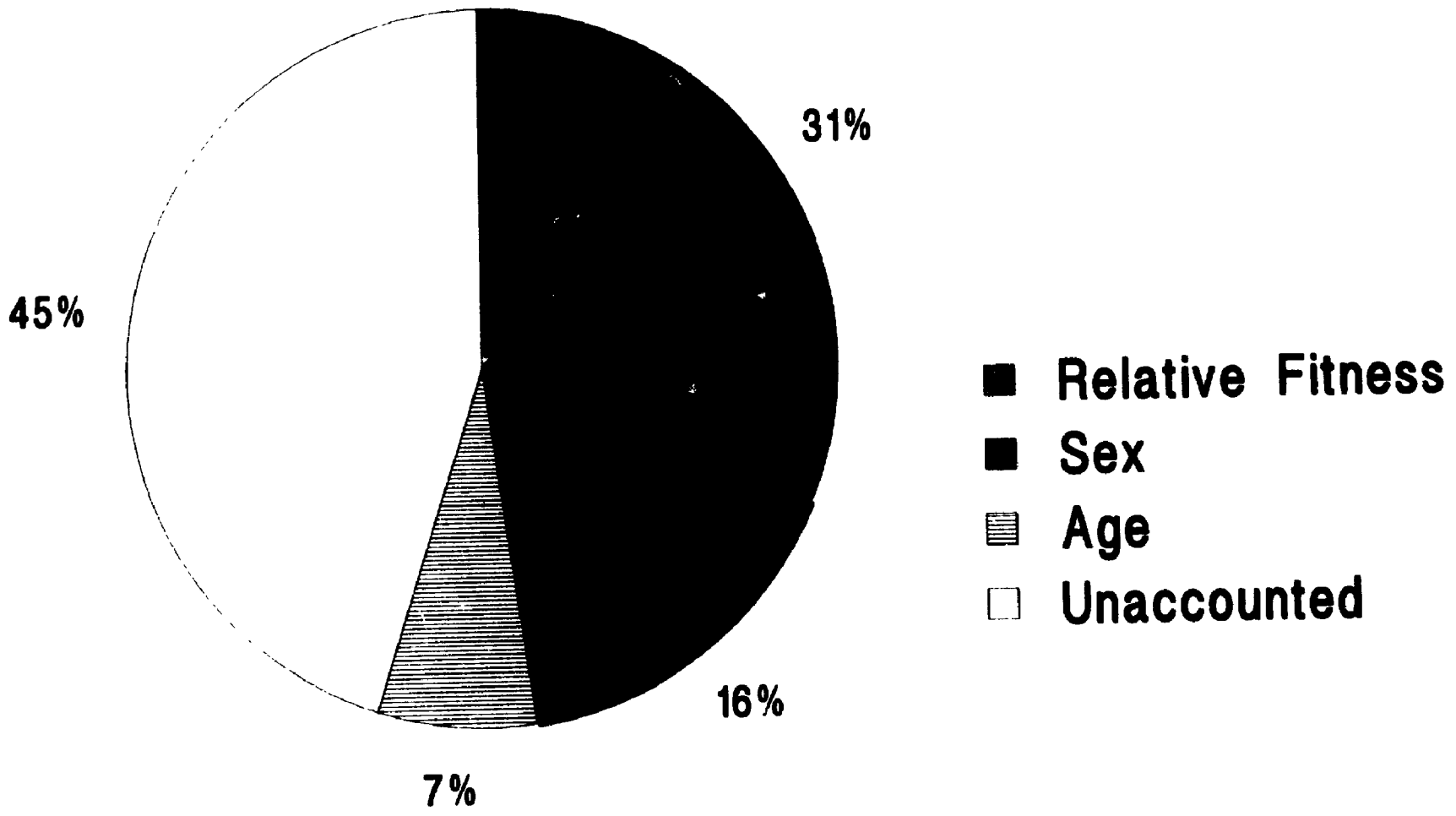
mid = 2

low = 3

Sex: females = 0, males = 1

Figure 20: Incremental proportion of variance for the dependent variable of τVO_2 accounted for by independent variables

Variance in Oxygen Uptake Kinetics



6.5 Discussion

The results of this cross-sectional study indicate that $\dot{V}O_2$ kinetics are slowed with age (Table 11), in agreement with previous studies from this laboratory for both older men (Babcock et al., 1994a) and women (Cunningham et al., 1993). It was also shown that within an age group, $\dot{V}O_2$ kinetics are faster with higher levels of cardiorespiratory fitness, similar to previous results with young (Powers et al., 1985; Zhang et al., 1991) and old groups (Babcock et al., 1994b). The regression equation for $\tau\dot{V}O_2$ versus $\dot{V}O_2$max in the young group (Figure 19) is similar to a regression for young subjects developed by Zhang et al. (1991), but different from one developed by Powers et al. (1985). For example, using the average $\dot{V}O_2$max of 43.0 $mL \cdot kg^{-1} \cdot min^{-1}$ for young subjects in this study (Table 11), the regression of this study predicts a $\tau\dot{V}O_2$ of 40 s, the regression developed by Zhang et al. (1991) predicts a $\tau\dot{V}O_2$ of 43 s, and the regression developed by Powers et al. (1985) predicts a $\tau\dot{V}O_2$ of 52 s. Methods used in the current study were similar to those of Zhang et al. (1991), who measured $\dot{V}O_2$ on a breath-by-breath basis, while Powers et al. (1985) collected data over 15 s intervals. The subjects of Zhang et al. (1991) had a wide range of cardiorespiratory fitness ($\dot{V}O_2$max ranging from 31 to 67 $mL \cdot kg^{-1} \cdot min^{-1}$), similar to the subjects of the current study ($\dot{V}O_2$max ranging from 25 to 59 $mL \cdot kg^{-1} \cdot min^{-1}$), while the subjects of Powers et al. (1985) were all well trained ($\dot{V}O_2$max ranging from 50 to 70 $mL \cdot kg^{-1} \cdot min^{-1}$).

The relationship of $\tau\dot{V}O_2$ versus $\dot{V}O_2$max for old and young groups

(Figure 19), indicates that for small differences in $\dot{V}O_2$max, older subjects have a larger difference in $\dot{V}O_2$ kinetics. This suggests that with small improvements in $\dot{V}O_2$max, older subjects can achieve substantially faster $\dot{V}O_2$ kinetics, which approach values of young fit subjects. Babcock et al. (1994b) found similar results with training of eight older men (72 y). Following 6 months of aerobic cycle training, $\dot{V}O_2$max increased approximately 21%, from 21.7 to 26.2 $mL \cdot kg^{-1} \cdot min^{-1}$, which is still lower than values for average young individuals (approximately 60% of $\dot{V}O_2$max for young persons of average fitness), but $\tau\dot{V}O_2$ decreased 48%, from 62 to 32 s, a value similar to that seen in fit young subjects. Although Babcock et al. (1994b) only found a weak relationship between changes in $\tau\dot{V}O_2$ and $\dot{V}O_2$max with training ($R=-0.31$, NS), which is in contrast to the significant cross-sectional results of this study, their subject numbers and ranges for $\dot{V}O_2$max with training were small.

Using the regression equations relating $\tau\dot{V}O_2$ and $\dot{V}O_2$max from the present study on the $\dot{V}O_2$max data from the Babcock study (1994b) would predict that $\tau\dot{V}O_2$ would improve by only 8 seconds (60 to 52 s). This suggests that the regression equations based on cross-sectional data (as in the present study) will underestimate the true training-induced changes of $\tau\dot{V}O_2$ found in older populations. This difference between cross-sectional and longitudinal studies is expected, as cross-sectional studies are based on a continuum of fitness levels rather than specific training-induced increases in fitness levels. In studies of young groups (Cerretelli et al., 1979; Hagberg et al., 1980; Hickson

et al., 1978; Yoshida et al., 1992), training of similar duration has resulted in average changes of $\dot{V}O_2$max of 24%, from approximately 42 to 52 mL·kg^{-1}·min^{-1}, and decreases in $\tau\dot{V}O_2$ of 25%, from 52 to 39 s. The regression equation of $\tau\dot{V}O_2$-$\dot{V}O_2$max for the young group would predict a change in $\tau\dot{V}O_2$ pre- to post-training of 33%, from 42 to 28 s; a change of similar magnitude to that predicted from the present data, although the kinetics are faster than expected for the $\dot{V}O_2$max. Thus, training of old and young groups which results in similar relative increases in $\dot{V}O_2$max (20-30%), results in similar absolute values for $\tau\dot{V}O_2$ (30-40 s).

Relative levels of fitness seem to influence $\dot{V}O_2$ kinetics to a greater extent than age: Multiple linear regression for $\tau\dot{V}O_2$ indicated that relative fitness significantly accounted for the greatest proportion of the variability in $\dot{V}O_2$ kinetics, followed by sex and age. Correlations between these explanatory variables were not significant (Table 14), indicating that collinearity was absent. V_ET, another measure of aerobic fitness, and τHR were significantly correlated with $\tau\dot{V}O_2$ for both young and old groups (Table 12), although neither was a significant independent variable in the multiple regression for $\tau\dot{V}O_2$.

The large differences in $\tau\dot{V}O_2$ that are associated with relatively small differences in $\dot{V}O_2$max of the old individuals (Figure 19) suggests that habitual physical activity may be a strong predictor of $\tau\dot{V}O_2$. Although data on habitual physical activity of these subjects was not collected, perhaps the inclusion of this variable would improve the regression model.

The finding that relatively small differences in cardiorespiratory fitness of old individuals are associated with substantially large differences in $\tau\dot{V}O_2$ can also provide some insight as to the factors which limit $\dot{V}O_2$ kinetics in old humans. $\dot{V}O_2$ kinetics may be limited by the rate of O_2 delivery from the heart to working muscle (for example, cardiac output kinetics or muscle capillarization), or by peripheral factors such as activity level of oxidative enzymes within muscle mitochondria, which may limit the rate of O_2 utilization by working muscle (Hughson, 1990).

Heart rate kinetics, as an estimate of cardiac output kinetics, were used as an indication of the rate of O_2 delivery to working muscle. τHR was significantly correlated with $\tau\dot{V}O_2$ for both young and old groups (Table 12). With training of older men, Babcock et al. (1994b) found a significant correlation ($r=0.78$) between changes in $\tau\dot{V}O_2$ and τHR. Thus, in older individuals, improvement in the rate of O_2 delivery to working muscle with training may account for their large improvement in $\dot{V}O_2$ kinetics.

On the other hand, studies of older individuals indicate that muscle oxidative enzyme activity and capillarization can also be improved to a great degree with training, approaching or surpassing values found in young groups. Cross-sectional studies show that masters athletes have similar or greater levels of oxidative enzyme activity and muscle capillarization when compared to young groups (Coggan et al., 1990; Houston & Green, 1981; Jakobsson et al., 1990), despite lower levels of $\dot{V}O_2$max (Coggan et al., 1990; Houston & Green, 1981).

Training of sedentary old individuals results in substantial increases in levels of oxidative enzyme activity (Coggan et al., 1992a; Orlander & Aniansson, 1980; Suominen et al., 1977), muscle capillarization (Coggan et al., 1992a), and leg vasodilatory capacity (Martin et al., 1990), which approach or surpass values reported for young individuals (Coggan et al., 1992b), despite the old failing to attain levels of $\dot{V}O_2$max similar to young comparison groups (Coggan et al., 1992a, 1992b). The only study to compare peripheral effects of similar endurance training programs (12 weeks at an intensity of 70% $\dot{V}O_2$max) in sedentary young and old subjects found that the old group increased muscle oxidative capacity by 128%, compared to only 28% in a young group (Meredith et al., 1989), such that levels of muscle oxidative capacity were similar between the old and young groups post-training, despite the old group having a significantly lower $\dot{V}O_2$max (Meredith et al., 1989). Older individuals also demonstrate a substantial improvement in muscle metabolic response to exercise, as assessed by ^{31}P-Magnetic Resonance Spectroscopy, following endurance training (Marsh et al., 1993). Although not measured in the study of Marsh et al. (1993), it might be anticipated that $\tau\dot{V}O_2$ would be similar to younger groups.

In summary, this study suggests that, with moderate changes in cardiorespiratory fitness, old individuals may be able to achieve values for $\dot{V}O_2$ kinetics similar to those of fit young subjects. Although $\dot{V}O_2$ kinetics are slowed with age, relative level of fitness has a substantial influence on kinetics. The

ability of old individuals to attain similar values for $\dot{V}O_2$ kinetics may be due to their capacity for improvement in cardiac output (as estimated by HR) kinetics or a greater capacity for changes in muscle oxidative enzyme activity and capillarization with changes in fitness level.

Chapter 7

General Summary and Conclusions

The overall purpose of these studies was to determine which factors are limiting to $\dot{V}O_2$ kinetics in old individuals and how kinetics may be affected by fitness level.

In the first study, citrate synthase (CS) activity of the lateral gastrocnemius was compared with kinetics of oxygen uptake ($\dot{V}O_2$), measured breath-by-breath at the lungs, and oxygen consumption at the muscle (estimated by ^{31}P-MRS-measured PCr kinetics), during plantar flexion exercise, in groups of old (n=10; 66.9y) and young (n=10; 27.5y) individuals. Time constants (τ) for $\dot{V}O_2$ and PCr adjustment to, and recovery from, exercise averaged ~43s and, along with CS activity, did not differ between old and young individuals. The CS activity, as a marker of mitochondrial capacity, was not significantly correlated with kinetic measurements. Whereas a high correlation between the kinetics and the rate of mitochondrial reactions would suggest peripheral O_2 utilization in control of kinetics, from the present finding this hypothesis cannot be rejected. CS may not be a representative enzyme of mitochondrial respiration (and is not a rate limiting enzyme) and variability in its measurement may have prevented significant correlations. It is concluded that for exercise of a muscle group (plantar flexors) that is active on a daily basis (i.e. during walking), PCr

and $\dot{V}O_2$ kinetics are maintained in old humans. Further, PCr kinetics measured at the exercising muscle, are similar to phase 2 $\dot{V}O_2$ kinetics, measured at the lung.

The purpose of the second study was to examine the relationship between muscle capillarization (which affects peripheral O_2 delivery) of the lateral gastrocnemius muscle and $\dot{V}O_2$ kinetics during plantar flexion in old (n=9; 66.0y) and young (n=11, 25.9y) individuals, from the same groups as study 1. $\dot{V}O_2$ kinetics and muscle capillarization were similar in these two groups. Capillarization measures were not significantly correlated with the on-transient kinetics. It is concluded that capillarization does not impose a limitation on kinetics, although other factors which influence peripheral O_2 delivery (i.e. capillary lengths capillary branching, capillary recruitment patterns and O_2 diffusion rate, including diffusion from adjacent muscle cells) cannot be eliminated. In this study it was also notable that in young individuals, recovery of $\dot{V}O_2$ following plantar flexion exercise was faster with a greater capillary supply over a given muscle fibre area, but this was not the case in the old individuals.

The third study examined the relationship between $\dot{V}O_2$ and HR (as an estimate of cardiac output) kinetics in old and young individuals, during an exercise that placed small demands on the cardiovascular system (unilateral ankle plantar flexion) and exercises that placed larger demands on the cardiovascular system (cycling and treadmill exercise). Twelve old (66.7y) and

16 young (26.3y) individuals (including those from studies 1 and 2) were compared during plantar flexion and cycling exercise, while separate groups of five old (69.6y) and five young (24.4y) subjects were compared during cycling and treadmill exercise. Old groups had slower $\dot{V}O_2$ and HR kinetics during cycling and treadmill exercise, but similar $\dot{V}O_2$ and HR kinetics during plantar flexion, when compared to the young groups. Old individuals had significantly faster kinetics during plantar flexion and treadmill walking, than during cycling. It is concluded that: 1) Differences with ageing between the modes of exercise may be related to the muscle mass involved, and the circulatory demands; and, 2) Slower $\dot{V}O_2$ kinetics occur to a greater extent in a mode (cycling) in which the muscles are not accustomed to the activity, while in a mode of normal activity (walking) and with muscle groups (plantar flexors) accustomed to the activity, $\dot{V}O_2$ kinetics are not slowed to the same degree with age.

From the above studies, it appears that old individuals can achieve kinetics similar to young subjects, for muscle groups that are moderately exercised on a daily basis. The purpose of the final study, therefore, was to determine the influence of ageing versus relative levels of cardiorespiratory fitness (adjusted for age and sex) on $\dot{V}O_2$ kinetics, measured during cycling in large groups of young (n=16, 26.3y) and old (n=29, 68.9y) individuals of varying fitness levels. Young individuals had a significantly faster $\tau\dot{V}O_2$ than the old group; however, the slope of the $\tau\dot{V}O_2$-$\dot{V}O_{2max}$ regression was steeper in the old group, indicating that fit old subjects had kinetics similar to fit young

individuals. A multiple linear regression indicated that relative fitness (VO_{2max} adjusted for age and sex) was a stronger predictor of $\tau\dot{V}O_2$, than was age. It is concluded that old individuals can achieve $\dot{V}O_2$ kinetics similar to fit young subjects, with moderate improvements in fitness.

7.1. List of Conclusions:

1) These studies show that old individuals maintain the capacity for oxidative metabolism and O_2 delivery at the periphery, with moderate levels of activity. For a muscle group (plantar flexors) used on a daily basis (i.e. for walking), the following characteristics are similar in old and young groups:

a) Rates of oxygen uptake and PCr kinetics measured during the transition to or recovery from plantar flexion exercise (with time constants $\sim$43s).

b) Levels of citrate synthase activity of the lateral gastrocnemius ($\sim$34 $mmol \cdot kg^{-1}$ protein $\cdot min^{-1}$).

c) Capillarization around muscle fibres of the lateral gastrocnemius, expressed as absolute capillary numbers (i.e. capillary to fibre ratio or capillary contacts per fibre) or number of capillaries serving a given fibre area (i.e. capillaries per mm^2, or capillary contacts per fibre area).

2) Old individuals have slower $\dot{V}O_2$ kinetics (than young groups) during an exercise (cycling) that places a greater challenge on the central cardiorespiratory system. Old subjects have slower heart rate kinetics which correlate with the $\dot{V}O_2$ kinetics. Heart rate kinetics during plantar flexion are

similar in old and young individuals, along with their similar $\dot{V}O_2$ kinetics; however, a correlation (of $\dot{V}O_2$ and HR kinetics) exists in the young group only. For the old individuals, $\dot{V}O_2$ and heart rate kinetics are also slower than for the young individuals during treadmill exercise, but significantly faster than during cycle exercise. With kinetics during plantar flexion and treadmill exercise being significantly faster than during cycling, it appears that kinetics during exercise of muscle groups, accustomed to daily activity are not affected to the same degree as those used during unfamiliar exercises.

3) Finally it is concluded that although $\dot{V}O_2$ kinetics are slower during cycling in old compared to young groups, moderately fit old have similar kinetics compared to moderately fit young individuals, implying that $\dot{V}O_2$ kinetics in old individuals can be greatly improved with small improvements in fitness.

With regard to factors that control $\dot{V}O_2$ kinetics in old individuals, it can be speculated that $\dot{V}O_2$ kinetics during exercise of a small muscle mass may be limited by factors at the periphery, related to capillarization and O_2 availability at the muscle cell, or to oxidative mitochondrial capacity. These measures (capillaries, CS) were similar in old and young individuals, along with their similar $\dot{V}O_2$ kinetics. During exercise of a larger muscle mass (i.e. cycling or treadmill walking), $\dot{V}O_2$ kinetics of old individuals appear to be limited by central O_2 transport (i.e. Q kinetics, as estimated by HR kinetics), as both $\dot{V}O_2$ and HR kinetics were slower, and correlated, in old individuals.

7.2. Limitations and Recommendations for Future Studies

All measures affecting $\dot{V}O_2$ kinetics during plantar flexion exercise were similar in old and young individuals (mitochondrial enzyme activity, capillarization, and HR kinetics), but definite conclusions cannot be made regarding which factors are dominant in the control of $\dot{V}O_2$ kinetics in old individuals, as none of these factors correlated with the $\dot{V}O_2$ kinetics, in the old group. Future research in this area should concentrate on isolating these potential controlling or limiting factors, so that one can be changed independently of the others. Changes in $\dot{V}O_2$ kinetics along with changes in each individual factor could then be followed and the controlling mechanisms identified. One method of studying the effects of central O_2 delivery would be to have old subjects breath hyperoxic gas mixtures during transitions to exercise, similar to studies recently performed with young individuals (Hughson & Kowalchuk, 1995). If central O_2 delivery is limiting, one would expect $\dot{V}O_2$ kinetics to be faster in this situation. A perturbation, which would change peripheral muscle characteristics (blood flow redistribution, capillarization, oxidative enzymes) but not affect central O_2 delivery to a great extent, could involve endurance training of small muscle groups (i.e. single leg training).

During cycling, the slower $\dot{V}O_2$ kinetics of old individuals were correlated with slower HR kinetics, which implies that central O_2 delivery may be limiting during this type of exercise; however, measurements of capillarization and enzyme activities of the quadriceps were not made; therefore,

conclusions about whether peripheral factors were impaired, cannot be made.

It is difficult to explain why τHR was slower during cycling, but not plantar flexion exercise in the old group. Increase in HR during a small muscle mass exercise may be caused primarily by parasympathetic withdrawal (which tends to be fast), while during exercise with a large muscle mass, with a greater increase in HR, there is a larger contribution from sympathetic activation (which is a slower process) [Maciel et al., 1986]. Perhaps sympathetic activation is slower in old individuals; this may be due to a diminished beta-adrenergic responsiveness (Seals et al., 1994). A study of kinetics of HR changes to different levels of HR at steady-state in old individuals is another area for future research.

Another limitation of this thesis is the comparison of PCr recovery kinetics to kinetics of VO_2 during the adjustment to exercise, in the review of literature and Chapter 3. It was stated that long PCr kinetics during recovery measured in old individuals (Keller et al., 1985; Hands et al., 1986; McCully et al., 1993) was similar to the long VO_2 kinetics measured during the adjustment to cycling exercise (Cunningham et al., 1993; Babcock et al., 1994a). Here, it was assumed that PCr kinetics during recovery would be similar to those measured during on-transients and that PCr kinetics measured at the muscle reflect VO_2 kinetics measured at the lung. Several investigators have found similarities between PCr on- and off- kinetics (Marsh et al., 1993; McCreary et al., 1996); therefore the former assumption may be valid. Although the kinetics

for PCr and VO_2 during the adjustment to the same exercise task have similar time courses (McCreary et al., 1996), a cause and effect relationship cannot be assumed. There is much evidence to show that VO_2 kinetics can be altered by various manipulations (Hughson and Kowalchuk, 1991, 1995), but very little (if any) evidence to show that these manipulations affect PCr kinetics. A study for the future could involve the simultaneous measurement of VO_2 and PCr kinetics, while breathing hypoxic gas mixtures or following the administration of beta-blockers, two perturbations shown to affect VO_2 kinetics. If PCr kinetics can be shown to be affected to the same degree as VO_2 kinetics, a stronger relationship between PCr and VO_2 kinetics may be inferred.

The use of a supine, as opposed to an upright exercise model (our plantar flexion model) throughout this thesis may have had an effect on VO_2 kinetics (Hughson et al., 1993) and the comparisons between old and young subjects. Kinetics of VO_2 are slower in the supine, compared to the upright position in young individuals (Hughson et al., 1993). The lack of differences between old and young subjects of this study, with respect to kinetics, may have been due to a greater slowing of kinetics in response to supine exercise in the young compared to the old subjects. A study for the future may involve the effects of supine exercise on VO_2 kinetics in old individuals and whether their already slow kinetics can be further slowed in this condition. There may be a limit as to how slow kinetics can get, but this possibility remains to be tested.

The statistical analyses throughout this thesis relied heavily on

correlation coefficients. When performing correlations between two variables which are both estimated with error, the slope of the regression is underestimated by a factor equal to the correlation coefficient (Anderson et al., 1986). The "reduced major axes regression model" proposed by Anderson et al. (1986) has been applied to the regressions performed throughout this thesis, to correct for this fault. The use of regressions between variables estimated with error is a limitation of the statistical analyses throughout this thesis.

Appendices

Appendix I
Characteristics of Subjects in Studies 1 to 3 (Chapters 3,4, and 5)

Old Group

Subject ID	Sex	Age (y)	Mass (kg)	Height (cm)	Cycle VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	Ventilation Threshold
1762	M	67	96.8	179	26.0	13.4
2461	M	64	75	180	21.2	15.0
2147	F	63	66.5	170	24.1	16.5
2381	F	72	65.2	167	20.0	14.9
2333	F	69	56	159	18.1	13.4
2082	F	73	63	167	18.3	13.2
2324	F	52	86.8	165	23.0	17.1
1621	F	65	60.6	161	19.5	16.5
2471	F	79	74	158	17.0	12.7
1978	F	69	63.8	157	19.6	13.2
2053	F	63	69.1	156	20.0	17.3
2226	F	64	60.0	166	18.5	15.2

Young individuals

Subject ID	Sex	Age	Mass	Height	Cycle VO_{2max}	Ventilatory Threshold
2007	M	26	80.0	184	59.0	31.0
2200	M	27	78.0	183	50.1	28.2
2299	M	25	59.5	161	40.0	26.1
2378	M	26	87.2	184	49.6	27.8
2258	M	25	78.5	181	48.0	29.8
1569	M	28	95	180	46.3	28.7
2243	M	29	77.4	177	46.0	28.1
2377	M	26	112	184	35.5	19.5
2283	M	31	89.4	182	35.0	24.3
1437	F	30	67.8	173	52.0	27.4
2376	F	24	67.0	183	44.8	26.8
2426	F	27	57.0	159	37.2	26.1
2410	F	24	50.0	159	37.1	24.2
2501	F	26	61.8	164	36.9	24.3
2557	F	22	64.5	165	45.0	22.5
2403	F	25	84.1	163	25.0	18.8

Appendix II

PCr, $\dot{V}O_2$ and HR Kinetics For Subjects in Studies 1-3

Old

Subject ID	Cycle square wave power (w)	Cycle τVO_2-on (s)	Cycle τVO_2-off (s)	Cycle τHR (s)	Plantar-flexion Square wave power (w)	Plantar-Flexion τVO_2-on (s)	Plantar-Flexion τVO_2-off (s)	Plantar-Flexion τHR (s)	Plantar-Flexion τPCr-on (s)	Plantar Flexion τPCr-off (s)
1762	65	60.7	29.2	-	2.5	42.7	29.3	25.7	34.6	26.5
2461	50	64.3	71.9	-	1.9	70.7	18.4	-	45.2	58.5
2147	45	39.7	38.6	55.7	2.2	31.5	15.9	13.8	14.5	8.1
2381	25	58.9	46.7	60.0	0.8	15.8	43.2	-	39.0	58.1
2333	20	51.0	49.9	102.5	1.6	50.8	50.3	44.9	57.9	82.2
2082	28	77.1	77.0	41.8	1.3	59.0	40.6	23.5	88.3	39.6
2324	40	43.2	64.3	57.7	1.5	40.2	32.1	102	-	-
1621	50	82.5	71	53.5	2.2	46.5	72.5	55.2	32.8	24.4
2471	20	89.0	17.5	149	0.8	38.2	63.3	8.2	18.4	26.2
1978	40	87.4	55.3	106.6	1.6	76.9	74.1	29.9	54.6	38.8
2053	55	65.6	35.0	64.1	1.7	30.9	36.5	5.0	1^.6	19.7
2226	24	43.4	35.1	26.6	1.3	38.2	25.9	11.0	18.8	19.8

Young Individuals

Subject ID	Cycle Square Wave Power (w)	Cycle τVO_2-on (s)	Cycle τVO_2-off (s)	Cycle τHR (s)	Plantar Flexion square wave power (w)	Plantar Flexion τVO_2-on (s)	Plantar Flexion τVO_2-off (s)	Plantar Flexion τHR (s)	Plantar Flexion τPCr-on (s)	Plantar Flexion τPCr-off (s)
2007	190	20.2	26.1	28.0	5.3	37.0	12.2	9.7	21.4	17.8
2200	140	35.0	35.1	40.1	6.0	37.1	38.2	25.0	41.7	26.8
2299	80	50.0	29.4	50.0	2.0	22.2	29.4	10.3	_	_
2378	132	34.9	27.8	42.2	3.8	34.9	27.8	42.2	-	-
2258	147	45.4	37.0	35.4	3.7	48.6	30.8	109	68.6	57.6
1569	140	44.0	37.5	31	3.2	40.0	59.4	10.0	23.0	25.6
2243	100	44.2	43.6	32.5	2.5	35.4	27.8	34.9	61.1	67.1
2377	123	48.1	42.6	65.5	3.8	47.2	49.2	21.9	69.4	58.4
2283	110	50.3	39.4	113	5.8	44.7	45.6	10.8	16.8	23.3
1437	130	25.2	27.6	30.5	3.7	35.6	13.6	18.9	42.3	57.5
2376	90	35.7	36	41.8	3.2	59.6	20.0	53.3	75.9	50.1
2426	79	44.4	32.0	43.9	2.2	56.9	46.0	80.0	57.3	40.4
2410	70	21.5	32.2	29.6	1.7	70.8	41.3	57.8	75.4	57.9
2501	54	46.4	34.3	57	1.8	45.1	45.0	24.3	89.7	54.2
2403	90	74.0	51.5	72.9	3.0	31.9	14.7	8.7	-	-
2557	70	25.6	28.7	32.3	1.9	55.7	45.0	55.7	-	-

Appendix III

Biopsy Data For Subjects in Studies 1 and 2

Old individuals: Capillaries, Citrate Synthase, Overall Mean Fibre Area

Subject ID	Capillary/ fibre	Capillary Contacts/ fibre	Capillary Density (caps·mm^{-2})	Capillary Contacts/ fibre area	Fibre Area (um^2)	Maximal diffusion distance (microns)	Average Diffusion distance (microns)	Citrate Synthase (mmol/kg /min)
1762	1.6	2.8	190	0.34	8150	64.4	31.8	50
2461	1.2	2.4	210	0.42	5694	61.3	30.2	51
2381	1.9	3.8	299	0.60	6353	53.1	26.2	36
2333	1.6	2.7	180	0.31	8596	66.1	32.6	-
2082	3.0	5.5	336	0.61	9022	54.5	27.0	34
2324	1.8	3.5	241	0.48	7295	58.1	28.7	24
1621	1.3	2.4	250	0.46	5263	56.7	27.9	19
1978	1.2	2.1	225	0.39	5352	59.5	29.3	39
2053	1.9	4.1	245	0.53	7699	58.4	28.9	7

Young individuals: Capillaries, Citrate Synthase, Overall Mean Fibre Area

Subject ID	Capillary/ fibre	Capillary Contacts/ fibre	Capillary Density (caps·mm ^{2})	Capillary Contacts/ Fibre Area	Fibre Area (um^{2})	Maximal diffusion distance (microns)	Average diffusion distance (microns)	Citrate Synthase (mmol/kg /min)
2007	2.6	4.2	513	0.84	5000	42.3	20.9	39
2378	2.1	4.1	295	0.58	7073	54.0	26.7	36
2258	2.1	4.4	196	0.40	10970	67.2	33.2	48
1569	1.9	3.1	181	0.29	10595	68.6	33.9	47
2377	2.0	4.2	230	0.48	8758	61.2	30.3	19
1437	1.5	3.5	261	0.59	5917	56.4	27.8	39
2376	1.8	3.3	335	0.63	5260	49.3	24.4	41
2426	1.5	2.3	218	0.33	6933	59.1	29.2	12
2501	2.1	3.9	229	0.42	9311	62.0	30.6	32
2557	1.7	2.7	352	0.56	4803	48.3	23.8	28
2403	1.9	3.7	434	0.86	4310	43.8	21.6	-

Appendix IV

Subjects and Results for Part 2 of Study 3 (Treadmill versus Cycle Kinetics)

Old individuals

Subject ID	Sex	Age (y)	Treadmill τVO_2 (s)	Cycle τVO_2 (s)	Treadmill τHR (s)	Cycle τHR (s)
1258	M	57	44.1	34.9	48.2	40.2
0326	M	72	36.1	49.3	32.3	42.7
0308	M	70	26.5	48.0	33.9	10.3
1239	M	64	36.6	41.5	34.7	32.5
0189	M	75	26.5	50.4	33.9	31.2

Young individuals

Subject ID	Sex	Age (y)	Treadmill τVO_2 (s)	Cycle τVO_2 (s)	Treadmill τHR (s)	Cycle τHR (s)
1213	M	27	26.1	30.2	18.0	19.6
1215	F	26	18.9	26.8	29.8	28.3
1216	F	24	23.1	17.2	14.8	13.8
1217	M	25	12.6	26.5	19.2	20.3
1218	F	19	34.2	26.9	14.1	34.3

Appendix V

Additional Old Subjects For Study 4 -Characteristics and Results

Subject ID	Sex	Age (y)	Mass (kg)	Height (cm)	VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	Ventilatory Threshold	τVO_2 (s)	τHR (s)
2176	M	65	72.0	184	21.0	16.4	38.2	37
1956	M	65	95 9	178	23.9	19.1	51.0	55
2164	M	67	75.0	-	31.2	22.5	41.0	43
1933	M	75	90.3	176	25.6	17.9	40.7	77
0189	M	78	79.0	178	25.2	17.8	58.3	-
0445	M	78	63.8	165	30.5	20.1	46.9	28
0957	M	69	86.5	186	28.3	16.9	44 3	63
2209	M	71	80 0	-	42.3	24.5	33.6	56
1239	M	64	78 0	181	30.5	11.5	26.4	10
1258	M	67	100.0	185	26.0	13.0	31.5	32
0308	M	70	71.1	171	31.4	18.3	69.0	10
2077	F	66	59.9	164	20.4	15.2	54.6	55
2087	F	65	68.0	-	17 5	12.3	40.0	30
2039	F	76	74.5	160	24.4	16 1	55.4	45
1440	F	74	67.2	172	37 8	24.9	26.6	28
2075	F	67	75.3	160	25.3	18.7	70.1	43
2188	F	75	57.4	164	17.5	11.6	91.1	55
2175	F	71	58.5	-	30.4	20.7	51.1	57

Appendix VI
Glossary of Terms and Abbreviations

Actin: The thin contractile filament. Part of the myofillament. Joins with myosin to form cross-bridges during muscular contraction.

ADP: Adenosine diphosphate

Adenine Nucleotide Translocase: An enzyme located on the inner mitochondrial membrane;involved in the transport of ADP into the mitochondria and ATP out to the space between inner and outer mitochondrial membranes.

Adrenoceptor: A receptor for either epinepherine or norepinephrine.

Amplitude: The difference between baseline and steady-state.

ATP: Adenosine triphosphate

ATPase: A term to describe a set of enzymes involved in the hydrolysis of ATP to ADP and Pi.

AVO_2 difference: The difference between the oxygen content of arterial and mixed venous blood.

Beta-Blocker: A beta-adrenergic blocking agent.

Citrate Synthase: An enzyme of the Krebs or Citric Acid Cycle, located in the mitochondria.

Cardiac Output (Q): The amount of blood pumped by the heart in one minute; the product of stroke volume and heart rate.

Central O_2 Transport: The transport of O_2 in the blood from the heart and lungs, through the arterial system, to the exercising muscle.

Cr: Creatine.

Creatine (Phospho-) Kinase (CPK or CK): The enzyme which catalyses the hydrolysis of PCr to Cr and Pi, and the reverse reaction.

Cryostat: An instrument with a cooled knife, used for cutting thin cross-sections of muscle samples, obtained by biopsies. Cross-sections can then be mounted on coverslips, stained for histochemical properties (i.e. fibre type, capillarization) and examined under microscope.

Cytochrome Oxidase: The terminal enzyme of the respiratory chain.

Cytoplasm: The portion of a cell's contents outside of the nucleus.

Cytosol: The watery medium inside cells but outside of cell organelles.

Dynamic Linearity: Where the time course of the system output scales everywhere with input (i.e. when there are no differences between on- and off-kinetics).

Dynamics: A synonym for kinetics or non-steady states.

First-Order: A first order system is one whose equations of motion are described by first derivatives only.

Fourier Transformation: Analyses of a complex waveform (which is the combination of a number of waves of different frequencies and amplitudes) and determines the amplitude, or amount of energy, in each frequency of the wave's components.

Frank-Starling Mechanism: With an increase in venous return and increased filling of the heart, there is an increase in the strength of the subsequent contraction and increased stroke volume.

Free Induction Decay (FID): The transient signal induced in the NMR coil after a radiofrequency pulse has excited the system. It will decay toward zero with a characteristic time constant.

Functional Residual Capacity (FRC): The volume of air in the lungs at resting expiratory level

Half Time: The time for half of a change (from baseline to steady state) in a variable to take place.

Heart rate kinetics: A term to describe the rate of heart rate adjustment to a change in exercise work rate.

Haemoglobin: A protein located in erythrocytes (red blood cells), which carries most of the oxygen in the blood.

Hexagonal Arrays: Used to describe the arrangement of capillaries around individual muscle fibres. A hexagonal array occurs when average capillary contacts = 3, and capillary to fibre ratio = 1.5.

Homogeneous Magnetic Field: Where the field is of constant strength when measured at various places with the volume.

Hyperoxia: A term to describe an excess of oxygen, or above normal amount of oxygen.

Hypoxia: A term used to describe a deficiency of oxygen.

Infrared Spectroscopy: A method by which haemoglobin and myoglobin oxygen saturation is measured, over a muscle bed.

Intracellular Threshold (IT): The point during a ramp test at which there is an accelerated rate of breakdown of PCr and build up of Pi, with a coincident acceleration in the decrease of pH.

In vitro: "In glass", i.e. in the test tube.

In vivo: "In life", i.e. in the cell or organism.

Isozyme: Multiple forms of an enzyme that differ from each other in their substrate affinity, in their maximal activity, or in regulatory properties.

Iterations: A process performed by a computer program when attempting to fit a curve to data collected during breathing experiments (i.e. when attempting to find the "best fit" exponential curve to on-transient VO_2 data). An initial estimate (first guess) of the value of each parameter of the VO_2 response must be provided by the computer operator (parameters for an exponential response include τ, TD, amplitude, and baseline of the VO_2 response). A non-linear regression procedure then adjusts these values to improve the fit of the curve to the data. It then adjusts those new values to improve the fit again. These "iterations" continue until negligible, if any, improvement occurs. This point occurs when there is no longer an appreciable reduction in residuals.

Lateral Gastrocnemius: The outside portion of the muscle on the posterior portion of the lower leg. It inserts into the calcaneus and is involved in plantar flexion.

Linearity: Direct proportionality between one variable and another. A linear system is one whose output variable values scale with input variable values.

Mass Spectrometer: Used for collecting breath-by-breath respiratory data. Monitors changes in gas concentrations, matched to changes in flow.

Michaelis-Menten Kinetics: Kinetics which are described by an equation

relating the velocity and the substrate concentration of an enzyme.

Myofibril: The portion of a muscle fibre containing the contractile filaments of actin and myosin; located in the cytoplasm of the muscle cell.

Myoglobin: A protein within muscle fibres, which binds oxygen. It serves the dual function of storing and transporting oxygen.

NAD: Nicotinamide adenine dinucleotide. A coenzyme functioning as a carrier of hydrogen atoms and electrons in some oxidation-reduction reactions.

NADH+: The reduced form of NAD (NAD after it has accepted a proton).

NMR: Nuclear magnetic resonance.

Old: The term "old" throughout this thesis refers to anyone over the age of 60 years, although we did have one old subject under 60 years of age.

Oxidative Phosphorylation: The process by which energy derived from the reaction between hydrogen and oxygen (to form water) is transferred to ATP during its formation from ADP and inorganic phosphate; occurs in the mitochondria.

Parameter: A quantity whose value characterizes the behaviour of a variable.

PCr: Phosphocreatine.

Periodic Acid-Schiff's: A stain used to mark capillaries in a muscle sample.

Peripheral O_2 Transport: The transport of O_2 through the microvascular net .ork (i.e. capillaries) surrounding muscle, to the exercising muscle.

$P_{ET}CO_2$: Partial pressure of end tidal CO_2.

$P_{ET}O_2$: Partial pressure of end tidal O_2.

pH: The negative logarithm of H+ concentration. It decreases as exercise intensity increases. It's decrease has been implicated as a cause of fatigue at various points in exercising systems.

Phosphate Energy Stores: The ATP and PCr stores within a muscle.

Pi: Inorganic phosphate.

PO_2: The partial pressure of oxygen.

Ramp: Used to describe an exercise test, where the work rate is progressively increasing.

Random Array: A term used to describe the pattern of arrangement of capillaries around muscle fibres. A random array occurs when capillary contacts and capillary to fibre ratio are intermediate to those which characterize hexagonal arrays (see above) and square arrays (where capillary contacts = 4 and capillary to fibre ratio = 2).

Reducing Equivalent: A term for an electron or an electron equivalent in the form of a hydrogen atom.

Residuals: The differences between measured response and modeled response.

Respiratory Chain: A sequence of electron-carrying proteins that transfer electrons from substrates to oxygen in aerobic cells.

Sarcolemma: The muscle cell membrane.

Shimming: A process by which magnetic field inhomogeneity (introduced by placing a sample in the field) is reduced.

Spectrometer: Portions of the NMR apparatus that produce the NMR phenomena and acquire the signals; for example the magnet, probe, etc.

Spectrum: The frequency components of the NMR signal. After Fourier transformation, the different compounds (ATP, PCr, etc.) will appear as a series of peaks called the spectrum.

Steady State: A period during an exercise test where the rate of measured variables (i.e. VO_2) are not changing. When measuring VO_2 during constant-load exercise tests, this is often referred to as "phase 3".

Stroke Volume: The volume of blood ejected by a ventricle during one beat of the heart.

Square Wave: Used to describe an exercise test performed at a constant intensity for a set amount of time. If graphing power output or workmate as a function of time, the shape of the graph would be square-like.

Succinate Dehydrogenase: An enzyme of the Krebs (Citric acid) Cycle.

Supine: A body position described as lying down (on one's back), with face upwards.

T1 Relaxation: Longitudinal relaxation. When putting a sample in a magnetic field, it takes time for that sample to acquire magnetization and align with the field. This is a relaxational process (the energy state is low) and is referred to as longitudinal relaxation, T1 relaxation, or spin-lattice relaxation. T1 relaxation is an exponential process. The T1 relaxation time of a sample = the time it takes for ~63% of the remaining relaxation to take place. The more "watery" a tissue is, the longer its T1 relaxation.

T2 Relaxation: Transverse or spin-spin relaxation. When a molecule with an odd number of protons (i.e. 31-Phosphorous) is placed in a magnetic field, the majority of protons align themselves with the field (in a "low energy state"), along a longitudinal plane. This forms a "vector" of magnetization aligned along the longitudinal plane. If one applies a 90 degree pulse of radiofrequency energy to this vector, the vector rotates (90 degrees) into the transverse plane. The individual vectors will spin around this transverse plane, emitting a radiofrequency signal which is characteristic of the molecule being studied. The individual vectors gradually fall out of line with each other due to interactions with other charged particles in the tissue and inhomogeneities in the field. This causes a gradual disappearance of the net transverse vector. The process by which the net magnetic vector in the transverse plane decreases is referred to as T2 relaxation. This is an exponential process, where the T2 relaxation time = the time in which ~63% of the remaining relaxation takes place. T2 relaxation becomes longer as a tissue becomes more "watery".

Telsa (T): Si unit used to describe magnetic field strength.

Tibialis Anterior: A muscle on the anterior portion of the lower leg. Involved in dorsiflexion.

Time Delay (TD or δ): The time delay before the onset of a response.

Total Lag Time (TLT): The sum of the time constant and time delay of a response. Sometimes referred to as "mean response time (MRT)".

Time Constant (τ): Refers to the amount of time for ~63% of change in a variable from baseline to steady state, during a constant-intensity exercise test.

Turbine: An instrument connected to the mouthpiece during breath-by-breath gas collection, used to measure the volume of air flow. Breathing into the turbine causes the spinning of a propeller, w[illegible] the amount of spin is proportional to the volume of gas passing thro[illegible]h the turbine.

Vagal Tone: Refers to the influence of the parasympathetic nervous system (i.e. this keeps heart rate low; removal of vagal tone upon exercise onset causes HR to increase).

Vascular Conductance: A term which refers to the ease with which blood can flow through the vascular system. Equal to cardiac output divided by mean arterial pressure.

Vastus Lateralis: Often referred to as the quadriceps, the muscle covering the anterior portion of the upper leg. Active mainly during knee extension and hip flexion (as occurs during cycling).

V_ET: Ventilatory threshold.

Ventilation-Perfusion Ratio: Describes the matching of ventilation to pulmonary blood flow. A mismatching of the two results in hypoxia.

VO_2: Oxygen uptake, measured over time.

VO_2max: Maximal oxygen uptake, measured in absolute terms as $L \cdot min^{-1}$ and in relative terms as $mL \cdot kg^{-1} \cdot min^{-1}$. The highest VO_2 attainable by an individual.

VO_2peak: Similar to VO_2max, except this term refers to the highest VO_2 attained on a specific exercise test, and is not necessarily the maximal VO_2 of an individual. VO_2peak and VO_2max have been used interchangeably throughout this thesis, but there is a subtle difference between the terms.

Watts (W): A unit of power determined by dividing work (joules) by time (seconds).

Young: The term "young" throughout this thesis refers to anyone under the age of 35 years.

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