Oxygen Uptake Response to Moderate and Very Heavy Intensity Exercise: relationship with muscle creatine kinase and uncoupling protein.

A thesis presented for the degree of
Masters of Science

by

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Abstract

During a square-wave increase in work rate (WR) there is an immediate and abrupt increase in energy (ATP) demand. The associated phase II kinetics of pulmonary oxygen uptake ($\dot{V}O_2$) are now known to reflect those of muscle oxidative phosphorylation ($\dot{Q}O_2$). The control of $\dot{V}O_2$ kinetics remains a topic of intense debate. One of the candidate mechanisms is feedback control via high-energy phosphate status, which is supported by a close matching of the response kinetics of phosphocreatine (PCr) breakdown and $\dot{V}O_2$ increase following exercise onset. Above the lactate threshold (θ_L) $\dot{V}O_2$ kinetics become more complex, with a delayed slow component which induces a greater O_2 cost which, as work rates exceed the critical power (CP), places $\dot{V}O_2$ on a trajectory for maximum (or peak) $\dot{V}O_2$. The mechanism(s) responsible for the $\dot{V}O_2$ slow component remain conjectural. One recently proposed mechanism might involves mitochondrial uncoupling protein expression.

The present study examined phase II $\dot{V}O_2$ kinetics during moderate and very heavy-intensity cycling exercise with respect to muscle creatine kinase (CK), the enzyme involved in PCr breakdown, whose activity may be important in dictating recruitment of muscle $\dot{Q}O_2$. Second, as mitochondrial uncoupling protein expression (UCP-2 and UCP-3) has been implicated in the compromised oxidative efficiency of the $\dot{V}O_2$ slow component, these kinetics were examined with respect to the degree of UCP expression. Finally, as $\dot{V}O_2$ kinetics above critical power (CP) are poorly characterised, repeats at

two exhausting WRs were used to appropriately characterise $\dot{V}O_2$ kinetics, placing emphasis on the degree of inter-subject variability, and to examine potential relationships of variability in kinetic parameters with CK activity, UCP expression and aerobic fitness.

In six subjects, gas exchange was measured breath-by-breath and $\dot{V}O_2$ kinetics were modelled using commercially available software. Muscle biopsy samples from the resting quadriceps femoris muscle were analysed for CK activity (gel electrophoresis and spectrophotometry). UCP mRNA was measured (reverse-polymerase chain reaction) and expressed relative to protein content (β-actin). Each subject performed an exhausting ramp exercise test for estimation of the lactate threshold (θ_L). Six square-wave exercise tests to 90% of θ_L were then performed on two separate days. The high-intensity power-duration relationship, determined from three to five fatiguing square-wave tests performed on different days, was used to estimate (a) CP (the asymptotic power) and (b) the WRs required to elicit a target duration for fatigue of 12 and 6 minutes.

CK activity ranged from 2.38 to 3.11 absorbance.min^{-1} and τ_{II} $\dot{V}O_2$ for moderate-intensity exercise ranged from 16 to 25s. A significant positive relationship was found between CK activity and τ_{II} during moderate intensity exercise ($p<0.01$). For very heavy-intensity exercise (supra-CP), appreciable inter-subject variability was evident for the $\dot{V}O_2$ kinetics: (a) τ_{II} ranged from 15-46s and showed a non-significant trend towards a positive association with CK activity; (b) the slow component amplitude (A_{sc})

ranged from 0.47 to 1.02 $l.min^{-1}$, but this variability did not significantly associate with UCP expression, which ranged from 2.2 to 5.4 O.D for UCP-2 and 1.2 to 3.0 O.D. for UCP-3; and (c) variability in the $\dot{V}O_2$ kinetic parameters was not significantly correlated with markers of aerobic fitness.

This study has shown that the phase II $\dot{V}O_2$ time constant related to CK activity, although a cause-and-effect relationship could not be determined. Furthermore, the amplitude of the $\dot{V}O_2$ slow component was not related to UCP mRNA expression (contrary to a recent report) and therefore elucidation of the mechanism causing the slow component awaits further investigation.

Declaration

I hereby declare that this thesis has been composed by myself, that all the work of which is recorded has been done by myself, except removal and storage of muscle biopsy samples, which was performed by Professor W. Behan and her technical staff, taking and analysis of blood lactate samples, which was performed technical staff, J. Wilson, assistance given by Dr. N. MacFarlane for creatine kinase analysis and Dr. M. MacEntergart for analysis of uncoupling protein expression.

The work of this thesis has not been submitted in any previous application for a higher degree and that all sources of information have been specifically acknowledged by means of referencing.

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A most sincere thank-you is extended to the volunteer subjects who not only made many visits to the laboratory, but also endured strenuous testing, and co-operated in every way.

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I would like to thank my mother and brother for always providing loving support and encouragement and to my father, to whom this thesis is dedicated; you inspire and motivate me - not only are you my father, you are my teacher and friend. I thank you for everything you have done for me over the years.

This experience would not have been possible without the support of the "Commonwealth Scholarship" and I am most grateful for the opportunity it afforded me.

Dedicated to my father

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Abbreviations

A_{II}	$\dot{V}O_2$ phase II amplitude
A_{sc}	$\dot{V}O_2$ slow component amplitude
$A_{sc\ cor}$	$\dot{V}O_2$ slow component amplitude "corrected"
$A_{sc\ 5\text{-}3}$	$\dot{V}O_2$ slow component rate of increase
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
CaO2	Arterial oxygen content
CK	Creatine kinase
CO_2	Carbon dioxide
C_vO_2	Mixed muscle venous oxygen content
Cr	Creatine
CP	Critical power
DCA	Dichloroacetate
DNA	Deoxyribonucleic acid
Δ	Delta
ΔG	Free energy
EMG	Electromyography
$FADH_2$	Flavin adenine dinucleotide
G_{II}	$\dot{V}O_2$ phase II gain

G_{sc}	$\dot{V}O_2$ slow component gain
$G_{sc\ cor}$	$\dot{V}O_2$ slow component gain "corrected"
H^+	Hydrogen ion
H_2O	Water
HCO_3^-	Bicarbonate ion
HR	Heart rate
MPF	Mean power frequency
mRNA	Messenger ribonucleic acid
NADH	Nicotinamide adenine dinucleotide
N_2	Nitrogen
NO	Nitric oxide
NOS	Nitric oxide synthase
[La]	blood lactate concentration
Km	Michaelis constant
O_2	Oxygen
P	Power
PCr	Phosphocreatine
$P_{ET}CO_2$	Partial pressure of carbon dioxide
$P_{ET}O_2$	Partial pressure of oxygen
P_i	Inorganic phosphate
P_iO_2	Intracellular partial pressure of oxygen
PDH	Pyruvate dehydrogenase
P_mO_2	Microvascular partial pressure of oxygen
RER	Respiratory exchange ratio

RT-PCR	Reverse transcriptase polymerase chain reaction
θ_L	Lactate threshold
t	Time
$\dot{Q}$	Muscle blood flow
$\dot{Q}O_2$	Muscle oxygen uptake
τ_{II}	Phase II time constant
UCP	Uncoupling protein
$\dot{V}CO_2$	Pulmonary carbon dioxide output
$\dot{V}_E$	Pulmonary ventilation
$\dot{V}O_2$	Pulmonary oxygen uptake
$\dot{V}O_2$ ee	End-exercise pulmonary oxygen uptake
$\dot{V}O_2$ peak	Peak pulmonary oxygen uptake
$\dot{V}O_2$ max	Maximal pulmonary oxygen uptake
W'	Anaerobic work capacity
WR	Work rate

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$A_{sc\ 5\text{-}3}$ represents the rate of slow component increase, A_{sc} is the amplitude of the slow component increase, G_{sc} is the gain of the slow component, $G_{sc\ cor}$ is the 'corrected' gain of the slow component, A_{sc} % A_{ee} is the proportional contribution of the slow component.

Table 3.5. Individual values for work rate, performance time, Δ[La] and phase II $\dot{V}O_2$ kinetics for the "6 minute" test.

WR represents work rate, Δ[La] represents the increase in blood [lactate], τ_{II} represents the phase II time constant, SD represents standard deviation, A_{II} represents the phase II amplitude, G_{II} represents the phase II gain and A_{II} % A_{ee} represents the proportional contribution of A_{II} to the total $\dot{V}O_2$ increase.

Table 3.6. Individual values for the slow component for "6 minute" test.

$A_{sc5\text{-}3}$ represents the rate of slow component increase, A_{sc} is the amplitude of the slow component increase, G_{sc} is the gain of the slow component, $G_{sc\ cor}$ is the 'corrected' gain of the slow component, A_{sc} %A_{ee} is the proportional contribution of the slow component

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CHAPTER 1

INTRODUCTION

1.1 ENERGY PRODUCTION DURING EXERCISE

The energy required for daily living and for increases in metabolic demand, such as with exercise, is supplied through the potential energy of the high-energy phosphate bonds in adenosine triphosphate (ATP). As the storage of ATP within the muscle is minimal (~5 mM wet/kg) (Hultman, 1967), ATP re-synthesis is provided through the anaerobic alactic, anaerobic lactic and aerobic energy producing systems. These processes of energy transfer in exercise or bioenergetics are detailed in textbooks of exercise physiology (McArdle et al., 1991; Wasserman et al., 1987; Brooks et al., 2000). This section briefly reviews energy production during exercise.

The anaerobic alactic systems involve the breakdown of stored muscle phosphocreatine (PCr) which then donates a high-energy phosphate to adenosine diphosphate (ADP) for ATP and creatine (Cr) production, as shown in equation 1.1:

$$PCr + ADP + H^{+} \leftrightarrow Cr + ATP \qquad [1.1]$$

The muscle can store approximately 15 mM wet/kg of PCr (Hultman et al., 1967) (250-400 mg total), therefore providing an extremely rapid but limited supply of ATP (~6s for "all out" exercise) (Pernow and Karlson, 1971).

The anaerobic lactic system produces ATP through the oxidation of carbohydrates by way of glycolysis and lactate and H^+ production (Gollnick and Hermansen, 1973). Glycolysis involves a series of chemical reactions which breakdown glucose (or glycogen) to the end-product pyruvate acid, and then pyruvate is converted to lactate as shown by equation 1.2. This yields a low ATP in comparison to the capacity of the aerobic system, but at a more rapid rate (Alpert, 1965).

$$\text{Glucose} + 2P_i + 2\text{ADP} \rightarrow \text{pyruvate} \leftrightarrow \text{lactate} + 2H_2O + 2\text{ATP} \qquad [1.2]$$

where P_i is inorganic phosphate, ADP is adenosine diphosphate and ATP is adenosine triphophate.

The aerobic system utilises the energy available through the oxidation of carbohydrates, fats and to a much lesser degree, protein. As described above, glucose or glycogen is broken down through glycolysis resulting in the production of pyruvate. As pyruvate is then converted to acetyl coenzyme A (acetyl CoA), it is available for the Krebs cycle within the mitochondria. Fats undergo β-oxidation leading to the production of acetyl CoA.

Proteins, depending on the specific amino acids, enter the energy production system at the level of pyruvate, acetyl CoA or directly within the Krebs cycle. Acetyl CoA undergoes further chemical reactions within the Krebs cycle, which most importantly lead to the generation of nicotinamide adenine dinucleotide (NADH) and, to a lesser degree, flavin adenine dinucleotide ($FADH_2$). These reduced substrates are then available to the electron transport system and go through a series of redox reactions, with O_2 being the final electron acceptor. The energy from these electron transfers pumps protons across the mitochondrial membrane and the electrical gradient then harnesses the energy required for ATP re-synthesis. The O_2 taken up per ATP produced is higher for fat than carbohydrate, although the CO_2 production per O_2 is less for fat than carbohydrate metabolism (e.g. Brooks et al., 2000) as shown by equations 1.3 and 1.4, respectively:

$$C_{16}H_{32}O_2 + 23O_2 \leftrightarrow 16CO_2 + 15H_2O + 130ATP \qquad [1.3]$$

$$C_6H_{12}O_6 + 6O_6 \leftrightarrow 6CO_2 + 6H_2O + 36/37ATP \qquad [1.4]$$

During rest and in the steady state of exercise, by definition the energy systems are in a state of equilibrium. It is during the transition from one work rate to another that the steady-state equilibrium is disrupted. With an instantaneous change from either rest or a low work rate to a higher work rate, the increased metabolic demands are met by the energy production systems working in combination. Ideally, during sustained exercise, the greater the aerobic contribution to energy production the better; i.e. most efficient mode of ATP production. This is because there is a limited supply of stored PCr and less fatigue will

ensue if the product of lactate (and therefore H^+) from the anaerobic lactic system is minimised. Therefore, routes of ATP re-synthesis determine the intensity and duration of exercise, and the rate of increase in oxygen utilisation at the onset of exercise is also important in relation to the requirement imposed on the other energy systems.

1.2 MUSCLE OXYGEN CONSUMPTION

As O_2 is the final electron acceptor for aerobic production of ATP, during exercise muscle O_2 uptake ($\dot{Q}O_2$) provides an indication of the rate of aerobic production of ATP. $\dot{Q}O_2$ is given by the Fick equation 1.5:

$$\dot{Q}O_2 = \dot{Q} \bullet (CaO_2\text{-}CvO_2) \qquad [1.5]$$

where $\dot{Q}$ is muscle blood flow, CaO_2 is arterial oxygen content and CvO_2 is muscle venous oxygen content.

In humans, measurements of $\dot{Q}$ can be made at the feed artery of the exercising muscle group indirectly using Doppler ultrasound (Radegren and Saltin, 1998) and directly using thermodilution techniques (Andersen and Saltin, 1985). The O_2 content in the femoral artery and vein of the exercising muscle group can be determined from blood samples by catheterisation, but this approach is highly invasive. In addition, the combination of the above measurements does not provide an exact reflection of the $\dot{Q}O_2$ for the exercising

portions of the muscle (or active motor units). This is because: i) the blood flow and the venous O_2 content measures also include non-exercising muscle and skin; ii) within the exercising muscle group $\dot{Q}$: $\dot{Q}O_2$ inhomogeneities exist (Richardson et al., 2001); and, iii) there is a temporal delay between $\dot{Q}O_2$ and the sample site for CvO_2. A useful expedient in humans that circumvents these approaches is to measure pulmonary oxygen uptake ($\dot{V}O_2$), which has been argued to provide a proxy for $\dot{Q}O_2$. For example, Barstow et al. (1990) have modelled the $\dot{V}O_2$ and $\dot{Q}O_2$ inter-relationships during moderate exercise and concluded that $\tau\ \dot{V}O_2$ is within 10% of $\tau\ \dot{Q}O_2$. In addition, Grassi et al. (1996) have shown a similarity in $\tau\ \dot{V}O_2$ and $\tau\ \dot{Q}O_2$, in humans when $\dot{Q}O_2$ is measured in humans via limb blood flow measurement and the arteriovenous O_2 difference across the exercising muscle group.

Thus, not only in the steady state but also in the transient, the exercise-induced increase in muscle oxygen uptake ($\Delta\dot{Q}O_2$) is reflected, with essentially no loss of dynamic fidelity, in a corresponding increase in pulmonary oxygen uptake ($\Delta\dot{V}O_2$). This is provided no additional movements of non-exercising muscles are incorporated (i.e. of the upper body). The pulmonary $\dot{V}O_2$ response to exercise will also include the oxygen cost of respiratory muscles and the heart, although these have been argued to be a minimal contribution in healthy subjects even at maximal-intensity exercise (Poole et al., 1994). In addition, it should be noted that an anatomical muscle-to-lung transit delay exists, such that blood from the exercising muscle does not appear instantaneously in the pulmonary capillaries (Krogh

and Linhard 1913; Whipp et al., 1982). Thus, the $\Delta\dot{Q}O_2$ response to exercise is reflected in the $\Delta\dot{V}O_2$ after an appropriate time delay (see section 1.5 for further discussion).

Over the last two decades or so, the development of breath-by-breath $\dot{V}O_2$ measurements using rapidly-responding gas concentration and volume transducers has resulted in a substantially improved resolution of response dynamics (Beaver et al., 1973 and 1981). Also, by performing multiple repeats, the signal-to-noise ratio can be appreciably increased (Lamarra, 1987).

1.3 EXERCISE INTENSITY DOMAINS

The characteristics of the temporal response profile of $\dot{V}O_2$ are dependent on the exercise intensity. This is well illustrated for a square-wave change in work rate (Whipp and Wasserman, 1972; Whipp and Mahler, 1980; Paterson and Whipp, 1991; Ozyener et al. 2001). Pulmonary gas exchange depends critically on whether ATP is re-synthesised solely from aerobic means or whether anaerobic lactate-producing mechanisms are also activated. It is useful therefore, to define exercise intensity in terms of arterial lactate concentration [La] (Whipp and Wasserman, 1972). There are two important functional demarcations that can be considered in this regard: the lactate threshold (θ_L) and the critical power (CP) [or fatigue threshold (θ_F)].

θ_L is a threshold $\dot{V}O_2$ above which there is a sustained increase in arterial [La]. It has been argued by Whipp (1994) that most of the evidence to date indicates anaerobiosis may be the most likely mechanism for the increased [La]. With a lack of O_2 supply, a limitation in the rate at which the pyruvate produced from glycolysis can be utilised by the Krebs cycle would occur and therefore the pyruvate would be converted to La. This suggestion is supported by the evidence that, when inspiring a hyperoxic mixture during progressive exercise, the onset of [La] increase was delayed, and similarly, with hypoxia, θ_L was reduced (e.g. Yoshida et al., 1987). Another suggested hypothesis is that there is an increase in the recruitment of type II fibres at the onset of θ_L. As type II fibres have less mitochondria, their oxidative potential is lower (Holloszy, 1967), and therefore their recruitment may result in an increased lactate production. Although the mechanism responsible for θ_L remains to be elucidated, it is clear that there is in an increase in [La]. This can be estimated non-invasively through gas exchange measurements as an increase in pulmonary carbon dioxide output ($\dot{V}CO_2$) relative to $\dot{V}O_2$ (V-slope) as shown in Figure 1.1 (Davis, 1985; Beaver et al., 1986; Whipp et al., 1986).

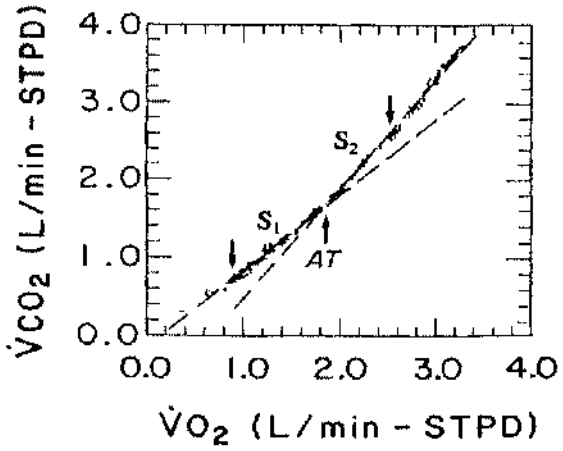

Figure 1.1 Non-invasive estimation of the lactate threshold (AT) using the V-slope technique (Beaver et al., 1986). Best-fit lines (S_1 & S_2) are plotted through the sub- and supra -θ_L(AT) data respectively, θ_L(AT) determined as the point of intersection of the two lines. Adapted from (Beaver et al., 1986).

This increased $\dot{V}CO_2$ is due to HCO_3^- buffering a proportion of the H^+ associated with the lactate that is produced in the conversion from pyruvate (see section 2.4.1 for details regarding identification of θ_L).

CP is the work rate above which the tolerable duration (t) of constant-load exercise decreases systematically with increased work rate (Monod and Scherrer, 1965; Moritani et al., 1981; Poole et al., 1988). The power asymptote of the hyperbolic power-duration relationship (P-t), or the y-intercept of its P-1/t linear transform, defines this work rate

(Poole et al., 1988; Hill, 1993) (see equation 1.6). In addition, the curvature constant of the P-t hyperbola, or the slope of the P-1/t relationship, represents a constant amount of work (W') that can be performed above CP (Moritani et al., 1981; Poole et al., 1988; Fukuba and Whipp, 1999) (see equation 1.7):

$$P = W' \cdot (1/t) + CP \qquad [1.6]$$

$$W' = (P - CP) \cdot t \qquad [1.7]$$

An example of the P-t curve and the corresponding P-1/t relationship is shown in Figure 1.2.

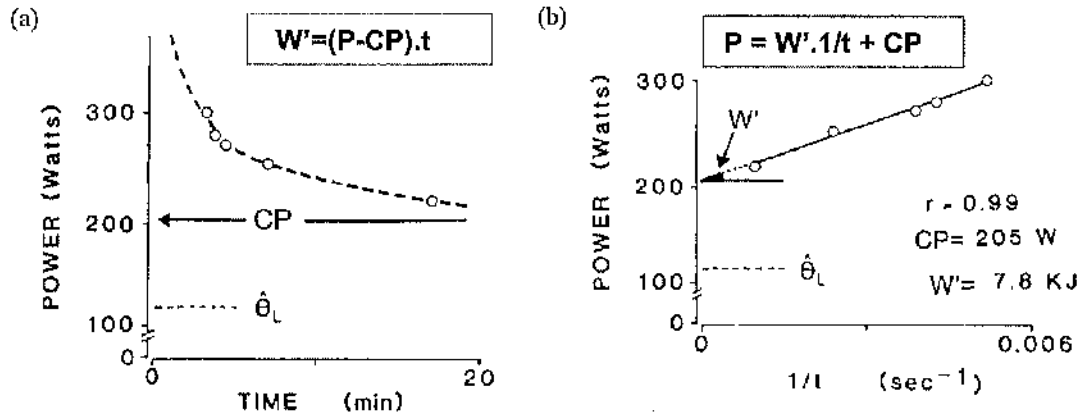

Figure 1.2. The power-duration relationship for x constant-load exercise bouts. (a) Shows the hyperbolic P-t relationship where the asymptote of the power axis is CP and the curvature constant is W'. (b) Shows the P-1/t relationship, where the intercept on the power axis is CP and the slope of the line is W' (adapted from Poole et al., 1988).

The mechanism of CP remains to be elucidated. However, it has been suggested CP coincides with the work rate equivalent to the maximal lactate steady state (MLSS), wherein there is an elevation in [La] due to production, but the level remains stable as the production is balanced by the rate of clearance (e.g. Poole et al., 1988). However, recently Pringle et al. (2002) have suggested that CP determined from the power-duration curve occurred at a WR ~6% higher than the WR corresponding to MLSS (defined as when a less than 1mmol.l^{-1} increase in [La] occurred across the last 20 min of exercise). Therefore, further research is required to elucidate the association between CP and MLSS as the mechanism of CP.

W' has been referred to as the 'anaerobic work capacity' and it has been suggested it is related to finite energy stores of high-energy phosphates, glycogen stores (for anaerobic glycolysis) and O_2 stores (Monod and Scherrer, 1965; Moritani et al, 1981; Hill 1993; Miura et al., 2002). More recently, it has also been suggested W' could be associated with the build up of fatiguing intramuscular metabolites (H^+ and P_i) reaching critical levels (Coates et al., 2003).

θ_L expressed as a percent of $\dot{V}O_{2\,peak}$ is variable between individuals (ranging by as much as 40-80%) (Davis et al., 1976; Reinhard et al., 1979). Likewise, CP when expressed as a percentage of WR peak is variable across subjects (Whipp and Ward, 1992). Thus, as will be discussed later, the WR normalisation to determine a similar relative stress for different

individuals cannot be done simply relative to $\dot{V}O_{2\,peak}$, as all fatiguing WRs at and above CP elicit a $\dot{V}O_2$ essentially equal to $\dot{V}O_{2\,peak}$ (see section 4.3). Therefore, expressing the WR as a percentage of the $\dot{V}O_{2\,peak}$ (or maximum heart rate, maximum work rate, or METs, which represent multiples of the resting metabolic rate, where 1 MET is 3.5 $ml.kg^{-1}.min^{-1}$) does not provide an appropriate method to define the exercise intensity. This is because it would not be known a priori (Whipp and Ward, 1992; Whipp et al., 2002) if the work rates prescribed would be above or below an individual's $0_{I,}$ and CP.

As these thresholds are closely associated (Pringle et al., 2002) with lactate status, the partitioning of the different exercise intensities has been based on arterial [La] (Wasserman et al., 1967). Moderate-intensity exercise is defined as those WRs that lie below θ_L, thus either no increase in [La] occurs or [La] increases initially but then returns to baseline values. Heavy-intensity exercise is defined as work rates that are above θ_L, and thus [La] increases initially but over time reaches a steady state level, or may even decline slightly. Very heavy exercise refers to work rates that are above CP, with [La] increasing throughout the work bout to the point of fatigue (Whipp and Ozyener, 1998; Ozyener et al., 2001) (see Figure 1.3).

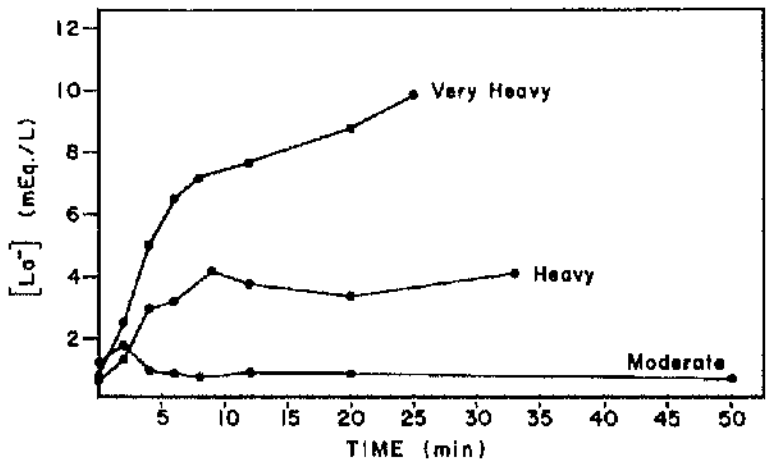

Figure 1.3. Assignment of exercise intensity based on the profiles of arterial [La] during constant-load exercise at different work rates. Adapted from Wasserman et al. (1967).

The $\dot{V}O_2$ response within each of the exercise domains will be discussed in detail below. However, briefly, it can be see in Figure 1.4 that, within the moderate intensity domain $\dot{V}O_2$ increases exponentially to attain a steady state; in the heavy domain $\dot{V}O_2$ continues to increase and eventually stabilises; in the very heavy intensity domain, $\dot{V}O_2$ continually increases and is truncated at $\dot{V}O_{2\,peak}$; and, in addition, a severe intensity domain is defined, which occurs when the $\dot{V}O_2$ requirement is greater than $\dot{V}O_{2\,peak}$ and therefore $\dot{V}O_2$ quickly impacts on $\dot{V}O_{2\,peak}$, resulting in a very short tolerable duration (Whipp and Ozyener, 1998; Ozyener et al. 2001)

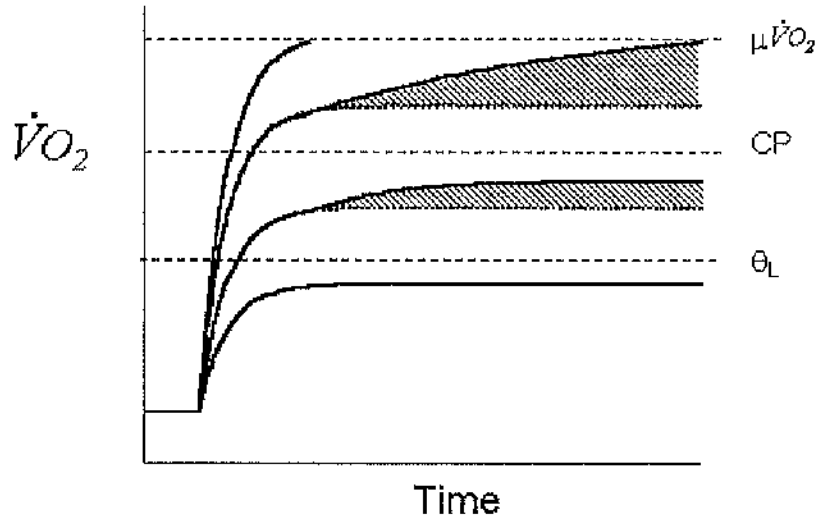

Figure 1.4. Illustration of the dependence of $\dot{V}O_2$ kinetics on exercise intensity domain. Thick solid lines represent the $\dot{V}O_2$ response, and dashed lines signify the intensity domain boundaries of lactate threshold (θ_L), critical power (CP) and $\dot{V}O_2$ peak ($\mu\dot{V}O_2$). Shaded areas reflect the additional $\dot{V}O_2$ above that predicted for the given work rate based on $<\theta_L$ equations. From Whipp & Ozyener (1998).

1.4 TEMPORAL DOMAINS FOR $\dot{V}O_2$

1.4.1 Moderate intensity exercise

Phase I

This period of time is due to the muscle-to-lung transit delay and lasts approximately 15-20s (Cummin et al., 1986; Casaburi et al., 1989). The blood returning from the muscle to

the pulmonary circulation still reflects the composition of the blood in the muscle prior to or very early in the transient, and thus the arterio-mixed venous O_2 content difference (CaO_2-CvO_2) will not yet be increased (i.e. the blood reflecting an increased oxygen extraction with exercise has not yet arrived at the lungs) (Krogh and Lindhard, 1913; Whipp et al., 1982). However, an increase in $\dot{V}O_2$ is apparent during this period due to the instantaneous increase in $\dot{Q}$, and therefore pulmonary blood flow, when the work rate is increased (Cummin et al., 1986). This initial phase is therefore termed the 'cardiodynamic phase' (Krogh and Lindhard, 1913; Whipp et al., 1982). A schematic of the phase I response is shown in Figure 1.5 (page 17).

Phase II

Phase II follows the cardiodynamic phase. The $\dot{V}O_2$ response in phase II represents the dominant component of the non steady-state response and has been argued to reflect $\dot{Q}O_2$, as noted earlier. The $\dot{V}O_2$ response increases mono-exponentially toward the steady state according to the equation:

$$\Delta \dot{V}O_2\ (t) = \Delta \dot{V}O_2\ (ss) \cdot [1\text{-}e^{-(\tau\text{-}\delta)/(t)}] \qquad [1.8]$$

where $\Delta\ \dot{V}O_2$ (t) is the $\dot{V}O_2$ increment at time t, $\Delta\ \dot{V}O_2$ (ss) is the steady state $\dot{V}O_2$ increment above baseline, τ is the time constant (i.e. the time to reach 63% of the steady-state $\dot{V}O_2$) and δ is the delay term reflecting the influence of the muscle-to-lung transit time

(Linnarsson, 1974; Casaburi et al., 1978; Whipp et al., 1982; Miyamoto et al., 1982; Barstow et al., 1994; Hughson and Kowalchuk, 1995). A schematic of the phase II response is shown in Figure 1.5 (page 17).

Both the amplitude of the steady-state response ($\Delta \dot{V}O_2$ (ss)) and the phase II time response (τ_{II}) of the increase are of much importance as they provide an indication of the amount of energy not supplied through oxidative phosphorylation, i.e. the 'oxygen deficit' (O_2def) (Whipp et al., 1982; Whipp and Ward, 1992). The equation for O_2def is:

$$O_2\text{def.} = \tau_{II} \cdot \Delta \dot{V}O_2 \text{ (ss)} \qquad [1.9]$$

For a given work rate, $\Delta \dot{V}O_2$ (ss) is fairly constant among individuals (Hansen et al., 1987). The more significant component affecting the magnitude of the O_2 deficit is τ_{II}, as it can vary appreciably among individuals, with typical τ_{II} values for young, healthy subjects of 25-35s (Whipp et al., 1982, 2002). From cross-sectional data, τ_{II} is longer in subjects with lower cardiorespiratory fitness (Hickson et al., 1978), although τ_{II} as a function of $\dot{V}O_{2\,peak}$ has also been shown to vary widely in the normal population (Whipp et al., 2002). τ_{II} is greater in older individuals (Babcock et al., 1994) and in cases of various chronic diseases (Sietsema, 1992; Hansen et al., 1987; Nery et al., 1982). From longitudinal studies, τ_{II} has been shown to be increased with age (Bell et al., 2001a) and decreased with training (or likewise increased with de-training) (e.g. Phillips et al., 1995).

To indicate whether the process involved in determining τ_{II} is a single rate-limiting step, a control systems perspective follows. In terms of control system theory, it was originally thought that $\dot{V}O_2$ kinetics conformed to a dynamically linear system within the moderate-intensity domain (Whipp, 1971; Whipp and Ward, 1990); that is, similar τ_{II} values would result for any change in work rate (e.g. Fujihara et al., 1973a). Additionally, there would be "on- off"symmetry of the time course of the phase II on-transient, and indeed the off-transient does appear to be similar to the on transient τ_{II} (Linnarsson, 1974; Whipp et al., 1982; Griffiths et al., 1986;Paterson and Whipp, 1991; Ozyener et al., 2001). Recently Rossiter et al. (1999, 2002b) have shown symmetry between the on- and off-transients for both $\dot{V}O_2$ and [PCr] for the moderate intensity domain.

This is consistent with the control system following first-order principles. Similarly, the principle of superposition would be expected to apply (Barstow et al., 1994; Rossiter et al., 2002), with estimates of τ being unaffected by the particular WR forcing function being used. Indeed, investigations have variously used not only square-wave WR forcing (see above), but also ramps, (e.g., Hughson et al., 1986; Babcock et al., 1992; Niizeki et al., 1995), impulses (e.g. Miyamoto et al., 1983; Hughson et al., 1988), sinusoids (e.g. Casaburi et al., 1977; Miyamoto et al., 1983, 1992; Cunningham et al., 1993) and pseudo-random binary sequence (PRBS) (e.g. Hughson et al., 1990, 1991; Essfeld et al., 1987; Edwards et al., 2001). However, arguing against a dynamically linear $\dot{V}O_2$ kinetics in moderate exercise, Hughson and Morrissey (1982) and more recently Brittain et al. (2001) and Paterson et al. (2003), have demonstrated that τ_{II} can differ with WR, or the prior exercise

condition. τ_{II} was longer in the upper region (in transition from the lower region) versus lower region (in transition from loadless exercise) of moderate-intensity exercise. In addition, Rossiter et al. (2002b) recently found that τ was significantly longer for the off-transient in the upper-moderate intensity region for both $\dot{V}O_2$ and [PCr], although the reason for these differing results is unclear at present.

Phase III

The phase III $\dot{V}O_2$ response represents the sum of the phase I and phase II responses. For cycle ergometry, it has an amplitude (or 'gain'), relative to work rate ($\Delta\dot{V}O_2/\Delta$WR) of ~10 ml.min^{-1}.watt^{-1} (Hansen et al., 1987). A schematic of the phase III response is shown in Figure 1.5.

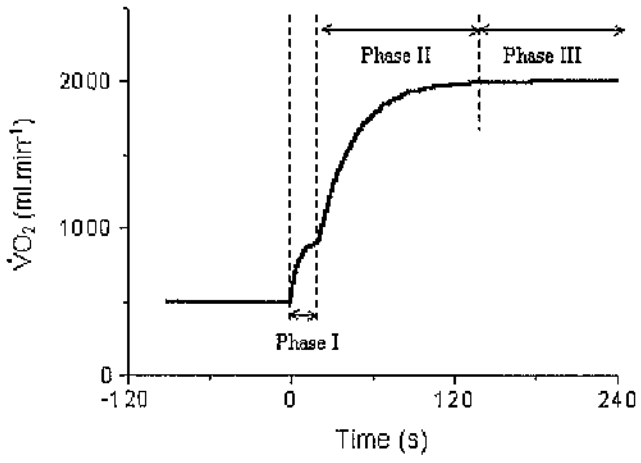

Figure 1.5. Illustration demonstrating the three phases of $\dot{V}O_2$ kinetics for moderate intensity exercise. Zero seconds represents the onset of exercise.

1.4.2 Heavy-Intensity Exercise

As for sub-θ_L WRs, a phase I $\dot{V}O_2$ is also evident for all intensities above θ_L.

However, the kinetics of the subsequent $\dot{V}O_2$ response become more complex because of the appearance of an additional component with delayed and slowly developing characteristics, termed the $\dot{V}O_2$ 'slow component' or 'excess' $\dot{V}O_2$ (Whipp, 1987). This additional component introduces non-linearity into the overall response. These system non-linearities therefore imply that WR forcing functions such as sinusoids and PRBS are not appropriate for > θ_T characterisations, as they results in "lumped" parameter values for the combined on off transition (Whipp and Ward, 1990).

Phase II

The phase II time constant (τ_{II}) for heavy-intensity exercise has been shown to be similar to that of moderate-intensity cycle ergometry (Barstow and Mole, 1991; Barstow et al., 1993; Ozyener et al., 2001; Paterson et al. 2003; Pringle et al., 2003) and treadmill running (Carter et al. 2002). However, it has also been shown that at WRs above θ_L, τ_{II} for cycling is marginally increased (~5-10s) in contrast to work in the moderate-intensity domain for cycling (Paterson and Whipp, 1991; MacDonald et al., 1997; Sheuermann et al, 1998; Koga et al., 1999; Jones et al., 2002).

Similar to most studies of moderate-intensity exercise, the on- and off-transient τ_{II} values in the heavy-intensity domain have been shown to demonstrate symmetry (Ozyener et al., 2001; Paterson and Whipp, 1991). The gain of the phase II response has been shown to be

similar to that of moderate-intensity exercise (Paterson and Whipp, 1991; Barstow and Mole, 1991; Ozyener et al., 2001) as well as lower than that for moderate-intensity exercise (Jones et al., 2002; Pringle et al., 2003).

Slow component

In the heavy-intensity domain, the $\dot{V}O_2$ response is delayed in reaching a new steady state. Furthermore, $\dot{V}O_2$ increases to a value beyond that predicted by the $\Delta\dot{V}O_2/\Delta$WR estimation for sub θ_L (therefore less efficient), to as much as 13 $ml.min^{-1}.W^{-1}$ (Whipp, 1987; Ozyener et al., 2001). The excess $\dot{V}O_2$ component can stabilise, given sufficient time eg. 10-20 minutes (Whipp and Mahler, 1980).

The amplitude of the slow component appears to be less in more fit individuals (Barstow et al., 1996), decreases with training (Carter et al., 2000) and increases with increases in work rate above θ_L (Whipp and Mahler, 1980). In the heavy intensity domain, a loss of "on-off" symmetry occurs as the slow component is apparent for the on transient but not for the off-transient response (Ozyener et al., 2001; Paterson and Whipp, 1991).

An empirical and useful indicator of the $\dot{V}O_2$ slow component magnitude is the $\Delta\dot{V}O_2$ between minute 3 and 6 of exercise (Roston et al., 1987; Whipp, 1987; Casaburi et al., 1987; Poole et al., 1991; Gerbino et al., 1996; Russell et al., 2002). This index makes no assumptions about underlying model structure.

However, controversy exists regarding the appropriate kinetic characterisation of the $\dot{V}O_2$ response of supra θ_L exercise. Paterson and Whipp (1991) used a model that omitted the phase I component and fitted the subsequent 3 minutes of exercise with a single exponential:

$$\Delta\dot{V}O_2\,(t) = \Delta\dot{V}O_2\,(ss) \bullet [1\text{-}e^{-(\tau\text{-}\delta)/t)}] \qquad 20s \leq t \leq 180s \qquad [1.10]$$

where $\Delta\dot{V}O_2$ (t) is the $\dot{V}O_2$ increment at time t, and $\Delta\dot{V}O_2$ (ss) is the asymptotic $\dot{V}O_2$ increment above baseline.

It has been demonstrated that the slow component is of delayed onset, beginning at approximately 90-160s following the exercise onset (Whipp et al., 1987; Paterson and Whipp, 1991; Barstow and Molc, 1991; Barstow et al., 1994; Scheuermann et al., 1998; Bell et al., 2001a; Ozyener et al., 2001). Some investigations have therefore used a two-component, double exponential (omitting phase I) to characterise the $\dot{V}O_2$ response (Barstow and Molc, 1991; Bearden and Moffat, 2000; Bell et al. 2001a; Ozyener et al., 2001):

$$\Delta \dot{V}O_2 (t) = \Delta\dot{V}O_2 (ss) \cdot [1\text{-}e^{-(\tau 1-\delta 1)/(t1)}] + \Delta\dot{V}O_2 (sc) \cdot [1\text{-}e^{-(\tau 2-\delta 2)/(t2)}] \qquad [1.11]$$

for $20s \leq t_1 < \delta_2$; $\delta_2 \leq t_2 \leq 360s$

where $\Delta\dot{V}O_2$ (t) is the $\dot{V}O_2$ increment at time t, $\Delta \dot{V}O_2$ (ss) is the asymptotic $\dot{V}O_2$ increment above baseline, τ_1 phase II time constant and $\delta_1$is the delay term reflecting the influence of the muscle-to-lung transit time, $\Delta \dot{V}O_2$ (sc) is the projected steady state $\dot{V}O_2$ increment above $\Delta \dot{V}O_2$ (ss), τ_2 is the slow component time constant and δ_2 is the delay term reflecting the onset of the slow component. A double exponential fit of the $\dot{V}O_2$ kinetics is shown in Figure 1.6.

On-transient $\dot{V}O_2$ slow component τ values obtained using the double exponential model have shown a range from 120-230s (Barstow and Mole, 1991; Bearden and Moffat, 2000; Ozyener et al., 2001; Bell et al., 2001a). However, to date, there is little physiological justification for an exponential fit of the slow component, as the mechanism of the slow component remains to be identified (Bearden and Moffatt, 2001; Whipp et al., 2002).

Rossiter et al. (2001) have provided a criterion for discerning the onset of the slow component, without recourse to an exponential fit for the $\dot{V}O_2$ slow component. An iterative process is used in which the fitting window of phase II was lengthened until the 'goodness' of the model fit deteriorated, indicating the onset of the slow component. This was indicated by a threshold in the summed square error value and visual inspection of the

residual profile for a consistent deviation from zero. The amplitude of the $\dot{V}O_2$ slow component can be derived as the $\Delta\dot{V}O_2$ between the asymptotic value for phase II and the end-exercise $\dot{V}O_2$.

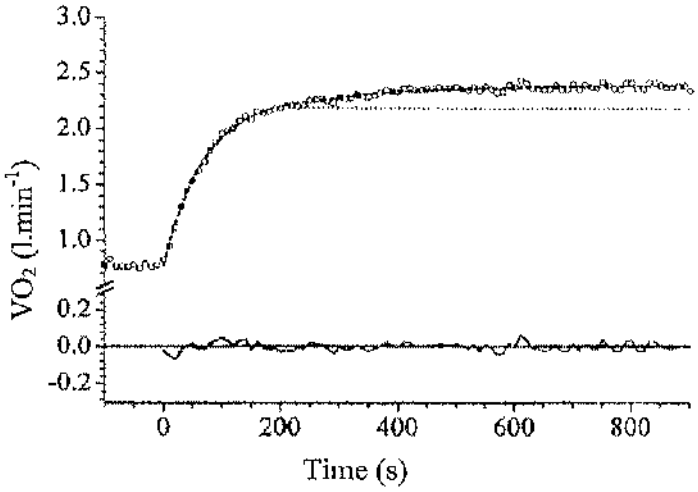

Figure 1.6. Modelling of the $\dot{V}O_2$ on-transient kinetics to heavy intensity exercise, including the double-exponential fit and residuals.

1.4.3 Very heavy exercise

Phase II

Relatively little research on $\dot{V}O_2$ kinetics has been conducted within the very heavy intensity domain. However, it was shown by Ozyener et al. (2001) that τ for very heavy intensity exercise was similar to that during all other domains of exercise (all 32-34s in this study). In addition, it was shown that the off-transient τ_{II} value after very heavy intensity

exercise was 33s, therefore indicating that within this exercise domain on–off symmetry prevails.

Slow Component

As very heavy exercise is above CP, the $\dot{V}O_2$ response continues to increase until $\dot{V}O_2$ peak is reached. Ozyener et al. (2001) showed that the $\dot{V}O_2$ slow component was apparent during both the on- and off-transient in this intensity domain. The on-transient response was modelled with the double exponential function with separate time delays for the two components, which resulted in a slow component τ of 163s. The off-transient response was also fit with a double exponential model but without a separate delay for the slow component, resulting in an off-transient slow component τ of 460s. Figure 1.7 shows the $\dot{V}O_2$ kinetics to very heavy-intensity exercise with a double exponential fit.

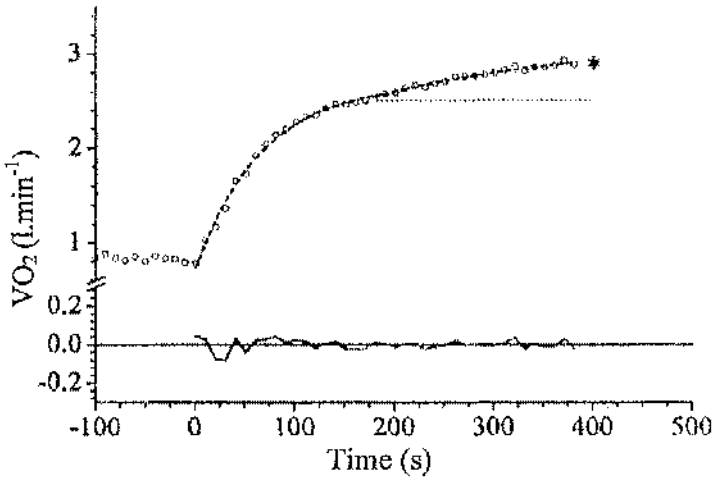

Figure 1.7. Modelling of the $\dot{V}O_2$ on-transient kinetics to very heavy intensity exercise, including the double-exponential fit and residuals. * indicates fatigue point.

1.4.4 Severe exercise

Phase II

Ozyener et al. (2001) demonstrated that the on-transient τ (35s) was similar to that found during the other exercise intensity domains. However, in contrast to the other intensity domains, the gain of the phase II response was found to be significantly lower than other intensities due to the response reaching $\dot{V}O_2$ max prior to attaining (or even approximating) a steady state (Ozyener et al., 2001). It was also demonstrated by Ozyener et al. (2001) that on-off symmetry existed with τ on=34s and τ off=35s.

Slow Component

The exercise durations in this domain are typically so short that a $\dot{V}O_2$ slow component is not induced to any discernible extent (Ozyener et al., 2001). However, the off transient demonstrated a slow component τ value of 539s (Ozyener et al., 2001). Figure 1.8 shows the $\dot{V}O_2$ kinetics to severe-intensity exercise, fit with a monoexpontial model.

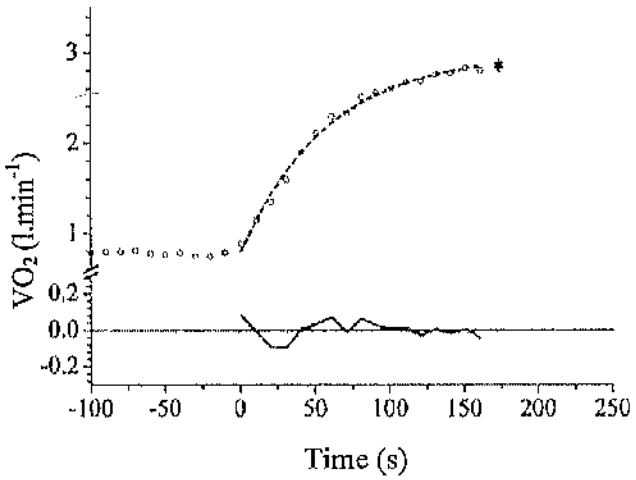

Figure 1.8. Modelling of the $\dot{V}O_2$ on-transient kinetics to severe intensity exercise, including the mono-exponential fit and residuals. * indicates fatigue point.

1.5 CONTROL OF $\dot{Q}O_2$ KINETICS

As discussed earlier (section 1.2), direct measurement of $\dot{Q}O_2$ (especially in the non-steady state) is technically difficult. However, the temporal response profile of $\dot{V}O_2$ measured

breath by breath at the mouth provides an acceptable surrogate. Recent research investigating the control of the phase II $\dot{V}O_2$ kinetics of the exercise on-transient has been focussed on three main, competing, hypotheses, namely:

(1) an O_2 delivery or diffusion limitation

(2) an intramuscular O_2 utilisation limitation operating via feed forward control; (or)

(3) an intramuscular O_2 utilisation limitation operating via feedback control.

Evidence to date suggests that the limiting factor may differ with different intensities, and therefore the intensity domains are treated separately.

1.5.1 Moderate intensity exercise

1.5.1.1 O_2 delivery

1.5.1.1.1 Evidence for O_2 delivery as a limitation

Studies from Hughson's group have demonstrated that when a slowing of $\dot{Q}$ occurs there is a corresponding slowing of $\dot{V}O_2$. Hughson and Kowalchuk (1991) and Hughson and Smyth (1983) showed that, with a β-blocker intervention (which impairs cardiac output and therefore, presumably, $\dot{Q}$), slower $\dot{V}O_2$ kinetics were seen compared to control. Alterations in body position have also been used to alter (decrease) $\dot{Q}$. With both supine knee-extension exercise (MacDonald et al., 1998) and forearm exercise above the heart (Hughson et al., 1996), $\dot{V}O_2$ kinetics were significantly slower. These studies, however,

only demonstrated that a slowing of $\dot{Q}$ slowed $\dot{V}O_2$, they do not provide convincing evidence that $\dot{Q}$ necessarily limits $\dot{V}O_2$ kinetics in the normal state.

1.5.1.1.2 *Evidence against O_2 delivery as a limitation*

Williamson et al. (1996) demonstrated that when blood flow was reduced with lower body positive pressure during exercise there was no change in the $\dot{V}O_2$ kinetics. In addition, studies have shown that the on-transient kinetics of $\dot{Q}$ are faster than those of $\dot{V}O_2$ during cycling exercise (Grassi et al. 1996), knee extension exercise (MacDonald et al., 1998; Bell et al., 2001b), and forearm exercise (Hughson et al., 1996), possibly suggesting that the transient $\dot{Q}$ response to the working muscles is sufficient. However, a recent study has shown evidence of blood flow heterogeneity within the exercising muscle. Richardson et al. (2001) were able to assess the distribution of perfusion and metabolic demand ($\dot{Q}/\dot{V}O_2$) in exercising human muscle using magnetic resonance imaging techniques to provide multiple, localised measurements of both $\dot{Q}$ and $\dot{V}O_2$. ^{31}P chemical shift imaging provided images of the phosphorus spectra in different locations of the muscle, yielding an indication of PCr breakdown as a surrogate of $\dot{Q}O_2$. Arterial spin labelling magnetically tags arterial blood flow, allowing observation of $\dot{Q}$ to different areas of the muscle. Large variations in $\dot{Q}/\dot{V}O_2$ were found, indicating the existence of areas of under- and over-perfusion relative to metabolic rate. Therefore, while studies demonstrating fast $\dot{Q}$ kinetics suggest that there

is excess O_2 availability for the requirements of the muscle group as a whole, they do not prove $\dot{Q}$ is not the limit in some constituent muscle fibres.

With an in situ canine gastrocnemius muscle preparation, Grassi et al. (1998b) used electrical stimulation of the muscle to increase metabolic rate, and a perfusion pump to increase $\dot{Q}$ to exercise steady-state levels prior to the onset of exercise, therefore eliminating $\dot{Q}$ as a limit to $\dot{Q}O_2$. During the condition of elevated $\dot{Q}$, $\dot{Q}O_2$ kinetics were not speeded, suggesting that $\dot{Q}$ kinetics were unlikely to have been limiting under control conditions.

Using another in situ muscle preparation, Grassi et al. (1998a) attempted to augment peripheral or diffusive flux by increasing the O_2 partial pressure gradient from capillary to mitochondria through imposing a hyperoxic inspirate and infusing a adenosine which increases haemoglobin O_2 off-loading. Under these conditions, again, the $\dot{Q}O_2$ kinetics were not speeded, despite the enhanced O_2 delivery. MacDonald et al. (1997) studied the effects of a hyperoxic inspirate on humans during cycling exercise and similarly showed no effect on the $\dot{V}O_2$ kinetic response.

It has been suggested that nitric oxide (NO) may inhibit enzymes involved in oxidative phosphorylation, such as pyruvate dehydrogenase (PDH), cytochrome oxidase and creatine kinase (CK). The influence of NO was examined by Kindig et al. (2002a) after inhibition of NO synthase (NOS) with N^G-nitro-L-arginine methyl ester (L-NAME) was in horses (i.e.

synthesis of NO catalysed by NOS). Thus, Kindig et al. (2002a) demonstrated that after L-NAME administration $\dot{V}O_2$ was accelerated (12s vs. 17s). These results therefore supported the hypothesis that O_2 delivery was not the limiting factor for $\dot{V}O_2$ kinetics; i.e. NO inhibition would, if anything, decrease vasodilation and therefore slow $\dot{Q}$ kinetics (Joyner and Dietz, 1997).

The effect of NOS inhibition in humans has not yet been studied in the moderate-intensity exercise domain and therefore it is unknown whether the results of Kindig et al. (2002a) apply to humans. Horses have much faster $\dot{V}O_2$ kinetics than humans suggesting that the physiological systems of the two species may not be identical.

The evidence to date therefore demonstrates that O_2 delivery, at least during moderate-intensity exercise, does not normally limit $\dot{V}O_2$ kinetics. It is clear, however, that techniques to measuring $\dot{Q}$ directly in the active muscle of human subjects are needed to resolve this issue.

1.5.1.2 Feedforward control by pyruvate dehydrogenase activation

Pyruvate dehydrogenase (PDH) is a multi-enzyme complex involved in the conversion of pyruvate to acetyl-CoA, a mitochondrial substrate for the Krebs cycle. It has been suggested that the lack of sufficient activation of PDH via PDH kinase may limit the

production of acetyl CoA, thereby limiting the production of the redox substrates NADH, and $FADH_2$ and, therefore, ATP production (Howlett et al., 1999; Timmons al., 1998).

It was shown by Howlett et al. (1999) that, with dichloroacetate (DCA) administration to activate PDH (by inhibiting PDH kinase), there was an increase in acetyl CoA and a corresponding reduction in PCr degradation. As PCr breakdown has been shown to be an indicator of oxidative phosphorylation increase (McCreary et al., 1996; Whipp et al., 1999; Rossiter et al., 1999, 2002b), Howlett et al. (1999) concluded that there must have been an increase in oxidative phosphorylation, although $\dot{Q}O_2$ was not measured in this study.

Campbell-O'Sullivan et al. (2002) increased acetyl group availability by utilising prior moderate-intensity exercise and found that $\dot{V}O_2$ kinetics were correspondingly accelerated. However, the $\dot{V}O_2$ response kinetics were modelled as a mean response time thus including the phase I response, which, as described in section 1.4.1, is not a part of the fundamental response. Therefore, the results from this study remain inconclusive.

1.5.1.3 Feedback control by high-energy phosphate status

As ATP is hydrolysed, Pi and ADP are released with free energy (ΔG). Utilising a model for skeletal muscle $\dot{Q}O_2$ control, it was proposed by Chance et al. (1962) that the concentration of cytosolic ADP acted as the control for controlling the rate of $\dot{Q}O_2$. This model suggestion initially arose from studies done by Chance and Williams (1956), in

which they supplied increased levels of ADP to isolated mitochondria. It was shown that as [ADP] increased O_2 consumption increased; the changes fit Michaelis-Menten kinetics, suggesting that a single control step was involved. Using ^{31}P-NMR, spectroscopy it was found that ADP levels were much lower than the K_m whereas P_i levels were in excess of their K_m (Michaelis constant) value. Therefore, the [Pi] would not be able to exert the many-fold increase required to account for the large increase in ATP hydrolysis with exercise, suggesting that [ADP] was the limiting substrate not [Pi] (Chance et al., 1985). The model suggested by Chance et al. (1985) is shown in figure 1.9.

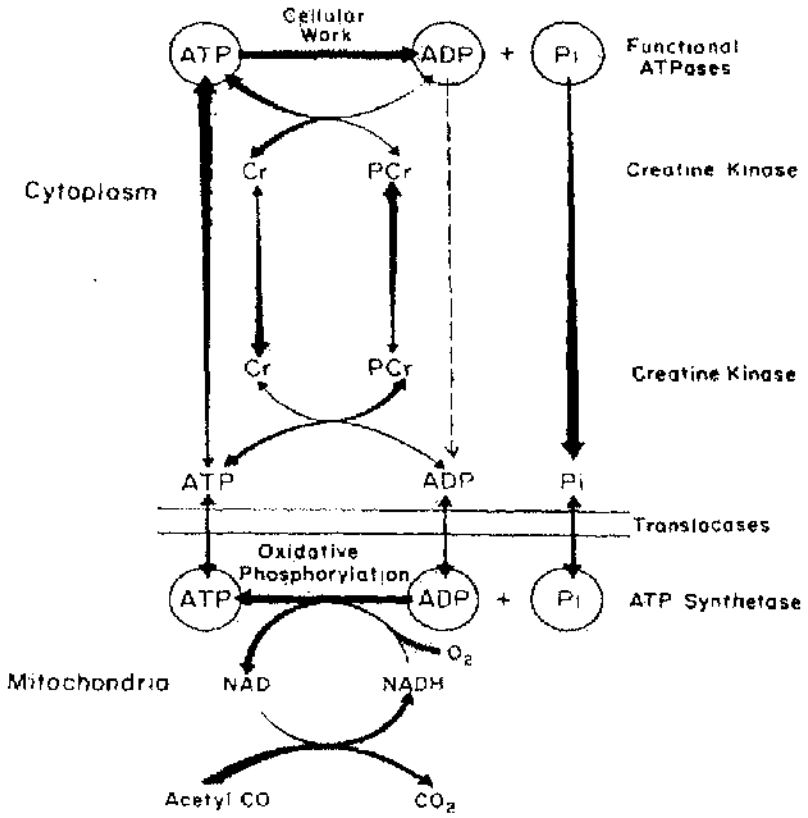

Figure 1.9. – Diagram summarising the cytosolic and mitochondrial reactions involved in ATP generation. Note the ATP and ADP are shuttled between the mitochondria and cytosol via the translocase. From Chance et al., 1985.

As the transport of ATP and ADP into and out of the mitochondrial matrix is via the transporter, adenine nucleotide translocase, Klingenberg (1980) suggested that although [ADP] was the main controller of the rate of oxidative phosphorylation, the competition that ATP employed towards [ADP] for the ADP transporter meant it was the ratio of [ATP]/[ADP] that was likely to be the important control. In addition, Hochachka and Matheson (1992) recognised that in intact, animal, skeletal muscle (rather than isolated mitochondria as used by Chance and colleagues, 1956, 1962, 1985), [ADP] at rest was not

in fact very far below the its K_m value. Therefore, they proposed the notion of additional involvement of other limiting factors, which would in turn affect the primary substrates (ATP and ADP) that were involved in oxidative phosphorylation.

The phosphorylation of ADP to ATP at the level of cytochrome c of the electron transport system (ETS) depends on the supply of reducing substrates (NADH and $FADH_2$) that are provided by the Krebs cycle. It has been suggested that the ratio of redox substrates (i.e. [NAD+]/[NADH], $FADH_2$ being negligible in comparison) may also provide further control of $\dot{Q}O_2$ at the onset of exercise. Meyer and Foley (1994) reviewed numerous sources that hypothesised a model referred to as "nonequilibrium thermodynamics" in which ATP synthesis is a function of the hydrolysis products ([ATP]/[ADP][Pi]), with ΔG driving ATP synthesis as well as the redox potential.

Erecinska et al. (1974), although using a slightly different model referred to as the 'near equilibrium hypothesis', also suggested that the phosphorylation potential ([ADP]/[ADP][Pi]) and redox potential ([NAD+]/[NADH]) were interrelated with muscle $\dot{Q}O_2$. This notion was based on evidence that the reduction of cytochrome c oxidase is irreversible, and so the overall $\dot{Q}O_2$ would have to be equal to the rate of oxygen reduction (therefore the rate-controlling step). Although this reaction is not dependent on redox potential, the reaction that supplies the substrate (reduced cytochrome c) is. Further discussion of this hypothesis was presented by Wilson (1994). Evidence was based on a number of sources in which it was observed that the phosphorylation potential could be

manipulated, but respiratory rate was maintained by adaptive changes in redox potential. As evidence emerged that the above models might not express the entire process controlling oxidative phosphorylation in vivo, investigations into the control of oxygen uptake turned to other potential high-energy phosphates involved in oxidative phosphorylation.

Bessman and Geiger (1981) proposed that, rather than the control of the rate of oxidative phosphorylation being signalled by the phosphorylation potential of ATP/ADP (or versions), the phosphorylation potential of [PCr]/[Cr] may provide the control to oxidative phosphorylation. Bessman and Carpenter (1985) and Bessman (1986, 1987) compiled evidence supporting this suggestion. In particular, it was demonstrated that an isoform of creatine kinase exists at the mitochondrial membrane (CK-mi) and that the location of the different CK isoforms was associated with the location of the nucleotide translocases for ADP/ATP. This "new" model, as shown in Figure 1.10, was termed the 'creatine shuttle' by Bessman and Geiger (1981). It suggests that the newly produced ATP is channelled from the mitochondrial matrix to CK-mi at the inner mitochondrial membrane, which phosphorylates Cr into PCr yielding ADP. The PCr then moves into the cytosol to rephosphorylate cytosolic ADP to provide ATP at sites of utilisation. In addition, Cr is produced and is therefore available to return to the mitochondria to continue the cycle. Meanwhile, the ADP that had been produced in the mitochondria is channelled back into the mitochondrial matrix for rephosphorylation by oxidative phoshporylation.

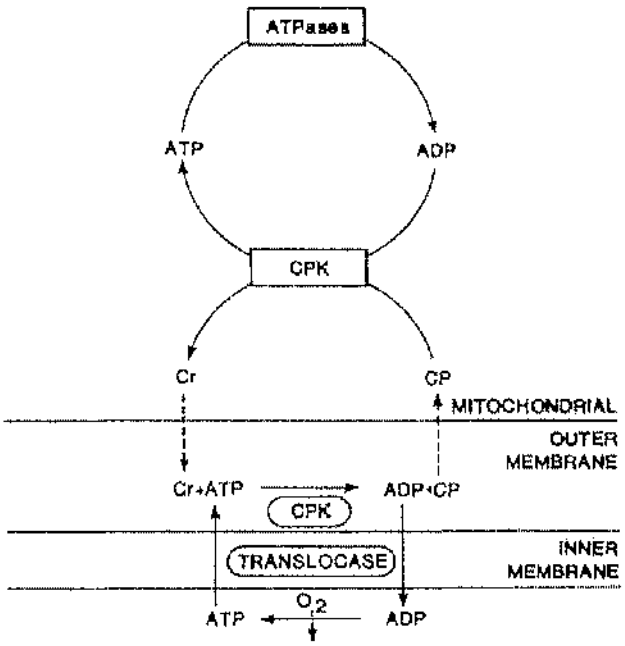

Figure 1.10. Diagram showing the role of phosphorylation potential and creatine kinase (CK) in the control of oxidative phosphorylation. From Whipp and Mahler, 1980.

Within this model, CK serves several purposes: a temporal buffer of ATP/ADP levels; a spatial buffer of ATP/ADP; maintaining pH (as H+ is used in rephosphorylating ADP); and protecting the cell from ADP accumulation (Wallimann et al., 1992).

This hypothesis gained support from Mahler (1985) who demonstrated that the old model by Chance et al. (1985) could only hold in isolated mitochondria. The demonstration that [ATP] levels stay constant and there is no cytosolic CK in isolated mitochondria would in

fact reduce dependence of $\dot{Q}O_2$ on [ADP] in a Michaelis-Menten fashion. Mahler (1985) therefore measured of $\dot{Q}O_2$, using intact frog sartorius muscle, when a twitch stimulus was applied. The muscle was then removed, frozen and examined for [PCr] and [Cr] (and in some cases [ATP] and [ADP]). It was observed (as previously reported) that $\dot{Q}O_2$ displayed an off-transient response consistent with first-order kinetics (mono-exponential for a step function). It was observed that the [PCr] and [Cr] displayed similar first-order responses which, when plotted against $\dot{Q}O_2$, also showed linear changes. This confirmed PCr's association with the rate of oxidative phosphorylation. Further, the ATP and ADP concentrations did, in fact, remain essentially constant in skeletal muscle. Taken together, these data when applied to the mathematical equations describing the two models (that of Chance compared with Bessman's creatine shuttle) showed that only the model of the creatine shuttle could hold.

Meyer et al. (1988) confirmed this conclusion using ^{31}P-NMR spectroscopy in intact skeletal muscle (rat gastrocnemius) to observe [PCr], thereby eliminating any experimental error involved in removal of the muscle and freezing as utilised by Mahler (1985). The results confirmed those of Mahler (1985), in that there was a mono-exponential off-transient increase for [PCr], and further showed that the [PCr] on-transient was also mono-exponential and that rate of PCr changes were independent of work rate. Both the independence of the rate of PCr change with the work rate and the symmetry of the on- and off-kinetics provided additional evidence that PCr displayed first order kinetics.

The natural extension of the PCr work by Mahler (1985) (as mentioned above) was to attempt these observations in humans. Yoshida and Watari (1993) using ^{31}P-NMR spectroscopy demonstrated that at the onset of femoral flexion exercise in humans, [PCr] decreased in an exponential fashion with a time course that was not dependent on exercise intensity and showed on-off symmetry. McCreary et al. (1996) used ^{31}P-NMR spectroscopy during calf exercise in human subjects to demonstrate that the kinetics of PCr degradation and $\dot{V}O_2$ increase were similar. However, $\dot{V}O_2$ and PCr were not measured simultaneously and, although many repeats were performed (12) the small muscle mass limited the ability to resolve kinetics with high confidence (Rossiter et al., 1999). Recently, the ability to simultaneously measure PCr breakdown and $\dot{V}O_2$ in a larger muscle mass (quadriceps exercise) in humans has shown that indeed the time constants of PCr and $\dot{V}O_2$ are the same during moderate-intensity exercise (Whipp et al., 1999; Rossiter et al., 1999, 2002).

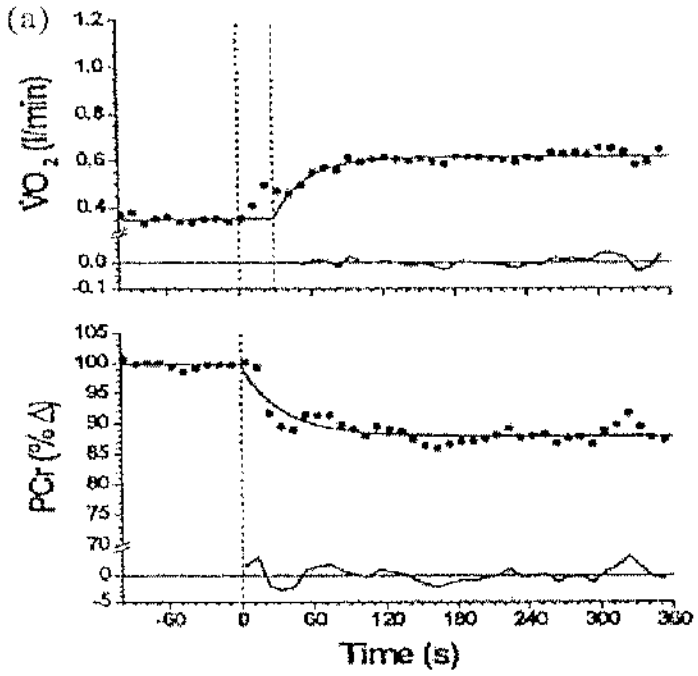

Figure 1.11 Similarity of the kinetics for $\dot{V}O_2$ and PCr degradation at the onset of moderate intensity exercise. From Rossiter et al., 2002.

1.5.2 Above the lactate threshold

1.5.2.1 O_2 delivery

1.5.2.1.1 Evidence for O_2 delivery as a limitation

A number of studies have shown that when muscle O_2 delivery is impaired, $\dot{V}O_2$ kinetics during heavy-intensity exercise were slowed. Hughson et al. (1996) altered forearm blood flow during 'high power output' arm exercise by utilising exercise above and below the

heart. It was found that forearm blood flow and $\dot{V}O_2$ kinetics were both slower when the arm was above the heart compared to below. Similarly it has been shown that $\dot{V}O_2$ kinetics are slowed when O_2 delivery is limited by breathing a hypoxic gas mixture (Hughson and Kowalchuk, 1995) or by breathing carbon monoxide (Koike et al., 1990).

In contrast, other studies have utilised different interventions in attempts to demonstrate that, with an increased muscle blood flow, $\dot{V}O_2$ kinetics are speeded. During supine cycling, with a decreased $\dot{Q}$ to the exercising limbs, Hughson et al. (1993) added lower body negative pressure to compensate for the decreased $\dot{Q}$, and noted that $\dot{V}O_2$ kinetics were accelerated to resemble those of upright exercise. It has also been shown that when breathing a hyperoxic gas mixture, faster $\dot{V}O_2$ kinetics ensue (Hughson and Kowalchuk, 1995; MacDonald et al., 1997). Utilising the same in situ muscle preparation method mentioned previously (Grassi et al., 1998b), but eliciting an exercise intensity of 100% $\dot{V}O_{2\,max}$, Grassi et al. (2000) showed that when $\dot{Q}$ was elevated to the exercise steady state level prior to the transition $\dot{Q}O_2$ was slightly faster.

Another method that has been used to increase $\dot{Q}$ is to perform a prior bout of heavy cycling exercise, which was suggested to result in an acidic-induced vasodilation and/or enhanced O_2 unloading from haemoglobin (Gerbino et al., 1996). In these studies there was a speeding of $\dot{V}O_2$ kinetics on the subsequent bout of heavy-intensity cycling exercise, suggesting O_2 delivery may play a role in the rate of oxidative phophorylation (Gerbino et

al., 1996; MacDonald et al., 1997; Bohnert et al., 1998;). However, it has also been suggested that prior exercise could modulate enzyme activity status, such as that of PDH (Gibala and Saltin, 1999). In addition, the effects of prior exercise on $\dot{V}O_2$ kinetics have been re-evaluated recently. The previously-mentioned studies characterised $\dot{V}O_2$ response in terms of a mean response time rather than discerning the fundamental phase from the other components of the response. Subsequently, Burnley et al. (2001) have demonstrated that during cycling exercise, with the different phases being modelled separately, the fundamental phase (phase II) was not faster. Rather, the amplitude of the slow component of the response was smaller, and this factor decreased the mean response time. Interestingly, with alternate leg knee extension exercise, it was found that the Phase II response was faster on the 2nd bout, and that the slow component was also reduced (Rossiter et al., 2001).

Therefore, in contrast to the previous suggestion that O_2 delivery may be limiting $\dot{V}O_2$ kinetics, as faster $\dot{V}O_2$ kinetics resulted after prior exercise (Gerbino et al, 1996; Bohnert et al., 1998; MacDonald et al., 1997) more recent results present conflicting evidence as to the impact of prior exercise on $\dot{V}O_2$ kinetics (Burnley et al, 2001; Rossiter et al., 2001). The results to date suggest that a muscle-specific response exists or that the response is dependent on the total muscle mass involved, as whole-body prior exercise did not elicit a speeding of $\dot{V}O_2$ kinetics, when modelled appropriately (Burnley et al., 2001), whereas alternate leg rhythmic knee extension did (Rossiter et al., 2001).

1.5.2.1.2 Evidence against O_2 delivery as a limitation

Similar to the study of moderate-intensity exercise as mentioned earlier (section 1.5.1.1.2), Grassi et al. (2000) utilised hyperoxic inspirate to increase the rate of peripheral O_2 diffusion by increasing the O_2 partial pressure gradient from capillary to mitochondria. This was done through inspiration of a hyperoxic gas mixture and by L-NAME administration. No effect on the $\dot{V}O_2$ kinetics at 100% max was found.

Utilising one-legged training, it was demonstrated that $\dot{V}O_2$ kinetics were accelerated in the trained leg (Bell et al., 2001b), despite an unchanged mean blood velocity measured at the femoral artery using Doppler ultrasound. However, citrate synthase activity was increased in the trained leg, suggesting that the acceleration of the $\dot{V}O_2$ kinetics was not due to increased O_2 delivery, but rather to an improved rate of O_2 utilisation.

Recently, Bangsbo et al. (2000) used thermodilution and indocyanine green techniques to measure $\dot{Q}$ while $\dot{Q}O_2$ was simultaneously measured across the femoral artery and vein.. The indocyanine green allowed calculation of the time delay from the site of blood-tissue exchange to the measurement site. It was demonstrated that leg $\dot{Q}$ increased immediately and in excess, whereas $\dot{Q}O_2$ did not increase until 6s later, indicating that $\dot{Q}$ was unlikely to be the limiting factor for the for $\dot{Q}O_2$ response.

As discussed in moderate intensity exercise (section 1.5.1.1.2), the effect of L-NAME on NOS and therefore NO was examined in the horse. Similar to the findings for moderate intensity exercise, $\dot{V}O_2$ kinetics in the horse were speeded with L-NAME infusion (Kindig et al., 2001). The role of NO in humans is questionable, however, as Frandsenn et al. (2001) did not find any effect of L-NAME in humans performing both submaximal and exhaustive knee extension exercise. Although, it is important to note that the kinetics of the $\dot{V}O_2$ response were not estimated, but rather $\dot{V}O_2$ was monitored at only 10 minute intervals.

1.5.2.2 Feedforward control by pyruvate dehydrogenase

Studies investigating the effect of PDH on $\dot{V}O_2$ kinetics, have utilised PCr breakdown measurements as a proxy of oxidative phosphorylation. Timmons et al. (1998) showed that after dichloroacetate (DCA) infusion to activate PDH kinase there was a decrease in PCr degradation of approximately 50% during submaximal exercise. This suggests a smaller O_2 deficit and therefore faster $\dot{V}O_2$ kinetics.

In contrast, Savasi et al. (2002) showed that with cycling exercise at 90% $\dot{V}O_{2\ max}$ DCA infusion had no effect on PCr breakdown or [La] (as an indicator of pyruvate build up). Grassi et al. (2002), using the in situ canine muscle preparation, also demonstrated that DCA had no effect on PCr breakdown, lactate production or $\dot{Q}O_2$ kinetics. Bangsbo et al. (2002) showed a similar result in humans during severe intensity knee extension exercise;

$\dot{V}O_2$ kinetics were not speeded despite elevated PDH levels within the first 15s of exercise, and neither were PCr degradation and blood lactate values reduced.

The most recent of these results therefore strongly suggest that PDH does not limit $\dot{V}O_2$ kinetics during heavy-intensity exercise.

1.5.2.3 Feedback control by high energy phosphate status

With the knowledge that $\dot{V}O_2$ kinetics in heavy intensity exercise do not display the same mono-exponential kinetics as shown for moderate exercise, reflecting existence of a slow component (see section 1.4.2), a recent study utilised ^{31}P-NMR spectroscopy during heavy-intensity exercise to simultaneously observe kinetics of $\dot{V}O_2$ increase and PCr breakdown (Rossiter et al., 1999). It was found that PCr and $\dot{V}O_2$ did not show similar phase II τ values. In addition, as briefly discussed above (section 1.5.2.1.1) this study also investigated the effects of a prior bout of heavy intensity exercise. It was found that when the $\dot{V}O_2$ kinetics were speeded on the second bout, there was a lesser decrease in [PCr] (for the second bout); however, the rate of PCr breakdown was not speeded. The observation that there was a modulation of PCr dynamics when $\dot{V}O_2$ was also modulated supports the involvement of the creatine shuttle model. However, the non-linearity between the amount of PCr breakdown for the same work rate increment, and the multi-compartment and

complex dynamics of the PCr response suggests that the relationship between the $\dot{V}O_2$ and PCr kinetics is more complex and may be modulated by other factors than just the rate of the creatine shuttle. However, the most recent work by Rossiter et al. (2002) has shown conflicting results, as PCr and $\dot{V}O_2$ kinetics were found to be similar in heavy-intensity exercise as shown in Figure 1.12. The reason for this discrepancy is unclear.

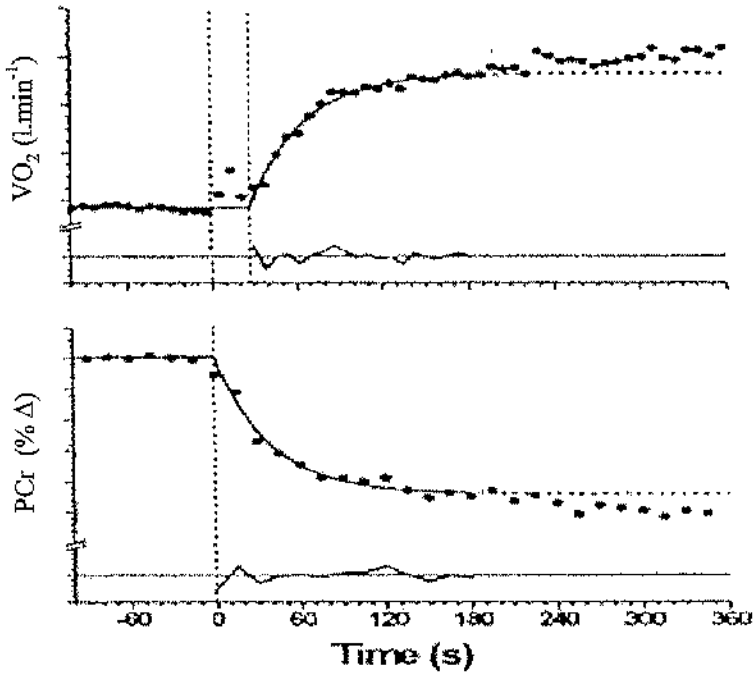

Figure 1.12. Similarity of the kinetics for $\dot{V}O_2$ and PCr degradation at the onset of heavy-intensity exercise. The solid line represents the model fit for phase II and the dashed line indicates the extension of that fit, therefore demonstrating the appearance of a slow component. From Rossiter et al., 2002b.

1.5.3 Muscle metabolic heterogeneity

It has been shown that there is significant heterogeneity of blood flow within the exercising muscle in animal models (e.g. Piiper, 1989, 1991, 1992, 1999, 2000). As discussed in section 1.5.1.1.2, Richardson et al. (2001) has recently confirmed this heterogeneity of blood flow and in addition demonstrated that heterogeneity of $\dot{Q}O_2$ exists with in the muscle in humans.

To address the issue of heterogeneity, the recent advance of phosphorescence quenching techniques has allowed measurement of the intracellular partial pressure of O_2 (P_iO_2) in an isolated single frog skeletal muscle fibre (Hogan et al., 1999, 2001), and microvascular partial pressure of O_2 (P_mO_2) in rat skeletal muscle (Behnke et al., 2001, 2002a, 2002b) in response to electrical stimulation. The intensities elicited by the electrical stimulation cannot be categorised, and therefore the results of these experiments are discussed without reference to a particular exercise intensity domain. Electrical stimulation elicits concurrent fibre activation and has the benefits over volitional muscle activation of eliminating variable factors such as motor unit activation patterns and muscle fibre recruitment. The quenching method measures the length of the phosphorescence lifetime of a porphyrin probe, when exposed to a flash of light. As the lifetime is dependent on PO_2, an index of the PO_2 in the environment is obtained.

The recent studies of Hogan (Hogan, 1999; Howlett and Hogan, 2001) have demonstrated a decrease in P_iO_2 when electrically stimulated contractions were initiated. The fall in P_iO_2 mirrored the intramuscular oxygen uptake kinetics and, in addition, a linear relationship existed between the decrease in P_iO_2 and stimulation frequency (measure of energy demand) (Howlett and Hogan, 2001), supporting its use as an index of $\dot{Q}O_2$. At the onset of contractions, there was evidence of a delay period of ~13s preceding a subsequent mono-exponential decrease in P_iO_2 to steady state (Hogan et al., 1999).

The measurement of P_mO_2 therefore provides an indication of the overall $\dot{Q}O_2/\dot{Q}$ ratio. That initial delay in the fall of P_mO_2 suggested therefore that $\dot{Q}O_2$ was proportionally matched by $\dot{Q}$; and, in fact, in some cases an initial increase in P_mO_2 was observed, suggesting a hyper-perfusion and therefore requiring a reduced O_2 extraction (Behnke at al., 2001). Further, Behnke at al. (2002b) demonstrated that a prior bout of contractions decreased the time delay from 12.2s to 5.7s therefore speeding the $\dot{Q}O_2$ response. This was despite no elevation in P_mO_2 baseline, which would have indicated increased $\dot{Q}$.

These results therefore suggested that O_2 delivery does not limit $\dot{Q}O_2$ at least in this model. Rather, the intracellular utilisation of O_2 in the process of oxidative phosphorylation would appear to be limiting. Recent work by Behnke et al. (2002a) combined P_mO_2 and capillary red blood cell flux (Kindig et al., 2002) to resolve the time course of microvascular $\dot{Q}O_2$. The results led to the conclusion that the apparent delay in P_mO_2 is related to sufficient

delivery compensating for the need for increased extraction, rather than an actual delay in oxidative phosphorylation occurring.

In addition, the method for measuring P_iO_2 was recently used by Howlett and Hogan (2002) to investigate the effect on P_iO_2 of increasing the activation of PDH via DCA administration (see section 1.5.1.1.2). It was found that, with DCA, the time delay was similar but the P_iO_2 dropped more rapidly, indicating a faster activation of oxidative metabolism.

The above evidence measuring P_iO_2 and P_mO_2 therefore lends support to the hypothesis of metabolic inertia as a limiting factor to $\dot{V}O_2$ kinetics, with PDH possibly playing a role. However, it is important to note that these results may not provide a direct indication of human muscle mechanisms in vivo.

1.6 CONTROL OF THE $\dot{V}O_2$ SLOW COMPONENT

The mechanisms responsible for the slow additional rise in $\dot{V}O_2$, seen in heavy-intensity exercise, remains to be elucidated (reviewed in Whipp, 1994; Poole et al., 1994). Several theories have been suggested over the past years, some of which have subsequently been disproved with some certainty; these are therefore discussed first.

1.6.1 Muscle temperature and increased respiratory, cardiac and upper body work

Two hypotheses that generally have been dispelled as possible causes of the $\dot{V}O_2$ slow component were that it was related to an increase in muscle temperature that occurs with heavy-intensity exercise (Hagberg et al., 1978; Poole et al., 1988), and the possibility of an increase in the O_2 cost due to the greater respiratory, cardiac and/or upper body work (Hagberg et al., 1978; Wasserman et al., 1995). Koga et al. (1997) and Poole et al. (1988) addressed the possibility of muscle temperature and demonstrated that exercising with an increased muscle temperature did not result in an increase in the slow component.

Recently, evidence from Ozyener et al. (2001) has demonstrated that a $\dot{V}O_2$ slow component is not discernable in the off-transient $\dot{V}O_2$ response of heavy-intensity exercise. If the above hypotheses were causes of the slow component, it would be expected that at the offset of exercise the cessation of these influencing mechanisms would be manifest in the $\dot{V}O_2$ response. In addition, it has been reported that the slow component resides within the muscle. Poole et al. (1991) reported a high correlation between the slow components of $\dot{V}O_2$ and of $\dot{Q}O_2$ measured across the exercising muscle and suggested that $\dot{Q}O_2$ could account for at least 86% of the $\dot{V}O_2$ slow component. In addition, Rossiter et al. (2002a, 2002b) have reported with ^{31}P-NMR spectroscopy that there is a slow component evident in the [PCr] decline at exercise onset, indicating it is likely there is an energetic manifestation of the slow component within the muscle.

1.6.2 Lactate concentration

Another intriguing hypothesis was that [La] may be responsible for the slow component as it was shown that there was an association between the arterial [La] and the $\dot{V}O_2$ slow component (Roston et al., 1987; Poole et al., 1988) and between the onset of the [La] increase and the slow component (Poole et al., 1988). In addition, after training, the decrease in the slow component was also associated with a decrease in [La] (Poole et al., 1990). However, the most recent evidence more likely reported that the [La] is not the cause of the slow component, but rather a coincidental association. For example, Gaesser et al. (1994) demonstrated that with training the decrease in the $\dot{V}O_2$ slow component was not compatible with the time course of the decrease in [La]. In addition, after prior infusion of adrenaline, an increase in [La] was seen but there was no change in the $\dot{V}O_2$ slow component (Gaesser et al., 1994). In a study in which the blood [La] was decreased by administration of acetazolamide (presumably slowing its removal from the muscle and thereby increasing muscle [La]), it was found that there was no corresponding alteration in the slow component magnitude (Sheuermann et al., 1998).

A more direct approach was taken by Poole et al. (1994). After infusion of [La] into the arterial blood supply of an isolated canine muscle preparation, there was an increase in intramuscular [La] but no additional increase in the $\dot{Q}O_2$ slow component.

1.6.3 Fibre type recruitment

The hypothesis that has received the most focus recently has been with regard to the possibility that the slow component is due to an increase in the recruitment of type II fibres. It has been demonstrated, although with *in vitro* animal preparations, that type II fibres are less efficient in terms of the ATP production per O_2 used (Crow and Kushmerick, 1982; Kushmerick et al., 1992). This is possibly due to type II fibres use of the α-glycerophosphate shuttle instead of the malate-aspartate for transport of NADH into the mitochondria (Crow and Kushmerick, 1982; Kushmerick et al., 1992). Therefore, if a greater proportion of type II fibres are recruited in heavy exercise, the aerobic inefficiency of these fibres may result in the additional $\dot{V}O_2$ apparent with the slow component. It was of interest therefore that Barstow et al. (1996) demonstrated a significant correlation between an individual's percentage of type II fibres and the $\dot{V}O_2$ slow component amplitude.

The most recent studies have therefore utilised electromyography (EMG) to determine whether the onset of the slow component might in fact be associated with an additional recruitment of type II fibres. It was shown by Shinohara and Moritani (1992) that at the onset of the slow component there was in fact an increase in the integrated EMG signal, indicating there had been an increase in overall motor unit recruitment. However, Scheuermann et al. (2001) suggested that the EMG signal expressed as a mean power frequency (MPF) spectrum was required to identify whether there was a change in the

proportion of type I and type II motor units recruited. It was found by Scheuermann et al. (2001) and Lucia et al. (2000) that, in contrast to the results of Shinohara & Moritani (1992), there was no increase in the integrated EMG or a change in the MPF throughout exercise despite evidence of a $\dot{V}O_2$ slow component. Burnley et al. (2002) used integrated EMG and MPF but studied more of the exercising muscle groups (as only two were studied in that of Scheuermann et al. (2001)). It was demonstrated that there was an increase in both signals at the onset of the $\dot{V}O_2$ slow component. Most recently, however, the use of MPF and integrated EMG to identify the recruitment of type II fibres has been questioned (Borrani et al., 2001).

Rossiter et al. (2002a) has used ^{31}P-NMR spectroscopy to discern intramuscular areas of high and low pH indicated by a splitting of the inorganic phosphate peak (P_i), which have been proposed to reflect the recruitment type I versus type II fibres (Yoshida and Watari, 1993). However, this split was only observed in some subjects and the split occurred between 35 and 235 s after exercise onset. This indicated that the P_i splitting did not reflect the $\dot{V}O_2$ slow component response, as the slow component has been shown to consistently occur at ~120s as described previously and this was observed in all of their subjects.

Therefore, whether the recruitment of type II fibres is responsible for the slow component remains to be elucidated.

1.6.4 Uncoupling proteins

The role of the mitochondrial uncoupling proteins (UCP) in the manifestation of the slow component was recently studied by Russell et al. (2002). It was suggested there may be a positive correlation between the expression of UCP-3 mRNA (but not UCP-2) and the amplitude of the slow component. Although the specific function of UCP-3 is still unknown, it has been suggested by Brand et al. (1994) that it may be involved in the leak of protons across the inner mitochondrial membrane at rest when ATPase at the inner mitochondrial membrane is not stimulated at a high rate. Although this allows for the large increases in ATP production required when performing exercise, it may be that the existence of high concentrations of UCP-3 may still allow a leak of protons even at high metabolic rates, therefore decreasing the ATP production for O_2 consumed i.e. reducing efficiency. In addition, it may be that the UCP-3 proton leaks may be more abundant in type II fibres as it was shown by Russell et al. (2002) that there was a positive correlation between UCP-3 and of the proportion type IIa muscle fibres.

1.7 CONCLUSIONS AND OBJECTIVES FOR THE PRESENT STUDY

It is apparent that despite the numerous studies in the area, the evidence to date regarding the control of $\dot{V}O_2$ kinetics during exercise in humans remains inconclusive. It appears that if O_2 delivery is a limiting factor, it may play more of a role in heavy-intensity exercise rather than in moderate-intensity exercise. The studies regarding metabolic inertia through

muscle PDH activation suggest it is probably not a limit at either intensity, at least in vivo in humans. Studies showing close matching of the kinetics of PCr breakdown with those of $\dot{V}O_2$ provide compelling evidence that the limit to $\dot{V}O_2$ kinetics may reside within the muscle and involve high-energy phosphate status, although the specific mechanism is not apparent from these studies.

The enzyme involved in the breakdown and rephosphorylation of PCr is CK. Many studies have attempted to investigate the role of the CK reaction in mitochondrial respiration. However, most of the studies have examined the steady-state response (Chance et al. 1985; Meyer 1988; Saks et al, 1995; Walsh et al., 2001a, 2001; Zoll et al., 2002). In addition, these measurements were made using isolated mitochondria (Chance et al., 1985; Saks et al., 1995) or isolated muscle (Saks et al., 1995; Tonkonogi et al., 1998; Walsh et al., 2001a, 2001b), and thus direct conclusions regarding human skeletal muscle in the in vivo state cannot be made. However, it is clear that the CK-catalysed reaction plays an important role in mitochondrial respiration. *Therefore, this study investigated whether the CK activity rate was associated with the rate of aerobic ATP production at the onset of exercise.*

It has been established that different isoforms of CK exist with in skeletal muscle (Wallimann et al., 1992). The cytosolic isoforms muscle (M) and brain (B) form dimeric isoenzymes MM, MB and BB. However, CK-B is only expressed during muscle cell differentiation until the myoblast undergoes a transitional development to the myotube (Trask and Billadello, 1990). The sarcomeric mitochondrial CK isoform in humans is an

octomeric isoenzyme located at the outer surface of the inner mitochondrial membrane. *Therefore, this study also attempted elucidated the CK isoform distribution and establish whether a correlation exists between an individual's isoform distribution and the* $\dot{V}O_2$ *kinetics during exercise.*

The mechanism of the $\dot{V}O_2$ slow component still remains to be elucidated. Although the recent study by Russell et al. (2002) suggested UCP-3 may have a role, several design shortcomings related to the selection of appropriate exercise intensities and the characterisation of the $\dot{V}O_2$ kinetics render the conclusions questionable. In Russell et al. (2002) the most reliable techniques for identification of the θ_L were not used, and thus the relative exercise intensities used in each individual to elicit the $\dot{V}O_2$ slow component may vary considerably. Breath-by-breath gas exchange with a ramp increase in WR provides many serial measurements allowing precise identification of the θ_L (Beaver et al., 1986; Whipp et al., 1986). However, Russell et al. (2002) sampled [La] every four minutes during an incremental WR test of square-wave WR increases every four minutes, initiated from a work rate of 40% of the individual's peak power output. Therefore, there would be relatively few data points below θ_L and the minimal sampling rate would result in a curvilinear response, making precise identification of the θ_L difficult. Further, measurement of [La] at the antecubital vein does not provide an appropriate reflection of [La] from the exercising legs as the muscle of the arm would consume the [La] (Brooks et al., 2000), especially as arterialisation of the blood was not performed. As a result of imprecise θ_L identification, the work rates performed may not have been of the same

relative intensity between subjects. As the $\dot{V}O_2$ slow component amplitude varies with relative intensity (Whipp and Ward, 1992: Whipp et al., 2002) it is imperative similar intensities be performed by the subjects if the goal is to relate the slow component amplitude to other factors. Further, in the study of Russell et al. (2002) the amplitude of the slow component amplitude to other factors. *Therefore, a further purpose of the present investigation was to examine the relationship between the amplitude of the slow component during very heavy intensity exercise and the expression of uncoupling proteins.*

A final purpose related to the identification of the parameters of the $\dot{V}O_2$ response during very heavy intensity exercise. Although Ozyener et al. (2001) studied the $\dot{V}O_2$ response within this exercise intensity domain, a double exponential model was used to fit the data, which has little a priori physiological justification (see section 1.4.2 for details). Therefore, the $\dot{V}O_2$ parameters within the very heavy intensity domain have not been sufficiently elucidated using the appropriate method of slow component identification described previously (Rossiter et al., 2001). *The present investigation thus also examined the existence of inter-subject $\dot{V}O_2$ parameter variability, in response to very heavy-intensity exercise, using appropriate modelling, to compare kinetic parameters for two different work rates, within the very heavy intensity domain.*

CHAPTER 2

METHODS

2.1 SUBJECTS

Six male subjects volunteered to take part in the study. The study protocol was approved by the University of Glasgow Ethics Committee (appendix i) and subjects provided written informed consent. All subjects were non-smokers and recreationally active. The subjects participating in this study had a mean age of 22±2 years, height of 179±5 cm and mass of 77±7 kg (Table 2.1). There was a variable range of aerobic fitness indicators demonstrated by a range of $\dot{V}O_{2\,peak}$ values from 45 to 56 ml.kg^{-1}.min^{-1}, θ_L values ranging from 1.40 to 2.55 l.min^{-1}, and θ_L expressed as a percent of $\dot{V}O_{2\,peak}$ ranging from 37 to 53%. Individual values are shown in Table 2.1.

Table 2.1. Individual subject characteristics and exercise intensity demarcations.

θ_L represents the anaerobic threshold, $\dot{V}O_2$ peak represents the peak O_2 uptake, CP represents critical power, W' represents the anaerobic work capacity, Δ'/Δ.

Subject	Age (years)	Height (cm)	Mass (kg)	θ_L ($l.min^{-1}$)	$\dot{V}O_{2\ peak}$ ($l.min^{-1}$)	$\dot{V}O_{2\ peak}$ ($ml.min^{-1}.kg^{-1}$)	θ_L % $\dot{V}O_{2\ peak}$ (%)	θ_L (W)	CP (W)	W' (KJ)	WR_{peak} (W)	Δ'/Δ (%)
1	21	180	80	1.60	3.63	45.3	44	100	207	14.3	275	61.1
2	20	182	88	2.55	4.80	54.5	53	190	262	25.6	345	46.5
3	19	180	68	1.65	3.57	52.9	46	105	183	15.1	270	49.1
4	26	182	79	1.65	4.43	56.1	37	115	281	25.5	377	63.4
5	22	170	71	1.40	4.43	49.6	39	108	198	19.4	279	52.6
6	21	177	76	1.90	4.43	52.6	48	130	243	13.0	310	62.8
Mean	22	179	77	1.79	3.99	51.8	45	125	229	18.8	309	55.9
SD	2	5	7	0.40	0.52	3.9	6	34	39	5.6	44	7.4

A medical questionnaire (appendix ii) was used to ensure there were no pre-existing health conditions which would affect the response to exercise, such as asthma or a family history of sudden death, which may increase the risk associated with performing strenuous exercise tests. In addition, a physician medically screened all subjects, including a resting 12 lead electrocardiogram and blood pressure measurement. To maximise controlled conditions as much as possible, subjects were instructed not to: perform strenuous exercise for 48 hours, consume alcohol for 24 hours, ingest caffeine for 4 hours, or consume food for 2 hours prior to exercise testing. If any subject was not able to adhere to the criteria on a particular visit or was feeling ill, injured or fatigued, the test was postponed until the subject recovered.

All subjects were familiarized to the laboratory, equipment and all experimenters involved. The cycle seat and handlebars were adjusted to maximise comfort and these settings were recorded and used for each test. Prior to each protocol the details of the test were explained to the subject. During the testing music was played, monitoring equipment was kept out of the subject's view, audible alarms were turned off and talking among experimenters was avoided. At the end of testing subjects were allowed to rest, re-hydrate and shower, after which they were thanked for their time and escorted from the laboratory.

2.2 MEASUREMENTS

2.2.1 Work rate

All exercise testing was performed on a computer controlled cycle ergometer (Excalibur Sport, Lode BV, Gronigen, Netherlands). The power output is linear over the range of 10-1000 W, independent of cadence between 30 and 120 rpm, and is accurate to ≤ 2% from 20 W (Lode, Excalibur Sport Operator Manual V2.0, 1994). Instantaneous changes in work rate occur at a rate of up to 1000 W/s and a constant work rate is ensured, independent of pedalling rate, by altering the instantaneous resistance in proportion to changes in cadence. An electrical signal proportional to the work rate was relayed to the computer for data output. In addition, work rate was recorded on a digital chart recorder run at 1mm/s for reference purposes when editing the data as described later. The ergometer was calibrated using a motor-driven torque calibrator (VacuMed, model 17800, Ventura, California, USA).

2.2.2 Gas exchange

Subjects breathed through a mouthpiece unit from which gas was drawn continuously by a capillary tube sample line. Respired [O_2], [CO_2] and [N_2] were measured every 20ms by quadrupole mass spectrometer (QP9000, Morgan Medical, Kent, UK), which was calibrated against two precision-analysed gas mixtures of concentrations which spanned the

range seen during exercise. A bi-directional, low resistance, low dead space (90ml) turbine and volume transducer (Interface Associates Inc., Laguna Niguel, California, USA) attached to the mouthpiece unit was used to measure inspired and expired flows and volume continuously. Prior to each test the turbine was calibrated using a 3.0 L syringe (Hans Rudolph, Kansas City, USA) and the measured value was accepted if it was within 0.2% of 3.0 L. The flow signal was relayed to the computer instantaneously. However, as there is a physical transit delay for the gas sample transport down the capillary line, the flow and gas concentration signal required time alignment. To measure the amount of time alignment necessary, the time delay for a high velocity, high concentration bolus of CO_2 gas to pass from the sample line to the analysis system was determined and recorded on chart paper at 100 mm/s. The delay was then calculated as the time from the voltage signal of the solenoid switch (time zero) to the time to reach 63% of the steady state increase of the CO_2 rise.

Immediately before the test the gas concentrations were checked to ensure they were within 1% of the values that had been accepted for calibration. At the end of the test the gas concentrations were re-checked to ensure they were within 1% of the gas concentration measured prior to the test. Respired gas concentration and volume profiles were also recorded on the digital chart recorder (Astro Med, Rhode Island, USA) to be used later for data editing purposes.

The signals collected throughout the tests from the mass spectrometer and turbine transducer were converted from analog to digital format. Breath-by-breath calculations for

$\dot{V}O_2$, $\dot{V}CO_2$, ventilation ($\dot{V}_E$), partial pressure of O_2 ($P_{ET}O_2$) and partial pressure of CO_2 ($P_{ET}CO_2$) and were performed based on the algorithms of Beaver et al. (1973). These algorithms calculate pulmonary gas exchange over the duration of a single breath, whereby the continuously-measured expired flow and gas concentration signals are divided into consecutive temporal samples (Δt) of 20ms, which is the mass spectrometer sampling interval. Therefore, $\dot{V}_E$ is measured by the equation:

$$\dot{V}_E = \sum_{t=0}^{T} Vexp(t + \Delta t) \bullet \Delta t \qquad [2.1]$$

where Vexp is the mean flow rate during the time interval $t + \Delta t$, minute ventilation ($\dot{V}_E$).

$\dot{V}O_2$ is calculated by the product of the gas concentration and the expired flow for each small sampling period by equation:

$$\dot{V}O_2 = \sum_{t=0}^{T} Vexp(t + \Delta t) \bullet \Delta t \bullet [(\Delta FO_2)true] \qquad [2.2]$$

where ΔFO_2 for true is given by:

$$[(\Delta FO_2)true] = \frac{(F_IO_2 - F_EO_2 - F_IO_2 \bullet F_ECO_2)}{(1-F_IO_2)} \qquad [2.3]$$

where F_IO_2 is the fraction of inspired O_2 and F_EO_2 is the fraction of expired O_2. Minute ventilation ($\dot{V}_E$) or $\dot{V}O_2$ is obtained by summing V_E or VO_2 across the duration of an expiration and dividing by the time for expiration. The calculation of $\dot{V}CO_2$ is based on the same principle as $\dot{V}O_2$.

2.2.3 Heart rate and arterial saturation

Heart rate (HR) was measured beat-by-beat throughout the test using a six lead ECG configuration (Quinton Medical 710, Kent, UK). Paper recordings were made at various times during the test to ensure no cardiac abnormalities occurred. Arterial O_2 saturation was measured continuously and non-invasively at the ring finger using pulse oximetery (Satellite Trans, Datex Engstrom, Helsinki, Finland) to ensure saturation did not fall below 85%; were this to occur (which it did not), the test would be stopped immediately.

2.2.4 Blood lactate samples

Blood lactate was measured from a ~30 μl fingertip blood sample using an automated lancet (Autoclix, Boehringer, Germany), collected in 50 μl capillary tubes prepared with heparin, fluoride and nitrite. Prior to the test, the subject's hand was bathed in hot water (42-44°C) to obtain vasodilation and thus to arterialise the capillary blood. The first sample of blood after the puncture could contain damaged cells and therefore was discarded. The

subsequent sample was capped and stored on ice until analysis, which was performed within 1 hour after sampling, using an automated lactate analyzer (Analox GM-7, Analox Instruments, London, UK). A calibration and re-calibration were performed using an 8mM standard. Blood samples were accepted when two measurements of the same sample were within 0.2 mM of each other

2.2.5 Muscle biopsies

Each subject had a resting muscle biopsy taken from the lateral head of the quadriceps femoris using the Bergstrom needle biopsy technique (Bergstrom, 1962) with suction. This biopsy was taken by an experienced physician. A local anesthetic (lidocaine) was used to decrease the discomfort of the subject. A small incision (4-8mm) was made into the skin, which allowed for entry of the fine needle, which took 50-150 mg of muscle. The incision was then covered with gauze for three days to avoid infection at the incision. Samples were immediately frozen in liquid nitrogen and subsequently stored at –84 °C.

2.3 PROTOCOLS

2.3.1 Familarisation

The first visit was a familiarisation protocol consisting of 4 minute step transitions in work rate from 20 W to 70 W to 20 W which was then repeated if the subject's breathing was

erratic, or it appeared that he had not adjusted to the novelty of the breathing apparatus. Subjects were instructed to pedal at a comfortable cadence of 45-55 rpm throughout the test. Subsequently, an incremental ramp test of 3W/12sec (15W/min) was performed to the limit of tolerance, determined by when subjects could no longer continue to pedal at a cadence of 60 rpm or greater. These familiarisation tests were designed to minimise the physiological response to stress and discomfort that could contaminate the response measured on subsequent trials.

2.3.2 Protocol for moderate-intensity exercise

A second incremental maximal exertion test as described above was performed for the non-invasive estimate of the lactate threshold (θ_L). On two subsequent visits subjects performed a total of 5-6 step transitions in work rate. Subjects were initially at rest on the cycle and were not instructed to begin pedalling until it was established that they were comfortable and no longer hyperventilating, indicated by a RER around 0.8, a $P_{ET}CO_2$ of 6% and a $\dot{V}_E$ of less than 10.0 L/min. Subjects then began cycling at a comfortable cadence between 45-55 rpm at 20 W to achieve unloaded pedalling (so the only energy expended was in rotating the legs). This was continued for a minimum of 3 minutes, until $\dot{V}O_2$ and RER were stable at which point the work rate increased instantaneously (1000W/s) to a work rate corresponding to 90% θ_L which was continued for 6 minutes. The work rate was then instantaneously returned to 20 W for a minimum of 6 minutes of recovery, until $\dot{V}O_2$ and RER were stable. A second transition similar to the first was then repeated. Subjects then

rested off of the cycle for a minimum of 15 minutes and, if they were then comfortable enough to do one more repeat, the same protocol was repeated but with only one transition (20 W to 90% θ_L to 20 W).

2.3.3 Protocol for power-duration tests

On differing days (with a minimum of 48 hours between tests), subjects performed 3-5 step tests of supra CP exercise (work rates estimated based on the maximum work rate from the incremental test) to the limit of tolerance, for determination of the power-duration curve (see section 2.4 for details). The order of the periods of exercise was randomised and subjects were not informed of the work rate that was to be performed. Subjects began on the cycle at rest and were not instructed to begin pedalling until breathing was normal as described above. Subjects then began pedalling at 20 W for a minimum of 3 minutes. Within one second before the instantaneous transition to the higher work rate, (i.e. insufficient time to allow for any "startle" response) the subjects were instructed to increase their pedalling rate to overcome the inertia associated with the large increase in resistance. Subjects then cycled at the given work rates, at a cadence comfortable for them (ranging from 65-85 rpm) to the limit of tolerance, determined when the cadence dropped more than 15 rpm below the pedal rate they had selected throughout the test despite verbal encouragement.

2.3.4 Protocol for very heavy exercise tests to 6 and 12 minutes of fatigue

Based on the power duration curve profile, work rates that would correspond to 6 minutes ("6 minute") and 12 minutes ("12 minute") of fatiguing exercise were estimated. Subjects then performed 2-4 repeats at each of these work rates (protocol as above for the power duration tests), until 2 repeats with similar times (within 30s of each other) and similar peak $\dot{V}O_2$ and HR values were obtained. Trials that did not meet these criteria were discarded. In addition, finger prick blood samples were taken for [La] on each of the tests. Samples were taken at rest, after 3 min of 20 W exercise and 10 sec before estimated exhaustion or at exhaustion if it ensued earlier than estimated.

2.4 ANALYSIS

2.4.1 Determination of the lactate threshold

Prior to analysis, the breath-by-breath gas exchange data were initially edited for 'bad' data points based on visual inspection of 'mis-triggers' of a true breath, due to coughing, swallowing, sighs or movement on the cycle. These responses result in mis-calculated values or values that were out of the range of the true $\dot{V}O_2$ response.

The lactate threshold (θ_L) was determined non-invasively from pulmonary gas exchange data. At the θ_L an additional, non-metabolic production of CO_2 occurs due to the buffering of lactic acid by the bicarbonate reaction:

$$La^{-}+H^{+}+Na^{+}\ (\text{or } K^{+})HCO_3^{-} \leftrightarrow H_2CO_3 \leftrightarrow CO_2+H_2O \qquad [2.4]$$

The increase in CO_2 production is also associated with an increase in $\dot{V}_E$. This increase in $\dot{V}_E$ is thereby out of proportion to $\dot{V}O_2$. Therefore the following gas exchange variables were plotted against $\dot{V}O_2$: $\dot{V}CO_2$ (V-slope), respiratory exchange ratio (RER), the ventilatory equivalents ($\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$) and end-tidal gas tensions ($P_{ET}CO_2$ and $P_{ET}O_2$). Averaging of the data over a variable number of breaths was performed to obtain the most appropriate visual image for determining the location of the θ_L, based on the following criteria (Beaver et al., 1985; Whipp et al., 1986):

(a) a more positive (increased) slope in $\dot{V}CO_2$ vs. $\dot{V}O_2$;

(b) an increase in $\dot{V}_E/\dot{V}O_2$ with no change in $\dot{V}_E/\dot{V}CO_2$;

(c) the onset of an increase in $P_{ET}O_2$ with $P_{ET}CO_2$ reaching the apex of its increase or remaining constant.

The θ_L identification for a representative subject is shown in Figure 2.1.

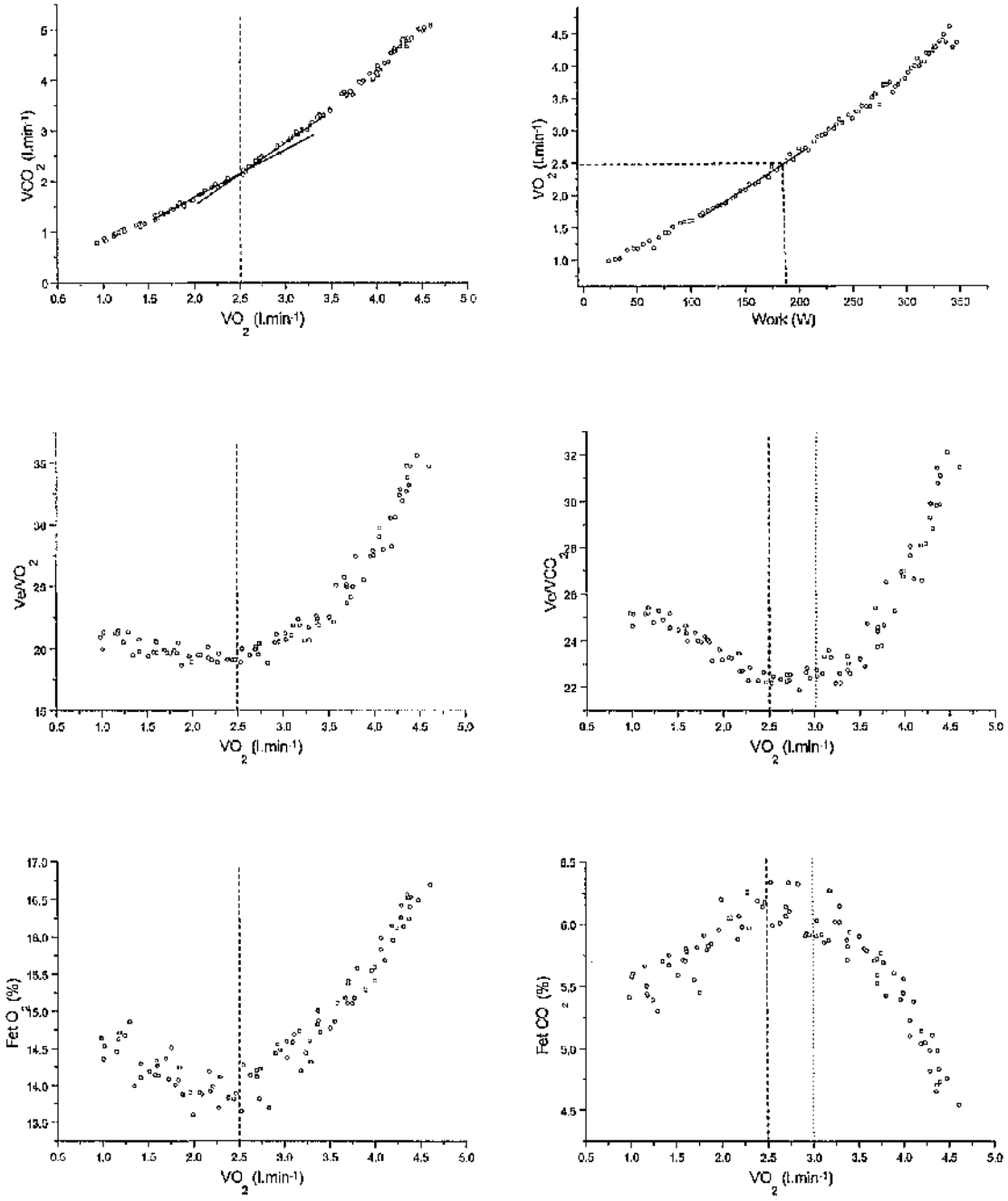

Figure 2.1. Identification of the lactate threshold (θ_L). The upper left panel shows identification of the θ_L based on the V-slope. Solid lines represent the sub - θ_L and supra - θ_L slopes and the intersection point representing θ_L. The upper right panel shows the conversion from $\dot{V}O_2$ at the θ_L to WR at the θ_L. In all other panels the thick vertical dashed line represents θ_L and the thin dotted line represents the respiratory compensation point.

The $\dot{V}O_2$ identified at the θ_L was then converted to an equivalent WR as determined from the incremental test $\dot{V}O_2$ to WR relationship. However, as an incremental ramp protocol was used, the $\dot{V}O_2$ response lags behind the steady state response for the associated WR. Therefore, to account for this lag an estimated 60s was used for the mean response time of the lag, and thus the WR at the desired $\dot{V}O_2$ was 15 W high (as the ramp was 15W/min) and this was subtracted from the determined WR.

2.4.2 Determination of the power-duration curve

There exists a hyperbolic relationship between the tolerable duration of high intensity constant load exercise and power output expressed by the equation:

$$W' = (P - CP) \cdot t \qquad [1.7]$$

where W' is the curvature constant, P the power output, CP is the critical power (of fatigue threshold) and t is the tolerable duration of exercise. (Moritani et al., 1981; Whipp et al., 1982b; Poole et al., 1988). The plot of power versus time was linearised by plotting power versus $1.time^{-1}$, the y-intercept (or power) then reflecting CP (Poole et al., 1988) (see section 1.3, Figure 1.2). The target WRs for the "6 minute" and "12 minute" tests for each subject were predicted from his power duration curve (Figure 2.2).

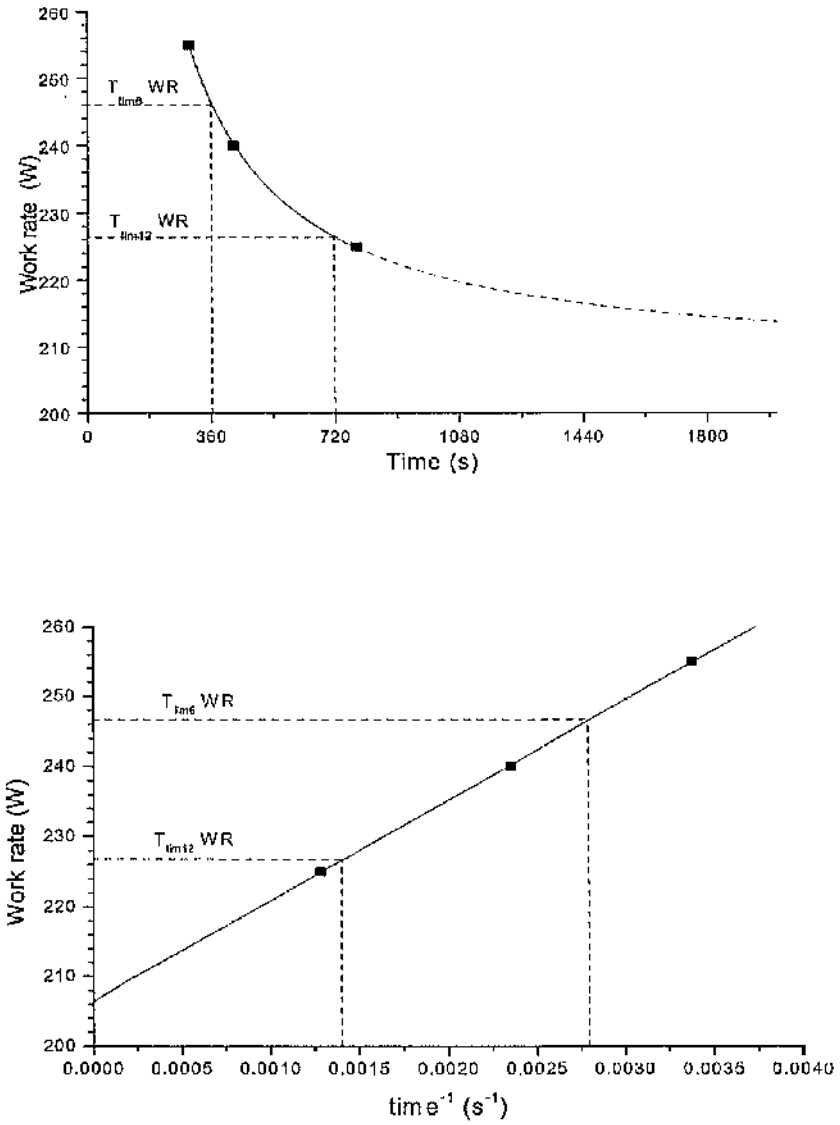

Figure 2.2. Estimation of the WRs required for the "12 minute" and "6 minute" tests, for a single representative subject.

2.4.3 **Modelling of the $\dot{V}O_2$ kinetics**

For each trial, 'bad' data points were removed as described above. In addition, data points lying outside a 99% prediction band (of a preliminary fit) were removed. Data were then interpolated to 1s, time-aligned to the onset of the work rate (set to 0 sec), and similar protocols for each subject (5-6 repeats of moderate-intensity, two fatiguing tests to approximately six minutes of >CP, and two fatiguing tests to 12 minutes of >CP) were ensemble-averaged. The data were then averaged over 10s intervals.

The phase II component of the $\dot{V}O_2$ response was modelled to estimate the parameters of the response using a mono-exponential model (Whipp et al., 1982; Paterson and Whipp, 1991):

$$\dot{V}O_2\ (t)=\Delta\ \dot{V}O_2\ (ss)+ \dot{V}O_2\ (1-e^{-(t-\delta/\tau)}) \qquad [1.8]$$

where $\dot{V}O_2$ (t) is $\dot{V}O_2$ at time t, $\Delta\ \dot{V}O_2$ (ss) is the baseline $\dot{V}O_2$, $\dot{V}O_2$ is the asymptotic value to which $\dot{V}O_2$ is assumed to project, τ is the time it takes to reach 63% of the response, and δ is the time delay.

This was done with non-linear, least-squares regression technique using Origin software (Origin, Microcal Software, USA). The best fit was determined when the sum of square of errors (χ^2) was not reduced with further iterations. The appropriate fitting 'window' for phase II was identified by:

(a) Iteratively increasing the fitting window from 35s back towards 0s until the τ value increased, indicating inclusion of phase I data

and/or

(b) when the RER began to drop (due to slower $\dot{V}CO_2$ kinetics compared with $\dot{V}O_2$ initially and therefore reflecting the point at which the muscle metabolic rate is being seen at the mouth) (Whipp and Ward, 1992).

The time taken to identify the start of phase II was taken as the 10s point after the occurrence of the above determinants. A fit was then performed from the identified beginning of the phase II to end of exercise at 6 minutes.

For the very heavy intensity exercise data, phase I was identified as described above and then a fitting 'window' from the start of phase II to initially 90s was used. To identify the start of the slow component, this 'window' was expanded iteratively towards end-exercise until the "goodness of the fit" deteriorated determined by three factors:

(a) the deviation of the residual plot deviated from the zero line;

(b) the attainment of a 'threshold' in the χ^2 value (sum squared error) indicating a consistent deviation from the exponential fit;

(c) when the value of τ began to systematically become larger.

A fit was then performed from the identified start of phase II to the 10s point before the occurrence of these determinants.

The phase II amplitude for the $\dot{V}O_2$ response (A_{II}) was calculated as the change in $\dot{V}O_2$ ($l.min^{-1}$) for each step from baseline to the end of 6 minutes for moderate intensity exercise, and to the extrapolated phase II steady state for very heavy intensity exercise (figure x). The A_{II} was expressed relative to WR by dividing the A_{II} by the change in work rate (ΔWR); this is termed the gain (G_{II}). The amplitude of the slow component (A_{sc}) in the very heavy intensity exercise was calculated as the difference between the end exercise $\dot{V}O_2$ (20s mean) and the identified $\dot{V}O_2$ at the start of the slow component ($\dot{V}O_2$ value taken from the end value of the phase II exponential fit) (Figure 2.3). Again, the slow component gain (G_{sc}) was given by $A_{sc}/\Delta WR$. The rate of change for the slow component is calculated as the difference in A from minute 3 to minute 5 (20s mean) expressed as $A_{sc\ 5\text{-}3}$ (Figure 2.3).

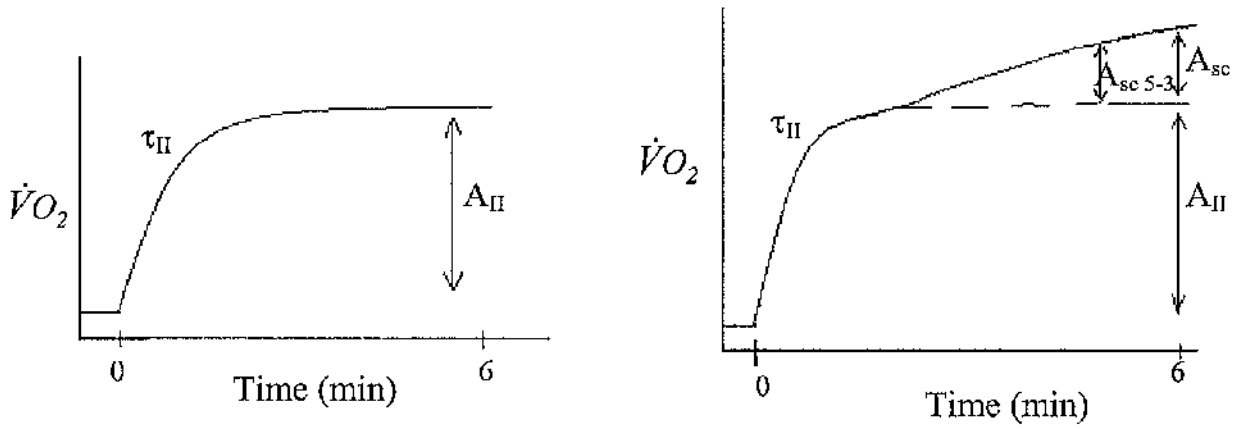

Figure 2.3. Schematic representation of the $\dot{V}O_2$ kinetic parameters used in the present study for (a) moderate intensity exercise (b) very heavy intensity exercise.

2.4.4 Lactate

Lactate values represent an average of the two values obtained from each sample. The amplitude of the lactate increase was taken as the difference between the 20 W sample and the end-exercise sample.

2.4.5 Creatine Kinase

For quantification of the total creatine kinase (CK) activity the muscle protein was extracted and placed in solutions of saturating substrate and enzyme concentrations so that the CK activity would be the rate limiting step. The maximal rate of CK activity was measured based on the production of nicotinamide diphosphate (NADPH) as it is proportional to the CK activity, this was done using (a) the absorbency of NADPH with spectrophotometery (b) the optical density of NADPH product on an electrophoresis gel.

2.4.5.1 Total CK activity by spectophotometry

Total CK activity was determined at 25°C using a hexokinase/glucose-6-phosphate dehydrogenase-coupled enzyme system, that ultimately yields a reduced nicotinamide diphosphate (NADPH) proportional to the total CK activity (Sigma Diagnostics, St. Louis, MO). A portion of the muscle biopsy was homogenized for 15 s in a 1:100 (wt/vol) dilution of CK extraction buffer containing 26mM Tris, 0.3M sucrose, 1% non-ionic detergent (NP-40) and 20mM mercaptoethanol at pH 8.0. Homogenates were then diluted

to 1:100 in extraction buffer. Spectrophotometery was performed at 25°C with 1 ml of CK assay buffer. The assay buffer contained 130mM potassium choloride, 10mM Tris, 1mM magnesium choloride, 2mM adenosine monophosphate (AMP), 50μM diadenosine pentaphosphate (ADP), 5mM glucose, 0.7mM nicotinamide diphosphate, 1.5mM adenosine diphosphate, 9mM PCr, 1.3 units of hexokinase, and 0.5 units of glucose-6-phosphate dehydrogenase at pH 7.4.

Spectrophotometry was performed using a Cecil 4400 double-beam spectrophotometer. Absorbance was measured at 340nm, and a baseline value obtained for the assay buffer was recorded and then 30 μl aliquots of diluted homogenate added to the assay buffer and mixed. The rate of absorbance, due to the production of NADPH through the coupled enzyme reaction, was obtained by measuring the absorbance each minute over five minutes. This rate, in arbitrary units of absorbance.min^{-1}, is proportional to the total CK activity.

2.4.5.2 CK isoform activity resolved electophoretically

Homogenized muscle tissue (as above) was centrifuged for 20 min at 14,000 revolutions/min (4°C). The supernatant was diluted 1:10 in extraction buffer and 1 μl of diluted supernatant was added to a 1% agarose gel (Helena BioSciences, Europe). Electrophoresis was performed at 120 V for 20 min at 4°C. The MM, MB and BB isoforms are separated by this electrophoretic technique due to the different isoelectric properties of the isoforms. CK activity was visualised by spreading CK isoenzyme reagent (90mM

phosphocreatine, 60 mM magnesium acetate, 60 m M glucose, 60 mM N-acetyl cysteine, 15 mM AMP, 12 mM ADP. 6 mM NAD, 10 μM diadenosine pentaphosphate, 9000 U/l hexokinase and 7500 U/l glucose-6-phosphate dehydrogenase) on the gel and incubating for 20 min at 37°C. The production of NADPH in the gel was visualized using ultraviolet light. Photographs of the gels were taken using a digital camera (Nikon Coolpix 990) and analyzed by using a scanning densitometer program (Quantiscan, Biosoft, UK) to quantify the relative isoform activity.

2.4.6 Uncoupling protein mRNA expression

To resolve the UCP messenger ribonucleic acid (mRNA) expression the muscle mRNA was extracted, a complementary DNA strand was annealed to the mRNA and the product was amplified using reverse transcriptase polymerase chain reaction (RT-PCR). The PCR products were quantified relative to each other based on the optical density of the product from gel electrophoresis. The details of the procedures follow below.

a) Homogenization and extraction

A sample of the tissue was frozen in liquid nitrogen and then ground to a powder using pestle and mortar. The sample was mixed with denaturing solution for five minutes until it was dissolved. mRNA extraction was performed using the commercially available Tripure isolation reagent (Roche Diagnostics, Indianapolis, IN). This reagent disrupts cells and

denatures endogenous nucleases thus preserving the integrity of deoxyribonucleic acid (DNA) and mRNA. 1 ml of Tripure was added to the homogenized tissue until it dissolved. The mixture was incubated at room temperature for five minutes, allowing dissociation of nucleoprotein complexes. To separate the mRNA from the organic phase and DNA, 0.2 ml of choloform was added to the mixture, shaken vigorously to 15s, left to incubate at room temperature for 2-15 minutes and centrifuged at 12 000g for 15 minutes at 4°C. The aqueous phase (containing the mRNA) was removed, mixed with 0.5 ml of isopropanol and left to incubate at room temperature for 5-10 minutes resulting in a precipitation of the mRNA from the aqueous layer. The mixture was centrifuged at 12 000g for 10 minutes at 4°C after which the supernatant was removed and discarded. The mRNA pellet was washed (for purification) with 1ml of 75% ethanol after which the ethanol was poured off. The dry pellet was put on ice for 10 minutes and the pellet was re-suspended with nuclease free water heated to 60°C, allowing the mRNA to dissolve into solution form.

b) Spectrophotometry of mRNA

The absorbency of the samples at 260 nm was obtained using a spectrophotometer (Cecil 4400 double-beam), allowing quantification of the amount of mRNA in the solution. Each sample was then diluted with nuclease free water to ensure the same concentration of mRNA between each sample analysed.

c) Complementary DNA

Complementary DNA (cDNA) was made by mixing 5 µl with RNA with 2 µl of Oligo dT's (12-18 bases long) (Amersham Biosciences, Piscataway, NJ). This mixture was incubated at 65°C for 5 minutes and then chilled on ice for longer than a minute, allowing for initial attachment to oligo dT to the RNA. To expand the cDNA strand on the RNA the following chemicals were added; a mixture of the four deoxynucleotide triphosphate (dNTP's) (1 µl of each), 4 µl of first strand buffer, 1 µl of 0.1M di-thio-threitol (DTT), 1 µl of RNAase out (which ensures no mRNA destroying RNAase is contaminating the sample) and 1 µl of superscript reverse transcriptase. The mixture was then incubated at 42°C for 5 minutes, 50°C for 50 minutes and 70°C for 15 minutes. The cDNA was stored at -20°C until use.

d) Polymerase Chain Reaction

Reverse transcriptase polymerase chain reaction (RT-PCR) was performed for the six tissue samples each with the following four primers added separately (i.e. 24 samples); PDK-4, UCP-3, UCP-2 and β-actin. To amplify the cDNA, 4 µl of cDNA was combined with 44 µl of a commercially available, pre made 'reddy mix' (ABgene, Surrey, UK), which was a mixture of Taq polymerase, dNTP's, reaction buffers and magnesium choloride. This mixture was also combined with 1 µl of an antisense and 1 µl of sense primer (TAGN, Newcastle Upon Tyne, UK; sequences shown in Table 2.2), which were diluted in 2x the volume in nuclease free water, making a solution from the powder form.

Primer	Sense	Antisense
UCP-3	AGAACCTCGCGAGGGAGGAAGGA	CAGCGGGGAGGCCACTGT
UCP-2	GACCTATGCCTCATCAAGG	ATAGGTGACGAACATCACCACG
PDK-4	CGGCTTGCCAATTTCTCGTCTGTA	TGATCCCGTAAAGTGTCCTGAGTG
β-actin	CCCAAGGCCAACCGCGAGAAGAT	GTCCCGGCCAGCCAGGTCCAG

Table 2.2. Sense and antisense primer sequences used for RT-PCR.

Each of the end mixtures were therefore a reaction mixture of 50 μl. PCR cycles were then performed using an automated cycler (Darwin, Gene Legends, East Sussex, UK) with the following protocol; 5 minutes at 94°C, 35 repeats of (a) 30s at 94°C for denaturing (cDNA/RNA double strand opens to a single strand) (b) 30s at 57°C for annealing of the primers to the cDNA (c) 30s cycles of 45s at 72°C for extension of the template strand with the dNTP bases and finally 7 minutes at 72°C. PCR products were stored at -20°C until analysis.

e) Electrophoresis

An agarose gel was made using 40 ml of 1x tris-borate-EDTA (TBE) buffer (Promega, Madison, US) and 0.4 g of agarose. The mixture was boiled for one minute and allowed to cool. Ethidium bromide (0.4 μl) was added to the solution as it labels the PCR product,

allowing visualisation under the ultra violet light later used for quantification. The solution was then added to a gel frame and allowed to set over night. 5 μl of each PCR product was mixed with 5 μl of the loading dye bromophenol, which allows visualization of the PCR product as it advances through the gel when electrophoresis is applied. The samples and a DNA ladder (providing a size reference) were added and loaded into the wells of the agarose gel. Electrophoresis was performed at 100V until the products had advanced 2/3 of the way up the gel. The bands were visualized using ultraviolet light. Photographs of the gels were taken using a digital camera (Nikon Coolpix 990) and analyzed by using a scanning densitometer program (Quantiscan, Biosoft, UK) to quantify the relative intensity.

2.5 STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation (SD). Tests to confirm that the data was normally distributed were performed using the Ryan-Joiner test. Comparisons of results under different experimental conditions performed by each of the subjects were made by paired t-tests. Relationships between variables were examined using Pearson's product-moment correlation. Statistical analyses were performed using Minitab (Minitab Inc., PA, US). Statistical significance was accepted at $P < 0.05$.

CHAPTER 3

RESULTS

3.1 DEMARCATIONS OF EXERCISE INTENSITY

The demarcations of exercise intensity are (a) the lactate threshold (θ_L), which demarcates the moderate from the heavy intensity exercise domain; (b) the critical power (CP) or fatigue threshold, which demarcates the heavy from the very heavy intensity domain; (c) and $\dot{V}O_{2\ peak}$, which demarcates the very heavy from the severe intensity exercise domain (see section 1.3 for details).

Above CP the tolerable duration of exercise decreased systematically with increasing work rate. As shown in the representative example (Figure 3.1), exercise duration decreased curve linearly with increased WR, with this relationship being well fit by a hyperbolic function (Monod and Scherrer, 1965; Moritani et al., 1981; Poole et al., 1988). The power asymptote of this hyperbolic power-duration (WR-t) relationship or alternatively the y-intercept of the transformed WR-1/t linear regression, defines this work rate. In addition, the curvature constant of the hyperbola or the slope of the WR-1/t relationship represents that constant amount of work that can be performed above CP (W'). An example of a power duration curve and a power versus 1/time plot for a representative subject is shown

in Figure 3.1. The WRs at each of the determined exercise intensities demarcations (θ_L, CP and $\dot{V}O_{2\,peak}$) can be found in section 2.1, Table 2.1. As θ_L and CP represent highly variable proportions of $\dot{V}O_{2\,peak}$ and WR_{peak} respectively (see section 1.3). Therefore, as well the CP is expressed as a percentage of the difference between the WR at θ_L and the WR_{peak} (Δ'/Δ).

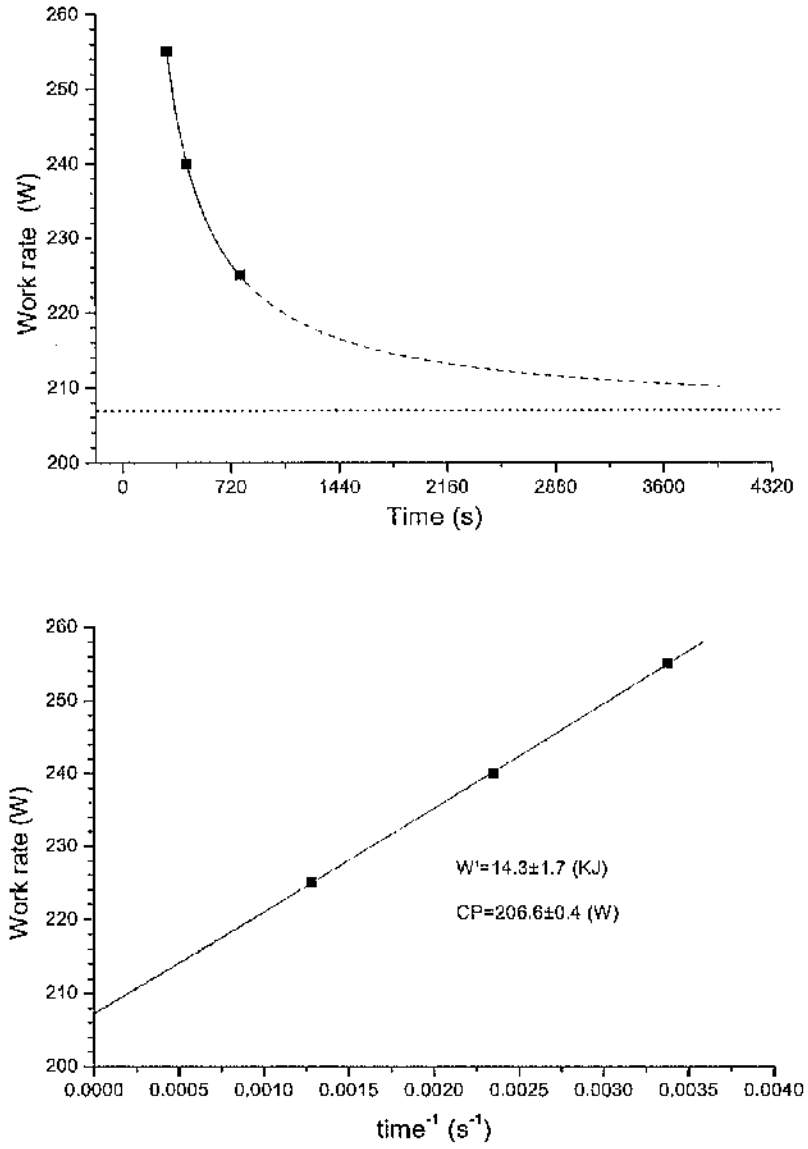

Figure 3.1. The power-duration relationship for a single representative subject (subject 1) with three tests to the limit of tolerance, (solid squares). The top panel shows the hyperbolic work rate versus time curve where the asymptotic value along the x-axis dashed line represents CP and the curvature constant equals W'. The lower panel shows the power-duration relationship linearised as a function of 1/time. The intercept value on the work rate axis represents CP and the slope equals W'.

3.1.1 Moderate-intensity exercise

a) $\dot{V}O_2$ response kinetics

Following exercise onset, $\dot{V}O_2$ increases instantaneously while RER remains stable for ~18s (Figure 3.2). RER then begins to drop and $\dot{V}O_2$ evidences a more marked increase. This transition point (arrow on Figure 3.2) represents the phase I-II transition (Whipp et al., 1982).

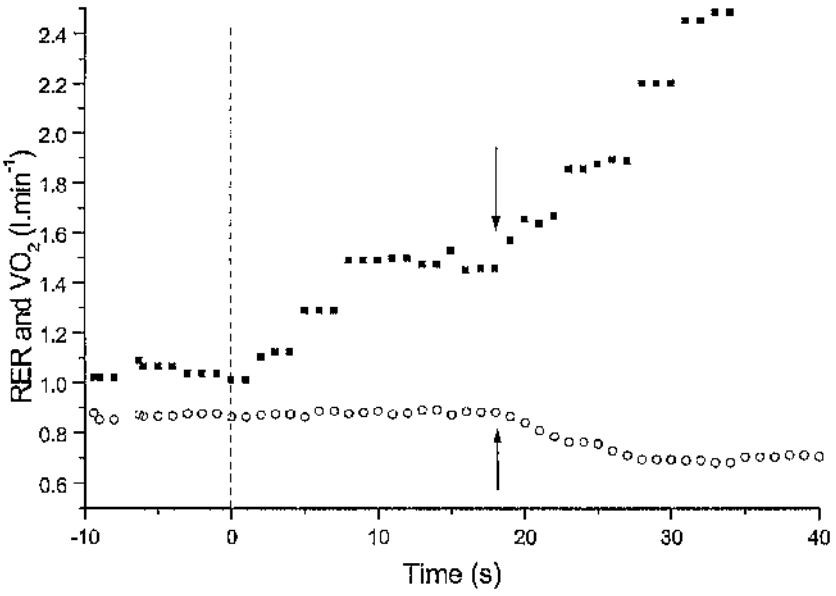

Figure 3.2. Expanded display of RER (open circles) and $\dot{V}O_2$ (solid squares) following the onset of exercise (dashed line). Arrows indicated the identified start of the phase II response. Each point represents 1s interpolated value (see methods, page 70).

Phase II

$\dot{V}O_2$ then continues to increase as an exponential function with no discernible delay, to reach a new steady state within ~2-3 min; a corresponding decline was evident at the off-transient. A representative example of a complete $\dot{V}O_2$-on-off-transient response is shown in Figure 3.3, together with the best fit monoexponential to phase II (see methods, page 70, equation 1.8);

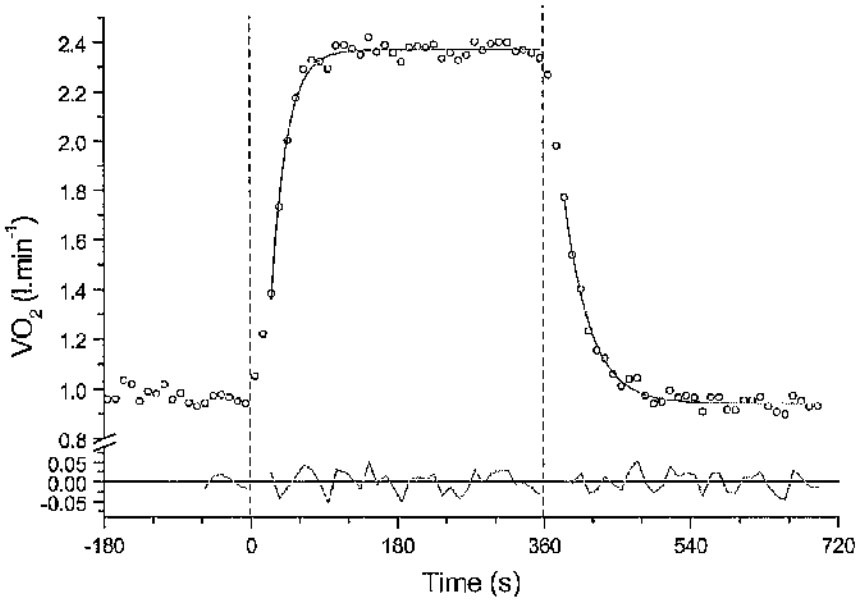

Figure 3.3. Averaged (10s) $\dot{V}O_2$ response (n=6), for an individual subject (subject 2), to a square wave change in WR. Dashed lines represents on onset and offset WR square-wave function. The residuals, shown in grey below the $\dot{V}O_2$ response, indicate the deviation of the fit from the actual response.

i.e. the initial phase I components were excluded based on a (a) visual identification, (b) lengthening of the τ_{II} estimate when phase I data points were included and (c) the RER profile (Figure 3.2).

What is clearly evident for this representative subject is that the residuals (a) scatter essentially randomly about zero (indicating a good fit to the model throughout the entire transient and (b) they are of small amplitude, i.e. < ± 0.05 $l.min^{-1}$ compared to the amplitude of the $\dot{V}O_2$ response itself (< ~3% deviation in the on transient, < ~2% in the steady state).

b) Individual variability

Phase II

The group-mean parameter values for the $\dot{V}O_2$ kinetics to the moderate exercise are presented in Table 3.1. The mean on-transient phase II time constant (τ_{II}) was 21.2±3.7s, which was not significantly different from the mean off-transient τ_{II} (25.3±5.1s). All subjects demonstrated this trend except one subject (subject 2) in whom the on-transient τ_{II} was 19.0s and the off-transient τ_{II} was 31.6s. However, we are confident in this response for subject 2 as (a) the τ_{II} for each individual trial were similar to that of his averaged response; (b) the trend for a longer off-transient τ was evident in every individual trial; and (c) the fit of this subject was 'good', with a SD of only 0.5s and 0.6s for the on and off transients τ_{II} values respectively. The τ_{II} on value across subjects ranged from 16s to 25s.

Because of the different WR amplitudes across subjects (reflecting their different θ_L values; Table 2.1), the corresponding steady-state change in $\dot{V}O_2$ (A_{II}) varied between individuals, being higher when the work rate was higher. $A_{II\ on}$ ranged from 0.5-1.41 $l.min^{-1}$ and was not different to $A_{II\ off}$, which ranged from 0.51-1.43 $l.min^{-1}$. However, when A_{II} was normalized to WR (i.e. to provide the gain (G_{II})) the $G_{II\ on}$ values were reasonably similar with a mean of 11.0±0.5 ml min^{-1} W^{-1}, and were not significantly different from $G_{II\ off}$ (mean=10.8±0.7 $ml.min^{-1}.W^{-1}$).

No relationship was found between τ_{II} and $\dot{V}O_{2\ peak}$ (r=0.528, p=0.221) or between τ_{II} and θ_L (r=-0.215, p=0.682).

Table 3.1. Individual subject on and off-transient $\dot{V}O_2$ kinetics for moderate intensity exercise.

WR represents work rate, τ_{II} represents the phase II time constant, SD represents standard deviation, A_{II} represents the phase II amplitude and G_{II} represents the phase II gain.

Subject	WR (W)	τ_{on} (s)	SD_{on} (s)	τ_{off} (s)	SD_{off} (s)	$A_{II\ on}$ ($l\ min^{-1}$)	$A_{II\ off}$ ($l\ min^{-1}$)	$G_{II\ on}$ ($ml\ min^{-1}\ W^{-1}$)	$G_{II\ off}$ ($ml\ min^{-1}W^{-1}$)
1	67	25.3	2.3	30.0	2.4	0.50	0.51	10.6	10.9
2	153	19.0	0.5	31.6	0.6	1.41	1.43	10.6	10.8
3	77	19.8	2.0	19.7	1.7	0.65	0.65	11.4	11.4
4	82	15.9	1.4	19.1	1.5	0.66	0.64	10.6	10.3
5	78	25.0	1.4	24.3	1.4	0.63	0.57	10.9	9.8
6	90	22.5	1.0	26.0	0.9	0.81	0.81	11.6	11.6
Mean	91	21.3	1.3	25.3	2.0	0.78	0.77	11.0	10.8
SD	31	3.7	0.6	5.1	0.5	0.33	0.34	0.4	0.7

3.1.2 Very heavy-intensity exercise

Above CP, a target of two repeat tests was selected this reflected necessary temporal constraints. However, it is important to emphasis that loss of statistical power resulting from a small "n" was likely to have been offset (to some extent) by the large amplitude of these supra-CP $\dot{V}O_2$ responses (Lamarra et al., 1987) (see section 4.5).

Despite the stringent criteria subjects followed so as to standardise conditions for testing (outlined in section 2.1) obtaining reproducible trials (in terms of tolerable duration) was difficult. This was perhaps not unexpected, given the challenging nature of these tests). In three cases (subject 2, 4, 5), only one trial for "12 minute" was used as the subjects were unable to perform a second trial with a time close enough to the first trial (i.e. differing by more than some 40 sec). This trend for "12 minute" to be less reliable than for shorter fatiguing tests has also been noted by Poole et al. (1988): these authors suggested that tests with a tolerable duration of ~8 min had increased variation (4-6%) compared to tests with a tolerable duration of ~4 min (2-4%). However, in the present study the $\dot{V}O_2$ response parameters could still be estimated with one trial i.e. with an acceptably small SD on the time constant.

In addition, the $\dot{V}O_{2\ ee}$ value was significantly lower for the "12 minute" test compared with "6 minute" test (3.89±0.55 and 4.00±0.52 respectively; p=0.014). The lower $\dot{V}O_{2\ ee}$ value was reached in the "12 minute" test despite randomisation of the tests, verbal

encouragement, repeated attempts and subject exhaustion at a similar HR values (mean "12 minute"=184±10 bpm, "6 minute" =181±8 bpm). The values for $\dot{V}O_{2\ cc}$ are shown in Table 3.2. Note that the inter-subject difference in $\dot{V}O_{2\ cc}$ ranged from 0.05 l.min^{-1} for subjects 1 and 2 to 0.25 l.min^{-1} for subject 3.

Subject	$\dot{V}O_{2\ ee}$ "6 minute" (l.min^{-1})	$\dot{V}O_{2\ ee}$ "12 minute" (l.min^{-1})	$\dot{V}O_{2\ ee}$ "6 minute - 12 minute" (l.min^{-1})
1	3.63	3.58	0.05
2	4.80	4.75	0.05
3	3.57	3.33	0.25
4	4.43	4.32	0.12
5	3.55	3.46	0.10
6	3.98	3.88	0.11
Mean	4.00	3.89	0.11
SD	0.52	0.55	0.07

Table 3.2. $\dot{V}O_{2\ cc}$ values for the "12 minute" and "6 minute" tests and the difference between them.

The analytical goals of the supra-CP component of the project was to examine the proportional contributions of phase II and the slow component to the vial $\dot{V}O_2$ response at the two selected WRs. It was therefore decided to submit the "12 minute" data to a normalisation strategy, to facilitate comparison with the "6 minute" data (i.e. to account for the $\dot{V}O_{2\ ee}$ being slightly less in the "12 minute" tests). Thus, the "12 minute" data were expressed in terms of the actual response achieved and as a corrected value ($A_{cc\ cor}$). A schematic representation of the correction is shown in Figure 3.4: the "12 minute" $\dot{V}O_{2\ cc}$ value is thus "extended" to the $\dot{V}O_{2\ ee}$ value for the "6 minute" test, allowing the amplitude

components of the "12 minute" $\dot{V}O_2$ response to be expressed relative to the new A_{ee} value that would have occurred, had subjects actually achieved the "6 minute" $\dot{V}O_{2\,ee}$ on the "12 minute" test.

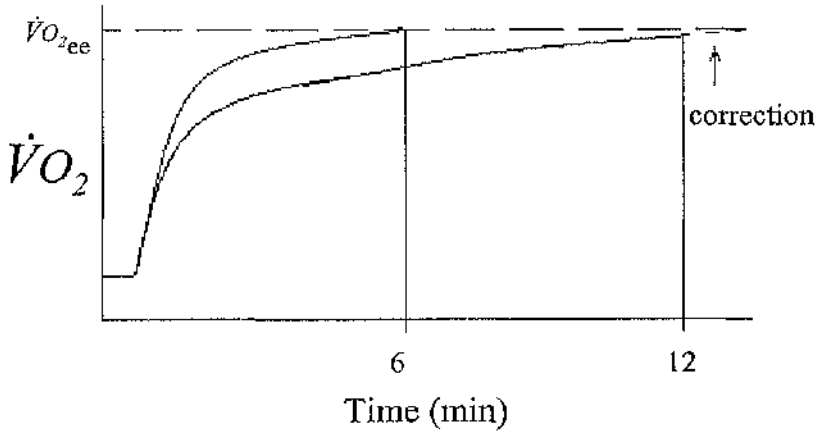

Figure 3.4. Schematic representation of the correction for $\dot{V}O_{2\,ee}$ for the "12 minute" test. The "12 minute" $\dot{V}O_{2\,ee}$ is extended (indicated by the arrow) to that of the "6 minute".

3.1.2.1 "12 minute" test

For the "12 minute" test, subjects cycled for a mean of 11min 52 s± 40s. The mean Δ[La] at the end of the test was 8.5±0.8 mmol.l^{-1}. Individual performance times and Δ[La] values are shown in Table 3.3.

a) $\dot{V}O_2$ response kinetics

Phase II

Similar to the moderate-intensity $\dot{V}O_2$ response profile, following phase I, the $\dot{V}O_2$ increased as an exponential function. However, a new steady state is not reached within ~2-3 min; rather, a slow component is superimposed on the phase II response, with $\dot{V}O_2$ continuing to increase until $\dot{V}O_{2\,peak}$ is reached (see section 1.3). A representative example is shown in Figure 3.5.

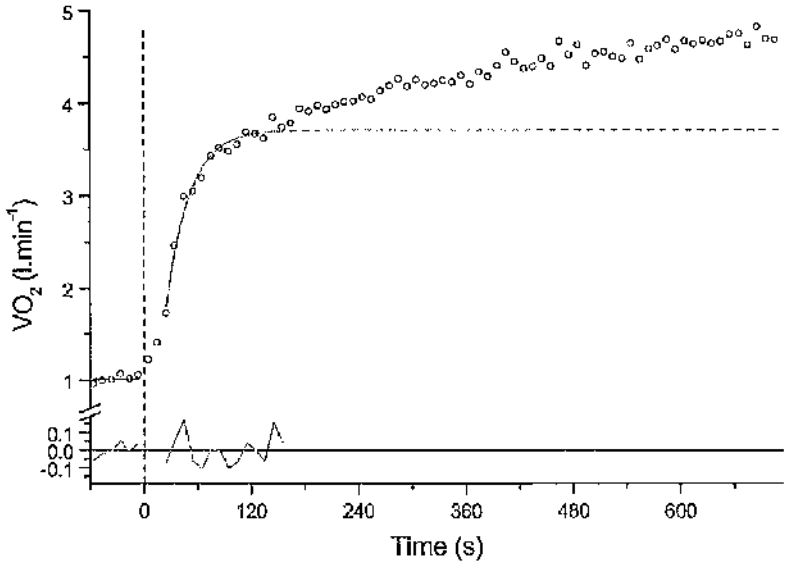

Figure 3.5. Averaged (10s) $\dot{V}O_2$ response (n=1), for an individual subject (subject 2), to a square-wave increase in WR. The vertical dashed line represents on onset of WR, the solid line represents the fitting region and the extending dashed line represents the projection of that fit.

The phase II response was modelled as a single exponential function (see section 2.4.3 for details) i.e. phase I and slow component were excluded (see section 2.4.3).

As described for moderate-intensity exercise (section 3.1.1, Figure 3.3) it is evident for this representative subject that (a) the residuals scatter apparently randomly about zero (although less so than shown for moderate-intensity exercise, indicating a slightly inferior fit) and (b) there is a small amplitude of the residuals e.g. < ± 0.15 l.min-1 when compared to the overall amplitude (< ~5% in the on transient, < ~4% in the steady state).

The mean phase II time constant (τ_{II}) was 26.9±10.3s. The phase II amplitude (A_{II}) was 2.32 ±0.39 l.min^{-1}: when expressed relative to the end-exercise amplitude (A_{ee}) the A_{II} contribution was 76.7±4.0%; and when expressed relative to WR, the phase II gain (G_{II}) was 9.8±0.3 ml.min^{-1}.W^{-1}. The individual values are shown in Table 3.3.

Slow component

The increase in $\dot{V}O_2$ between ($A_{sc\ 5\text{-}3}$) was 0.29±0.06 l.min^{-1}, the slow component amplitude (A_{sc}) was 0.70±0.19 l.min-1 and the corrected slow component amplitude ($A_{sc\ cor}$) was 0.81±0.16 l.min-1. Therefore, when expressed relative to A_{ee}, A_{sc} was 23.3±4.0% and $A_{sc\ cor}$ was 27.2±4.9%. The slow component gain (G_{sc}) was 3.0±0.7 ml.min^{-1}.W^{-1} and the corrected slow component gain ($G_{sc\ cor}$) was 3.5±.7 ml.min^{-1}.W^{-1}. The individual values are shown in Table 3.4.

Table 3.3. Individual values for work rate, performance time, blood [lactate] and phase II $\dot{V}O_2$ kinetics for the "12 minute" test.

WR represents work rate, Δ[La] represents the increase in blood [lactate], τ_{II} represents the phase II time constant, SD represents standard deviation, A_{II} represents the phase II amplitude, G_{II} represents the phase II gain and A_{II} % A_{ee} represents the proportional contribution of A_{II} to the total $\dot{V}O_2$ increase.

Subject	WR (W)	Performance time (s)	Δ [La] ($mmol.l^{-1}$)	τ_{II} (s)	SD (s)	A_{II} ($l.min^{-1}$)	G_{II} ($ml.min^{-1}.W^{-1}$)	A_{II} as % A_{ee} (%)	A_{II} as % A_{ee} (corrected) (%)
1	227	758	8.73	24.2	0.7	2.09	10.1	77.6	75.6
2	298	714	8.40	26.1	1.0	2.69	9.7	71.9	70.6
3	208	731	8.50	23.2	0.7	1.83	9.7	76.3	66.0
4	315	684	8.60	15.0	0.8	2.81	9.5	81.0	77.7
5	226	735	9.65	46.1	1.6	2.03	9.9	72.6	69.2
6	260	647	7.10	27.0	0.7	2.43	10.1	81.1	77.5
Mean	256	712	8.50	26.9	0.5	2.52	9.8	76.7	72.8
SD	39	40	0.82	10.3	0.2	0.36	0.3	4.0	4.9

Table 3.4. Individual values for the slow component for the "12 minute" test.

$A_{sc\ 5\text{-}3}$ represents the rate of slow component increase, A_{sc} is the amplitude of the slow component increase, G_{sc} is the gain of the slow component, $G_{sc\ cor}$ is the 'corrected' gain of the slow component, A_{sc} %A_{ee} is the proportional contribution of the slow component.

Subject	$A_{sc\ 5\text{-}3}$ ($l.min^{-1}$)	A_{sc} ($l.min^{-1}$)	$A_{sc\ cor}$ ($l.min^{-1}$)	G_{sc} ($ml.min^{-1}.W^{-1}$)	$G_{sc\ cor}$ ($ml.min^{-1}.W^{-1}$)	A_{sc} as % A_{ee} (%)	$A_{sc\ cor}$ as % A_{ee} (%)
1	0.22	0.60	0.66	2.9	3.2	22.4	24.4
2	0.29	1.05	1.10	3.8	4.0	28.1	29.4
3	0.31	0.57	0.82	3.0	4.3	23.7	34.0
4	0.24	0.66	0.77	2.2	2.6	18.9	22.3
5	0.40	0.77	0.86	3.7	4.2	27.4	30.8
6	0.29	0.57	0.67	2.4	2.8	18.9	22.5
Mean	0.29	0.70	0.81	3.0	3.5	23.3	27.2
SD	0.06	0.19	0.16	0.7	0.7	4.0	4.9

b) Individual variability

Phase II

τ_{II} ranged from 15.0 to 46.1 s. However, four of the subjects (subjects 1,2, 3,6) showed similar τ_{II} values (23-27s), whereas subject 4 had faster (15±0.8s) and subject 5 had slower (46.1±1.6s) values. A comparison between individuals shows that τ_{II} was not associated with G_{II}; i.e. subjects who had very similar τ_{II} values (subject 1 compared with 2, or subject 2 compared with 6) showed a G_{II} difference of 0.4 ml.min-1.W^{-1}. Similarly, subjects with very different τ_{II} values (subject 4 compared with 5) also showed a difference of 0.4 $ml.min^{-1}.W^{-1}$. In addition, the τ_{II} values did not show a relationship with any indicators of the slow component response.

Furthermore, the exercise-induced increase in blood [lactate] (Δ[La]) was not associated with the phase II $\dot{V}O_2$ response parameters. For example subjects 4 and 5 had very different τ_{II} values and also different Δ[La] values; subjects 4 and 3 also had different τ_{II} values, but similar Δ[La]; and subjects 2 and 6 had very similar τ_{II} values but appreciably different Δ[La] values.

Slow component

Subject 4 (with a fast τ_{II}) showed a smaller % $A_{sc\ cor}$, G_{sc} and A_{sc5-3} compared to subject 5 (slower τ_{II}). In contrast, there were subjects who had very similar τ_{II} values i.e. subject 1

compared with 3 or 2 compared with 6 but appreciably different % $A_{sc\ cor.}$, G_{sc} and $A_{sc\ 5\text{-}3}$ (although $A_{sc\ 5\text{-}3}$ was not different for subjects 1 and 3).

Δ[La] also was not associated with any of the slow component indicators. Although Δ[La] was lower in subject 4 compared with subject 5, A_{sc} as % A_{ee} was similar. Likewise, for subject 3 compared with 4, A_{sc} as % A_{ee} was appreciably different but Δ[La] was similar. For G_{sc}, subjects 3 and 4 showed different values but similar Δ[La] values, while subjects 3 and 5 had similar G_{sc} values but appreciably different Δ[La] values. Finally, although $A_{sc\ 5\text{-}3}$ was very different between subjects 1 and 2, Δ[La] was similar; conversely subjects 4 and 6 had similar $A_{sc\ 5\text{-}3}$ values, but very different Δ[La] values.

Therefore, the individual variability in $\dot{V}O_2$ response parameters for the "12 minute" test did not demonstrate any overall trends or relationships between each other or with Δ[La].

c) Comparison to moderate-intensity exercise

The "12 minute" τ_{II} (26.9±10.3s) was not statistically different from that for moderate-exercise (21.3±3.7s, p=0.150). However, it can be seen in Figure 3.6 that there was a trend for the "12 minute" τ_{II} to be slightly slower than the moderate τ_{II} in 3 subjects (subjects 2,3,5) and appreciably slower in subject 6.

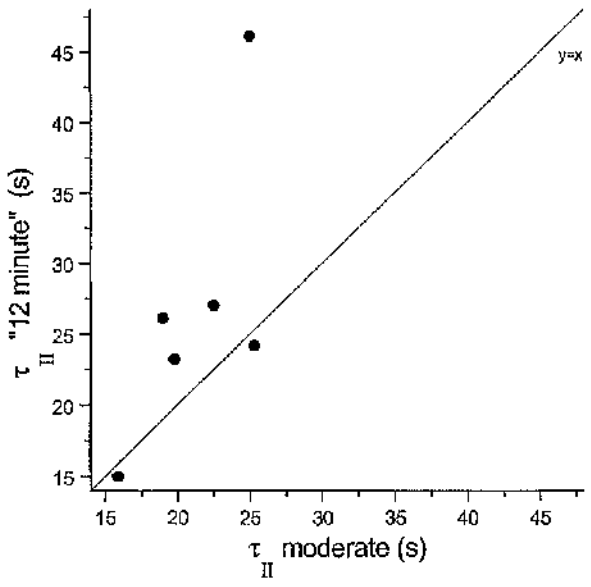

Figure 3.6. τ_{II} values for "12 minute" compared with moderate-intensity exercise.

The G_{II} for moderate intensity exercise (11.0±0.4 $ml.min^{-1}.W^{-1}$) was significantly greater than that for the "12 minute" test (9.8±0.2 $ml.min^{-1}.W^{-1}$, p=0.001) in all subjects as shown in Figure 3.7.

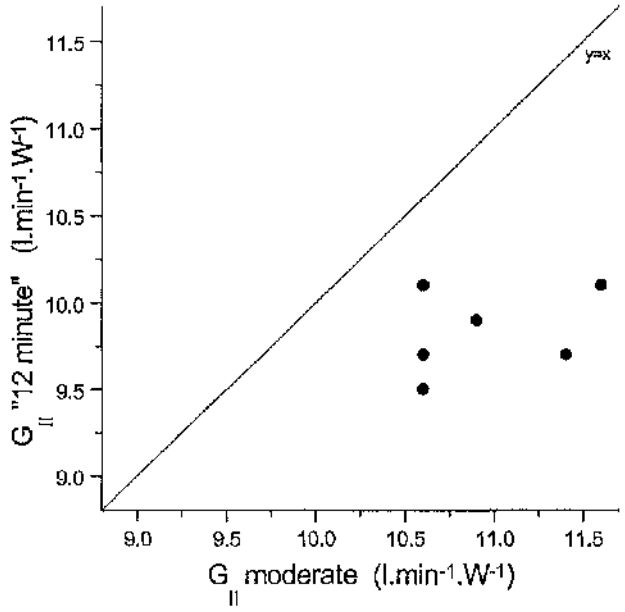

Figure 3.7. Gain for "12 minute" compared with moderate-intensity exercise.

3.2.2.2 "6 minute" test

For the "6 minute" test, subjects cycled for a mean of 6 min 8s ±53s. The mean Δ[La] was 7.9±1.2 mmol.l^{-1}. Individual performance times and Δ[La] values are shown in Table 3.5.

a) $\dot{V}O_2$ response profile

Phase II

As described for the "12 minute" test, following the phase I, the $\dot{V}O_2$ increased as an exponential function. The slow component is superimposed on the phase II response, with

$\dot{V}O_2$ continuing to increase until $\dot{V}O_{2\,peak}$ is reached (see section 1.3). A representative example is shown in Figure 3.8.

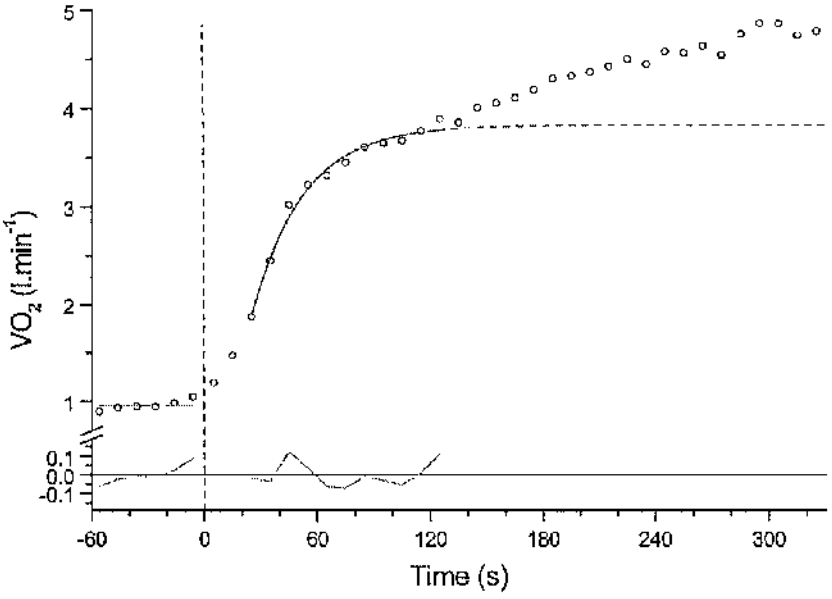

Figure 3.8. Averaged (10s) $\dot{V}O_2$ response (n=2), for an individual subject (subject 2), to a square-wave increase in WR. The dashed line represents on onset of WR, the solid line represents the fitting region and the extending dashed line represents the projection of that fit. The residuals, shown in grey below the $\dot{V}O_2$ response, indicate the deviation of the fit from the actual response.

The phase II response was modelled as a single exponential function (see section 2.4.3 for details) i.e. phase I and the slow component were excluded (see section 2.4.3).

It is evident for this representative subject that (a) the residuals scatter apparently randomly about zero (although less so than shown for moderate intensity exercise, indicating a slightly inferior fit) and (b) there is a small amplitude of the residuals e.g. $< \pm 0.10$ l.min^{-1} when compared to the overall amplitude (< ~3% in the on transient, < ~2.5% in the steady state) .

The mean τ_{II} was 29.3±9.2 s. A_{II} averaged 2.52±0.36 l.min^{-1}: when expressed relative to A_{ee} the A_{II} contribution was 79.8±3.4%; and when expressed relative to WR G_{II} was 9.7±0.6 ml.min^{-1}.W^{-1}. The individual values are shown in Table 3.5.

Slow component

The mean $A_{sc\ 5\text{-}3}$ was 0.38±0.15 l.min^{-1} and A_{sc} was 0.65±0.20 l.min^{-1}. Therefore when expressed relative to A_{ee}, A_{sc} was 20.2±3.4% and when expressed relative to WR, G_{sc} was 2.6±0.5 ml.min^{-1}.W^{-1}. Individual values are shown in Table 3.6.

Table 3.5. Individual values for work rate, performance time, Δ[La] and phase II $\dot{V}O_2$ kinetics for "6 minute" test.

WR represents work rate, Δ[La] represents the increase in blood [lactate], τ_{II} represents the phase II time constant, SD represents standard deviation, A_{II} represents the phase II amplitude, G_{II} represents the phase II gain and A_{II} % A_{ee} represents the proportional contribution of A_{II} to the total $\dot{V}O_2$ increase.

Subject	WR (W)	Performance time (s)	Δ[La] ($mmol.l^{-1}$)	τ_{II} (s)	SD (s)	A_{II} ($l.min^{-1}$)	G_{II} ($ml.min^{-1}.W^{-1}$)	A_{II} as % A_{ee} (%)
1	247	401	7.55	29.1	0.73	2.27	10.0	82.9
2	333	330	7.93	27.3	0.77	2.87	9.2	73.8
3	230	457	8.45	27.1	0.87	2.16	10.3	79.2
4	352	318	9.85	17.4	0.37	2.98	9.0	81.4
5	257	337	6.63	45.7	0.62	2.22	9.4	78.9
6	279	365	6.73	28.9	0.67	2.64	10.2	82.8
Mean	283	368	7.85	29.3	0.67	2.52	9.7	79.8
SD	45	53	1.20	9.2	0.17	0.36	0.6	3.4

Table 3.6. Individual values for the slow component for the "6 minute" test.

$A_{sc\ 5\text{-}3}$ represents the rate of slow component increase, A_{sc} is the amplitude of the slow component increase, G_{sc} is the gain of the slow component, $G_{sc\ cor}$ is the 'corrected' gain of the slow component, A_{sc} $\%A_{ee}$ is the proportional contribution of the slow component

Subjects	A_{sc} ($l.min^{-1}$)	$A_{sc\ 5\text{-}3}$ ($l.min^{-1}$)	G_{sc} ($ml.min^{-1}.W^{-1}$)	A_{sc} as % A_{ee} (%)
1	0.47	0.22	2.1	17.0
2	1.02	0.64	3.3	26.2
3	0.57	0.29	2.7	20.8
4	0.68	0.36	3.0	18.6
5	0.60	0.43	2.5	21.1
6	0.55	0.31	2.1	17.2
Mean	0.65	0.38	2.4	20.2
SD	0.20	0.15	0.5	3.4

b) Individual variability

Phase II

τ_{II} ranged from 17.4 to 45.7 s. However, four of the subjects (subjects 1,2, 3,6) showed similar τ_{II} values (27-29s), whereas subject 4 and 5 had faster (17±0.4s) and slower (46.1±0.6s) values, respectively. A comparison between individuals shows that τ_{II} was not associated with G_{II}; i.e. subjects who had very similar τ_{II} values (subjects 2 compared with 3) showed a G_{II} difference of 0.9 $ml.min^{-1}.W^{-1}$, whereas, subjects with very different τ_{II} (subjects 4 compared with 5) showed a difference of only 0.4 ml.min-1.W^{-1}. In addition, the τ_{II} values did not show a relationship with any indicators of the slow component response. Furthermore, Δ[La] was not associated with the phase II $\dot{V}O_2$ response parameters. For example subjects 4 and 5 had very different τ_{II} values and also different Δ[La] values; subjects 2 and 6 had very similar Δ[La] values but appreciably different τ_{II} values.

Slow component

Subject 4 and 5 had very different τ_{II} values but similar A_{sc} as % A_{cc}, G_{sc} and $A_{sc\ 5\text{-}3}$ values. In contrast, subjects 2 and 6 had very similar τ_{II} values but appreciably different % $A_{sc\ cor}$, G_{sc} and $A_{sc\ 5\text{-}3}$ values.

Δ[La] was also not associated with any of the slow component indicators. Although Δ[La] was higher in subject 4 compared with subject 6, A_{sc} as % A_{cc} was very similar. Likewise,

for subjects 1 compared with 2, A_{sc} as % A_{ee} was appreciably different but Δ[La] was similar. For G_{sc} subjects 1 and 2 showed different values but similar Δ[La] values while subjects 2 and 4 had similar G_{sc} values but appreciably different Δ[La] values. Finally, although $A_{sc\ 5\text{-}3}$ was very different between subjects1 and 2, Δ[La] was similar; conversely, subjects 4 and 6 had similar $A_{sc\ 5\text{-}3}$ values but very different Δ[La] values.

Therefore, as found for the "12 minute" test, the individual variability in the $\dot{V}O_2$ response parameters for the "6 minute" test did not demonstrate any overall trends or relationships between each other or with Δ[La].

c) Comparison to moderate-intensity

The τ_{II} (29.3±9.2s; p=0.033) was slower compared with moderate intensity (21.3±3.7 s) for all subjects, as shown in Figure 3.9.

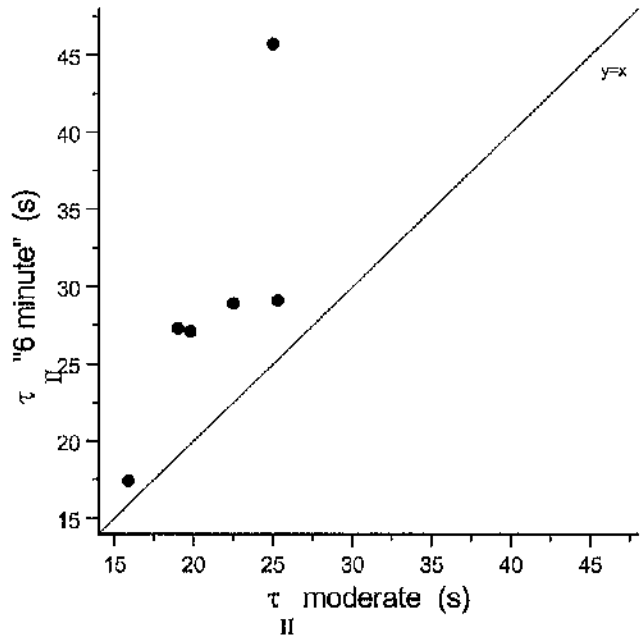

Figure 3.9. τ_{II} value for "6 minute" compared with moderate intensity exercise.

G_{II} for moderate intensity exercise (11.0±0.4 ml min^{-1} W^{-1}) was significantly greater than "6 minute" for all subjects (mean =9.7±0.6 ml min^{-1} W^{-1}, $p<0.0001$), as shown in Figure 3.10.

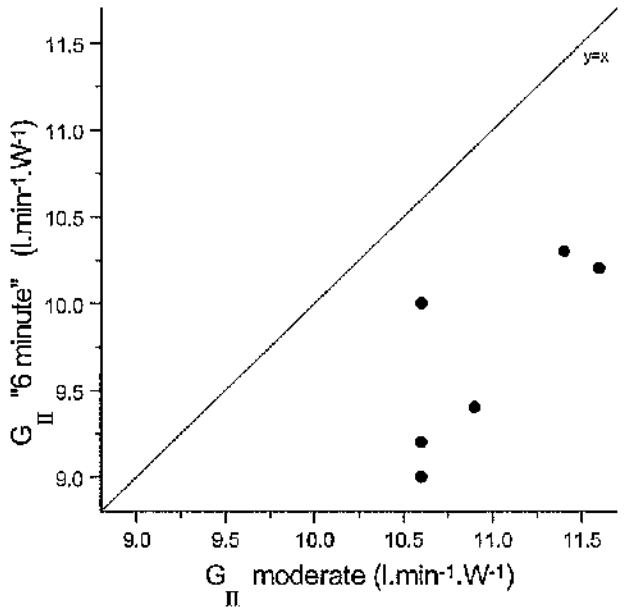

Figure 3.10. G_{II} for "6 minute" compared with moderate-intensity exercise

3.1.2.3 Comparison of "12 minute"and "6 minute" tests

a) [Lactate]

The [La] at rest, 20W, and end-exercise were not significantly different between the "6 minute" and "12 minute" tests. Mean values are shown in Table 3.7.

	[La] for "12 minute" ($mmol.min^{-1}$)	[La] for "6 minute" ($mmol.min^{-1}$)
Rest	1.21 ± 0.29	1.11 ± 0.22
20w	1.26 ± 0.30	1.33 ± 0.20
End exercise	9.11 ± 1.12	9.82 ± 0.95

Table 3.7. [La] at rest, 20w and end exercise for "6 minute" and "12 minute" tests.

Therefore, the Δ[La] from 20w to end exercise was not significantly different the tests ("6 minute" 7.85±1.20, "12 minute" 8.50±0.82). Individual values for Δ[La] are shown in Table 3.3 and 3.5.

b) Temporal parameters

Phase II

The phase II fitting window, discerned from the criteria outlined in chapter 2, resulted in a mean fitting window ranging from 25 to 154 s for the "12 minute" test, and from 25 to 127s for the "6 minute" test. τ_{II} was faster for "12 minute" than for "6 minute" (26.9±10.3 and 29.3±9.16, respectively), as shown in Figure 3.11. Although the mean difference was only 2 seconds, the trend was seen in all but one subject (subject 5) and was therefore statistically significant (p=0.03).

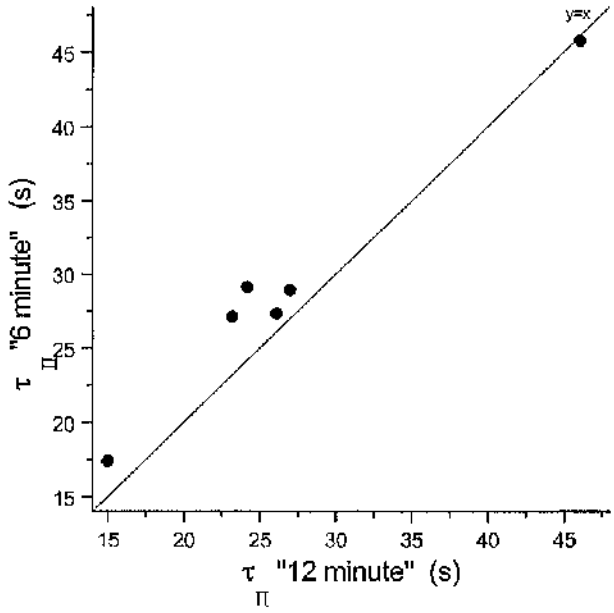

Figure 3.11. τ_{II} value for "6 minute" compared with "12 minute"

Slow component

The rate of increase of the slow component, demonstrated by the index $A_{sc\ 5\text{-}3}$, was similar ($p=0.203$) for the "12 minute" test (0.29 ± 0.06 $l.min^{-1}$) and the "6 minute" test (0.38 ± 0.15 $l.min^{-1}$) (Figure 3.12). However, 3 of the subjects (4, 5, 6) showed slightly greater $A_{sc\ 5\text{-}3}$ for the "6 minute" test and subject 2 showed an appreciably greater $A_{sc\ 5\text{-}3}$.

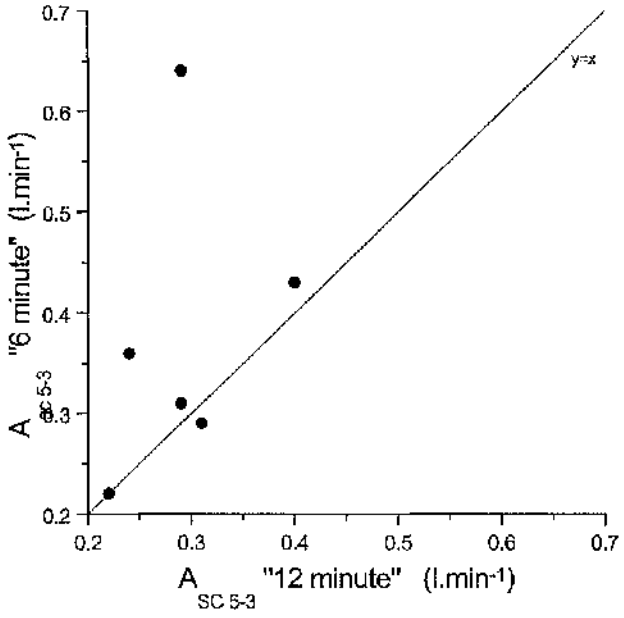

Figure 3.12. $A_{sc\ 5\text{-}3}$ for "6 minute" compared with "12 minute".

c) Amplitude and gain parameters

Phase II

As expected (i.e. as the WR is lower), A_{II} was significantly less for the "12 minute" compared with the "6 minute" test (2.32±0.39 and 2.52±0.36, respectively; p=0.000). However, the magnitude of this difference varied between subjects: subject 2 showed a small difference (0.18 l.min^{-1}), whereas subject 3 showed a larger difference (0.32 l.min^{-1}). The relationship between the "6 minute" – "12 minute" difference in A_{II} and $\dot{V}O_{2\ ee}$ (Figure 3.13) demonstrated a significant correlation (r=0.914, p=0.03).

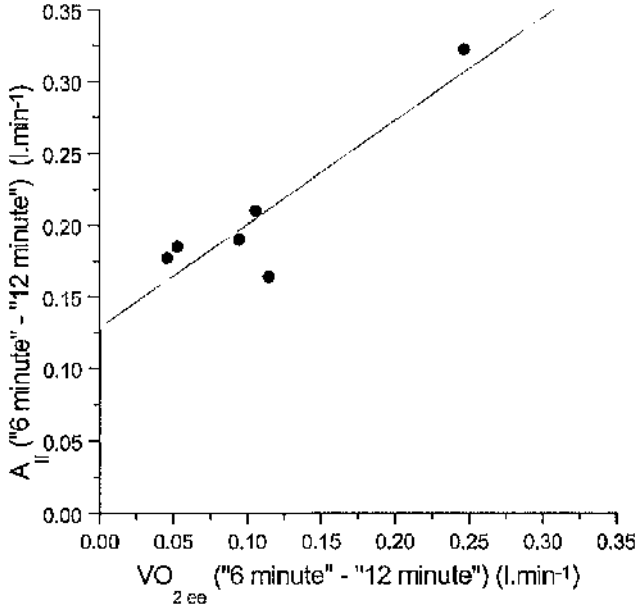

Figure 3.13. Relationship between "6 minute" -"12 minute" difference in A_{II} and A_{ee}.

This correlation is further illustrated by the $\dot{V}O_2$ responses to the "12 minute" and "6 minute" tests for individual subjects. A subject who reached a similar $\dot{V}O_{2\ ee}$ (subject 2) is shown for the "6 minute" and "12 minute" is shown, demonstrating that the A_{II} was also similar (Figure 3.14). In contrast a subject (subject 3) who did not reach a similar $\dot{V}O_{2\,ee}$ during the "12 minute" similar to that for the "6 minute" is also shown, demonstrating that A_{II} was appreciably different (Figure 3.15).

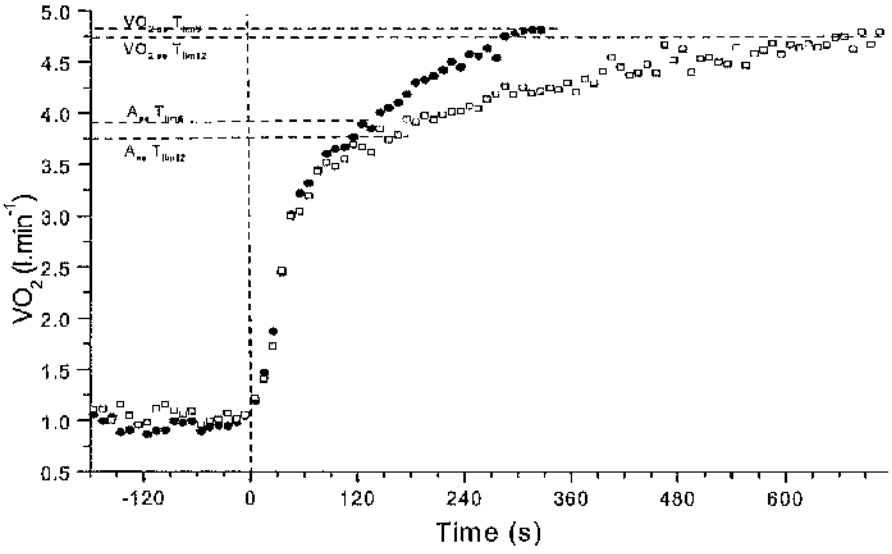

Figure 3.14. $\dot{V}O_2$ profiles for the "12 minute" and "6 minute" tests for subject 2, who reached similar A_{ee} values for the "12 minute" and "6 minute" tests. Solid circle indicates "6 minute" data, open square indicates "12 minute" data.

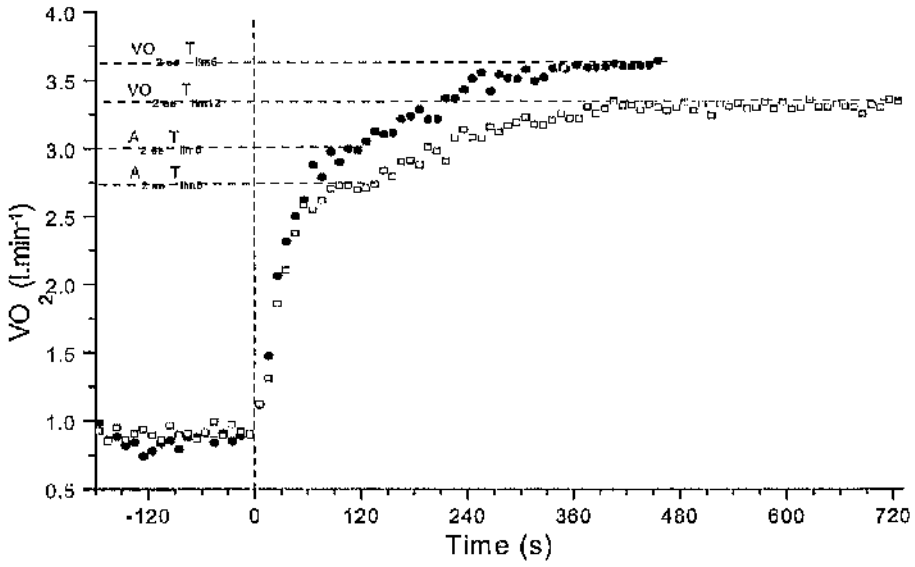

Figure 3.15. $\dot{V}O_2$ profiles for the "12 minute" and "6 minute" tests for subject 3, who did not reach a similar A_{ee} values for the "12 minute" and "6 minute" tests. Solid circle indicates "6 minute" data, open square indicates "12 minute" data.

As A_{II} was similar for "12 minute" and "6 minute2 in subject 2, when expressed relative to WR the G_{II} was lower for the "6 minute" test compared with "12 minute" (i.e. A_{II} normalised for WR). In subject 3, A_{II} was much higher for the "6 minute" test compared with "12 minute", and therefore G_{II} was larger for "6 minute" compared with "12 minute". However, there was no significant difference between G_{II} for the two tests with mean G_{II} being similar for the "12 minute" and "6 minute" trials (9.8±0.2 and 9.7±0.6, respectively). Individual data are shown in Figure 3.16.

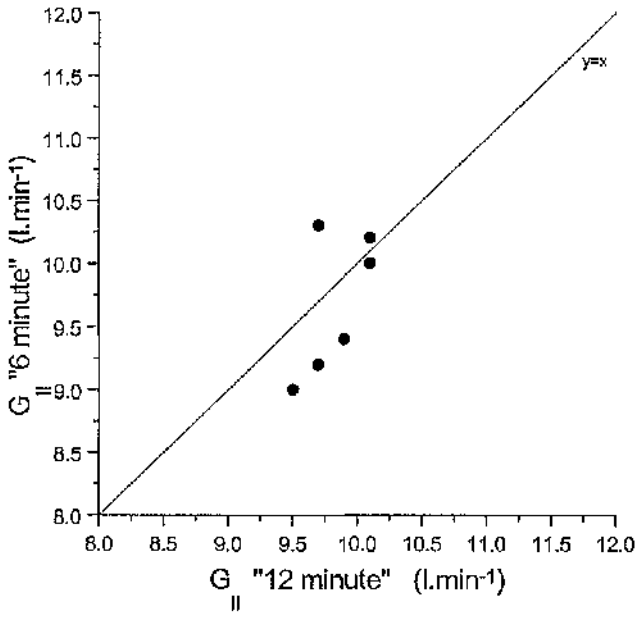

Figure 3.16. G_{II} for "6 minute" compared with G_{II} for "12 minute".

Slow component

A_{sc} for the "12 minute" and the "6 minute" tests were similar (0.70±0.19 and 0.64±0.20 l.min-1, respectively, p=0.129) (Figure 3.17). However, $A_{sc\ cor}$ for the "12 minute" test was significantly greater than that for the "6 minute" test (0.81±0.16 and 0.65±0.20 l.min-1, respectively; p=0.004) (Figure 3.18).

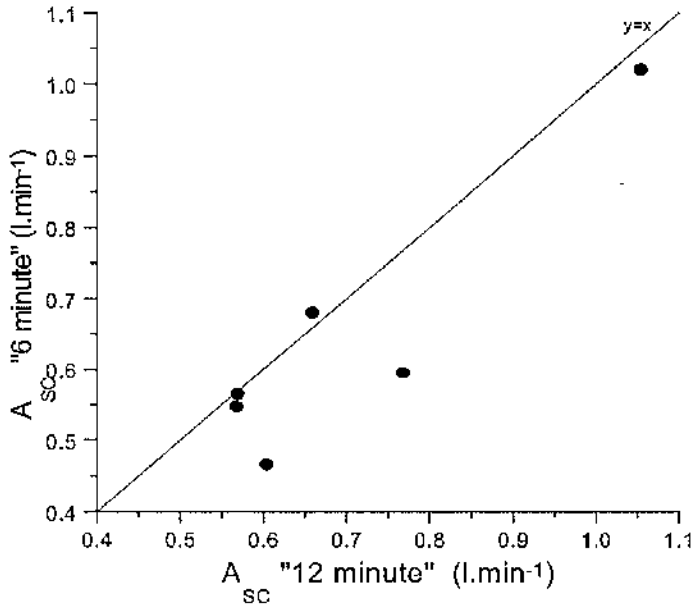

Figure 3.17. A_{sc} for "6 minute" compared with that A_{sc} for "12 minute".

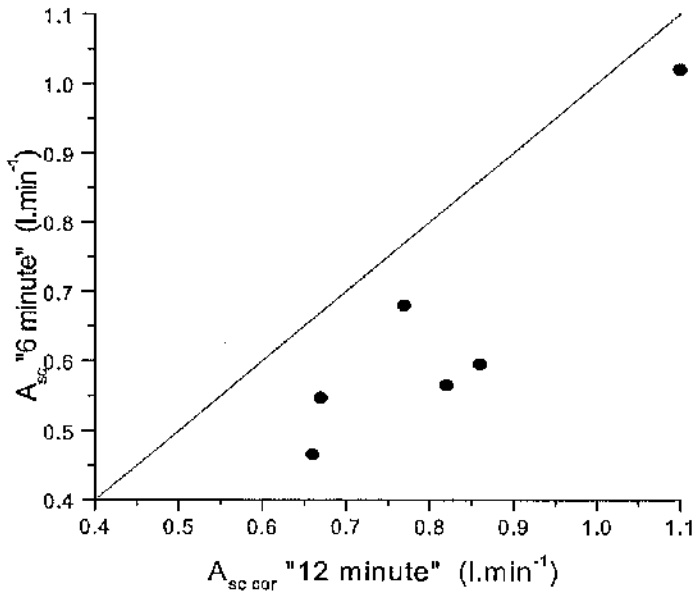

Figure 3.18. A_{sc} "6 minute" compared with $A_{sc\,cor}$ "12 minute".

Similarly, $G_{sc\ cor}$ for the "12 minute" test was significantly greater than that for the "6 minute" test ($p=0.035$) as shown in Figure 3.19.

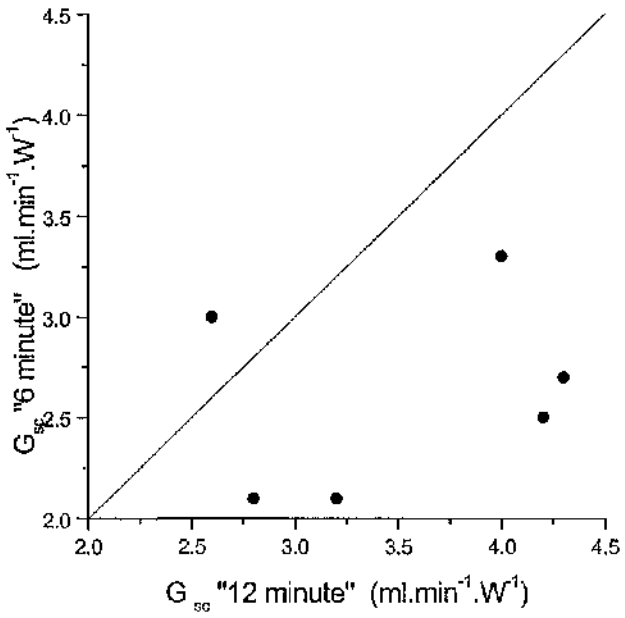

Figure 3.19. G_{sc} "6 minute" compared with $G_{sc\ cor}$ "12 minute".

Proportional contribution of phase II and the slow component

To compare the amplitude profiles of each of the components of the "12 minute" and "6 minute" response, the data have been expressed as percentages of the corresponding end-exercise amplitude (A_{ee}) obtained from each of the tests. The slow component amplitude at end-exercise was therefore 23.3% and 20.4% of the total A_{ee} for the "12 minute" and "6 minute" tests, respectively; with the phase II amplitude of the "12 minute" test was only

being 76.7% and 79.6%, respectively. Following 'correction', the phase II amplitude of the "12 minute" test was only 74.0% while the slow component increased to 26%. This comparison is shown in Table 3.8.

	A_{II} (%)		A_{sc} (%)	
	"12 minute"	"6 minute"	"12 minute"	"12 minute"
Actual value	76.7	79.6	23.3	20.4
Corrected value	74.0	79.6	26.0	20.4

Table 3.8. Comparison between the mean proportional contribution of A_{II} and A_{sc} for "12 minute" and "6 minute". Expressed as 'actual' values and as the 'corrected values' for the "12 minute" data.

3.2 RELATIONSHIP BETWEEN CREATINE KINASE AND $\dot{V}O_2$ KINETICS

What controls $\dot{V}O_2$ kinetics during exercise remains to be elucidated. However, evidence has suggested a relationship between the intramuscular PCr status (or some surrogate of this) and $\dot{V}O_2$ (see section 1.5.1.3). Therefore, intramuscular (vastus lateralis) CK activity rates, were measured to determine whether there was a relationship between CK activity and $\dot{V}O_2$ kinetics.

3.2.1 Creatine kinase activity

To increase the reliability of the total CK activity measured from spectrophotometry, the absorbency rate at two concentrations of homogenate was performed. A proportional increase in the CK activity rate at the higher concentration of homogenate (30 µl of non-diluted homogenate) occurred for all subjects, indicating good reliability.

The relationship between the absorbency rate at 300 µl of diluted homogenate and 30 µl of non-diluted homogenate, for each subject, is shown in Figure 3.20.

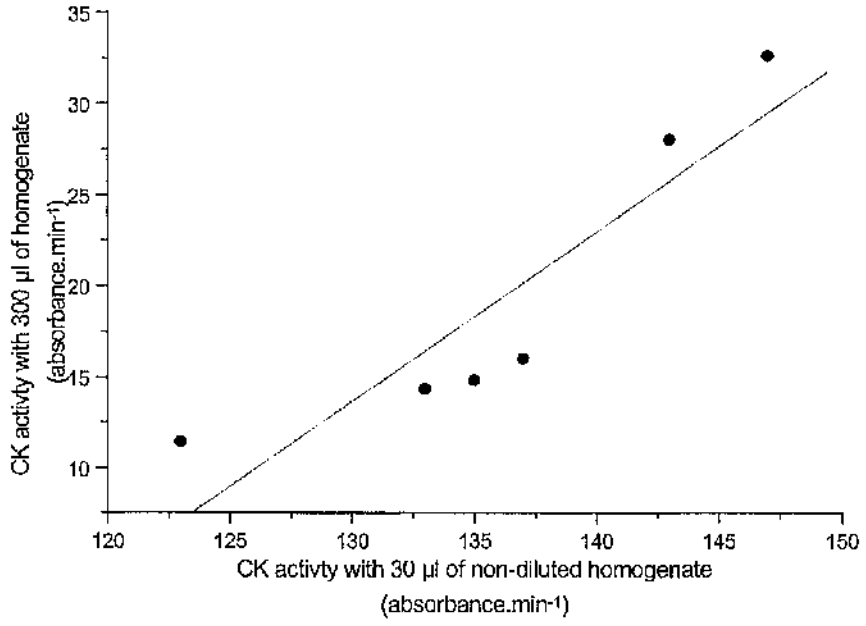

Figure 3.20. Relationship between CK activity with 300 µl of diluted (1:100) homogenate and 30 µl of non-diluted homogenate.

As 30 μl of non-diluted homogenate was the more-concentrated solution, it was used for the expression of CK activity rates by spectrophotometry. These values ranged from 11.4 to 32.6 absorbance.min^{-1}.

The isoform distribution of CK resolved using electrophoresis showed a predominance of CK-MM activity in all subjects, with undetectable levels of the other isoforms (Figure 3.21). That is, the CK-MM activity (e.g. Figure 3.21) was essentially entirely reflective of total CK activity levels. With electrophoresis, the CK activity ranged from 2.38 to 3.11 O.D.

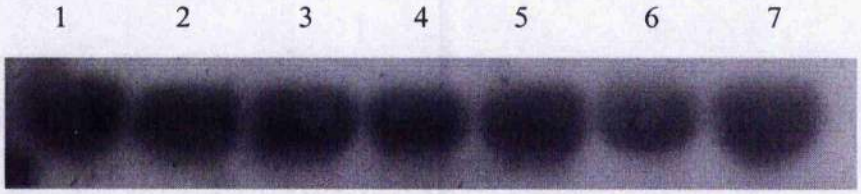

Figure 3.21. Picture of the electrophoresis gel, showing the absorbency of NADPH, a marker of CK activity. Lane 2-7 represents the optical density for each subject. Lane 1 served as a control as it was from the same subject as that in lane 2 and had the same optical density.

3.2.2 Creatine kinase activity and moderate-intensity exercise

a) Temporal parameters

Phase II

The CK activity level based on spectrophotometry and the phase II time constant (τ_{II}) values for moderate intensity exercise showed no correlation (r=0.027; p=0.959) (Figure 3.22).

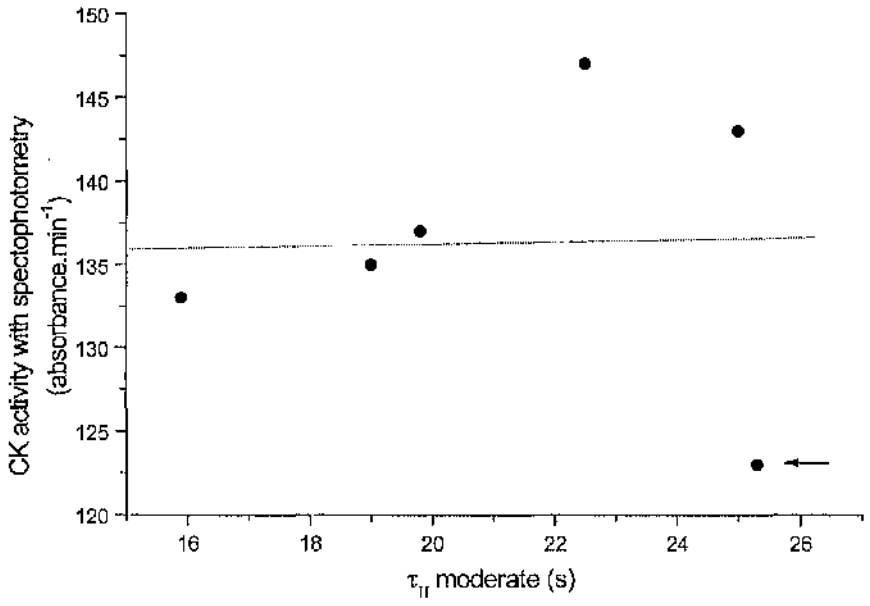

Figure 3.22. Relationship between CK activity rates from spectrophotometery and τ_{II} for moderate intensity exercise. Arrow indicates possible outlier.

However, it is apparent that an outlier (indicated by the arrow) may have skewed, an otherwise trend towards a of lower CK activity being associated with a faster τ_{II}.

This trend became statistically significant when CK activity was resolved with electrophoresis, the CK-MM activity showed a significant correlation with τ_{II} for (r=0.964, p=0.002) (Figure 3.23).

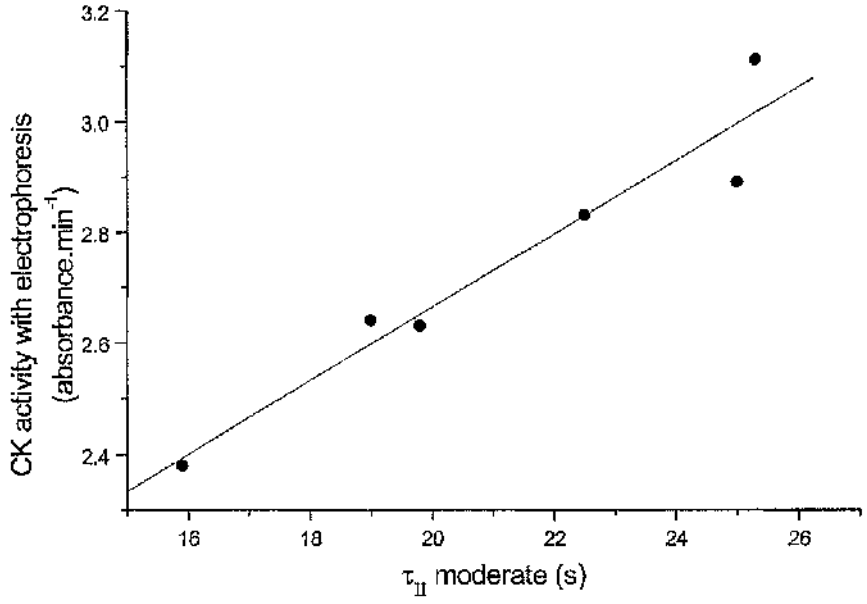

Figure 3.23. Relationship between CK activity from electrophoresis and τ_{II} for moderate intensity exercise.

With spectrophotometry, normalisation for protein concentrations is done relative to the weight of the whole muscle tissue, which includes connective tissue (whereas CK is only in

skeletal muscle protein). Therefore, if a particular muscle sample contains a large amount of connective tissue compared with other samples, then the CK level could appear lower than it should be, i.e. it would have been normalised 'excessively' for the actual amount of muscle protein in the sample. With electrophoresis, however, only the supernatant is used (i.e. free of connective tissue). And thus, as subject 1 showed a significantly lower CK activity with spectrophotometry compared to electrophoresis, it was decided to characterise the CK activity profiles using only the electrophoresis measurements; ensuring that CK expression was normalised to the appropriate protein concentration for all subjects.

b) Amplitude and gain parameters

Phase II

However, no relationship was found between CK activity and the phase II gain (G_{II}) for moderate-intensity exercise (r=0.133, p=0.801) as shown in Figure 3.24.

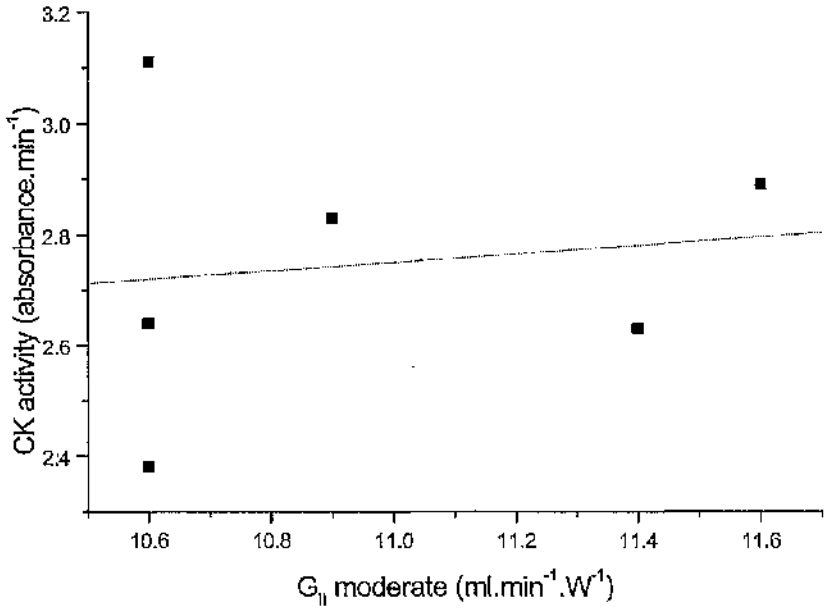

Figure 3.24. Relationship between CK activity and G_{II} for moderate intensity exercise.

3.2.3 Creatine kinase activity and very heavy-intensity exercise

a) Temporal parameters

Phase II

Similar to moderate-intensity exercise, a trend towards lower CK activity with a faster τ_{II} was evident. However, the strength of this relationship was less than that for moderate-intensity exercise, i.e. no significant correlation was found between CK activity and τ_{II} for

the 12 minute test (Figure 3.25) or the 6 minute test (Figure 3.26) ("12 minute" r=0.423, p=0.403; "6 minute" r=0.525, p=0.285).

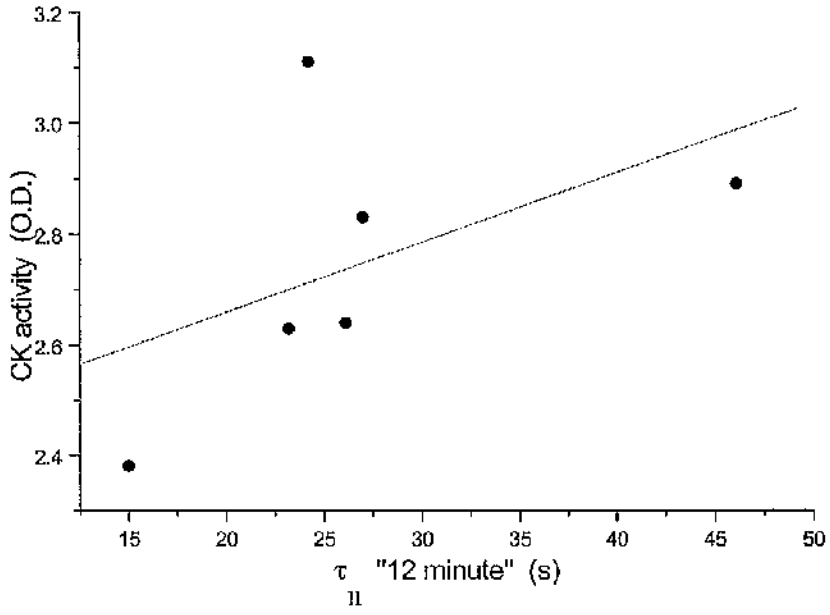

Figure 3.25. Relationship between CK activity and τ_{II} for "12 minute"

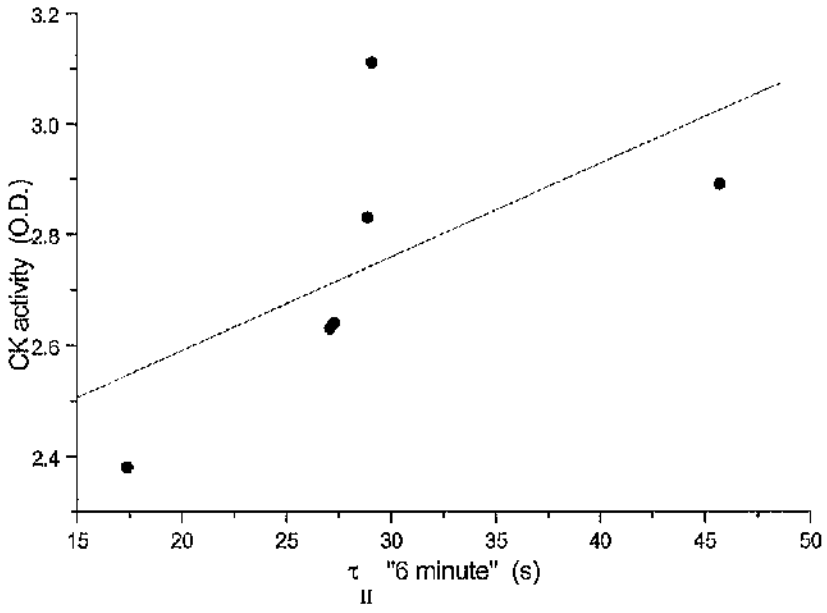

Figure 3.26. Relationship between CK activity and τ_{II} for "6 minute"

Slow component

The CK activity was not associated with the rate of slow component increase ($A_{sc\ 5\text{-}3}$) for "12 minute" as shown in Figure 3.27 (r=-0.003, p=0.995). And although a positive trend was observed in the corresponding relationship for he "6 minute" test, this was not significant (r=-0.396, p=0.436) (Figure 3.28).

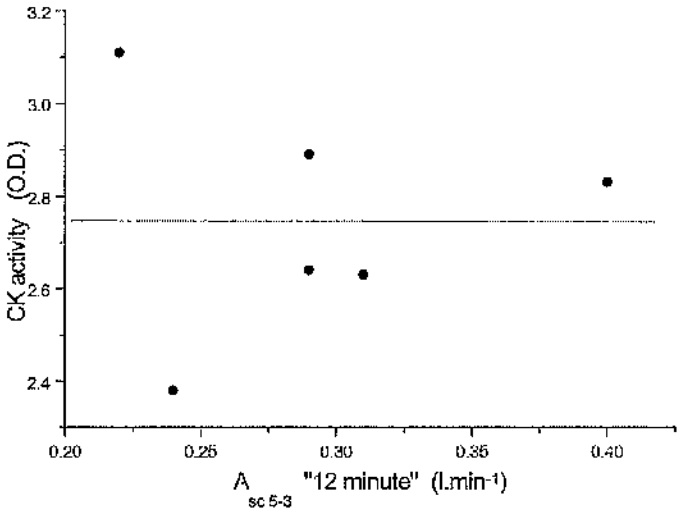

Figure 3.27. Relationship between CK activity (with electrophoresis) and $A_{sc5\text{-}3}$ for "12 minute".

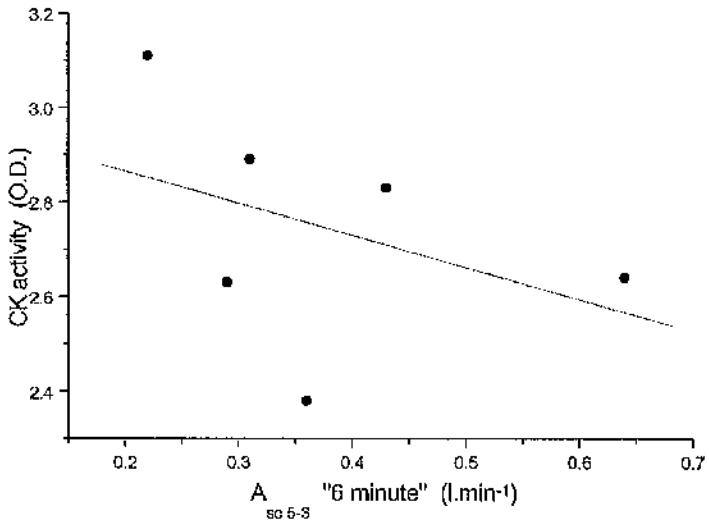

Figure 3.28. Relationship between CK activity and $A_{sc\ 5\text{-}3}$ for "6 minute".

c) *Amplitude and gain parameters*

Phase II

A significant positive relationship was found between CK activity and the phase II gain (G_{II}) for the "12 minute" test (r=0.957, p=0.003), as shown in Figure 3.29. A similar trend was evident for the "6 minute" test, but this was not significant (r=0.567, p=0.241) as shown in Figure 3.30.

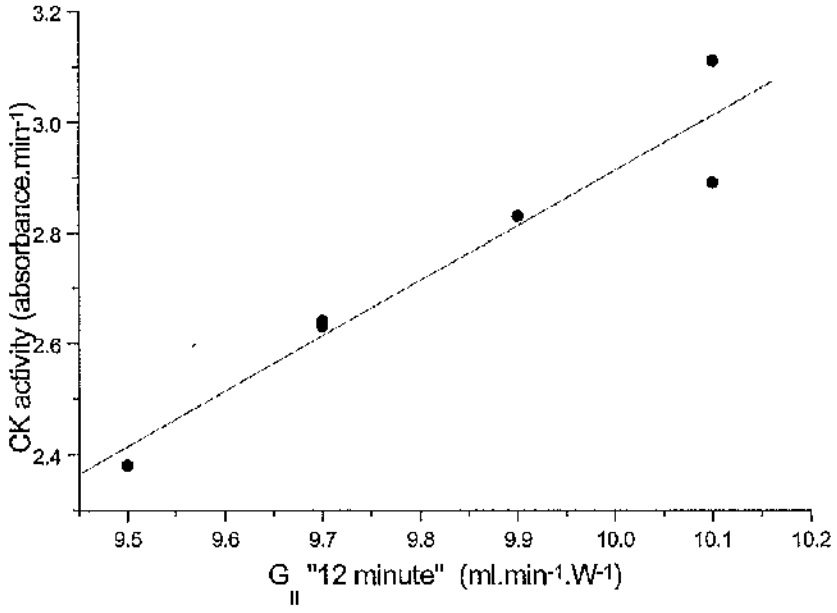

Figure 3.29. Relationship between CK activity and G_{II} for "12 minute".

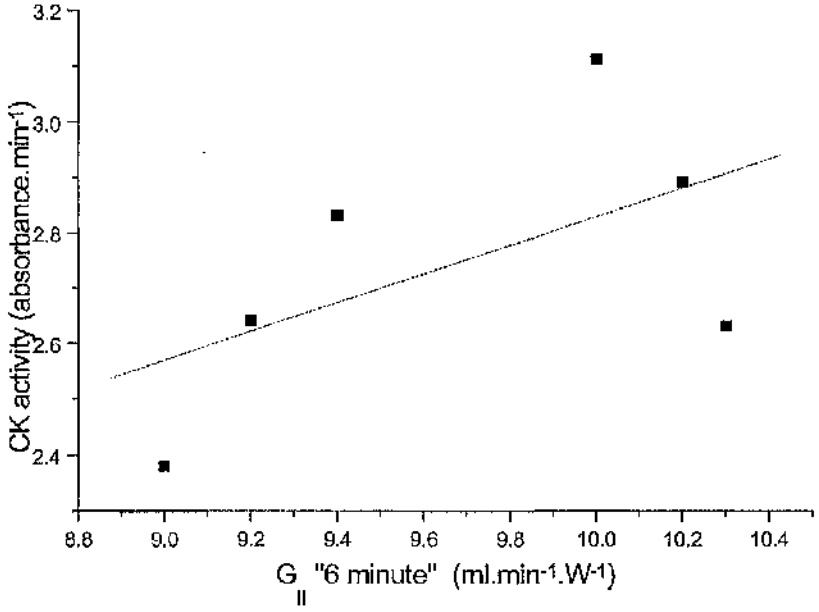

Figure 3.30. Relationship between CK activity and G_{II} for "6 minute".

Slow component

A negative trend was evident between CK activity and the slow component amplitude (A_{sc}) for "6 minute"; however it was not significant (r=-0.519, p=0.302) as shown in Figure 3.31. In contrast, the slow component gain (G_{sc}) showed an essentially significant trend with CK activity (r=-0.810, p=0.051; shown in Figure 3.32); i.e. an increased CK activity was associated with a smaller G_{sc}.

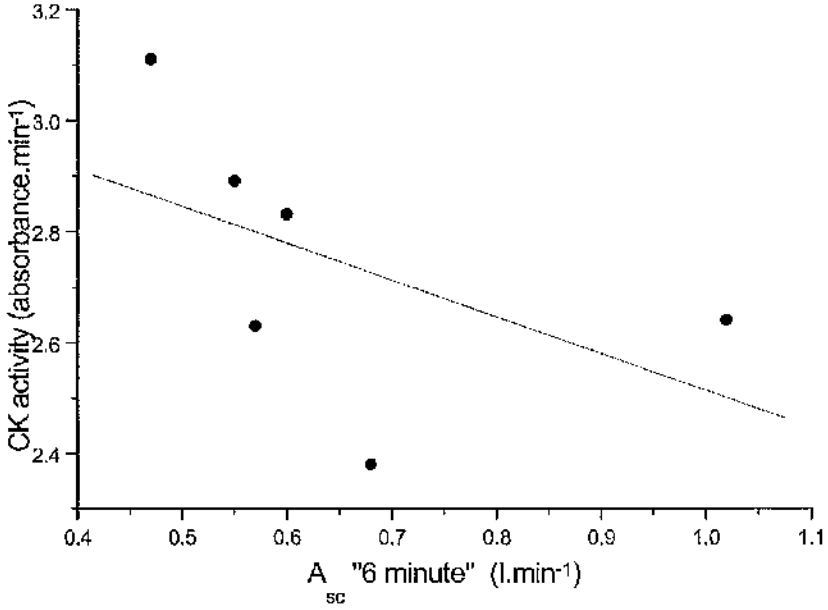

Figure 3.31. Relationship between CK activity and A_{sc} for "6 minute".

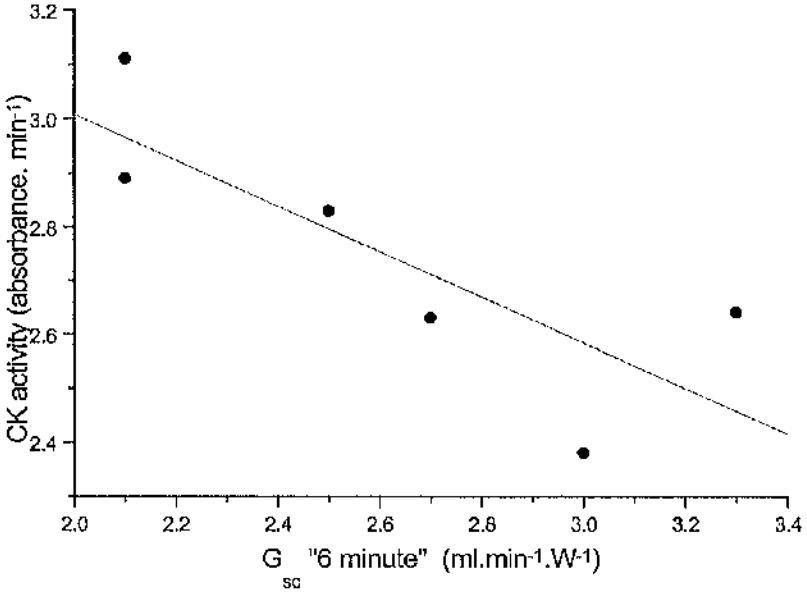

Figure 3.32. Relationship between CK activity and G_{sc} for "6 minute".

3.2.4 Creatine kinase activity and fitness level

No relationship was found between CK activity and any indicator of fitness level; θ_L (r=-0.194, p=0.206; Figure 3.33), CP (r=-0.542, p=0.266; Figure 3.34), W' (r=0.775, p=0.103; Figure 3.35) and $\dot{V}O_2$ peak (r=-0.560, p=0.247; Figure 3.36). However, there was a negative trend between CK activity and W'.

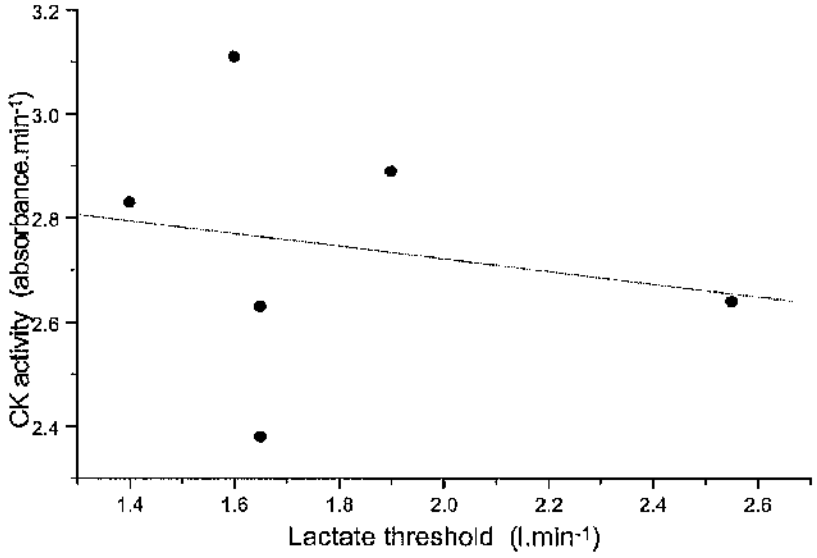

Figure 3.33. Relationship between CK activity with electrophoresis and the lacate threshold.

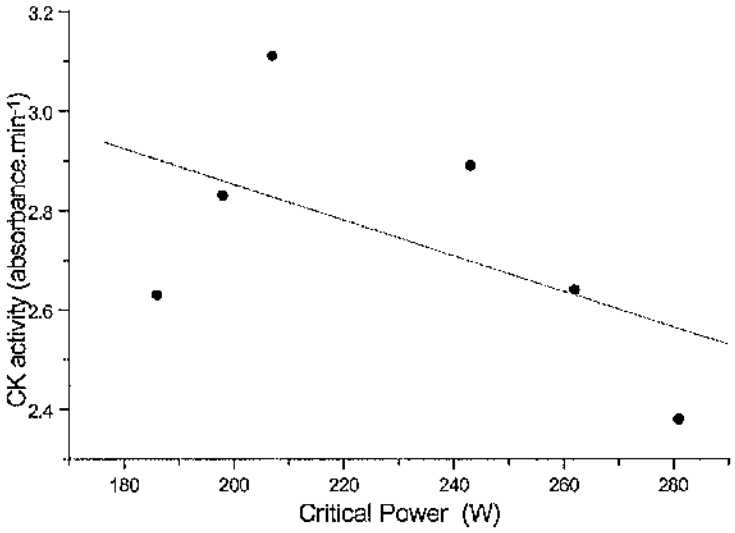

Figure 3.34. Relationship between CK activity with electrophoresis and critical power.

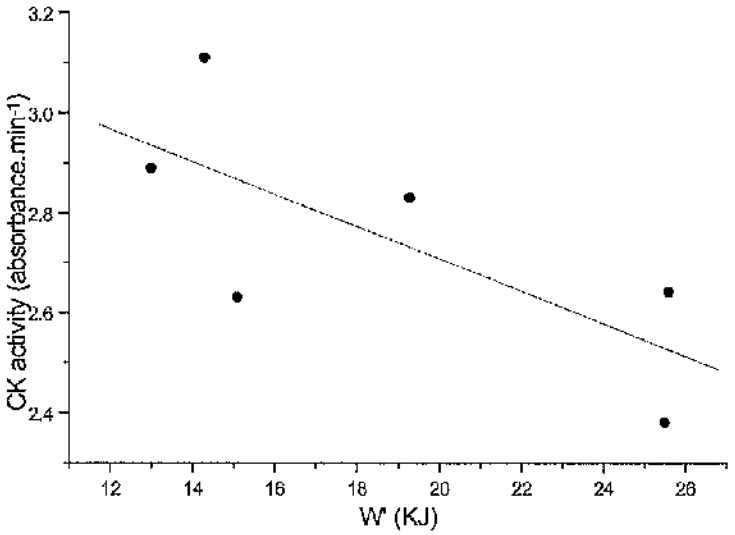

Figure 3.35. Relationship between CK activity with electrophoresis and anaerobic work capacity (W').

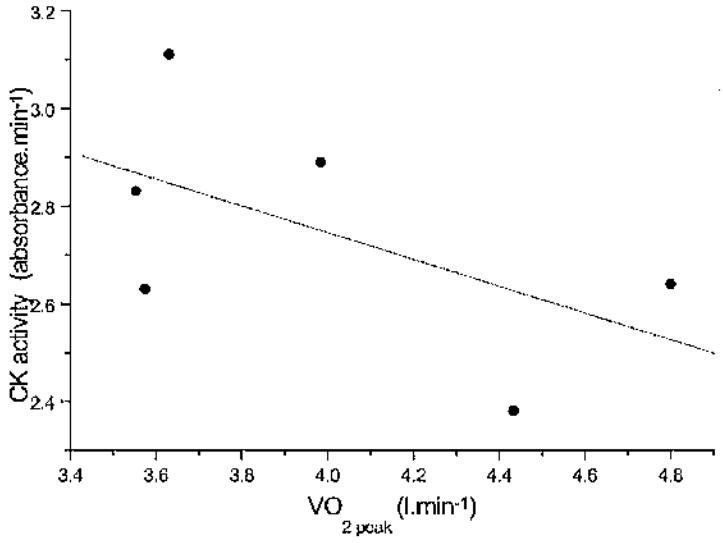

Figure 3.36. Relationship between CK activity and $VO_{2\ peak}$

3.3 RELATIONSHIP BETWEEN UNCOUPLING PROTIENS AND $\dot{V}O_2$ KINETICS

The mechanism responsible for the $\dot{V}O_2$ slow component at above θ_L remains to be elucidated. However it was recently suggested that expression of UCP-3 (but not UCP-2) might be involved. Therefore, the present study measured UCP-2 and UCP-3 mRNA expression in the vastus lateralis muscle sampled to determine whether a relationship between UCP and the slow component exists when appropriate modelling and quantification of the slow component is used (see page 169 for discussion). In addition, as

the mechanisms responsible for other $\dot{V}O_2$ kinetic parameters was unknown, their relationship with UCP has also been investigated.

As the $\dot{V}O_{2\,cc}$ value for the "12 minute" test was lower than that for the "6 minute" test (see section 3.1.2), for the purposes of correlating the slow component with the expression of UCP-2 and UCP-3, only the slow component data for the "6 minute" test were used, i.e. ensuring that only a true slow component amplitude is used in analysis.

3.3.1 Uncoupling protein mRNA expression

The photograph of the gel, for the intensity of UCP-3, pyruvate dehydrogenase kinase (PDK), β-actin and UCP-2 of each individual, is shown in Figure 3.37.

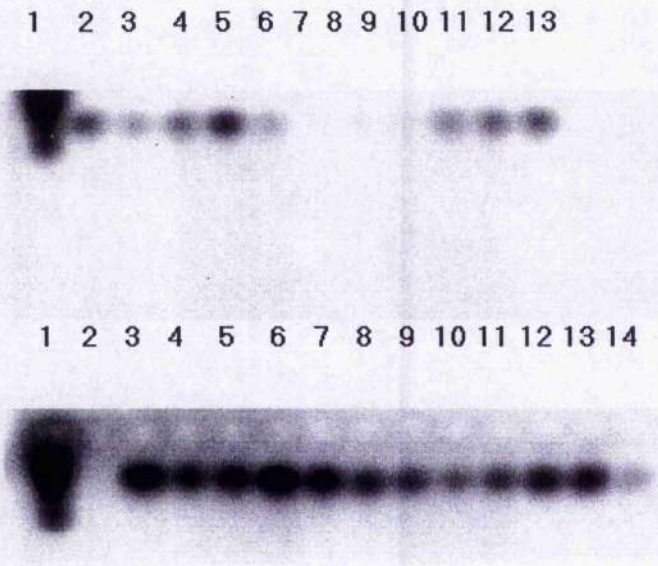

Figure 3.37. Polymerase chain reaction products on the electrophoresis gel. Lane 1 top and bottom are DNA ladders; lane 2, bottom is a control; lane 2-8, top are UCP-2; lane 9-13, bottom are PDK, lane 3-8, bottom are β-actin; lane 9-14, bottom are UCP-3 expression.

The expression of PDK-4 (indicator of mitochondrial content) was minimal-to-undetectable in most subjects. As the PCR worked for the other primers this low level of expression is probably due to the quality of the primer or the amount of magnesium required for PDK-4 primer annealing is greater than that of the other primers. Therefore, UCP-2 and UCP-3 are expressed relative to a "house keeping" gene i.e. β-actin. It was found that the UCP-3 mRNA expression ranged from 1.227 to 2.955 O.D. units (mean=1.802±0.683 O.D.). For UCP-2 mRNA expression, one subject showed undetectable levels of UCP-2 mRNA; therefore, this subject (subject 6) was excluded from the analysis of UCP-2. Reasons for this response are unknown. The UCP-2 expression for

the remaining five subjects ranged from 2.234 to 5.412 O.D. units (mean=3.571±1.278 O.D.). As only five subjects were available for UCP-2 analysis, the results should be interpreted with even greater caution.

3.3.2 Uncoupling protein expression and moderate-intensity exercise

a) Temporal parameters

Phase II

No significant relationship was found between UCP-3 and the phase II time constant (τ_{II}) (r=0.044, p=0.934), although a positive trend (but non-significant) was evident in the case of UCP-2 (r=0.709, p=0.180) as shown in Figure 3.38.

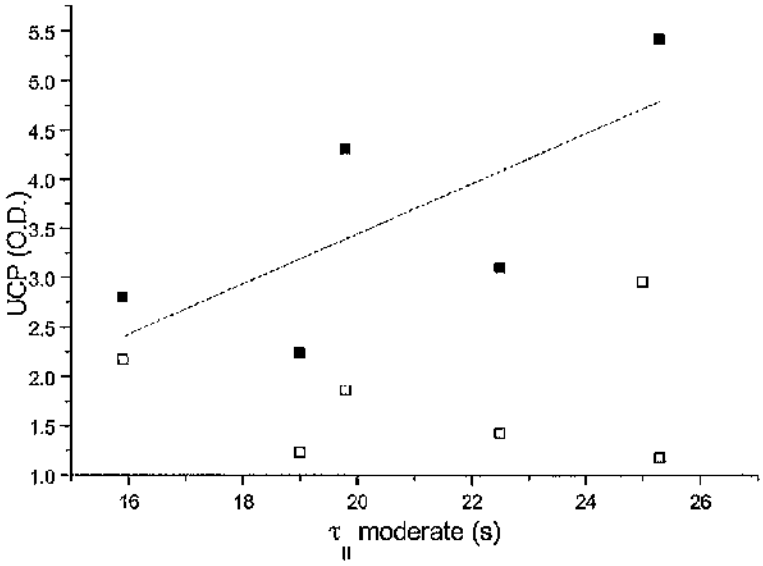

Figure 3.38. Relationship between τ_{II} for moderate intensity exercise and UCP-3 (open circle) and UCP-2 (solid square). Five subjects for UCP-2 and six subjects for UCP-3 shown.

a) Amplitude and gain parameters

No significant relationship was found between either UCP-2 or UCP-3 and the phase II gain (G_{II}) (UCP-2 r=0.251, p=0.684; UCP-2=0.713, p=0.112) as shown in Figure 3.39.

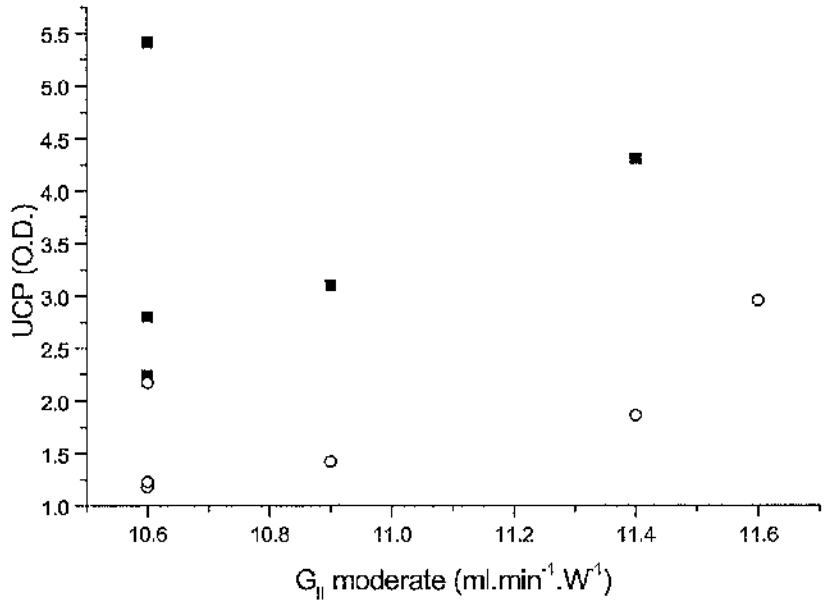

Figure 3.39. Relationship between G_{II} for moderate intensity exercise and UCP-3 (open circle) and UCP-2 (solid square). Five subjects for UCP-2 and six subjects for UCP-3 shown.

3.3.3 Uncoupling protein and very heavy-intensity exercise

a) Temporal parameters

Phase II

No relationship was evident for UCP-2 or UCP-3 and phase II time constant (τ_{II}) for "12 minute" as shown in Figure 3.40 (UCP-2 r=0.643, p=0.242; UCP-3 r=0.074, p=0.889) or "6 minute" as shown in Figure 3.41 (UCP-2 r=0.643, p=0.242; UCP-3 r=0.297, p=0.568).

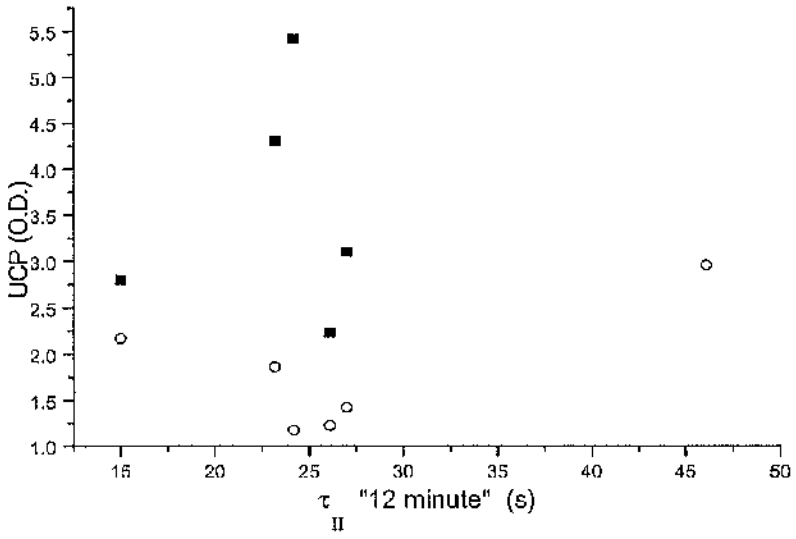

Figure 3.40. Relationship between τ_{II} for "12 minute" and UCP-2 (solid square) and UCP-3 (open circle). Five subjects for UCP-2 and six subjects for UCP-3 shown.

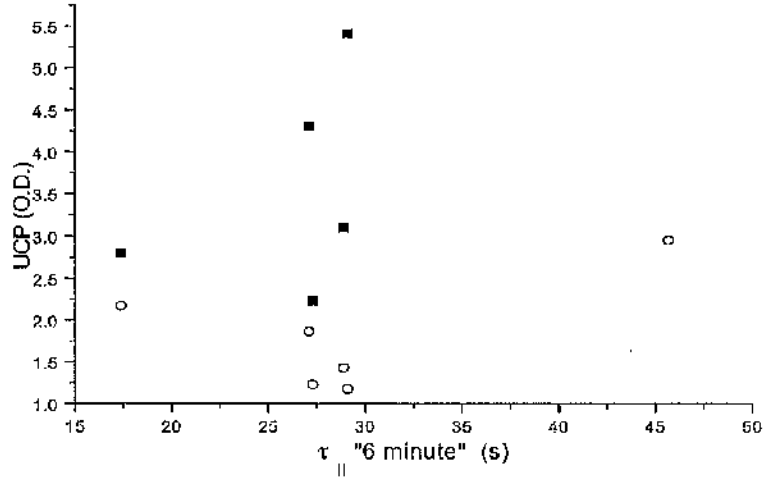

Figure 3.41. Relationship between τ_{II} for "6 minute" and UCP-2 (solid square) and UCP-3 (open circle). Five subjects for UCP-2 and six subjects for UCP-3 shown.

Slow component

No correlation was found between UCP-3 and the rate of the slow component increase (A_{sc5-3}) for "12 minute" (r=-0.071, p=0.894; shown in Figure 3.42) or A_{sc5-3} for "6 minute" (r=-0.321, p=0.535, shown in Figure 3.43). However, a non significant negative trend was evident for UCP-2 and Asc 5-3 for "12 minute" (r=-0.353, p=0.560) as shown in Figure 3.42, and an even stronger trend was evident between UCP-2 and $A_{sc\ 5-3}$ for "6 minute" as shown in Figure 3.43 (UCP-2, r=-0.870, p=0.055)

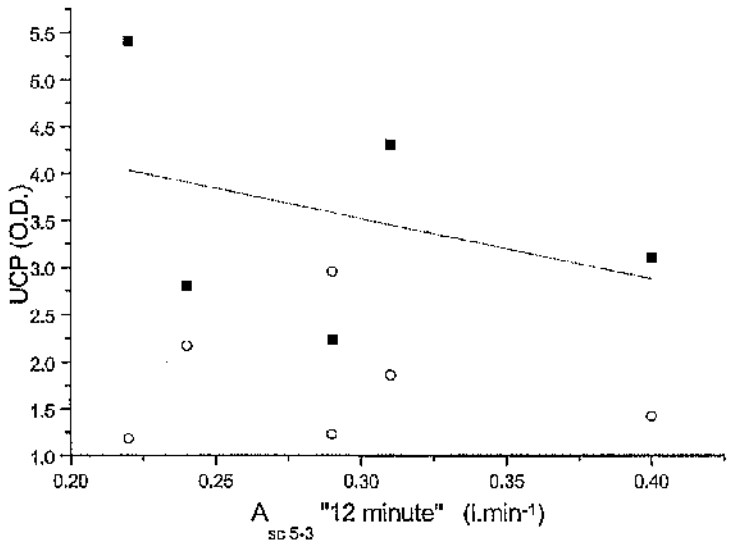

Figure 3.42. Relationship between $A_{sc\ 5-3}$ for "12 minute" and UCP-2 (solid square) and UCP-3 (open circle). Five subjects for UCP-2 and six subjects for UCP-3 shown.

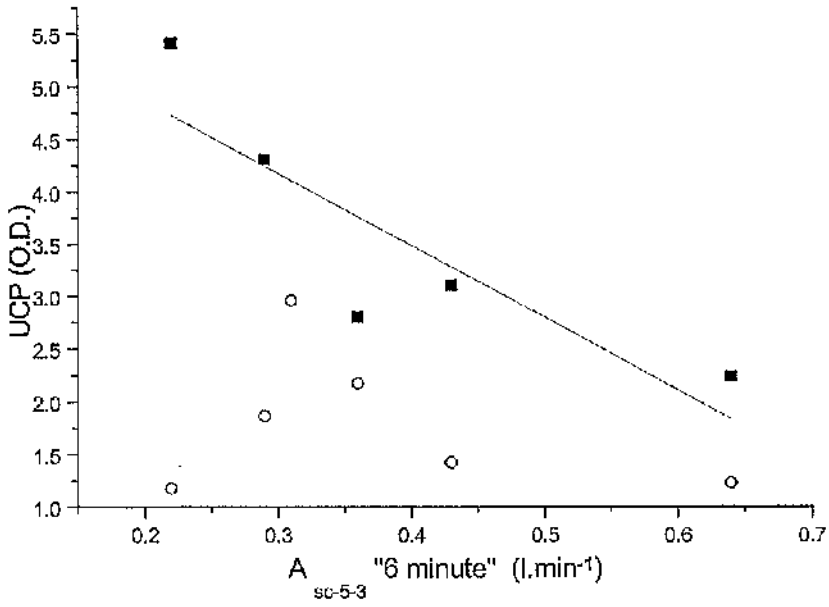

Figure 3.43. Relationship between $A_{sc\ 5\text{-}3}$ for "6 minute" and UCP-2 (solid squares) and UCP-3 (open circles). Five subjects for UCP-2 and six subjects for UCP-3 shown.

b) Amplitude and gain parameters

Phase II

No relationship was found between UCP-3 and G_{II} for "12 minute" (r=0.074, p=0.889; shown in Figure 3.44) or "6 minute" (r=0.297, p=0.568; shown in Figure 3.45). A slight but non-significant positive trend was evident between UCP-2 and G_{II} for "12 minute" (r=0.643, p=0.242; shown in Figure 3.44) and "6 minute" (r=0.512, p=0.242; shown in Figure 3.45).

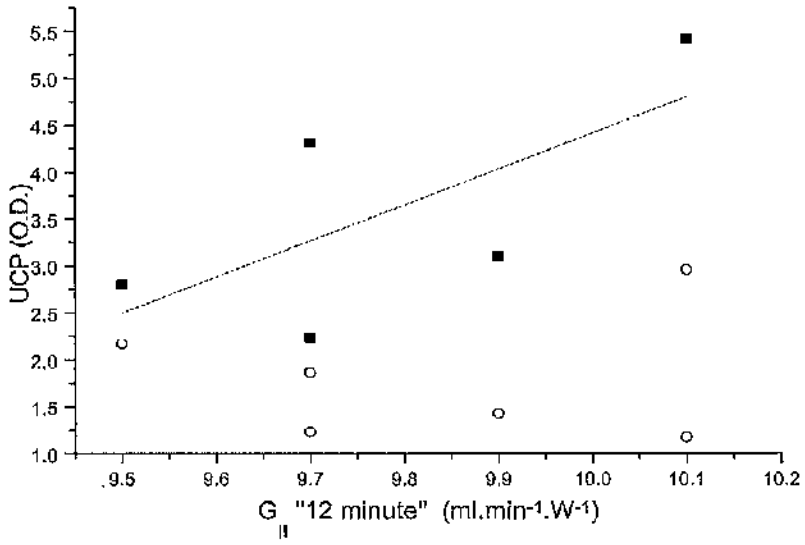

Figure 3.44. Relationship between G_{II} for "12 minute" and UCP-2 (solid squares) and UCP-3 (open circles). Five subjects for UCP-2 and six subjects for UCP-3 shown.

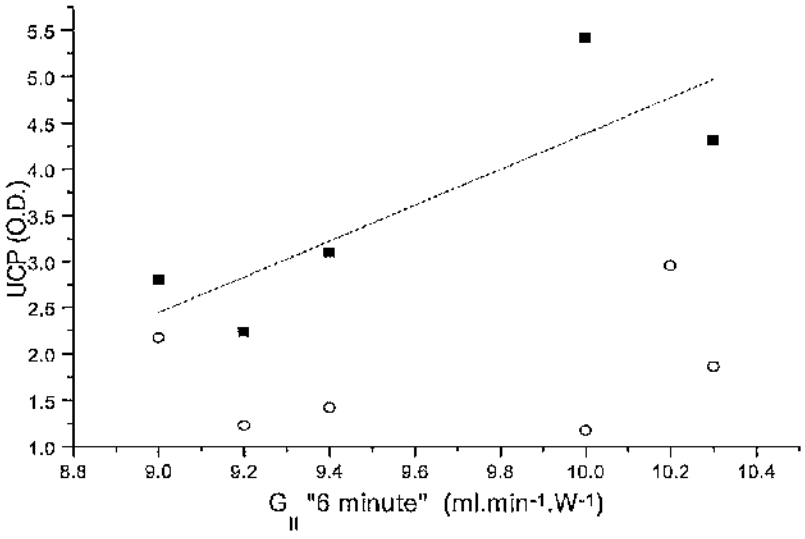

Figure 3.45. Relationship between G_{II} for "12 minute" and UCP-2 (solid squares) and UCP-3 (open circles). Five subjects for UCP-2 and six subjects for UCP-3 shown.

Slow component

No significant relationship was found between UCP-3 and the end-exercise slow component amplitude (A_{sc}) as shown in Figure 3.46 (UCP-3, r=-0.283, p=0.587). There was however, the suggestion of a negative trend between UCP-2 expression and A_{sc} as shown in Figure 3.46; however, this was not significant (UCP-2, r=-0.822 p=0.088).

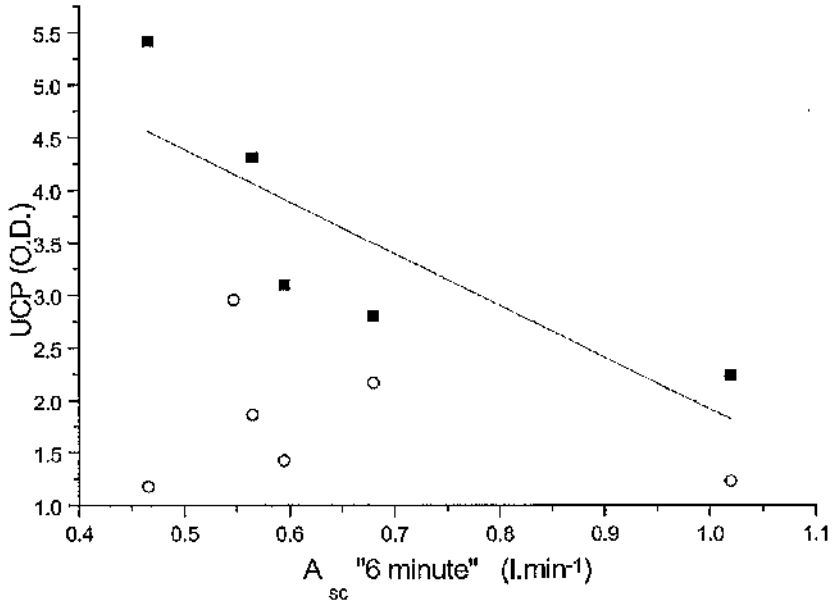

Figure 3.46. Relationship between UCP-2 (solid squares) and UCP-3 (open circles) and Asc for "6 minute". Five subjects for UCP-2 and six subjects for UCP-3 shown.

No relationship was found between UCP –3 and G_{sc} for "6 minute" (r=-0.288, p=0.580) as shown in Figure 3.47. Again, a negative trend was evident for UCP-2; however, this was not significant (r=-0.868, p=0.056) as shown in Figure 3.47.

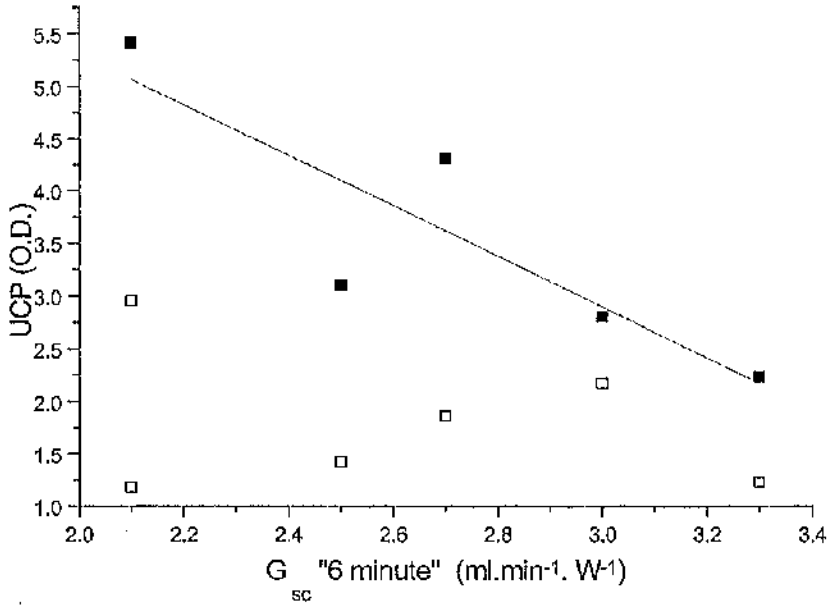

Figure 3.47. Relationship between UCP-2 (solid squares) and UCP-3 (open circles) and Gsc for "12 minute". Five subjects for UCP-2 and six subjects for UCP-3 shown.

3.3.4 Uncoupling protein expression and fitness levels

A significant relationship was found between UCP-2 and W' (r= -0.918, p=0.028; Figure 3.50). However, UCP-3 and UCP-2 were not correlated with any of the other indicators of fitness θ_L; (UCP-3 r=-0.075, p=0.888; UCP-2 r=-0.643, p=0.242; Figure 3.48), CP (UCP-3

r=0.301, p=0.562; UCP-2 r=-0.643, p=0.242; Figure 3.49), W'(UCP-3 r=-0.025, p=0.96; Figure 3.50) and $\dot{V}O_{2\ peak}$ (UCP-3 r=0.029, p=0.956; UCP-2 r=0.695, p=0.395; Figure 3.51).

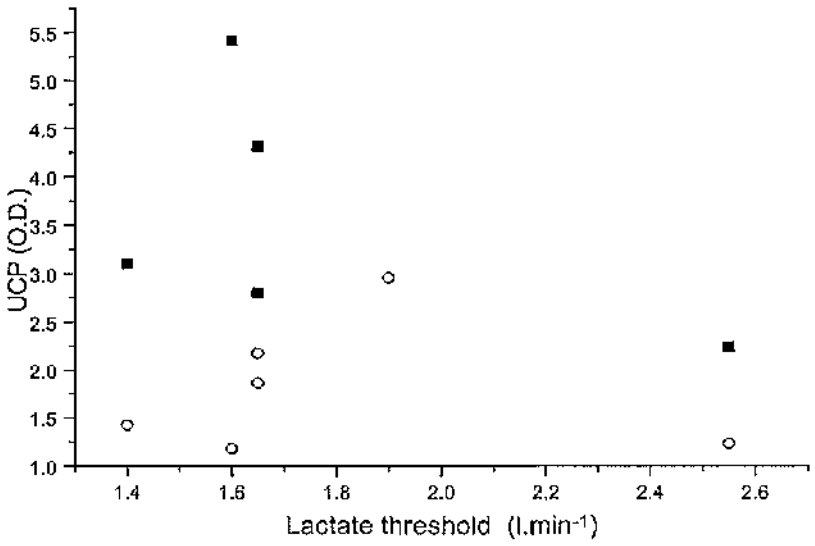

Figure 3.48. Relationship between UCP –2 (solid square) and UCP-3 (open circle) and the lactate threshold. Five subjects for UCP-2 and six subjects for UCP-3 shown.

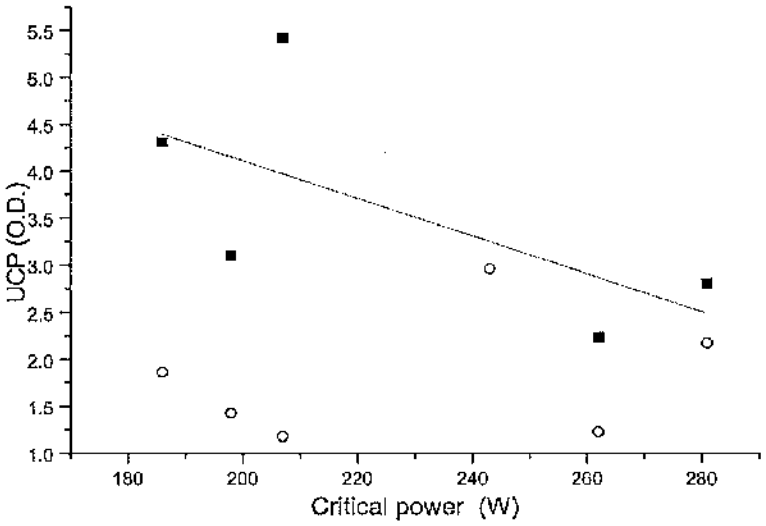

Figure 3.49. Relationship between UCP –2 (solid square) and UCP-3 (open circle) and critical power. Five subjects for UCP-2 and six subjects for UCP-3 shown

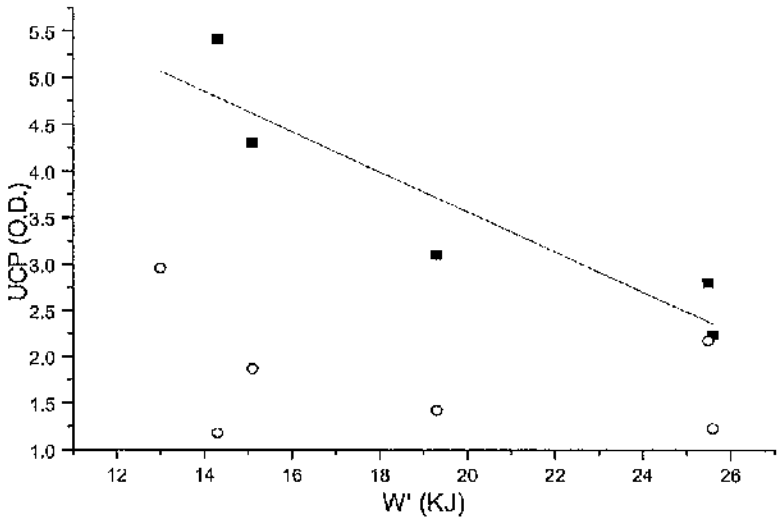

Figure 3.50. Relationship between UCP –2 (solid square) and UCP-3 (open circle) and W'. Five subjects for UCP-2 and six subjects for UCP-3 shown.

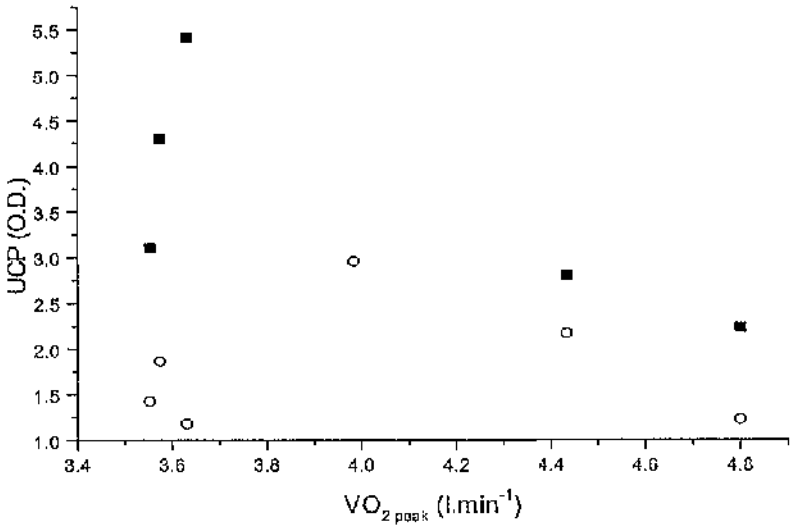

Figure 3.51. Relationship between UCP –2 (solid square) and UCP-3 (open circle) and $VO_{2\,peak}$. Five subjects for UCP-2 and six subjects for UCP-3 shown.

CHAPTER 4

DISCUSSION

4.1 INTRODUCTION

The control of $\dot{V}O_2$ kinetics during exercise remains inconclusive. However, the demonstration of a close matching between the kinetics of phosphocreatine (PCr) breakdown and $\dot{V}O_2$ increase at the exercise onset (and the converse at exercise offset) provides compelling evidence that the control processes reside within the muscle and involve some aspect of high-energy phosphate turnover (Mahler, 1985; Rossiter et al., 1999). It has also been suggested that the mechanisms controlling $\dot{V}O_2$ kinetics are intensity-dependent, with O_2 delivery asserting a greater role during heavy-intensity exercise (e.g. Grassi et al., 2000). During exercise performed above the lactate threshold (θ_L) an additional delayed component of 'excess' $\dot{V}O_2$ is manifest (the 'slow component') the mechanism of which remains to be elucidated. In addition, the $\dot{V}O_2$ kinetic parameters during very heavy-intensity exercise (above critical power) have not been investigated using the most appropriate modelling procedures.

Therefore, the main objectives of the present study were to investigate: (1) the relationship between creatine kinase (CK) and $\dot{V}O_2$ kinetics during moderate- and very heavy-intensity

exercise; (2) the $\dot{V}O_2$ kinetics to repeats at two different exhausting WRs in the very heavy-intensity exercise domain, placing emphasis on the degree of inter-subject variability; (3) the relationship between uncoupling protein expression and the $\dot{V}O_2$ slow component of very heavy-intensity exercise.

4.2 MODERATE INTENSITY EXERCISE

4.2.1 Phase II

4.2.1.1 Temporal parameters

The results from the present study for $\dot{V}O_2$ kinetics in the moderate-intensity exercise domain are consistent with previous investigations on cycle ergometry. It has been shown that the on-transient phase II time constant (τ_{II}) for young healthy subjects averages approximately 25-30 s (e.g. Whipp et al., 1982; Hughson et al., 1988; Paterson and Whipp, 1991; Whipp and Ozyener, 1998; Ozyener et al., 2001). In the present study, the τ_{II} ranged from 16 s to 25s across subjects, and the faster than average $\dot{V}O_2$ kinetics is consistent with the aerobic fitness levels being in the "above average to fit" range (i.e. $\dot{V}O_{2\,peak}$ ranging from 45.3 to 56.1 $ml.kg^{-1}.min^{-1}$). In addition, consistent with the literature, the off-transient τ_{II} was not different to the on-transient τ_{II} (Linnarsson, 1974; Whipp et al., 1982; Griffiths et al., 1986; Paterson and Whipp, 1991; Ozyener et al., 2001).

Creatine kinase

Despite the narrow range of τ_{II} values in moderate-intensity exercise a significant and negative correlation was found between τ_{II} and vastus lateralis CK activity (section 3.2.2, Figure 3.23). Evidence in the literature regarding the role of CK activity in oxidative phosphorylation is confounding, with many suggestions leading to different hypothesis of how CK activity might influence muscle $\dot{Q}O_2$ and hence $\dot{V}O_2$ in the exercise on-transient. Roman et al. (1996) demonstrated in mice that, when skeletal muscle CK activity was increased by 47% there was no difference in the rate of PCr breakdown (using ^{31}P-NMR spectroscopy) at the onset of twitch contractions, compared to control mice. This would suggest that the control level of CK activity was in excess of the normal metabolic rate; otherwise, the rate of PCr breakdown would have been faster as a result of the increased activity. Based on these findings, the present investigations in humans might have been expected to reveal no correlation between CK activity and τ_{II}. At odds with this view are the demonstrations in the horse, which have reported a speeding of $\dot{V}O_2$ kinetics when nitric oxide, a putative inhibitor of CK (Brown et al., 2000), is inhibited by L-NAME administration (Kindig et al., 2001, 2002a). This would suggest a faster τ_{II} would be associated with an increased CK activity (at least in the horse).

More recently, however, Roman et al. (2002) used ^{31}P-NMR spectroscopy with an increased time resolution compared to that they had used previously (Roman et al., 1996) and showed that at the onset of twitch stimulation slower PCr breakdown occurred in CK-

deficient mice. Recognising that [ADP] is a candidate controller of mitochondrial oxidative phosphorylation (e.g. Jeneson et al., 1996; Conley et al., 2001) these authors used modelling techniques to predict the influence of the CK-knockout paradigm on [ADP] kinetics and therefore $\dot{Q}O_2$. That is, in the CK-deficient condition, muscle [ADP] cannot be calculated from the assumed equilibrium of CK-catalysed PCr hydrolysis. It was of interest, therefore, that the model predicted a dissociation of [PCr] and [ADP] kinetics in exercise for the CK-deficient mice; i.e. [ADP] increased more rapidly than in the controls, suggesting therefore that $\dot{Q}O_2$ would also increase more rapidly. This proposed dissociation of $\dot{Q}O_2$ kinetics from [PCr] kinetics runs counter to observations in animals (Mahler et al., 1985) and humans (Rossiter et al., 1999, 2002) for submaximal sustained exercise. It should be pointed out that there has yet been no formal report of the degree to which [ADP] kinetics associate with those of $\dot{V}O_2$ for sustained exercise. It will be of considerable interest, therefore, to explore these issues in the CK-deficient mouse.

The implications of the model predictions of Roman et al. (2002) in the context of the present investigation would appear to be clear, however. The CK-deficient condition can be viewed as the extreme of a spectrum of CK activity. Thus, the prediction of fast $\dot{Q}O_2$ and therefore, presumably $\dot{V}O_2$ kinetics early in exercise with CK deficiency coheres with our demonstration of a progressive slowing of the phase II $\dot{V}O_2$ kinetics with increasing CK activity. We might, therefore predict that the slower phase II kinetics are accompanied by slower [ADP] kinetics but not necessarily slow [PCr] kinetics. This exciting prediction awaits investigation.

As Pringle et al. (2002) have shown that the τ_{II} is related to muscle fibre type, it would be interesting to examine the relationship between CK activity and fibre type profiles in the context of the phase II $\dot{V}O_2$ kinetics. It has been shown that the highest CK concentrations are found in muscles with a high glycolytic potential (Newsholme et al., 1978) and that, with the conversion of type II to type I fibres with training, there is an associated significant decrease in total CK activity (Apple and Rogers, 1986). However, Zoll et al. (2002) found no significant difference between total CK activity and training status, despite significantly higher type I profiles in trained subjects. With the present results, it may be hypothesised that increased CK activity would be associated with an increased incidence of type II fibres; however, this awaits further investigation.

Previous studies have shown a positive correlation between both τ_{II} and $\dot{V}O_{2\,peak}$ and τ_{II} and θ_L (Hagberg et al., 1980; Chilibeck et al., 1996). However, more recently it has been demonstrated by Whipp et al. (2001) that considerable variation exists in these inter-relationships: "if $\tau\ \dot{V}O_2$ is a stamen of stamina, it appears to be frail one". The results of the present study are consistent with this suggestion, as no relationship was found between τ_{II} and $\dot{V}O_{2\,peak}$ or θ_L. In addition, CK activity was not associated with $\dot{V}O_{2\,peak}$ or 0_L (section 3.2.4, Figure 3.36,3.33). The lack of correlation with $\dot{V}O_{2\,peak}$ and θ_L strongly suggests that the relationship between τ_{II} and CK activity is not simply due to associated differences in aerobic fitness levels, but rather provides more weight for a "cause and effect" relationship.

Uncoupling proteins

Although the mechanisms of action of UCP-2 and UCP-3 are unknown, the suggested function is similar to that found for UCP-1, namely acting as a leakage route in the inner mitochondrial membrane for protons to pass through without translating into an increased ATP production. Therefore, there would be an increased O_2 utilisation without ATP production. Therefore, as a slower τ reflects less instantaneous O_2 utilisation for the ATP requirement, it would be expected that UCP would not have any association with the phase II τ. This was confirmed in the present study as indeed no relationship was found between UCP mRNA expression and τ_{II} for moderate-intensity exercise (section 3.3.2, Figure 3.38). It is possible that there may be a trend for lower UCP-2 levels with faster τ_{II} values. However, as only five subjects were analysed the confidence in this trend is minimal and requires further investigation to confirm the existence of any relationship.

4.2.1.2 Amplitude and gain parameters

In the present study it was found that the phase II on-transient gain ($G_{II\,on}$ i.e. $\Delta \dot{V}O_2/\Delta WR$) averaged 11.0±0.5 $ml.min^{-1}.W^{-1}$ and was similar for the off-transient, which is consistent with other literature (Linnarsson, 1974; Whipp et al., 1982; Griffiths et al., 1986; Paterson and Whipp, 1991; Ozyener et al., 2001). In the present study, the range among individuals for $G_{II\,on}$ was relatively minor i.e. from 10.6 to 11.6 $ml.min^{-1}.W^{-1}$. It has been suggested by Mallory et al. (2002) that a greater gain during moderate-intensity exercise was associated

with a higher $\dot{V}O_{2\,max}$. This relationship was not observed here and therefore differences between studies may indicate that individual variability exists (i.e. different subject pools measured) for the relationship between $\dot{V}O_{2\,peak}$ and G_{II}.

Creatine kinase

It has been suggested by Wallimann et al. (1992) that the efficiency of ATP hydrolysis is increased when ATP resynthesis from PCr is catalysed by CK-MM, as it maintains a high ATP/ADP near the actomyosin ATPase and therefore, as demonstrated in equation 4.1, the ΔG available is increased as:

$$\Delta G=\Delta Gi-RT*\ln[ATP]/\{[ADP].[Pi]\} \qquad [4.1]$$

where ΔG is the free energy available from ATP hydrolysis, ΔGi is the initial free energy available, R and T are constants, and ln[ATP]/{[ADP].[Pi]} represents the inverse logarithmic function of [ATP]/[ADP].[P_i].

Therefore, it could be hypothesised that decreased CK activity would result in a lower [ATP]/[ADP] ratio, which (based on equation 4.1) would mean that a decreased ΔG is available. This would result in a slightly higher ATP requirement to perform a given work rate and, thereby an increased O_2 cost. It might therefore be expected that a greater gain ($\Delta\dot{V}O_2/\Delta WR$) might be expected to occur in association with a lower CK-MM activity.

Studies have shown a positive effect of increased [Cr] or decreased [PCr] on mitochondrial respiration (e.g. Walsh et al, 2001a; Walsh et al., 2001b). This may indicate that CK activity is not limiting in the exercise steady state, as the rate of mitochondrial respiration can be increased when the CK activity rate is caused to be increased. However, it is noteworthy that the effect of increased [Cr] on mitochondrial respiration has been shown to be greater in trained individuals (Tonkonogi & Sahlin, 1997 in isolated mitochondria; Zoll et al., 2002 and Walsh et al., 2001a; in skinned human skeletal muscle fibres). Furthermore, Walsh et al. (2001b) reported that in trained muscle fibres the positive effect of decreased [PCr] on mitochondrial respiration was greater. As the substrate concentrations (Cr and PCr) could influence the rate of respiration to a greater degree in trained muscle fibres, it might mean that the potential CK activity was greater than that for sedentary individuals. Nevertheless, Roman et al. (2002) reported that the PCr depletion after 60s was similar between CK-MM deficient and control mice. These authors therefore suggested that, in the steady state, cytoplasmic CK activity does not influence mitochondrial respiration. The results from the present study are in agreement with the suggestion of Roman et al. (2002), as no correlation was evident between the moderate-intensity phase II gain (G_{II}) and CK activity (section 3.2.2, Figure 3.24).

Uncoupling proteins

As the suggested role of UCP is as an 'inefficiency' in ATP production per O_2 molecule taken up, it may have been expected that a relationship between UCP expression and the

phase II gain in moderate-intensity exercise (G_{II}) would exist. However, in the present study no relationship was found between UCP-3 and UCP-2 mRNA expression and G_{II} (section 3.3.2, Figure 3.39).

UCP should not be regarded only as a deleterious mechanism. For example, it has been suggested that a leakage of H^+ through UCP at rest may facilitate the many-fold increases in ATP production required during subsequent exercise (Boss et al., 2000). At rest, high H^+ flux rates may be maintained without ATP production, by a substantial portion of the H^+ leaking through UCP rather than ATPase. Once the ATP demand is increased, the H^+ ions are then diverted to the ATPase so that sufficient ATP is produced. Therefore, the expression of UCP may not result in inefficient H^+ flux when it is not required (i.e. a higher $\dot{V}O_2$ gain), but rather may allow a precise and rapid switching between H^+ leak and ATP synthesis in response to changes in ATP requirement. The present results found no relationship between the G_{II} and UCP, an observation which is consistent with this suggestion.

4.3 VERY HEAVY-INTENSITY EXERCISE

4.3.1 $\dot{V}O_{2\,peak}$ correction

An interesting and important result in the present study that must be addressed prior to discussion of the $\dot{V}O_2$ response is that the end-exercise $\dot{V}O_2$ for the 12 minute fatiguing test was slightly but significantly lower than that for the 6 minute fatiguing test. This finding is not, at first sight, consistent with the literature, which suggests that the $\dot{V}O_2$ response to all fatiguing WRs above the CP will reach $\dot{V}O_{2\,peak}$ (e.g. Whipp and Mahler, 1980; Poole et al., 1988; Whipp and Ozyener, 1998; Ozyener et al., 2001).

Comprehensive individual data are not available in the studies that have examined the $\dot{V}O_2$ response to different WRs above the CP (Whipp and Mahler, 1980; Poole et al., 1988; Pringle and Jones, 2002; Miura et al., 2002; Coates et al., 2003). However, a single individual's $\dot{V}O_2$ response profiles to multiple, square-wave exercise tests of different WRs are shown in Whipp and Mahler (1980). Close visual examination of each $\dot{V}O_2$ shows that, as the tolerable exercise time decreased (and therefore $\dot{V}O_{2\,peak}$ should have been attained), the actual end exercise $\dot{V}O_2$ achieved was marginally lower for those WRs which resulted in a tolerance time of ~10 min and ~7 min (~3.7 $l.min^{-1}$ and ~3.6 $l.min^{-1}$, respectively), compared with the WR that lasted ~4 min (~3.8 $l.min^{-1}$) (shown in appendix iii). In addition, Poole et al. (1988) suggested that, for tests performed slightly above CP (i.e.

closer to the "12 minute" test versus the "6 minute" test of the present study), individual variability in terms of achieving a similar end-exercise $\dot{V}O_2$ occurred. Thus, it was stated that in three subjects end-exercise $\dot{V}O_2$ slightly exceeded $\dot{V}O_{2\,peak}$, whereas in the remaining five, it was either equal or *slightly lower* than $\dot{V}O_{2\,peak}$. In light of the data from these studies, the lower $\dot{V}O_{2\,peak}$ in the "12 minute" versus the "6 minute" constant work rates may represent a true physiological outcome. Further investigation is warranted to resolve this issue.

In the present study, all experiments were conducted under rigorously controlled conditions; i.e. a controlled laboratory environment, consistency of the subject's physical condition, randomisation of trials, a consistent amount of verbal encouragement, retrials to attempt to obtain a similar end-exercise $\dot{V}O_2$, and evident subject exhaustion on each test. In addition, the end-exercise HR was the same for the "6 minute" and "12 minute" tests, which strongly suggests that a maximum effort had been given; although it is noted that HR peak is not always a reliable indicator of having reached $\dot{V}O_{2\,peak}$ as it has been shown (at least in highly-trained endurance athletes) stroke volume may continue to increase at maximum exercise (Gledhill et al., 1994). Thus, using HR peak as an indicator of reaching $\dot{V}O_{2\,peak}$ must be approached with caution.

However, until further investigation with more subjects reveals whether or not the lower end-exercise $\dot{V}O_2$ found in the "12 minute" test versus the "6 minute" test is consistent, for

the purposes of discussion the $\dot{V}O_2$ response for the "12 minute" test was expressed as a corrected value; that is, to quantify the $\dot{V}O_2$ amplitude, the "12 minute" end-exercise $\dot{V}O_2$ ($\dot{V}O_{2\,ee}$) was extrapolated to the $\dot{V}O_{2\,ee}$ value for the "6 minute" test. It is of note that this correction does not change any trends reported in the present study, but only reflects the differences to a greater degree.

Previous studies have investigated the temporal features of the $\dot{V}O_2$ response to exercise above the lactate threshold (θ_L). However, as discussed in section 1.4.3, it is clear that exercise above critical power (CP) results in a $\dot{V}O_2$ kinetic response, which is qualitatively quite different from that for WRs lying just above θ_L (Ozyener et al., 2001). That is, above CP $\dot{V}O_2$ continues to increase throughout the test to attain $\dot{V}O_{2\,peak}$ and, therefore, to the limit of tolerance. Although Ozyener et al. (2001) have examined the $\dot{V}O_2$ response to exercise which was clearly above CP (as $\dot{V}O_{2\,peak}$ was reached), the exercise intensity was prescribed as 80% ($\dot{V}O_{2\,peak}$-θ_L). In addition, Pringle et al. (2003) examined $\dot{V}O_2$ kinetics at 70% ($\dot{V}O_{2\,peak}$-θ_L), which may have been above CP but this cannot be confirmed as the duration of exercise was not continued to the limit of tolerance. As CP occurs at variable percentages of ($\dot{V}O_{2\,peak}$-θ_L) (Poole et al., 1988), prescribing exercise intensity based on ($\dot{V}O_{2\,peak}$-θ_L) does not necessarily ensure that WRs of similar metabolic intensity are being performed between individuals. The present study is the first (to the author's knowledge), which has formally explored $\dot{V}O_2$ kinetics in the supra-CP range; i.e. by utilising each

subject's power-duration curve to estimate the CP. Furthermore, also for the first time, more than one work rate was investigated in this intensity domain i.e. two WR's were used and importantly, were standardised across the subjects in terms of predicted exercise durations (from the P-t relationship) of 6 and 12 minutes.

In addition, in the study of Pringle et al. (2003), a triple exponential model was used to fit each of the $\dot{V}O_2$ phases (i.e. phase I, phase II, and the slow component), while in the study of Ozyener et al. (2001) a double exponential model (omitting the phase I response) was used. However, as suggested by Rossiter et al. (2000), conferring a discriminable model structure on the $\dot{V}O_2$ slow component cannot be justified, given the short duration and small amplitude of response which compromises confidence in the associated fit. Therefore, in the present study, only the phase II response was fit (as a simple exponential), and identification of the onset of the slow component was discerned utilising the strategy described by Rossiter et al. (2000) (see section 2.4.3). In addition, individual parameter values have been reported rather than simply mean values (e.g. Ozyener et al., 2001; Pringle et al., 2002) in order to investigate the individual variation in the supra-CP $\dot{V}O_2$ kinetics; i.e. examining the proportional contributions to the end-exercise $\dot{V}O_2$ of phase II and the slow component.

4.3.2 Phase II

4.3.2.1 Temporal parameters

It was found in the present study the supra-CP phase II time constant (τ_{II}) value varied between individuals from 15 to 46 s and that this variability was not associated with any of the other $\dot{V}O_2$ response parameters or with $\dot{V}O_{2\,peak}$ or Δ[La].

Creatine kinase

In the present study, consistent with the results discussed earlier for moderate-intensity, there was trend for lower CK activity to be associated with faster τ_{II} values although this did not reach significance (section 3.2.3, Figure 3.25, 3.26). This suggests that CK activity may also be involved in the control of $\dot{V}O_2$ at the onset of supra-CP exercise (i.e. it is still manifest during heavy-intensity exercise), although this does not preclude involvement of other factors whose significance may vary across subjects. This suggestion might explain the discrepant results of Rossiter et al. who reported that during heavy-intensity exercise the rate of PCr breakdown is both associated (Rossiter et al., 2002) and dissociated (Rossiter, 2001) with $\tau_{II}\dot{V}O_2$. The reasons for the difference between these two studies is unclear but would be consistent with the suggestion that some other factor may limit $\dot{V}O_2$ during heavy-intensity exercise in some subjects (but not others) and, hence, the difference between studies may be related to characteristics of the subject pool.

Fibre-type distribution is known to vary widely across subjects. It is of interest, therefore, that Pringle et al. (2002) have reported that, in the very heavy-intensity domain no relationship existed between the τ_{II} and the incidence of type IIx fibres. One may speculate, therefore, that subjects having increased proportions of type IIx fibres may have differential $\dot{Q}O_2$ (and therefore $\dot{V}O_2$) control systems acting, compared with subjects with lower type IIx fibre profiles. This is interesting proposal requires further investigation.

Uncoupling proteins

As described for moderate-intensity exercise, if the proposed mechanism of UCP is an increased O_2 without ATP production rather than an inefficiency in the $\dot{V}O_2$ response, no association between UCP's and τ_{II} would be expected. We can confirm that, in the present study there was no correlation between UCP expression and the τ_{II} for supra-CP exercise (section 3.3.3, Figure 3.40, 3.41).

4.3.2.2 Amplitude and gain parameters

The proportion of the $\dot{V}O_2$ increase that phase II contributed to the overall $\dot{V}O_2$ response for supra-critical power (i.e A_{II} expressed as a % of A_{cc}) varied by up to 9% between individuals, and the phase II gain (G_{II}) ranged from 9.0 to 10.2 $ml.min^{-1}.W^{-1}$.

It was shown by Pringle et al. (2002) that subjects with a high type II fibre content had a low G_{II}, longer τ_{II} and a greater proportional contribution from the slow component to the end-exercise $\dot{V}O_2$ value. Barstow et al. (1996) reported a similar relationship between type II fibres incidence and G_{II}, but not τ_{II}. In the present study no relationship was found between the supra-CP τ_{II} and corresponding G_{II}. Therefore, it would be interesting to observe which $\dot{V}O_2$ kinetic parameter (if any) might correlate with a subject's fibre type profile.

Creatine kinase

It was of surprise that the G_{II} for very heavy-intensity exercise was correlated with CK activity, with lower CK activity being associated with a smaller G_{II} .(section 3.2.3). This relationship was significant for "12 minute" (Figure 3.29) and there was a similar trend for "6 minute" (Figure 3.30). Barstow et al. (1996) and Pringle et al. (2002) have shown that a high incidence of type II fibres was associated with a lower G_{II} for heavy-intensity exercise. Based on their results, it may be expected that lower CK activity would be associated with an increased type II fibre profile. However, as type II fibres have also been associated with slower τ_{II} (Pringle et al., 2002) and, in the present study, increased CK activity was associated with a slower τ_{II}, the relationship with CK activity probably is not only simply one involving fibre-type profile.

Uncoupling proteins

As the very heavy-intensity domain was associated with a decreased O_2 cost, there would be no a priori expectation that UCP levels would be related to the phase II gain (G_{II}); and indeed, in the present study, no significant relationship was found between UCP expression and G_{II} (section 3.3.3, Figure 3.44, 3.45). However, there was a statistically insignificant trend for a lower G_{II} with lower UCP-2 expression, which requires further investigation with a much larger subject group (i.e. only five subjects were available for UCP-2 analysis).

4.3.3 Slow component

4.3.3.1 Temporal parameters

In the present study the rate of slow component increase ($\Lambda_{sc\ 5\text{-}3}$) ranged widely, from 0.22 to as much as 0.64 $l.min^{-1}$.

Creatine kinase

The rate of the slow component increase was not significantly related to CK activity (section 3.2.3, Figure 3.27, 3.28). However, it should be pointed out that a nonsignificant trend was evident for the "6 minute" test, with a low rate of slow component increase

tending to occur with an increased level of CK activity (Figure 3.28). This issue should receive further investigation with an increased subject pool.

Uncoupling proteins

In the present study, we have shown that there was no relationship between UCP expression and the rate of the slow component increase (A_{sc5-3}) (section 3.3.3, Figure 3.42, 3.43). It has been suggested that UCP-2 and UCP-3 may act at the inner mitochondrial membrane to channel the leak of H^+ from the inner membrane spaces to the matrix rather than through ATPase reactions, therefore resulting in inefficient ATP production. The $\dot{V}O_2$ slow component seen at work rates above θ_L is an inefficiency with respect to O_2 cost, compared to moderate-intensity work rates (Paterson and Whipp, 1991). In the present study there was the suggestion of a trend for UCP-2 to be related to the early rate of the $\dot{V}O_2$ slow component increase; i.e. with increased UCP-2 expression being associated with a slower (rather than faster) rate of slow component increase. Because of the small number of subjects (n=5), these data should be interpreted with caution.

4.3.3.2 Amplitude and gain parameters

In the present study there was a range in the size of the slow component, expressed at end-exercise, with the slow component amplitude (A_{sc}) ranging from 0.47 to 1.05 $l.min^{-1}$. A_{sc} expressed as a proportion of the total in increase in $\dot{V}O_2$ (Asc as a % of Aee) ranged from

17 to 34%, and the slow component gain (G_{sc}) ranged from 2.1 to 3.8 $ml.min^{-1}.W^{-1}$. It was also of interest that the start of the slow component occurred later during the "12 minute" test (154±10s) versus the "6 minute" test (127±18s) in all subjects. When the calculation of [(four x τ) + time for phase I) is performed to approximate when the phase II steady state should occur, it is estimated that phase II for the "6 minute" test should have ended at ~137s which is just after the time point at which the slow component was discerned (i.e. 127 s). However, for the "12 minute" test the phase II steady state should occur at 125s, which is earlier than the discerned start of the slow component (i.e.154 s). This suggests that in the "12 minute" test the slow component is of delayed onset occurring just after phase II steady state is reached, whereas in the "6 minute" test the slow component occurs at, if not simultaneous with the end of the phase II response.

A similar trend for the $\dot{V}O_2$ slow component to emerge later at a higher WR was found by Ozyener et al. (2001) in which the time delay for the slow component was 154±55s in heavy exercise but only 137±28s in very heavy exercise. The reasons for the earlier emergence of the slow component at a higher work rate is unknown, but it may provide additional information to help resolve the mechanisms of the slow component, as factors hypothesised to affect the slow component can be studied within an individual rather than across individuals, to see if the factors hypothesised are also manifest earlier at higher work rates.

Creatine kinase

No significant relationship was found between the end-exercise magnitude of the $\dot{V}O_2$ slow component and CK activity. However, there was a trend for increased CK activity with a smaller slow component amplitude (section 3.2.3, Figure 3.28). The results of the present study have also shown a relationship of increased CK activity with slower τ_{II} values (section 3.2.2, Figure 3.23; section 3.2.3, Figure 3.25 and 3.26), and others have shown an association between slower τ_{II} values with increased type II fibre profiles (Pringle et al., 2002). Therefore, if CK activity is associated with fibre type and if the $\dot{V}O_2$ slow component is related to type II fibre recruitment as some studies have suggested (Shinohara and Moritani, 1992; Barstow et al., 1996; Burnley et al., 2002; Pringle et al, 2002), it might have been expected that if there was a relationship between CK activity and the slow component it would be *increased* CK activity with a *greater* slow component amplitude. However, this was not the case in the present study. Therefore, further investigation with an increased subject pool is required to confirm whether a relationship is evident between the amplitude of the $\dot{V}O_2$ slow component and CK activity, and further to determine whether there is a relationship with fibre type.

Uncoupling proteins

In the present study no relationship was found between UCP expression and either the slow component amplitude or gain (A_{sc}, G_{sc}) (section 3.3.3, Figure 3.46, 3.47). It was evident that a trend may have existed for increased UCP-2 to occur with a smaller slow component. However, this trend is not consistent with the suggested mechanism of UCP (which would suggest increased UCP-2 expression occurring with an increased slow component amplitude) The extent to which this represents the small subject number (n=5) requires further investigation.

In this context, the study of Russell et al. (2002), which reported a relationship between UCP-3 (but not UCP-2) and the $\dot{V}O_2$ slow component is demanding of some discussion. There are several major shortcomings in experimental design and data analysis. It is possible that the relationship was an artefact of the effect of the subjects working at different relative exercise intensities.

Firstly, as evident from the present experiments in the very heavy domain, it is critical in establishing the amplitude of the slow component that the work rate for each individual be established relative to his/her own markers of exercise intensity domain. Russell et al. (2002) established the work rates for each subject relative only to the lactate threshold (θ_L).

However, the accuracy of the θ_L determination should be considered. The identification of θ_L was based on directly measured blood [La] profiles. However, the sample site (the

antecubital vein) was entirely inappropriate. Because of substantial and variable lactate clearance by the relatively "non-exercising" forearm muscles (Brooks et al., 2000), especially when the venous blood is not "arterialised" the primary lactate accumulation profile will necessarily be contaminated and thus lead to underestimation of θ_L (Yoshida, 1986). Further, the degree of forearm lactate clearance may vary with fitness levels, affecting the estimated threshold in the two groups differently.

In addition, because Russell et al. (2002) used a slow ramp test of step increases every fourth minute and sampled blood only every four minutes, the resulting data density will be poor. Thus, there would be minimal data in the moderate region with which to establish the baseline, against which judgements of the subsequent onset of blood [lactate] increase would be made. Also [La] typically does not generate a precise breakpoint, in the way that the widely-used gas exchange markers do (Jones, 1982; Beaver et al., 1985; Whipp et al., 1986).

These several factors, perhaps not surprisingly, conspire to generate clearly different exercise intensities in the two subject groups. Thus, Russell et al. (2002) state that their recreationally active subjects were exhausted or reached $\dot{V}O_{2\,max}$, whereas in the trained subjects $\dot{V}O_2$ was able to stabilise - suggesting that the work rates were lower than CP. As the $\dot{V}O_2$ slow component amplitude and its ability to attain a steady-state varies with exercise intensity, it is imperative that identical intensities are used to be able to interpret meaningfully the relative differences in the slow component amplitude with UCP data. As

the trained subjects were at a lower relative exercise intensity it would be expected that - had they performed a relative intensity equivalent to that of the recreational group - the slow component would have been larger than actually reported. This assertion agrees with the statement by Russell et al. (2002) that their trained subjects showed a smaller slow component.

Further, criticism of the study of Russell et al. (2002) is that the amplitude of the slow component was calculated as an arbitrary increase in $\dot{V}O_2$ from 3 to 6 minutes (e.g. Roston et al., 1987), rather than discerning the onset of the slow component from modeling analyses (see section 1.4.2). Again, this approach may also result in differences in the slow component amplitude among individuals, as the slow component may emerge in the range of 90 to 160 s (Whipp et al., 1987; Paterson and Whipp, 1991; Barstow and Mole, 1991; Barstow et al., 1994; Scheuermann et al., 1998; Bell et al., 2001; Ozyener et al., 2001). Therefore, taking these several criticisms together, the report of Russell et al. (2002) of a smaller $\dot{V}O_2$ in association with a slow component with lower UCP-3 expression has to be viewed with considerable skepticism.

Rossiter et al. (2002) have shown that slow component was also evident in the PCr breakdown for exercise on-transients supra θ_L. This was taken to indicate that there is an increased phosphate cost of force production rather than an increased O_2 cost for phosphate production. The results of the present study, showing no correlation between UCP and the $\dot{V}O_2$ slow component amplitude, are consistent with this suggestion. That is, the suggested

mechanism of UCP as a proton leak would only result in an increased O_2 uptake for the ATP produced, rather than an increased phosphate cost for force production.

Many investigators at present favour an involvement of recruitment of less efficient type II fibres in the mechanism of the $\dot{V}O_2$ slow component (Shinohara and Moritani, 1992; Barstow et al., 1996; Burnley et al., 2002). The extent to which UCPs are selectively expressed in different fibre types in humans is equivocal however. Russell et al. (2002) demonstrated a positive correlation between the proportion of type IIa fibres and UCP-3 in their recreationally active but not their trained subjects, whereas Calsbeek et al. (2002) showed a significant positive correlation between type I fibre content and UCP-3. In contrast, the relationship between UCP-2 and fibre type profile was not reported in the study of Russell et al. (2002); neither was UCP-2 measured in the study of Calsbeek et al. (2002). Therefore, the relationship between UCP levels and fibre type remains to be clearly established. Without this link between vital information, any putative recruitment of type II fibres and the $\dot{V}O_2$ slow component through a mechanism related to greater UCPs expression must remain only speculative.

The literature to date is also equivocal regarding the relationship between UCP expression and aerobic fitness levels in humans. Both UCP-2 and UCP-3 have been reported to be lower in subjects with a higher $\dot{V}O_{2\,peak}$ (Russell et al., 2002) and after training (Schrauwen et al, 2001; Tonkonogi et al, 2000). However, Calsbeek et al. (2002) showed no correlation between UCP-3 and $\dot{V}O_{2\,peak}$ in a study of 24 human subjects. The results from the present

study are in agreement with those of Calsbeek et al. (2002), as no relationship was found between UCP expression and indicators of aerobic fitness (θ_L, CP and $\dot{V}O_{2\,peak}$) (section 3.3.4, Figures 3.48, 3.49, 3.51). However, it is interesting to note that a relationship was evident between the anaerobic capacity parameter of the P-t relationship (W') and UCP-2 (section 3.3.4, Figure 3.50). While this is suggestive of a predisposition towards anaerobic metabolism (perhaps because of cellular inefficiencies in O_2 utilisation), resolution of this issue is beyond the scope of present investigation.

In the present study, it must be acknowledged that the interpretation of UCP measured from mRNA expression is not without problems. UCP mRNA expression was measured, rather than UCP protein expression, and it should be recognised that mRNA expression does not necessarily correlate with protein levels as the regulation of protein translation can be complex. A valid technique for UCP protein analysis from human muscle biopsy material is not currently available; this reflects the small muscle volume taken. Therefore, mRNA expression is the best indicator until further methodological advancements are made. *Thus, it cannot be categorically stated that the UCP-3 is not a mechanism involved in development of the $\dot{V}O_2$ slow component and its differential prominence among different individuals differentiating the magnitude of the slow component among individuals. The data from the present study do suggest that this explanation of the slow component, as proposed by Russell et al. (2002), is questionable.*

4.4 COMPARISON OF $\dot{V}O_2$ KINETICS FOR MODERATE– AND VERY HEAVY-INTENSITY EXERCISE

4.4.1 Temporal parameters

The present study found that the τ_{II} $\dot{V}O_2$ value was significantly faster during moderate exercise (21±4s) than that of the "6 minute" very heavy-intensity exercise (29±9s) (section, 3.2.2.2, Figure 3.9) and, although not significant, a trend to be faster than "12 minute" very heavy-intensity exercise (27±10s) (section 3.1.2.1, Figure 3.6).

The results from other studies to date have shown that, in comparison to moderate intensity exercise τ_{II} above θ_L was significantly longer (Jones et al., 2002; Koga et al., 1999; Paterson and Whipp, 1991; Jones et al., 2002), showed a trend to be longer (Barstow and Molé, 1991; Paterson et al., 2003), or showed no significant difference (Ozyener et al., 2001; Carter et al., 2000; Pringle et al., 2003). However, the exercise intensities utilised in each of the studies are likely to have varied to an unknown extent, as the WRs were assigned as a percentage of the difference between $\dot{V}O_{2\,peak}$ and θ_L. Therefore, whether the work rates elicited were heavy-intensity or very heavy-intensity exercise cannot be determined with high confidence. The present results suggest that the severity of the intensity of exercise may be important to the degree of difference observed between the τ_{II} values. In addition, the above studies used different modelling techniques to identify the appropriate phase II component (some fitting the slow component response; some not),

which may have also contributed to the conflicting results. Whether the phase II $\dot{V}O_2$ response conforms to a dynamically-linear system above 0_L and above CP has important implications regarding the possible control mechanisms of $\dot{V}O_2$. If τ_{II} values are independent of work rate, then it suggests that the system is a first order system, limited by a single control mechanism.

However, recent studies by and Brittain et al. (2001) and Paterson et al. (2003) have reported that τ_{II} is faster for an equal change in work rate into the lower region versus the upper region of moderate-intensity exercise (Brittain et al., 2001; Paterson et al., 2003) and further slower into the lower region of heavy-intensity exercise (Paterson et al., 2003). In addition, a single work rate step to the lower region of heavy-intensity exercise resulted in a phase II $\dot{V}O_2$ response that was not significantly slower than for a single step into the upper region of moderate-intensity exercise (there was, however, a trend for τ_{II} to be longer); however, τ_{II} was significantly slower than for a single step into the lower region of moderate-intensity exercise. These results suggest the phase II $\dot{V}O_2$ response does not conform to a dynamically linear system.

The mechanism responsible for the slower τ_{II} is unknown. However, as the present study demonstrated a trend for CK activity to be negatively associated with τ_{II} in the very heavy-intensity domain, it might be expected that a mechanism affecting CK activity could be involved in controlling the phase II $\dot{V}O_2$ response profile.

It has been suggested that intramuscular pH and Pi may dissociate CK-mi from the inner mitochondrial membrane, which may decrease the rate of mitochondrial respiration (Walsh et al., 2002). As a substantial accumulation of H^+ and Pi can occur during very heavy-intensity exercise it could be hypothesised that the acute effect of inhibiting CK-mi activity is a slowing of τ_{II} during very heavy-intensity exercise compared with that of moderate exercise in which there is a relative lack of increase of H^+ and Pi. In the present study, the phase II moderate-intensity $\dot{V}O_2$ response was faster than that of the very heavy 6 minutes exercise test (as discussed above). However, whether this was due to an acute decrease in CK-mi activity at the higher work rates cannot be determined in the present study, as biopsy samples would need to have been taken during exercise. In addition, the ability to accurately quantify CK-mi from human tissue samples is still debatable due to the very low proportion CK-mi compared to CK-MM) (Wallimann, 1992). The finding that the phase II $\dot{V}O_2$ τ was slower in the "6 minute" than versus the "12 minute" test would be consistent with the effect of pH and/or Pi, as once above CP, H^+ and Pi continually accumulate which would be hypothesised to have an increasingly potent effect on τ_{II}.

It has also been suggested that the slower $\dot{V}O_2$ kinetics during heavy-intensity exercise may be because O_2 delivery becomes limiting (Gerbino et al., 1996; Hughson et al., 1996; MacDonald et al., 1997; Bohnert et al., 1998; Grasssi et al., 2001). However, the evidence from Rossiter et al. (2002) that the kinetics of [PCr] breakdown and $\dot{V}O_2$ are so similar

during heavy-intensity exercise suggests that this may not be the case, but rather an intramuscular limit may exist such as inhibition of CK-*mi*.

As it has been argued that the slower τ_{II} values are associated with increased type II fibre content (Pringle et al., 2003), individual differences in fibre type profiles might well contribute to the degree of phase II slowing which occurs at the higher WRs; i.e. individuals who have more type II fibres may therefore show a greater degree of slowing from moderate to $>\theta_L$ exercise as proportionally more type II fibres would be recruited above θ_L and, as importantly, above CP. This trend can, in fact, be seen in the study of Pringle et al. (2003), in which there appears to have been a trend for subject groups with the highest amount of type II fibres to show the greatest slowing at the higher work rates (i.e. an 8s difference, compared with 3 s for those with a low percentage of type II fibres). Previous studies in which individual data were available showed similar inter-subject variability in the degree of τ_{II} slowing (Ozyener et al., 2001; Paterson and Whipp, 1991; Barstow and Molé, 1991). Therefore, it is possible this effect of inter-subject variability of the τ_{II} above CP may be dependent on fibre type, which could perhaps explain the differences seen in some studies but not others.

In the present study, one subject (subject 6) showed a significantly slower τ_{II} value for the very heavy intensity exercise (average 46s) compared with moderate (25s); others however, showed a minimal difference (i.e. subject 5: very heavy average=16s, moderate=16s). It would be interesting, therefore, to see if a relationship exists, on an individual basis,

between the amount of slowing that occurs from moderate- to heavy- intensity exercise and the proportion of type II fibres.

4.4.2 Amplitude and gain parameters

The present study found that the phase II gain (G_{II}) for moderate-intensity exercise (11.0±0.4 $ml.min^{-1}.W^{-1}$) was significantly higher than that for very heavy-intensity exercise (average 9.7±0.4 $ml.min^{-1}.W^{-1}$) (section 3.1.2.1, 3.7; section 3.2.2.2, Figure 3.10). These results differed from those of Paterson and Whipp (1991) and of Barstow and Mole (1991). They are, however, in accord with those of Jones et al. (2002) and Pringle et al. (2003): in both of these studies a significantly smaller G_{II} was found for exercise above θ_L (9.9 and 9.1 $ml.min^{-1}.W^{-1}$, for the studies, respectively) than below (10.8 and 10.6 $ml.min^{-1}.W^{-1}$, respectively). Although Ozyener et al. (2001) did not report a significant difference, the moderate-intensity G_{II} was 11.5$ml.min^{-1}.W^{-1}$ compared with 10.7 $ml.min^{-1}.W^{-1}$ for very heavy-intensity exercise. Similarly, Carter et al. (2002) showed a trend for a decreased G_{II} with increased running speed. As previously mentioned it has been shown that subjects with a high proportion of type II fibres evidence a lower gain (Pringle et al., 2003; Barstow et al., 1996). The finding in the present study that the phase II gain was lower in very heavy-intensity compared with moderate-intensity exercise is compatible with this suggestion as it would be expected that more type II fibres are recruited at higher intensities (Essen, 1977; Jones and Round, 1990). The mechanisms causing a greater oxidative efficiency in type II fibres remain unknown (Heglund & Cavagna, 1987; Pringle et al.,

2003). Regardless, a lower phase II gain for very heavy-intensity exercise provides further evidence that the $\dot{V}O_2$ response above θ_L and particularly above CP does not conform to a dynamically linear system. This suggests that multiple factors, dependent on exercise intensity, are involved in the control of $\dot{V}O_2$ and therefore muscle O_2 consumption.

4.5 CONCLUSIONS

With regard to objective 1, the present study has shown for the first time that the phase II $\dot{V}O_2$ time constant (τ_{II}) is related, inversely, to CK activity in the vastus lateralis. Although the mechanism(s) through which this interaction occurs could not be elucidated, the results concur with recent literature (Roman et al., 2002) which suggests decreased CK activity would be associated with faster $\dot{V}O_2$. A limitation of the present study is that the small biopsy samples taken may not be representative of the entire muscle group and that samples were taken only from one of the muscle groups involved in cycling. Therefore, we cannot preclude the possibility that these factors could potentially lead to outliers in the results. Further investigation with an increased sample size is required to firmly elucidate the consistency and nature of the relationship between CK activity and τ_{II}. In addition, it was of interest that the phase II gain (G_{II}) for the supra-CP test was positively related to the CK activity. In light of recent suggestions of a significant role for fibre type on $\dot{V}O_2$ kinetics (τ_{II} and G_{II}), it would be interesting to investigate the relationship between (a) CK activity and fibre type, and (b) CK activity and τ_{II} following training-induced changes in fibre-type profile.

With regard to objective 2, the present study demonstrated that inter-subject variability exists in the $\dot{V}O_2$ kinetic parameters to very heavy-intensity exercise, when appropriate exercise intensity definition and modelling are prescribed. No relationship was found between the $\dot{V}O_2$ kinetic parameters and blood [La] or with fitness levels. In addition no significant relationship was found between either CK activity or UCP mRNA expression and $\dot{V}O_2$ kinetics (except G_{II} for "12 minute") above CP. Therefore, future studies are needed to investigate relationships underlying this kinetic variability. It will be particularly interesting to investigate the relationship between $\dot{V}O_2$ kinetics among individuals having a wide distribution of muscle fibre type profile.

In addition, the $\dot{V}O_2$ kinetics for moderate- and very heavy-intensity exercise were compared, as the literature remains equivocal on this point. It was shown that the phase II (τ_{II}) was slower above CP compared with moderate-intensity exercise and, also that the phase II gain was lower above CP. These observations clearly highlight the fact that $\dot{V}O_2$ does not conform to a dynamically linear system, and argue that a spectrum of mechanism(s) controlling the $\dot{V}O_2$ (and therefore $\dot{Q}O_2$) kinetics varies and also probably differs with intensity.

In this context, the design limitation of the present study should be noted, in which only two (or, on occasion one) repeat were undertaken, (whereas the moderate-intensity exercise

used six repeats). Although the supra-CP $\dot{V}O_2$ responses had a large asymptotic (or predicted steady-state) and therefore provided parameter estimates with a sufficiently small SD, it would have been preferable to have been able to increase the number of repeats so as to optimise the signal-to-noise ratio. However, multiple repeats were not feasible in the present study due to temporal constraints as subjects had each been asked to perform ~14 tests prior to the 6 and 12 minute supra-CP tests. Future studies should therefore strive to ensure that the most appropriate number of repeats is performed for high resolution of the $\dot{V}O_2$ kinetic response. This is particularly important if functional significance of the individual differences in $\dot{V}O_2$ kinetic responses and their potential relationships with other variables (as highlighted in the present study) are to be resolved convincingly.

With regard to objective 3 it was shown that, unlike the conclusions of Russell et al. (2002), there was no relationship between UCP-3 mRNA expression and the slow component amplitude for supra-CP exercise. The present study did suggest that there may be a trend between UCP-2 mRNA expression and the slow component amplitude. However, as data from only five subjects were available, more comprehensive investigations will be required to firmly elucidate any such relationship between UCP-2 expression and the $\dot{V}O_2$ slow component.

Another limitation of the present study is the recognition that interpretation of relationships involving UCP mRNA expression must be approached with caution, as mRNA expression does not necessarily reflect protein expression, owing to the complexities of translation

control. However, methodological constraints currently preclude analysis of UCP protein expression due to the small volume of muscle that can be obtained with a muscle biopsy sample. Therefore, mRNA expression is presently the best indicator available indicator of UCP levels until further methodological advancements are made.

The present study has examined the $\dot{V}O_2$ kinetics during moderate- and very heavy-intensity exercise and shown significant relationships with creatine kinase but not uncoupling protein. However, as some trends were evident between $\dot{V}O_2$ kinetics and CK and between $\dot{V}O_2$ kinetics UCP, more investigation with an increased subject pool needs to be performed to firmly elucidate the role of creatine kinase and it association with $\dot{V}O_2$ kinetics and muscle fibre-type profiles and activation patterns.

REFERENCES

Alpert, N.R. (1965). Lactate production and removal and the regulation of metabolism. *Ann. NY Acad. Sci.* **119**, 995.

Andersen, P. & Saltin, B. (1985). Maximal perfusion of skeletal muscle in man. *J.Physiol* **366**, 233-249.

Apple, F. S., Rogers, M. A., & Ivy, J. L. (1986). Creatine kinase isoenzyme MM variants in skeletal muscle and plasma from marathon runners. *Clin.Chem.* **32**, 41-44.

Babcock, M. A., Paterson, D. H., & Cunningham, D. A. (1992). Influence of ageing on aerobic parameters determined from a ramp test. *Eur.J.Appl.Physiol Occup.Physiol* **65**, 138-143.

Babcock, M. A., Paterson, D. H., & Cunningham, D. A. (1994). Effects of aerobic endurance training on gas exchange kinetics of older men. *Med.Sci.Sports Exerc.* **26**, 447-452.

Bangsbo, J. (2000). Muscle oxygen uptake in humans at onset of and during intense exercise. *Acta Physiol Scand.* **168**, 457-464.

Bangsbo, J., Gibala, M. J., Krustrup, P., Gonzalez-Alonso, J., & Saltin, B. (2002). Enhanced pyruvate dehydrogenase activity does not affect muscle O_2 uptake at the onset of intense exercise in humans. *Am.J.Physiol* **282**, 273-280.

Barstow, T. J., Lamarra, N., & Whipp, B. J. (1990). Modulation of muscle and pulmonary O2 uptakes by circulatory dynamics during exercise. *J.Appl.Physiol* **68**, 979-989.

Barstow, T. J. & Molé, P. A. (1991). Linear and nonlinear characteristics of oxygen uptake kinetics during heavy exercise. *J.Appl.Physiol* **71**, 2099-2106.

Barstow, T. J., Casaburi, R., & Wasserman, K. (1993). O_2 uptake kinetics and the O_2 deficit as related to exercise intensity and blood lactate. *J.Appl.Physiol* **75**, 755-762.

Barstow, T. J., Buchthal, S. D., Zanconato, S., & Cooper, D. M. (1994). Changes in potential controllers of human skeletal muscle respiration during incremental calf exercise. *J.Appl.Physiol* **77**, 2169-2176.

Barstow, T. J., Jones, A. M., Nguyen, P. H., & Casaburi, R. (1996). Influence of muscle fiber type and pedal frequency on oxygen uptake kinetics of heavy exercise. *J.Appl.Physiol* **81**, 1642-1650.

Bearden, S. E. & Moffatt, R. J. (2000). VO_2 kinetics and the O_2 deficit in heavy exercise. *J.Appl.Physiol* **88**, 1407-1412.

Bearden, S. E. & Moffatt, R. J. (2001). VO_2 slow component: to model or not to model? *Med.Sci.Sports Exerc.* **33**, 677-680.

Bearden, S. E. & Moffatt, R. J. (2001). Leg electromyography and the VO_2-power relationship during bicycle ergometry. *Med.Sci.Sports Exerc.* **33**, 1241-1245.

Beaver, W. L., Wasserman, K., & Whipp, B. J. (1973). On-line computer analysis and breath-by-breath graphical display of exercise function tests. *J.Appl.Physiol* **34**, 128-132.

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

Beaver, W. L., Wasserman, K., & Whipp, B. J. (1986). A new method for detecting anaerobic threshold by gas exchange. *J.Appl.Physiol* **60**, 2020-2027.

Behnke, B. J., Kindig, C. A., Musch, T. I., Koga, S., & Poole, D. C. (2001). Dynamics of microvascular oxygen pressure across the rest-exercise transition in rat skeletal muscle. *Respir.Physiol* **126**, 53-63.

Behnke, B. J., Barstow, T. J., Kindig, C. A., McDonough, P., Musch, T. I., & Poole, D. C. (2002). Dynamics of oxygen uptake following exercise onset in rat skeletal muscle. *Respir.Physiolo.Neurobiol.* **133**, 229-239.

Behnke, B. J., Kindig, C. A., Musch, T. I., Sexton, W. L., & Poole, D. C. (2002). Effects of prior contractions on muscle microvascular oxygen pressure at onset of subsequent contractions. *J Physiol* **539**, 927-934.

Bell, C., Paterson, D. H., Kowalchuk, J. M., Padilla, J., & Cunningham, D. A. (2001). A comparison of modelling techniques used to characterise oxygen uptake kinetics during the on-transient of exercise. *Exp.Physiol* **86**, 667-676.

Bell, C., Paterson, D. H., Kowalchuk, J. M., Moy, A. P., Thorp, D. B., Noble, E. G., Taylor, A. W., & Cunningham, D. A. (2001). Determinants of oxygen uptake kinetics in older humans following single- limb endurance exercise training. *Exp.Physiol* **86**, 659-665.

Bessman, S. P. & Geiger, P. J. (1981). Transport of energy in muscle: the phosphorylcreatine shuttle. *Science* **211**, 448-452.

Bessman, S. P. & Carpenter, C. L. (1985). The creatine-creatine phosphate energy shuttle. *Annu.Rev.Biochem.* **54**, 831-862.

Bessman, S. P. (1986). The physiological significance of the creatine phosphate shuttle. *Adv.Exp.Med.Biol.* **194**, 1-11.

Bessman, S. P. (1987). The creatine phosphate energy shuttle--the molecular asymmetry of a "pool". *Anal.Biochem.* **161**, 519-523.

Bohnert, B., Ward, S. A., & Whipp, B. J. (1998). Effects of prior arm exercise on pulmonary gas exchange kinetics during high-intensity leg exercise in humans. *Exp.Physiol* **83**, 557-570.

Borrani, F., Candau, R., Millet, G. Y., Perrey, S., Fuchslocher, J., & Rouillon, J. D. (2001). Is the VO2 slow component dependent on progressive recruitment of fast- twitch fibers in trained runners? *J.Appl.Physiol* **90**, 2212-2220.

Boss, O., Hagen, T., & Lowell, B. B. (2000). Uncoupling proteins 2 and 3: potential regulators of mitochondrial energy metabolism. *Diabetes* **49**, 143-156.

Brand, M. D., Chien, L. F., Ainscow, E. K., Rolfe, D. F., & Porter, R. K. (1994). The causes and functions of mitochondrial proton leak. *Biochim.Biophys.Acta* **1187**, 132-139.

Brittain, C. J., Rossiter, H. B., Kowalchuk, J. M., & Whipp, B. J. (2001). Effect of prior metabolic rate on the kinetics of oxygenuptake during moderate-intensity exercise. *Eur.J.Appl.Physiol Occup.Physiol* **86**, 125-134.

Brooks, G.A., T.D. Fahey, T.P. White, K.M. Baldwin. (2000). *Exercise Physiology. Human Bioenergetics and Its Applications, 3rd edition.* Mayfield Publishing, Mountain View, California, Mayfield Publishing. ISBN 0-767-1024-6.

Brown, G. C. (2000). Nitric oxide as a competitive inhibitor of oxygen consumption in the mitochondrial respiratory chain. *Acta Physiol Scand.* **168**, 667-674.

Burnley, M., Doust, J., Carter, H., & Jones, A. (2001). Effects of prior exercise and recovery duration on oxygen uptake kinetics during heavy exercise in humans. *Exp.Physiol* **86**, 417-425.

Burnley, M., Doust, J. H., Ball, D., & Jones, A. M. (2002). Effects of prior heavy exercise on VO_2 kinetics during heavy exercise are related to changes in muscle activity. *Journal of Applied Physiology* **93**, 167-174.

Calsbeek, D. J., Thompson, T. L., Dahl, J. A., Stob, N. R., Brozinick, J. T., Jr., Hill, J. O., & Hickey, M. S. (2002). Metabolic and anthropometric factors related to skeletal muscle UCP3 gene expression in healthy human adults. *Am.J Physiol Endocrinol.Metab* **283**, E631-E637.

Campbell-O'Sullivan, S. P., Constantin-Teodosiu, D., Peirce, N., & Greenhaff, P. L. (2002). Low intensity exercise in humans accelerates mitochondrial ATP production and pulmonary oxygen kinetics during subsequent more intense exercise. *J.Physiol* **538**, 931-939.

Carter, H., Jones, A. M., Barstow, T. J., Burnley, M., Williams, C., & Doust, J. H. (2000). Effect of endurance training on oxygen uptake kinetics during treadmill running. *J.Appl.Physiol* **89**, 1744-1752.

Carter, H., Pringle, J. S., Jones, A. M., & Doust, J. H. (2002). Oxygen uptake kinetics during treadmill running across exercise intensity domains. *Eur.J.Appl.Physiol* **86**, 347-354.

Casaburi, R., Whipp, B. J., Wasserman, K., Beaver, W. L., & Koyal, S. N. (1977). Ventilatory and gas exchange dynamics in response to sinusoidal work. *J.Appl.Physiol* **42**, 300-301.

Casaburi, R., Whipp, B. J., Wasserman, K., & Stremel, R. W. (1978). Ventilatory control characteristics of the exercise hyperpnea as discerned from dynamic forcing techniques. *Chest* **73**, 280-283.

Casaburi, R., J. Daly, J.E. Hansen, and R.M. Effros. (1989). Abrupt changes in mixed venous gas composition after the onset of exercise. *J.Appl.Physiol.* **67**, 1106-1112.

Chance, B. & Williams, G.R. (1956). The respiratory chain and oxidative phosphorylation. *Adv.Enzymol.Relat.Subj.Biochem* **17**, 65-134.

Chance, B., Mauriello, G., & Aubert, X. (1962). ADP arrival at muscle mitochondria following a twitch. In: *Muscle as a Tissue.* Rodahl, K., & Horvath, S. (eds.). McGraw, New York.

Chance, B., Leigh, J. S., Jr., Clark, B. J., Maris, J., Kent, J., Nioka, S., & Smith, D. (1985). Control of oxidative metabolism and oxygen delivery in human skeletal muscle: a steady-state analysis of the work/energy cost transfer function. *Proc.Natl.Acad.Sci.U.S.A* **82**, 8384-8388.

Chilibeck, P. D., Paterson, D. H., Petrella, R. J., & Cunningham, D. A. (1996). The influence of age and cardiorespiratory fitness on kinetics of oxygen uptake. *Can.J.Appl.Physiol* **21**, 185-196.

Coates, E., Rossiter, H., Day J., Miura A., Fukuba Y., & Whipp B. (2003). Intensity dependent tolerance to exercise after training VO_2max in humans. *J. Appl. Physiol* (in press).

Conley, K. E., Kemper, W. F., & Crowther, G. J. (2001). Limits to sustainable muscle performance: interaction between glycolysis and oxidative phosphorylation. *J.Exp.Biol.* **204**, 3189-3194.

Crow, M. T. & Kushmerick, M. J. (1982). Chemical energetics of slow- and fast-twitch muscles of the mouse. *J.Gen.Physiol* **79**, 147-166.

Cummin, A. R., Iyawe, V. I., Mehta, N., & Saunders, K. B. (1986). Ventilation and cardiac output during the onset of exercise, and during voluntary hyperventilation, in humans. *J.Physiol* **370**, 567-583.

Cunningham, D.A., J.E. Himann, D.H. Paterson, and J.R. Dickinson. (1993). Gas exchange dynamics with sinusoidal work in young and elderly women. *Respir. Physiol.* **91**, 43-56.

Davis, J. A., Vodak, P., Wilmore, J. H., Vodak, J., & Kurtz, P. (1976). Anaerobic threshold and maximal aerobic power for three modes of exercise. *J. Appl. Physiol* **41**, 544-550.

Davis, J. A. (1985). Anaerobic threshold: review of the concept and directions for future research. *Med.Sci.Sports Exerc.* **17**, 6-21.

Edwards, A. M., Challis, N. V., Chapman, J. H., Claxton, D. B., & Fysh, M. L. (2001). The test-retest reliability of gas exchange kinetics in humans using a pseudo random binary sequence exercise test. *Eur.J.Appl.Physiol* **85**, 333-338.

Erecinska, M., Veech, R. L., & Wilson, D. F. (1974). Thermodynamic relationships between the oxidation-reduction reactions and the ATP synthesis in suspensions of isolated pigeon heart mitochondria. *Arch.Biochem.Biophys.* **160**, 412-421.

Essen, b. (1977). Intramuscular substrate utilization during prolonged exercise. *Ann.N.Y.Acad.Sci.* **301**, 30-44.

Essfeld, D., Hoffmann, U., & Stegemann, J. (1987). VO2 kinetics in subjects differing in aerobic capacity: investigation by spectral analysis. *Eur.J.Appl.Physiol Occup.Physiol* **56**, 508-515.

Frandsenn, U., Bangsbo, J., Sander, M., Hoffner, L., Betak, A., Saltin, B., & Hellsten, Y. (2001). Exercise-induced hyperaemia and leg oxygen uptake are not altered during effective inhibition of nitric oxide synthase with N(G)-nitro-L- arginine methyl ester in humans. *J.Physiol* **531**, 257-264.

Fujihara, Y., Hildebrandt, J., & Hildebrandt, J. R. (1973). Cardiorespiratory transients in exercising man. II. Linear models. *J.Appl.Physiol* **35**, 68-76.

Fukuba, Y. & Whipp, B. J. (1999). A metabolic limit on the ability to make up for lost time in endurance events. *J.Appl.Physiol* **87**, 853-861.

Gaesser, G. A. (1994). Influence of endurance training and catecholamines on exercise VO2 response. *Med.Sci.Sports Exerc.* **26,** 1341-1346.

Gerbino, A., Ward, S. A., & Whipp, B. J. (1996). Effects of prior exercise on pulmonary gas-exchange kinetics during high-intensity exercise in humans. *J.Appl.Physiol* **80**, 99-107.

Gibala, M. J. & Saltin, B. (1999). PDH activation by dichloroacetate reduces TCA cycle intermediates at rest but not during exercise in humans. *Am.J Physiol* **277**, E33-E38.

Gledhill, N., Cox, D., & Jamnik, R. (1994). Endurance athletes' stroke volume does not plateau: major advantage is diastolic function. *Med.Sci.Sports Exerc.* **26**, 1116-1121.

Gollnick, P.D., and L. Hermansen. (1973). Biochemical adaptations to exercise: Anaerobic metabolism. In: *Exercise and Sport Science Reviews*, Vol. 1. Edited by J.H. Wilmore, New York, Academic Press.

Grassi, B., Poole, D. C., Richardson, R. S., Knight, D. R., Erickson, B. K., & Wagner, P. D. (1996). Muscle O_2 uptake kinetics in humans: implications for metabolic control. *J.Appl.Physiol* **80**, 988-998.

Grassi, B., Gladden, L. B., Stary, C. M., Wagner, P. D., & Hogan, M. C. (1998). Peripheral O2 diffusion does not affect VO_2 on-kinetics in isolated insitu canine muscle. *J.Appl.Physiol* **85**, 1404-1412.

Grassi, B., Gladden, L. B., Samaja, M., Stary, C. M., & Hogan, M. C. (1998). Faster adjustment of O2 delivery does not affect VO_2 on-kinetics in isolated in situ canine muscle. *J.Appl.Physiol* **85**, 1394-1403.

Grassi, B., Hogan, M. C., Kelley, K. M., Aschenbach, W. G., Hamann, J. J., Evans, R. K., Patillo, R. E., & Gladden, L. B. (2000). Role of convective O_2 delivery in determining VO_2 on-kinetics in canine muscle contracting at peak VO_2. *J.Appl.Physiol* **89**, 1293-1301.

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

Grassi, B., Hogan, M. C., Greenhaff, P. L., Hamann, J. J., Kelley, K. M., Aschenbach, W. G., Constantin-Teodosiu, D., & Gladden, L. B. (2002). Oxygen uptake on-kinetics in dog gastrocnemius in situ following activation of pyruvate dehydrogenase by dichloroacetate. *J.Physiol* **538**, 195-207.

Griffiths, T. L., Henson, L. C., & Whipp, B. J. (1986). Influence of inspired oxygen concentration on the dynamics of the exercise hyperpnoea in man. *J.Physiol* **380**, 387-403.

Hagberg, J. M., Mullin, J. P., & Nagle, F. J. (1978). Oxygen consumption during constant-load exercise. *J.Appl.Physiol* **45**, 381-384.

Hagberg, J. M., Hickson, R. C., Ehsani, A. A., & Holloszy, J. O. (1980). Faster adjustment to and recovery from submaximal exercise in the trained state. *J.Appl.Physiol* **48**, 218-224.

Hansen, J. E., Sue, D. Y., Oren, A., & Wasserman, K. (1987). Relation of oxygen uptake to work rate in normal men and men with circulatory disorders. *Am.J.Cardiol.* **59**, 669-674.

Heglund, N. C. & Cavagna, G. A. (1987). Mechanical work, oxygen consumption, and efficiency in isolated frog and rat muscle. *Am.J.Physiol* **253**, C22-C29.

Hickson, R. C., Bomze, H. A., & Hollozy, J. O. (1978). Faster adjustment of O_2 uptake to the energy requirement of exercise in the trained state. *J.Appl.Physiol* **44**, 877-881.

Hill, D. W. (1993). The critical power concept. A review. *Sports Med.* **16**, 237-254.

Hochachka, P. W. & Matheson, G. O. (1992). Regulating ATP turnover rates over broad dynamic work ranges in skeletal muscles. *J. Appl.Physiology* **73**, 1697-1703.

Hogan, M. C. (1999). Phosphorescence quenching method for measurement of intracellular PO_2 in isolated skeletal muscle fibers. *J.Appl.Physiol* **86**, 720-724.

Hogan, M. C. (2001). Fall in intracellular PO(2) at the onset of contractions in Xenopus single skeletal muscle fibers. *J Appl.Physiol* **90**, 1871-1876.

Holloszy, J. O. (1967). Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J.Biol.Chem.* **242** , 2278-2282.

Howlett, R. A., Heigenhauser, G. J., Hultman, E., Hollidge-Horvat, M. G., & Spriet, L. L. (1999). Effects of dichloroacetate infusion on human skeletal muscle metabolism at the onset of exercise. *Am.J.Physiol* **277**, E18-E25.

Howlett, R. A. & Hogan, M. C. (2001). Intracellular PO_2 decreases with increasing stimulation frequency in contracting single Xenopus muscle fibers. *J Appl.Physiol* **91**, 632-636.

Hughson, R. L. & Morrissey, M. (1982). Delayed kinetics of respiratory gas exchange in the transition from prior exercise. *J.Appl.Physiol* **52**, 921-929.

Hughson, R. L. & Smyth, G. A. (1983). Slower Adaptation of VO2 to steady state of submaximal exercise with beta-blockade. *Eur.J Appl Physiol Occup.Physiol* **52**, 107-110.

Hughson, R. L. & Inman, M. D. (1986). Oxygen uptake kinetics from ramp work tests: variability of single test values. *J.Appl.Physiol* **61**, 373-376.

Hughson, R. L., Sherrill, D. L., & Swanson, G. D. (1988). Kinetics of VO2 with impulse and step exercise in humans. *J.Appl.Physiol* **64**, 451-459.

Hughson, R. L., Winter, D. A., Patla, A. E., Swanson, G. D., & Cuervo, L. A. (1990). Investigation of VO2 kinetics in humans with pseudorandom binary sequence work rate change. *J.Appl.Physiol* **68**, 796-801.

Hughson, R. L. & Kowalchuk, J. M. (1991). Beta-blockade and oxygen delivery to muscle during exercise. *Can.J Physiol Pharmacol.* **69**, 285-289.

Hughson, R. L., Cuervo, L. A., Patla, A. E., Winter, D. A., Xing, H. C., Dietrich, B. H., & Swanson, G. D. (1991). Time domain analysis of oxygen uptake during pseudorandom binary sequence exercise tests. *J.Appl.Physiol* **71**, 1620-1626.

Hughson, R. L., Cochrane, J. E., & Butler, G. C. (1993). Faster O2 uptake kinetics at onset of supine exercise with than without lower body negative pressure. *J.Appl.Physiol* **75**, 1962-1967.

Hughson, R. L. & Kowalchuk, J. M. (1995). Kinetics of oxygen uptake for submaximal exercise in hyperoxia, normoxia, and hypoxia. *Can.J.Appl.Physiol* **20**, 198-210.

Hughson, R. L., Shoemaker, J. K., Tschakovsky, M. E., & Kowalchuk, J. M. (1996). Dependence of muscle VO2 on blood flow dynamics at onset of forearm exercise. *J.Appl.Physiol* **81**, 1619-1626.

Hultman, E., Bergstrom, J., & Anderson, N. M. (1967). Breakdown and resynthesis of phosphorylcreatine and adenosine triphosphate in connection with muscular work in man. *Scand.J Clin.Lab Invest* **19,** 56-66.

Jeneson, J. A., Wiseman, R. W., Westerhoff, H. V., & Kushmerick, M. J. (1996). The signal transduction function for oxidative phosphorylation is at least second order in ADP. *J.Biol.Chem.* **271**, 27995-27998.

Jones, A. M., Carter, H., Pringle, J. S., & Campbell, I. T. (2002). Effect of creatine supplementation on oxygen uptake kinetics during submaximal cycle exercise. *J.Appl.Physiol* **92**, 2571-2577.

Jones, D. A. & Round, J. M. Skeletal muscle in health and disease. (1990). *Muscle Physiology.* Manchester, United Kingdom, Manchester University Publishing.

Jones, N. L. & Ehrsam, R. E. (1982). The anaerobic threshold. *Exerc.Sport Sci.Rev.* **10,** 49-83.

Joyner, M. J. & Dietz, N. M. (1997). Nitric oxide and vasodilation in human limbs. *J Appl.Physiol* **83**, 1785-1796.

Kindig, C. A., McDonough, P., Erickson, H. H., & Poole, D. C. (2001). Effect of L-NAME on oxygen uptake kinetics during heavy-intensity exercise in the horse. *J.Appl.Physiol* **91**, 891-896.

Kindig, C. A., McDonough, P., Erickson, B. K., & Poole, D. C. (2002). Nitric oxide synthase inhibition speeds oxygen uptake kinetics in horses during moderate domain running. *Respiratory Physiology & Neurobiology* **132**, 169-178.

Kindig, C. A., Richardson, T. E., & Poole, D. C. (2002). Skeletal muscle capillary hemodynamics from rest to contractions: implications for oxygen transfer. *J.Appl.Physiol* **92**, 2513-2520.

Klingenberg, M. (1980). The ADP-ATP translocation in mitochondria, a membrane potential controlled transport. *J Membr.Biol.* **56**, 97-105.

Koga, S., Shiojiri, T., Kondo, N., & Barstow, T. J. (1997). Effect of increased muscle temperature on oxygen uptake kinetics during exercise. *J.Appl.Physiol* **83**, 1333-1338.

Koga, S., Shiojiri, T., Shibasaki, M., Kondo, N., Fukuba, Y., & Barstow, T. J. (1999). Kinetics of oxygen uptake during supine and upright heavy exercise. *J.Appl.Physiol* **87**, 253-260.

Koike, A., Wasserman, K., McKenzie, D. K., Zanconato, S., & Weiler-Ravell, D. (1990). Evidence that diffusion limitation determines oxygen uptake kinetics during exercise in humans. *J Clin.Invest* **86**, 1698-1706.

Krogh, A. & Lindhard, J. (1913). The regulation of respiration and circulation during the initial stages of muscular work. *J.Physiol* **47**, 112-136.

Kushmerick, M. J., Meyer, R. A., & Brown, T. R. (1992). Regulation of oxygen consumption in fast- and slow-twitch muscle. *Am.J.Physiol* **263**, C598-C606.

Lamarra, N., Whipp, B. J., Ward, S. A., & Wasserman, K. (1987). Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. *J.Appl.Physiol* **62**, 2003-2012.

Linnarsson, D. (1974). Dynamics of pulmonary gas exchange and heart rate changes at start and end of exercise. *Acta Physiol Scand.Suppl* **415**, 1-68.

Lucia, A., Hoyos, J., & Chicharro, J. L. (2000). The slow component of VO2 in professional cyclists. *Br.J.Sports Med.* **34**, 367-374.

Macdonald, M., Pedersen, P. K., & Hughson, R. L. (1997). Acceleration of VO2 kinetics in heavy submaximal exercise by hyperoxia and prior high-intensity exercise. *J.Appl.Physiol* **83**, 1318-1325.

MacDonald, M. J., Shoemaker, J. K., Tschakovsky, M. E., & Hughson, R. L. (1998). Alveolar oxygen uptake and femoral artery blood flow dynamics in upright and supine leg exercise in humans. *J.Appl.Physiol* **85**, 1622-1628.

Mahler, M. (1985). First-order kinetics of muscle oxygen consumption, and an equivalent proportionality between QO2 and phosphorylcreatine level. Implications for the control of respiration. *J.Gen.Physiol* **86**, 135-165.

Mallory, L. A., Scheuermann, B. W., Hoelting, B. D., Weiss, M. L., McAllister, R. M., & Barstow, T. J. (2002). Influence of peak VO2 and muscle fiber type on the efficiency of moderate exercise. *Med.Sci.Sports Exerc.* **34**, 1279-1287.

McArdle, W.D., F.I. Katch, V.L. Katch. (1991). In: *Exercise Physiology. Energy, Nutrition, and Human Performance, Third edition.* Lea and Febiger, Philadelphia,.

McCreary, C. R., Chilibeck, P. D., Marsh, G. D., Paterson, D. H., Cunningham, D. A., & Thompson, R. T. (1996). Kinetics of pulmonary oxygen uptake and muscle phosphates during moderate-intensity calf exercise. *J Appl.Physiol* **81**, 1331-1338.

Meyer, R. A. (1988). A linear model of muscle respiration explains monoexponential phosphocreatine changes. *Am.J.Physiol* **254**, C548-C553.

Meyer, R. A. & Foley, J. M. (1994). Testing models of respiratory control in skeletal muscle. *Med.Sci.Sports Exerc.* **26**, 52-57.

Miyamoto, Y., Y. Nakazono, T. Hiura, Y. Abe. (1983). Cardiorespiratory dynamics during sinusoidal and impulse exercise in man. *Jap. J. Physiol.* **33**: 971-986.

Miyamoto, Y. (1992). Kinetics of respiratory and circulatory responses to step, impulse, sinusoidal and ramp forcings of exercise load in humans. *Front. Med.*Biol. Eng. **4**(1): 3-18.

Miura, A., Endo, M., Sato, H., Sato, H., Barstow, T. J., & Fukuba, Y. (2002). Relationship between the curvature constant parameter of the power- duration curve and muscle cross-sectional area of the thigh for cycle ergometry in humans. *Eur.J.Appl.Physiol* **87**, 238-244.

Monod, H. & Scherrer, J. (1965). The work capacity of a synergic muscle group. *Ergonomics* **8**, 329-338.

Moritani, T., Nagata, A., deVries, H. A., & Muro, M. (1981). Critical power as a measure of physical work capacity and anaerobic threshold. *Ergonomics* **24**, 339-350.

Nery, L. E., Wasserman, K., Andrews, J. D., Huntsman, D. J., Hansen, J. E., & Whipp, B. J. (1982). Ventilatory and gas exchange kinetics during exercise in chronic airways obstruction. *J.Appl.Physiol* **53**, 1594-1602.

Niizeki, K., Takahashi, T., & Miyamoto, Y. (1995). A model analysis of asymmetrical response of pulmonary VO2 during incremental and decremental ramp exercise. *J.Appl.Physiol* **79**, 1816-1827.

Ozyener, F., Rossiter, H., Ward, S., & Whipp, B. (2001). Influence of exercise intensity on the on- and off-transient kinetics of pulmonary oxygen uptake in humans. *J.Physiol* **533** , 891-902.

Paterson, D. H. & Whipp, B. J. (1991). Asymmetries of oxygen uptake transients at the on- and offset of heavy exercise in humans. *J.Physiol* **443**, 575-586.

Paterson, N.D., D.H. Paterson, J.M. Kowalchuk. (2003). VO_2 kinetics are progressively slowed in regions of upper moderate- and heavy-intensity exercise domains. *Med. Sci. Sports Exerc.* **35,** (Suppl.): S336.

Pernow, B., and J. Karlson. (1971). Muscle ATP, CP and lactate in submaximal and maximal exercise. In: *Muscle Metabolism During Exercise.* Edited by B. Pernow and B. Saltin. New York, Plenum Press.

Phillips, S. M., Green, H. J., MacDonald, M. J., & Hughson, R. L. (1995). Progressive effect of endurance training on VO_2 kinetics at the onset of submaximal exercise. *J.Appl.Physiol* **79**, 1914-1920.

Piiper, J., Marconi, C., Heisler, N., Meyer, M., Weitz, H., Pendergast, D. R., & Cerretelli, P. (1989). Spatial and temporal variability of blood flow in stimulated dog gastrocnemius muscle. *Adv.Exp.Med.Biol.* **248**, 719-728.

Piiper, J. & Haab, P. (1991). Oxygen supply and uptake in tissue models with unequal distribution of blood flow and shunt. *Respir.Physiol* **84**, 261-271.

Piiper, J. (1992). Modeling of oxygen transport to skeletal muscle: blood flow distribution, shunt, and diffusion. *Adv.Exp.Med.Biol.* **316**, 3-10.

Piiper, J. & Scheid, P. (1999). Modeling oxygen availability to exercising muscle. *Respir.Physiol* **118**, 95-101.

Piiper, J. (2000). Perfusion, diffusion and their heterogeneities limiting blood-tissue O_2 transfer in muscle. *Acta Physiol Scand.* **168**, 603-607.

Poole, D. C., Ward, S. A., Gardner, G. W., & Whipp, B. J. (1988). Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics* **31**, 1265-1279.

Poole, D. C., Ward, S. A., & Whipp, B. J. (1990). The effects of training on the metabolic and respiratory profile of high-intensity cycle ergometer exercise. *Eur.J Appl Physiol Occup.Physiol* **59**, 421-429.

Poole, D. C., Schaffartzik, W., Knight, D. R., Derion, T., Kennedy, B., Guy, H. J., Prediletto, R., & Wagner, P. D. (1991). Contribution of excising legs to the slow component of oxygen uptake kinetics in humans. *J.Appl.Physiol* **71**, 1245-1260.

Poole, D. C., Barstow, T. J., Gaesser, G. A., Willis, W. T., & Whipp, B. J. (1994). VO_2 slow component: physiological and functional significance. *Med.Sci.Sports Exerc.* **26**, 1354-1358.

Poole, D. C. (1994). Role of exercising muscle in slow component of VO_2. *Med.Sci.Sports Exerc.* **26**, 1335-1340.

Pringle, J. S. & Jones, A. M. (2002). Maximal lactate steady state, critical power and EMG during cycling. *Eur.J.Appl.Physiol* **88**, 214-226.

Pringle, J. S., Doust, J. H., Carter, H., Tolfrey, K., Campbell, I. T., & Jones, A. M. (2003). Oxygen uptake kinetics during moderate, heavy and severe intensity 'submaximal' exercise in humans: the influence of muscle fibre type and capillarisation. *Eur.J.Appl.Physiol* **89**, 289-300.

Radegran, G. & Saltin, B. (1998). Muscle blood flow at onset of dynamic exercise in humans. *Am.J.Physiol* **274**, H314-H322.

Reinhard, U., Muller, P. H., & Schmulling, R. M. (1979). Determination of anaerobic threshold by the ventilation equivalent in normal individuals. *Respiration* **38**, 36-42.

Richardson, R. S., Haseler, L. J., Nygren, A. T., Bluml, S., & Frank, L. R. (2001). Local perfusion and metabolic demand during exercise: a noninvasive MRI method of assessment. *J Appl.Physiol* **91**, 1845-1853.

Roman, B. B., Foley, J. M., Meyer, R. A., & Koretsky, A. P. (1996). Contractile and metabolic effects of increased creatine kinase activity in mouse skeletal muscle. *Am.J.Physiol* **270**, C1236-C1245.

Roman, B. B., Meyer, R. A., & Wiseman, R. W. (2002). Phosphocreatine kinetics at the onset of contractions in skeletal muscle of MM creatine kinase knockout mice. *Am.J.Physiol Cell Physiol* **283**, C1776-C1783.

Rossiter, H. B., Ward, S. A., Doyle, V. L., Howe, F. A., Griffiths, J. R., & Whipp, B. J. (1999). Inferences from pulmonary O_2 uptake with respect to intramuscular phosphocreatine kinetics during moderate exercise in humans. *J.Physiol* **518 (Pt 3)**, 921-932.

Rossiter, H. B., Howe, F. A., Ward, S. A., Kowalchuk, J. M., Griffiths, J. R., & Whipp, B. J. (2000). Intersample fluctuations in phosphocreatine concentration determined by 31P-

magnetic resonance spectroscopy and parameter estimation of metabolic responses to exercise in humans. *J.Physiol* **528**, 359-369.

Rossiter, H. B., Ward, S. A., Kowalchuk, J. M., Howe, F. A., Griffiths, J. R., & Whipp, B. J. (2001). Effects of prior exercise on oxygen uptake and phosphocreatine kinetics during high-intensity knee-extension exercise in humans. *J.Physiol* **537**, 291-303.

Rossiter, H. B., Ward, S. A., Howe, F. A., Kowalchuk, J. M., Griffiths, J. R., & Whipp, B. J. (2002). Dynamics of intramuscular 31P-MRS Pi peak splitting and the slow components of PCr and O_2 uptake during exercise. *J Appl.Physiol* **93**, 2059-2069.

Rossiter, H. B., Ward, S. A., Kowalchuk, J. M., Howe, F. A., Griffiths, J. R., & Whipp, B. J. (2002). Dynamic asymmetry of phosphocreatine concentration and O_2 uptake between the on- and off-transients of moderate- and high-intensity exercise in humans. *J Physiol* **541**, 991-1002.

Roston, W. L., Whipp, B. J., Davis, J. A., Cunningham, D. A., Effros, R. M., & Wasserman, K. (1987). Oxygen uptake kinetics and lactate concentration during exercise in humans. *Am.Rev.Respir.Dis.* **135**, 1080-1084.

Russell, A., Wadley, G., Snow, R., Giacobino, J. P., Muzzin, P., Garnham, A., & Cameron-Smith, D. (2002). Slow component of VO_2 kinetics: the effect of training status, fibre type, UCP3 mRNA and citrate synthase activity. *Int.J Obes.Relat Metab Disord.* **26**, 157-164.

Saks, V. A., Kuznetsov, A. V., Khuchua, Z. A., Vasilyeva, E. V., Belikova, J. O., Kesvatera, T., & Tiivel, T. (1995). Control of cellular respiration in vivo by mitochondrial outer membrane and by creatine kinase. A new speculative hypothesis: possible involvement of mitochondrial-cytoskeleton interactions. *J Mol.Cell Cardiol.* **27**, 625-645.

Savasi, I., Evans, M. K., Heigenhauser, G. J., & Spriet, L. L. (2002). Skeletal muscle metabolism is unaffected by DCA infusion and hyperoxia after onset of intense aerobic exercise. *Am.J Physiol Endocrinol.Metab* **283**, E108-E115.

Scheuermann, B. W., Kowalchuk, J. M., Paterson, D. H., & Cunningham, D. A. (1998). O2 uptake kinetics after acetazolamide administration during moderate- and heavy-intensity exercise. *J.Appl.Physiol* **85**, 1384-1393.

Scheuermann, B. W., Hoelting, B. D., Noble, M. L., & Barstow, T. J. (2001). The slow component of O_2 uptake is not accompanied by changes in muscle EMG during repeated bouts of heavy exercise in humans. *J.Physiol* **531**, 245-256.

Schrauwen, P., Troost, F. J., Xia, J., Ravussin, E., & Saris, W. H. (1999). Skeletal muscle UCP2 and UCP3 expression in trained and untrained male subjects. *Int.J Obes.Relat Metab Disord.* **23**, 966-972.

Sietsema, K. E. (1992). Oxygen uptake kinetics in response to exercise in patients with pulmonary vascular disease. *Am.Rev.Respir.Dis.* **145**, 1052-1057.

Timmons, J. A., Gustafsson, T., Sundberg, C. J., Jansson, E., & Greenhaff, P. L. (1998). Muscle acetyl group availability is a major determinant of oxygen deficit in humans during submaximal exercise. *Am J Physiol* **274**, E377-E380.

Tonkonogi, M. & Sahlin, K. (1997). Rate of oxidative phosphorylation in isolated mitochondria from human skeletal muscle: effect of training status. *Acta Physiol Scand.* **161**, 345-353.

Tonkonogi, M., Harris, B., & Sahlin, K. (1998). Mitochondrial oxidative function in human saponin-skinned muscle fibres: effects of prolonged exercise. *J Physiol* **510**, 279-286.

Tonkonogi, M., Krook, A., Walsh, B., & Sahlin, K. (2000). Endurance training increases stimulation of uncoupling of skeletal muscle mitochondria in humans by non-esterified fatty acids: an uncoupling-protein-mediated effect? *Biochem.J* **351**, 805-810.

Trask, R. V. & Billadello, J. J. (1990). Tissue-specific distribution and developmental regulation of M and B creatine kinase mRNAs. *Biochim.Biophys.Acta* **1049**, 182-188.

Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K., & Eppenberger, H. M. (1992). Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. *Biochem.J.* **281**, 21-40.

Walsh, B., Tonkonogi, M., Soderlund, K., Hultman, E., Saks, V., & Sahlin, K. (2001). The role of phosphorylcreatine and creatine in the regulation of mitochondrial respiration in human skeletal muscle. *J.Physiol* **537**, 971-978.

Walsh, B., Tonkonogi, M., & Sahlin, K. (2001). Effect of endurance training on oxidative and antioxidative function in human permeabilized muscle fibres. *Pflugers Arch.* **442**, 420-425.

Walsh, B., Tiivel, T., Tonkonogi, M., & Sahlin, K. (2002). Increased concentrations of P_i and lactic acid reduce creatine-stimulated respiration in muscle fibers. *Journal of Applied Physiology* **92**, 2273-2276.

Wasserman, K., Van Kessel, A. L., & Burton, G. G. (1967). Interaction of physiological mechanisms during exercise. *J.Appl.Physiol* **22**, 71-85.

Wasserman, K., Stringer, W. W., & Casaburi, R. (1995). Is the slow component of exercise VO2 a respiratory adaptation to anaerobiosis? *Adv.Exp.Med.Biol.* **393**, 187-194.

Whipp B.J. & Mahler M. Dynamics of Pulmonary Gas Exchange during Exercise. Pulmonary Gas Exchange II. (1980). United States, Academic Press Incorporated. Ref Type: Generic

Whipp, B. J. (1971). Rate constant for the kinetics of oxygen uptake during light exercise. *J.Appl.Physiol* **30**, 261-263.

Whipp, B. J. & Wasserman, K. (1972). Oxygen uptake kinetics for various intensities of constant-load work. *J.Appl.Physiol* **33**, 351-356.

Whipp, B. J. & Casaburi, R. (1982). Characterizing O_2 uptake response kinetics during exercise. *Int.J.Sports Med.* **3**, 97-99.

Whipp, B. J., Ward, S. A., & Wasserman, K. (1986). Respiratory markers of the anaerobic threshold. *Adv.Cardiol.* **35**, 47-64.

Whipp, B. J. (1987). Dynamics of pulmonary gas exchange. *Circulation* **76**, VI18-VI28.

Whipp, B. J. & Ward, S. A. (1990). Physiological determinants of pulmonary gas exchange kinetics during exercise. *Med.Sci.Sports Exerc.* **22**, 62-71.

Whipp, B. J. & Ward, S. A. (1992). Pulmonary gas exchange dynamics and the tolerance to muscular exercise: effects of fitness and training. *Ann.Physiol Anthropol.* **11**, 207-214.

Whipp, B. J. (1994). The bioenergetic and gas exchange basis of exercise testing. *Clin.Chest Med.* **15**, 173-192.

Whipp, B. J. & Ozyener, F. (1998). The kinetics of exertional oxygen uptake: assumptions and inferences. *Medicina Dello Sport* **51**, 139-149.

Whipp, B. J., Rossiter, H. B., Ward, S. A., Avery, D., Doyle, V. L., Howe, F. A., & Griffiths, J. R. (1999). Simultaneous determination of muscle ^{31}P and O_2 uptake kinetics during whole body NMR spectroscopy. *J.Appl.Physiol* **86**, 742-747.

Whipp, B. J., Rossiter, H. B., & Ward, S. A. (2002). Exertional oxygen uptake kinetics: a stamen of stamina? *Biochemical Society Transactions* **30**[2], 237-247.

Williamson, J. W., Raven, P. B., & Whipp, B. J. (1996). Unaltered oxygen uptake kinetics at exercise onset with lower-body positive pressure in humans. *Exp.Physiol* **81**, 695-705.

Wilson, D. F. (1994). Factors affecting the rate and energetics of mitochondrial oxidative phosphorylation. *Med.Sci.Sports Exerc.* **26**, 37-43.

Yoshida, T. (1986). A comparison of lactate threshold and onset of blood lactate accumulation during two kinds of duration of incremental exercises. *Ann.Physiol Anthropol.* **5**, 211-216.

Yoshida, T., Chida, M., Ichioka, M., Makiguchi, K., & Suda, Y. (1987). Effect of hypoxia on lactate variables during exercise. *J Hum.Ergol.(Tokyo)* **16**, 157-161.

Yoshida, T. & Watari, H. (1993). 31P-nuclear magnetic resonance spectroscopy study of the time course of energy metabolism during exercise and recovery. *Eur.J Appl Physiol Occup.Physiol* **66**, 494-499.

Zöll, J., Sanchez, H., N'Guessan, B., Ribera, F., Lampert, E., Bigard, X., Serrurier, B., Fortin, D., Geny, B., Veksler, V., Ventura-Clapier, R., & Mettauer, B. (2002). Physical activity changes the regulation of mitochondrial respiration in human skeletal muscle. *J.Physiol* **543**, 191-200.

Appendix i

Information and consent form

University of Glasgow
Institute of Biomedical and Life Sciences
University of Glasgow

INFORMATION SHEET

TITLE OF INVESTIGATION: **Influence of creatine kinase and mitochondrial uncoupling proteins on the control of muscle oxygen consumption during exercise in healthy human subjects**

You have been invited to take part in a research study looking at the influence of muscle proteins on exercise performance. In order to help you to understand what the investigation is about, please read the following information carefully. If there are any points that need further explanation, please ask a member of the research team. It is important that you understand what you are volunteering to do and are completely happy with all the information before you sign this form.

What is the purpose of the study? It has been proposed that two muscle proteins (creatine kinase and mitochondrial uncoupling proteins) and one gene (the angiotensin-converting enzyme or ACE gene) may influence how quickly oxygen is consumed during exercise and may therefore dictate how well an individual may exercise before fatiguing. This study aims to investigate this possibility by asking fourteen healthy volunteers to exercise. The results obtained could be important in both health (to aid sporting performance) and disease (where exercise can prove very difficult).

Why have I been chosen? You have been selected as a possible participant in this investigation because you are aerobically trained and in good health. Before you become a subject, you will complete a medical questionnaire. People who have asthma, heart-related and/or circulatory problems, hypertension or any other contraindicated condition will not be allowed to take part in the study.

Do I have to take part? It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and you will be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part? You will be asked to visit the laboratory on no more than nineteen separate days over a three-month period. This will provide us with up to four "repeat" responses for each of the exercise protocols described below. However, in our experience, two to three repeats are normally sufficient to provide clear response profiles. Each visit will last no more than 2 hours.

On your first visit, you will be introduced to the laboratory personnel and familiarised with all the equipment used. You will be asked to complete two confidential questionnaires: the first will allow us to obtain information related to your general health; and the second will allow us to quantify your past exercise/activity involvement. You will then be asked to perform what is called a "progressive exercise test" on a cycle ergometer (exercise bike). During this test, the workload will start at a very low level and increase gradually every minute. The idea is that you keep going until you can do no more. The test will be stopped when you decide you can go no longer.

On your second visit, you will repeat the "progressive exercise test". This will allow us to determine your fitness and therefore to select the exercise levels for the remaining tests. In addition, we will ask you to take a small sample of the cells that line the inside of your cheek, with a small disposable spatula. We will subsequently analyse these cells for ACE gene function.

On the third visit, while you are lying down, a small sample of tissue (approximately 100 milligrams, 2 mm long and 2 mm diameter) will be taken from one of your thigh muscles, using a fine needle to be inserted into one of your thigh muscles. To minimise any discomfort, a local anaesthetic will be injected into the sample site prior to the sample being taken.

On each subsequent visits, the exercise testing set-up remains the same but the exercise protocol changes. Instead of the workload gradually rising constantly throughout the test, the workload will change instantaneously between one level and another - we call this "constant-load exercise". You will exercise from a period of very light exercise (almost no resistance in the pedals) to either "moderate" exercise (for no more than 10 minutes) or to "heavy" exercise (for no more than 20 minutes or until you cannot continue, whichever comes sooner). We would like you to complete a total four "moderate exercise tests, and no more than twelve "heavy" exercise tests (four at each of three different work rates)

In all tests we will ask you to breath through a "snorkel" type mouthpiece with a noseclip in place. This is so we can monitor the air you breathe in and out.

We will be taking blood from you at various times during all tests. With the use of a surgical needle, a small plastic tube (called a catheter) will be inserted into a vein on the back of your hand. This is common practice and will cause only a mild "prick" of the skin. A small tap is connected to the inserted tube, which allows repeated samples of blood to be taken without having to use a needle each time. No more than 60 ml (about 6 dessert spoons) of blood is taken in any one test.

During the test, a strapping (bandages and elastic tape) will be placed around your right thigh. This is used to keep in place two optodes (these emit and detect light). You will not feel the light emitted from the optodes. It is used to estimate the amount of oxygen in your thigh muscles. The strapping will also be used to keep in place two surface electrodes, that will be used to monitor the electrical activity in your thigh muscle. Great care will be taken when putting on the strapping and you should feel no discomfort. A similar, but much smaller, device will be placed on your finger to measure the amount of oxygen in your blood. Each of these devices are harmless. In order to calibrate the optode device on your leg, you will asked to perform a maximal contraction of your thigh muscles (lasting 10-20 seconds).

On the handlebars of the cycle ergometer is a small knob that controls an illuminated scale extending from 0% to 100%. By turning the knob, you can progressively illuminate the scale until you reach the desired point on the scale. At various points in the test, you will be asked to rate the difficulty of your breathing and the tiredness in you legs. In response to this, you will turn the knob until the illumination reaches the point you think corresponds to how you are feeling. 0% equals no sensation, and 100% equals very severe sensation.

Finally, you will not be able to consume any alcohol 48 hours prior to each lab visit. You will be excluded from participating in this study if you take drugs (recreational or performance-enhancing drugs).

What are the side effects of taking part? There are none.

What are the possible disadvantages and risks of taking part? Exercise has a negligible risk in healthy adults, although maximal exercise does carry a small risk of inducing myocardial ischaemia ("heart attack"). The primary symptom of myocardial ischaemia is chest pain on exertion. If you experience any unusual sensations in your chest during the experiment, you should cease exercising immediately. Your heart rate will be monitored via adhesive electrodes placed at points on the chest (an "electrocardiogram" or ECG). In the unlikely event you experience serious problems during the exercise, medically-qualified personnel are on call at all times during the test and approved emergency procedures are in place.

At the end of the tests you will be very tired (exhausted), your legs will be very heavy and you will be out of breath. It is also not uncommon to feel a little light-headed and sometimes nauseous. As the "constant-load" tests will last approximately 1 hour in total, it is possible that you may feel some discomfort after sitting on the bike for this long period.

You may experience difficulty swallowing while breathing through a mouthpiece and wearing a noseclip; this is due to a slight but transient pressure build up in your ears. Also, some subjects experience increased salivation while breathing through a mouthpiece.

When the catheter is removed from the back of the hand, a little bruising may occur. A small wound may also be evident. This may take a few days to heal.

When a muscle biopsy is taken, there is a small risk of inducing local pain, bruising, infection or damage to adjacent tissue These may take a few days to subside. In the event of any problem being experienced after the muscle biopsy, we recommend that you seek medical advice by either contacting your GP or Professor Wilhelmina Behan (0141-211-2206) who took the biopsy. You should also notify Professor Susan Ward (0141-330-6287), the senior principal investigator, that a problem has occurred for which you have sought medical assistance.

What are the possible benefits of taking part? The results from the exercise tests will give you a good idea of how fit you are. The research team will take the time to explain these results to you. The information from these tests will provide us with valuable information on the effect of muscle protein function on exercise performance.

What if something goes wrong? If you are harmed by taking part in this research project, there are no compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. The principal investigators, although not medically qualified, are fully trained

in Advanced Life Support. In the event of an untoward incident, the principal investigator(s) will provide basic life support including chest compressions and ventilation, and will apply an advisory defibrillator (if necessary) until emergency medical staff are on hand.

Will my taking part in this study be kept confidential? All information which is collected about you during the course of the research will be kept strictly confidential.

What will happen to the results of the research study? Results will be published in a peer-reviewed scientific journal once the study is completed. You will automatically be sent a copy of the full publication. You will not be identified in any publication.

If you are worried about any unwanted side effects from any of the above procedures, you should contact:

Professor Susan A Ward, Director
Director, Centre for Exercise Science and Medicine
Institute of Biomedical and Life Sciences
West Medical Building
University of Glasgow,
Glasgow G12 8QQ
Phone: 0141 330 6287
Fax: 0141 330 6345
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Influence of creatine kinase and mitochondrial uncoupling proteins on the control of muscle oxygen consumption during exercise in healthy human subjects (Approved: October 30, 2002; Revised Approval: January 06, 2003)

Consent Form

I .....

give my consent to the research procedures which are outlined above, the aim, procedures and possible consequences of which have been outlined to me

Signature .....

Date .....

Appendix ii

Medical Questionaire

CENTRE FOR EXERCISE SCIENCE AND MEDICINE

MEDICAL HISTORY

(CONFIDENTIAL)

Please read.

It is important to take a record of your medical history. You may have, or may have once had a condition that would make this type of testing unsuitable for you. For this reason we ask you to be as truthful and detailed as possible. At no point will this information be made available to any one other than the principal investigators for this study. If you have any doubts or questions, please ask.

SUBJECT DETAILS:

NAME:

AGE: D.O.B:

SEX (M/F):

GP NAME & ADDRESS:

SMOKING:

Never Smoked .....
Not for >6 months .....
Smoke <10 per day .....
Smoke >10 per day .....

ILLNESSES:

ALLERGIES:

HOSPITALISATIONS:

MUSCULO-SKELETAL DISORDER:
(Arthritis, Joint Pain, Fractures, Sports injury, Others)

CARDIOVASCULAR DISORDER: (Fever, Heart Murmurs, Chest Pain, Palpitations, High Blood Pressure, Others)

RESPIRATORY DISORDER: (Asthma, SOB, Cough, URTI, Others)

GASTROINTESTINAL DISORDER: (Jaundice, Bleeding, Others)

DIABETES:

CNS DISORDER: (Fits, Blackouts, Tremor, Paralysis, Epilepsy, Other)

PSYCHIATRIC TREATMENT:

FAMILY HISTORY: (Sudden death in a first degree relative under the age of 35 years)

ARE YOU CURRENTLY TAKING ANY MEDICATION? No / Yes*

(*Please specify)_____

ARE YOU CURRENTLY TAKING ANY SUBSTANCES TO HELP IMPROVE YOUR TRAINING OR CONTROL YOUR WEIGHT i.e. CREATINE, PROTEIN SUPPLEMENT? No / Yes*

(*Please specify) _____

ARE YOU CURRENTLY TAKING ANY OTHER SUPPLEMENTS i.e. FOOD SUPPLEMENTS, VITAMINS? No / Yes*

(*Please specify) _____

CAN YOU THINK OF ANY OTHER REASON WHY YOU SHOULD NOT TAKE PART IN ANY OF OUR TESTS?

_____

SYMPTOMS:

Do you experience any of the following, particularly on exercise?

Breathlessness	No / Yes
Chest Pain	No / Yes
Dizzy Fits/Fainting	No / Yes
Palpitations	No / Yes

Please note that if you feel unwell on the day of the proposed test, or have been feeling poorly over the preceding day or two, please inform the investigators and DO NOT TAKE PART in the exercise test.

DECLARATION:

I have completed this questionnaire fully and truthfully. I have not kept any information from the investigators that may put myself at risk during high-intensity exercise, or affect the results that they obtain. I understand that I may withdraw from any one test or the study as a whole if I feel unwell, or feel uncomfortable with any part of the testing procedure.

(Signature).....

(Date) .....

PHYSICAL EXAM:

WEIGHT: _____ HEIGHT: _____

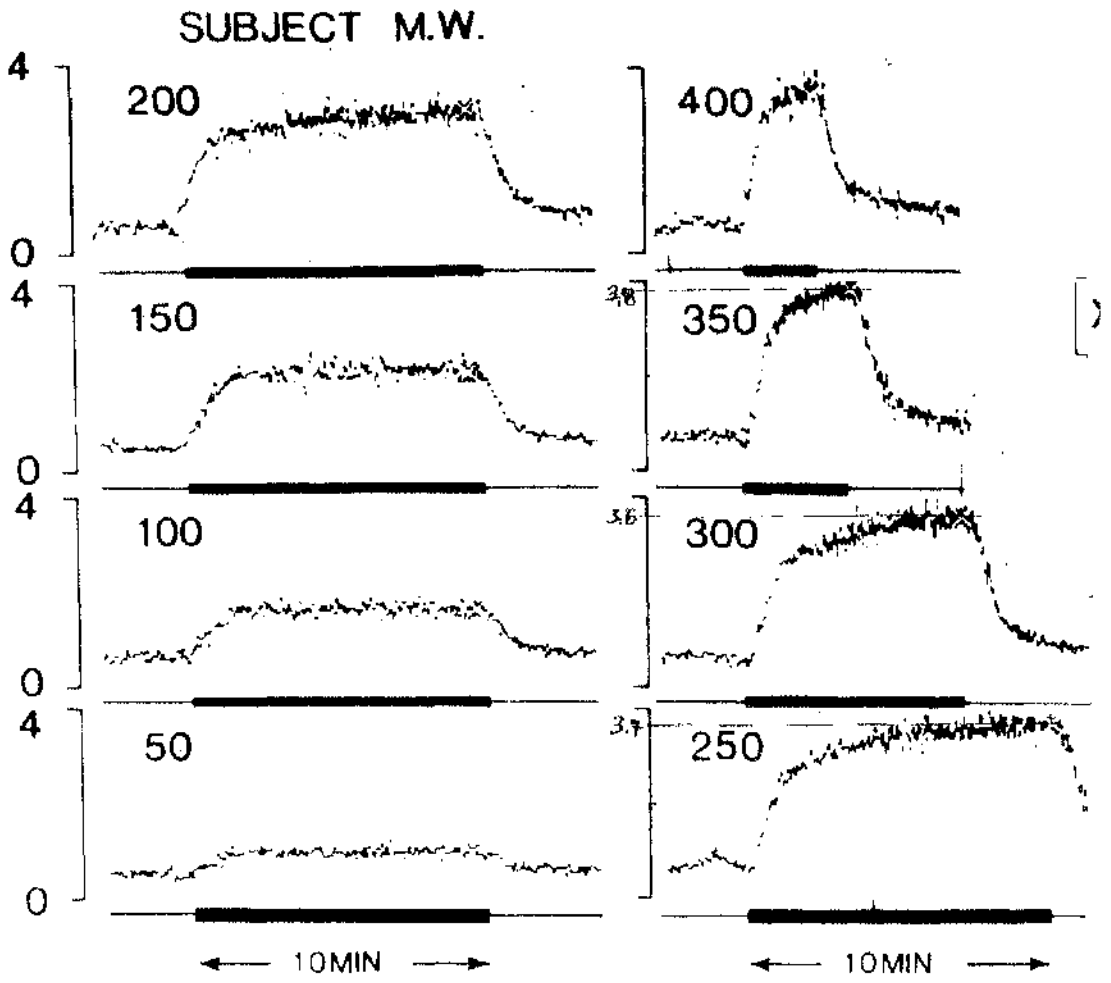
PULSE (Resting): _____ BP (Resting): _____

Screened by: .....

(Signature) ..... (Date) .....

Appendix iii

End-exercise $\dot{V}O_2$ estimation



Identification of the end-exercise $\dot{V}O_2$ at work rates above the critical power. From